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ASPECTS OF ANTHELMINTIC

RESISTANCE IN

NEMATODES OF SHEEP

This thesis is presented in partial fulfillment
of the requirements of the degree
of Master of Veterinary Science
at Massey University

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FEBRUARY 1989

ABSTRACT

The increasing prevalence of anthelmintic resistance in nematodes of sheep is causing concern among animal scientists and farmers. In Australia anthelmintic resistance has become widespread since the first case was reported in 1968, and in some districts up to 68% of farms are affected. Benzimidazole resistance is most common, but levamisole and morantel resistance also occurs, and some farms have nematodes resistant to both major anthelmintic groups. Strains of the following species have shown resistance: Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus spp. and, least commonly, Nematodirus spp.

In New Zealand, anthelmintic resistance was first reported in 1980, and subsequent surveys found that its prevalence was generally low except on farms with above average anthelmintic usage. The same anthelmintics and nematodes as in Australia were implicated, although resistant Nematodirus spp. were reported more frequently in New Zealand.

The most common test used to identify anthelmintic resistance - the faecal egg count reduction (or depression) test - was used in the survey of 15 Manawatu sheep farms which is the subject of this report. On each farm the anthelmintics oxfendazole, ivermectin and levamisole were given by mouth to groups of 12 young sheep, at dose rates of 5.0, 0.2 and 8.0 mg/kg, respectively. Twelve additional sheep were designated as untreated controls. Faecal samples for egg counting were taken from all sheep on the day of treatment and 7 days later. Composite faecal samples from each farm were cultured for identification of larvae present before treatment and, when anthelmintic treatments were deemed unsatisfactory, post-treatment faeces were also cultured for larval identification.

Using the simplest method of calculation of faecal egg count reduction, and a cut-off point of 90% or below, there were (respectively) 4,2 and 0 farms with oxfendazole, ivermectin and levamisole resistant strongylate nematodes (excluding Nematodirus spp.). However, supporting evidence for the existence of ivermectin resistant nematodes was weak, and no claim is made that true ivermectin resistance has been detected. On the 4 farms with oxfendazole resistant nematodes, resistant Trichostrongylus spp. were the most common (4 farms), followed by H. contortus (3 farms), O. circumcincta (2 farms) and Oesophagostomum spp. (2 farms).

Oxfendazole resistant Nematodirus spp. were very common, but it was not possible to reach any conclusion about the susceptibility of the Nematodirus spp. present on each farm because of the low numbers of sheep passing Nematodirus spp. eggs and the small numbers of those eggs. Resistant Nematodirus spp. have probably been overlooked in the past and methods to reduce the likelihood of this are suggested.

The faecal egg count reduction test is a useful field screening test, but it is difficult to interpret when only a slight degree of anthelmintic resistance is encountered. This is partly due to uncertainty in where the cut-off point should be, and partly to variation in calculation methods. The acceptance of recently formulated standard test protocols for Australia and New Zealand should reduce this problem, but supplementary tests will still be needed in many cases of anthelmintic resistance testing.

ACKNOWLEDGEMENTS

I wish to thank my wife Judith whose encouragement and support sustained me in many ways during the course of this work and our stay in New Zealand.

To my supervisor, Dr David West, I am grateful for his guidance and many perceptive suggestions and criticisms made throughout the planning and conduct of the survey and the writing of this thesis.

Barbara Adlington, Shirley Calder and Bill Pomroy gave me the essential help and advice that I needed in the laboratory and I am most grateful to them.

To the farmers, managers and stockmen on the farms I visited, my thanks for the friendly assistance and cooperation I always received.

I am sincerely grateful to Cheryl Cooper who typed this thesis with speed, accuracy and good humour, and also helped me on some farm visits.

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PREFACE

The increasing prevalence of anthelmintic resistant nematodes in sheep has aroused considerable interest and concern, particularly in Australia and New Zealand. An almost total reliance on anthelmintics for the control of internal parasites means that a decline in the efficacy of these normally highly effective compounds could have serious consequences for the profitability of a sheep enterprise. Such a decline in efficacy can occur when nematodes become resistant to the effects of anthelmintics.

The work described in the following report was undertaken primarily to determine the extent and degree of anthelmintic resistance on sheep farms in a limited area of New Zealand (the Manawatu), and to identify the nematode genera and anthelmintics involved. A secondary aim was to develop a practical, inexpensive and uncomplicated method for veterinary clinicians to test flocks for the presence of anthelmintic resistant nematodes. There is an obvious need for greater uniformity in the conduct of these tests so that results can be compared and changes in prevalence and degree of anthelmintic resistance noted.

CHAPTER 1

REVIEW OF THE LITERATURE

INTRODUCTION

DEFINITION OF ANTHELMINTIC RESISTANCE

ANTHELMINTICS AND THEIR MODES OF ACTION

THE OCCURRENCE OF ANTHELMINTIC RESISTANCE IN SHEEP

CAUSES OF ANTHELMINTIC RESISTANCE

REVERSION - RETURN TO ANTHELMINTIC SUSCEPTIBILITY

DIAGNOSIS OF ANTHELMINTIC RESISTANCE

STRATEGIES TO MINIMISE THE IMPACT OF ANTHELMINTIC RESISTANCE

INTRODUCTION

Because modern broad spectrum anthelmintics are safe, highly effective and relatively cheap, farmers have relied almost entirely on them to limit the ill effects caused by nematodes in their sheep. Frequent use of anthelmintics, often combined with specific recommended management practices, has given very good control of nematodes. However an undesirable side-effect of this control procedure has emerged in recent years: the increased prevalence of anthelmintic resistant nematodes (Prichard et al., 1980; Donald, 1983; Martin, 1987).

In this review of the literature, the story of anthelmintic resistance in sheep nematodes is traced from the early scattered reports to the present time. The existence and importance of anthelmintic resistance is now generally accepted and research has turned toward methods of circumventing the problem of resistance while still using the best means of nematode control - the anthelmintics. Other possible methods of helminth control are also mentioned as these may, in the long term, replace anthelmintics.

Methods of testing for anthelmintic resistance are discussed and compared. These have been widely, but not uniformly, applied in the monitoring of helminth control programmes and in surveys to determine the prevalence of resistant nematodes.

DEFINITION OF ANTHELMINTIC RESISTANCE

"Anthelmintic resistance is the ability of an individual to survive the lethal effects of a chemical and is the result of selection acting upon the genetic variation within the population." (Martin, 1987)

Side resistance is present when a nematode displays resistance to a different anthelmintic with the same mode of action as that of the anthelmintic against which resistance developed originally (Prichard et al., 1980). Side resistance is usual among the benzimidazole group, although the degree of resistance displayed by one nematode strain to each benzimidazole compound may vary (Hotson et al., 1970; Theodorides et al., 1970; Berger, 1975; Hall et al., 1978a and 1978b; Boersema et al., 1982).

Side resistance also occurs between levamisole and morantel (Le Jambre and Martin, 1979; Whitlock et al., 1980a), but it may not be reciprocal: a levamisole resistant nematode strain will also be morantel resistant, but a morantel resistant strain may be susceptible to levamisole (Martin, 1988a).

Cross resistance is said to be present when a nematode is resistant to an anthelmintic with a different mode of action from that against which the nematode originally became resistant (Prichard et al., 1980). Cross resistance is rare, but a case has been reported where thiabendazole resistant Trichostrongylus colubriformis (T. colubriformis), Ostertagia spp. and Haemonchus contortus (H. contortus) showed apparent cross resistance to phenothiazine when low daily doses of phenothiazine to sheep did not completely inhibit hatching of nematode eggs. (Kelly et al. 1981a and 1981b)

Multiple resistance occurs when a nematode shows resistance to more than one anthelmintic group, either as a result of cross resistance or, usually, as a result of selection by each anthelmintic group (Prichard et al., 1980). It is incorrect to use the term, multiple resistance, to describe the situation where two or more nematode genera are resistant to one anthelmintic group (Charleston, per. comm.).

ANTHELMINTICS AND THEIR MODES OF ACTION

When buying an anthelmintic, sheep farmers have many commercial products from which to choose: in Australia there are more than 25 and in New Zealand more than 34 such products, although many of these are simply different brands containing the same active ingredient.

The properties, uses and modes of action of anthelmintics have been reviewed by numerous authors (Kelly et al., 1976; Rew, 1978; Arundel, 1985a and 1985b; Behm and Bryant, 1985; Bogan and Armour, 1987; Prichard, 1987).

The mode of action of an anthelmintic is important in relation to the occurrence of resistance. Table 1.I lists the anthelmintics available in Australia and New Zealand, grouped according to their modes of action and spectrum of activity (Arundel, 1985a and 1985b).

"Broad (or wide) spectrum" is a general and imprecise term used to describe those anthelmintics which are effective against many gastrointestinal nematodes and also, in some cases, against cestodes, trematodes and nematodes in other organs. Narrow spectrum anthelmintics, in contrast, are effective against only one or two internal parasites, particularly H. contortus or Fasciola hepatica.

Group 1 anthelmintics (tubulin binding)

These are the benzimidazoles and probenzimidazoles. Thiabendazole was the first of this group (Brown, et al., 1961). These compounds bind to tubulin in the cytoplasm of intestinal cells of susceptible nematodes, thus preventing polymerisation of tubulin to form microtubules (Borgers et al., 1975). Susceptible nematodes usually die of starvation, due to reduced glucose uptake, but they may survive if the exposure to an adequate level of drug is less than 20-24 hours (Prichard, 1978b; Le Jambre, 1985a). The benzimidazoles are also potent inhibitors of egg hatching (Southcott, 1963).

Group 2 anthelmintics (ganglion blocking agents)

This group contains two chemically dissimilar compounds, levamisole and morantel, which both act on nematode ganglia but in different ways as described by Arundel (1985b). Levamisole stimulates ganglia, causing sustained muscle contraction and reversible paralysis of rapid onset. While paralysed, the worms are expelled; an increase in the host's bowel motility caused by levamisole assists in the removal of paralysed worms in 24 to 36 hours after treatment. Anthelmintics in this group are not ovicidal (Prichard, 1978a).

Levamisole also paralyses nematode larvae, and this effect is utilized for the in vitro test for levamisole resistance described by Martin and Le Jambre (1979) and refined

by Dobson et al., (1986).

Like levamisole, morantel causes paralysis of adult and larval nematodes, but does so by depolarising the nematode's neuromuscular system (Coles et al., 1974).

Table 1.I

Anthelmintics grouped according to mode of action (after Arundel, 1985b)

GROUP 1	Mode of action:	Tubulin binding	
	Spectrum:	Wide	
	Members:	Thiophanate Febantel Netobimin*	pro-benzimidazoles
		Thiabendazole Albendazole Fenbendazole Mebendazole Oxfendazole Rycobendazole	benzimidazoles
GROUP 2	Mode of action:	Ganglion blocking	
	Spectrum:	Wide	
	Members:	Levamisole Morantel	
GROUP 3	Mode of action:	Acetylcholinesterase antagonist	
	Spectrum:	Narrow	
	Members:	Dichlorvos Trichlorphon Napthalophos	
GROUP 4	Mode of action:	Uncouple oxidative phosphorylation	
	Spectrum:	Narrow	
	Members:	Oxyclozanide Bromsalans Nitroxynil Niclosamide Closantel Rafoxanide	
GROUP 5	Mode of action:	Gamma aminobutyric acid agonist	
	Spectrum:	Wide	
	Members:	Ivermectin	

* Netobimin (Hapadex[R], Schering Corporation) has been recently released for commercial use in Britain and the United States (Richards et al., 1987a and 1987b) but is not available in Australia or New Zealand. Although not itself a benzimidazole, it is metabolised in vivo to albendazole so is classified as a probenzimidazole (Prichard, 1987).

Group 3 anthelmintics (acetylcholinesterase antagonist)

These are organophosphate compounds which act by inhibiting acetylcholinesterase. The degradation of the neuromuscular transmitter acetylcholine is prevented, resulting in continual stimulation of the nematode's nerve endings or muscle (Rew, 1978).

Members of this group are narrow spectrum compounds, and currently little used in the sheep industry in Australia or New Zealand.

Group 4 anthelmintics (uncouple oxidative phosphorylation)

Members of this group - substituted phenols and salicylanilides - uncouple mitochondrial reactions involved in electron transport and associated phosphorylation (Van den Bossche, 1972). They are effective against cestodes and trematodes, but not nematodes and are therefore classified as narrow spectrum anthelmintics.

Group 5 (gamma aminobutyric acid [GABA] agonist)

Ivermectin is the only commercially available member of this group of broad spectrum anthelmintics. Campbell (1981) concluded from a series of earlier studies that the avermectins stimulate the release of GABA in nerve synapses and enhance its binding to post synaptic receptors, thus resulting in paralysis of nematodes and arthropods, but not trematodes or cestodes.

Ungrouped

Phenothiazine was the first broad spectrum anthelmintic to be introduced (Gordon, 1945). It is thought to interfere with the anaerobic energy metabolism of nematodes and is ovicidal (Prichard, 1978a). Forsyth et al., (1961) reported that particle size and chemical purity affected the activity of the drug. Phenothiazine was released in 1938 and was gradually superceded by the benzimidazoles in the 1960's (Le Jambre, 1978); it is now rarely used except as a low concentration component of molasses blocks ("Wormolas", Animeals Pty. Ltd. N.S.W.) in which only its ovicidal activity is utilised.

THE OCCURRENCE OF ANTHELMINTIC RESISTANCE IN SHEEP

Australia

Although the first reports of anthelmintic resistance in sheep originated from the United States of America and Brazil (Drudge et al., 1957; Drudge et al., 1964; Santos and Franco, 1967), it now seems, judging by the number of publications on the subject, that anthelmintic resistance is most common and serious in Australia and New Zealand. This may however be an incorrect assumption and simply reflect a greater interest in anthelmintic resistance in these countries (Waller, 1985a).

Phenothiazine was widely used in Australia after its release in 1938, and it was known that resistance could occur (Drudge et al., 1957), but there have been no Australian reports of phenothiazine resistance (Le Jambre, 1978).

Thiabendazole was released in Australia in 1962 (Arundel 1985a) and resistance to it, by H. contortus on 3 farms in northern New South Wales, was discovered within a few years (Smeal et al., 1968). All 3 farms had been experiencing sheep deaths from Haemonchosis despite recent drenching with thiabendazole.

Most of the early reports of anthelmintic resistance concerned research farms or research projects in which drenching frequency was high. Trichostrongylus spp. resistant to benzimidazoles were detected on 2 research farms near Sydney where thiabendazole had been used, every 3 or 4 weeks, for several years (Hotson et al, 1970). On another research farm, on the Northern Tablelands of New South Wales, thiabendazole resistant H. contortus, O. circumcincta and T. colubriformis were identified. Thiabendazole had been used frequently in sheep on parts of this farm (Le Jambre et al., 1976, 1977 and 1978a).

Two trials, designed to compare the merits of various drenching frequencies on sheep production, resulted also in the appearance of drench resistant nematodes. In southern New South Wales Donald et al., (1980) detected oxfendazole resistance in O. circumcincta and, in South Australia, Martin et al., (1982) reported the development of thiabendazole resistance in Ostertagia spp., over the 3 years of their trial.

In a Victorian trial in which various drenching frequencies with thiabendazole (44 mg/kg) had been used with the primary aim of assessing the effect on the development of drench resistance, (rather than animal performance), H. contortus, highly resistant to thiabendazole, appeared after one year in the groups drenched 9 and 52 times. A small proportion of resistant H. contortus was thought to have been present at the beginning of the trial in December 1979 (Barton, 1980 and

1983).

Resistance to the probenzimidazole, thiophanate, by H. contortus was reported on a farm in Western Australia. Benzimidazoles had been used on the farm "for many years" to control Haemonchosis, but thiophanate had been given only 3 times, over a 6 month period, to all the sheep. Resistance was discovered when the authors investigated continuing weight loss and death of lambs following thiophanate drenching. Side resistance to thiabendazole was not exhibited by the thiophanate-resistant H. contortus on this farm (Edwards and de Chaneet, 1980).

As with benzimidazole resistance, the early cases of levamisole or morantel resistance (or both) were usually associated with a research station or research project. Le Jambre and Martin (1979) reported the field occurrence of levamisole and morantel resistant O. circumcincta on the research station on the Northern Tablelands of New South Wales where they had previously selected - in the laboratory -strains of H. contortus, O. circumcincta and T. colubriformis with slight resistance to levamisole or morantel or both.

Barton (1983) found that some Ostertagia spp. had become highly resistant to levamisole at the end of a 3 year drenching frequency trial. In this trial thiabendazole had been replaced by levamisole after the first year because thiabendazole resistance had developed in H. contortus. Resistance to levamisole was high in Ostertagia spp. from sheep which had been drenched either 49 or 11 times annually for 2 years, and resistance was moderate in Ostertagia spp. from sheep drenched 5 times a year for 2 years.

