Epidemiological studies of parasitism in sheep and reproduction in horses

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Abstract

Anthelmintic resistance is a well-recognised problem for control of nematodes in sheep in most countries of the world. The climatic conditions in New Zealand are particularly favourable to the survival and development of gastrointestinal nematodes. As a consequence, gastrointestinal parasitism is a major impediment to profitable sheep raising in New Zealand.

A random postal survey of 300 sheep farmers in the southern North Island region of Manawatu was conducted with the purpose of examining current farming and drenching practices and investigating possible risk factors in the development of anthelmintic resistance. The results of this study, reported in Chapter 2, revealed a high degree of awareness and concern about the problem of resistance, but also a lack of understanding on how grazing management strategies should be combined in order to achieve integrated control over gastrointestinal nematodes while minimising the use of anthelmintic drugs. Only 31% of respondents had performed at least one drench test on their property. Among testing farms, prevalence of resistance approached 70% and involved benzimidazole products in all but one case.

Subsequently, a trial was undertaken to investigate the economic consequences of anthelmintic resistance in growing lambs on commercial farms (Chapters 3 and 4). Five farms with a history of resistance to benzimidazole drenches were selected. The effects of three treatment strategies (partially ineffective, effective and suppressive) on nematode egg counts, bodyweight gains and susceptibility to diarrhoea were compared between groups of ewe lambs. Suppressively treated lambs performed significantly better than effectively treated lambs, which in turn performed better than ineffectively treated lambs. However, a partial budgeting analysis carried out by means of a stochastic simulation model (Chapter 4) indicated that effective treatment yielded the highest net returns. The model also showed that the range of possible outcomes oscillated substantially around the mean, reflecting the degree of uncertainty about the outcome on any single farm due to variation between farms.

Chapter 5 describes a study which was carried out with the objective of evaluating two management strategies for breeding mares after foaling. Mares were examined on day 7-9 postpartum by palpation and ultrasound. Group 1 mares were bred at foal heat provided that they met predetermined criteria and Group 2 mares were treated with a $PGF_{2\alpha}$ analogue. Pregnancy rates, pregnancy loss rates and time from foaling to conception in the two groups were compared. Pregnancy rate at first served oestrus was 58.3% and 71.4% for Group 1 and 2 respectively. However, the statistical power of the study as determined by power analysis, was insufficient for the observed differences to reach statistical significance.

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When I came to New Zealand in February 1995, I was a newly graduated veterinarian who knew little about anthelmintic resistance in sheep and pregnancy diagnosis in mares, nothing about epidemiology and even less about computers: let alone statistics! During these two years, many people have worked hard in order to teach me something on all these subjects. Thanks to their patience and understanding I am now able to appreciate the difference between an equine embryo, a *Nematodirus* egg and a computer virus, as well as have a better idea of the risk factors associated with each of them.

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Table of contents

List of Tables

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

List of Figures

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 1

A review of the epidemiology and control of anthelmintic resistance in gastrointestinal nematodes of sheep

Epidemiology of gastrointestinal parasitism of sheep in New Zealand

General strongylid life cycle

Members of the Trichostrongylidae and Strongylidae families are widespread among ruminants in most countries of the world. The adult forms of the parasites colonise different locations around the gastrointestinal tract, with their presence resulting in variable damage to the host. Table 1 shows the nematode species recorded for sheep in New Zealand according to their localisation and importance. The life cycle is similar for most trichostrongylid species. All have a direct cycle, not requiring an intermediate host. Sexually mature females live in the abomasum or small intestine of sheep and lay eggs which are expelled with the host's faeces. When optimal climatic conditions are met, larvae develop in the eggs in 15-20 hours, hatching as larvae (with the exception of *Nematodirus*) which ingest faecal bacteria and, after two moults, develop into third stage larvae which are still covered by their previous stages' sheath. The lag between hatching and developing to the infective stage is dependent upon temperature, humidity, and other environmental factors and varies according to the nematode species[36]. Under field conditions development is essentially confined to the months of the year with a mean air temperature above 10°C[40].

Table I. Gastrointestinal nematodes recorded in New Zealand sheep (from Charleston, 1982)[36]

	Major importance	Secondary or occasional importance	Little or no importance
Abomasum	*Haemonchus contortus*		
	Ostertagia(O.) circumcincta*		*O. crimensis*
	O. trifurcata		*O. ostertagi*
			O. pinnata
	Trichostrongylus(T.) axei		
Small intestine		*Cooperia curticei*	*C. oncophora*
			C. punctata
			N. abnormalis
			N. furcatus
			N. helvetianus
		Strongyloides papillosus	
	T. colubriformis		*T. capricola*
	T. vitrinus	*Bunostomum trigonocephalum*	
Large intestine		*Oesophagostomum venulosum*	
		Chabertia ovina	*Trichuris ovis*

Note: *Ascaris suum* has been recorded rarely from the small intestine of lambs (the pig is the normal host).

Oesophagostomum columbianum has been recorded from imported sheep in quarantine

* =*Teladorsagia*

A studyconducted by Larsen[84] between 1967 and 1969, investigating the seasonal fluctuations in numbers of infective larvae on herbage of two pastures suggested a fairly regular annual pattern, with a small peak of larvae in the spring and a larger peak in the autumn. The nematode larvae recovered were *Trichostrongylus spp., Ostertagia spp., Haemonchus contortus, Cooperia spp., Nematodirus filicollis, N. spathiger, Bunostomum trigonocephalum, Chabertia ovina* and *Oesophagostomum venulosum*; all tended to follow a basic pattern with a small spring peak and a larger autumn peak. The spring peak was numerically dominated by *N. filicollis* and *Ostertagia* with smaller numbers of *Trichostrongylus*, whereas the autumn peak was dominated by *Trichostrongylus* followed by *N. filicollis*; the other genera were less consistent in their order of occurrence. The larval peaks occurred in the periods when the mean air temperature was over 10° C; the autumn peaks occurred during or just after the first periods of heavy rainfall at the end of each summer, and were also correlated with the soil moisture status. According to the results of this study, all genera recovered with the exception of *Nematodirus*, developed 2 generations of infective larvae on the pasture per year, one in the spring derived from the post parturient rise of the ewes, and the second in the autumn, derived from eggs deposited by lambs during the summer and early autumn. However, it should be noted that this simplifies the most commonly observed situation, in which several generations of larvae develop on pasture over the same year.

While most larvae deposited during spring and summer die after a short time because of exposure to high temperatures, direct sunlight or desiccation, a proportion survives until the following summer, ensuring the survival of the species.

After ingestion by the host, third stage larvae exsheath and colonise the gut -the particular location depending on the species-.These larvae subsequently develop to the fourth stage in the mucosal crypts. The prepatent period -from infection to production of eggs- typically varies from 2 to 3 weeks[36].

As an example, survival times for both free-living and parasitic stages of the nematode *Ostertagia circumcincta* are shown in Table II.

Table II. The free-living and parasitic stages of the life cycle of *O. circumcincta* (from Callinan *et al.*, modified)[35]

Type	Stage	Site	Duration (days)
Free-living	Eggs	Faeces	1-17
	Pre-infective larvae	Faeces	1-15
	Infective	Faeces, soil herbage	1-235
Parasitic	Larvae	Abomasum	4-60
	Adults	Abomasum	16+

Length and survival rates of both free-living and parasitic stages of strongylids are influenced by enviromental factors, which will be discussed in some detail in the next section.

Although the basic life cycle is similar for all nematode species, there are some important differences regarding their fecundity[43][114], ability to survive in the environment and the rate at which the adults lay eggs. *Haemonchus*, for instance, whose larvae are relatively vulnerable, has a very high reproductive rate; *Trichostrongylus axei*, on the contrary, lays very few eggs, but offers a greater endurance to adverse climatic conditions. In the field, the percentage of eggs that develop to the infective stage usually vary between 1% and 10%, but can reach peaks as high as 25%[40].

Nematodirus represents a special case. The larvae of this genus deposited in spring develop to the infective stage within the egg and normally do not hatch until late summer/autumn when conditions are cooler. Considerable numbers of larvae of this genus survive until the following summer and small numbers have been found to survive over 24 months[40], although there are within-genus differences, *N. spathiger* behaving more similarly to the other strongylid genera.

Vlassoff[155] investigated the contribution made by the ewe and lamb to the trichostrongyle larval population on pasture over a period of 3 years. He outlined that lambs grazing the year round acquire one generation of nematodes in the spring, from residual larvae and/or those that develop from eggs passed by the ewe during the postpartum period, and a second generation in the autumn from eggs passed by the lambs. The results of his study suggest that in most years the residual pasture infestation is the more important source of infection for lambs in the spring. The source of the initial infection to lambs, the number of generations of larvae that develop on the pasture in one grazing season, and the particular generation of worms that causes clinical disease in lambs will vary from area to area, depending on the climate and the species of nematodes present.

Recent studies have indicated that adult sheep have a greater role in contaminating pastures than was previously thought. Even when the faecal egg output is low, subsequent L3 populations found on pasture can be significant if the grazing pressure has been high(53) (i.e., high stocking rates). The rate of pasture growth also influences pasture larval populations: in the winter months, as the growth rate of pasture slows down, lack of a dilution factor allows larvae to reach higher levels(54).

Environmental factors

The environment plays a considerable role in the magnitude of the effects of parasitism by acting both upon the helminths, influencing their rate of survival and development, and upon the host and its general health status. The term environment comprises not only weather conditions and type of soil, but also a variety of other factors, controllable by the farmer, such as stocking rates, proportions of various stock classes, grazing and pasture management practices employed, timing of parturition, duration and retention of young stock, frequency and timing of drench treatments(28).

Climatic variations between farming regions in New Zealand are relatively small. Seasonal conditions for at least part of each year are favourable for the development of the free-living stages of most parasites in all districts(153). Consequently, all the nematode parasites of sheep may be found throughout the country, although their relative abundance varies between districts(108). Of the most economically important genera, *Ostertagia* and *Trichostrongylus* tend to predominate in all areas, while *Haemonchus* and *Cooperia* are more abundant in northern than in southern districts. Outbreaks of *Nematodirus* infection, on the other hand, have been reported more frequently in the South Island than in the North[158]. Within this genus, *N. filicollis* is known to prefer wetter areas, while *N. spathiger* predominates in dry areas[27].

The seasonal/age-related patterns of gastrointestinal nematode infections in sheep tend to follow a relatively constant pattern from year to year. Worm numbers build up slowly in lambs during spring and early summer, reaching a peak in autumn, followed by a rapid decline in winter when they are 10-12 months of age[158]. Infections are usually mixed, although there may be some seasonal changes in the relative abundance of each. *Nematodirus* spp. tend to predominated in late spring, followed by *Ostertagia* spp., *Haemonchus contortus,* and small intestinal *Trichostrongylus* spp. in late summer/autumn[158]. *Cooperia* spp. and *T. axei* usually form a major part of the worm burden of animals in their second year. A similar succession of genera has been shown to occur in the populations of infective larvae on pasture[50][154][155]. Temperature and moisture appear to be the two most critical factors influencing the development and survival of nematode larvae[54].

The role of the host

A feature of the ruminant host response to gastrointestinal nematode infection is the between-animal variation in response. The distribution of gastrointestinal parasites in the sheep population has been reported to be overdispersed, meaning that a few sheep carry most of the parasites while most of them only carry a small proportion of the total worm load. Negative binomial distributions have been fitted to faecal egg counts in sheep by a number of authors[15][144]. This undoubtedly has a significant genetic component. The amount of variation in worm egg counts appears to increase as the lambs mature. This could be due to differences in the rate at which effective immunity develops. Development of immunity has both age and experience components[146]. Age has been shown to have a direct effect on immune response and on survival of nematodes in the host[25][90][142]. Older lambs also show an increase in variation of parasite burdens, which means that a smaller proportion of individuals will produce a greater proportion of the total pasture contamination. Studies suggest that the factors accounting for the majority of the variation in faecal egg counts in older lambs are early egg counts, sex[17], date of birth, sire, dam, year and month of sampling[143]. However, a study performed on lambs which were matched for breed, sex, age and farm of origin[145], showed that following infection with *O. circumcincta* there was extensive variation among sheep in both parasitological (worm burdens, worm size and number of inhibited larvae) and immunological (concentration of mast cells, globule leucocytes, eosinophils, IgA-positive plasma cells and parasite-specific Ig-A in the abomasal mucosa) variables. Research with a variety of parasites in several species has shown that much of the variation in parasite burdens following infection is under genetic control by the host, being moderately inheritable. Breed variation in severity of infection and nematode egg output has been extensively investigated. For example, a study showed that fewer worms became established and less severe clinical and pathophysiological changes were observed in Scottish Blackface than in Finn Dorset sheep[2]. Another trial found significant variation in faecal egg counts, which were lower in Perendale and Crossbred lambs than in Romneys[169]. Some authors have suggested that selection of lines of resistant sheep would result in animals carrying worm burdens around 10%-20% of those of unselected animals[14].

A study examining associations between counts of nematodes of different genera in lambs supported the hypothesis that they are influenced by common host mediated factors. In the same trial it was also found that the correlations between genera parasitising the same gastrointestinal organ were significantly stronger than the correlations between genera parasitising different gastrointestinal organs, suggesting the existence of common regulatory factors that tend to be organ specific[13]. The time needed for lambs to develop immunity to nematodes is usually fairly long, although it varies according to the species. It can vary from as little as 5 months to 18 and more months. Following the establishment of immunity to these worms, sheep need to be constantly reinfected in order to maintain this resistant status. In general, animals which are exposed daily to low numbers of infective larvae, while being adequately fed, gradually develop resistance to parasites and never develop overt clinical signs of parasitism.

Larval inhibition (hypobiosis) is an interruption of larval development which occurs at a specific stage of development (usually as early as fourth stage larvae) and can last for several months; larvae can then revert to the actively developing stage and become sexually mature[120]. Some of the factors which have been postulated to initiate this developmental arrest include the host's immunity mechanisms, the effect of climate on developing larvae on pasture, and the presence of adult worms in the gut[37]. However, the phenomenon still needs further clarification.

Spring rise is a term used to describe a relaxation of immunity which is usually observed in ewes during the periparturient period, and allows a marked increase in parasite egg production. Late pregnancy, parturition, lactation and the associated hormonal changes, a certain decrease in the immunological defences, nutritional deficiencies, and new infections acquired in spring have been advocated as possible causes of such phenomenon. In strains of sheep selected for resistance to nematode infection, principally through a more rapidly acquired immune response, resistant ewes still undergo a periparturient loss of immunity but retain their relative superiority over unselected or susceptible ewes. The mechanisms are not completely understood, but probably involve effects of hormones associated with lactation on the immune system[17].

Following the post-parturient peak, egg counts in the ewes decrease to low levels by weaning and remain low for the rest of the year[148]. The egg count of lambs, on the other hand, increases gradually from birth, reaches a peak in autumn and, after remaining at a high level for 2-3 months and subsequently decreases rapidly during late autumn/early winter[34].

In order to optimise the use of the information available and reach a better understanding of the epidemiology of gastrointestinal nematodes of sheep, several computer models have been developed[20][35][134][149]. The results of such mathematical exercises are discussed in the following sections.

Effects of parasitism on sheep health and production

Nematodiosis in sheep is an epidemiologically complex disease, controlled by the effects of weather on the development, migration and survival of the free-living stages of the life cycle and by the sheep's resistance to the parasitic stages, which in turn will vary with the species of nematode, the age and physiological and nutritional status of the sheep and its previous experience of infection. This explains the great variability of effects of parasitism on sheep health and productivity.

Most cases of parasitism in sheep in New Zealand involve the simultaneous presence of several species of nematodes. Both absolute numbers (the total number of adult worms present in the gastrointestinal tract) and the relative proportion of each species greatly influence the severity of the disease. Virulence of nematodes depends greatly upon their feeding habits, as well as on the host response that they initiate. In the abomasum for example *Haemonchus* is considered highly pathogenic due to its haematophagous habits. The adult form of *Ostertagia circumcincta* lives in the superficial mucus layer, being a surface browser, and causes profound changes in pH, which some experts believe to have implications for solubility of nutrients and their absorption in subsequent sections of the tract[(148)]. Other abomasal dwellers such as *Trichostrongylus axei* appear to have less intimate contact with tissues and less effect on pH[(148)]. On the other hand, small intestinal species (*Trichostrongylus* spp., *Nematodirus* spp.) often induce atrophy of the mucosal villi and morpho-functional changes in the intestinal crypts. There is controversy about how severely these changes affect normal digestive processes, as distant parts of the small intestine have been shown to compensate for losses in absorption from the parasitised tract.

Other effects of gastrointestinal parasitism include influences on appetite, skeletal growth, hematopoiesis (particularly *Haemonchus contortus*[(1)]) and mineral and protein metabolism. For example, a trial[(41)] conducted infecting lambs with *Ostertagia circumcincta* showed extensive abomasal damage with mucosal hypertrophy and depletion of parietal cells. Growth rates appeared to be depressed, the deposition of protein, calcium and phosphorous in the carcass was considerably reduced and plasma pepsinogen concentrations were consistently elevated.

Although digestion of food *per se*, with regard to energy- and protein-yielding constituents is generally not now considered the major impact of infection, recent studies have underlined the importance of the marked reduction in feed intake which is observed in parasitised sheep, as well as the increase in body protein synthesis aimed at repairing the gastrointestinal tissue and increasing mucus and plasma protein secretions into the tract as a part of the host's immune response[(146)]. A study[(76)] comparing the effects of four different treatment policies (salvage, curative, preventive, and suppressive) showed a dramatic effect of parasite control on appetite as well as on grazing behaviour, as uncontrolled parasite infection resulted in patchy grazing.

Economic consequences of parasitism

A number of trials[(22)(84)(131)(156)(41)(76)(33)] have been carried out in order to estimate the effects of parasitism and different control measures on the productivity of sheep. In New Zealand[(34)(122)(119)] it was shown that even relatively small differences in the levels of larvae recorded on pasture resulted in significant differences in liveweight gain and wool production. All studies indicated that parasitism has severe consequences on both liveweight and wool growth of sheep, lambs being the most susceptible category.

Parasitism also has indirect effects on productivity, by increasing susceptibility of lambs to flystrike[(85)]. The response by affected sheep to the ingestion of trichostrongylid larvae has been shown to be the major risk factor for this syndrome[(83)], which consists in the invasion of the body tissues by the larvae of a number of species of blowflies. The costs associated with flystrike are the sum of control measures, production loss (wool and liveweight as well as death) and by-product damage (pelts), and implicit in all of these are labour, material and opportunity costs[(64)].

In New Zealand it has been estimated that, despite present levels of parasite control, production losses as a result of internal parasite infections of sheep amount to NZ$275 million per year. These losses comprise $27.7 million in lost meat production; $149.2 million in lost wool production; $92.3 million as a result of reduced fertility resulting from reduced live weight in ewes at first mating and $5 million as the overall estimated cost of dag removal. On top of these losses the cost of the anthelmintics used is conservatively put at about $26 million[(55)]. According to a later report from Farm Market Index, farmers are spending $29.3 million on anthelmintics to control sheep parasites, for a total value of sheep products for export of $2193.3 million[(158)].

Control of gastrointestinal parasitism

Anthelmintic drugs

Control of parasitism, to date, relies basically upon anthelmintics and the application of particular management practices. The main role of antiparasitic drugs is to reduce worm load to an acceptable degree. Frequency of anthelmintic administration depends upon climatic and other epidemiological factors, which ultimately determine the amount of time during which animals are exposed to high levels of infective larvae. Therefore, any anthelmintic program will have to take into account the specific situation of each region and farming system. Within particular regions of the world, attempts have been made to determine the most favourable antiparasitic strategy.

In an attempt to evaluate some nematode control programmes in western Victoria, Callinan *et al.*[(35)] developed a mathematical model called *NEMAT*, which estimated the development and death rates of *Ostertagia* and *Trichostrongylus*. Using observed weather data, it simulated the continuous development of nematode populations over a 20 year period on pastures set stocked on the 1st of January each year with recently drenched weaner sheep. The effects of nematodiosis, the effects of drenches given at fixed and at variable times of the year, and the effects of shifting stock on to 'clean' pasture were evaluated and combined. The results showed substantial variation between years. The model confirmed the value of a drench in February and another at the autumn break for weaner sheep drenched in the previous December. These drenches minimised the total nematode population before the period of best translation of the free-living stages. A drench and shift on to 'clean' pastures (see next section) in July was considered necessary if the administration of extra drenches was to be avoided. The number and timing of these drenches after the autumn break depended on the daily effects of weather on the nematode population. Comparable models[(19)(126)] have yielded similar results.

In many farming situations, particularly in intensive rearing systems, grazing management is aimed at optimal pasture utilisation and there may be only limited scope for change to aid in the control of internal parasites. Under such conditions, pasture larval infestations can be maintained at a lower level by the strategic use of anthelmintics to suppress pasture contamination during the period of optimal conditions for development and translocation of larvae. In Australia, natural discontinuities in pasture infestation, resulting from unfavourable climatic conditions, have been enhanced by the use of 1 or 2 strategic drenches[4] which ensure that immediate reinfection is low and that the pasture provides 'safe' grazing for the remainder of the season.

The summer discontinuity in pasture infestation in New Zealand is not so pronounced, and a larger number of anthelmintic treatments is required to reduce the pasture contamination sufficiently to ensure that pastures are 'safe' for grazing by lambs in the autumn. Vlassoff and Brundson in 1981[156] compared two anthelmintic drenching programmes in New Zealand and concluded that a 'preventive' versus 'protective' approach yielded better results. The preventive programme consisted of drench at weaning in December followed by 3 further treatments at 28 day intervals, whereas the protective programme comprised a drench at weaning and at 28 day intervals in March, April and May. It was shown that preventive drenching resulted in an advantage in live weights and fleece weight.

Management strategies

In recent years, epidemiologically based strategic drenching programs have been developed, which aim to minimise drenching frequency, in an attempt to both achieve lower costs and reduce the risk of introducing drench resistance. In Australia, the *Wormkill* program was readily adopted in the summer-rainfall and coastal regions, and led to a sensible drop in the use of broad-spectrum anthelmintics. Similar programs were developed for the winter rainfall regions of Australia (e. g. *Drenchplan, Wormplan*) and for other areas of the world. These programs achieved an equal or better control of worm populations at a reduced cost[12].

Management strategies include alternation of grazing management by hosts of different species, cropping and fodder crops, timing of reproduction and variation in the proportion of young, dry and lactating stock.

Pasture spelling

Rotational grazing has been recommended for years to control nematodes of domesticated animals in many regions of the world. Depending on environmental conditions, nematode larvae remain infective for days, weeks, or even months. The rationale behind pasture spelling is to leave susceptible animals on a pasture only so long as there are no infective larvae on it and then to move the animals to fresh pasture, not returning them to an infected pasture until the infective larvae have died or until there are so few present that they will do no harm. This strategy led to variable, sometimes[91] even negative results. Its value is questionable because larvae can survive a long time in pasture. If rotation intervals only last 6 to 8 weeks, as is often the case, it is possible that the animals are exposed to even higher levels of infective larvae[29].

Strategic decontamination of pastures

Another widely adopted measure is to provide 'safe' (or clean) pastures for lambs at weaning and at other strategic times. These habitually consist of hay aftermaths, new pasture or fodder crops. Residual infestation in these pastures is usually low enough to avoid significant impairment of productivity in susceptible animals. The value of such control measure has been emphasised on the basis of both trial data[28] and mathematical models[35][19]. For example, a model[19] developed in Australia with the aim to investigate the effects of different strategies on the production of lambs found that management practices that did not move weaners from the lambing paddock failed to control parasitism. According to this model, drenching lambs with an effective product and moving them to a safe pasture at weaning was the best strategy, and caused a substantial reduction in worm population. However, the area available on most farms is rarely enough to provide continuous safe grazing for all susceptible animals, and specially sown fodder or browse crops are not always practical or economic[29].

A modification of the concept of safe pastures is mixed grazing, a strategy which uses either heterologous host species or resistant hosts of the same species. This ensures lower pasture infectivity overall through simple dilution of larval density and hence reduced intake by susceptible animals. The first procedure (use of heterologous species) has a considerably higher degree of efficacy and can also have additional beneficial effects, such as efficient utilisation of pasture production with only minimal further homologous contamination and improvement of pasture quality due to complementary grazing habits[29].

Stocking rates

One of the recommendations traditionally given to farmers with the aim of decreasing internal parasite problems is lowering stocking rates. The value of this procedure, however, has recently been questioned[125]. The idea behind this belief is that the additional sheep increase contamination of the pasture; also, at higher stocking rates, the amount of pasture on offer decreases and hence worm larvae are more concentrated. However, stocking rates are not normally increased unless there is a proportional increase in pasture production, so pasture contamination may not increase at higher stocking rates. Similarly, it is unlikely that by lowering stocking rates, a better control of the parasites will be achieved.

Pasture composition

The impact of pasture composition on survival and development of nematode larvae has not yet been intensively investigated, although some of the work that has been undertaken suggests that larval dynamics and migration do vary between different grasses[123][122]. One study[118], for example, found that chicory swards offered the best opportunity to reduce larval intake in grazing animals as they had the lowest populations per unit of herbage mass, compared with grass based and lucerne swards. This was, however, associated with a lower herbage mass, which led the authors to the conclusion that lucerne was the most suitable forage for growing lambs, as it combined a low larval population with high nutritive value and herbage mass[118].

Performance of parasitised lambs has been shown to be substantially increased by a range of forages which contain condensed tannins, such as sulla (*Hedysarium coronarium*), Goldie lotus (*Lotus corniculatus*) and Maku lotus (*Lotus pedunculatus*). Lambs grazed on such pastures also showed a reduced degree of scouring compared to controls[123]. Hypotheses regarding the mechanisms involved in the increased growth rates include decreased protein degradation in the rumen leading to increased protein availability in the small intestine, while the way these forages affect dagginess is unknown(122).

The degree of acceptance of any of the management strategies described will always be determined by their economical benefits. For example, in mixed grazing the balance between sheep and cattle stock is greatly determined by the profitability of each species. Ideally, the ratio of cattle to sheep stock units should approach 50:50(123).

Genetic selection

Since moderate heritability of faecal egg counts in lambs has shown that breeding for enhanced host resistance is feasible, several trials have been carried out to further investigate the genetic control of resistance of sheep to gastrointestinal nematode infections(14)(2). One study concluded that selection of lines of resistant sheep should result in animals carrying worm burdens around 10%-20% of those of unselected animals. According to the authors, the use of genetically resistant hosts should permit a reduced frequency of anthelmintic treatment, which might delay the development of anthelmintic resistance in the parasite(14). Other researchers postulated culling of highly parasitised lambs as a measure of nematode control(143) on the basis of genetically determined between animal variation, which means that a small proportion of individuals produces most of the total pasture contamination. A mathematical model indicated that if parasites are highly overdispersed, selective treatment of the most heavily infected 8% of the population will cause a 50% reduction in population mean worm burdens(126). Different selection lines of lambs have been shown to have different FEC, dag scores, weight gains, wool growth and blood plasma parameters; these findings also indicate the possibility for selection(173). While it has been shown that greatly decreased worm burdens and worm egg contamination rates can be achieved with resistant lambs without compromising productivity, it is necessary to simultaneously select for both traits in order to obtain increased growth rates under untreated challenge. Ewes selected for enhanced host resistance as lambs show reduced peri-parturient FEC(28).

The use of genetically resistant hosts has also been advocated by the results of simulation models(19).

In a recent paper[172], Woolaston and Baker reviewed the issue of breeding for resistance to internal parasites. They described three different approaches: breeding for resistance, resilience, or number of treatments required during parasitism. Resistance was defined as 'the ability of a host to initiate and maintain responses to suppress the establishment of parasites and/or eliminate the parasite load', as indicated by the animal's faecal egg count. The authors' definition for resilience, on the other hand, was 'the ability of the host to maintain a relatively undepressed production level under parasite challenge'. Finally, the approach of selecting animals that least required treatment for internal parasites was considered a more subjective way of estimating the animal's health status. Breeding for resistance is probably the most extensively investigated of the three methods. It involves the advantage of reducing parasite contamination of pasture, which should, in turn, result in a decreased need for anthelmintic treatment. Bisset and Morris, however[30], argue that genetically low FEC lambs have not shown significant production advantages over their high FEC counterparts. They suggest that resilience, assessed by comparing growth under standard challenge, is a better alternative breeding option under New Zealand conditions, as it appears to be the most direct route to develop lambs with the ability to cope with the effects of nematode challenge while grazing. On the other hand, according to Woolaston and Baker, the theoretical bases of breeding for resilience seem to be weaker, and it has been argued that the disadvantages of selecting for resilience may in fact outweigh its advantages[173]. The issue is even more controversial regarding breeding for reduced number of treatments, as it is dependent upon frequent careful and 'objective' inspection of the stock.