By the late 1970's, reports had appeared of nematodes showing multiple anthelmintic resistance. On a farm on the Southern Slopes of New South Wales, Hall et al. (1979) identified a strain of O. circumcincta which was not only resistant to thiabendazole but also to levamisole. Treatment with levamisole gave erratic egg count reductions and only 94% removal of adult Ostertagia spp.; the larvae also displayed moderate tolerance to a levamisole paralysis test. Nematodes with multiple resistance were also detected on a farm near Sydney and one in Queensland. On the former farm, both T. colubriformis and O. circumcincta were resistant to levamisole, morantel and (slightly) to thiabendazole (Sangster et al., 1979 and 1980; Whitlock et al., 1980a). On the latter farm, H. contortus was resistant to a range of benzimidazoles, levamisole and morantel, and slightly resistant to napthalophos (Green et al., 1981).

With the increasing number of reports of anthelmintic resistance which had developed under experimental conditions or under isolated commercial conditions, the curiosity of many workers was aroused regarding the prevalence of anthelmintic

resistance in various areas of Australia. Beginning in the late 1970's field surveys for anthelmintic resistance were conducted in New South Wales, Victoria and Western Australia.

The first survey, of 40 farms on the Northern Tablelands of New South Wales, used only thiabendazole as a test anthelmintic. Resistant H. contortus were detected on 22 farms (55%), but this may have been an under-estimate, as the dose rate of thiabendazole used (66 mg/kg) was 1.5 times the recommended therapeutic rate (Webb et al., 1979).

In the west and southwest of Victoria, 132 sheep farms were surveyed. Twenty eight of the farms were selected on the basis of one or more of the following criteria: suspected anthelmintic failure; consistent, frequent long-term use of benzimidazoles; high stocking rate (over 13 dry sheep equivalents per hectare). On these selected farms only thiabendazole (44 mg/kg) was tested. The remaining 104 farms were randomly chosen and on them both thiabendazole (44 mg/kg) and levamisole (8 mg/kg) were tested. Unfortunately, pretreatment mean faecal egg counts on most of the farms were less than 100 eggs per gram so faecal egg count reduction tests were conducted on only 17 of the 28 selected farms and 10 of the 104 randomly chosen farms. On 5 of 17 selected farms and 3 of the 10 randomly chosen farms thiabendazole resistance was diagnosed because faecal egg count reductions were below 90%. The benzimidazole resistant nematode on 2 of the selected farms was Teladorsagia (Ostertagia) circumcincta. No levamisole resistance was detected on any farm. The researchers concluded that "anthelmintic resistance was not of practical importance to the majority of sheep producers in the region." (Riffkin et al., 1984).

Cameron et al., (1984) disputed the conclusion of Riffkin and co-workers and interpreted their results instead as showing that anthelmintic resistance already constituted "...a formidable problem on perhaps 30% of farms." Benzimidazole resistance was present in approximately 30% of the flocks of the Victorian clients of Cameron and co-workers and resistance to levamisole, and to both levamisole and benzimidazoles, had also been detected.

In a survey of 116 randomly selected sheep farms in the south west of Western Australia, both thiabendazole (44 mg/kg) and levamisole (7.5 mg/kg) were used as test anthelmintics (Edward et al., 1986a). Tests were completed on 86 farms and 68% of these had resistant worm populations. Thiabendazole resistance was found in Trichostrongylus, Teladorsagia (Ostertagia) and Haemonchus contortus; levamisole resistance was found in Teladorsagia (Ostertagia), Trichostrongylus, Haemonchus contortus and Nematodirus. Multiple resistant populations were found on 18% of farms.

A survey of 46 farms in the Gippsland, Central District and Western Plains district of Victoria, found that anthelmintic resistance was present on 29 farms (63%) (Vizard, 1986). This prevalence is comparable with that reported by Edwards et al., (1986a) from Western Australia, but is much higher than levels found earlier in Victoria by Riffkin et al., (1984) and even higher than that reported by Cameron et al., (1984). Benzimidazole resistance predominated on the 29 affected farms. Teladorsagia (Ostertagia) circumcincta was the most common resistant species, and it was the only species showing resistance to levamisole. Two cases of Trichostrongylus spp. and one of Nematodirus spp. resistant to thiabendazole were detected and one farm had multiply resistant Trichostrongylus spp. It was concluded, not surprisingly, that anthelmintic resistance was widespread in Victoria and that there was a high prevalence of affected farms.

Forty farms in the Central Tablelands of New South Wales were surveyed for anthelmintic resistance, using both thiabendazole (44 mg/kg) and levamisole (8 mg/kg) (Webb and Ottaway, 1986). On 3 farms the mean pre-drench faecal egg counts were less than 30 e.p.g., but of the remaining 37, thiabendazole resistant nematodes were present on 21 (57%), levamisole resistant nematodes on 4 others (11%), and nematodes resistant to both anthelmintics on a further 4 farms (11%). Ostertagia spp. and Trichostrongylus spp. were the predominant resistant species. H. contortus burdens were present on only 8 of the 37 farms, but 3 of the 8 showed resistance to thiabendazole. Nematodirus spp. were present on all farms but thiabendazole resistant Nematodirus spp. were suspected (but not proven) on only one farm.

There seem to be no published reports of surveys for anthelmintic resistance on sheep farms in the states of Queensland, South Australia or Tasmania. However, the presence of multiple resistant H. contortus in Queensland (Green et al., 1981), thiabendazole resistant Ostertagia spp. in South Australia (Martin et al., 1982), and the widespread occurrence of benzimidazole resistant Nematodirus spp. in Tasmania (Obendorf, pers. comm.), all indicate that the prevalence of anthelmintic resistance in these states is likely to be similar to that reported from comparable areas elsewhere in Australia.

New Zealand

As in Australia, there have been no confirmed cases of phenothiazine resistance in New Zealand. Sinclair (1953), in response to "...unsubstantiated complaints (of resistance) from farmers and Field Officers of the Department of Agriculture" attempted to cause the development of phenothiazine resistance in T. colubriformis but he failed. He concluded that the dose of 20g of phenothiazine, which was commonly given to lambs, was simply too low and that underdosing, and not resistance, was the reason for phenothiazine's poor activity against T. colubriformis. In addition Douglas et al., (1959) found that commercially available phenothiazine was less active than pure phenothiazine.

The first case of anthelmintic resistance in New Zealand was reported in 1980, 18 years after the commercial release of thiabendazole, and 12 years after the first report in Australia. Resistance was detected in H. contortus during the course of a parasite control trial using albendazole. The nematode was resistant to both albendazole and thiabendazole (Vlassoff and Kettle, 1980).

Beginning shortly after the first report of resistance, a national survey and two restricted surveys were undertaken to ascertain the prevalence of anthelmintic resistance in New Zealand. In the national survey of 97 farms, thiabendazole (66 mg/kg) and levamisole (8 mg/kg) were used, and a diagnosis of resistance made if a faecal egg count depression was less than 90% (Kettle et al., 1981). Only 5 farms were considered to have resistant nematodes and of these farms 4 had Trichostrongylus spp. resistant to levamisole and one had Haemonchus spp. resistant to thiabendazole. This may have underestimated resistance to thiabendazole because the dose rate used was 50% greater than the recommended dose rate. The researchers compared these findings with those which had been recently published in Australia by Webb et al., (1979), and concluded that anthelmintic resistance in New Zealand sheep was not a severe problem, although the methods used in the New Zealand survey "...could miss low levels of resistance."

In a North Island survey, an in vitro egg hatch technique (Whitlock et al., 1980b) was used by which only benzimidazole resistance in strongylate nematodes (other than Nematodirus spp.) could be detected (Kemp and Smith, 1980 and 1982). Of 52 farms surveyed, benzimidazole resistant H. contortus or Trichostrongylus spp., or both, were detected on 11 (21.2%).

Vlassoff and Kettle (1986), believing that the likelihood of anthelmintic resistance was greater with more frequent use of anthelmintics, surveyed 37 farms on which lambs received 7 or more doses of anthelmintic in their first year, compared with the average frequency in New Zealand of 6.3 (Brunsdon et al., 1983). Resistant nematodes were present on 7 farms (19%). Six of these had benzimidazole resistant Haemonchus, Nematodirus or Trichostrongylus spp. present, and one had levamisole resistant Ostertagia spp.

During the period that surveys for anthelmintic resistance were being undertaken in New Zealand, and afterwards, there were also reports of anthelmintic resistance resulting in clinical disease on individual farms, and many veterinary practitioners were encountering the problem although few reported their findings.

Benzimidazole resistant Ostertagia spp. were found to be responsible for poor growth and diarrhoea in a group of young rams on a North Island farm. It was believed that high drench usage, a high concentration of young animals and set stocking of (largely) one class of animal had predisposed to the development of resistance (Hughes and Seifert, 1983).

Nematodirus spathiger (N. spathiger) resistant to oxfendazole was identified on a Hawkes Bay farm. Benzimidazole anthelmintics had been used exclusively and at high frequency in sheep on this farm for the previous 6 years (Middleberg and McKenna, 1983). In the same area of New Zealand benzimidazole resistant Nematodirus had been recognised on other farms in the summer and autumn (Quinlivan and Middleberg, 1983). In 1984, Mason reported the identification of benzimidazole resistant Nematodirus spp. on 2 farms in Otago and Southland, and recently it was stated that Nematodirus spp. were the most common anthelmintic resistant parasites in South Island sheep (Rutherford, 1988).

A strain of H. contortus resistant to the probenzimidazole, thiophanate, but susceptible to albendazole, was identified on a farm where thiophanate had been used exclusively for the previous summer (1983/84). Prior to that time a variety of anthelmintics had been used, including albendazole. As with the earlier reports, anthelmintic resistance was revealed on this farm during an investigation into poor performance and deaths in sheep despite apparently adequate anthelmintic treatment (Pomroy et al., 1985).

By 1986 there had been 21 instances of benzimidazole resistance reported in sheep, involving Haemonchus, Nematodirus, Ostertagia and Trichostrongylus, and only two instances of levamisole resistance, involving Haemonchus, Ostertagia and Trichostrongylus (Vlassoff and Kettle, 1986). Ivermectin resistance had not been reported but it had been available in New Zealand for use in sheep only since October 1982 (McPherson pers. comm.).

From the reports cited, it is clear that anthelmintic resistant nematodes are relatively common in New Zealand sheep and that they are sometimes responsible for illness and death of sheep. The number of farms affected by anthelmintic resistance is increasing.

South Africa

Anthelmintic resistance in South Africa has mostly been reported in strains of Haemonchus contortus. Benzimidazole resistance is most common (Berger, 1975; Van Schalkwyk, 1984), but there have also been reports of H. contortus resistant to rafoxanide (Van Wyk and Gerber, 1980), closantel (Van Wyk et al., 1982) and ivermectin (Carmichael et al., 1987; Van Wyk and Malan, 1988).

Ostertagia spp. resistant to benzimidazoles were identified on two farms (Van Schalkwyk et al., 1983). Although the efficacies of levamisole and morantel against adult Ostertagia spp. from one of those farms were only 53.9% and 87.0% respectively, it has been stated recently that levamisole resistance has not been proven in South Africa (Van Wyk and Malan, 1988).

The United States of America

As previously noted, the first cases of both phenothiazine and thiabendazole resistance were detected in the United States of America (Drudge et al., 1957; Drudge et al., 1964) but, since then, reports of anthelmintic resistance have not been numerous.

During the course of a trial comparing anthelmintics, thiabendazole resistant H. contortus were detected in sheep after only 3 treatments, at monthly intervals, with thiabendazole (44 mg/kg). After 6 treatments, at monthly intervals, thiabendazole was having no effect on H. contortus worm numbers or egg output (Drudge et al., 1964). This rapid development of such a high degree of resistance has not been equalled in any subsequent reports either in the United States or elsewhere in the world. Although other research using thiabendazole in sheep had been conducted on the same research station, Drudge and co-workers stated that their trial sheep could not have been infected with resistant nematodes at the commencement of the trial and that "...the selection (for resistance) was effected by 3 drenches".

Perhaps the rapid development of resistance which occurred was related to the dose rate of thiabendazole which was used (44 mg/kg). This dose rate may be inadequate for H. contortus, even though it was commonly used and recent authorities have recommended it for sheep (Arundel, 1983 and 1985b; Prichard, 1978a). However Hebden (1961) and Smeal et al. (1968) recommended a dose rate of 50 mg/kg, and Conway (1964) considered that 80 mg/kg was the necessary dose rate of thiabendazole to treat severe Haemonchosis.

Benzimidazole resistant H. contortus strains are widely distributed in the United States, with occurrences in Kentucky, Massachusetts, New Mexico, Oregon and Texas (Theodorides et al., 1970; Andersen and Christofferson, 1973; Coles et al., 1986;

Resistance to levamisole and morantel was reported in strongylates (species not identified) after sheep were drenched with these anthelmintics at intervals of 2 weeks for a period of 5 months (Le Marie et al., 1987).

Other Countries

Only benzimidazole resistance has been reported in Great Britain - in O. circumcincta and H. contortus. In one survey, 7 of 52 farms (13.5%) in the south-east of England had benzimidazole resistant H. contortus (Britt, 1982; Cawthorne and Whitehead, 1983; Cawthorne and Cheong, 1984).

In Switzerland, resistance to benzimidazoles has been reported in strains of H. contortus and T. colubriformis (Jordi, 1980).

In Brazil, strains of H. contortus have been identified which are resistant to thiabendazole (Santos and Franco, 1967) and levamisole (Santiago et al., 1978).

Benzimidazole resistant H. contortus is the predominant resistant nematode in The Netherlands, but small numbers of benzimidazole resistant Ostertagia spp. and Trichostrongylus spp. have also been detected. A survey, using an in vitro egg hatch assay, found benzimidazole resistant nematodes on 28 of 59 farms (49%) but these results are not comparable with those of most other surveys which use the in vivo method of faecal egg count reduction (Boersema et al., 1982; Eysker et al., 1983; Boersema et al., 1987).

CAUSES OF ANTHELMINTIC RESISTANCE

The development of resistance can be viewed from 3 different points of view: those of a geneticist, a toxicologist or a farmer (McKenzie, 1985). Research into anthelmintic resistance similarly falls into one of 3 (overlapping) categories of genetics, toxicology or biochemistry, and field or operational factors.

A detailed discussion of the genetic background to anthelmintic resistance was given by Kelly and Hall (1979a and 1979b). Original nematode populations invariably contain a few individuals able to survive any anthelmintic. When anthelmintic treatment has killed all the susceptible nematodes in sheep, their faeces will contain only eggs of the surviving nematodes. Therefore, a small proportion of the infective larvae subsequently ingested with pasture will have inherited an ability to survive the anthelmintic. If treatment of the sheep continues using the same, or a related anthelmintic, then the proportion of infective larvae which have originated from resistant nematodes in the sheep will steadily increase.

Development of resistance in a population will occur rapidly if resistance is controlled by a single major gene, and more slowly if the resistance is polygenic. The rate of development of resistance in the worm population is however also influenced by frequency of anthelmintic treatment and persistence of the anthelmintic (Le Jambre, 1985a).

From a biochemical viewpoint, anthelmintic resistance is an inevitable consequence of chemotherapy: the chemotherapeutic agents are the anthelmintics which have selective toxicity for nematodes. An understanding of the biochemical basis of resistance - beyond the identification of the anthelmintic's mode of action - could lead to manipulation of anthelmintic structure to overcome biochemical changes in the nematode population associated with resistance (Lacey, 1985).

Farmers and field researchers have described the causes of anthelmintic resistance in broader terms than have geneticists or biochemists, although the underlying causes of resistance must be both genetic and biochemical. From a field, or operational viewpoint there seem to be two major causes of anthelmintic resistance: high drenching frequency and under-dosing of anthelmintic.

Frequency of Anthelmintic Treatment

The work of Johnstone et al., (1976, 1979) convinced many farmers that production per sheep, and especially wool production, increased in direct proportion to the number of drenches given. It is now clear however that frequent drenching at intervals close to nematode prepatent periods (3-4 weeks) is one of the most important factors in the development of anthelmintic resistance (Donald, 1983). Field trials showed a direct relationship between drenching frequency and rate of development of resistance (Barton 1980 and 1983; Martin et al., 1982 and 1984). A survey of flocks in Western Australia showed

this same relationship: resistance was more prevalent in flocks where more drenches were given (Edwards et al., 1986b). It is impossible however to predict the rate of development of resistance in different worm populations given the same rate of anthelmintic administration, because of differences in nematode population dynamics (Martin, 1985a).

Although frequent (suppressive) drenching is most likely to predispose to the development of drench resistance, it is also possible that some control strategies, such as "summer drenching", treating-and-shifting, and peri-parturient treatment of ewes, which all rely on infrequent but carefully timed treatments, may also increase the probability of anthelmintic resistance.

On the basis of his epizootiological finding, Anderson (1972 and 1973) suggested that all sheep in winter rainfall areas of temperate Australia should be given two anthelmintic drenches in summer. Economic analyses (Anderson et al., 1976; Morris et al., 1977) supported Anderson's recommendations and the practice, termed summer drenching, has been widely recommended to farmers in appropriate areas of Australia (Edwards et al., 1986b; Dash 1988; Napthine and Callinan, 1983). The practice has, however, been suspected of selecting for resistant nematodes (Prichard et al., 1980), and these suspicions were reinforced when it was shown that the rate of development of resistance varies inversely with the proportion of the worm population in "refugia", (or places where anthelmintic treatment is ineffective), at the time of treatment (Martin et al., 1981). In a hot, dry summer, relatively few infective larvae, other than Nematodirus spp., survive on pasture (in refugia) and this is the reason for the success of summer drenching (Anderson, 1972). In support of summer drenching, Martin (1988a) considered that it caused little selection for resistance, provided the anthelmintic used is highly efficient, because any larvae that survive the summer will have originated from eggs passed in the spring - before the summer treatments. Although under suspicion, the role of summer drenching in the development of anthelmintic resistance is unproven (Donald, 1983).

Another system of helminth control, which involves limited anthelmintic usage and which may predispose to resistance, is the strategy of drenching with anthelmintic and moving sheep immediately to pasture of low infectivity (Le Jambre, 1978; Michel et al., 1983). In benzimidazole resistant Ostertagia spp. the level of resistance increased when sheep were drenched with a benzimidazole anthelmintic and moved to clean pasture (Martin et al., 1985; Martin, 1987). It is likely that a programme of anthelmintic treatment followed by a transfer of sheep to clean pasture and repeated 3 or 4 times in a year, would select for helminth resistance as strongly as monthly anthelmintic treatment of set-stocked sheep (Prichard et al., 1980).

The practice of drenching ewes with an anthelmintic shortly before or after lambing, (or at both times), with the aim of reducing pasture contamination for their lambs, is reasonably common. Although its value has been questioned (Brunsdon and Adam, 1975; Anderson et al., 1978; Dash, 1988) in some circumstances it has been shown to be beneficial (Brunsdon and Vlassoff, 1985). When peri-parturient drenching is effective, however, it is also likely to increase the prevalence of resistant nematodes (Michel, 1982).

Anthelmintic Dosage

Various extension programmes have emphasized to farmers the importance of giving the correct dose of anthelmintic to sheep. High dose rates will retard the development of resistance if used infrequently, but not necessarily if used frequently, for suppression (Donald, 1983; Anderson, 1985).

The amount of anthelmintic to which nematodes in the gastrointestinal tract are exposed depends on the dose the farmer decides to give the sheep, the amount successfully administered and the effectiveness of its absorption.

All labels of drench containers indicate the dose rate of their contents. For correct dosing, farmers must know the weights of their sheep and, when weights are known, be able to calculate the correct dose volumes. It seems that farmers are often mistaken in both weight estimations and calculations of dose volume. In a survey in Western Australia, 86% of farmer estimates of sheep body weights were below the correct weights and when given the sheep's weight, nearly 30% of farmers calculated a dose volume 10% or more different from the correct dose (Besier and Hopkins, 1988).

An obvious but often overlooked cause of under-dosage is faulty equipment or drenching technique. Drench gun faults, such as weak return springs, faulty inlet valves, faulty assemblies, inaccurate calibration and air in the barrel or tubing, as well as failure to shake drench containers sufficiently to re-suspend some anthelmintics have all lead to under-dosing of sheep (Napthine and Callinan, 1983).

Even when the required volume of anthelmintic is correctly delivered into a sheep's mouth, closure of its oesophageal groove could still result in an inadequate concentration of anthelmintic in the intestinal tract and bloodstream. The oesophageal (or reticular) groove in young suckling ruminants closes when the sucking reflex is initiated, and diverts milk directly into the abomasum (Church, 1979). The reflex closure of the groove is necessary for normal growth in suckling lambs (Lawlor et al., 1971) but redundant in older sheep, although it may still occur in a significant proportion of them at drenching (Prichard and Hennessy 1981). The significance of the oesophageal groove in anthelmintic resistance relates to the variable efficacy of some anthelmintics, depending on whether they are swallowed in to the rumen or pass directly to the abomasum (Kelly et al., 1976). Phenothiazine, thiabendazole and levamisole are equally effective, regardless of whether

they are swallowed into the rumen or abomasum (Gibson, 1980). In contrast, the efficacies of fenbendazole and parbendazole (Kelly et al., 1977) and oxfendazole (Prichard and Hennessy, 1981) against thiabendazole resistant H. contortus or T. colubriformis were found to be diminished if the anthelmintic passed directly to the abomasum. The efficacy of the probenzimidazole, thiophanate, is also markedly reduced if it is not swallowed into the rumen (Arundel, 1983).

To avoid stimulating oesophageal groove closure, paste or bolus formulations of anthelmintic may be used (Prichard and Hennessy, 1981). When using liquid formulations the dose volume should not exceed 10 ml (Ross, 1936) and it should be deposited at the rear rather than the front of the mouth (Watson, 1944) and squirted quickly into the mouth (Gibson, 1980). Intraruminal injection of oxfendazole, as used in cattle, eliminates entirely the risk of oesophageal groove closure (Bairden et al., 1983), but this method of anthelmintic administration in sheep is probably impractical (Hotson, 1985).

REVERSION - RETURN TO ANTHELMINTIC SUSCEPTIBILITY

Most farmers and researchers now accept that totally new anthelmintics will, in future, appear only rarely in the marketplace. Consequently there is interest in whether resistance to a currently available anthelmintic group will "go away" if a farmer stops using all members of that group for a period of time, thus allowing eventual re-use of the anthelmintics with their original high efficacy restored. This is probably a vain hope, judging by evidence, reviewed by Martin (1987), from a small number of field observations and a slightly greater number of experimental observations.

In most cases, reversion of a resistant strain of helminth to susceptibility - after use of the responsible anthelmintic group has ceased - has either not occurred during the period of observation, or has only occurred slowly over 5 or more nematode generations (Le Jambre et al., 1978a; Kelly and Hall, 1979b; Prichard et al., 1980; Le Jambre, 1982; Le Jambre, 1985a; Martin et al., 1988a). Even if resistance levels do decline, they are likely to rise again when use of the offending anthelmintic resumes, as occurred within 3 nematode generations with a strain of H. contortus resistant to benzimidazoles (Kelly and Hall, 1979b).

Attempts have been made to hasten the normally slow return of anthelmintic susceptibility by selection with another anthelmintic. Selection with levamisole on a benzimidazole resistant strain of Ostertagia spp. apparently did speed the process of reversion (Donald et al., 1980), but this effect does not always occur (Donald, 1983). The reverse selection, using thiabendazole against a levamisole resistant strain of T. colubriformis, may be more successful (Waller, 1985b).

McKenzie (1985) discussed reversion to anthelmintic susceptibility in relation to the fitness of susceptible and resistant nematodes to survive and reproduce. He speculated that, if resistance was controlled by multiple genes, there would not be a marked difference in fitness between susceptible and resistant nematodes and therefore reversion to susceptibility would be relatively slow; if resistance was controlled by a single gene however, reversion would usually be rapid because there would be a relatively greater difference in fitness between the resistant and susceptible populations. Kelly et al., (1981a and 1981b) and Martin (1988a) also discussed reversion in relation to fitness, but from a different viewpoint than McKenzie's, and both considered that failure of some benzimidazole resistant nematodes to revert to susceptibility meant that they must therefore have been "more fit" than susceptible strains.

While there is still much to be learnt, the best current advice is that, in the case of high levels of anthelmintic resistance, reversion to susceptibility will be too slow and

reoccurrence too rapid for reversion to be of practical use to a farmer when planning a helminth control programme (Donald, 1983; Martin, 1985b).

DIAGNOSIS OF ANTHELMINTIC RESISTANCE

If nematodes resistant to a particular anthelmintic are present on a farm, then continued use of that anthelmintic, or members of the same group, will cause an increase in the level of resistance to it, with a concomitant reduction in the effectiveness of the farm's helminth control programme. Hence it is important that a farmer should know whether resistant nematodes are present on his farm, and what anthelmintic is involved, so that the best level of helminth control can be achieved.

A variety of tests may be used to determine the type and degree of anthelmintic resistance (Presidente, 1985; Martin, 1988a). Of these tests, only the faecal egg count reduction and controlled anthelmintic efficiency tests are performed in vivo, whereas egg hatch assays, larval paralysis tests, larval tubulin binding tests and a number of enzyme assays are conducted entirely in the laboratory.

The faecal egg count reduction (or depression) test

This is the most commonly used test for anthelmintic resistance (Prichard et al., 1980; Arundel 1985b; Presidente, 1985; Vizard, 1986). There is no agreed standard procedure for this test, hence there are many variations of detail in its application in the field and the laboratory, and in the calculation and interpretation of results. Nevertheless, because the test is relatively simple to perform and requires only basic laboratory equipment, it has been commonly used in surveys for anthelmintic resistance, and it is recommended as the first diagnostic procedure to use in field investigations of anthelmintic efficacy (Prichard et al., 1980; Arundel, 1985b).

Despite the many possible variations, all faecal egg count reduction tests adhere to the same general procedure. A group of naturally infected sheep is drenched with an anthelmintic and fresh faeces for nematode egg counts are collected from all sheep, on the day of treatment (usually) and again between 4 and 14 days later. An untreated control group of sheep may also be sampled at the same times. The faecal egg count reduction achieved is expressed as a percentage and is compared with an arbitrarily decided minimum satisfactory level.

The faecal egg count reduction test is not a direct test for anthelmintic resistance. The test assesses only the effect of an anthelmintic on nematode egg production and not its effect on the nematodes themselves. Since there are many other factors, in addition to anthelmintics, which affect nematode egg output and faecal egg counts, this test alone can only provide presumptive evidence of anthelmintic resistance (Arundel, 1985b; Presidente, 1985). Egg production of nematodes varies greatly between different nematode genera (Soulsby, 1965; Reinecke, 1984), and it is also influenced by sheep breed (Sangster et al., 1979; Le Marie et al., 1987), age

(Soulsby, 1965) and (for ewes) reproductive status (Salisbury and Arundel, 1970). Even the time of year (Anderson, 1972 and 1973), time of day (Soulsby, 1965), and length of time that sheep have been off pasture (Presidente, 1985) influence nematode egg output. Faecal egg counts are obviously also affected by the consistency (moisture content) of the faeces collected.

It is not surprising that faecal egg counts of individual sheep are not closely related to the numbers of nematodes present. Guidelines have however been developed for the assessment of helminthosis in a young flock, based on faecal egg counts from a sample of that flock (Kingsbury, 1965; McKenna, 1981; Tarazona, 1986; McKenna, 1987a and 1987b).

To minimise the number of variables in a faecal egg count reduction test, sheep should be matched evenly and samples collected at the same time of day on each visit (Presidente, 1985). The interval between the two collections of faecal samples should be between 7 and 14 days. Levamisole and the benzimidazoles may temporarily reduce nematode egg output, so sampling before 7 days could give a falsely favourable impression of an anthelmintic's efficacy (Martin et al., 1985; Dash et al., 1988). Because the prepatent periods for abomasal and small intestinal nematodes can be as short as 14 days (Charleston and Pomroy, 1984), faecal samples taken later than 14 days after treatment may contain eggs from nematodes which were ingested as larvae on the day of treatment or shortly after.

For statistically significant analyses of results, at least 10 animals are necessary in each group and the mean pretreatment faecal egg count of the group should be at least 200 eggs per gram (Presidente, 1985; Vizard, 1986).

An unresolved question is whether or not an untreated control group is essential for the conduct of a faecal egg count reduction test. Presidente (1985) and Martin (1988a), in Australia, stipulated that control groups should be used, and that their mean faecal egg counts be included in the final calculations, but in New Zealand MAFQual laboratories do not require faecal samples from control sheep (McKenna, pers. comm.). All 5 surveys, discussed earlier, in which faecal egg count reductions were used to assess anthelmintic resistance, used control groups (Kettle et al., 1981 and 1982; Cawthorne and Cheong, 1984; Riffkin et al., 1984; Edwards et al., 1986a; Webb and Ottaway, 1986), but only one (Edwards et al., 1986a) used the control egg counts when calculating faecal egg count reductions.

In a variation of the usual test, a control group is used but egg counts are only performed on faeces collected 10 days post-treatment, from the control and treated groups. This simplified procedure has given results comparable to those obtained using pre- and post-treatment mean faecal egg counts, and is less expensive to

perform (Vizard and Wallace, 1987; Bell, 1988).

Laboratory methods used to determine faecal egg counts have varied between different workers, but all used some modification of the McMaster technique (Gordon and Whitlock, 1939; Whitlock, 1948). The use of a correction factor, based on a subjective assessment of faecal moisture content, was widespread in Australia, but it is now considered not worthwhile (Arundel, pers. comm.).

There are at least 3 methods used to calculate the faecal egg count reduction achieved with anthelmintic treatment. In the simplest method, the average (or arithmetic mean) of all group members' post-treatment faecal egg counts is subtracted from the average egg count pre-treatment and expressed as a percentage of the pre-treatment average (Webb and Ottaway, 1986). This is the calculation method used by MAFQual laboratories in New Zealand (McKenna, pers. comm.). In a more complex calculation, Presidente (1985), Edwards et al. (1986a) and Vizard (1986), used the geometric means of both treated and control groups, pre-and post-treatment. A third method of calculation also uses geometric means, but only those of the control and treated groups after treatment (Vizard, 1986). Dash et al. (1988) supported the use of the arithmetic mean for three reasons: it is simple to calculate, it relates to the total egg output of a group of sheep more closely than does the geometric mean, and it usually results in a lower estimate of the faecal egg count reduction than that obtained using geometric means and therefore gives a more cautious result.

When the faecal egg count reduction is calculated, it is compared with an arbitrarily determined cut-off point and resistance is declared to be present, absent or uncertain in relation to the anthelmintic used. If geometric mean egg counts are used in the calculation, a reduction of less than 90% is generally accepted as indicating resistance (Edwards et al., 1986a; Vizard, 1986; Martin, 1988b), and reductions of 90-96% suggest emerging resistance and the need for further testing (Vizard, 1986; Martin, 1988b). The same cut-off point of 90% is usually applied when arithmetic mean egg counts are used (Riffkin et al., 1984; Webb and Ottaway, 1986; McKenna, pers. comm.).

Faecal cultures for larval identification have been commonly performed as a supplement to faecal egg count reduction tests (Presidente, 1985). Faecal culturing allows the genus or species of each resistant nematode to be determined by examination of larvae which are easier to differentiate than are the eggs of many strongylate nematodes. A composite sample of faeces from sheep treated with anthelmintic 7 to 14 days previously is incubated at 27°C for 7 days. Larvae are then collected and identified using standard criteria. It is desirable to also identify the nematodes present in untreated sheep, using faecal cultures, so that any change in

relative numbers of nematode larvae following treatment is apparent.

The controlled anthelmintic efficiency test

This test requires the slaughter of a number of sheep and is expensive to perform; it is rarely done and only after a faecal egg count reduction test has indicated the presence of resistance (Turton and Clark, 1974; Le Jambre, 1978; Arundel, 1985b).

In this test groups of worm free sheep are dosed with infective larvae cultured from eggs passed in faeces of sheep previously treated with an anthelmintic to which resistance is suspected. One group of sheep will be untreated controls and the other groups are given varying doses of the suspect anthelmintic. When slaughtered 30-35 days after dosing, total worm counts are performed and an LD90 calculated for the anthelmintic and compared with that for known susceptible nematode strains.

Post mortem examination

A simpler and cheaper alternative to the controlled anthelmintic efficiency test has been recommended (Vizard, 1986). At least 2 sheep are selected from the control and treated groups of a faecal egg count reduction test when anthelmintic resistance is suspected. The sheep are slaughtered, 5 to 15 days after treatment, and total worm counts (including abomasal digests) are performed. If a mixed population of nematodes in the control sheep has reverted to a single species population in the treated sheep, then resistance is strongly suspected (Prichard et al., 1980; Arundel, 1985b; Vizard, 1986).

In vitro tests for anthelmintic resistance

The most commonly used laboratory technique for assessing benzimidazole resistance is the egg hatch assay, which tests the ability of benzimidazoles to prevent egg embryonation and hatching (Le Jambre, 1976; Coles and Simpkin, 1977; Hall et al., 1978a; Whitlock et al., 1980b). It is considered by Presidente (1985) to be a more rapid, sensitive and economical test than the faecal egg count reduction test. A modification of this test, suitable for Nematodirus spathiger, has also been developed (Obendorf et al., 1986).