It should be noted that none of these strategies will necessarily result in a decreased incidence of scouring, as it has not been demonstrated that sheep which are resistant to gastrointestinal nematodes are also less likely to develop diarrhoea[173].

While there seem to be little doubts about the feasibility of a breeding program aimed at selecting sheep with increased resistance to parasites, only a comprehensive economic analysis will be able to assess the profitability of such an approach.

Vaccination

Nematodes do not produce an immune response in the host comparable to that of many bacteria. In most cases there is no rapidly developing and lasting immunity. Even resistant animals -i. e., able to stop the worms establishing or to restrict the growth of a worm population[153]- are not exempt from some of its pernicious effects. In these animals in general, the number of new larval infestations is balanced by an approximately equal number of adults being eliminated. Following the findings on the previously described natural resistance to parasites, many studies have been undertaken to investigate the possibility of artificially immunising animals[23]. This, so far, has led to inconsistent results in gastrointestinal nematodiosis. The problem is partly due to the immunological inadequacy of young animals, which are the ones that mostly need to be protected. Another important element is the antigenic variability among different larval and adult stages, which complicates the making of an effective vaccine[32]. So far, investigations have not been able to identify and isolate nematode antigens that are able to induce a protective immune response during natural infection in all susceptible host categories[116]. One additional problem is the supply of large numbers of nematodes as the source of functional antigen[38]. Nevertheless, the development of effective vaccines is still being actively investigated[59], and recent advances in basic immunology have brought new hopes on the possibility to develop more specific vaccine antigens[116]. A recently developed mathematical model designed for *Trichostrongylus*[20] also supported the indication that efficacies required for nematode vaccines are well below those required for anthelmintics.

Biological methods

Free-living stages of parasites on pasture are vulnerable to a range of both abiotic factors, such as extremes in temperature and dessiccation[118], and biotic factors, including macro- and microorganisms. Only in recent years research has started investigating the practical consequences and possible use of some of these factors. At present, greatest interest lies with the finding that many predacious fungi may grow in faecal material where, upon contact with nematodes, they capture and kill nematode larvae through a variety of mechanisms. Some produce trapping devices, which include specialised structures such as adhesive networks or knobs or constricting or non-constricting rings(174). Within a short time after it captures the worm, the fungus generally penetrates and destroys it. *In vitro* and *in vivo* screening procedures have been developed for selecting fungal candidates with a strong nematode-killing effect as well as the ability to maintain viability after passage through the gastrointestinal tract of host animals(168). Studies have detected a few promising candidates for biological control of nematodes, such as *Duddingtonia flagrans*. This fungus has been shown to survive gut passage of domestic animals and to subsequently destroy nematode larvae in their faeces. In temperate regions, dosing with fungal material may be used as an additional strategic measure which would replace early season anthelmintic dosings(123). This hypothesis, however, needs to be further tested for animal and human health, as well as for safety and assessment of environmental impact. Furthermore, elaboration of production technologies for fungal spore, and dosing devices and procedures have not yet reached a stage that allows for commercial, widespread application; a thorough cost-effectiveness analysis also needs to be performed.

In practice, at present, farmers tend to adopt a critical combination of anthelmintics and management, based on insight into local epidemiological patterns and risk factors.

Control of dagginess

Diarrhoea, with the associated increased risk of flystrike, as previously mentioned, is one of the undesirable consequences of parasitism. Unfortunately, studies have shown that it is unlikely that commonly used worm control programs will be able to reduce the numbers of pasture worm larvae below the level at which susceptible sheep will not develop diarrhoea and dag(58). A better control of dag has been achieved through the administration of slow-release ruminal capsules, which by their sustained action are able to stop the establishment of new incoming larvae, thereby preventing the host's reaction which would lead to diarrhoea(83). A different strategy has been suggested by Leathwick and Atkinson(88), who found that the use of the tanning containing pasture species *Lotus corniculatus* resulted in a lower incidence of flystrike in lambs.

Anthelmintic drugs

Ideally, an antiparasitic drug should have the following properties:

- Reach the proper site in gastrointestinal tract to contact the specific worm(s)
- Be effective in removing the worm(s) at any developmental stage
- Have a wide safety margin, being highly toxic to the parasite but safe to the host
- Be easily administered, causing minimal upset in the animals' routine
- Not leave potentially toxic amounts of residues in the animal and environment

Anthelmintics are commonly categorised into action families, based on related mode of action. Broad spectrum products currently available for anthelmintic use belong to one of three action families: benzimidazoles, levamisole/morantel, and macrocyclic lactones, also known as avermectins/milbemycins.

Benzimidazoles

Thiabendazole was the first anthelmintic that satisfied to a considerable degree the above-described requirements of an anthelmintic drug. It appeared to have a wide spectrum of action, to be both larvicidal and ovicidal, to be easily administered *per os*, and to have wide safety index margins for the host.

Following the fortunate discovery of thiabendazole, a number of similar compounds were synthesised, of which only a few were selected and developed into commercial products.

All modern benzimidazole drugs have the same central structure (1,2-diaminibenzene) and are weakly soluble in water; they differ from thiabendazole in that they have a substitution in the benzene ring. They act by inhibiting tubulin polymerisation into microtubules, a process which is reversible and saturable. Failure in microtubule function causes various consequences, among which interference with the generation of ATP in the parasites' mitochondria. Since they progressively deplete energy reserves, an important factor in efficacy of the benzimidazole is prolongation of contact time between drug and parasite[56].

Some benzimidazoles possess activity against hypobiotic larvae of *Ostertagia*; this may be related to the degree of hypobiosis of the larvae (i.e., those with low metabolic rate have a low energy requirement and thus are not very susceptible to disruption by the benzimidazoles)[56].

In both nematodes and fungi resistance to benzimidazoles appears to be associated with an alteration in beta-tubulin genes which reduces or abolishes the high affinity binding of benzimidazoles for tubulin in these organisms. More than one genetic locus appears to be involved in the fullest expression of resistance to the benzimidazoles[48][141]. It has been found to be incompletely recessive in *Ostertagia, Trichostrongylus colubriformis*[100] and *Haemonchus contortus*[87].

Levamisole/Morantel

Levamisole is a wide-spectrum anthelmintic which belongs to the family of Imidothiazoles and is commercially found in the form of hydrochloride or phosphate. Levamisole paralyses nematodes stimulating their ganglia through a cholinemimetic action. At high concentrations it also inhibits the enzyme fumarate reductase. Its safety margin is not as wide as for benzimidazoles, and symptoms of toxicity reflect its cholinergic activity[31].

Levamisole has limited efficacy against *Ostertagia*, has no activity against flukes and tapeworms, and is not ovicidal. Morantel[132] has a similar mode of action, in that it paralyses nematodes, but it does so by depolarising their neuromuscular system. Because of their mechanism of action, the peak blood concentration is more relevant to its antiparasitic activity than the duration of concentration[56].

The efficacy of levamisole against inhibited or immature stages of sheep parasites is variable, resulting in susceptible worms surviving treatment but not necessarily contributing to the development of resistance[104][68].

Levamisole also possesses immunostimulant effects and has been used in several diseases both in humans and in animals.

In the levamisole/morantel drug group, resistance appears to be associated with alterations in cholinergic receptors in resistant nematodes. It has been shown to be sex-linked and recessive[102] in *Trichostrongylus colubriformis*, while no evidence of sex link has been shown for *Haemonchus contortus*[48]. This would account for the more rapid selection for levamisole resistance, which has been observed in *Trichostrongylus colubriformis* in areas of Australia in which *Haemonchus contortus* was equally present[164].

A study was conducted in Australia[162] to investigate the rate of development of resistance to three anthelmintics (thiabendazole, levamisole and morantel) in three different strains of *Trichostrongylus colubriformis* used to infect groups of worm-free sheep. Resistance to morantel appeared to develop at a much higher rate compared with levamisole resistance. The results of this study indicate that the mechanism of levamisole resistance covers a wide spectrum, and embraces that for morantel. The authors suggested that in order to conserve the effectiveness of the levamisole/morantel group of broad spectrum anthelmintics, morantel should be used to the exclusion of levamisole until resistance is detected, at which time levamisole may be introduced to re-establish high levels of control.

Macrocyclic lactones

Avermectins and milbemycins are chemically related compounds (having all a macrocyclic lactone structure) produced by the fermentation of the soil-dwelling actinomycetes from the genus *Streptomyces*, all of them having anthelmintic properties. They appear to display identical mode of action against a wide range of both internal and external parasites at very small doses, which have a very low degree of toxicity towards the host. They exert no activity against cestodes or trematodes. The avermectins have a disaccharide substituent on carbon 13, which is absent in the milbemycins. The best known compounds are, for the avermectins, ivermectin, abamectin and doramectin, and for the milbemycins milbemycin, nemadectin and moxidectin.

These compounds exert their anthelmintic effect by irreversibly opening chloride channels in muscle membranes. Several studies have been conducted to investigate the effects of avermectins on different parasite genera, such as *Ascaris*(103) and *Caenorhabditis elegans*(8). These works have demonstrated that avermectins interact stereoselectively and with high affinity to a nematode specific glutamate-gated chloride channel distinct from GABA-sensitive chloride channels(8)(140). The subsequent chloride ion flux into neurons is presumed to cause the observed paralysis and death in nematode and arthropod species, by hyperpolarising the resting potential of the neuron.

The avermectins were discovered in 1975, and 6 years later the semi-synthetic ivermectin was first introduced commercially for animal use. In New Zealand, ivermectin was introduced in 1982. Since then, it has been used extensively in a number of animal species including sheep, cattle, goats, swine, dogs, etc. Intensive use has resulted in development of a limited number of nematode strains which show anthelmintic resistance in sheep and goats(140) and, although infrequently, also in cattle(152). A study was carried out in New Zealand to evaluate the persistence of the anthelmintic activity of ivermectin in sheep in both injectable and oral formulations(107). It was shown that ivermectin given by injection had a statistically significant persistent anthelmintic effect only against *Cooperia* five days after treatment.

The best known milbemycin is moxidectin. It is a relatively new semi-synthetic compound, which has been found to have a high efficacy against benzimidazole-resistant isolates of different nematode genera(77)(150). It has been shown by several studies(150)(133)(170) to have a persistent effect, which varies according to the nematode species, and has been proposed to be due to a slower degradation in the rumen compared with ivermectin(150). Apparent efficacy of moxidectin against ivermectin-resistant strains of *Ostertagia* spp. in goats(124)(171)and *Haemonchus* in sheep(150) was probably dose-related. In other words, these studies did not compare susceptible and resistant strains and used only a single dose level, thereby missing the shift in drug susceptibility to moxidectin which had occurred[140]. Differential potencies may also originate from different kinetics parameters resulting ultimately from different formulations and/or different metabolism[140]. Several studies reported greater persistency of moxidectin compared with ivermectin at comparable dose rates[10], which may account for its greater potency and efficacy against several sheep nematodes[67]. The persistent activity of moxidectin against Haemonchus contortus, Trichostrongylus colubriformis and Ostertagia circumcincta was shown by a study to be 5, 2 and 5 weeks, respectively, while ivermectin efficacy lasted 1 week or less[10]. Other workers found moxidectin to be ineffective against ivermectin-resistant parasites in sheep[128][140], goats and cattle[152].

A secondary effect of the macrocyclic lactones is suppression of reproductive function. Although it has been studied in ticks, it is poorly defined in gastrointestinal nematodes. According to some authors[72][152][170], it can last for up to 10 days, while one study reported intervals as long as 21 days[72].

The glutamate receptor to which ivermectin appears to bind has been expressed *in vitro* so that further studies of the interaction of ivermectin with this receptor and its possible alteration in ivermectin resistance will be feasible[130].

In a study investigating the characteristics of ivermectin-resistant *Haemonchus contortus*[74] it was found that the males of this isolate were more sensitive to ivermectin than the females, implying the possibility of a sex-linked toxicity or inheritance of resistance. Also, the inheritance of ivermectin resistance appeared to be dominant. This suggests that once present ivermectin resistance will spread fast. Finally, the authors found that cross resistance existed to other members of the ivermectin/milbemycin action family[74].

Anthelmintic resistance

Definition of resistance

The definition of anthelmintic resistance accepted by the W.A.A.V.P. (World Association for the Advancement of Veterinary Parasitology)[39] is the one given by Prichard *et al.* (1980): "resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and is heritable"[129]. In the same paper, Prichard gave the following definitions for other related terms.

Side-resistance exists where the resistance to a compound is the result of selection by another compound with a similar mode of action.

Cross-resistance resembles side-resistance but involves compounds with different modes of action.

Multiple resistance occurs when individuals are resistant to two or more different anthelmintic groups either as a result of selection by each group independently or as a result of cross-resistance.

Reversion is a decrease in the frequency of resistant individuals in a population following removal of the selecting agent[129].

A revised definition of *drug resistance* was recently given by Shoop[139], who stated that "resistance is a change in gene frequency of a population, produced by drug selection, which renders the *minimal*, effective dosage previously used to kill a defined portion (e. g. 95%) of the population no longer equally effective".

Other related definitions were also modified and amplified by Shoop[139] as follows:

Side resistance: a state in which a drug-selected population has gene(s) coding for a mechanism that defeats the toxicity of drugs within a mode of action family.

Cross-resistance: a state in which a drug-selected population has a gene(s) coding for a mechanism that defeats the toxicity of drugs from different mode of action families.

Multiple resistance: a state in which a population has been selected independently by drugs from different mode of action families to produce different but concurrent mechanisms of evasion, as used herein, or it is sometimes used as a synonym of cross-resistance.

Reversion: a state in which there is a return to or toward drug susceptibility.

Tolerance: innate lack of susceptibility that did not result from drug selection.

According to Shoop, it seems preferable to think of drug resistance as having occurred 'when the minimal, effective dosage previously used to kill a defined portion (e.g. 95%) of a population is no longer effective'[139].

Attempts have been made to quantify the definition of anthelmintic resistance, in order to give a standard interpretation to the tests used to assess efficacy of anthelmintic drugs. When describing the diagnostic methods available for detecting the presence of resistance, Prichard[129] gave some general recommendations and precautions, but did not provide any standards to be followed. The Australian Working Party recommended some criteria for defining anthelmintic resistance by the faecal egg count reduction test. Resistance was said to be present if the percentage reduction was less than 95%, together with a lower 95% confidence limit of less than or equal to 90%. This definition, however, has been questioned by researchers[111] who found that lower confidence limits of 90% or less were always associated with mean percentage reductions of less than 95%. The authors suggested that little practical purpose was likely to be served by consideration of the lower confidence limits of the estimates.

The general procedures for anthelmintic resistance tests and anthelmintic resistance trials (including trial design and statistical procedures) have been set by the W.A.A.P.[172]. The design suggested involves one trial with 7 groups of sheep, and aims to enable statistical evaluation of the efficacy of a product against a resistant strain, verification that the strain was resistant to the commercial anthelmintic and confirmation of efficacy of the commercial anthelminthic against a susceptible strain.

History of drench resistance and current trends

The first report of anthelmintic resistance dates back to the mid-1950s as a result of the failure of phenothiazine to control haemonchosis in a flock of sheep kept at a research farm in Kentucky, USA. Similar reports followed in the early 1960s incriminating the then still new drug, thiabendazole. Today, anthelmintic resistance has become a widespread phenomenon, which appears to be complicated by several factors.

In New Zealand, anthelmintic resistance was reported for the first time in 1980(80). In 1980-81, a survey was conducted on 90 randomly selected sheep farms throughout the country(81)(82), but the extent of the problem was not exactly defined. *Haemonchus contortus* was the chief species responsible for lack of effectiveness of thiabendazole in cases of resistance emerged from a survey of 54 randomly selected farms in the North Island of New Zealand and Nelson region, while only *Trichostrongylus* eggs were recovered from Levamisole-resistant strains(82). The same trial procedure, carried out on 43 South Island farms, only evidenced thiabendazole resistance on one farm and resistance to levamisole on 7 farms(81). Numerous reports have followed ever since, concerning all domestic animals but more frequently sheep and goats, and involving many species of nematodes. All nematodes usually express side resistance. Multiple resistance is also becoming increasingly common(109). Multigeneric resistance, in which various species constituting the host animal's worm burden show resistance to the same drug, whilst comparatively rare, further complicates the problem(136). Resistance in Nematodirus was reported for the first time in New Zealand in 1983(117), and has been increasing steadily ever since.

An examination of practitioner initiated investigations carried out from 1986 to 1988 indicated that drench resistance was relatively common on sheep farms in the southern North Island of New Zealand, with a prevalence of 21 to 45%. Most of the resistance was to benzimidazoles, but the data indicated the emergence of resistance to levamisole/morantel. Resistance occurred in at least 5 nematode genera (*Haemonchus, Ostertagia, Trichostrongylus, Oesophagostomum* and *Nematodirus*), sometimes involving mixtures of a number of them. The level of resistance in many of the parasite populations was relatively high(105).

Between 1989 and 1990, a survey was carried out on a national level on 168 cases of suspected drench ineffectiveness confirmed resistance in just over 50% of them. It appeared to be predominantly a problem with sheep and goats, and in 95% of confirmed cases in sheep it involved benzimidazoles, whereas in goats it was spread more evenly among the three drench families[9].

An analysis of 295 veterinary diagnostic cases submitted to the Batchelar Animal Health Laboratory (Palmerston North) for faecal egg count reduction testing in sheep between 1986 and 1991[113] showed that 63% of them originated from properties carrying anthelmintic resistant worms. Most of these cases involved resistance to a single drench type only, with the benzimidazoles being implicated most frequently. Overall, the figures suggested a frequency of resistance to benzimidazole anthelmintics of 74%, to levamisole type anthelmintics of 23% and to benzimidazole/levamisole combinations of 30%. No cases of resistance to milbemycin/avermectin type drenches were recorded. Resistance to levamisole and benzimidazole/levamisole combination drenches mainly involved strongyle genera only, and resistance to benzimidazoles occurred mainly in Nematodirus spp.

In 1994 larval cultures and FECR were used to determine the identities and frequency of occurrence of nematode genera involved in 102 cases of ovine anthelmintic resistance submitted to the Batchelar Animal Health Laboratory between 1992 and 1994[113]. In 68% of cases benzimidazole drugs were the only action family involved, while in 27% levamisole was responsible for the resistance problem, and in the remaining five cases resistance had developed to both action families. The survey evidenced that in the southern North Island the genera most frequently involved were *Trichostrongylus, Ostertagia* and *Nematodirus.* Also implicated, although less commonly, were infections of *Oesophagostomum/Chabertia, Haemonchus* and *Cooperia.* The majority of cases involved resistance in two or more genera, with resistance in a single nematode genus occurring in 45% of cases.

Even more alarming figures have been reported for Australia, where resistance to anthelmintics was first notified in 1968. The prevalence of benzimidazole resistance rapidly increased in the following decades, particularly in the summer rainfall regions of New South Wales[166]. A survey[53] carried out between July 1981 and December 1983 on 116 randomly selected sheep farms in the south west of Western Australia showed a prevalence of anthelmintic resistance of 68%. *Haemonchus contortus, Ostertagia circumcincta* and *Trichostrongylus colubriformis* were the species involved in the cases of benzimidazole resistance, while populations resistant to levamisole included *Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus colubriformis* and *Nematodirus* spp. Multiple resistant populations were found on 17% of farms. Data collected during 1991 and 1992 on 881 sheep farms throughout Australia(125)(11) revealed a prevalence of anthelmintic resistance of 91%, of which 85% to benzimidazoles, 65% to levamisole and 34% to combination (benzimidazoles + levamisole) products. Resistance to ivermectin was not detected. The culture of faeces from untreated sheep showed *Ostertagia circumcincta, Trichostrongylus* spp., *Chabertia ovina* and *Haemonchus contortus* to be the principal species.

Other parts of the world in which anthelmintic resistance represents a major problem for the sheep industry include South Africa and most of South America. During April to September 1994, large scale surveys were conducted to assess the status of anthelmintic resistance in nematodes among sheep flocks of northern Argentina, the southern state of Brazil, all of Paraguay and all of Uruguay. The results of the survey revealed a degree of resistance to benzimidazole ranging from 40% (Argentina) to 90% (Brazil), and variable but lower degrees of resistance to levamisole and ivermectin(167).

In Europe, a survey conducted in 1990(69) showed that Southern England had a degree of anthelmintic resistance ranging from 35 to 61%, the main species involved being *Ostertagia circumcincta* and *Haemonchus contortus*. In 1992 a new survey showed that 44% of the sheep farms tested in the southwest and 15% of those in the north east of England had parasitic nematodes which were resistant to benzimidazole anthelmintics, *Ostertagia circumcincta* still being the main species implicated(69). Resistance was restricted to the benzimidazole action family.

The above figures describe a rather dramatic situation in all sheep-rearing countries. Moreover, there is evidence that the incidence of anthelmintic resistance is increasing in livestock in countries throughout the world(71). In New Zealand, a definite trend is apparent. Anthelmintic resistance has been increasing ever since it was first recorded in 1980(9). Although to date only resistance to benzimidazole products appears to have reached alarming levels, current knowledge, including understanding of the mechanisms of resistance at biochemical levels and the inadequacy of current control measures, prevents us from being optimistic about future trends.

Causes of resistance

The causes of anthelmintic resistance have been extensively investigated both by means of field trials and with the aid of computer modelling. Mathematical models integrate factors such as parasite strain, geographic location, management practice and genetic fitness to identify effective control regimens which do not lead to the development of resistance over a predetermined period. Several models have been developed over the last decade, aimed at investigating the importance of each of these factors and the likely impact of new technologies on drug resistance and how efficient they need to be to sustain good worm control[(57)(19)(20)(47)(89)(131)(47)]. The results of such exercises usually agree on the importance of the better known factors, although they do show some contradictions when trying to explain the role played by each putative risk factor.

This section outlines the factors which have been most commonly associated (both by field data and by computer simulation models) with the development of anthelmintic resistance.

<u>Genetics</u>

Anthelmintic resistance in nematodes is thought to be a pre-adaptive phenomenon[(148)]. Studies on selection and genetics of resistance suggest that nematode genotypes capable of resistance to anthelmintics are probably present in any population of parasites at a very low frequency[(71)(137)]. As a general rule, there appear to be three phases in the selection process. At first, the frequency of resistant individuals within the population is very low, and anthelmintics provide a very satisfactory control of parasitism. Given continued exposure to a drug, an intermediate phase then develops in which the frequency of heterozygous resistant individuals within the population increases. Finally, sustained selection pressure results in a resistant phase where homozygous resistant individuals predominate within the population. However, there are differences between nematode genera as to the number and nature of genes involved in anthelmintic resistance.

Knowledge of the genetics of anthelmintic resistance could help determine the sensitivity of *in vitro* tests for anthelmintic resistance and predict its rate of development in the field using computer models. The degree of dominance of a resistance trait will determine at what frequency in the population resistance alleles will begin to affect control, as well as determine what proportion of resistance alleles is removed by treatment[48] (pages 21-24).

Frequency of treatment

The importance of drenching frequency in the aetiology of anthelmintic resistance has been extensively investigated and documented[62][135][160][97]. A trial by Martin *et al.* (1982)[96] compared the rate of development of anthelmintic resistance in groups of sheep with three different treatment strategies ('nil', 'planned' and 'regular') and demonstrated by means of egg hatch assays a level of resistance for *Ostertagia* spp. which was proportional to the frequency of thiabendazole treatment. Numerous theoretical models have also underlined the role of drenching frequency on the development of resistance[19][89][20][47]. Recently, Dobson *et al.*[48] investigated the combined effects of anthelmintic treatment frequency, efficacy and persistency, using a modified version of a model by Barnes and Dobson[19]. They showed that the role of the number of drenches administered on the rate of development of anthelmintic resistance varied greatly according to other factors, including persistence of declining drug levels after treatment. Other authors also came to the conclusion that frequency of treatment, although crucial in determining the rate of onset of anthelmintic resistance, had different consequences depending on the presence of other variables. A model by Leathwick[89], for example, suggested that the proportion of the total parasite population exposed to the drench and the survival of eggs produced by those worms which were not removed by the drench played a crucial role in determining the rate of resistance build-up.

Underdosing

Since the selection of resistance is most rapid when both heterozygous and homozygous individuals survive treatment, underdosing, which enables survival of the former, can play a key role in influencing the rate of development of resistance. Suppressive regimens, however, such as overdosing and treatment of animals close to the prepatent period of the parasite population still allow homozygous resistant individuals to survive, while at the same time removing natural competition between these and naturally susceptible worms. Therefore, they do not offer the answer to the problem(71).

Underdosing is probably still more frequent than most people think. It comprises factors such as lack of attention to the setting of drench guns and underestimation of the live weight of animals, all of which reduce effective blood concentration and therefore exposure of parasites to the anthelmintic. The ability of farmers to estimate sheep body weights was tested at 3 meetings in Western Australia(26). Of 237 farmers who estimated the body weight of a single sheep in groups of 10-20 animals, only 27% estimated accurately to within 20%, and 86% of estimates were lower than the actual weight. Nearly 30% of farmers miscalculated the correct dose of anthelmintic for an animal of given weight by > 10%. Other factors which can contribute to the administration of an insufficient dose of anthelmintic include drench gun faults, failure to shake drench containers sufficiently before dosing the animals, and closure of the oesophageal groove(36)(66)(65).

In contrast to current recommendations on administration of a correct dose, some authors suggest, on the basis of mathematical models, that underdosing plays a very small role in selecting for anthelmintic resistance, and that a very effective anthelmintic treatment is, during the persistency phase, very selective. These results were explained on the basis of a greater reproductive advantage for adult survivors of anthelmintic treatment during a phase in which resistant larvae only are able to establish(48).

Timing of treatment

Another critical factor is the timing of use of anthelmintic. When evaluating drenching policy, the ratio between free-living larvae (on pasture) and within-host nematodes should be taken into account. If the larval population on pasture is small, larvae from the surviving resistant worms are likely to comprise a larger proportion of the population. Mathematical models suggest that early season treatment would select for anthelmintic resistance rapidly, whereas late season treatments would not, owing to large numbers of untreated parasites accumulating at the beginning of the season[(52)(131)(89)]. There appears to be still considerable confusion among experts regarding the role played by the timing of anthelmintic on the development of anthelmintic resistance. Some authors suggest that the use of clean pasture enhances selection for resistance since larval populations on pasture become, increasingly, survivors of the drenching programme[(86)(130)]. However, according to some Australian experts, where this management practice has been followed the development of resistance does not appear to have accelerated[(162)]. In an Australian trial, Martin *et al.*[(99)] tested the speed of development of resistance in *Haemonchus* given different proportions of larvae in *refugia* (defined as an area on pasture where individual members of a population can escape exposure to a drug). The results indicated that *refugia* delayed the development of resistance in *Haemonchus contortus*. Where none or a small proportion of larvae was in *refugia*, a rapid increase in resistance occurred. With an increased proportion of larvae in *refugia*, resistance was slower to develop[(99)].

According to an *Ostertagia* model developed by Gettinby *et al.*[(57)], which also took into account mortality of larvae in *refugia* and probability of infecting new hosts, dose and move strategies appeared to select less heavily for resistance. This model was later retested to consider mixed infection, yielding similar results[(126)].

A 'nematode model' designed to study nematode control and anthelmintic resistance in an 'average New Zealand situation'[(89)] evidenced that the contribution to resistance buildup by any individual drench is not always equal. The proportion of the total population exposed to the product (i.e., the ratio between parasitic and free-living stages) and the survival of eggs produced by worms not removed by the drench will ultimately determine the rate at which resistance will become apparent. Decreasing the number of treatments, according to this model, will always reduce the rate of anthelmintic resistance, but the magnitude of this reduction depends on factors such as the species of worms involved, the timing of drenching and the weather pattern.