In the egg hatch assay, eggs of a single species of nematode suspected of benzimidazole resistance are incubated in various concentrations of thiabendazole. The hatching percentages are compared with those of known benzimidazole susceptible strains.

The egg hatch assay has been used as the primary test in some surveys for benzimidazole resistance (Jordi, 1980; Kemp and Smith, 1982; Boersema et al., 1987), and it has also been used as a check test in other surveys which have used faecal egg count reduction as their primary test (Kettle et al., 1981 and 1982; Britt, 1982; Cawthorne and Cheong, 1984).

An in vitro biochemical test for benzimidazole resistance has been developed as an alternative to the egg hatch assay. This test measures the reduction in the ability of

benzimidazoles to bind to tubulin in resistant nematodes and larvae (Lacey and Snowden, 1988), and is believed by its developers to be superior to the egg hatch assay, although others have cast doubts on its value for field investigations of resistance because of its cost, complexity and the time required for its performance (Campbell, 1985; Sutherland et al., 1988).

The effect of levamisole and morantel of causing paralysis in both adult and larval nematodes is utilised in the in vitro larval paralysis test (Martin and Le Jambre, 1979). An additional, and possibly superior test has been developed which also determines the effect of levamisole on larval movement, but uses first stage (unhatched) larvae rather than free living third stage larvae (Dobson et al., 1986).

Four enzyme assays which detect benzimidazole resistance in third stage larvae have been briefly described. These tests are said to be less expensive and more reliable than other in vitro methods for testing benzimidazole resistance (Sutherland et al., 1988).

Comparability between tests for anthelmintic resistance

No precise numerical relationships have been reported between the various tests for anthelmintic resistance, however direct relationships must exist since resistant adult nematodes produce eggs and larvae which are themselves resistant (Le Jambre, 1985b). Boersema et al. (1987) considered the faecal egg count reduction test to be less sensitive than the egg hatch assay in detecting benzimidazole resistant nematodes because in their survey, only 4 of 19 flocks where resistance had been identified by the egg hatch assay also showed resistance in vivo to a faecal egg count reduction test. The same conclusion about relative sensitivities could also be drawn from the results of 2 New Zealand surveys, (although not of the same farms), in which one survey - using the egg hatch assay - found benzimidazole resistance on 21.2% of farms surveyed, and the other - using the faecal egg count reduction test - found benzimidazole resistance on only one of 93 farms (Kemp and Smith, 1980 and 1982; Kettle et al., 1981 and 1982). However, an alternative interpretation of all these results could be that the egg hatch test is less specific than the faecal egg count reduction test.

The biochemical tests are considered to be the most sensitive and specific of the tests for anthelmintic resistance (Donald, 1985).

STRATEGIES TO MINIMISE THE IMPACT OF ANTHELMINTIC RESISTANCE

When helminth control programmes rely solely on the use of anthelmintics, as most do in Australia and New Zealand (Donald and Waller, 1982), an increase in the level of resistance is probably inevitable (Dash et al., 1985). Consequently, effective sustained helminth control systems are needed which will minimise the impact of helminths on productivity, while allowing for the presence, or emergence of anthelmintic resistance, and which are less reliant on anthelmintics than present practices. Such systems are likely to be more complex than those presently in use, and will probably combine specific grazing management practices with more logical use of anthelmintics - or other substances - and the utilization of natural or artificially acquired resistance to infection (Hall and Kelly, 1979; Brunsdon, 1980; Morley and Donald, 1980; Barger, 1982; Donald, 1985).

More effective use of anthelmintics

It is unlikely that manufacturers will continue to develop and market new, unrelated anthelmintics whenever those which are presently available lose their value because of the increasing prevalence of resistant nematodes (Bogan and Armour, 1985; Hotson, 1985). Even when new anthelmintics are released, there is every reason to expect that resistance will appear to them also, as it has in South Africa to ivermectin, only two years after its release there (Carmichael et al., 1987). Anthelmintics will continue to be the mainstay of helminth control schemes for some years to come (Waller, 1985a).

Donald (1985) suggested that, to use an anthelmintic most effectively, it should be given at the highest economically possible dose rate, providing resistance is not already detectable, but once benzimidazole resistance is present, Martin (1988a) considered that even doubling the dose rate would be of little value, although levamisole resistance could be partially and temporarily overcome with doubled dose rates of levamisole.

Although the dose rate used for single treatments should be high, the frequency of treatment should be minimised (Morley and Donald, 1980; Dash et al., 1985; Martin, 1988a). It has also been suggested that treatment might be given to only a portion of a flock, or that an anthelmintic with lower efficacy be used to treat the entire flock (Donald, 1985). These suggestions are based on the fact that selection for anthelmintic resistance will be less if most of the nematodes are not simultaneously exposed to effective anthelmintic treatment (Donald, 1983; Martin, 1985a).

The efficiency of benzimidazoles in killing nematodes can be improved by extending, for as long as possible, the time that nematodes are in contact with the anthelmintic (Prichard et al., 1978). There are many ways to do this, including water and feed supplementation, multiple oral dosing, subcutaneous, intramuscular or intravenous administration, and intraruminal controlled release devices (Anderson, 1985; Hotson, 1985).

Most of the methods used for prolonging the exposure of nematodes to anthelmintics have practical or economic limitations or both, but the controlled release devices appear to have the most practical value, and one containing morantel and one containing oxfendazole are already available in Europe for use in cattle. Reservations have been expressed about the potential of controlled release technology to exacerbate the occurrence of anthelmintic resistance (Prichard et al., 1980; Donald and Waller, 1982; Le Jambre, 1982; Arundel, 1985a), however Martin (1988a) has recommended that these devices, when available, should be incorporated into Australian worm control programmes, providing they release a constant level of anthelmintic which stops abruptly when exhausted.

The common practice of alternating anthelmintic groups within a single year is considered probably the worst use of anthelmintics if a farmer wishes to avoid anthelmintic resistance in his flock (Brunsdon et al., 1983). Instead, the slow rotation of anthelmintic groups - with one to two year cycles for each - has been almost universally recommended in Australia and New Zealand as the best way to delay the occurrence of anthelmintic resistance (Prichard et al., 1980; Dash et al., 1985; Kettle and Vlassoff, 1985; Riffkin et al., 1984; Martin, 1988a). Rotation of anthelmintic groups at a slow frequency means that proportionately few worms are exposed to more than one anthelmintic because one year is about the maximum generation interval for sheep nematodes, including both the free living and parasitic phases (Le Jambre et al., 1978b). It is still largely a matter of speculation however whether the practice achieves this goal (Donald, 1983; Dash et al., 1985).

Another practice, favoured by Le Jambre et al. (1977, 1978a, 1978b) is to continue to use anthelmintics from one group until treatment failure is clinically apparent and then change to another group. A high level of resistance could develop with this regime and, if so, reversion to susceptibility to the first anthelmintic group used would be very slow (Donald, 1983).

Mixtures of anthelmintics may be used, either to delay the appearance of resistant nematodes, as in the "Wormkill" programme for the summer rainfall areas of New South Wales (Dash et al., 1985), or to regain control once resistance has arisen (Anderson et al., 1988). In some cases, the effect of a mixture is simply additive, and each anthelmintic is given at its normal dose rate, so that all nematodes are killed by one or the other anthelmintic (Martin, 1987). Alternatively, the mixture may have a synergistic or potentiating effect, as with levamisole (at low dose rate) and any benzimidazole against H. contortus (Behm and Bryant, 1985; Bryant, 1985), or parbendazole (at low dose rate) and oxfendazole against H. contortus and T. colubriformis (Hennessey et al., 1985).

Integrated control of nematodes, by the combination of grazing practices which minimise intake of infective larvae with strategic, minimal anthelmintic usage, should be the ideal way to reduce the effects of parasites on sheep (Gardiner and Butler, 1964; Barger, 1978; Morley and Donald, 1980). The effects of the recommended practices on the development of resistance have not been assessed (Martin, 1985a), and the correct application of these practices is difficult for many farmers without major changes in their farming systems (Dash, 1988).

Alternatives to anthelmintics

There are various compounds which are not strictly anthelmintics but which could be useful to control nematodes in sheep, either as alternatives or adjuncts to anthelmintics. Generally, research into the uses of such compounds for nematode control has been minimal, or non-existent.

Hormones which interfere with growth and development, and pheromones which interfere with reproduction, are successfully used to control some insect pests. It has been suggested that such compounds would also be of value in nematode control, particularly if used in conjunction with periodic doses of anthelmintic (Waller and Lacey, 1985).

Cimetidine, used in human medicine to raise gastric pH, was tested in sheep and all H. contortus and 70% of O. circumcincta were removed. Although the method of administration used in the trial was highly impractical, further investigation was considered worthwhile (Hall and Oddy, 1984).

Prostaglandins, by increasing gastrointestinal secretions and peristalsis, create an environment inhospitable to nematodes, but a practical anthelmintic application of these effects has not been developed (Kelly et al., 1976).

The biological control of nematode parasites by their own parasites, if such exist, is an interesting approach suggested by Kelly and Hall (1979b), but there seems to have been no research in this field.

Pasture larvicides

In theory, if the infective larvae of nematodes could be killed when they were on pasture, then the need to treat sheep with anthelmintics would be largely or entirely eliminated and anthelmintic resistance would cease to cause any concern. Various chemicals, including copper sulphate, lime and bleaching powder, and chloro-picrin and calcium cyanide gases have been tried as larvicides, but all had disadvantages, including that of cost. Burning of pasture to kill larvae has also been tried but, although cheaper than chemicals, it had little effect on the larval population (Soulsby, 1965). It seems that treatment of pastures to control sheep helminths is not a viable practice.

Enhancement of the sheep's resistance to nematodes

If sheep were unaffected by helminths there would be no need to use anthelmintics, and hence no concern about anthelmintic resistance. This is an attractive idea and research, reviewed at a Workshop held in 1983 (Dineen and Outteridge, 1984), is active in two major fields, both aimed at producing sheep resistant to the effects of nematodes. Some researchers are investigating ways of increasing resistance to nematodes by vaccination of sheep presently bred on farms (Mathews, 1979; Dineen, 1985), and others are attempting to selectively breed sheep with high innate resistance (Riffkin and Dobson, 1979; Albers et al., 1984; Bissett, 1988; Vlassoff, 1988; Watson, 1988). The breeders of resistant sheep have so far been more successful than those developing vaccines but when genetically engineered antigens become available in the next few years it is hoped that vaccination will become economically viable (Dineen, 1985; Riffkin, 1988).

Planning, modelling and monitoring helminth control programmes

Strategic use of anthelmintics on a particular farm depends on detailed knowledge of the epidemiology of the major nematodes of sheep in that area, and the management practices on that farm. Other epidemiological information, whether obtained in another or the same country, and even if from a similar climatic zone, may not be applicable; indeed, there may even be significant differences between worm populations on adjacent farms if stock management differs (Michel, 1982).

Beginning with Gordon (1948), there have been many epidemiological studies of sheep nematodes in Australia (Salisbury and Arundel, 1970; Anderson, 1972 and 1973; Anderson et al., 1978; Donald et al., 1978), and in New Zealand (Brunsdon, 1960; Vlassoff, 1982; Familton et al., 1986). Utilising studies such as these, various regional helminth control programmes have been promulgated (Brunsdon and Adam, 1975; Dash et al., 1985; Dash, 1988), but there is still a need for more "tailor-made" programmes for individual farms (Morley and Donald, 1980; Riffkin, 1988).

More precise forecasting of the optimum times for anthelmintic treatment may be possible using computer models of sheep-nematode systems. These models can take into account pasture and animal production data as well as epidemiological information (Callinan and Napthine, 1983; Kettle and Vlassoff, 1985; Paton and Thomas, 1987).

Whether a farm helminth control programme is based on regional recommendations or on more specific advice, some form of monitoring is desirable; this is primarily to check that optimum helminth control is being achieved and also to give early warning of the development of resistance (Cooper, 1982; Watt, 1983; Vizard and Wallace, 1987; Bell, 1988). An additional benefit of monitoring is that it can aid research into anthelmintic resistance relative to on-farm operational factors (Donald, 1985).

Anthelmintic resistance is more likely to develop on farms with control programmes reliant on anthelmintics (Prichard et al., 1980), and on such farms monitoring for resistance is recommended. Farms on which only occasional curative treatments are used might dispense with monitoring since their risk of developing anthelmintic resistance is minimal (Prichard et al., 1980); monitoring would however show that the economic benefits of such programmes are also minimal (Johnstone et al., 1976 and 1979), except in arid zones (Gray and Kennedy, 1981; Donald and Waller, 1982).

Treatment of introduced sheep

It has been shown that there are many areas of Australia and New Zealand where a significant minority or even a majority of sheep farms have anthelmintic resistant nematodes. Farmers in these areas whose sheep are not affected may be at considerable risk of bringing resistant nematodes onto their farms in purchased or agisted sheep. This risk is acknowledged in the various helminth control programmes advocated in Australia, and farmers are advised to treat all introduced sheep with an effective anthelmintic on arrival (Waller, 1988). The "effective" anthelmintic is usually ivermectin, levamisole, or a combination of levamisole and a benzimidazole, administered separately.

CHAPTER 2

ANTHELMINTIC RESISTANCE SURVEY - MATERIALS AND METHODS

THE AREA AND THE FARMS

ANTHELMINTIC RESISTANCE TEST PROCEDURE

CALCULATIONS

FIGURES

THE AREA AND THE FARMS

A survey for anthelmintic resistance was conducted on 15 sheep farms in the Manawatu region between February 9 and May 24 1988. The Manawatu region in the lower North Island of New Zealand is the broad coastal plain running north and west from the Tararua Range up to the southern end of the Ruahines and across to the Rangitikei River (Figure 2.1).

The region has approximately 4 million sheep, which constitute 11% of the North Island sheep population and 6% of the total New Zealand flock. The predominant breed is the Romney.

Annual rainfall in the Manawatu region ranges from 760 to 1500 mm, and it is evenly distributed throughout the year and reliable. Summers in the region are warm and winters mild.

The farms to be surveyed were chosen from clients of the Massey University veterinary practice, on the basis of each farmer's interest and willingness to cooperate in the survey. The approximate location of the surveyed farms, numbered in the order that they were visited, is shown in Figure 2.2.

ANTHELMINTIC RESISTANCE TEST PROCEDURE

On each farm 48 young sheep, which had not been treated with anthelmintic for at least the previous month, were used for the test.

The sheep were divided into 4 groups of 12 in each, and each sheep was individually eartagged and weighed. For additional identification, all members of each group were marked with a coloured spray, using a different colour for each group.

One group of sheep was left as untreated controls. All sheep in each of the other groups were treated orally with one of 3 anthelmintics, using a drenching gun which had been checked for accuracy. Each sheep was dosed according to its body weight, using commercially recommended dose rates of anthelmintic.

FIGURE 2.1

Map of New Zealand showing the location of the Manawatu region

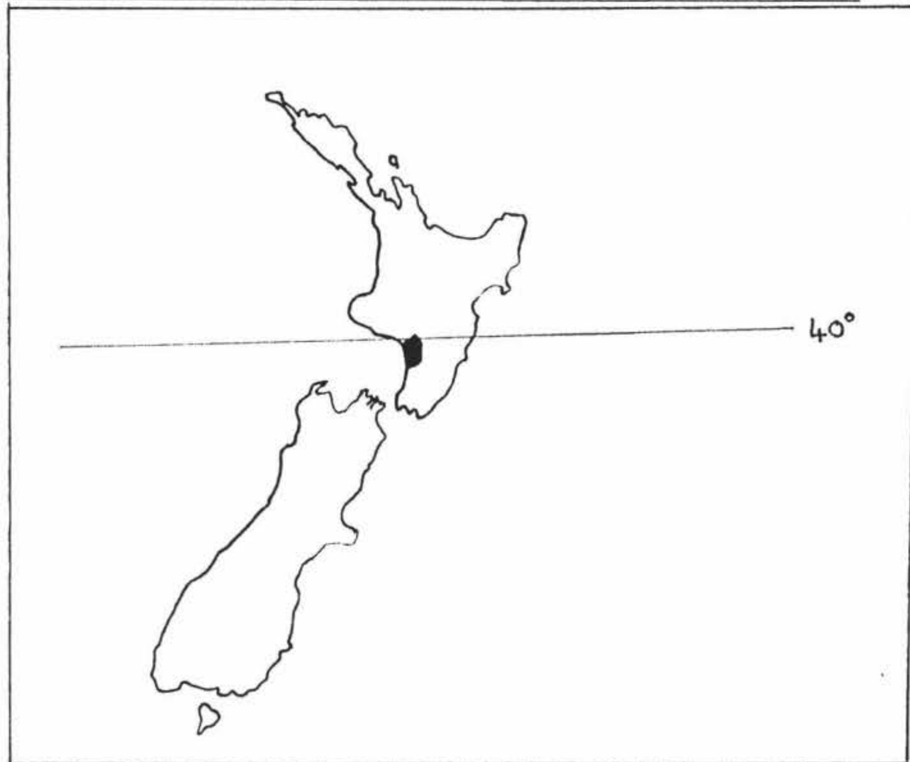

FIGURE 2.2

Map of the Manawatu region showing the locations of the surveyed farms

N
Scale: 1 cm = 2.26 km
BULLS
FEILDING
RUAHINE RANGE
RANGITIKEI RIVER
MANAWATU GORGE
PALMERSTON NORTH
MANAWATU RIVER
TARARUA RANGE
7
12
14
4
10
13
8
6
5
3
1
15
2
11
9

The anthelmintics used, and their dose rates, were as follows:

oxfendazole[1].......................5.0 mg/kg
levamisole[2].........................8.0 mg/kg
ivermectin[3]..........................0.2 mg/kg

Each of the anthelmintics chosen is in common use in New Zealand, and is representative of the action family to which it belongs. Ivermectin was included because it has been marketed in New Zealand for use in sheep since October 1982 and it is possible that nematodes resistant to ivermectin are now present.

After treatment, all sheep were returned to pasture.

Seven days later faecal samples were again collected from most of the treated and control sheep. (Samples could not be obtained from a small number of sheep).

Faecal egg counts were performed on all samples using a modified McMaster technique (Gordon and Whitlock, 1939; Whitlock, 1948) in which one egg counted represented 50 eggs per gram (Appendix I). Counts of Nematodirus spp. eggs and other Strongylate eggs were recorded separately. Any eggs of Moniezia spp., Trichuris ovis and Strongyloides papillosus, or oocysts of Eimeria spp. were disregarded.

For all farms, a composite sample of faeces from untreated sheep was incubated and the resultant larvae identified to determine the nematode genera present in the sheep at the time of treatment (Appendix II).

If anthelmintic treatment produced an unsatisfactory reduction in faecal egg counts on any farm, then faeces from the sheep which had been treated with the anthelmintic in question were cultured as above for identification of the possibly resistant larvae.

---

1 Synanthic[R] Coopers Animal Health (NZ) Ltd., Upper Hutt

2 Nilverm[R] Coopers Animal Health (NZ) Ltd., Upper Hutt

3 Ivomec[R] Merck Sharp and Dohme (NZ) Ltd., Auckland

CALCULATIONS

When anthelmintic treatment did not cause complete cessation of nematode egg production, the reduction in the faecal egg count for the treated group (FECR) was calculated and expressed as a percentage. Three methods of calculation were used, as follows:

Method 1. Simple difference of arithmetic mean egg counts.

$$FECR_1 = 100\left(\frac{Ta_1 - Ta_2}{Ta_1}\right)$$

Where

Ta_1 = arithmetic mean faecal egg count of all treated sheep before treatment
Ta_2 = arithmetic mean faecal egg count of all treated sheep 7 days after treatment

Method 2. Relative change in geometric mean egg counts.

$$FECR_2 = 100\left(1 - \frac{Tg_2}{Tg_1} \times \frac{Cg_2}{Cg_1}\right)$$

Where

Cg_1 = geometric mean of control faecal egg counts on day 0
Cg_2 = geometric mean of control faecal egg counts on day 7

Tg_1 = geometric mean of treated faecal egg counts on day 0 (pre-treatment)
Tg_2 = geometric mean of treated faecal egg counts on day 7 (post-treatment)

Only the results of valid pairs of faecal egg counts were used. A valid pair is one in which

(i) both a pre- and post-treatment (day 0 and day 7) faecal sample is obtained,

(ii) the pre-treatment (day 0) egg count is greater than zero.

Because a geometric mean for a column of numbers cannot be calculated if any number is zero, 25 e.p.g. was added to all egg counts if any control or post-treatment egg count was zero and then 25 e.p.g. was subtracted from the calculated mean before the $FECR_2$ was calculated.

Method 3. Post-treatment geometric mean egg count relative to control (day 7) geometric mean egg count.

$$FECR_3 = 100\left(1 - \frac{Tg_2}{Cg_2}\right)$$

CHAPTER 3

ANTHELMINTIC RESISTANCE SURVEY - RESULTS

FAECAL EGG COUNT REDUCTIONS (NOT INCLUDING NEMATODIRUS SPP.)

ANTHELMINTIC EFFICACY AGAINST NEMATODIRUS SPP.

NEMATODE IDENTIFICATION: PRE-TREATMENT

NEMATODE IDENTIFICATION: POST-TREATMENT

OXFENDAZOLE RESISTANT NEMATODE IDENTIFICATION - EXCLUDING NEMATODIRUS SPP.

MULTIPLE ANTHELMINTIC RESISTANCE

TABLES AND FIGURES

The detailed results for each farm are presented in Appendix III and the following is a summary of those results.

FAECAL EGG COUNT REDUCTIONS (NOT INCLUDING NEMATODIRUS SPP.), 7 DAYS AFTER TREATMENT WITH THE FOLLOWING ANTHELMINTICS:

(i) Oxfendazole (Table 3.I)

Using calculation methods 1, 2 and 3 faecal egg count reductions following oxfendazole treatment were less than 95% on 7, 6 and 6 of the farms and less than 90% on 4, 5 and 5 of the farms respectively. On the basis of these results, there was no evidence of oxfendazole resistant nematodes on farms 1, 3, 6, 9, 10 and 12, but resistant nematodes were present on farms 2, 5, 8, 14 and 15. A decision on the oxfendazole resistance status of sheep nematodes on farms 4, 7, 11 and 13 could not be made because of the equivocal results obtained on these farms.

TABLE 3.I

Percentage faecal egg count reduction - 7 days after the administration of oxfendazole (5.0 mg/kg)

FARM	$FECR_1$%	$FECR_2$%	$FECR_3$%
1	99	>99	>99
2	84	86	86
3	98	99	99
4	97	90	90
5	78	86	86
6	99	>99	>99
7	98	95	96
8	93	71	78
9	100	100	100
10	99	>99	>99
11	94	99	98
12	100	100	100
13	93	99	98
14	85	84	60
15	55	69	70

(ii) Ivermectin (Table 3.II)

Faecal egg count reductions were less than 95% on 3, 0 and 0 of the farms, and less than 90% on 2, 0 and 0 of the farms (methods 1, 2 and 3 respectively). There was no evidence of ivermectin resistance on farms 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15, but on farms 1, 3 and 5 the results were equivocal and a confident decision regarding ivermectin resistance on these farms could not be made.

TABLE 3.II

Percentage faecal egg count reduction - 7 days after the administration of ivermectin (0.2 mg/kg)

FARM	$FECR_1$%	$FECR_2$%	$FECR_3$%
1	88	97	96
2	100	100	100
3	94	98	99
4	100	100	100
5	89	97	98
6	99	>99	>99
7	100	100	100
8	100	100	100
9	100	100	100
10	100	100	100
11	97	99	99
12	100	100	100
13	100	100	100
14	100	100	100
15	100	100	100

(iii) Levamisole (Table 3.III)

Faecal egg count reductions were less than 95% on 2, 0 and 0 of the farms and on no farm was the reduction below 90%. There was no evidence of levamisole resistance on farms 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14 and 15, but on farms 3 and 13 equivocal results were obtained and no decision could be made regarding levamisole resistance on these farms.

TABLE 3.III

Percentage faecal egg count reduction - 7 days after the administration of levamisole (8.0 mg/kg)

FARM	$FECR_1$%	$FECR_2$%	$FECR_3$%
1	99	>99	>99
2	100	100	100
3	93	97	99
4	99	97	98
5	96	98	99
6	>99	>99	>99
7	99	99	98
8	100	100	100
9	100	100	100
10	100	100	100
11	100	100	100
12	>99	>99	>99
13	92	98	98
14	100	100	100
15	100	100	100

EFFICACY OF OXFENDAZOLE AGAINST NEMATODIRUS SPP.

On each farm, of the total number of sheep sampled prior to treatment, relatively few were passing Nematodirus spp. eggs (mean 4 sheep per treatment group, range 0-10), and the Nematodirus spp. faecal egg counts prior to treatment were mostly low (mean 67 e.p.g., range 0-1850). It was therefore considered that the percentage Nematodirus spp. egg count reduction for each anthelmintic on each farm had little meaning. However it was apparent, when all the individual farm results were combined, that there were Nematodirus spp. resistant to oxfendazole on many of the farms. Table 3.IV compares the effects of each anthelmintic in reducing the numbers of sheep passing Nematodirus spp. eggs and the percentage reduction achieved with each treatment. Both ivermectin and levamisole treatment markedly reduced the percentage of sheep passing Nematodirus spp. eggs, (reductions of 96.9 and 94.9%, respectively), but oxfendazole treatment was only 24.1% effective in reducing infected sheep numbers. The number of untreated (control) sheep declined by 13.4%.

Table 3.IV

A comparison of the efficacies of three anthelmintics in reducing the number of sheep infected with Nematodirus spp. (Combined results from 15 farms).

	No. sheep passing Nematodirus spp. eggs		Reduction (%)	Difference between Control & Treatment (χ^2)	Significance
	Pre-treatment	7 days Post-treatment			
Control	67	58	13.4		
Oxfendazole (5.0 mg/kg)	58	44	24.1	0.24	p=0.62
Ivermectin (0.2 mg/kg)	65	2	96.9	38.27	p<0.01
Levamisole (8.0 mg/kg)	59	3	94.9	32.57	p<0.01

IDENTIFICATION OF LARVAL GENERA IN FAECAL CULTURES

(i) Nematode genera present before anthelmintic treatment (Table 3.V)

Ostertagia and Trichostrongylus spp. larvae were most common, being present in faeces from all farms. Nematodirus spp. (identified by their distinctive eggs, and not by their larvae), were present on 14 farms. Haemonchus spp. were present on 12 farms, and Cooperia, Oesophagostomum and Chabertia spp. were present on 11, 8 and 4 farms, respectively. On each farm at least 4, but not more than 7 nematode genera were identified.

Table 3.V

Percentage nematode genera present - before anthelmintic treatment

	FARM														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Haemonchus	+	38	22	6		14	45	21		7	38	19	10	42	
Ostertagia	+	4	54	46	14	11	26	56	11	4	4	5	39	2	4
Trichostrongylus	+	40	23	44	85	75	28	18	21	57	19	66	34	46	82
Cooperia	+	2					1	4	6	11	37	4	17	4	14
Chabertia		1							1				3		6
Oesoph.		15	2	4	1					62	21	2	3		
Nematodirus	+	+	+	+	+	+	+	+	+	+	+	+		+	+

+ Genus present but percentage not recorded

(ii) Nematode genera present - 7 days after treatment with the following anthelmintics:

(a) Oxfendazole (Table 3.VI)

Faecal cultures for larval identification were not performed after oxfendazole treatment on farms 3, 6, 7, 9, 10 and 12 because, on the basis of the faecal egg count reductions achieved, it was believed that there were no oxfendazole resistant nematodes present.

Of the 9 farms from which faeces, collected after oxfendazole treatment, were cultured for larval identification, Trichostrongylus spp. were present on 8 farms, Oesophagostomum spp. on 6, Haemonchus spp. on 5, Ostertagia spp. on 4, Cooperia spp. on 2, and Chabertia spp. on one farm.

Table 3.VI

Percentage nematode genera present after oxfendazole treatment

	FARM														
	1	2	3*	4	5	6*	7*	8	9*	10*	11	12*	13	14	15
Haemonchus		2		11	11			2						28	
Ostertagia					1			9					55		2
Trichostrongylus		98		68	67			32			+		44	71	96
Cooperia	+												1		
Chabertia															2
Oesoph.	+			21	21			57			+			1	
Nematodirus	+	+	+	+	+	+		+	+	+	+	+		+	+

+ Genus present but percentage not recorded
* No faecal culture performed

The criteria used for separating Ostertagia and Trichostrongylus larvae was overall length with the former being longer and the latter shorter than 725 μm. It is known however that there is an overlap in size between these two genera (Vlassoff, pers. comm.). Thus the small numbers of Ostertagia larvae noted on farms 5 and 15 may have been Trichostrongylus. On these two farms this genus was not considered to be demonstrating resistance to

oxfendazole. For other genera, if there were less than 5% present this was considered to be insufficient evidence to declare resistance to that genus. These genera considered to be resistant are shown in figure 3.1. Resistant Trichostrongylus were found on all 5 farms, Haemonchus and Oesophagostomum on two each and Ostertagia on one. Two farms (5 and 8) had 3 resistant genera present.

FIGURE 3.1

Identification of oxfendazole resistant nematode genera (excluding Nematodirus spp.) on 5 farms

	FARM				
	2	5	8	14	15
Haemonchus		+		+	
Ostertagia			+		
Trichostrongylus	+	+	+	+	+
Oesophagostomum		+	+		

(b) Ivermectin

On farms 3, 5, 6 and 11 faeces from one or two sheep treated with ivermectin contained small numbers of Strongylate eggs (maximum 250 eggs per gram). Culture of faeces after ivermectin treatment on these farms was not attempted as it was considered that too few larvae would develop for identification.

On farm one, the faeces of 4 of the 12 sheep treated with ivermectin contained small numbers of Strongylate eggs (maximum 350 eggs per gram) so faecal culture was performed but only 2 or 3 larvae (Ostertagia and Trichostrongylus spp.) were recovered. On this farm the faecal egg count reductions achieved with ivermectin treatment were 88, 97 and 96% (methods 1, 2 and 3 respectively) and were considered equivocal.

(c) Levamisole

Small numbers of Strongylate eggs (maximum 150 eggs per gram) were present in faeces of one or two sheep 7 days after treatment with levamisole on farms 1, 3, 4, 5, 6, 7 and 12 but faecal culture was not attempted as it was considered that too few larvae would develop.

On farm 13, the faeces of 3 levamisole treated sheep contained very small numbers of Strongylate eggs (50, 50 and 55 eggs per gram), and the faeces of the entire group were cultured for larval identification. The larvae which developed comprised Haemonchus spp. (39%), Ostertagia spp. (40%) and Trichostrongylus spp. (57%). The relative proportions of these nematode genera were compared with those in faeces from untreated sheep on the same farm. This is futher evidence to support the probability that levamisole resistant Ostertagia and Trichostrongylus spp. were present on farm 13, even though the faecal egg count reduction achieved using levamisole on this farm had been considered equivocal.

MULTIPLE ANTHELMINTIC RESISTANCE

Although on farm 13 faecal egg count reduction tests yielded equivocal results for both oxfendazole and levamisole resistance, the results of faecal cultures on this farm provide further evidence that there may have been two genera of anthelmintic resistant nematodes present (Ostertagia and Trichostrongylus spp.), and that both genera were resistant to oxfendazole and levamisole (Table 3.VII).

Table 3.VII

Farm 13 - Larval culture results prior to treatment and 7 days after oxfendazole and levamisole treatments

	Larvae present in faeces (%)		
Nematode genus	Before treatment	7 days after:	
		Oxfendazole (5.0 mg/kg)	Levamisole (8.0 mg/kg)
Haemonchus	10	0	3
Ostertagia	39	55	40
Trichostrongylus	34	44	57
Cooperia	17	1	0

CHAPTER 4

DISCUSSION

Anthelmintic resistance in sheep nematodes is an important, but not the only possible cause of anthelmintic failure on sheep farms. When testing for anthelmintic resistance or investigating reports of unsatisfactory anthelmintic performance, it is important that other common causes of apparent anthelmintic failure, such as human error or drench gun faults, should also be considered.

In this survey, efforts were made to minimise the effects of any possible causes of anthelmintic failure other than resistance by nematodes to the anthelmintics used. Even so, interpretation of the results - of inherently imprecise field and laboratory tests - was dependent on arbitrarily determined levels of satisfactory performance and, for many of the farms, decisions had to be made on the basis of low egg counts in small numbers of sheep.

It is possible to reach some important general conclusions from this survey. Benzimidazole resistance was very common, and between 20 and 47% of farms had strongylates (other than Nematodirus spp.) resistant to benzimidazoles. Benzimidazole resistant Nematodirus spp. were present on the majority of farms. Levamisole resistance was possibly present on up to 13% of farms and nematodes resistant to ivermectin were possibly present on up to 20% of farms. Most farms had more than one nematode genus resistant to an anthelmintic, and one farm had nematodes resistant to more than one anthelmintic. Only one of the 15 farms surveyed had no resistant nematodes in their sheep.

The findings of this survey may seem surprising in relation to most previously published reports of anthelmintic resistance in New Zealand and the common belief that anthelmintic resistance in sheep nematodes is an Australian, and not a New Zealand problem. In fact, the results of earlier surveys do not conflict with the results reported in this paper, but it is apparent that the prevalence of affected farms has increased in New Zealand over the last 7 or 8 years.