Diagnosis of anthelmintic resistance

The ideal test for detection of anthelmintic resistance should be inexpensive, fast, and sensitive. The World Association for the Advancement of Veterinary Parasitology has been giving guidelines for evaluating the efficacy of anthelmintics in ruminants since 1982, the last edition having been released in 1995[(172)]. Table IV shows a summary of the *in vivo* and *in vitro* methods that may be used to detect resistance[(71)].

Table III. ***In vivo*** **and** ***in vitro*** **bioassays (BA), biochemical assays (BC), and genetic assays (G) used in the detection of anthelmintic resistance** ***(from Jackson, 1993)***[71]

Assay	*Spectrum*	*Assay type*	*Application*	*Author(s)*
Controlled test	All drugs	*In vivo* BA	Widespread	Powers *et al.* (1982)
Egg count reduction	All drugs	*In vivo* BA	Widespread	Presidente (1985)
Egg hatch assay(1)	BZ	*In vitro* BA	Widespread	Le Jambre (1976)
Egg hatch assay(2)	BZ	*In vitro* BA	Research	Hunt &Taylor (1989)
Larval paralysis(1)	LV	*In vitro* BA	Research	Martin & Le Jambre (1979)
Larval paralysis(2)	IV	*In vivo* BA	Research	Gill *et al.* (1991)
Larval development(1)	BZ, IV	*In vitro* BA	Research	Coles & Simpkin (1977)
Larval development(2)	BZ, LV	*In vitro* BA	Research	Taylor (1990)
Larval development(3)	BZ, IV, LV	*In vitro* BA	Research	Lacey *et al.* (1990)
Larval development(4)	BZ, IV, LV	*In vivo* BA	Research	Hubert & Kerboeuf (1992)
Tubulin binding	BZ	*In vitro* BC	Research	Lacey & Snowden (1988)
Esterase activity	BZ	*In vitro* BC	Research	Sutherland *et al.* (1989)
Tubulin probe(1)	BZ	*In vitro* G	Research	Roos *et al.* (1990)
Tubulin probe(2)	BZ	*In vitro* G	Research	Le Jambre (1990)

In vitro assays

Some *in vitro* assays which can be used for the diagnosis of anthelmintic resistance are specific for a particular class of anthelmintic, whilst others such as larval development assays can be used with most anthelmintics. Three *in vitro* assays (the egg hatch assay[(73)], tubulin binding assay, and a larval development assay), were evaluated by one trial to detect benzimidazole resistance. All 3 tests showed similar, consistent results. The authors hypothesised greater sensitivity than the *in vivo* assay and suggested that selection of an *in vitro* technique to determine benzimidazole resistance should be based on considerations other than precision, such as technical expertise, availability of equipment, cost and speed at which diagnosis is required[(75)].

Egg hatch assay

In the egg hatch assay, the ability of benzimidazoles to inhibit the embryonation and hatching of freshly collected nematode eggs is used to calculate the 50% lethal dose (LD_{50}) of the drug[(39)(73)]. It is a fast, inexpensive, sensitive and repeatable method when a single species is involved, and yields reliable results on nematode species in which eggs hatch rapidly[(39)]. For mixed infections differentiation of larvae is necessary. The egg hatch assay must be performed on freshly collected eggs because as they develop, aerobic metabolism predominates and this is unaffected by benzimidazoles[(129)].

Microagar larval development test

Sensitive inexpensive tests are required not only for monitoring the extent of anthelmintic resistance in the field, but also for laboratory based research (e. g., genetics of anthelmintic resistance). A microagar larval development test (MALDT), able to detect resistance to benzimidazole, levamisole and ivermectin drugs, has shown to provide an accurate, sensitive and easy to carry out test for the routine detection of resistance[(70)]. The test has been used so far to investigate field isolates from farms with problems of anthelmintic resistance as revealed with faecal egg count reduction tests.

Polymerase chain reaction.

A very sensitive PCR test was developed that could detect benzimidazole resistance in *Haemonchus contortus*. The usefulness of this test relies on the fact that it allows the population genetics of benzimidazole susceptible and resistant worms to be studied in more detail, under different conditions of selection. According to some authors, this may then lead to a better control and a delay in the development of anthelmintic resistance[135].

Larval paralysis.

A method has also been described for detecting levamisole and morantel tartrate resistance by determining the percentage of paralysed third stage larvae in serial dilutions of anthelmintic. Levamisole resistant *Ostertagia* spp. had a smaller proportion of larvae undergoing tonic paralysis in either levamisole or morantel tartrate than did a non resistant strain. The dose response thus obtained for a strain of worms could be tested statistically against strains which are known to be resistant or non resistant[98].

Controlled test

Controlled trials involve killing a sample of treated and untreated parasitised sheep and counting the number of adult worms present in its gastrointestinal tract at a suitable interval after treatment. The World Association for the Advancement of Veterinary Parasitology[172] recommends controlled test for dose titration and dose confirmation trials and gives outlines on how it should be carried out. This test has been shown to be a reliable method for evaluating anthelmintic activity in sheep. However, it is expensive and cannot be used routinely on farms.

Faecal egg count reduction test (FECRT)

The faecal egg count reduction test is by far the most widely adopted technique for detection of anthelmintic resistance. It involves the treatment of naturally infected animals and provides an estimate of anthelmintic efficacy by comparing worm egg counts from animals before and after treatment. In a controlled test, a group of animals treated with the anthelmintic is compared with a group of untreated animals. In New Zealand, this test takes the name of '*Drenchtest*'. Most authors agree that sensitivity of the FECRT is reasonably satisfactory, as it reflects consistently enough the adult worm population in the host. However, it appears to vary with the age of the sheep and the nematode species involved. McKenna(112) pointed out that field infections usually comprise a mixture of parasite genera which vary considerably in their egg-laying capacities.

Nevertheless, so long as the egg counts are not systematically biased, the estimated values for the dependent variables should indicate the size of the effect, although they may underestimate its statistical significance(143). This has been well documented for *Ostertagia*, where the results of a study(95) showed that it was possible to predict worm counts from the geometric mean egg counts from about 10 animals. However, some studies indicate that high gene frequencies exist before resistance to benzimidazoles becomes easily detected by a FECRT(100). A trial carried out in South Africa(51), found a poor correlation between the number of *Haemonchus* eggs per gram of faeces and adult worm counts at post-mortem, which led the authors of this study to conclude that egg counts are not good indicators of the degree of anthelmintic efficacy.

Interpretation of the FECRT is dependent on allowing sufficient (so as to allow the expulsion of worms) but not excessive (so as to prevent the development of new patent infections) time to elapse between administration of anthelmintic and faecal collection(147). According to most specialists, the interval between dosing of sheep and collection of faecal samples should be 10-14 days(95). It has been shown that benzimidazoles can inhibit egg production for up to 10 days. Ivermectin also appears to cause a temporary suppression in egg production, although its duration is somewhat uncertain. Some authors found that it lasts between 7 and 10 days(138), others for as long as 21 days(72). On the other hand, presence of nematode eggs in samples collected more than 14 days after treatment could represent a new generation of adult worms, as the pre-patent period can be as short as two weeks. As for levamisole, studies(61)(60) showed that cases of apparent drug failure, in which egg counts were quite substantial 14(61) and 11(60) days post-treatment, in reality were due to either reinfestation or the maturation of immature stages. This is because levamisole lacks effectiveness towards immature parasitic stages. Pre-adult stages which were present at the time of treatment may therefore have developed to egg-laying adults by 10 days post-infection.

The reason why it is considered important that diagnostic tests be able to detect low levels of resistance relies on the fact that although resistance at these low levels is unlikely to present any immediate problems of nematodiosis in sheep, the frequency of resistance genes in the parasite population may nevertheless still be high. Therefore, the speed with which an anthelmintic will become ineffective is likely to be high, with control failures becoming apparent within approximately a year(106)(114)(6)(172).

Statistical issues in analysing egg counts

Analysis and interpretation of faecal egg counts and the faecal egg count reduction test still need to be standardised. There is a debate among different authors, concerning principally the logarithmic transformation of counts and the use of either arithmetic or geometric means for comparison of pre-and post-treatment egg counts.

Since the mean is positively correlated with the variance (greater means are accompanied by greater variances), the logarithmic transformation is needed to make the variance independent of the mean, so that different means can be compared by parametric tests such as Student's t-test or analysis of variance.

One problem associated with egg counts, however, is that they often include a high proportion of zeros. It is therefore necessary to add a number to all counts. At present, however, there is no standard procedure. In most cases the transformation y = log (egg count +1) is used, but sometimes the number added to each egg count value is equal to half the dilution factor. For example, if the minimum detection level is 50 eggs per gram of faeces, the transformation is y = log (egg count + 25). These two transformations can produce quite different geometric means from the same set of data, leading to different FECR estimates. In general, estimates of FECR derived from geometric means are higher than the one derived from the arithmetic mean. When the issue is the assessment of the efficacy of an anthelmintic, and not only comparison of different groups of animals, the arithmetic mean appears to be more appropriate, as it is directly proportional to the total egg output of the group[46].

In the interpretation of data, the 1992 W.A.A.V.P. suggested that the arithmetic mean should be calculated, as "it provides a better estimate of the worm egg output", while at the same time being "a more conservative measure of anthelmintic efficacy"[39]. However, the 1995 edition of W.A.A.V.P guidelines for evaluating the efficacy of anthelmintic, states that "the geometric mean should be used, as it more accurately represents the distribution of nematode populations within a group of animals and would give a more accurate indication of the degree of efficacy of a product"[172].

Calculation of confidence limits around the mean, and their usefulness in defining nematodes as either resistant or susceptible, is another source of controversy. The Australian Working Party recommended that they should be calculated in order to achieve a standard diagnosis of anthelmintic resistance. However, McKenna found that the recommended lower confidence limits of 90% or less were always associated with mean percentage reductions of less than 95%, which alone could suffice for the definition of resistance[111]. Nevertheless, overdispersion has been shown to be a feature common to all nematode genera[15][143]. Therefore, it can be argued that confidence limits still provide an estimate of the dispersion of egg counts and a better description of the actual on-farm situation. A simple procedure for the calculation of confidence limits around the arithmetic mean has been described by Anderson *et al.* (1991)[5].

The results of many studies have to be interpreted with care, because the design of trials has to be taken into account. A study investigating the likelihood of detecting differences in parasitised sheep[147] outlined that the high degree of variability in egg counts between sheep implies that the power of statistical tests is likely to be poor. Therefore, large sample sizes are usually needed for parasitology trials.

Limitations of the FECRT

There is still controversy about sensitivity of the FECRT as an indicator of the true resistance status of a farm[100]. It appears that low levels of anthelmintic resistance may not be detected by the FECRT[94]. This may frequently be the case in mixed infections in which only one species may be resistant. Field infections usually comprise a mixture of parasite genera, which can only be differentiated as third stage larvae. Therefore, the FECRT should always be followed by culturing and identification of larvae. By providing information concerning the identity of any resistant genera present, larval culturing can help decide on possible future control options[106].

Another reason why low or nil FECs do not always indicate the absence of resistance is the great variability in their egg laying capacities[112], and especially the poor fecundity of some nematode species (e. g. *Ostertagia)*[128]. In these worms, the relationship between egg production and numbers of worms present is not always linear. Also, worm egg counts will not detect immature worms, which may survive the treatment, develop and contribute to post treatment egg count. Moreover, it has been shown that in some instances egg production by resistant worms is reduced or suspended for a period after treatment[157][72][152][74].

According to one study[94] neither the FECRT nor the *in vitro* egg hatch assay (page 37) appear to be reliable if the proportion of resistant worms is less than 25%. The authors of this study prepared composite strains of known resistance of *Trichostrongylus colubriformis* and *Ostertagia* spp. consisting of different percentages of known resistant strains and tested them for benzimidazole resistance using faecal egg count reduction tests, *in vitro* egg hatch assays and tubulin binding assays. All tests detected resistance where the proportion of the resistant strain in the composite was 50% or more, whereas none of the tests unequivocally detected resistance below 25%. Egg count reduction tests were no less sensitive than the *in vitro* tests in detecting low levels of resistance but the egg hatch and tubulin binding assays provided a better quantitative estimate of moderate to high levels of resistance. The overall conclusion of the study was that FECs provide a suitable means of detecting resistance in the field, but there is a need for developing new tests, more sensitive to low levels of resistance. When reviewing the available tests for anthelmintic resistance, Jackson suggested that such tests may need to be host and drug-specific[72].

Processing of faecal samples, although a simple and cheap technique, can be remarkably time-consuming. An attempt was made to simplify egg counting procedures for diagnostic purposes by using composite, instead of individual, faecal samples[121]. This technique, although encouraged by the authors of this study, has not found widespread acceptance to date.

Prevention of drench resistance

Despite the current knowledge on the aetiology of anthelmintic resistance, several problems prevent the prompt achievement of an effective control strategy worldwide. Unfortunately, there clearly is a conflict between the degree of worm control which the farmer wants to achieve and the urgency to prevent anthelmintic resistance. As was concluded by one study [22], "the five drench preventive programme ceases too early to prevent the winter build-up of *Trichostrongylus* infections in lambs". 1 or 2 extra drenches would be needed to reduce winter infections and prevent production losses, with the effect, however, of accelerating the onset of anthelmintic resistance. At the same time, it is now clear that any drenching is going to increase the proportion of worm resistant to a single drench family; therefore, the aim should be to delay the development of resistance. Sykes *et al.* stated that "the escalating development of anthelmintic resistance and the difficulty of unravelling the complexity of the host immune response mean that the major production and economic losses caused by nematode endoparasites will persist in the foreseeable future."[148]

Basically, these strategies rely upon an integrated approach to control[72] and include the following factors.

Minimal drenching

Most experts believe that drenching frequency is the single most crucial factor in the development of anthelmintic resistance[96][97][47][44][45] (page 31). Therefore preventive strategies must inevitably incorporate minimal chemoprohylaxis, thus optimising the use of existing anthelmintics and minimising the number of parasite generations exposed to a drug. The optimal number and interval between anthelmintic treatments depends on the specific epidemiological situation (e. g., the climatic region in which the farm is located) meaning by 'optimal' the achievement of a good control of nematodes and associated economic losses, at the same time minimising the risk of bringing resistance on the farm[78]. Several strategic programmes, as was mentioned in a previous section, have been developed and experimented throughout the world. In New Zealand, the generally advised programme involves the administration of five drenches at 21-28-days intervals, starting from weaning. Generally, drenching adult sheep should be avoided[78] as it is unlikely that the benefits will outweigh the costs when costs are measured in terms of production responses and reduction in the usefulness of an anthelmintic[78]. Another reason for not drenching adult sheep is that the relative increase in resistance gene frequency is likely to be greater than it would be following treatment of lambs[45], as any resistant survivors will not be as readily diluted by new incoming worms.

Strategic schemes for the control of sheep nematodiosis in view of delaying the onset of anthelmintic resistance were developed in Australia during the last decade, starting in 1984 with the widely adopted *Wormkill* plan[45]. This was aimed at controlling parasites (particularly *Haemonchus*) in the summer rainfall area of northern New South Wales by means which allowed a decrease in drenching frequency. Such plan involved a switch towards the use of closantel, as well as an overall decrease in the number of anthelmintic treatments. It was followed in 1986 by its modified version *Drenchplan*, aiming to control nematodes in winter rainfall areas. Both strategies were highly successful in controlling parasitism and reducing overall costs; however, their impact on the development of anthelmintic resistance cannot be quantified, and it is not known how effectively they slowed down the rate of resistance as compared to the use of a higher number of anthelmintic treatments[166]. Moreover, *Wormkill* had the undesired side-effect to hasten the development of closantel resistance[166].

Under New Zealand conditions, the general recommendation regarding frequency of treatment is to make use of a strategic plan, which will vary according to each farm's situation, followed by the use of faecal egg counts to determine the need for more treatments[127]. Leathwick[89], on the basis of his previously described model, while also pointing out that the contribution to resistance build-up by any individual drench is not always equal, stated that decreasing the number of treatments will always reduce the rate of anthelmintic resistance

Quarantine drenching

Treatment of purchased animals prior to introducing them onto the farm has been recommended as one of the key procedures that farmers need to follow in order to avoid bringing resistant nematode genotypes on to their property(148). The drug used should be one to which resistance is least likely to have developed, such as ivermectin or moxidectin.

Effective dosage

Most experts stress the instruction to treat animals with the full recommended dose of anthelmintic. This is done with the aim not to allow heterozygous resistant individuals to survive(78)(100) (page 32). It is considered important that the dose given to be lethal to the heterozygotes as this should slow the increase in gene frequency and reduce the chance for homozygous strains to appear(127).

Barnes *et al.*(20) argue that, while underdosing allows more heterozygotes to survive, gross underdosing may also allow more homozygous susceptible nematodes to survive, with the expected final effect of delaying the onset of resistance. On the basis of a mathematical model, they hypothesise not treating a portion of the flock so that the surviving nematodes will have a similar resistance gene frequency as the parent population.

Narrow-spectrum drenches

The use of narrow spectrum drenches (closantel, rafoxinide for *Haemonchus* control) tends to extend the useful life of the currently available broad spectrum drenches(44). This was one of the key points of the Australian *Wormkill* plan (page 13). In New Zealand, however, *Haemonchus contortus* is less dominant, with most infections being multigeneric. Therefore, narrow spectrum drenches have not been used widely in this country (Pomroy, *pers.comm.*).

<u>*Alternation of action families*</u>

Already in 1978 it was suggested by Le Jambre[86] that alternation within a single generation may hasten the development of resistance to both action families used. The author proposed that one anthelmintic family should be used until it fails, followed by a change to an alternative. The suggestion, however, did not find unanimous approval[129][62][93]. Prichard *et al.*[129] discussed the drawbacks of such strategy (failure to detect resistance in its early stages and high build-up of resistance to each action family) and proposed that a slow rotation policy be followed instead, in which anthelmintics from different action families are used between, but not within, a single generation. According to their suggestion, the maximum generation interval of common ruminant trichostrongylids being about a year, only broad spectrum anthelmintics from one group should be used within this interval[129]. This suggestion has found wide, though not universal, approval[78][163][127]. Some authors recommend instead that the change of action family should coincide with the end of a 3-4 week interval drenching programme or occur at a time when the change to safe pasture is most important, such as weaning[148].

The effect of different rotation strategies in a New South Wales environment were explored by Barnes *et al.*[20] by use of a modified model, originally built with the purpose of simulating grazing systems and the evolution of anthelmintic resistance in *Trichostrongylus colubriformis*[19]. In the modified model, development of resistance was investigated over a period of 20 years for two drugs to which resistance was assumed to be determined by codominant alleles. Four rotation strategies were compared: rotation of drug at each successive treatment, annual rotation, rotation every five years and rotation every ten years. It was shown that, although the strategy of rotating drenches annually resulted in the slowest rate of onset of anthelmintic resistance, after 20 years the levels of resistance were high (60-80%) and similar for all rotation strategies.

Another *Trichostrongylus* model[47] also indicated that the programme that minimised the development of levamisole resistance involved the alternating the drugs (levamisole and thiabendazole) between each worm generation.

Trial data have confirmed these findings. Anderson *et al.*[6] conducted a study to evaluate the efficacy of a mixture of albendazole and levamisole against *Ostertagia* and *Trichostrongylus* spp. in sheep. Based on the results of the study, the authors concluded that a preventive control programmes should include annual rotation of effective anthelmintics, including combination drenches.

Combination drenches

In recent years, the use of combinations of benzimidazole and levamisole drugs has been recommended with the aim of maximising their efficacy even in the presence of resistance to both drugs. Their efficacy has been tested by a number of trials[5][110][7][115], with generally encouraging results. The success of combination drenches depends on the degree of resistance present on the farm[161] as well as on the strains which are resistant to either drug[109]. For instance, a combination drench would not be expected to work as effectively if strains resistant to compounds from both classes predominated on the farm[5]. In 1990, multiple resistance in New Zealand was still uncommon in sheep[109], but it appears to be increasing steadily. It is important, therefore, to perform species differentiation when testing for resistance. While previous studies had postulated a synergistic interaction between the two compounds[24], in more recent works[5][6] the efficacy of the mixture was shown to be due solely to the additive effect of each anthelmintic. The authors of these works advised that the dose rates for the mixtures should not be less than the recommended dose rates for the single components[5]. They also suggested that the long-term use of mixtures, where high frequencies of resistance genes exist, is likely to favour selection for multiple resistance. To minimise this risk, it has been recommend that mixtures of anthelmintics be used in an annual rotation with other classes of effective compounds[109][6].

The use of two or more chemically different anthelmintics simultaneously rather than in rotation has been confirmed by computer simulation models to be one of the most effective ways to slow down selection for resistance. A previously described model [20] examined the option of using two drugs in a mixture over 20 years, and came to the conclusion that this option selected less heavily for resistance compared with the use of each drench individually in alternate years.

Controlled-release capsules (CRC)

A new form of anthelmintic delivery for sheep has become available in the last few years, which consists of capsules which release the product (albendazole) at a daily rate which is about one tenth of the recommended dose. The period of action of such capsules varies from 90 to 100 days. The rationale behind use of ruminal slow-release anthelmintic devices is to extend the period of contact between drug and worm. In the case of benzimidazoles, the result is an increased efficacy because it inhibits the dissociation of the tubulin-benzimidazole complex, thereby resulting in death of those nematodes whose tubulin does not bind as strongly to the benzimidazole. According to Barger(16), the greatly extended period of contact not only increases efficacy against already resident resistant worms(3) but offers protection against new infection by the more sensitive larval stage. However, the efficacy of such devices and ability to delay development of anthelmintic resistance needs to be further investigated. Theoretically, these capsules are effective against incoming third stage larvae at a very much lower drug concentration than against adult worm populations and may lead to a less rapid development of resistance(151) and have a positive effect on nematode contamination of pasture and ewe productivity(43). Several trials have underlined the advantages of controlled-release capsules (CRCs), in terms of ovicidal, larvicidal and adulticidal activity, and of increased productivity(84)(21)(18)(16)(92)(3)(42) as well as their efficacy in many cases of established resistance to benzimidazoles(151). A mathematical model(19) also indicated that the use of such devices will not cause a substantial increase in anthelmintic resistance for a period of five years. However, there have also been reports of unsatisfactory results with the use of such products (Macchi *et al.*, in press).

Grazing management

Since early times, the use of pasture management strategies(78) has been advocated to minimise the larval challenge of sheep, with particular attention to lambs. This would in turn allow the administration of a smaller number of drenches and, according to many, delay the onset of resistance. Grazing management strategies include the previously described strategies of rotating pastures, decreasing stocking rates, grazing sheep and cattle (or other naturally resistant species) together, weaning lambs on to pasture with low numbers of nematode larvae, and possibly using pasture species which are least favourable to larval survival and development (pages 13-16).

In more recent years, the practice of treating animals grazing minimally contaminated pastures, in an effort to delay the onset of anthelmintic resistance, has been questioned. Already in 1985, a trial conducted in Central Victoria selected benzimidazole resistance in a strain of *Ostertagia* spp. by anthelmintic treatment and movement on to pasture not previously grazed by sheep(95). Likewise, there is no evidence that programmes aimed at controlling parasitism while not selecting for resistant nematodes, such as the Australian *Wormkill* actually resulted in a delay of the onset of resistance. The integration of anthelmintic treatment and stock management has in fact been shown to result in selection of resistance(71)(101), depending on the frequency of treatment and movement as well as on the number of residual larvae on the pasture and the success of the resistant progeny in contributing to subsequent generations(49). A study by Martin(101) suggested that the selection pressure imposed by three anthelmintic treatments followed by relocation to worm-free pasture imposed a greater selection pressure than that imposed in the field by five-six treatments for 4 years. The author's conclusion was that in a situation in which post treatment contamination is likely to contribute substantially to future generations of worms, intense selection for resistance is to be expected unless a very efficient anthelmintic treatment is carried out. Based on previous findings, he suggested that an effective way to avoid selection for resistance (at least under Australian summer conditions) would be to use efficient treatment in summer, which would allow the eggs deposited in spring (prior to treatment) to contribute in a greater proportion to the autumn larval pool(101).

Vaccination

A medium-term measure of control of nematodiosis could be vaccination of either all or some of the susceptible animals. It has been suggested that even a vaccine of reduced effectiveness could result in a protection similar to that given by the Wormkill scheme(166). Difficulties and hopes associated with the development of effective vaccines have been discussed (page 18).

Breeding sheep for resistance

The search for methods of internal parasite control that are less dependant on anthelmintics has lead to a growing interest for measures which involve the identification and use of genetically resistant hosts. Some authors have hypothesised the application of current knowledge of genetic control of resistance in some species using them as models for sheep nematodes[159]. As was previously discussed (pages 16-17), resistance to gastrointestinal parasites appears to have moderate heritability and not to be correlated with production traits[58]. Sheep selected for on their ability to suppress the establishment of parasites will not significantly contaminate pasture, which should, in turn, result in a decreased need for anthelmintic treatment[171]. This was confirmed by a mathematical model created by Barger[14], who concluded that the use of genetically resistant hosts should eventually reduce the rate of development of anthelmintic resistance in the parasite.

One objection that has been addressed to breeding sheep for resistance to parasites is that the latter would readily adapt to their 'new' hosts and increase their virulence. At present, this has not been shown to occur[30], but the possibility has to be taken into serious account. Bisset and Morris[30] suggest that if adaptation of parasites to genetically resistant sheep did prove to be a problem, breeding for resilience, as opposed to resistance, would still be a viable option for reducing reliance on drenches.

Biological control

Certain fungi show great potential as biological control agents against animal worm parasites. As was previously discussed (pages 18-19), more studies need to be undertaken before the use of nematophagus fungi becomes a practical option in the control of gastrointestinal nematodes.

Susceptible strains

A relatively new strategy aimed at overcoming the problem of anthelmintic resistance was investigated in South Africa, where attempts to control a resistant strain of

Haemonchus contortus on pasture were made by replacing it at various times of the year with a susceptible strain. A reversion to susceptibility occurred in 3 of the 5 camps. These included both of the camps infested with the susceptible strain in the spring and one of the 2 infested in the autumn[175].

Education

The importance of education of veterinary students and graduates, as well as of farmers, is probably underestimated by many as a means of limiting the spread of anthelmintic resistance. Refresher courses should be made available for veterinarians and information should be disseminated to farmers by means of leaflets, papers, videos or other methods.

Reversion of resistant strains to susceptibility and control of anthelmintic resistance

Once anthelmintic resistance is present in a nematode population it has been shown to persist for several years even in the absence of continuous selection pressure by the specific anthelmintic[151][95][130]. This is partly due to the fact that very few producers routinely screen for anthelmintic resistance and to the lack of sensitive tests, which ensures that most cases of resistance are not detected at an early stage[71]. According to Prichard[129], depending on the relative fitness of susceptible and resistant individuals, some reversion towards susceptibility to the original action family may take place while an alternate family is being used. The degree and rate of reversion also appears to depend on the nematode species involved.

Most studies aimed at investigating the pattern of reversion to susceptibility, generally involving benzimidazole drugs, have so far yielded disappointing results. In 1982 a study was conducted in Australia[63] to investigate the potential for benzimidazole resistant strains of *Haemonchus contortus* and *Trichostrongylus colubriformis* to revert towards anthelmintic susceptibility when not exposed to anthelmintic treatments. Changes in anthelmintic resistance status were monitored throughout 12 generations. No reversion towards susceptibility was recorded for either nematode species, irrespective of the dose rate used. Some experts suggested that in the field, where selection is likely to be less intense, a benzimidazole anthelmintic could still be of practical value if it was reintroduced for a limited period, for example, in a slow rotation, while being closely monitored[129]. However, studies so far have not confirmed this hypothesis. In 1984, a trial[97] investigating changes in resistance status of a thiabendazole-resistant strain of *Ostertagia circumcincta* found no significant reversion when thiabendazole treatment was discontinued, or in the laboratory when the strain was either passaged in the absence of anthelmintic, or selected with levamisole. The authors suggested that anthelmintic strategies should aim at preserving nematode susceptibility through alternation of anthelmintics before any resistance develops, a recommendation shared by other experts[162][130]. Another trial[165] aimed at investigating the dynamics of resistance to benzimidazole in a mixed population of *Trichostrongylus colubriformis* and *Haemonchus contortus* spp. found that a change to levamisole for two years resulted in a reversion towards benzimidazole susceptibility. However, benzimidazole resistance increased rapidly following the re-introduction of oxfendazole into the anthelmintic treatment program. Based on similar findings, some authors[145][62] have concluded that, once resistance has been established in the population withdrawal of the offending anthelmintic and a subsequent return later, offers no useful method of control.