Of 3 previous New Zealand surveys for anthelmintic resistance, 2 used faecal egg count reduction tests and a reduction of 90% or below as an indicator of resistance as was used in this survey. One of these surveys found considerably lower prevalences of benzimidazole resistance (1%), compared with those reported here (Kettle et al., 1981). The second survey, conducted on selected farms with high anthelmintic usage, found 16% of farms with benzimidazole resistance and 3% with levamisole resistance (Vlassoff and Kettle, 1986). These levels are comparable with those detected in this survey, perhaps because the normal frequency of anthelmintic usage now is similar to that which was earlier considered to be a high rate of use. Farmers in this survey treated their sheep with anthelmintics between 7 and 11 times during their first year of life.

A third New Zealand survey used the in vitro egg hatch test and detected benzimidazole resistance nematodes on 21% of farms (Kemp and Smith, 1980 and 1982) but this would probably equate to a much lower prevalence if a faecal egg count reduction test had been used as the latter test appears to be much less sensitive than the egg hatch test (Boersema et al., 1987).

More recently in New Zealand, McKenna (1988) reported that 74% and 15% of laboratory accessions for investigation of anthelmintic effectiveness showed evidence of definite or suspected benzimidazole and levamisole resistance, respectively. These figures are certainly based on a biased sample, but may represent the current upper limits of anthelmintic resistance prevalence on New Zealand sheep farms.

In comparison with the results of recent Australian surveys, all but 2 of the findings of this survey are not unexpected. The exceptions were the high prevalence found here of benzimidazole resistant Nematodirus spp., which has been scarcely mentioned in Australian literature, and the possible discovery of ivermectin resistance here when it has previously been reported in the field only in South Africa. Both of these findings warrant further consideration.

Nematodirus spp. were the most commonly detected resistant nematodes in this survey. This was unexpected as no other survey, in New Zealand or elsewhere, had reported finding resistant Nematodirus spp., although some practitioners and parasitologists considered that they were common, particularly in the Hawkes Bay and Otago-Southland areas of New Zealand and in Tasmania.

Nematodirus spp. are notorious for the small number of eggs they lay and for the very poor correlation between faecal egg counts and the number of adult nematodes present in sheep. In addition, it has been suggested that anthelmintic resistant strains of N. spathiger lay even fewer eggs than do susceptible strains, (Chalmers, 1985) thus making the difficult task of detecting these nematodes by faecal examination even more difficult. Egg counting alone is therefore considered an inadequate method of detecting anthelmintic resistant Nematodirus spp. and the results of this survey support that opinion.

It is probable that the occurrence of anthelmintic resistant Nematodirus spp. has been under-diagnosed both in Australia and New Zealand when only the faecal egg count reduction test has been used. The usual in vitro egg hatch test, sometimes used in surveys, does not even allow for the presence of Nematodirus spp. although a modified form, developed by Obendorf et al., (1986) does. When anthelmintic efficacy is investigated by faecal egg count reduction testing on individual farms, the chances of diagnosing resistance in Nematodirus spp. will be even smaller than in a survey of farms. This can be seen in the results of this survey in which the occurrence of benzimidazole resistant Nematodirus spp. only became apparent when the results from all farms were considered together: for most individual farms

the small numbers of Nematodirus spp. eggs present before and after treatment allowed only a suspicion of anthelmintic resistance in Nematodirus spp.

Although there are some conflicting opinions about the relative importance of Nematodirus spp., it is certain that at least their larvae can be important pathogens in young sheep under some circumstances (Soulsby, 1965). Therefore in areas where Nematodirus spp. are considered to be important pathogens in sheep, any test for anthelmintic resistance should be conducted so that resistant Nematodirus spp., if present, are likely to be detected. The faecal egg count reduction test is the one most likely to be used as a field test. It is important that sheep used in this test should be less than 9 months old as the development of resistance by sheep to Nematodirus spp. is age related and immunity is acquired relatively quickly after exposure to Nematodirus larvae (Brunsdon, 1960, 1962 and 1963; Soulsby, 1965). The number of sheep in the treated group (or groups) should not be less than 10, otherwise there is a possibility that the presence of Nematodirus spp. infection in the flock will be missed. In the laboratory, some consideration might be given to methods of increasing the sensitivity of the faecal egg counting technique in detecting small numbers of Nematodirus spp. eggs. If Nematodirus spp. egg counts are low and only a few sheep are infected before and after treatment, it is advisable to kill at least 2 sheep, 5 to 15 days after treatment, for total worm counts (including larval counts).

Although the unsatisfactory faecal egg count reductions achieved on farms one and five indicated that ivermectin resistance may have been present, it is not claimed that ivermectin resistant nematodes have been positively identified in this survey. Further investigations, including slaughter of ivermectin treated sheep, would be necessary before such a claim could be substantiated. It is possible that ivermectin resistant nematodes are present in sheep in New Zealand and even in the sheep on these two farms as ivermectin has been used on both, (and also on 9 of the other 13 farms). Strains of H. contortus have become resistant to ivermectin in South Africa, and in the United States of America a resistant strain of T. colubriformis developed under experimental conditions of 4 serial subcutaneous injections of lambs with ivermectin (Giordano et al., 1988). Ivermectin resistance has not been detected in sheep nematodes in Australia, but this is not surprising since the anthelmintic has been in use in Australian sheep only since early 1988. In New Zealand, ivermectin has been used in sheep since 1982.

Benzimidazole resistant Oesophagostomum spp. had not been reported in New Zealand or elsewhere until 1988 when Hughes (1988) and McKenna (pers. comm.) reported its presence in mixtures of anthelmintic resistant nematode genera from some farms in the Taihape district, approximately 100 km north of Palmerston North. In this survey benzimidazole resistant Oesophagostomum spp. were identified on 3 farms. The species was presumably Oe. venulosum which is common in sheep in New Zealand but not very pathogenic. Oe. columbianum, which is highly pathogenic, does not occur in New Zealand (Charleston and Pomroy, 1984). In this survey, wherever resistant Oesophagostomum spp.

were present, so too were one or more other more pathogenic species so a recommendation to change to an anthelmintic of another group was obviously correct. If only resistant Oesophagostomum spp. had been present, anthelmintics of the group against which resistance had developed could still have been used provided that farms were monitored to check that no other more pathogenic nematodes also developed resistance.

Although time consuming, faecal cultures for larval identification yield worthwhile additional information if the presence of anthelmintic resistant nematodes has been clearly established according to a faecal egg count reduction test, but if the diagnosis is equivocal then faecal culture may not contribute to a more definite diagnosis, since only small numbers of eggs (and hence larvae) will be present in faeces after anthelmintic treatment. If resistant strains of relatively non-pathogenic nematodes, such as Oe. venulosum, become more common then the need for larval culturing may increase.

On most of the farms where resistance was suspected, there appeared to be more than one resistant nematode genus involved. It has been suggested that this may now be the more common situation on North Island sheep farms (Hughes, 1988; McKenna, pers. comm.). Identification of the resistant genera depended on interpretation of the relative proportions of the different larvae present before and after treatment, and it was sometimes difficult to confidently identify all resistant nematode genera. However, when mixed nematode populations pre-treatment were replaced by only one genus post-treatment, as occurred with oxfendazole treatment on farms 2 and 15, identification of the resistant genus was relatively simple.

There was no attempt made in this survey on any farm to relate the degree of anthelmintic resistance present (as determined by a faecal egg count reduction test) to any measure of sheep productivity. To do so would have required a far more complex experimental design and a great deal more time. It was noted however, on farms 5, 14 and 15, where oxfendazole treatment gave faecal egg count reductions (method 1) of 78, 85 and 55% respectively, that stockmen had previously observed clinical signs of parasitism (including some deaths) which had not been alleviated by treatment with a benzimidazole anthelmintic. On these farms, anthelmintic resistance had apparently become severe enough to be an observable problem. The generally mild degree of anthelmintic resistance found in this survey, despite its relatively high prevalence, is consistent with a commonly held belief in New Zealand that anthelmintic resistance in sheep nematodes is not a problem - that is, it is not seen to be a problem because anthelmintics still appear to be fully effective on most farms.

Because moderate to high prevalences of oxfendazole resistance were detected in nematodes of the genera Haemonchus, Ostertagia, Trichostrongylus, Oesophagostomum and Nematodirus, the normal efficacy of oxfendazole against these parasites might be questioned. Oxfendazole has been described as "One of the most efficient of the

benzimidazoles... producing good results against a broad spectrum of parasites" (Arundel, 1985b) and most reports of controlled tests of efficacy concur with this opinion, although its efficacy at a dose rate of 5 mg/kg against fourth stage larval Nematodirus spp. was reported to be only 91% (Baker and Fisk, 1977; Chalmers, 1977 and 1979). An unfavourable report from the U.S.S.R. stated that oxfendazole, at a dose rate of 4.6 mg/kg, was only 75.5% and 57.1% effective against Nematodirus spp. and "other strongyles" of sheep, respectively; at 15 mg/kg it removed all Nematodirus spp. but was only 87.5% effective against "other strongyles" (Magomedov et al., 1986). Despite this one report to the contrary, it is generally accepted that the efficacy of oxfendazole is normally very high in the absence of resistant nematodes, and that egg count reductions of less than 95% following oxfendazole treatment indicate the presence of resistant nematodes.

There are many possible variations of the faecal egg count reduction test, both in its field and laboratory aspects but, because it is a relatively simple and widely used test, there have been calls for its improvement and standardisation (Donald, 1985; Waller, 1988). For this survey a version of the test was chosen which was thought to be simple and practical yet capable of yielding valid results. However it was found that, even though it was used in conjunction with larval cultures and identification, the test did not always give unequivocal results, especially when faecal egg count reductions of 91-96% were obtained. In these cases, additional confirmatory tests, such as total worm counts of treated sheep or appropriate in vitro tests were needed. Therefore, although a good, standard faecal egg count reduction test is needed, it must be emphasized that such a test, used alone, could only be regarded as a screening test for anthelmintic resistance in sheep nematodes.

Arising out of this survey, a recommended standard protocol for the conduct of a faecal egg count reduction test was produced (Appendix IV), and submitted for discussion to the 18th Seminar of the Sheep and Beef Cattle Society of the New Zealand Veterinary Association (July, 1988). Subsequently, and almost simultaneously in Australia and New Zealand, two different standardised procedures for this test have been produced and recommended for adoption in their respective countries (Charleston, 1988; Martin, 1988b).

There is not unanimous agreement on the best method for calculation of the faecal egg count reduction. The use of geometric means had some support in Australia, but it seems now that most scientists favour the use of arithmetic means because of their unambiguous simplicity. Both protocols recommended for national application in Australia and New Zealand use arithmetic means to calculate faecal egg count reductions.

Although anthelmintic resistance on New Zealand sheep farms may be generally less severe than in Australia, there should be no complacency about its potential importance. Many farms already have resistant genera present and it is inevitable that they will suffer increased losses if their helminth control practices continue unchanged. Veterinary practitioners have an important role in both diagnosing anthelmintic resistance and advising farmers on

effective methods of helminth control that take anthelmintic resistance into account. As the faecal egg count reduction test is the field test for resistance most likely to be used, practitioners should acquaint themselves with the recently recommended standard procedure for this test, as well as its limitations, particularly in regard to the identification of resistant Nematodirus spp. and the interpretation of equivocal results.

APPENDIX I

METHOD OF COUNTING NEMATODE EGGS IN SHEEP FAECES

(1) Weigh 2 gm of faeces on a plastic spoon.

(2) Fill a Universal bottle to capacity (28 ml) with saturated salt (NaCl) solution and empty the solution into a small bowl.

(3) Tip weighed faeces into a small sieve (375 μm aperture) and rub through the sieve, into the salt solution, using the spoon.

(4) Discard spoon, sieve and faecal residue.

(5) Mix contents of bowl thoroughly and while mixing remove a sample with a pipette.

(6) Quickly transfer the contents of the pipette to one chamber of a McMaster egg counting slide.

(7) Repeat mixing and sampling and fill the other chamber of the counting slide.

(8) Allow the slide to stand for at least 2 minutes to allow eggs to rise in the salt solution.

(9) Count eggs in both chambers of the slide systematically, using a 10x objective lens.

(10) The number of eggs per gram of faeces (e.p.g.) equals the total number of eggs counted in both chambers of the slide, times 50.

APPENDIX II

METHOD OF FAECAL CULTURE AND RECOVERY OF LARVAE FOR IDENTIFICATION USING A MODIFIED BAERMANN TECHNIQUE

(1) Weigh the same quantity of faeces (0.5-2.0 gm) from all sheep in one group and mix all samples together using a mortar and pestle.

(2) Add vermiculite and water as required and blend until the mixture just sticks together and a small amount of water can be squeezed from it between the fingers.

(3) Place all of the mixture loosely in a suitable sized glass jar, leaving an air space at the top of at least 30% of the jar's volume. Screw a lid on loosely.

(4) Place the jar in an incubator at 27°C for 7 days. Ensure during this period that adequate moisture levels are maintained in the mixture.

(5) Empty the contents of the jar onto a double thickness of fine paper tissue in a sieve and fold the tissue over the mixture.

(6) Place sieve and contents on the top of a glass funnel which has clamped rubber tubing attached.

(7) Fill the funnel to within 2 cm of the top with water at room temperature, and leave overnight or for 8 hours.

(8) Release the clamp and draw off approximately 50 ml of water (containing larvae) into a suitable container.

(9) Centrifuge 5-10 ml of the water for 1-2 minutes and discard all but 0.5 ml.

(10) Add one drop of Lugol's iodine to the residue to kill larvae, then place one or two drops on a microscope slide, add a coverslip and identify all larvae present, up to a total of 100, under low power magnification, (Anon, 1977).

Larvae were identified by their general morphological characteristics (Anon, 1977). Ostertagia and Trichostrongylus larvae were differentiated on the basis of size; the former being longer and the latter being shorter than 725 μm (Vlassoff, pers. comm.). Oesophagostomum and Chabertia larvae were also differentiated on the basis of size; the former being longer and the latter being shorter than 790 μm (Soulsby, 1965).

APPENDIX III INDIVIDUAL FARM RESULTS - FARMS 1 to 15

Abbreviations used:

e.p.g.	Nematode eggs per gram of sheep faeces
S	Strongylate species, not including *Nematodirus*
N	*Nematodirus* species
N S	Not sampled
$FECR_1$, $FECR_2$, $FECR_3$	Faecal egg count reduction calculated by methods 1, 2 or 3 (see Materials and Methods)
Mean body weight	Arithmetic mean body weight

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 1

Faecal egg counts (epg)

CONTROL

	9/ 2/88		16/ 2/88	
	S	N	S	N
Mean body weight: 31 kg (28.5-35.5)	150	0	400	0
	850	0	650	0
	50	0	350	0
	0	0	950	0
	100	50	850	0
	950	0	1250	0
	800	0	1250	0
	550	0	1250	0
	0	0	400	0
	350	0	200	0
	0	0	500	0
	150	0	N S	
MEAN	329	4	732	0

IVERMECTIN (0.2) mg/kg

	9/ 2/88		16/ 2/88	
	S	N	S	N
Mean body weight: 32 kg (28.5-36.0)	150	0	150	0
	200	0	0	0
	500	0	0	0
	250	50	50	0
	0	0	0	0
	300	0	350	0
	1050	0	0	0
	450	0	100	0
	1200	0	0	0
	680	0	0	0
	150	0	0	0
	450	0	0	0
MEAN	448	4	54	0

$FECR_1$: 88% $FECR_2$: 97% $FECR_3$: 96%

OXFENDAZOLE (5.0 mg/kg)

	9/ 2/88		16/ 2/88	
	S	N	S	N
Mean body weight: 32 kg (27.5-34.5)	0	0	0	0
	150	50	0	50
	200	50	0	200
	650	50	0	0
	750	0	0	0
	450	0	50	0
	1000	0	0	0
	450	0	0	0
	650	0	0	0
	150	0	0	0
	200	0	0	0
	250	0	0	0
MEAN	408	8	4	21

$FECR_1$: 99% $FECR_2$: >99% $FECR_3$: >99%

LEVAMISOLE (8.0 mg/kg)

	9/ 2/88		16/ 2/88	
	S	N	S	N
Mean body weight: 31 kg (28.5-35.5)	550	50	0	0
	150	0	0	0
	450	0	N S	
	350	0	50	0
	1400	0	0	0
	1650	50	0	0
	0	0	N S	
	150	50	0	0
	650	0	0	0
	350	0	0	0
	400	0	0	0
	400	0	0	0
MEAN	542	13	5	0

$FECR_1$: 99% $FECR_2$: >99% $FECR_3$: >99%

Larval Cultures

Before treatment: Haemonchus, Ostertagia, Trichostrongylus, Cooperia.
After Oxfendazole treatment: Cooperia, Oesophagostomum (only 2-3 larvae total)
After ivermectin treatment: Ostertagia, Trichostrongylus (only 2-3 larvae total)

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 2

Faecal egg counts (epg)

CONTROL

Mean body weight: 31 kg (22.0-38.5)

	15/ 2/88		22/ 2/88	
	S	N	S	N
	200	0	750	0
	750	250	1050	500
	300	150	200	0
	450	50	2100	100
	1450	200	600	0
	100	0	50	0
	450	200	N S	
	550	0	550	100
	200	50	150	0
	100	0	300	0
	550	100	800	0
	250	50	100	50
MEAN	446	88	605	68

IVERMECTIN (0.2) mg/kg

Mean body weight: 32 kg (21.5-39.0)

	15/ 2/88		22/ 2/88	
	S	N	S	N
	250	150	0	0
	100	0	0	0
	50	50	0	0
	700	350	0	0
	200	50	0	0
	200	50	0	0
	350	0	0	0
	150	100	0	0
	150	200	N S	
	0	0	0	0
	650	100	0	0
	150	50	0	0
MEAN	246	92	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

OXFENDAZOLE (5.0 mg/kg)

Mean body weight: 34 kg (24.5-40.0)

	15/ 2/88		22/ 2/88	
	S	N	S	N
	300	0	50	150
	100	0	0	0
	1400	0	150	0
	300	50	200	50
	300	300	150	0
	150	0	0	0
	150	0	50	0
	350	0	100	0
	2000	50	250	350
	800	0	0	0
	200	0	50	0
	100	0	0	0
MEAN	513	38	83	46

$FECR_1$: 84% $FECR_2$: 86% $FECR_3$: 86%

LEVAMISOLE (8.0 mg/kg)

Mean body weight: 33 kg (25.0-37.0)

	15/ 2/88		22/ 2/88	
	S	N	S	N
	550	50	0	0
	200	50	0	0
	700	0	0	0
	550	150	0	0
	400	0	0	0
	500	0	0	0
	350	0	0	0
	50	0	0	0
	50	0	0	0
	1000	150	0	0
	750	150	0	0
	600	100	0	0
MEAN	475	54	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

Larval Cultures

Before treatment: Haemonchus 34%; Ostertagia 4%; Trichostrongylus 36%; Cooperia 2%; Chabertia 1%; Oesophagostomum 14%.
After oxfendazole treatment: Haemonchus 2%; Trichostrongylus 98%.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 3

Faecal egg counts (epg)

CONTROL

Mean body weight: 29 kg (25.5-32.0)

	18/ 2/88		26/ 2/88	
	S	N	S	N
	100	0	200	0
	600	0	450	0
	450	100	900	100
	200	0	0	0
	100	100	700	0
	0	0	0	0
	500	0	750	50
	400	0	1250	50
	1150	0	1350	50
	750	50	1200	50
	400	0	750	50
	150	0	150	0
MEAN	400	21	642	29

IVERMECTIN (0.2) mg/kg

Mean body weight: 28 kg (23.0-31.0)

	18/ 2/88		26/ 2/88	
	S	N	S	N
	0	0	0	0
	100	0	0	0
	250	0	0	0
	700	50	0	0
	1200	0	0	0
	200	0	0	0
	0	0	0	0
	100	0	0	0
	200	0	200	0
	100	0	0	0
	200	50	0	0
	150	0	0	0
MEAN	267	8	17	0

$FECR_1$: 94% $FECR_2$: 98% $FECR_3$: 99%

OXFENDAZOLE (5.0 mg/kg)

Mean body weight: 30 kg (25.5-32.5)

	18/ 2/88		26/ 2/88	
	S	N	S	N
	750	0	N S	
	950	0	0	0
	250	0	0	0
	50	0	0	0
	150	0	N S	
	1150	0	0	0
	150	0	100	0
	100	0	0	0
	0	50	0	0
	350	0	0	50
	850	0	N S	
	600	0	0	0
MEAN	446	4	11	6

$FECR_1$: 98% $FECR_2$: 99% $FECR_3$: 99%

LEVAMISOLE (8.0 mg/kg)

Mean body weight: 29 kg (26.5-31.0)

	18/ 2/88		26/ 2/88	
	S	N	S	N
	100	0	0	0
	150	0	0	0
	450	0	150	0
	200	0	0	0
	150	0	0	0
	200	0	0	0
	650	0	0	0
	50	0	0	0
	550	0	50	0
	150	0	0	0
	100	50	0	0
	200	0	0	0
MEAN	246	4	17	0

$FECR_1$: 93% $FECR_2$: 97% $FECR_3$: 99%

Larval Cultures

Before treatment: *Haemonchus* 22%; *Ostertagia* 54%; *Trichostrongylus* 23%; *Oesophagostomum* 2%.
No post-treatment cultures performed.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 4

Faecal egg counts (epg)

CONTROL

	23/ 2/88		1/ 3/88	
	S	N	S	N
	750	0	50	0
	450	700	200	550
Mean body	450	0	100	0
	1500	200	150	0
weight:	400	100	150	0
	200	0	100	0
30 kg	50	0	0	50
	50	50	200	0
(25.5-33.0)	200	100	150	300
	200	350	250	450
	1500	400	250	250
	400	950	100	400
MEAN	513	238	142	167

IVERMECTIN (0.2) mg/kg

	23/ 2/88		1/ 3/88	
	S	N	S	N
	150	150	0	0
	200	200	0	0
Mean body	100	50	0	0
	400	0	0	0
weight:	450	0	0	0
	1150	250	0	0
30 kg	350	50	0	0
	850	400	0	0
(26.0-32.5)	150	1250	0	0
	150	0	0	0
	50	0	0	0
	650	400	0	0
MEAN	388	221	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

OXFENDAZOLE (5.0 mg/kg)

	23/ 2/88		1/ 3/88	
	S	N	S	N
	100	0	0	0
	800	350	50	50
	150	0	50	0
	200	0	0	0
Mean body	1650	1600	0	850
	450	250	0	50
weight:	200	600	100	200
	500	1050	0	0
29 kg	100	0	0	0
	1000	200	N S	
(23.0-34.5)	0	50	0	0
	1000	200	0	0
MEAN	513	358	18	105

$FECR_1$: 97% $FECR_2$: 90% $FECR_3$: 90%

LEVAMISOLE (8.0 mg/kg)

	23/ 2/88		1/ 3/88	
	S	N	S	N
	100	0	0	0
	1000	0	0	0
	400	1850	0	0
	550	350	0	0
Mean body	0	0	0	0
	250	0	0	0
weight:	500	0	0	0
	300	100	50	0
30 kg	250	0	0	0
	350	0	0	0
(22.0-34.5)	300	250	0	0
	150	0	0	0
MEAN	346	213	4	0

$FECR_1$: 99% $FECR_2$: 97% $FECR_3$: 98%

Larval Cultures

Before treatment: Haemonchus 6%; Ostertagia 46%; Trichostrongylus 44%; Oesophagostomum 4%.

After oxfendazole treatment: Haemonchus 11%; Trichostrongylus 68%; Oesophagostomum 21%

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 5

Faecal egg counts (epg)

CONTROL

	29/ 2/88		7/ 3/88	
	S	N	S	N
	550	500	350	400
	750	100	950	250
Mean body	100	50	350	0
	850	200	750	300
weight:	300	50	500	0
	0	0	0	0
33 kg				
(28.0-45.0)				
MEAN	425	150	483	158

IVERMECTIN (0.2) mg/kg

	29/ 2/88		7/ 3/88	
	S	N	S	N
	350	300	150	0
	600	100	0	0
	250	0	0	0
Mean body	300	200	0	0
	550	550	0	0
weight:	400	50	0	0
	250	350	250	200
33 kg	500	150	0	100
	100	0	0	0
(29.0-42.0)	100	100	0	0
	0	100	0	0
	100	0	0	0
MEAN	292	158	33	33

$FECR_1$: 89% $FECR_2$: 97% $FECR_3$: 98%

OXFENDAZOLE (5.0 mg/kg)

	29/ 2/88		7/ 3/88	
	S	N	S	N
	50	0	0	0
	550	250	150	100
	550	350	100	300
	300	0	0	150
Mean body	500	250	100	350
	500	150	N	S
weight:	0	150	0	200
	850	200	100	550
32 kg	0	100	0	150
	350	50	50	0
(30.0-34.5)	400	200	100	150
	1000	150	400	0
MEAN	421	154	91	177

$FECR_1$: 78% $FECR_2$: 86% $FECR_3$: 86%

LEVAMISOLE (8.0 mg/kg)

	29/ 2/88		7/ 3/88	
	S	N	S	N
	200	300	50	0
	250	250	0	0
	600	0	0	0
	100	50	0	0
Mean body	350	0	0	0
	700	400	0	0
weight:	200	300	0	0
	0	0	0	0
32 kg	800	300	0	0
	0	150	0	50
(24.0-43.5)	0	100	0	0
	650	200	100	0
MEAN	321	171	13	4

$FECR_1$: 96% $FECR_2$: 98% $FECR_3$: 99%

Larval Cultures

Before treatment: Ostertagia 14%; Trichostrongylus 85%; Oesophagostomum 1%.
After oxfendazole treatment: Haemonchus 11%; Ostertagia 1%; Trichostrongylus 67%; Oesophagostomum 21%.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 6

Faecal egg counts (epg)

CONTROL

	4/ 3/88		11/ 3/88	
	S	N	S	N
	650	0	N S	
	4200	0	9400	0
Mean body	1250	0	7800	50
	2750	0	12450	50
weight:	100	0	N S	
	1400	0	N S	
28 kg	550	0	4100	0
	2650	0	10250	0
(23.0-33.0)	4300	0	19600	0
	4050	150	21650	100
	2900	50	19700	100
	1350	0	10300	0
MEAN	2179	17	12806	33

IVERMECTIN (0.2) mg/kg

	4/ 3/88		11/ 3/88	
	S	N	S	N
	1550	0	0	0
	3950	0	0	0
Mean body	3700	0	0	0
	2300	0	0	0
weight:	1650	0	250	0
	5850	0	0	0
28 kg	0	0	N S	
	600	0	0	0
(23.0-31.5)	4000	0	0	0
	1450	0	0	0
	1700	50	0	0
	2300	0	N S	
MEAN	2421	4	25	0

$FECR_1$: 99% $FECR_2$: >99% $FECR_3$: >99%

OXFENDAZOLE (5.0 mg/kg)

	4/ 3/88		11/ 3/88	
	S	N	S	N
	3800	100	200	0
	800	0	0	0
	1400	0	0	100
	1300	0	N S	
Mean body	10850	0	0	0
	4250	0	50	50
weight:	350	0	0	0
	2700	0	0	150
26 kg	900	0	0	0
	4350	50	0	100
(22.0-32.0)	5100	50	50	0
	2200	0	N S	
MEAN	3167	17	30	40

$FECR_1$: 99% $FECR_2$: >99% $FECR_3$: >99%

LEVAMISOLE (8.0 mg/kg)

	4/ 3/88		11/ 3/88	
	S	N	S	N
	1050	50	0	0
	6600	0	0	0
	1300	0	0	0
	3300	0	N S	
Mean body	900	0	0	0
	1100	0	0	0
weight:	1900	0	0	0
	450	0	0	0
26 kg	1550	0	0	0
	1750	0	0	0
(24.5-29.5)	2550	0	50	0
	1150	0	50	0
MEAN	1967	4	9	0

$FECR_1$: >99% $FECR_2$: >99% $FECR_3$: >99%

Larval Cultures

Before treatment: Haemonchus 14%; Ostertagia 11%; Trichostrongylus 75%.
No post treatment cultures performed.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 7

Faecal egg counts (epg)

CONTROL

Mean body weight: 29 kg (27.0-36.0)

	8/ 3/88		15/ 3/88	
	S	N	S	N
	400	0	200	0
	100	0	250	50
	100	100	200	0
	3050	50	50	0
	250	0	500	100
	0	50	400	0
	750	50	1050	0
	3800	0	N S	
	200	0	50	0
	1200	50	200	50
	250	0	50	0
	650	50	950	50
MEAN	896	29	355	23

IVERMECTIN (0.2) mg/kg

Mean body weight: 33 kg (27.0-39.0)

	8/ 3/88		15/ 3/88	
	S	N	S	N
	350	0	0	0
	1550	0	0	0
	2450	50	0	0
	100	0	0	0
	300	0	0	0
	100	0	0	0
	150	0	0	0
	200	0	0	0
	300	0	0	0
	650	50	0	0
	350	0	0	0
	300	0	0	0
MEAN	567	8	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

OXFENDAZOLE (5.0 mg/kg)

Mean body weight: 33 kg (30.0-37.0)

	8/ 3/88		15/ 3/88	
	S	N	S	N
	150	0	0	0
	200	0	0	0
	650	50	0	0
	1400	100	N S	
	100	0	0	0
	700	0	0	0
	1950	50	50	0
	200	0	50	0
	100	0	50	0
	1550	0	0	0
	300	0	0	0
	50	0	0	0
MEAN	613	17	14	0

$FECR_1$: 98% $FECR_2$: 95% $FECR_3$: 96%

LEVAMISOLE (8.0 mg/kg)

Mean body weight: 32 kg (27.0-35.0)

	8/ 3/88		15/ 3/88	
	S	N	S	N
	800	0	0	50
	550	0	100	0
	1600	0	0	0
	50	100	0	0
	3000	100	0	0
	0	0	0	0
	350	0	0	0
	1450	50	0	0
	0	0	0	0
	600	50	0	0
	100	0	0	0
	0	0	0	0
MEAN	708	25	8	4

$FECR_1$: 99% $FECR_2$: 99% $FECR_3$: 98%

Larval Cultures

Before treatment: Haemonchus 45%; Ostertagia 26%; Trichostrongylus 28%; Cooperia 1%.
No post treatment cultures performed.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 8

Faecal egg counts (epg)

CONTROL

	9/ 3/88 S	9/ 3/88 N	16/ 3/88 S	16/ 3/88 N
Mean body weight: 44 kg (36.0-55.0)	150	0	100	0
	0	0	50	0
	700	100	50	0
	100	50	100	100
	600	0	400	0
	250	0	N S	
	200	0	100	0
	200	0	100	0
	0	100	50	100
	50	0	N S	
	200	200	200	100
	250	150	0	50
MEAN	225	50	115	35

IVERMECTIN (0.2) mg/kg

	9/ 3/88 S	9/ 3/88 N	16/ 3/88 S	16/ 3/88 N
Mean body weight: 42 kg (36.0-48.0)	950	50	0	0
	900	50	0	0
	50	0	0	0
	450	0	0	0
	450	0	0	0
	50	0	0	0
	50	50	0	0
	50	0	0	0
	50	0	0	0
	150	0	0	0
	150	0	0	0
	350	0	0	0
MEAN	304	13	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

OXFENDAZOLE (5.0 mg/kg)

	9/ 3/88 S	9/ 3/88 N	16/ 3/88 S	16/ 3/88 N
Mean body weight: 45 kg (38.0-56.0)	1150	0	50	0
	100	50	0	150
	250	0	50	0
	50	100	0	200
	50	50	50	50
	0	0	0	0
	100	0	50	0
	1200	0	0	0
	100	50	0	0
	50	0	0	0
	950	0	50	0
	100	0	50	0
MEAN	342	21	25	33

$FECR_1$: 93% $FECR_2$: 71% $FECR_3$: 78%

LEVAMISOLE (8.0 mg/kg)

	9/ 3/88 S	9/ 3/88 N	16/ 3/88 S	16/ 3/88 N
Mean body weight: 41 kg (33.0-46.0)	450	0	0	0
	450	0	0	0
	0	0	0	0
	100	0	0	0
	900	0	0	0
	600	0	0	0
	600	50	N S	
	100	0	0	0
	350	50	0	0
	800	150	0	0
	150	50	N S	
	0	50	0	0
MEAN	375	29	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

Larval Cultures

Before treatment: Haemonchus 21%; Ostertagia 56%; Trichostrongylus 18%; Cooperia 4%; Chabertia 1%.