Based on current knowledge, it appears therefore unlikely that farms on which resistance to any drench family has developed, will still be able to make effective use of products of this family. However, some recommendations have been given for dealing with these situations[130]. These include alternation between the remaining drench families, and frequent testing for resistance to them.

In order to try to contain the anthelmintic resistance problem, surveillance is crucial[78]. Early detection of resistant worms will allow to suspend the use of a product before it becomes highly ineffective, which should in turn allow hopes for its future re-inclusion in the action family slow-rotation strategy. Specific recommendations given by a Task Force established in New Zealand in 1990 included frequent (at least annual) checking of drench efficacy by means of a *drenchcheck* (faecal egg count examination 7 days after drenching), followed by a proper FECRT (*Drenchtest*) in case of suspicion of resistance[130].

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CHAPTER 2

Anthelmintic resistance: a questionnaire study of farmer practices*

* This is a slightly edited version of a paper submitted for publication to the New Zealand Veterinary Journal in January 1997 as: "Anthelmintic resistance: a questionnaire study of farmer practices", C. Macchi, W. E. Pomroy, D. U. Pfeiffer, R. S. Morris, D. M. West, R. L. Sanson.

Abstract

A random postal survey of 300 sheep farmers in the Manawatu region of New Zealand was conducted with the purpose of determining current drenching practices and the factors associated with the development of drench resistance. The high response rate (65.7%) and the many comments indicated a high degree of farmer interest and concern. Farmers drenched their sheep at a higher frequency than would generally be considered advisable with regard to the development of drench resistance. The average number of anthelmintic treatments administered to lambs, two-tooths and ewes between 1 July 1994 and 30 June 1995 was respectively 6.16, 2.44, and 2.30. Grazing management as a measure to control larval infestation was routinely adopted by only a small proportion of farmers. There appeared to be a lack of understanding on how grazing management strategies should be combined in order to achieve integrated control over gastrointestinal nematodes while minimising the use of anthelmintic drugs. Fifty-three percent of farmers reported that they weaned their lambs on to a *clean* pasture at least occasionally, but only 24% of these waited more than two months before shifting the lambs back on to a contaminated pasture. Although in most cases (94%) farmers treated their sheep according to the weight of either the heaviest individual or the heaviest group of sheep in the mob, 43% of them estimated bodyweights based on their personal experience, and 12% never checked the accuracy of their drench gun. Only half of respondents who had purchased sheep during the last year quarantine drenched them upon arrival on the farm. Although all farmers appeared to be aware of the problem of anthelmintic resistance, only 31% of them had performed at least one drench test on their property. Among testing farms, prevalence of resistance was 69.6%, and involved products of the benzimidazole action family in all but one case in which levamisole was the ineffective product. Most farmers (76% of 166) reported that they were satisfied with their overall deworming programme; however, 14 farmers specifically drew attention to the lack of clear information concerning ways to prevent the establishment of anthelmintic resistance.

Introduction

Development of anthelmintic resistance is a continuing problem for control of nematodes in sheep. In the North Island of New Zealand, an analysis of faecal samples submitted to the Batchelar Animal Health Laboratory (Palmerston North, New Zealand) between 1986 and 1992 indicated a prevalence of resistance to benzimidazoles of 74%, to levamisole of 23%, and to benzimidazole-levamisole combinations of 30%[19]. A second extensive analysis of the results of diagnostic cases submitted to animal health laboratories in both the North Island and the South Island between 1992 and 1994[20] yielded similar results. According to this survey, resistance typically concerned a single drench type, the benzimidazoles, and the problem appeared to be more common in the South Island than in the North Island. Several studies have been carried out in order to investigate the risk factors associated with the development of anthelmintic resistance. Such factors include frequency of treatment[10][23][27][17][16], timing of use of anthelmintics in relation to season and life stage of the sheep[29][22][24][22][24][28], dosage[14], rotation of drench families[22][24][28][21], and management of pastures[12]. The aim of this questionnaire was to reach a better understanding of how New Zealand sheep farmers are controlling parasites, and how this compares with current recommendations for both the control of nematode parasites and the prevention of anthelmintic resistance. Personal opinions of farmers were also taken into account in the analysis.

Materials and Methods

Selection of farms and questionnaire design

In September 1995 a questionnaire was sent to 300 sheep farmers in the Manawatu region of New Zealand. The farms represented a random subset of all sheep farms registered in the 'Agribase' database, which contains most farms in the region. The subset was randomly selected among the farms registered as having at least 500 ewes, although some of them later appeared not to meet the criterion. A letter was attached to the questionnaire, explaining its purpose and asking for the farmers' co-operation. A reminder letter was sent to all non-respondents four weeks later, followed by a second reminder plus a duplicate copy of the original questionnaire three weeks later. The questionnaire comprised 38 closed-ended questions and a final open-ended question for those who wanted to add any personal comments. The 5-page questionnaire included four sections, respectively asking information about details of farm and livestock numbers, management of lambs, drenching policy and procedures, and the farmer's own opinion on issues related to anthelmintic resistance.

The information concerning the classes of animals present on the farms was used to determine stocking rates, by converting the numbers of each class to stock units, according to a previously described procedure[24].

Statistical analyses

A broad descriptive analysis was initially performed, aimed at outlining the general characteristics of the farms included in the survey and the management and deworming strategies adopted by the respondents. Subsequently, more in-depth statistical analyses were performed. The data were first screened by means of univariate analyses, which included χ^2 tests for dichotomous variables and Mann-Whitney U tests for continuous variables. Farms were compared according to whether or not a drench test had been performed, the outcome of the test, and the number of anthelmintic treatments administered to the lambs. Two more grouping variables were created based on subjective assessment of some answers given by the farmers. The first of these variables represented the quality of the farmer's drenching policy. Farmers who reported that they followed a pre-determined drenching policy, by treating their lambs every 3-4 weeks from weaning on 5-6 occasions according to the recommendations typically given by veterinarians and researchers were assigned a 1 ('good policy'). The same score was given to farmers who periodically monitored the nematode burden of their sheep and drenched them when their egg counts were high. On the other hand, farmers who did not follow a sound deworming strategy (e.g. they only drenched their lambs when they showed signs of scouring), were allocated to group 2 ('poor policy'). Personal satisfaction of the farmer was also scored. If the farmer considered the overall deworming programme either very effective or effective (1 or 2 on a scale from 1 to 5), he/she was assigned a 1 ('happy'), otherwise he/she was assigned a 2 ('unhappy'). All univariate analyses were conducted using Statistica® 5.1 for Windows (StatSoft Inc., Tulsa, OK, U.S.A.) except for 2x2 Tables, which were carried out in Statcalc, which is a component of EpiInfo® version 6.04 (Centers for Disease Control & Prevention, U.S.A./ World Health Organisation, Geneva, Switzerland).

All the independent variables which had a p-value < 0.20 were further investigated by means of a multivariable analysis. Initially, consideration was given to constructing a path model aimed at explaining the impact of each factor on the occurrence of anthelmintic resistance, but too few variables reached statistical significance, and such a model was therefore not achieved. However, it was judged feasible to conduct a forward stepwise logistic regression analysis, with the variable *TEST* (completion of a drench test on the farm) as the outcome variable. Variables showing at least a moderate association ($p<0.20$) with the outcome were included in the model as independent variables. Criteria for model selection included a p-value of 0.10 for entry of new variables and of 0.15 for removal of variables; goodness of fit was evaluated by means of a Pearson's χ^2 test. The stepwise selection method was based on the significance of the score statistic, and removal testing on the probability of a likelihood ratio statistic based on the maximum partial likelihood estimates. Logistic regression was conducted using SPSS® version 6.1 (SPSS Inc., Chicago, IL, U.S.A.).

Results

The response rate to the first distribution of the questionnaire was 38%, which was raised to 65.7% by the two reminders given during the following two months. After eliminating non-usable responses 178 (59.3%) questionnaires were available for analysis.

Details of farm and livestock numbers

Romneys were by far the most prevalent breed for both ewes (74%) and rams (65%). The frequency of the most prevalent ewe and ram breeds by farm is shown in Figure 1.

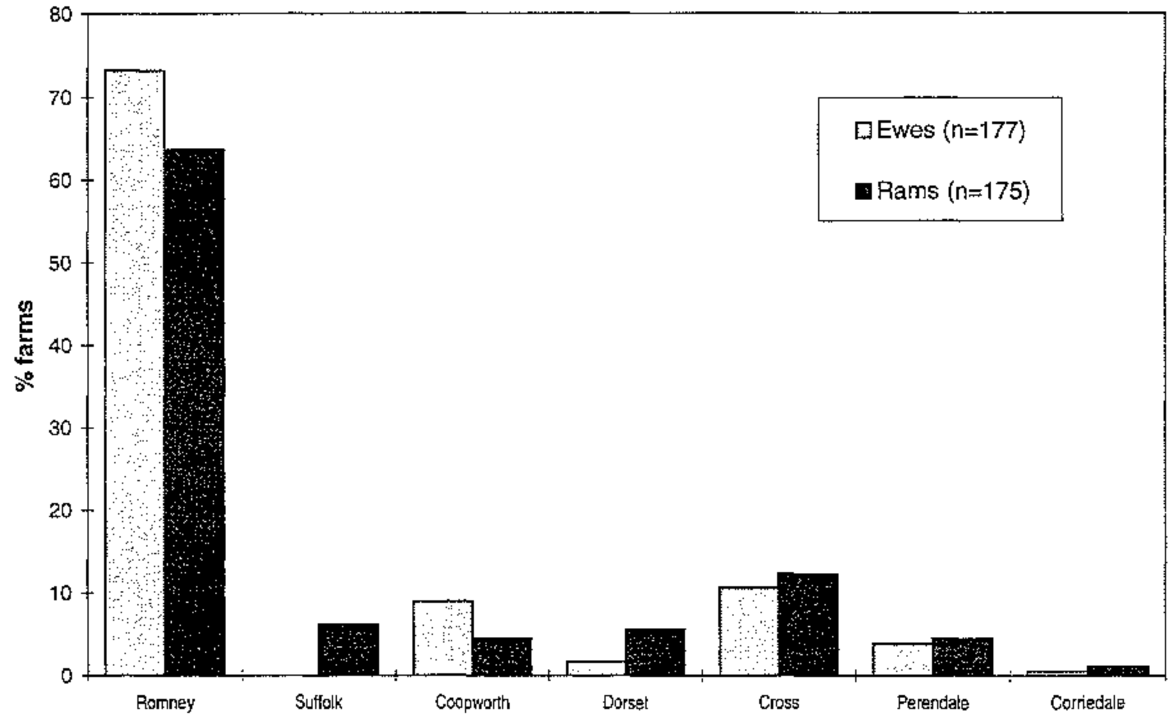

Figure 1. Main breed of ewes and rams kept on the farm

Details of the farms are shown in Table 1. The farms included in the survey had very variable areas and flock sizes, with most of them being between 100 and 400 hectares in area and farming between 1000 and 2000 ewes. The lambing percentage varied considerably among farms, although the majority of respondents reported a figure between 100 and 115%. Weaning was typically carried out during the first half of December.

Table I. General farm information

Variable	No. farms	Mean	Min	25th percentile	Median	75th percentile	Max
Grazing area (ha)	179	457.1	36.4	196.0	295.0	440.0	9308.1
No. ewes	176	2178	60	1000	1500	2400	32000
No. rams	171	51	2	12	20	34	2034
No. ewes/ram	176	71.6	30.0	54.0	68.7	82.7	218.2
Total stock units (SU)	179	5027	242	2053	3110	4538	140508
Sheep:Total SU	179	0.7	0.2	0.6	0.7	0.8	1.0
Stock. density (SU/ha)	179	11.5	3.6	9.0	10.9	12.8	51.4
Lambing%	167	110.7	82.0	100.0	110.0	118.0	180.0
Start of weaning	162	9/12/94	1/9/94	1/12/94	10/12/94	20/12/94	16/2/95

When asked whether they had purchased any animals during the last year, 71% of farmers indicated they had bought some cattle (of any age or sex category), and 80% of farmers had purchased at least some sheep (mostly rams) during the same time (Table II).

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Table II. Purchase of animals between 1 July 1994 and 30 June 1995

Animals purchased	No. farms	Mean	Min.	25th percentile	Median	75th percentile	Max.
Ewes	43/179	373	28	100	206	500	1500
Lambs	29/179	1427	60	300	600	1300	12000
Rams	121/179	6.6	1	4	5.0	9	30
Stock Units	179/179	660.7	0.0	11.0	188.0	664.0	9105.0

Management of lambs

During the first two months after weaning, according to the 172 respondents, lambs were either set stocked (41%) or shifted at intervals greater than 10 days (46%). Only the 22 remaining farmers (13%) shifted the animals every 2-10 days. Forty-seven respondents indicated that they never made use of uncontaminated pastures to graze their lambs at weaning, while the remainder used them at least occasionally (Figure 2). For the lambs which were weaned on to a 'clean' pasture (i. e., a pasture not grazed by lambing ewes since 1 June 1994), the majority were subsequently moved to a pasture previously grazed by ewes and lambs after a short time, i.e. less than one month in 34% of cases (n=85) and between one and two months in 42% of cases. Only nine of the replying farmers (10%) left the lambs on the clean pasture for more than two, and 12 (14%) for more than three months (Figure 3).

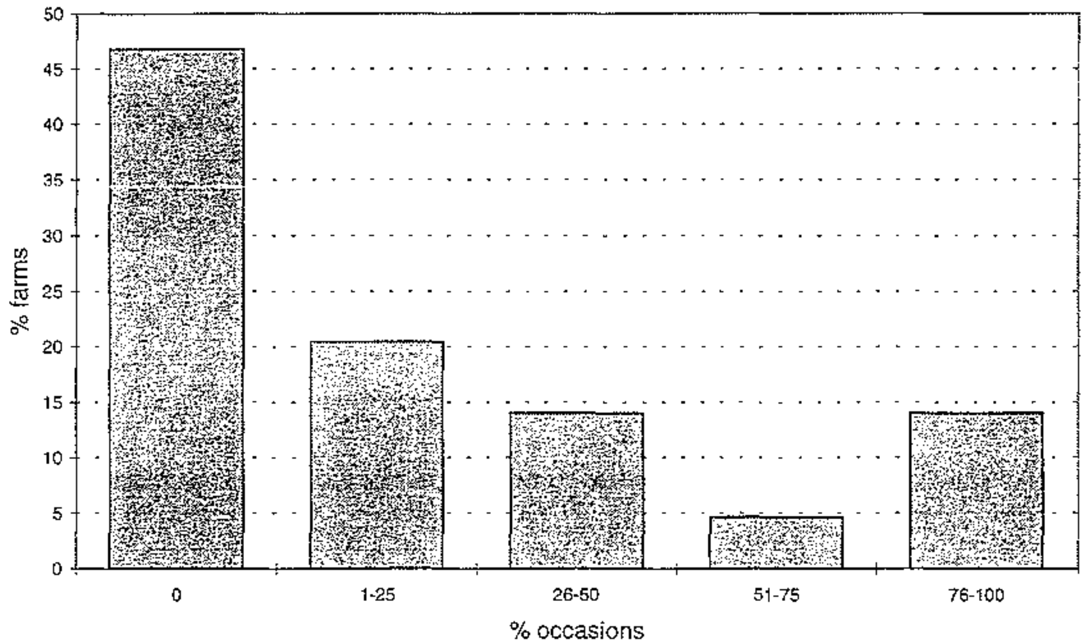

Figure 2. Frequency of weaning lambs on to a paddock not grazed by lambing ewes since 1 June 1994 (n=172)

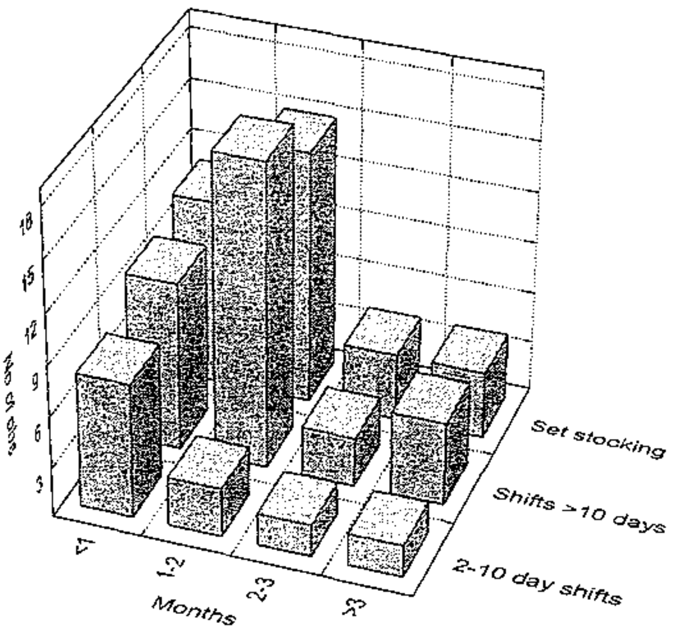

Figure 3. Association between grazing management of lambs and time spent on *clean* pasture.

The chart refers only to lambs which were weaned on to a 'clean' pasture. The x-axis shows the amount of time they spent on this pasture, while the z-axis categorises them into three groups according to how often they were shifted during the first two months after weaning (n=85).

Figure 4 indicates the relative numbers of farmers who attempted to create safe pastures for lambs by grazing cattle or deer, making hay, or by different means, in the intervals between sheep and lamb grazing, and the frequency of occasions in which they carried out such strategy. Figure 5 compares these findings with the frequency of occasions in which farmers weaned their lambs on to a safe pasture.

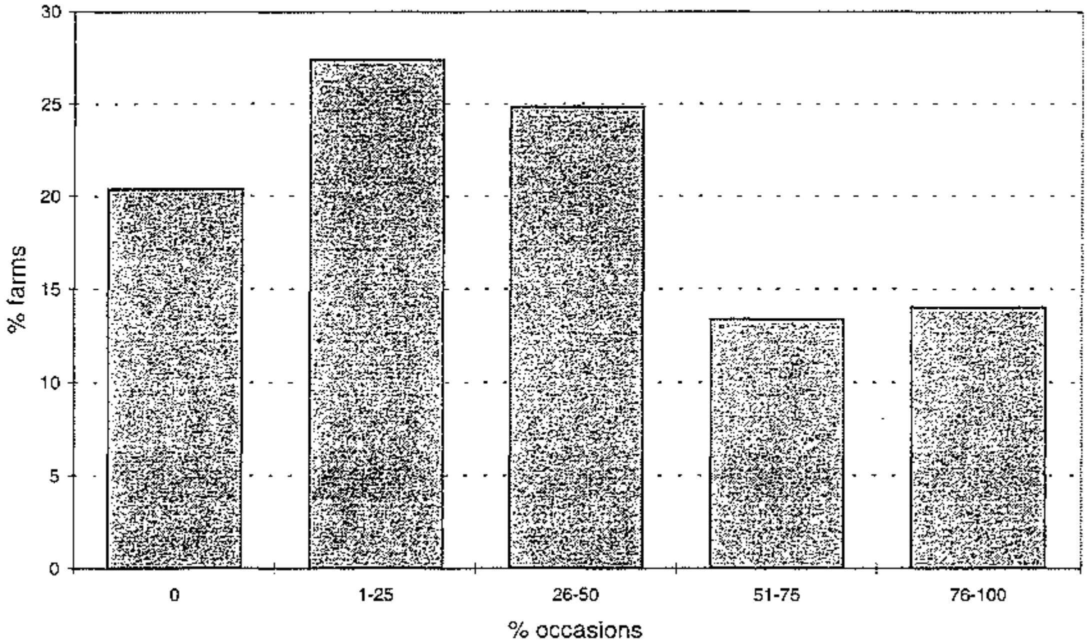

Figure 4. Percentage of occasions in which farmers attempted to create safe pastures for lambs by grazing animals of a different species or spelling paddocks (n=157).

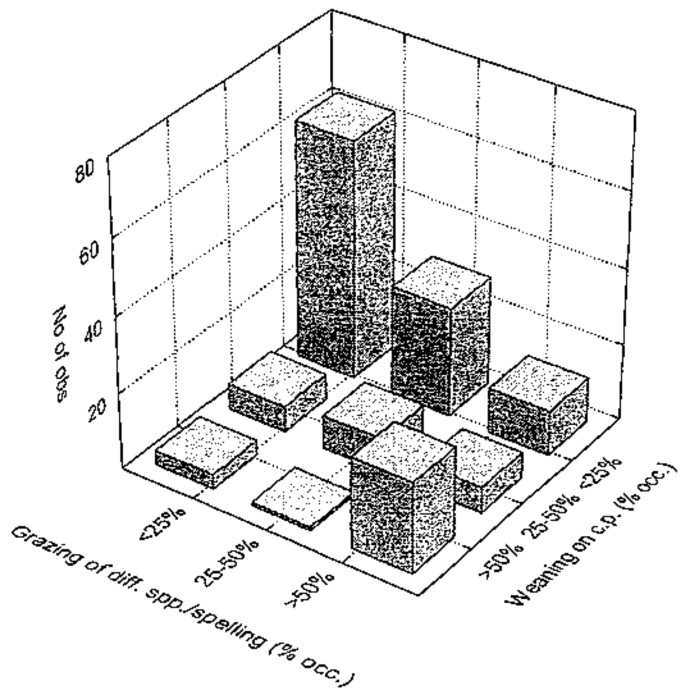

Figure 5. Association between weaning on to clean pasture and frequency of use of animals of a different species or spelling pastures (n=156).

The chart shows the relationship between the number of occasions (%occ.) in which lambs were weaned on to a clean pasture and the frequency with which farmers made use of alternative grazing or spelling strategies.

c.p.=clean pasture; occ.=occasions

Drenching policy and procedures

Lambs which remained on the property for the first year of their lives received, on average, 6.16 anthelmintic treatments during this year. However, there appeared to be a great deal of variability in the number of treatments administered (see Figure 6). As lambs were being sold at intervals following weaning it was not possible to determine how many individual lamb drenches were given, as information on dates of sale were often incomplete. A relatively high proportion of respondents used an anthelmintic which included praziquantel for their first lamb drench (50% of 121) in order to control tapeworm infection.

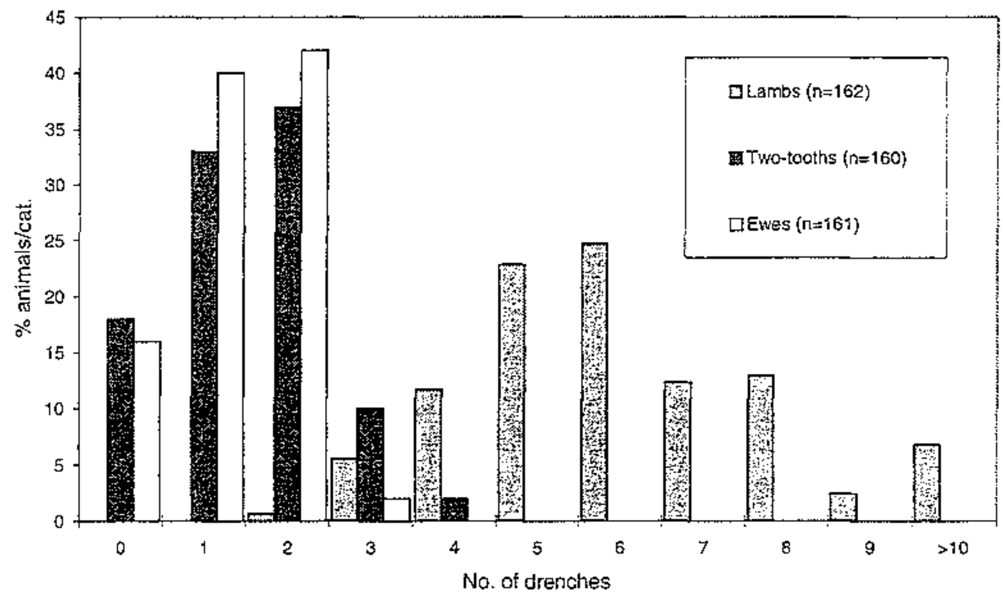

Figure 6. Drenching frequency for three categories of sheep (number of drenches administered between 1 July 1994 and 30 June 1995)

Lambs=all sheep born in 1994

Two-tooths=ewes from one year of age until first mating

Ewes=mixed-age ewes having been mated at least once

Of the 94 farmers (n=178) who changed drench between 1 July 1994 and 30 June 1995, 66% were following a pre-determined action family rotation. Of the remaining respondents who made a change, 12 (13%) did so on the advice of their veterinarian, while 3 had followed the advice given by a person other than a veterinarian. Three farmers indicated price as one of the factors implicated in their choice, two believed that the former drench was not working properly, and the remaining respondents suggested various reasons for using a different drench, such as the control of tapeworms in young lambs, use of ivermectin for nasal bot, or a different strategy for replacement ewe hoggets from that used for ram lambs. When asked whether or not they intended using a different drench the following year, 28% of farmers (n=170) answered they did, while 68% said they would not change, and 4% had not yet decided. A χ^2 test was performed to determine if an association existed between rotation of drench family in the previous year and the intention of using a different drench the following year. Farmers who had changed drenches in the previous year appeared to be more likely to change drenches the following year (72% of 64), compared with farmers who had not rotated action families (62% of 24). The test yielded an odds ratio (OR) of 1.53, although the difference was not statistically significant ($p=0.396$, $\chi^2=0.72$, 1 df).

Figure 7 shows the products used by farmers to treat their lambs during the first year of their lives. When the names of the products were investigated, it appeared that 29% of respondents were using drugs from more than one action family. Respondents were further investigated according to whether or not they had said that they were on a rotation programme. It appeared that as many as 37% of farmers who believed they were rotating drenches yearly (n=57) had actually used anthelmintics of different action families on the same generation of lambs within the same year.

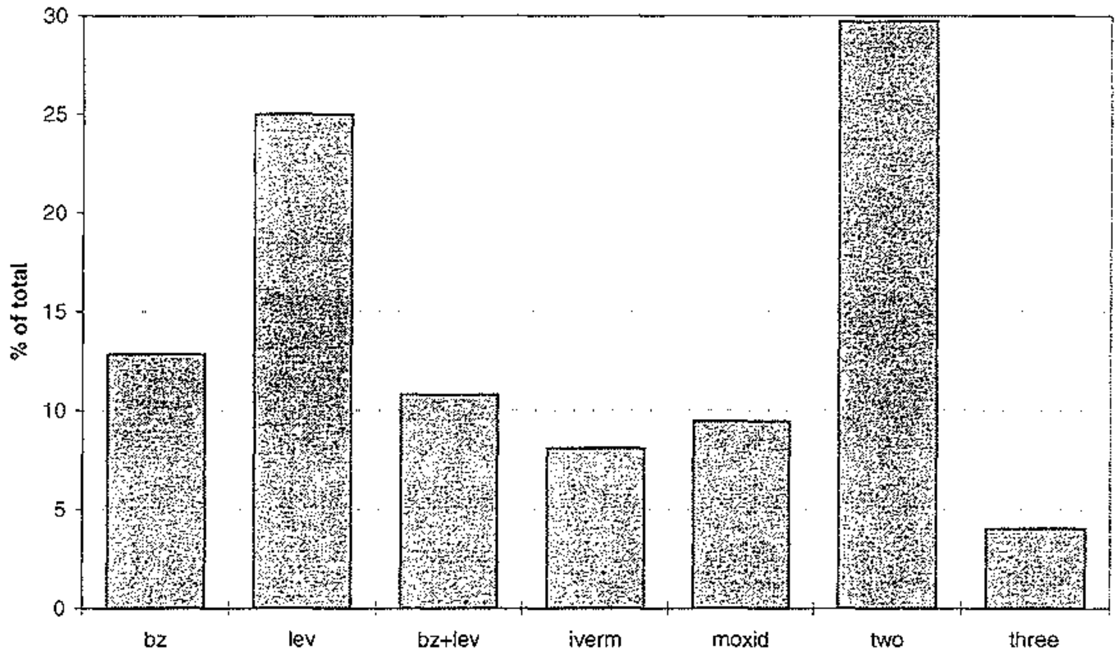

Figure 7. Products used on lambs between July 1994 and June 1995 (n=148)

bz=benzimidazole; lev=levamisole; bz+lev=combination drench; iverm=ivermectin; moxid=moxidectin; two=use of drenches belonging to two different action families; three=use of drenches belonging to three different action families.

When asked about their drenching policy, 37% of farmers (n=174) indicated that they followed a 5-6 drench programme by drenching every 3-4 weeks from weaning, regardless of the weather or other factors, although for some of these (15% of 65) other factors were also involved in determining the drenching times. Several farmers (13% of 174) only treated their lambs during periods of risk (e.g. rainy seasons); twenty farmers also, or only, indicated that they drenched when the animals showed signs of scouring (11%). Others (18%) said that they drenched the lambs regularly throughout the year. However, there appeared to be no significant difference in the actual number of treatments administered to the lambs by the latter compared with the farmers who stated that they were following a 5-6 drench programme. Faecal egg counting was used as a criterion to help determine the timing of drenching by 12% (n=174) of the farmers; of these, 33% (a total of 7 farmers) only drenched their lambs when the faecal egg counts were considered high (the definition of a high egg count was not specified in the questionnaire).

After drenching the lambs, 34% of 165 farmers returned them to a fresh pasture, not recently grazed by other lambs, on more than half of the occasions and 11% did so on an irregular basis, while the majority (54%) returned the lambs to the same paddock. The association between weaning onto a 'clean' pasture and returning drenched lambs to a 'clean' pasture was investigated. It was found that on those farms where they had been weaned onto a 'clean' pasture, it was more likely (OR=2.06) that lambs were also returned to a different pasture every time they were drenched. However, the χ^2 test was not statistically significant ($\chi^2 = 2.73$, 1 df, p=0.0987)

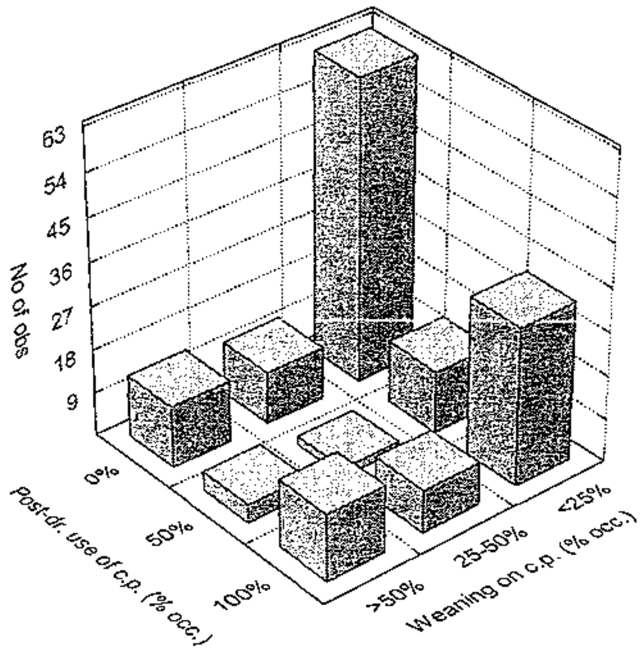

Figure 8. Association between use of clean pastures after weaning and after drenching lambs (n=162)

c.p.=clean pasture; occ.=occasions

Most farmers (70% of 160) drenched their two-tooth ewes either 1 or 2 times, and 12% 3 or more times between 1 year of age and mating. They (n=135) usually followed (on 67% of occasions) a pre-determined drenching programme. In other cases they drenched them because they looked daggy (18%) or had high egg counts (13%). Of 161 respondents, 26 (16%) never drenched their ewes, while 63 and 68, respectively (summing to 82%), drenched them (or treated them with sustained-release capsules) once or twice, and only three drenched them more than twice. When only one drench was administered, the time was commonly either at docking or before lambing, while in the cases in which the ewes were drenched more than once, they were most commonly (55% of 93) treated at mating and, later, at lambing. The frequency of treatments in three different categories of sheep is illustrated in Figure 6.

Among farmers who had treated their ewes before lambing (n=89), 34% had used a levamisole-based product, in many (53%) cases (n=30) combined with a clostridial vaccine (Nilvax®), 16% a benzimidazole, 3% a benzimidazole/levamisole combination drench, and 12% had used ivermectin. The remaining group had used long-acting products, such as benzimidazole sustained-release capsules (13%) and moxidectin (17%).

The pattern of drenching purchased sheep differed according to the category of sheep that were purchased. Forty-seven percent of the respondents who had bought more than 50 ewes (n=40) stated that they drenched all purchased sheep upon arrival on their property, while 93% of those who had bought more than 50 lambs (n=28) did so. The results of the 2x2 table showed that farmers who had purchased lambs were 14.37 times as likely to drench the purchased animals as farmers who had bought adult ewes (χ^2=15.14, $p<0.001$). Among all farmers who drenched purchased sheep, in 60% of cases the anthelmintic used was different from the one they were using on the rest of their animals. When asked about the product used and the reason for choosing the product, sixty-three percent of farmers (n=63) answered that they used either ivermectin or moxidectin, with the specific intention of minimising the risk of introducing resistant worms on to their property.

The volume of drench released by the gun was usually checked before (73% of 172) drenching the animals, sometimes (6%) both before and after drenching or (9%) repeatedly after treating a predetermined number of sheep. A proportion of farmers

(12%), however, said they relied on the gun being accurate. Underdosing appeared to be uncommon, as in the majority of cases (n=173) the dose rate was calculated according to the weight of either the heaviest individual (52%) or the heaviest group of animals (40%) in the mob. However, when asked how they evaluated live weight of sheep, 43% of them (n=171) stated they estimated it based on their personal experience; 49% said they weighed some animals before drenching the mob, while 8% always weighed all sheep prior to treatment.

Although the presence of drench resistance had been assessed on 53 farms (31% of respondents), only 46 answers were able to be analysed because they provided sufficient information about the test and its results. In these cases, a full drench test was usually undertaken (63%), with collection of faeces both before and after treatment, while in the remainder only post-treatment samples were examined. Of the 46 usable replies, 10 stated they had tested their drench efficacy on more than one occasion. In 73% of cases, only benzimidazole drenches were evaluated; in 15% levamisole was the product whose efficacy was being assessed, in 10% of cases both action families were evaluated, and only in one case was ivermectin included in the test along with the other two drugs. Although a test for anthelmintic resistance was first conducted on one of the responding farms in 1984, very few farmers had assessed their resistance status before 1990. For the 32 farms diagnosed as having drench resistance, this involved benzimidazoles in 29 cases, levamisole in 1, and a combination of the two in another case (the product was not specified in the remaining case).

The farmers' opinion

When asked how effective they judged their deworming programme to be, 76% of farmers (n=166) said they considered it effective, 20% satisfactory, and only 2% were not satisfied with the results. Gastrointestinal nematodes were considered a very important cause of economic loss by 70% of respondents (n=169) and fly strike by 50%. Other diseases such as pneumonia and facial eczema were not believed by respondents to cause major problems on their farms. Most farmers felt very strongly that action needs to be taken against the problem of drench resistance, especially by farmers themselves (87%, of 160) and drench companies (86%), but also by veterinarians (63%) and government agencies (62%). When asked whether they believed drench resistance was a problem for the industry, 58% of the 170 replying farmers said that it is already a very serious problem, 33% thought that, although present, it is not a major issue, 5.8% believed that it will be a problem in the next 5 years, and 3.5% of farmers stated that resistance, while not being a problem yet, is likely to become of concern in the next 20 years.

A relatively high number of respondents (14/67 expressing their opinion) also expressed disappointment with the lack of clear information about how to prevent, delay and/or fight drench resistance.

Further investigation into statistical relationships

When drenching frequency in lambs was analysed by means of crosstabulations, the results suggested that it was significantly positively associated with the size of the farm ($p=0.004$, $\chi^2=15.391$, 4 df), indicating that larger properties drenched more often. A 2x2 table indicated that farmers were more likely to be pleased with the results of their policy, if the adopted policy was a sound one ($OR=2.61$, $\chi^2=7.05$, 1 df, $p=0.006$).

A comparison of farmers -made by means of Mann-Whitney U tests and crosstabulations- according to whether or not a drench test had been performed on their property, revealed minor non-significant differences in the general pattern of farms (size, sheep/total stock units ratios, etc.) and in the drenching policies used. A significantly greater proportion (86%) of the 21 farmers who used faecal nematode egg counts in the decision on when to drench the lambs had also performed a drench test on their farm, compared with those who had not used egg counting ($n=156$), of whom only 19% had performed a drench test ($OR=0.004$, $\chi^2=41.74$, $p<0.001$). The difference was more striking if farms had previously been diagnosed as having a resistance problem: 52% of such farms ($n=27$) drenched their lambs based on the results of their egg counts, while only 20% of farms where a drench test had yielded a negative result for anthelmintic resistance (n=20) used egg counts as a basis for their drenching programme (OR=4.31, χ^2 =4.93, p=0.0263).

Table III shows the results of the univariate analyses carried out to select the variables for multivariable analysis. The factors included into the multivariable analysis were the use of egg counts as a diagnostic aid (*egg counts)*, the use of an overall sound anthelmintic strategy *(policy)*, the area size of the farm *(size*, categorised into <200 ha, 200-400 ha, and >400 ha), and the ratio between sheep stock units and total number of stock units present on the farm *(sheep:tot SU*, categorised into <0.6, >0.6 and <0.75, and >0.75). After performing a forward stepwise logistic regression, using *test* (whether or not a drench test had been carried out on the farm) as the dependent variable, the final model which offered the best fit included only two main effects: use of egg counts and sheep:total SU ratio (Table IV). The results suggest that farms on which faecal egg counting was carried out regularly appeared to be 24.63 times as likely to test for anthelmintic resistance as those where drenching of lambs was carried out irrespectively of their worm loads . Farmers with a sheep:total SU ratio comprised between 0.6 and 0.75 were shown to be more than twice as likely to perform a drench test as farmers with either higher or lower sheep:total SU ratios.

Table III. Results of univariate analyses showing the variables significantly associated with completion of a drench test on the farm (variable *TEST)*

Variable	**N**	χ^2	**df**	**p-value**
Size	179	8.90	2	0.012
Sheep:Tot SU	179	7.86	2	0.020
Policy	174	7.05	1	0.008
Egg counts	174	41.74	1	<0.001

Table IV. Final logistic regression model for the dependent variable *TEST*

Variable	OR	Lower 90% CI	Upper 90% CI	Wald	df	p-value
Egg counts	24.627	8.160	74.330	22.759	1	<0.001
Sheep:Tot SU				4.698	2	0.095
1	1.119	0.381	3.286	0.029	1	0.864
2	2.557	1.111	5.884	3.432	1	0.064

Discussion

New Zealand sheep farmers appear to be increasingly aware of the problem of drench resistance. This was confirmed by the high response rate that was reached by this questionnaire, as well as by the high percentage of farmers who added a comment at the end of it, usually reinforcing their point of view on the situation and how it should be dealt with. They claimed that media, veterinarians and drench companies all give too many conflicting suggestions and explanations. Still, most farmers said that they were satisfied with the results of their own anthelmintic strategy. Such strategy appeared to be mainly based on the farmers' past experience and subjective evaluation of the performance of animals. When asked if they believed that anthelmintic resistance was a serious problem for the industry, all respondents gave an affirmative answer, although only 58% of them saw it as a real threat today. The analysis of this questionnaire reveals the urgent need to provide new specific guidelines to farmers, aimed at minimising the risk of selecting for drench resistance, while still maintaining an effective worm control. However, this is a very controversial issue and there appears to be no perfect way of dealing with this double-folded problem. There is increasing evidence that any measure undertaken in order to control parasitism will ultimately result in a higher selection pressure for anthelmintic resistance(29)(12)(20). Farmers often receive different advice from experts according to whether the emphasis is being put on the achievement of optimal performance by sheep or on the avoidance of selecting for anthelmintic resistance. However, most strategies tend to rely upon an integrated approach to control(12) and generally include factors such as the use of grazing management to minimise the larval challenge of sheep, the use of a correct dose of anthelmintic and a strategic timing of treatments(29)(28).

Nevertheless, the results of this questionnaire indicate that most farmers do not appear to follow consistently these basic principles. As Figures 2-4 clearly show, the use of preventive control measures such as weaning of lambs on to pastures of low infectivity and use of specific strategies to lower the larval burden of pastures is still not widely adopted by farmers. Moreover, in most reported cases even the use of grazing management strategies to minimise the larval challenge to sheep is unlikely to yield satisfactory results in terms of either control of parasitism and delaying of anthelmintic resistance. The vast majority of farmers indicated that they returned their lambs to a contaminated pasture after a period of less than two months, in which case the use of *clean*, or *safe*, pastures will have little beneficial effect. Management of pastures has always been considered one of the most effective ways to control parasites without increasing drenching frequency[24][22][21][17]. However, some researchers have hypothesised that the use of *safe* pastures might actually accelerate the development of anthelmintic resistance[28][12].

The average number of anthelmintic treatments administered to lambs (6.16), two-tooths (2.44) and ewes (2.30) between 1 July 1994 and 30 June 1995 does not appear to have changed greatly in the last decade. The figures emerging from this questionnaire are not dissimilar from those found by a survey conducted in 1982, which aimed at investigating the control measures adopted by sheep farmers in the North Island of New Zealand[14]. Such a high frequency of treatments is likely to accelerate the development of drench resistance. Frequency of treatment, in fact, has long been claimed to be the most important factor associated with the development of resistance[23][27][17][21][29][11][29], although it has been more recently claimed that the intensity of selection for resistance really depends on the degree of control obtained that is attributable to the anthelmintics, rather than on the sheer number of drenches required to obtain it[20].

Even in the light of the most recent developments in the understanding of anthelmintic resistance, many questions still need to be carefully examined and answered. For example, in most drenching programs aimed at reducing the number of treatments, drenching times are selected so that larval numbers on pasture are at a minimum. This might ultimately attain the effect of accelerating the development anthelmintic resistance, instead of delaying it[29][12][20].

Annual rotation of drenches is also typically recognised as one of the components of any antiparasitic policy aimed at delaying the onset of resistance[28][28], although there is no unequivocal proof that this would select less rapidly for resistance[29]. The questionnaire revealed that only 66% of respondents specifically followed a drench family rotation.

Another factor which has been blamed as one of the main causes of drench resistance is underdosing[(23)(27)(19)(25)]. The results of this questionnaire suggest that farmers are aware of this problem and try to avoid it by basing the dosage on the weight of the heaviest animals that are being drenched. However, few of them systematically use scales to weigh their animals before drenching them. Previous studies have shown that farmers are often mistaken in both weight estimations and calculations of dose volume[(23)]. Another frequently overlooked cause of under-dosage is faulty equipment or drenching technique. The survey reveals a lack of attention to the setting of guns and imprecise estimation of the live weight of animals.

Experts agree in recommending quarantine drenching of all purchased sheep prior to introducing them on to a property in order to avoid bringing in resistant nematode genotypes. Treatment should be done with an anthelmintic to which drug resistance is least likely to have developed[(29)(25)], such as a product of the avermectin/milbemycin action group. This needs to be emphasised to farmers, as it appears that a considerable proportion of them do not routinely quarantine drench purchased sheep. Interestingly, farmers are more scrupulous when they buy lambs, as the questionnaire reveals that 98% of these do drench their animals upon arrival on to their property.

The results of logistic regression show that the use of faecal egg counting as a diagnostic tool and the ratio between sheep and total stock units have the greatest impact in predicting whether or not a farmer is likely to perform a drench test.

The analyses did not detect any significant differences in anthelmintic strategy between farms where resistance had been diagnosed and the other farms. This, however, may reflect the low power of the analyses, due to the fact that only a small proportion of farmers had performed a drench test, that only about half of them had yielded a positive result, and that not all of the farmers answered all questions. However, even significant differences would be difficult to explain solely on the basis of the information gathered through this questionnaire. It would be necessary to collect more data describing the history of each farm prior to the diagnosis of drench resistance, in order to draw any defensible inferences as to either causes or consequences of resistance. The primary objective of this questionnaire was to describe the present situation on a representative sample of sheep farms and to reach a better understanding of both perceived and real problems associated with drench resistance in this region of New Zealand.

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CHAPTER 3

An evaluation of the effects of anthelmintic resistance on the productivity of lambs*

* This is a slightly edited version of a paper submitted for publication to the New Zealand Veterinary Journal in January 1997 as: "An evaluation of the effects of anthelmintic resistance on the productivity of lambs", C. Macchi, R. S. Morris, W. E. Pomroy, D. U. Pfeiffer, D. M. West.

Abstract

This trial aimed to estimate the productivity consequences of anthelmintic resistance in growing lambs on commercial farms, by quantifying its effects on weight gains and susceptibility to diarrhoea. Five farms with a history of resistance to benzimidazole drenches were selected. On each farm, 150 ewe lambs were randomly allocated to one of three treatment groups. Groups 1 and 2 were treated five times at 28-day intervals, according to the recommendations typically given by veterinarians and researchers, with oxfendazole (the ineffective drench) and levamisole (later changed to a combination drench because it was found not to be fully effective) respectively. Lambs from Group 3 received a suppressive antiparasitic treatment, consisting of a combination of slow-release albendazole capsules and moxidectin. Parasitological comparisons included differentiated nematode egg counts and faecal egg count reductions, while weight gains and dag scores were the production parameters assessed and analysed. The results show that lambs from Group 3 had lower egg counts, better dag scores and better growth rates than those from Group 2, which in turn gained more weight than lambs from Group 1. The overall live weight gain during the five months of the study was 7.17 kg for Group 1, 8.35 kg for Group 2, and 9.88 kg for Group 3.

Introduction

Anthelmintic resistance is among the most serious problems modern sheep producers have to face. The Manawatu region of New Zealand, with its moist and temperate climate, offers nematodes an ideal habitat during most of the year. The presence of a substantial proportion of nematodes resistant to commonly used anthelmintics appears therefore particularly threatening in this area of the world. Most people acknowledge that resistance to anthelmintics is a major limiting factor for increasing sheep performance; however, the impact that reduced effectiveness of drenches can have on the productivity of sheep cannot be easily quantified. This trial compares the use of three different treatment policies -partially ineffective, effective, and suppressive drenching- on typical commercial farms, and assesses their effects on nematode egg counts, weight gains and susceptibility to diarrhoea in growing lambs.

Materials and Methods

Study design and selection of farms

The trial was conducted over a 5-month period between January and June 1996. Power analysis determined a sample size of 200 randomly allocated animals per group, in order to detect a 2 kg difference in the weight gain of lambs at the end of the trial with a power of 0.80 at the 5% significance level (α=0.05). This was met by 4 farms with 50 lambs for each treatment group. Only ewe lambs were included.

Criteria for inclusion of farms in the trial included a history of resistance to anthelmintics of the benzimidazole action family, interest of farmers and willingness to cooperate in the study, and location within a 100 km range from Massey University, Palmerston North, where all analyses were carried out. An outline of the history of each farm is given in Table I. On none of the farms had efficacy of non-benzimidazole products ever been tested by the egg count reduction method.

Table I. Detection of anthelmintic resistance on the trial farms.

FARM	Year of diagnosis	Product used	Details
1	1989	FBZ	Identification of resistant genera: *Haemonchus, Ostertagia, Nematodirus*
2	1992	ABZ	No details available
3	1991	BZ	Resistance not detected in 1992; resistance confirmed in 1993
4	1991	BZ	Resistance diagnosed in capsule-treated ewes (drench check)
5	1989	MBZ	Identification of resistant genera: *Haemonchus, Trichostrongylus, Nematodirus*

FBZ=fenbendazole; ABZ=albendazole; MBZ=mebendazole; BZ=non-specified benzimidazole

Experimental units and treatment protocol

On each farm, 150 ewe lambs born between August and September 1995 were randomly selected, individually identified and allocated into three groups of 50 lambs each. All were treated according to the weight of the heaviest lamb in their group. The trial lambs were run as one individual mob and kept separate from the rest of the sheep on the farm, but otherwise received the same type of management.

Table II shows the treatment protocol and dose rates for the three groups. According to the original trial design, all lambs from Groups 1 and 2 were to be drenched every four weeks with the same product that was used on the first visit, while those from Group 3 would receive only a second controlled-release capsule (CRC) 90 days after the first was introduced. However, an unexpected rise in the number of strongylid eggs in the faeces of lambs from both Group 2 and Group 3 on two farms prompted us to change our drenching policies slightly. Therefore, on visit 5 (March 1996) and all subsequent treatment visits, all lambs from Group 2 were treated with a combination oxfendazole/levamisole drench. Similarly, all lambs from Group 3 received moxidectin in addition to the CRCs.

Table II. Treatment schedule

VISIT NO	GROUP 1	GROUP 2	GROUP 3
1	OX (5 mg/kg)[†]	LEV (8 mg/kg)[‡]	OX/LEV (4.5/6.9 mg/kg)[°], CRC (0.5-1 mg/kg/day)[◊]
3	OX	LEV	
5	OX	OX/LEV	MOX (0.2 mg/kg)[*]
7	OX	OX/LEV	MOX, CRC[×]
9	OX	OX/LEV	MOX

OX=Oxfendazole ;LEV=levamisole; OX/LEV=combination drench; CRC=Controlled-release albendazole capsule; MOX=moxidectin

[†] Systamex®, Suntex Co., Pitman-Moore New Zealand Ltd., 33 Wakatiki St. Upper Hutt, New Zealand
[°] Scanda®, Pitman-Moore Australia Ltd., 71 Epping Road, North Ryde, NSW 2113, Australia
[‡] Nilverm®, Mallinckrodt Vet.Ltd., 33 Wakatiki St., Upper Hutt, New Zealand
[◊] Captec Extender Jnr®, Captec Pty.Ltd., 103 Pipe Road, Laverton, Vic. 3028, Australia
[*] Cydectin®, American Cyanamid Company, Wayne, USA
[×] Captec Extender 100®, Nufarm Ltd., 2 Sterling Avenue, Manurewa, Auckland, New Zealand

Measurements

Weights and dag scores

All lambs were individually weighed on the first visit and at subsequent 28-day intervals prior to anthelmintic dosing. The type of weigh scale used differed among farms, but their accuracy was checked at regular intervals during the weighing operation.

All animals were visually examined to assess the accumulation of faeces around the breech (dag). The presence of dag was scored on a scale from 0 (no dag) to 5 (heavy dag), following the procedure described by Larsen et al.[18]. Scores were always assigned by the same person.

Nematode egg counts and larval differentiation

A subset of 15 lambs from each group was randomly selected. Faecal samples were collected from the rectum of these lambs at 14-day intervals. Egg counts were estimated using a modified McMaster technique where each egg counted represented 50 eggs per gram (epg). Composite faecal samples from each group were subsequently cultured for larval identification. The percentage composition of these cultures was then used to calculate group mean faecal egg counts and reductions of the individual nematode genera. Samples were classified as either positive or negative for *Nematodirus* spp.

Faecal egg count reductions (FECRs) following treatment were estimated for Groups 1 and 2 in order to assess and monitor the efficacy of the products used throughout the trial by comparing pre-treatment and post-treatment egg counts.

Statistical analyses

Body weights and weight gains were analysed by repeated measures analysis of variance (ANOVA) using the statistical software Statistica® 5.1 for Windows (StatSoft Inc., Tulsa, OK, U.S.A.). Strongylid egg counts (excluding Nematodirus spp.) were first transformed to the log (count +1) in order to normalise distributions and stabilise variances, then analysed by repeated measures ANOVA. Pre-planned comparisons between means were conducted to asses the effectiveness of each treatment for each subsequent month of the trial. Least squares means were estimated for fixed categorical main and interaction effects. Egg count reductions (both total and relative to each genus) were calculated on the arithmetic means and the 95% confidence limits obtained by a previously described method[1]. The effect of each treatment policy on parameters measured on a categorical scale (such as dag scores and the number of lambs in each group shedding *Nematodirus* eggs) was analysed using χ^2 tests. The software used for all χ^2 analyses was Statcalc, which is a component of EpiInfo® version 6.04 (Centers for Disease Control & Prevention, U.S.A./World Health Organisation, Geneva, Switzerland).

Dag scores of 4 and 5 were classified as severe. The effect of capsule administration on the occurrence of severe dag at subsequent visits was assessed by means of stratified χ^2 analyses. A χ^2 test for trend in proportions was also performed to further investigate the trend in time for each treatment group.

Statistical significance was determined at $p<0.05$.

Results

Body weights

There was pronounced heterogeneity in the distribution of bodyweights both between and within farms. The smallest lambs, on average, were found on Farm 1, whereas Farm 2 had the heaviest animals. Lambs on Farm 3 showed the greatest variation in body weights, due to the presence of two different cross-breeds of sheep. Figure 1 shows the live weights, categorised by group, on each farm at the beginning and at the end of the trial. The histogram provides information both about the shape of the distribution of weights and about the differences between treatment groups. Where a considerable overlapping of the two distributions occurs, as in Group 1, the weight gain at the end of the trial was not very substantial; the opposite can be said for Group 3, where a marked divergence between the two histograms can be observed.

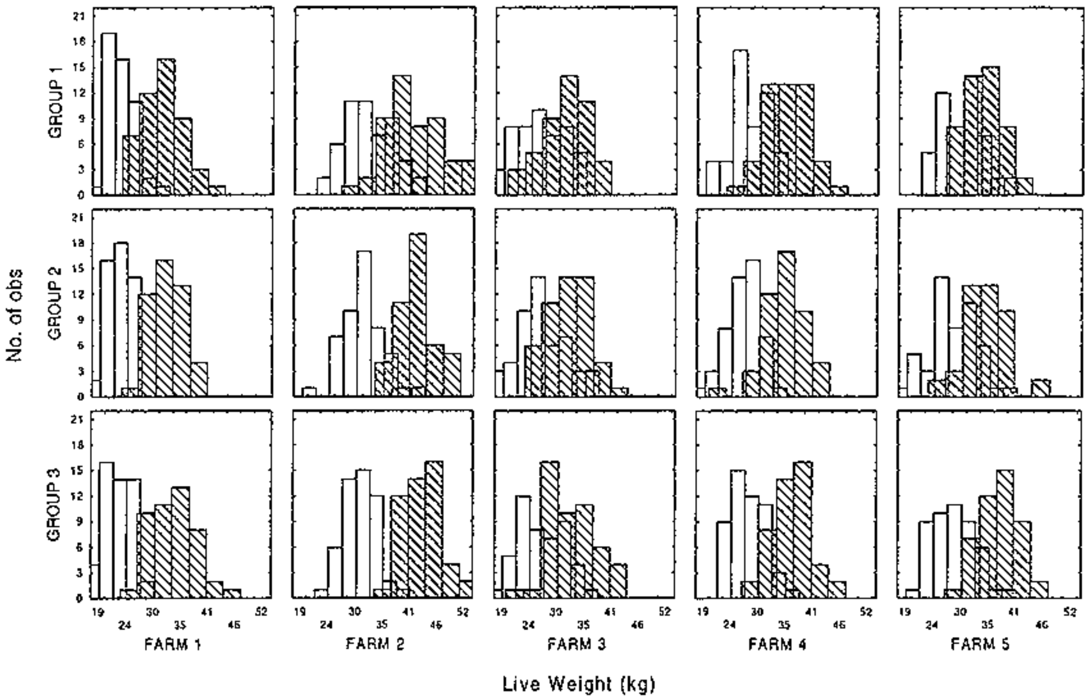

Figure 1. Distribution of live weights of lambs at the beginning (white bars) and at the end (striped bars) of the trial.

Figure 2 shows the change in body weights throughout the trial. Although there are differences between the five farms, the pattern of weight gains always shows Group 3 as performing best, usually followed by Group 2.