After oxfendazole treatment: Haemonchus 2%; Ostertagia 9%; Trichostrongylus 32%; Oesophagostomum 57%.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 9

Faecal egg counts (epg)

CONTROL

	21/ 3/88		28/ 3/88	
	S	N	S	N
	850	300	1150	150
	50	0	150	0
Mean body	200	200	550	200
	550	350	1150	200
weight:	250	150	800	100
	800	100	1450	150
27 kg	200	0	550	0
	400	50	650	0
(24.5-32.5)	700	50	1600	100
	0	0	450	0
	1250	200	800	300
	100	150	850	250
MEAN	446	129	842	121

IVERMECTIN (0.2) mg/kg

	21/ 3/88		28/ 3/88	
	S	N	S	N
	50	200	0	0
	100	50	0	0
Mean body	250	50	0	0
	450	200	0	0
weight:	700	100	0	0
	250	0	0	0
29 kg	0	0	0	0
	50	0	0	0
(26.0-33.0)	1050	250	0	0
	0	0	0	0
	150	50	0	0
	400	0	0	0
MEAN	288	75	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

OXFENDAZOLE (5.0 mg/kg)

	21/ 3/88		28/ 3/88	
	S	N	S	N
	350	50	0	50
	800	50	0	150
	100	0	0	0
	50	100	0	100
Mean body	400	0	0	0
	650	0	0	0
weight:	250	200	0	100
	450	50	N S	
27 kg	2050	0	0	50
	400	50	0	150
(24.0-30.0)	1050	200	0	100
	0	0	0	0
MEAN	546	58	0	64

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

LEVAMISOLE (8.0 mg/kg)

	21/ 3/88		28/ 3/88	
	S	N	S	N
	500	150	0	0
	1000	100	0	0
	150	100	0	0
	100	0	0	0
Mean body	0	0	0	0
	0	0	0	0
weight:	350	0	0	0
	250	0	0	0
27 kg	100	0	0	0
	100	0	0	0
(22.0-30.5)				
MEAN	255	35	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

Larval Cultures

Before treatment: Ostertagia 11%; Trichostrongylus 21%; Cooperia 6%; Oesophagostomum 62%.

No post treatment cultures performed.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 10

Faecal egg counts (epg)

CONTROL

Mean body weight: 33 kg (27.0-41.5)

	24/ 3/88		31/ 3/88	
	S	N	S	N
	2550	1300	1600	600
	1200	50	1450	0
	1100	0	400	0
	100	0	200	0
	850	0	1050	0
	1450	50	2450	50
	900	100	900	0
	1050	0	2050	0
	2800	200	2000	200
	400	50	900	0
	700	250	500	0
	1150	350	3200	550
MEAN	1188	196	1392	117

IVERMECTIN (0.2) mg/kg

Mean body weight: 31 kg (24.0-36.5)

	24/ 3/88		31/ 3/88	
	S	N	S	N
	1750	300	0	0
	1450	0	0	0
	800	550	0	0
	2800	300	N S	
	2000	0	0	0
	1000	0	0	0
	750	100	N S	
	1150	400	0	0
	600	100	0	0
	400	700	0	0
	2400	800	0	0
	1250	250	0	0
MEAN	1363	292	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

OXFENDAZOLE (5.0 mg/kg)

Mean body weight: 29 kg (25.0-34.0)

	24/ 3/88		31/ 3/88	
	S	N	S	N
	1150	0	0	0
	900	0	0	0
	5400	200	50	0
	500	250	0	0
	900	350	0	0
	500	0	50	0
	150	0	0	0
	700	350	0	50
	700	0	0	0
	1200	350	0	0
	2400	50	0	0
	650	0	0	0
MEAN	1263	129	8	4

$FECR_1$: 99% $FECR_2$: >99% $FECR_3$: >99%

LEVAMISOLE (8.0 mg/kg)

Mean body weight: 32 kg (25.0-38.5)

	24/ 3/88		31/ 3/88	
	S	N	S	N
	1350	50	0	0
	1650	0	0	0
	1050	0	0	0
	1950	0	0	0
	550	200	0	0
	800	0	0	0
	200	0	0	0
	1900	200	0	0
	1350	600	0	0
	2400	500	0	0
MEAN	1320	155	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

Larval Cultures

Before treatment: *Haemonchus* 7%; *Ostertagia* 4%; *Trichostrongylus* 57%; *Cooperia* 11%; *Oesophagostomum* 21%.

No post treatment cultures performed.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 11

Faecal egg counts (epg)

CONTROL

	7/ 4/88 S	7/ 4/88 N	14/ 4/88 S	14/ 4/88 N
	150	0	350	0
	50	0	250	0
Mean body	550	0	1000	0
	650	0	2700	0
weight:	100	0	N S	
	100	0	700	0
35 kg	50	0	300	0
	1150	150	1200	150
(25.5-47.0)	350	0	850	0
	450	0	1450	0
	200	0	350	0
	250	0	1350	0
MEAN	338	13	955	14

IVERMECTIN (0.2) mg/kg

	7/ 4/88 S	7/ 4/88 N	14/ 4/88 S	14/ 4/88 N
	100	100	0	0
	300	0	0	0
Mean body	0	0	0	0
	1650	50	150	0
weight:	0	0	0	0
	0	0	0	0
36 kg	250	0	0	0
	550	100	0	0
(25.5-45.0)	1450	0	0	0
	450	0	0	0
	100	0	0	0
	50	0	0	0
MEAN	408	21	13	0

$FECR_1$: 97% $FECR_2$: 99% $FECR_3$: 99%

OXFENDAZOLE (5.0 mg/kg)

	7/ 4/88 S	7/ 4/88 N	14/ 4/88 S	14/ 4/88 N
	350	0	0	50
	600	300	0	0
	850	0	50	0
	100	0	0	50
Mean body	250	0	N S	
	450	0	250	0
weight:	1600	250	0	250
	900	100	0	0
36 kg	250	0	50	50
	100	0	0	0
(27.0-43.5)	700	100	0	100
	750	0	0	0
MEAN	575	63	32	46

$FECR_1$: 94% $FECR_2$: 99% $FECR_3$: 98%

LEVAMISOLE (8.0 mg/kg)

	7/ 4/88 S	7/ 4/88 N	14/ 4/88 S	14/ 4/88 N
	550	0	N S	
	250	0	0	0
	400	50	0	0
	0	0	0	0
Mean body	300	500	0	0
	150	400	0	100
weight:	0	0	0	0
	350	0	0	0
33 kg	550	0	0	0
	350	100	0	0
(28.5-40.0)	600	150	0	0
	250	0	0	0
MEAN	313	100	0	9

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

Larval Cultures

Before treatment: Haemonchus 38%; Ostertagia 4%; Trichostrongylus 19%; Cooperia 37%; Oesophagostomum 2%.
After oxfendazole treatment: Only 2 larvae found - Trichostrongylus and Oesophagostomum.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 12

Faecal egg counts (epg)

CONTROL

	12/ 4/88		19/ 4/88	
	S	N	S	N
Mean body weight: 32 kg (28.0-33.0)	900	200	600	100
	200	0	470	0
	3300	200	1850	300
	1000	0	800	0
	400	200	250	100
	2700	600	2250	200
	400	50	50	50
	400	50	550	50
	880	0	650	50
	400	0	850	0
	1600	0	1100	0
	1000	0	250	50
MEAN	1098	108	806	75

IVERMECTIN (0.2) mg/kg

	12/ 4/88		19/ 4/88	
	S	N	S	N
Mean body weight: 32 kg (28.0-36.0)	700	0	0	0
	800	0	0	0
	850	0	0	0
	500	0	0	0
	850	0	0	0
	250	0	0	0
	1250	50	N S	
	2750	950	0	0
	650	50	0	0
	280	0	0	0
	100	0	0	0
	2600	0	0	0
MEAN	965	88	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

OXFENDAZOLE (5.0 mg/kg)

	12/ 4/88		19/ 4/88	
	S	N	S	N
Mean body weight: 30 kg (27.0-33.5)	1050	0	0	0
	750	0	0	0
	800	0	0	150
	50	0	0	0
	3050	0	0	50
	1450	350	0	0
	750	0	0	0
	280	0	0	0
	1950	500	0	0
	0	0	0	0
	1950	0	0	0
	600	0	0	0
MEAN	1057	71	0	17

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

LEVAMISOLE (8.0 mg/kg)

	12/ 4/88		19/ 4/88	
	S	N	S	N
Mean body weight: 33 kg (28.0-35.0)	1150	800	50	0
	1750	50	0	0
	500	450	0	0
	1100	0	0	0
	600	0	0	0
	650	400	0	0
	1650	0	0	0
	450	150	0	0
	1800	0	0	0
	1050	0	0	0
	950	200	0	0
	1450	0	0	0
MEAN	1092	171	4	0

$FECR_1$: >99% $FECR_2$: >99% $FECR_3$: >99%

Larval Cultures

Before treatment: Haemonchus 19%; Ostertagia 5%; Trichostrongylus 66%; Cooperia 4%; Chabertia 3%; Oesophagostomum 3%.
No post treatment cultures performed.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 13

Faecal egg counts (epg)

CONTROL

Mean body weight: 30 kg (26.0-32.0)

	29/ 4/88		6/ 5/88	
	S	N	S	N
	50	0	50	0
	200	0	600	0
	200	0	350	0
	200	0	400	0
	200	0	530	0
	50	0	300	0
	50	0	400	0
	50	0	1500	0
	50	0	350	0
	500	0	600	0
	400	0	400	0
	200	0	200	0
MEAN	179	0	473	0

IVERMECTIN (0.2) mg/kg

Mean body weight: 32 kg (25.5-36.0)

	29/ 4/88		6/ 5/88	
	S	N	S	N
	150	0	0	0
	0	0	0	0
	170	0	0	0
	250	0	0	0
	50	0	0	0
	200	0	0	0
	300	0	0	0
	200	0	0	0
	100	0	0	0
	0	0	0	0
	100	0	0	0
	250	0	0	0
MEAN	148	0	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

OXFENDAZOLE (5.0 mg/kg)

Mean body weight: 28 kg (23.0-32.5)

	29/ 4/88		6/ 5/88	
	S	N	S	N
	200	0	0	0
	200	0	0	0
	600	0	100	0
	100	0	N	S
	200	0	50	0
	200	0	0	0
	100	0	N	S
	250	0	0	0
	250	0	0	0
	100	0	0	0
	50	0	0	0
	300	0	0	0
MEAN	213	0	15	0

$FECR_1$: 93% $FECR_2$: 99% $FECR_3$: 98%

LEVAMISOLE (8.0 mg/kg)

Mean body weight: 30 kg (26.5-36.0)

	29/ 4/88		6/ 5/88	
	S	N	S	N
	50	0	0	0
	50	0	0	0
	100	0	50	0
	400	0	55	0
	300	0	0	0
	450	0	50	0
	100	0	0	0
	50	0	0	0
	100	0	0	0
	50	0	0	0
	250	0	0	0
	150	0	0	0
MEAN	171	0	13	0

$FECR_1$: 92% $FECR_2$: 98% $FECR_3$: 98%

Larval Cultures

Before treatment: *Haemonchus* 10%; *Ostertagia* 39%; *Trichostrongylus* 34%; *Cooperia* 17%.
After oxfendazole treatment: *Ostertagia* 55%; *Trichostrongylus* 44%; *Cooperia* 1%.
After levamisole treatment: *Haemonchus* 3%; *Ostertagia* 40%; *Trichostrongylus* 57%.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 14

Faecal egg counts (epg)

CONTROL

Mean body weight: 28 kg (25.0-32.0)

	9/ 5/88		16/ 5/88	
	S	N	S	N
	300	0	N S	
	450	0	150	0
	600	0	750	0
	350	0	500	0
	100	0	250	0
	150	0	N S	
	750	100	800	50
	1800	0	1400	100
	2250	0	875	0
	400	0	350	0
	1300	0	1150	150
	150	50	150	100
MEAN	717	13	638	40

IVERMECTIN (0.2) mg/kg

Mean body weight: 30 kg (25.0-35.0)

	9/ 5/88		16/ 5/88	
	S	N	S	N
	550	50	N S	
	1100	50	0	0
	650	0	0	0
	50	0	0	0
	2750	50	0	0
	1350	0	0	0
	600	0	N S	
	1000	50	N S	
	550	50	0	0
	0	0	0	0
	400	150	0	0
	0	0	0	0
MEAN	750	33	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

OXFENDAZOLE (5.0 mg/kg)

Mean body weight: 28 kg (20.0-35.0)

	9/ 5/88		16/ 5/88	
	S	N	S	N
	1500	0	200	0
	2500	0	500	0
	1350	50	250	0
	4600	0	100	0
	4250	0	600	0
	1550	0	200	0
	1350	0	500	0
	0	0	0	0
	350	0	N S	
	50	0	0	0
	1800	100	150	0
	850	150	250	300
MEAN	1679	25	250	27

$FECR_1$: 85% $FECR_2$: 84% $FECR_3$: 60%

LEVAMISOLE (8.0 mg/kg)

Mean body weight: 29 kg (25.0-35.0)

	9/ 5/88		16/ 5/88	
	S	N	S	N
	1400	0	0	0
	850	50	0	0
	450	50	0	0
	550	0	0	0
	1300	100	0	0
	600	0	0	0
	1250	100	0	0
	950	200	0	0
	150	0	0	0
	0	0	0	0
	3150	0	N S	
	2750	0	0	0
MEAN	1117	42	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

Larval Cultures

Before treatment: Haemonchus 42%; Ostertagia 2%; Trichostrongylus 46%; Cooperia 4%; Chabertia 6%.

After oxfendazole treatment: Haemonchus 28%; Trichostrongylus 71%; Oesophagostomum 1%.

APPENDIX III INDIVIDUAL FARM RESULTS - FARM 15

Faecal egg counts (epg)

CONTROL

Mean body weight: 37 kg (32.0-43.0)

	17/ 5/88		24/ 5/88	
	S	N	S	N
	1650	50	1600	100
	0	0	50	0
	2650	150	2900	50
	2050	50	2200	0
	1250	0	2000	0
	1550	0	1150	0
	900	0	1000	0
	1450	0	850	0
	500	0	400	0
	250	0	1200	0
	900	0	1900	0
	800	0	1250	0
MEAN	1163	21	1375	13

IVERMECTIN (0.2) mg/kg

Mean body weight: * kg

	17/ 5/88		24/ 5/88	
	S	N	S	N
	1400	0	0	0
	600	0	N S	
	1700	50	0	0
	100	0	0	0
	800	0	0	0
	450	0	0	0
	700	0	0	0
	800	0	0	0
	350	0	0	0
	1200	100	0	0
	150	0	0	0
	2100	0	0	0
MEAN	863	13	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

OXFENDAZOLE (5.0 mg/kg)

Mean body weight: 37 kg (34.0-41.0)

	17/ 5/88		24/ 5/88	
	S	N	S	N
	200	0	0	0
	1700	0	1350	0
	650	0	350	0
	3180	200	1100	50
	1550	0	1250	0
	1100	0	450	0
	1800	0	500	0
	1600	50	600	50
	250	0	100	0
	650	0	500	0
	3050	0	450	50
	550	0	N S	
MEAN	1357	21	605	14

$FECR_1$: 55% $FECR_2$: 69% $FECR_3$: 70%

LEVAMISOLE (8.0 mg/kg)

Mean body weight: * kg

	17/ 5/88		24/ 5/88	
	S	N	S	N
	550	0	0	0
	1450	0	0	0
	450	0	0	0
	900	0	0	0
	2250	250	0	0
	750	100	0	0
	1260	0	0	0
	1000	0	0	0
	350	0	0	0
	0	0	0	0
	0	0	0	0
	300	0	0	0
MEAN	772	29	0	0

$FECR_1$: 100% $FECR_2$: 100% $FECR_3$: 100%

* Unable to weigh sheep; all assumed to weigh 40 kg.

Larval Cultures

Before treatment: Ostertagia 4%; Trichostrongylus 82%; Cooperia 14%.
After oxfendazole treatment: Ostertagia 2%; Trichostrongylus 96%; Chabertia 2%.

APPENDIX IV

A SUGGESTED PROTOCOL FOR THE DETECTION OF ANTHELMINTIC RESISTANCE BY FAECAL EGG COUNT REDUCTION TESTING

A discussion paper for the 18th Seminar of the Sheep and Beef Cattle Society of the New Zealand Veterinary Association, Lincoln, July 1988.

(1) Standard Protocol

If there is doubt about the presence of a suitable worm burden for the test to proceed then 5-10 faecal samples should be checked prior to commencement. An average of 200 eggs per gram is a minimal figure.

Day 0 Allocate sheep at random to control and treatment groups - at least 12 sheep per group.
Identify groups by colour mark or tag.
Weigh all sheep, record weights.
Check drench gun(s) for function and accuracy.
Dose each sheep according to its weight.

Day 7 Collect faecal samples from all sheep per rectum into individually labelled containers.
Drench control sheep.

Note:

1. Any young sheep are suitable providing they have sufficiently high faecal egg counts.

2. Nematodirus resistance can be difficult to prove due to often low egg counts. If suspected on the basis of numbers of sheep infected pre and post drenching, sacrifice of selected sheep for total worm counts may be necessary.

3. In this protocol faecal samples are collected only once - seven days post drenching. This reduces the time taken on the farm, can halve laboratory costs and is highly correlated to the reduction calculated using both pre and post treatment egg counts. The formula to use is:

$$FECR = 100 \quad 1- \frac{\text{geometric mean drenched}}{\text{geometric mean controls}}$$

4. When resistance is suspected (FECR < 96%), a larval culture should be performed to identify the resistant species. This is not necessary for Nematodirus.

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