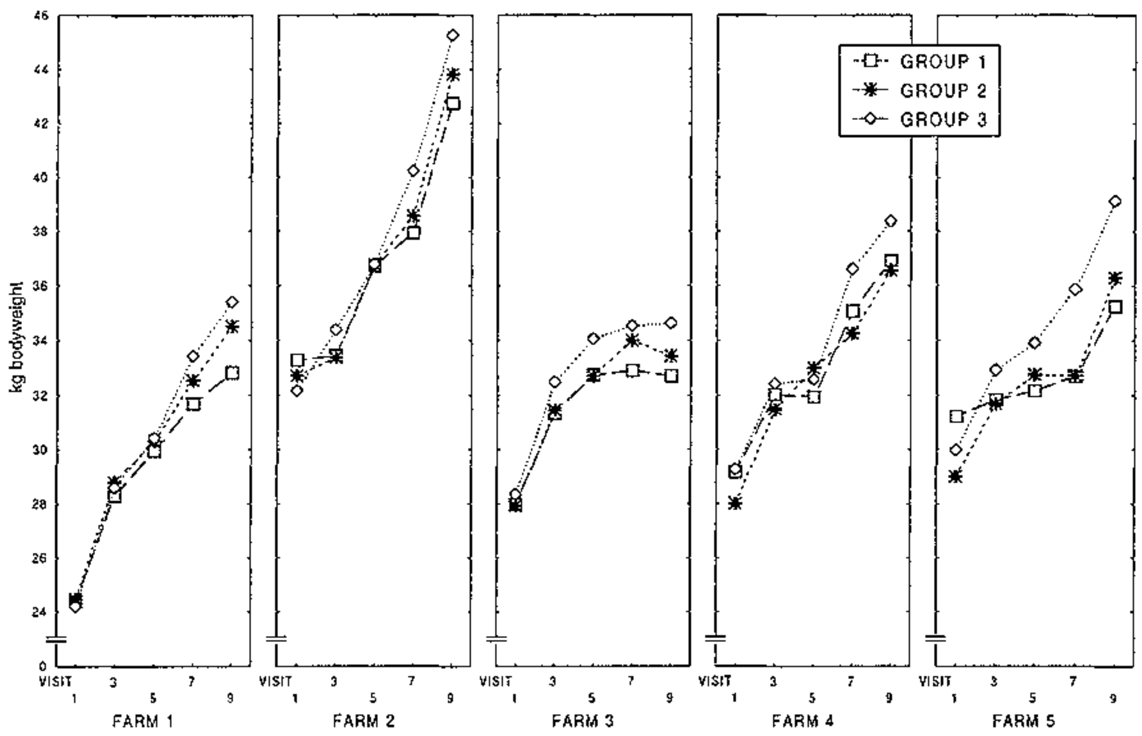

Figure 2. Mean change in live weights of lambs for each treatment group on individual farms

The differences are particularly striking towards the end of the trial. This is best assessed visually by examining Figure 3, in which weight changes, by group, on each farm, are expressed as percentage of initial weight.

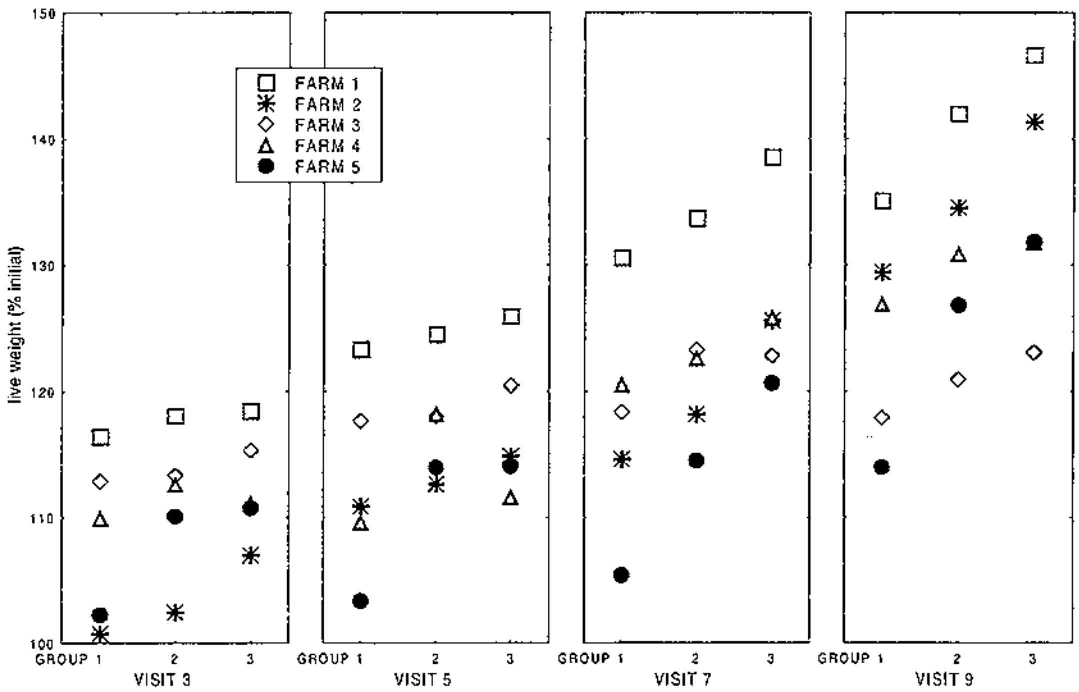

Figure 3. Mean weight change for each treatment group on subsequent visits, expressed as % of initial weight

The repeated measures ANOVA model for the dependent variable *weight gain* included the main effects *farm, treatment group, visit number*, and all interaction terms. The results are reported in Table III. All main effects were highly significant ($p<0.001$), as were the two-way interactions *farm x visit number* and *group x visit number* , and the three-way interaction *farm x group x visit number*.

Table III. Results of the repeated measures ANOVA model for the dependent variable *weight gain*

Source Term	DF	Mean Square	F-Ratio	p-value
A (FARM)	4	183.120	38.67	<0.001
B (GROUP)	2	101.097	21.35	<0.001
AB	8	8.569	1.81	0.071
C (VISIT)	3	249.165	52.62	<0.001
AC	12	256.139	54.09	<0.001
BC	6	35.568	7.51	<0.001
ABC	24	17.677	3.73	<0.001

Table IV shows the differences in weight gain and outlines the ones that reached statistical significance. Average weight gains were usually significantly higher for Group 3 on all farms during the whole study period. An exception was the lower live weight gained by lambs from this treatment group on Farm 4 during the first two months of the trial. This coincided with an unanticipated high number of nematode eggs being found in the faeces of capsule-treated lambs. Administration of moxidectin caused a reversal in the trend with Group 3 lambs already performing significantly better than lambs from both Groups 1 and 2 on visit 7. On average, the overall weight gains from the beginning of the trial to its end were, for Groups 1, 2 and 3 respectively, 7.17 kg, 8.35 kg, and 9.88 kg.

Table IV. Weight gains for each treatment group at subsequent visits.

VISIT NO	FARM	MEAN WEIGHT GAIN (STD DEV)		
		GROUP 1	GROUP 2	GROUP 3
3	1	3.89 (1.79)	4.23 (1.71)	4.41 (1.35)
	2	0.18[a] (3.71)	0.80[a] (2.71)	2.26[b] (2.23)
	3	3.39 (2.53)	3.53 (2.12)	4.22 (2.85)
	4	2.86 (1.64)	3.20 (2.32)	3.18 (1.35)
	5	0.57[a] (2.52)	2.69[b] (2.24)	2.92[b] (2.94)
5	1	1.58 (1.52)	1.49 (1.68)	1.85 (2.54)
	2	3.26[a] (1.55)	3.31[a] (1.74)	2.42[b] (3.17)
	3	1.28 (2.44)	1.27 (1.64)	1.64 (2.13)
	4	-0.09[a] (2.41)	1.72[b] (1.80)	0.25[a] (2.02)
	5	0.33 (1.46)	1.05 (1.57)	1.04 (2.60)
7	1	1.79[a] (1.61)	2.22 (1.20)	3.01[b] (1.38)
	2	1.24[a] (2.10)	1.77[a] (1.89)	3.44[b] (3.31)
	3	0.01 (1.84)	1.29 (2.23)	0.61 (1.90)
	4	3.12[a] (2.19)	1.26[b] (1.30)	4.07[c] (2.76)
	5	0.53[a] (1.22)	-0.06[a] (3.70)	1.96[b] (2.16)
9	1	1.11[a] (2.11)	1.96[b] (1.41)	1.98[b] (1.52)
	2	4.87 (2.17)	5.28 (2.04)	5.02 (2.00)
	3	0.06 (2.35)	-0.44 (2.32)	-0.03 (1.54)
	4	1.86 (1.19)	2.35 (2.02)	1.76 (2.15)
	5	2.57[a] (2.11)	3.65[b] (3.44)	3.27 (1.27)

Means in the same row with different superscript letters differ significantly ($p<0.05$)

Dag scores

A bar chart (Figure 4) was chosen to summarise the findings concerning dag scores on subsequent farm visits. Initially, only a few lambs were assigned to the 'severe dag' category, which comprised scores of 4 and 5. The proportion of daggy lambs, however, increased on subsequent visits. This finding was confirmed by the outcome of the χ^2 test for linear trend (χ^2=15.847, p<0.001), which showed that having severe dag was 10.5, 9.2, 21.7, and 7.5 times as likely at visits 3, 5, 7, and 9 respectively, as at the first visit. However, the odds ratio (OR) on visit 5 is likely to be an underestimate of the real situation, because on all farms lambs were crutched shortly after visit 3. Capsule-treated lambs had less dag than lambs from Groups 1 and 2. The difference was significant at visits 7 (χ^2=8.16, 1 df, p=0.004) and 9 (χ^2=7.43, 1 df, p=0.006). In the first case the OR was 4.31, indicating that 3 months after receiving the first sustained-release albendazole capsule, lambs were 4.31 times less likely to develop diarrhoea than lambs receiving a different anthelmintic treatment. On visit 9 the odds ratio was still in the same range (OR=3.93).

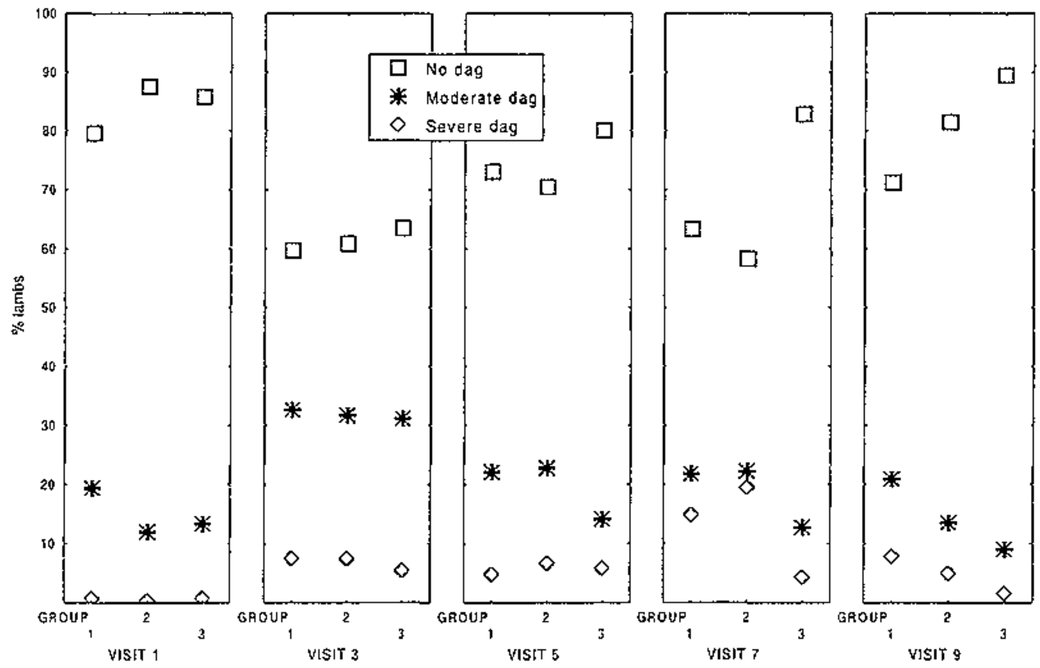

Figure 4. Distribution of lambs from each treatment group according to dag score at subsequent visits.

Parasitology

<u>Parasite burdens</u>

Faecal egg counts were low (<150 epg) at the beginning of the experiment on three of the five trial farms. This prevented us from drawing immediate conclusions about the farms' actual resistance status, although the percentage egg reduction in the benzimidazole-treated group (Group 1) varied between 93 and <0 (as on one farm the egg counts increased, instead of decreasing, 14 days after treatment). Table IV shows the results of the FECRT in Group 1 for each individual farm, as well as indicating the nematode genera which appeared to be resistant. Only the results for Group 1 are shown, since very few or no eggs were found in post-treatment samples from Group 2. As previously mentioned, however, on some occasions levamisole was unable to completely suppress egg counts (Figures 6 and 10). For this reason, on visit 5 it was replaced by a more effective combination drench.

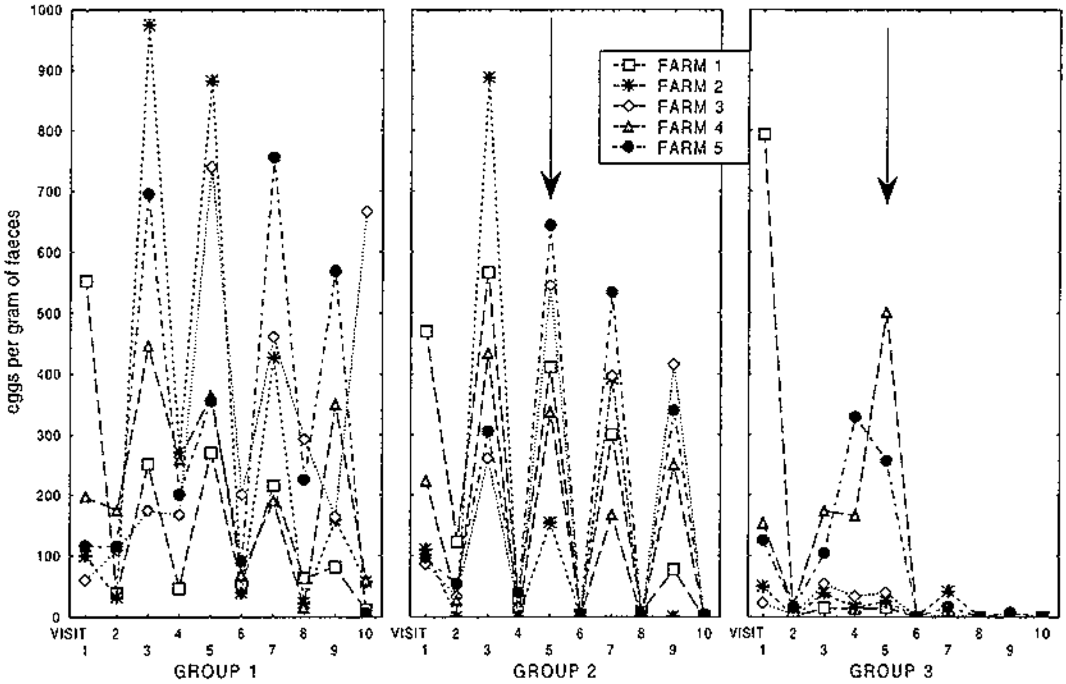

Figure 5. Mean change in Strongylid faecal egg counts excluding *Nematodirus spp.* Arrows indicate the change in treatment

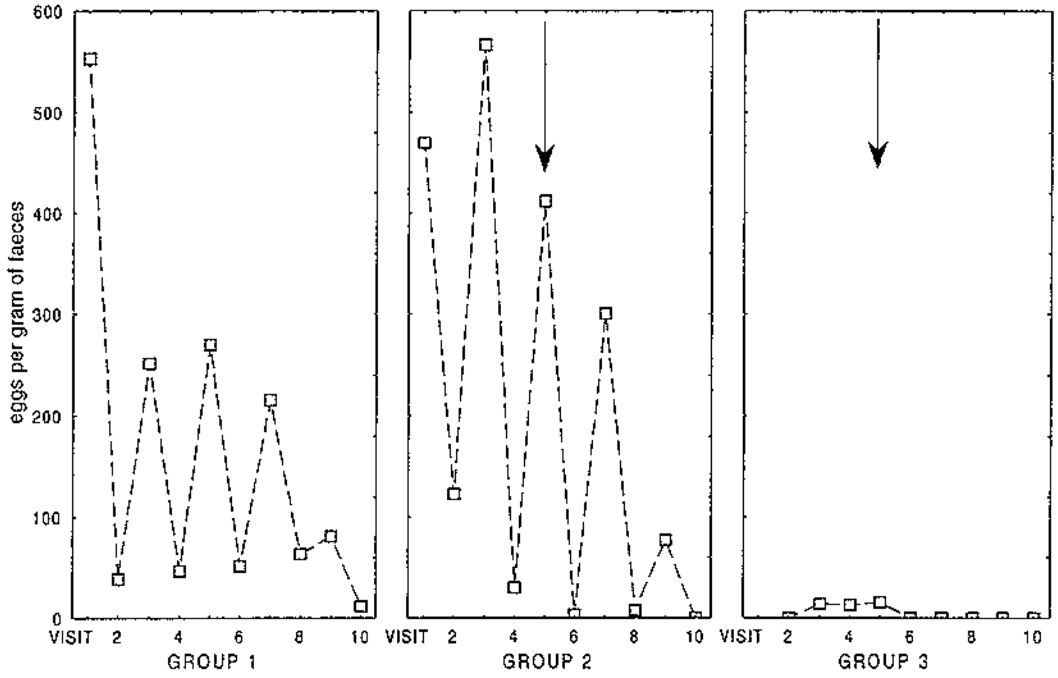

Figure 6. Farm 1. Mean change in Strongylid faecal egg counts.

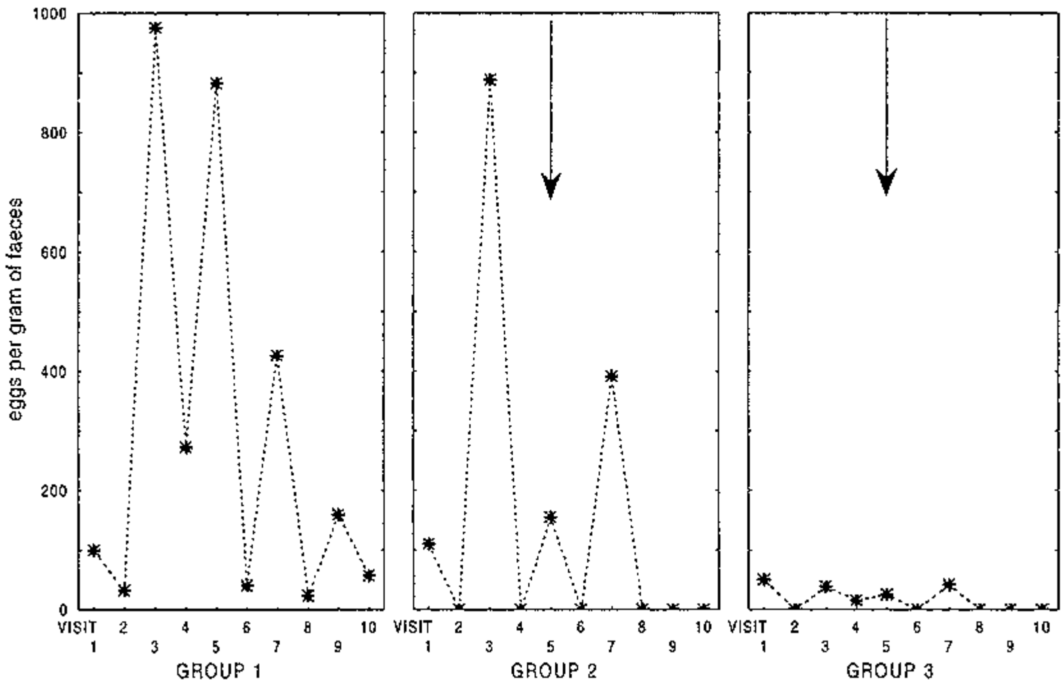

Figure 7. Farm 2. Mean change in Strongylid faecal egg counts

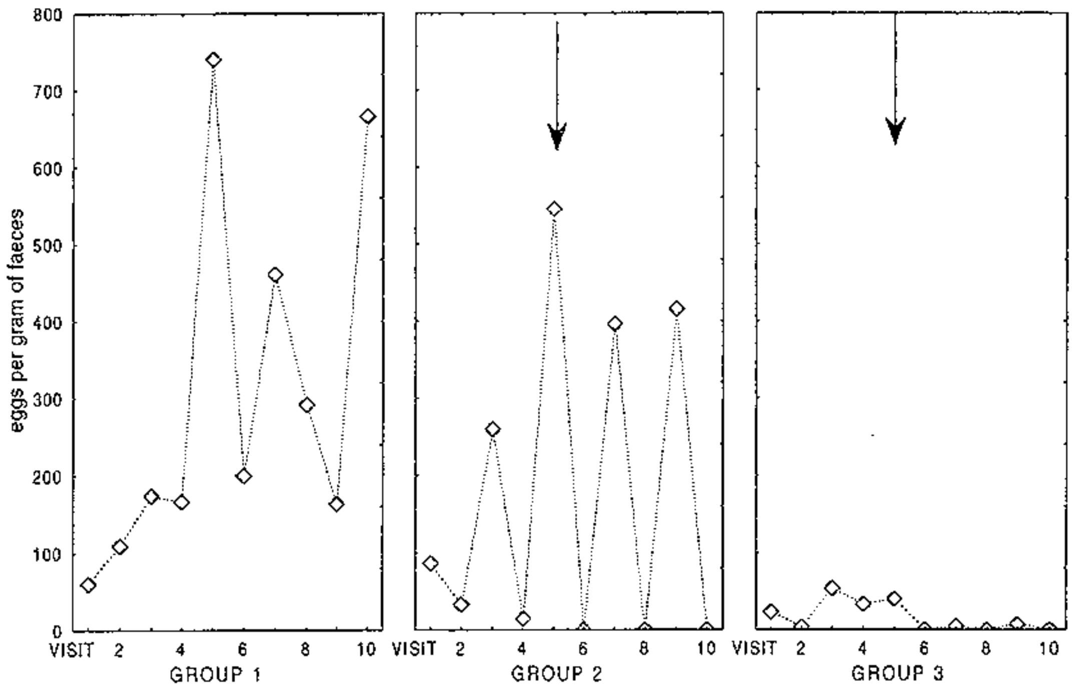

Figure 8. Farm 3. Mean change in Strongylid faecal egg counts

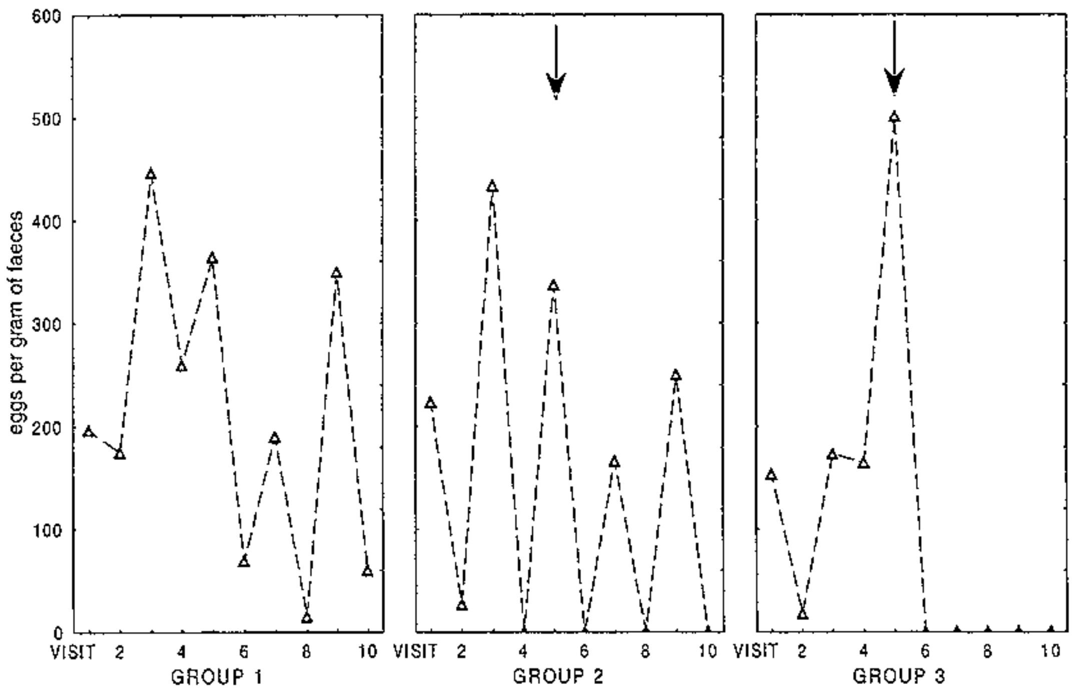

Figure 9. Farm 4. Mean change in Strongylid faecal egg counts

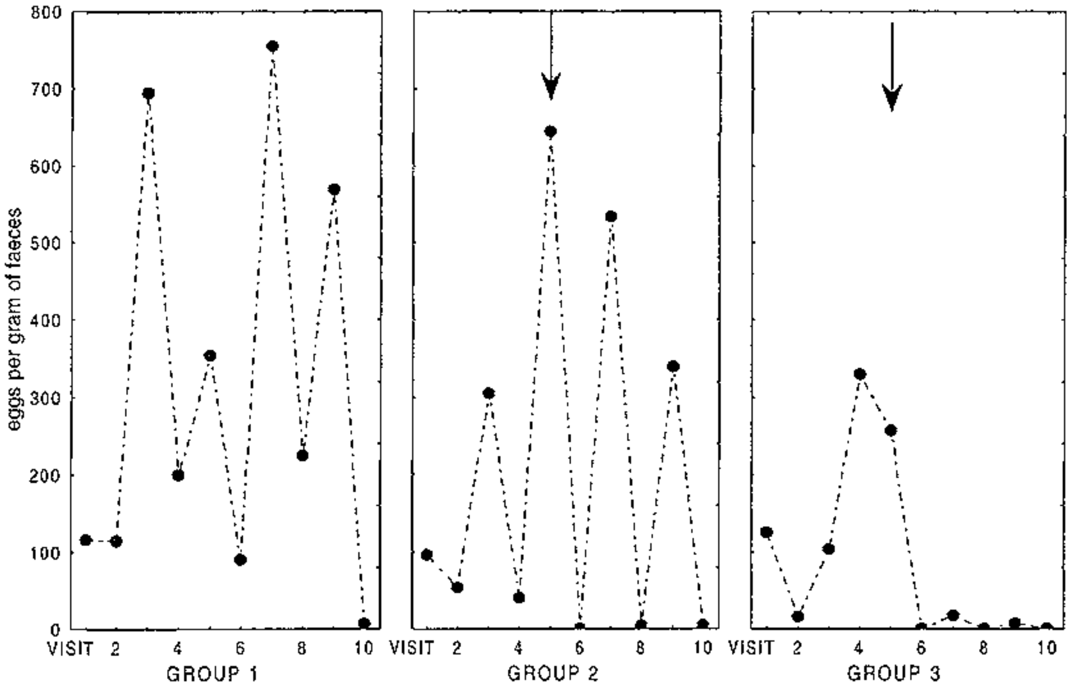

Figure 10. Farm 5. Mean change in Strongylid faecal egg counts

Figure 5 shows the pattern of strongylid egg output for each treatment group. All farms are represented for direct comparison. Separate trends for each individual farm are outlined in Figures 6-10. The graphs offer a clear indication of the efficacy of each treatment strategy,. In Group 1, mean egg counts never reach zero in the post-treatment samples. Treatment with an effective product (Group 2) appeared to eliminate all adult worms, as indicated by the zero egg counts 14 days after treatment. However, 28 days after treatment egg counts in lambs had gone back to substantially high levels, although usually lower than the corresponding levels in Group 1 lambs. The CRCs administered to lambs from Group 3 failed to keep the animals worm-free. However, as clearly shown in Figures 9 and 10, on farms 4 and 5 the capsules proved non-effective for this purpose, and as a consequence, all animals from Group 3, besides receiving a second CRC 90 days after the first one, were drenched with moxidectin, on visits 5, 7 and 9.

The final model resulting from the repeated measures ANOVA is shown in Table V. All variables and their interaction terms were statistically significant.

Table V. Results of the repeated measures ANOVA model for the dependent variable *epg* (log *epg+1*)

Source Term	DF	Mean Square	F-Ratio	p-value
A (FARM)	4	19.853	2.696	0.037
B (GROUP)	2	457.605	62.141	<0.001
AB	9	118.594	46.237	<0.001
C (VISIT)	8	21.696	2.946	0.007
AC	36	11.040	4.304	<0.001
BC	18	21.477	8.374	<0.001
ABC	72	5.563	2.169	<0.001

Prevalence of nematode genera

All four major strongylid genera (*Haemonchus, Ostertagia, Trichostrongylus,* and *Cooperia*) were initially represented on farms 1, 3, and 5, while only *Ostertagia* and *Trichostrongylus* were found on farm 2 and *Haemonchus, Ostertagia* and *Trichostrongylus* on farm 4. Large intestinal genera (*Oesophagostomum* and *Cooperia*) appeared to be a problem only on later visits (4, 5 and 7) on farm 2. *Ostertagia* was the only parasite responsible for the incomplete effectiveness of levamisole on farms 1 and 5. The benzimidazole resistant genera in Group 1 varied between the five farms and are shown in Table VI.

Table I. Results of FECRT and larval differentiation for lambs from Group 1

VISIT NO.	FARM 1		FARM 2		FARM 3		FARM 4		FARM 5	
	%red (95%CL)	Resistant genera	%red (95%CL)	Resistant genera	%red (95%CL)	Resistant genera	%red (95%CL)	Resistant genera	%red (95%CL)	Resistant genera
2	93 (98,72)	H	68 (91,-)*	O	0 (30,-)*	-	11 (54,-)	H	1 (73,-)*	O
4	81 (91,62)	-	72 (92,-)	O,T,Oe	4 (44,-)	-	42 (95, 26)	H,O	71 (94,-)	H
6	81 (90,62)	H,O	95 (99,86)	O,T	73 (90,30)	-	81 (95,26)	-	75 (93,1)	H,T
8	71 (91,1)	C	95 (98,83)	O,T	37 (66,-)	H,T	92 (98,61)	-	70 (90,10)	H,T
10	86 (97,32)*	-	64 (84,20)	-	0	T	83 (94,47)	H,T	99 (100,91)	-

Percentage reduction from previous(treatment) visit and 95% confidence limits calculated on arithmetic means. The asterisks indicate occasions in which pre-treatment egg counts were <150 epg. The column 'resistant genera' lists the individual nematode genera which had a pre-treatment mean of at least 50 epg that treatment failed to reduce by at least 95% (McKenna, 1995)[24].

H: Haemonchus; O: Ostertagia; C: Cooperia; T: Trichostrongylus; Oe: Oesophagostomum/Chabertia

Faecal samples were classified as being either positive or negative for *Nematodirus*. The analyses therefore assessed the prevalence of *Nematodirus* on different farms and treatment groups rather than quantifying mean burdens. Table VII shows the total number of positive samples for each treatment group throughout the trial and outlines statistically significant differences. The proportion of sheep eliminating *Nematodirus* is depicted in Figure 6.

Table VII. Number of sheep excreting *Nematodirus* spp. eggs.

VISIT NO	GROUP 1	GROUP 2	GROUP 3
1	8	6	5
2	8^a	1^b	0^b
3	25^a	20	12^b
4	23^a	0^b	22^a
5	30^a	16^b	29^a
6	25^a	1^b	0^b
7	17^a	11	5^b
8	18^a	0^b	0^b
9	10^a	3^b	1^b
10	6^a	0^b	0^b

Different superscript letters for numbers in the same row indicate a statistically significant (P<0.05) difference between the groups.

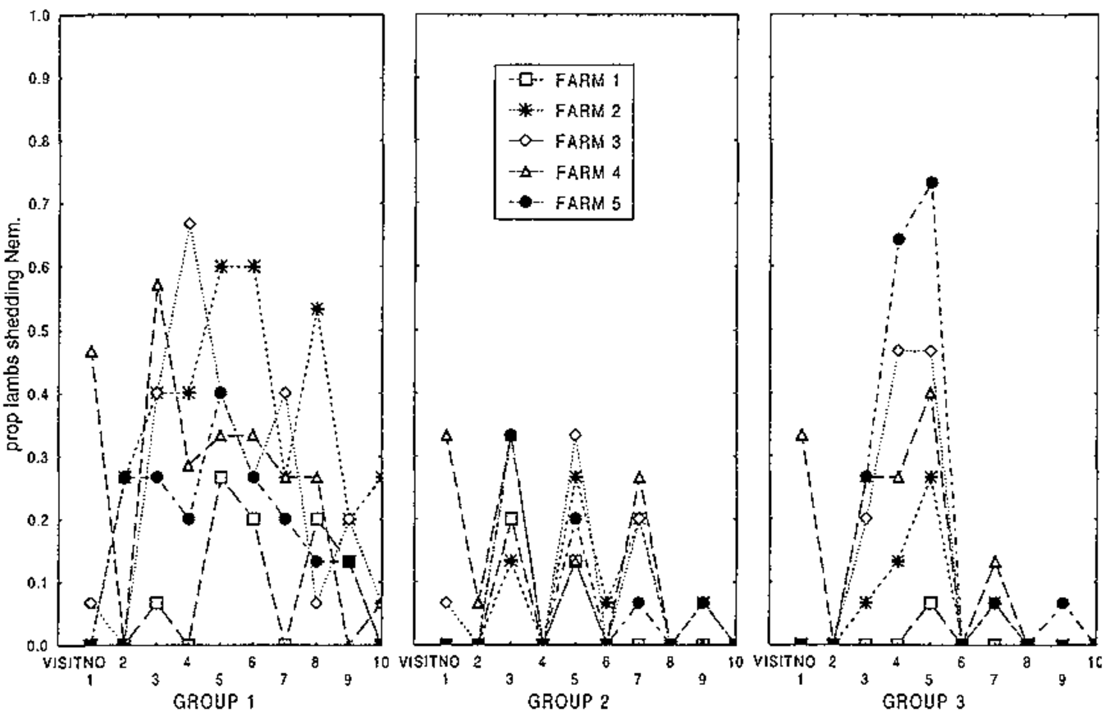

Figure 11. Prevalence of *Nematodirus* spp.

Proportion of lambs in each treatment group excreting Nematodirus eggs at subsequent visits.

There appears to be a definite resistance of this nematode to benzimidazoles, both administered in the form of oral drench and of slow-release capsules. Levamisole -alone or combined with oxfendazole-, on the other hand, was fully effective against *Nematodirus* spp., except on two occasions, as was moxidectin. The percentage reduction for Group 1 oscillated considerably both between and within farms. On farm 2 it was zero throughout the trial, while on farm 4 it went from an initial 100% effectiveness to complete ineffectiveness and on the other farms it usually took intermediate values.

Discussion

The objective of this trial was to assess the impact of drench resistance on naturally infected lambs in a true commercial situation. Every effort was made, therefore, to keep unaltered normal farming procedures. However, it was not possible to graze the three treatment groups separately and hence the results represent the impact of worm burden *per se* and not the additive effect of reduced or increased pasture larval levels resulting from good or poor parasite control. The presence of effectively de-parasitised animals constituted a diluting factor for pasture contamination, with the greatest role being played by Group 3 lambs. On the other hand, it has long been reported that, with experimental designs where treatment groups graze together, the full effect of any treatment program will be eroded by the effects of continuous reinfestation(17). Lambs from Group 3 carried very low (close to zero) worm burdens throughout the trial. Such a situation is expected to have a beneficial impact not only directly on health and productivity of sheep, but also on the degree of pasture contamination. A study conducted in South Africa(20) showed that treating ewes with slow-release capsules reduced infective potential of pasture by 71.6%. This can be viewed as a long-term benefit of suppressive treatment policy, as it is likely to play an important role in reducing parasite exposure in the next generation of lambs.

The mode of action of slow-release albendazole capsules is no different from the general mechanism by which benzimidazole drugs eliminate gastrointestinal nematodes. However, capsules have been advocated to be effective also against resistant worms because the continued presence of even quite low concentrations of benzimidazole shifts the equilibrium towards a prolonged binding of the drug to tubulin, which ultimately results in the death or elimination of the worm from the host(3).

The results of our trial, however, where on two of five farms capsules failed to attain a satisfactory control of parasitism, indicate that farmers should not rely on CRCs without first testing to assess their efficacy. The optimism of previous studies(4)(5)(19)(20) showing excellent results regarding both parasitological and production data, even in the presence of resistant worms(19), should therefore be reviewed. Barger(3) postulated a relationship between the degree of susceptibility to benzimidazoles and the efficacy of capsules. He showed that on farms where the FECR was 58%, CRCs took around 50 days to exert their full effect. In our trial however, the degree of resistance on farms 4 and 5 was similar to that found on the other farms: therefore, other factors are likely to contribute to the partial failure of these devices.

Mean pre-treatment egg counts differed considerably among farms and between consecutive treatment visits. Objective measurement of the actual resistance status was therefore difficult, because mean pre-treatment egg counts were often lower than the 150 epg recommended by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.)[(10)]. This can be partly explained by the different composition of nematode populations, since different genera have distinct fecundity patterns. A study by Coyne et al.[(13)] showed that average fecundities varied widely among nematode species, being more than 11000 eggs/female/day for *Oesophagostomum venulosum*, 6582 for *Haemonchus contortus* and 262 for *Trichostrongylus* spp. The differences in the relative composition of worm burdens are also likely to be responsible for the variability in the response to treatment, which is quantified by the percentage faecal egg reduction.

For all analytical purposes, samples were categorised into positive and negative for *Nematodirus* spp., instead of comparing the actual egg counts, following previous findings that outlined this parasite's extremely low fecundity and the poor correlation between faecal egg counts and the number of adult nematodes present in sheep[(9)]. Percentage reductions were also calculated on number of positive samples before and after treatment, as it is often difficult to fulfil the requirements for a mean pre-treatment egg count of >150 epg[(25)]. In recent years *Nematodirus* spp. has been commonly associated with anthelminitic resistance in New Zealand[(9)]. In our study, the efficacy of benzimidazoles against *Nematodirus* was extremely poor. Only on Farm 1 did they show any consistent effect on parasite burdens of lambs. Even prolonging the time of exposure to benzimidazoles through the use of capsules appears not to be an effective way of dealing with resistance, if *Nematodirus* is one of the nematodes involved in the problem. Quite surprisingly too, the administration of moxidectin to lambs from Group 3 did not achieve the expected long-term *Nematodirus* control.

The results from weight gain data confirm previous findings on the subclinical effects of parasitism, which can be severe and lead to major production losses. The degree of harm caused by gastrointestinal nematodes depends on a number of factors related to the host (age, health status, immunity)[28] , the parasites involved, and multiple environmental factors. The overall effects are difficult to assess, but include influences on appetite, skeletal growth, hematopoiesis, mineral and protein metabolism[12][28]. A number of trials[6][10][11][17][26][29] have been carried out in order to estimate the effects of parasitism and different control measures on the productivity of sheep. One of them[17], which compared the effects of four different treatment policies (salvage, curative, preventive, and suppressive) showed a dramatic effect of parasite control on both appetite and grazing behaviour. Parasite suppression led to uniform grazing across the entire paddock, while with uncontrolled parasite infection grazing was patchy. In New Zealand it was shown that even relatively small differences in the levels of larvae recorded on pasture resulted in significant differences in liveweight gain and wool production. A study[8] showed that suppressively treated lambs (which would model good parasite control) gained, on average, 2.71 kg more bodyweight than lambs which received a partially ineffective drench, and 1.53 kg more than those which were treated with an effective product, while the latter gained 1.18 kg more than ineffectively-treated lambs.

In New Zealand it has been estimated that, despite present levels of parasite control, production losses as a result of internal parasite infections of sheep amount to $275 million per year, including those due to lost meat and wool production, reduced fertility resulting from reduced live weight in ewes at first mating, dag removal, and excluding the cost of the anthelmintics used[15]. According to a later report from Farm Market Index, farmers are spending $29.3 million on anthelmintics to control sheep parasites, for a total value of sheep products for export of $2193.3 million[30].

In the present study, weight gains appeared to vary significantly among farms, visits, and treatment groups. The significant interactions outlined in the ANOVA model (Table III) indicate that the rate of increase in weight gain differed between farms and groups, as well as between different visits. Figure 3 is particularly helpful in visualising the latter information. It shows that a net separation between the three groups occurs mainly towards the end of the trial. This is when suppressive treatment was reached on all farms through the use of moxidectin in addition to the capsule. In other words, treatment had a significant effect on weight gains of lambs, which was not constant over time (interaction *group x visit*). The three-way interaction implies that the different weights gained by lambs throughout the trial were not explained by farm-associated factors alone, nor by the group allocation of the animals or the time of the year. Rather, these three main effects interacted in such a way that each combination gave a different outcome.

The findings associated with the dependent variable *epg* (Table V) can be explained in a similar way. The main effect *farm* was only marginally (p=0.037) significant, while all other main effects and interaction terms were highly significant. This suggests that parasite burdens of lambs were the result of a combination of factors: farm and time of the year (which together influence the degree of pasture contamination) and degree of parasite control in the animals, and that egg counts from different groups of lambs could really be compared only within each farm and for each treatment visit.

Parasitism also has indirect effects on productivity, by increasing susceptibility of lambs to flystrike[16]. The relationship between parasitism and dagginess (and the associated increased risk of flystrike) has not been completely clarified. Several studies have shown that there is no correlation between worm egg output and the severity of dag. A common belief is that diarrhoea and consequent dag formation is nutritionally induced. Results from a study conducted by Larsen et al.[18] suggested that scouring is associated with a host response to challenge with trychostrongylid larvae, since removal of adult worms through ivermectin treatment did not account for the reduction in dag in treated ewes. This would explain the fact that use of a controlled-release capsule has the capacity to significantly reduce dagginess after three months of continued use, whereas a drench, however effective (see Group 2), does not decrease dag formation, since it is unable to avoid incoming larvae to continually stimulate the host's immune system.

Arithmetic, rather than geometric, means, were used to calculate percentage egg reductions because they provide a more accurate estimate of the overall worm load and pasture contamination. This follows the general recommendations provided by the W.A.A.V.P. in 1992[10], as well as the opinion of many Authors[7][14][22], who believe that arithmetic means provide a better estimate of the actual number of nematode eggs being eliminated on to the pasture. Confidence limits were also reported because, while not crucial for the diagnosis of anthelmintic resistance[23], they provide a more accurate description of the situation[21]. In our trial, confidence intervals helped to estimate the amount of variability within each group of sheep. This confirms previous findings describing the very skewed distribution of nematode egg output in sheep[2][27].

Overall, the findings emerging from this study underline the importance of parasitism as a limiting factor for achieving optimal performance, and the necessity to carefully plan effective strategies in order to minimise the losses.

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CHAPTER 4

Evaluation of anthelmintic strategies: a stochastic partial budgeting analysis

Abstract

A partial budgeting analysis was undertaken in order to evaluate and compare the profitability of three anthelmintic strategies in growing lambs. The analysis was carried out by means of a spreadsheet-based stochastic simulation model, which allows inputs to be described as distributions rather than as fixed values.

The results show that control of parasitosis by use of an effective drench provides the highest net returns, yielding a margin over ineffectively-treated lambs of 133 New Zealand dollars (NZ$) per 100 lamb on average. However, stochastic analysis indicates that the expected value oscillates greatly around the mean and that negative outcomes are not unlikely. This reflects the degree of uncertainty about the outcome on any single farm due to variation between farms. Suppressive treatment based on the administration of two controlled-release capsules resulted in an average loss of NZ$ 151/100 lambs over ineffectively treated animals. In this case, however, the range of possible results oscillated considerably, comprising between -NZ$ 841 (5^{th} percentile) and NZ$ 545 (75^{th} percentile). Moreover, analysis of the results from capsule-treated lambs did not take into account the non-measurable benefits associated with lower pasture contamination. Sensitivity analysis indicates that carcass price greatly influences the profitability of any parasite control program, while the cost of anthelmintic influences the marginal profitability of each control option.

Introduction

It is universally accepted that gastrointestinal parasitism causes important losses in ruminants. Both its economic effect on ruminant production and the efficacy of various control strategies have been widely observed and documented (Chapter 1). However, the comparative effects of different parasite control measures in the presence of resistant nematodes have never been fully investigated.

Measuring the economic benefits of parasite control in sheep on pasture presents a number of problems. The inherent variability associated with sheep, parasite infections, weather, managerial conditions, etc, are often obstacles to the interpretation of data. The most obvious and frequently measured benefit of parasite control is gain in body weight. However, measurement of body weight alone may not accurately reflect the value of parasite control. Water intake and water retention appear to be increased in parasitised ruminants. In addition, it has been noted that anthelmintic treatment may also affect carcass yield and quality[(4)].

The objective of this partial budget was to determine the economic merit of each of the three parasite control strategies described in Chapter 3. A stochastic simulation approach was chosen in order to realistically model variation inherent in each of the input variables. This approach also enabled conclusions regarding each anthelmintic treatment strategy to be based on a combination of their expected results and the manner in which economic benefit is likely to vary around this mean, due to factors beyond the farmer's control.

Materials and Methods

This partial budget analysis was based on the results of the anthelmintic resistance trial documented in Chapter 3. In this trial, recently weaned ewe lambs were randomised to one of three treatment groups and followed for five months. Productivity was determined by measuring weight gain and the proportion of 'daggy' lambs in each treatment group. Initially, a deterministic model was built in Microsoft Excel©+; this model is illustrated in Figure 1 and the spreadsheet is shown in Tables 4 and 5. Two groups of input variables were included: costs and returns (Figure 1).

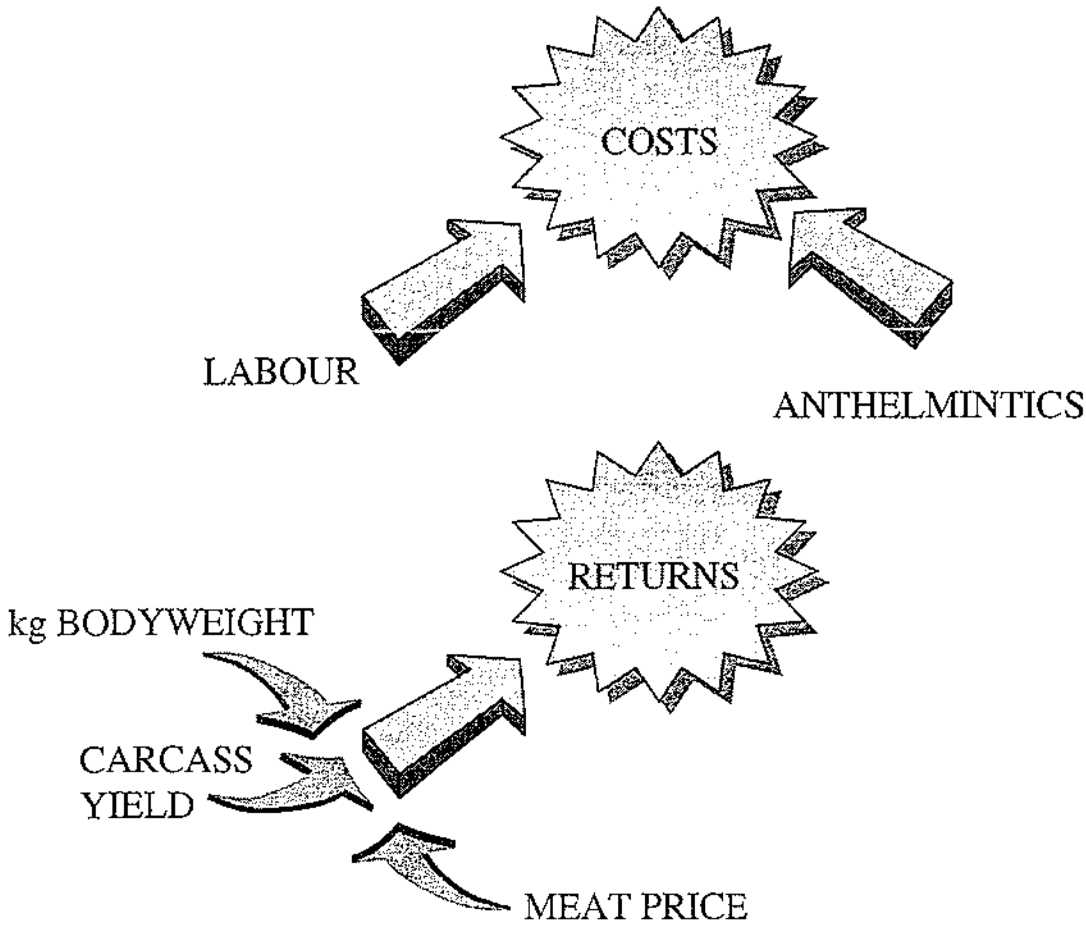

Figure 1. Input variables for economic analysis.

+ Microsoft Excel for Windows 95 Version 7.0. Copyright 1985-1995 Microsoft Corporation

Subsequently, a stochastic economic analysis was performed using a risk analysis and simulation add-in for Microsoft Excel*. This Monte-Carlo simulation package allows inputs (both costs and benefits) to be modelled as distributions rather than as deterministic fixed values. Input distributions are sampled at each iteration and the corresponding result for each output recorded. This simulation was conducted using 1000 iterations generated by Latin hyper-cube sampling[2].

Costs

Anthelmintics: The cost of anthelmintics was determined through a survey of retailers. This group of variables appeared to have a most commonly observed value and a limited range. On this basis, triangular distributions with parameters (maximum, minimum and mode) supplied by the survey results were used (Table 1).

Labour costs: The hourly cost of labour was determined by questioning farmers enrolled in the trial. Labour costs appeared to be distributed normally about a mean although minimum and maximum hourly rates were reported. These values were modelled as a truncated normal distribution (Table 1).

Mustering, drenching, crutching and *capsule administration:* The amount of time spent on each of these tasks was modelled as a series of triangular distributions (Table 1). These variables were not extensively documented although maximum, minimum and most likely values could be supplied by farmers in the trial.

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Table I. Input variables for @RISK model. *Anthelmintics, labour costs, labour time.*

VARIABLE	MEAN	STD DEV	MODE	MIN	MAX
SYSTAMEX®†			130	80	150
NILVERM®†			90	70	120
SCANDA®†			340	300	370
CYDECTIN®†			320	300	380
EXTENDER CRC®‡			200	180	230
LABOUR*	10	2		7	20
MUSTERING§			0.5	0.4	1
DRENCHING§			0.4	0.5	1
CRUTCHING§			2	2.5	3
CAPSULES§			2.2	1.8	2.7

†*NZ$/10 L; triangular distribution*

‡*NZ$/100 units; triangular distribution*

**NZ$/hour; truncated normal distribution*

§*Time (hours) required to perform the job; triangular distribution*

Dagginess: The proportion of daggy lambs in each group was reported in the trial (Chapter 3). A standard error was obtained for these statistics and, in consideration of the size of the trial, normal distributions were generated. These were subsequently multiplied by 100 in order to model the percentage of daggy lambs in each group (Table 2). The cost attributed to daggy lambs was estimated as the sum of the fixed cost of mustering plus the cost associated with crutching the proportion of lambs that were daggy (a 'dag score' > 1).

Table II. Input variables for @RISK model. *Dagginess*. The variable is normally distributed.

VARIABLE	GROUP 1		GROUP 2		GROUP 3	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
PERCENT DAGGY	28.95	3	18.06	2	10.64	2

Returns

Carcass price: The price per 15kg of carcass weight was determined by assessing variation in the slaughter value of lambs, as reported in the Meat Board Bulletin[1]. Carcass price per 15kg was modelled using a triangular distribution (Table 3).

Carcass yield (%): It was considered unlikely that carcass yield would vary substantially from an expected value of 43%[7] and for this reason a beta distribution was chosen in favour of the heavier tailed triangular distributions used elsewhere in the analysis (Table 3).

Table III. Input variables for @RISK model. *Meat Price* and *Carcass yield.*

VARIABLE	DISTRIBUTION	MODE	MIN	MAX	MEAN	STD DEV
MEAT PRICE*	Triangular	37.66	32	50		
CARCASS YIELD %	Beta				0.42	0.03

**NZ$/15 kg carcass*

Wool growth was not assessed in this trial. It was considered unlikely that skin quality would vary substantially between treatment groups and this was also ignored. No deaths due to intestinal parasitism were recorded in the trial.

Output variables considered in this analysis included returns minus costs, margins over Group 1 and margins over Group 2. Outputs were calculated for each treatment group. The spreadsheet used in the analysis is presented as Tables 4 and 5. Sensitivity analyses were performed for each output in order to determine the most significant input variables.

Table IV. Spreadsheet showing input variables *costs*. Prices are adjusted to 100 lambs of 35kg live weight.

	COSTS		
ANTHELMINTICS	*NZ$/10 L*	*ml/35 kg*	*NZ$/100 lambs*[*]
SYSTAMEX	120.00	7.00	8.40
NILVERM	93.33	7.00	6.53
SCANDA	336.67	3.50	11.78
CYDECTIN	333.33	7.00	23.33
CAPSULES			203.33
LABOUR	*NZ$/Hour*		
	10.28		
TIME	*HOURS/100 lambs*	*NZ$/100 lambs*[†]	
MUSTERING	0.63	6.51	
DRENCHING	0.63	6.51	
CRUTCHING	2.50	25.69	
CAPSULES	2.23	22.95	
CRUTCHING	*PERCENT DAGGY*	*NZ$/100 lambs*	
GROUP 1	29	13.96	
GROUP 2	18	11.13	
GROUP 3	11	9.33	
	TOTAL COSTS		
	GROUP 1	*GROUP 2*	*GROUP 3*
DRENCH	42.00	48.42	488.45
LABOUR	79.04	76.21	97.96
TOT	121.04	124.63	586.41

[*] *calculated as average dose (ml/35 kg) times cost per ml [(NZ$/10 L)/100]*
[†] *calculated as cost of labour/hour times number of hours spent/group*

Table V. Spreadsheet showing input variables *returns*. Prices are adjusted to 100 35 kg lambs.

RETURNS

$/15 kg carcass	*$/kg carcass*	*carc yield %*	*$/kg bw* *
39.89	2.66	43.50	1.16
WEIGHT GAIN (KG)			
GROUP 1	*GROUP 2*	*GROUP 3*	
7.17	8.35	9.88	
	GROSS RETURNS/100 lambs		
GROUP 1	*GROUP 2*	*GROUP 3*	
829.36	965.86	1142.83	
	RET-COSTS		
GROUP 1	*GROUP 2*	*GROUP 3*	
708.33	841.23	556.42	
	MARGIN OVER GROUP 1		
GROUP 1	*GROUP 2*	*GROUP 3*	
	132.90	-151.90	
	MARGIN OVER GROUP 2		
GROUP 1	*GROUP 2*	*GROUP 3*	
		-284.81	

* *calculated as price of kg carcass times carcass yield*

Results

Output distributions obtained from the simulation are presented individually. Expected values, 5th and 95th percentiles obtained from output distributions were compared graphically in order to determine whether the differences observed between groups was likely to reflect true difference in economic merit or random variation.

Returns minus costs (NZ$/100 lambs):

Figure 2 shows the output distribution obtained for Group 1. This distribution appears to be bell-shaped and reasonably symmetrical with the following parameters:

Mean	$707.62
Maximum	$2145.15
Minimum	-$225.343
Median	$705.43
5th percentile	$225.33
95th percentile	$1194.33

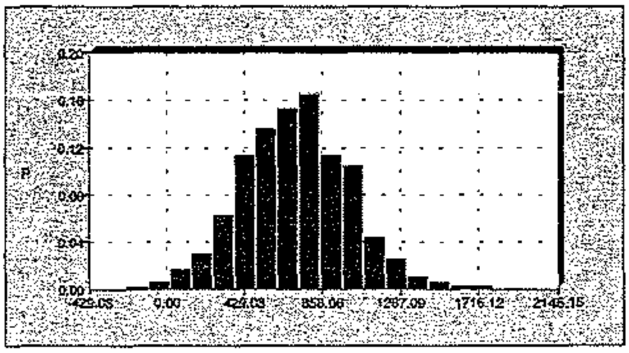

Figure 2. Returns minus costs for Group 1

(P=probability)

Figure 3 shows the output distribution obtained for Group 2. This distribution appears to be bell-shaped and is broadly symmetrical with the following parameters:

Mean	$840.49
Maximum	$2160.52
Minimum	-$40.81
Median	$828.66
5th percentile	$365.79
95th percentile	$1332.73

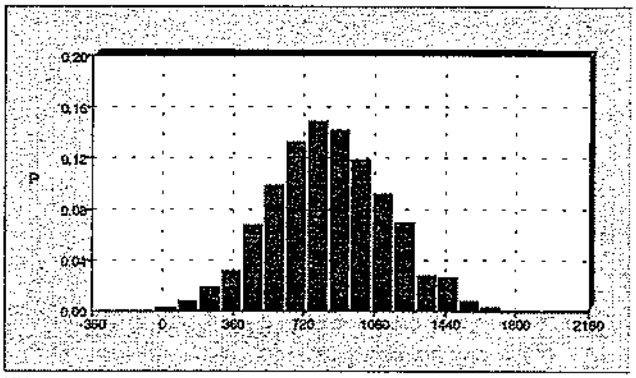

Figure 3. Returns minus costs for Group 2

(P=probability)

Figure 4 shows the output distribution obtained for Group 3. This distribution appears to be bell-shaped and is slightly right skewed. The following parameters were reported:

Mean	$554.75
Maximum	$1771.40
Minimum	-$447.99
Median	$556.95
5th percentile	$71.51
95th percentile	$1056.28

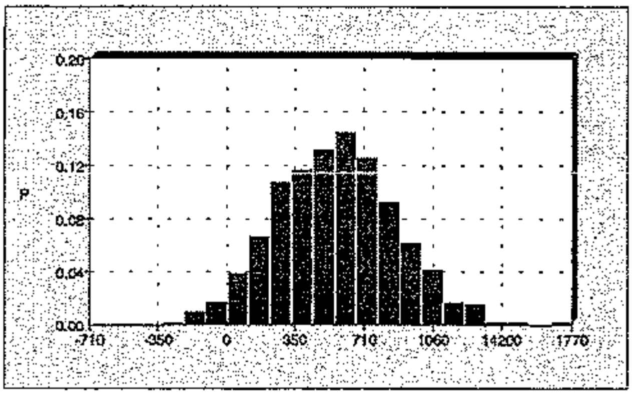

Figure 4. Returns minus costs for Group 3

(P=probability)

Returns minus costs obtained from Groups 1 to 3 appeared to be most sensitive to changes in carcass price and carcass yield. This was followed by the cost of labour and the time spent mustering. As an example, the tornado graph obtained for Group 1 is presented as Figure 5.

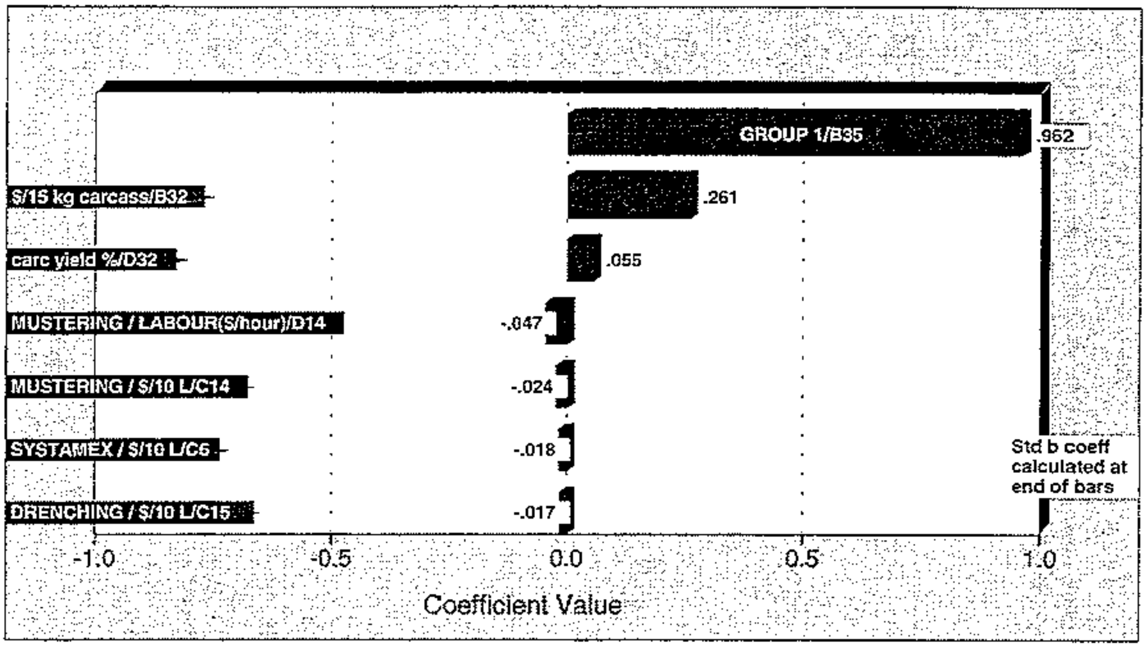

Figure 5. Tornado graph showing the results of the sensitivity analysis for Group 1.

Figure 6 compares returns minus costs across the three treatment groups. It can be seen from this graph that while mean returns appear to be different across the three groups, their 5th and 95th percentiles quite clearly overlap. This suggests that in a single iteration or 'random sample' of the trial, any one of the treatments may lead to the most favourable return.

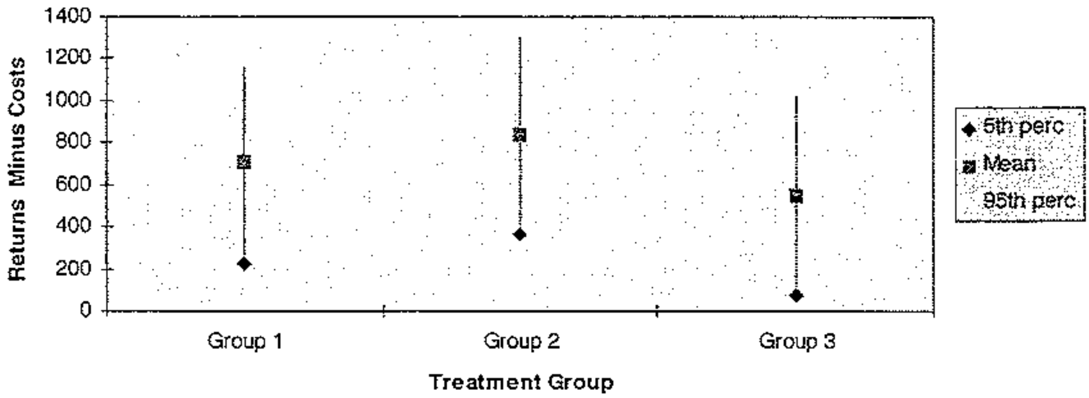

Figure 6. Comparison of *returns minus costs* for three treatment groups.

Margins over Group 1 (NZ$/100 lambs):

Figure 7 shows the output distribution obtained for Group 2. This distribution appears to be bell-shaped and quite markedly right-skewed. The distribution has the following parameters:

Mean	$132.87
Maximum	$1574.96
Minimum	-$1276.41
Median	$128.91
5th percentile	-$523.40
95th percentile	$785.71

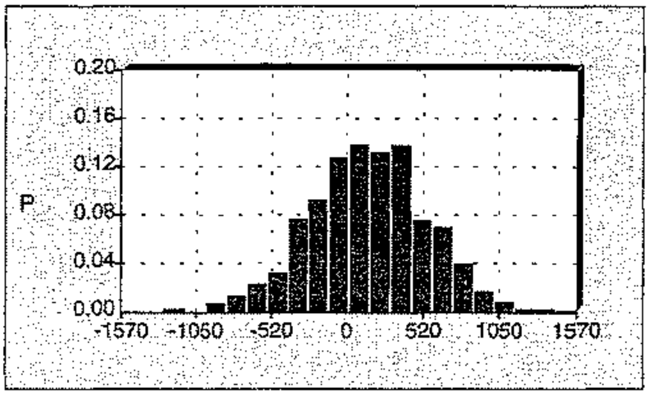

Figure 7. Margins over Group 1 for Group 2
(P=probability)

Figure 8 shows the output distribution obtained for Group 3. This distribution appears to be bell-shaped and symmetrical. The distribution has the following parameters:

Mean	-$151.31
Maximum	$1075.28
Minimum	-$1450.85
Median	-$149.65
5th percentile	-$841.61
95th percentile	$544.91

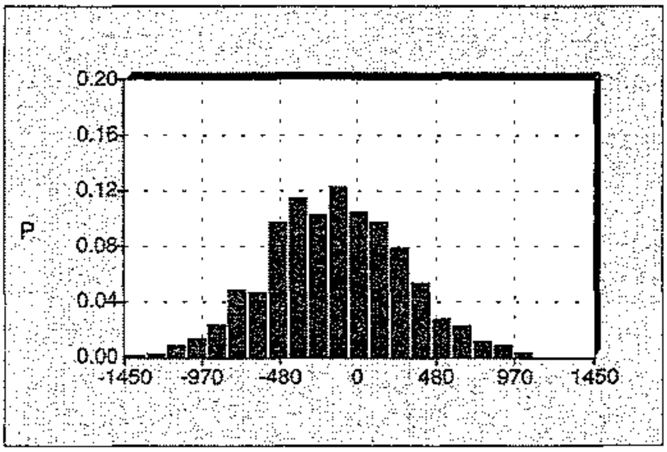

Figure 8. Margins over Group 1 for Group 3
(P=probability)

Margins over Group 1 were particularly sensitive to changes in carcass price and to changes in the costs of their respective anthelmintic treatments. Other important variables included carcass yield and the hourly cost of labour.

Figure 9 compares margins over Group 1 for the remaining two treatment groups. It can be seen from this graph that while the treatment given to Group 2 produces a higher mean margin than that obtained from Group 3, 5th and 95th percentiles for the two groups quite clearly overlap. This suggests that in a single iteration or 'random sample' of the trial, either of the treatments given to Group 2 or Group 3 may lead to a greater margin over Group 1.

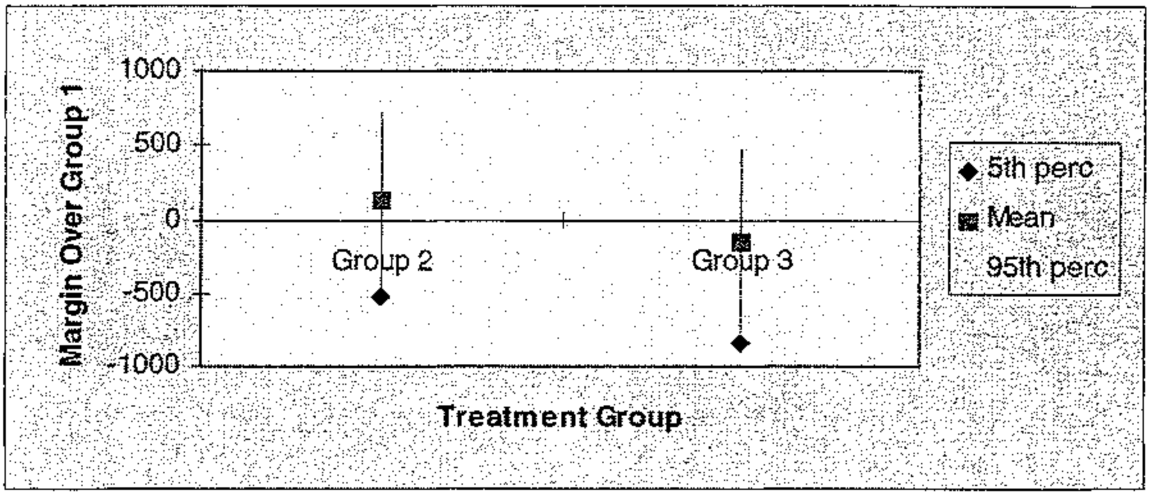

Figure 9. Comparison of *margin over Group 1* for Groups 1 and 2.

Margins over Group 2:

Figure 10 shows the output distribution obtained for Group 3. This distribution appears to be bell-shaped and symmetrical. The distribution has the following parameters:

Mean	-$284.19
Maximum	$1099.89
Minimum	-$1775.34
Median	-$292.08
5th percentile	-$981.94
95th percentile	$375.00

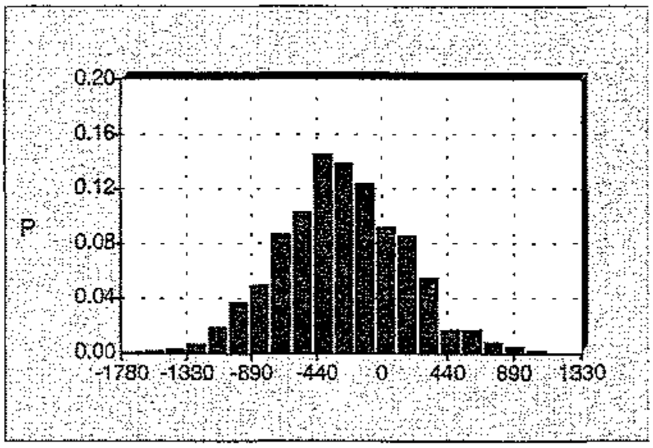

Figure 10. Margins over Group 2 for Group 3

(P=probability)

Margins over Group 2 were particularly sensitive to changes in the price of anthelmintic treatment and to changes in carcass price. Other important variables included the hourly cost of labour and the cost attributed to the time spent treating lambs.

Discussion

This exercise was undertaken with the aim of investigating profitability of good parasite control. However, there are other benefits associated with the control of parasitism, which cannot be easily quantified, including lower pasture contamination, which results in a decreased risk of infecting other animals. This is likely to play a very substantial role in the control strategy adopted for Group 3, whose measurable returns appeared to be quite low. It should also be noted that on all farms the three treatment groups were run together as one individual mob. In a real situation all lambs on farm would receive the same treatment. Therefore, suppressive treatment would result in lower pasture contamination and future larval challenge than would have been shown by this trial. On the other hand, it appears that one of the causes of reduced weight gain in parasitised lambs is anorexia. It could be argued, therefore, that this anorectic effect increases the amount of residual food available[(6)], so part of the economic impact of parasitism might be overcome by increasing stocking rates, although in practice this is unlikely to occur.

Factors which contribute to the variability of the results of a partial budget analysis of anthelmintic resistance include differences in the cost of drenches. Drenching costs differ greatly between different farms and are in constant flux. Another contentious variable is the question of labour costs for mustering and drenching[(3)].

The results of this analysis show that it is not advisable to continue the use of a product to which resistance has been diagnosed, as this will lead to considerable losses as financial returns foregone. The simulation model indicates that the economic return from treating lambs with an effective product (Group 2) are considerably higher than those from animals treated with a product towards which strains of parasites have become resistant. The benefit of a suppressive treatment (Group 3), as assessed by this analysis, appears to be outweighed by the costs, yielding a negative net return. In fact, CRC are expensive devices and require high labour costs.

This economic analysis confirms previous findings regarding the profitability of the control of helminthiasis in sheep[5] and underlines the importance of testing for the presence of anthelmintic resistance in order to avoid unnecessary losses.

Tables 4 and 5 show the deterministic outcomes of the analysis. By comparing these results with the ones obtained with the simulation model, it is possible to appreciate the benefits of such a procedure. The histograms (Figures 2-4, 7-8, 10) show that, while mean figures are those most likely to occur, a real situation would not exclude the possibility of very different outcomes. In fact, stochastic simulation modelling provides a useful tool which allows great flexibility in the analysis of economic data. Distributions may be specified not only on the basis of trial results, but also according to the biology of the variable concerned.

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CHAPTER 5

Studies on foal heat breeding in the mare

Abstract

The objective of this study was to evaluate two management strategies for breeding mares after foaling. Foaling mares (n=26) were randomly assigned to groups after stratification by age and foaling date. All mares were examined on day 7-9 postpartum by rectal palpation and ultrasound. Group 1 mares were bred at foal heat if they met the following criteria: unassisted foaling, live healthy foal, passage of placenta within 3-hrs of foaling, normal uterine involution and first ovulation after 9-d post foaling. All mares in group 1 met these criteria. Group 2 mares were treated with a PF2α analogue 6-d after their first ovulation and mated on the second oestrus. Pregnancy diagnosis was performed at days 14, 25 and 45 post ovulation. Pregnancy rate (PR) at first served oestrus was 58.3% and 71.4% for group 1 (P1) and 2 (P2) respectively ($P>0.05$). Cumulative, seasonal PR was 75% and 78.6% for group 1 and 2 respectively ($P>0.05$). The statistical power of the study results (Ha: $P1<P2$; $\alpha = 0.05$) was 0.17 and 0.08 for first oestrus and cumulative PR respectively. Using the PRs observed in the current study, group sizes of 167 and 1700 mares would be required to identify the differences recorded for first oestrus and cumulative PR respectively as being significant (Ha: $P1<P2$; $\alpha = 0.05$; Power = 0.80). This indicates that it would be feasible to conduct a study able to detect differences in PR at first served oestrus. The results suggest that on farms with high mare:stallion ratios, the practice of breeding at foal heat be avoided due to the risk of lowered pregnancy rates at this oestrus.

Introduction

The production cycle in the equine is traditionally treated as though it were precisely annual. However, the long gestation length of mares (340 days on average) combined with a breeding season restricted to a maximum of 4 ½ months for most breeds places tremendous strain on breeders attempting to maintain such a cycle length. The age of racing horses, in the Southern Hemisphere, is calculated from the 1st August. Therefore, it is considered advantageous to have foals born as soon after this date as possible. Foals born soon after the 1st August then have maximal time to grow relative to their group of peers in preparation for a racing career. Even a few months difference in birthdate may have an impact on physical growth and subsequent athletic performance as 2 and even 3 year olds.

The first postpartum oestrus is commonly referred to as foal heat, and is characterised by normal follicular development and ovulation within the first 18(12)-20(8) days after foaling, usually within 5 to 12 days. The length of the interval from foaling to onset of foal heat does not appear to be related to season(12). However, both duration of foal heat and interval from parturition to ovulation have been shown to decrease as day length increases(2)(8).

Foal heat breeding has been utilised in an attempt to save valuable reproductive time, especially in mares foaling late in the season. However, it is a controversial management practice, as it is commonly believed to be associated with the following problems.

Lower pregnancy rates.

Pregnancy rates have been found to average between 10%(8) and 16%(6) lower in mares bred on foal heat as compared to those bred on the second heat post-foaling.

In six of nine studies reviewed by Ginther(2), the difference was statistically significant, ranging from 11% to 34%. A study by Lieux(6) showed pregnancy rates of 39% and 55% for mares mated at the first oestrus versus the second oestrus. A trial conducted in Finland(3) reported figures of 47% and 67% respectively for foal-heat bred and second-heat bred mares. However, these findings may represent an overestimate of the true situation. In fact, it is a common procedure to breed at foal heat mares which foal late in the breeding season[9]. It has been shown that the interval from foaling to ovulation tends to shorten as the season advances[3][8]. Other studies have shown that pregnancy rate in mares which are bred and conceive to an ovulation less than 10 days after foaling is lower than in mares which ovulate later than 10 days after foaling. Therefore it seems probable that mares which foal late and are bred on foal heat are more likely to ovulate early and have poorer pregnancy rate[8].

Higher pregancy loss rates.

Early embryonic death rates and abortion rates have been shown to be higher in foal heat bred mares [6][7][8][19][21]. However, cumulative season pregnancy and foaling rates are similar between mares first bred on the foal heat and mares first bred on later post-partum oestrous periods, and the time-saving advantage is, therefore, the primary impetus for breeding mares during their foal heat[6][8].

Higher risk of metritis.

A common belief is that mares bred at foal heat are more susceptible to uterine infection; this, however, has never been unequivocally demonstrated[9].

Different explanations have been given in an attempt to explain the decreased fertility observed at foal heat. The following are the most widely accepted hypotheses.

- Poor mating management
- Incomplete uterine involution

Great morphologic changes in size and structure of the uterus occur gradually during the 11-month gestation. During the puerperium, however, the uterus is restored to nearly the prepregnancy condition within approximately three weeks of foaling[2][18]. It has been suggested that at the time of the first oestrus postpartum the repair of the endometrium is incomplete, and that the effectiveness of the uterine defence mechanisms is reduced . It appears therefore that the uterus may not be ready to support a developing embryo[2].

Some studies, however, suggest that by the time the embryo reaches the uterine horn, involution is often sufficient to allow its fixation.

- Bacterial contamination of the uterus and/or impaired uterine defence mechanisms against bacteria introduced at breeding.

Methods employed to manage breeding in the postpartum period in an attempt to achieve normal pregnancy rates include:

Shorten the interval to the second postpartum oestrus

This is usually achieved by administering prostaglandin ($PGF_{2\alpha}$) 6 to 7 days after the first postpartum ovulation. Endometrial $PGF_{2\alpha}$ has been shown to be the luteolytic agent in mares[20]. Administration of exogenous $PGF_{2\alpha}$, therefore, causes regression of the corpus luteum and a more rapid return to heat. The procedure has been shown to increase pregnancy rates compared with foal heat breeding. One study[19] found that breeding at the induced oestrus resulted in an 81% pregnancy rate compared with a 60% rate after breeding at the foal heat and a 61% rate for mares bred which were bred for the first time later than 25 days postpartum, regardless of whether they had a foal heat or not. However, time is lost relative to breeding on foal heat and the time saved, relative to second-heat breeding, is only 7 days approximately.

Delay the onset of the foal heat in order to allow more complete uterine involution to occur.

This is generally achieved by hormonal therapy

a) Progesterone daily for 8 days followed by prostaglandin

b) Daily treatment with a combination of progesterone and oestadiol-17β for 5 days[10][11].

The rationale behind this strategy is that delaying the first postpartum oestrus and ovulation will allow time for improvement of the uterine environment before ovulation, thereby leading to higher pregnancy rates[14].

Progesterone treatment alone does not inhibit follicular development uniformly. When progesterone given to block ovulation is withdrawn in a group of mares, follicles in a wide range of developmental stages may exist, resulting in ovulation of more mature follicles in a short time and a much longer time to ovulation in cases of the most immature follicles. Combined progesterone and estradiol-17β treatment, on the other hand, appeared to result in inhibition of follicular development at more uniformly early stages, and withdrawal of the treatment resulted in a narrower interval during which ovulations occurred and an apparently more normal distribution of ovulations within that interval(11). Overall, the practice of delaying the first oestrus results in improved fertility, but the advantage seems to be trivial from a practical viewpoint(10), and the method is therefore seldom used in practice. Objections to the use of steroid therapy for several consecutive days include cost, labour, the fact that the treatment delays the onset of the first postpartum oestrus sufficiently so that foaling intervals are not significantly reduced, and possible detrimental effects on subfertile mares.

Encourage involution by treatment with $PGF_{2\alpha}$, oxytocin or oestrogen.

Repeated injections of oxytocin, $PGF_{2\alpha}$, or oestrogens post-foaling have been used also with the aim of improving uterine involution time. In one study(1), mares were injected with microspheres containing 100 mg oestradiol-17β or no hormone, on the day of foaling. The treatment period was considered to last for 12-15 days. No difference was detected between groups for length of oestrus or interval to ovulation, recovery of potential bacterial pathogens, presence of endometritis, and presence of intrauterine fluid 11-16 days post partum. Pregnancy rate of mares treated with oestradiol (5 of 11; 45%) was not significantly different from that of controls (9 of 11; 82%). The authors concluded that oestradiol treatment did not decrease uterine involution time. Better results have been reached by the use of prostaglandin analogues to improve pregnancy rates at foal heat breeding(5).

Shorten pregnancy length by artificial lighting.

By artificially increasing daylight length it has been possible to decrease the duration of gestation by approximately 10 days(2).

Selectively choose mares to breed on foal heat based on examination of the genital tract after foaling.

Several authors have recommended that the judged normality of parturition and condition of the reproductive tract at the postpartum oestrus should be considered in deciding whether to breed mares on foal heat or not[(2)(14)]. Suggested factors to consider include time of ovulation relative to foaling, history of problem (dystocia, retained foetal membranes, metritis) associated with the puerperal period, uterine involution, presence or absence of uterine fluid at foal heat.

Presence of fluid in the uterine lumen during the first postpartum ovulatory period has been associated with decreased pregnancy rates[(14)]. It has been observed that fewer mares become pregnant if they are mated when uterine fluid was detected during the 1st oestrus postpartum. A recent study[(17)] extended this finding to mares mated at different oestrous cycles. The authors of this study therefore suggested that ultrasonographic finding of uterine fluid at foal heat should discourage breeding of mares. Other studies[(13)(4)(16)], however, found that only a small proportion of mares showed signs of uterine inflammation at the time of foal heat, and concluded that the uterus returns rapidly to a cyclic condition.

The objective of this study was to evaluate two different management strategies for mares after foaling, in order to provide some useful advice to commercial breeding farmers.

Materials and methods

The study was conducted at the Flock House thoroughbred stud, Bulls, Manawatu, between September and December 1995. A total of 90 foaling mares were initially included in the study and randomly assigned to one of two treatment groups after stratification according to age and foaling date. However, in the early phase of the trial most animals had to be withdrawn from the study and the final sample sizes were 12 mares in Group 1 and 14 in Group 2. The reasons for the withdrawal are summarised in the discussion section of this paper.

All mares were examined on day 7-9 postpartum to monitor uterine involution and ovarian activity. Examination of the genital tract was carried out by palpation *per rectum* and ultrasound. The parameters assessed are shown in Table I.

Table I. Parameters assessed by palpation per rectum and ultrasound

Palpation	Ultrasound
Turgidity of follicle	Diameter of largest follicle
Uterine tone	Shape of largest follicle
Degree of cervical relaxation	Thickness and echogenicity of the wall of the largest follicle
	Presence and intensity of endometrial oedema
	Presence of one or more CLs

Following the first examination, a decision was made and the mares were treated according to their group allocation.

Group 1

Mares were bred at foal heat if they met the following criteria:

- normal unassisted foaling with a live, healthy foal
- no evidence of uterine infection and normal uterine involution at day 7-9
- passing of placenta within 3 hours of foaling
- first ovulation after day 9 postpartum

Mares that did not meet the above criteria were treated with the prostaglandin F2α analogue dinoprost (Lutalyse®, Upjohn Inter-America Corporation, Auckland) at a dose rate of 7.5 mg/mare six days after ovulation and mated on the following oestrus.

Group 2

All group 2 mares were treated with dinoprost six days after ovulation and mated on the second oestrus following parturition.

Mares were teased every second day by exposure to a pony stallion. On the basis of teasing responses and breeding records mares were then selected for further examination.

Mares were mated to one of two stallions when the following parameters indicated imminent ovulation[15]:

- teasing behaviour
- palpation findings:
  - cervical relaxation greater than 50% (on a scale 0-100%)
  - declining uterine tone
  - increasing softness of follicle wall

- ultrasound parameters:

    * presence of one or more follicles with a diameter ≥ 30 mm
    * change in follicle shape from primarily spherical to nonspherical
    * increase in thickness of the follicle wall
    * appearance of echogenic spots within the follicular lumen
    * absence of a corpus luteum (CL)
    * endometrial folds present and declining in intensity

Ovulation was confirmed by the ultrasonographic finding of a rapid decrease in diameter of the non-echogenic follicle and the development of a very irregular shape or, at a later stage, by the finding of a developing corpus haemorrhagicum/corpus luteum.

Accurate records were kept for each mare. Two weeks after mating mares were examined again for pregnancy diagnosis. Following a positive diagnosis, the progress of pregnancy was monitored through regular ultrasound scanning. Pregnant mares were usually checked at days 25 and 42-45. Final test and certification of pregnancy was performed at day 42-45.

In case of twin conception, one of the twins was manually crushed at an early stage of pregnancy (16-19 days post-ovulation) after manipulating it to the end of the uterine horn; the development of the remaining embryo was monitored. If the twins were fixed unilaterally (i.e. adjacent vesicles in the same uterine horn) and their size differed by at least 4 mm, they were left to reduce spontaneously.

Breeding was terminated on 30 December 1995.

Ultrasound examination was performed using a scanner 200 (Pie Medical Ltd.) with a 5/7.5 mHz, linear array, rectal transducer.

Pregnancy rates were compared by means of crosstabulations, which were carried out in Statcalc, a component of EpiInfo® version 6.04 (Centers for Disease Control & Prevention, U.S.A./ World Health Organisation, Geneva, Switzerland). Statistica® 5.1 for Windows (StatSoft Inc., Tulsa, OK, U.S.A.) was used to conduct survival analysis, and Pass® 6.0 (Kaysville, UT, U.S.A.) to perform power analysis and determination of sample size. One-sided tests were used because the study was aimed at determining if breeding 'normal' mares at foal-heat and only treating 'problem' mares with prostaglandin was better than treating all mares with prostaglandin regardless of their reproductive status.

Results

The age of mares varied between 5 and 25 years, with most of them being between 10 and 12 years old (Figure 1).

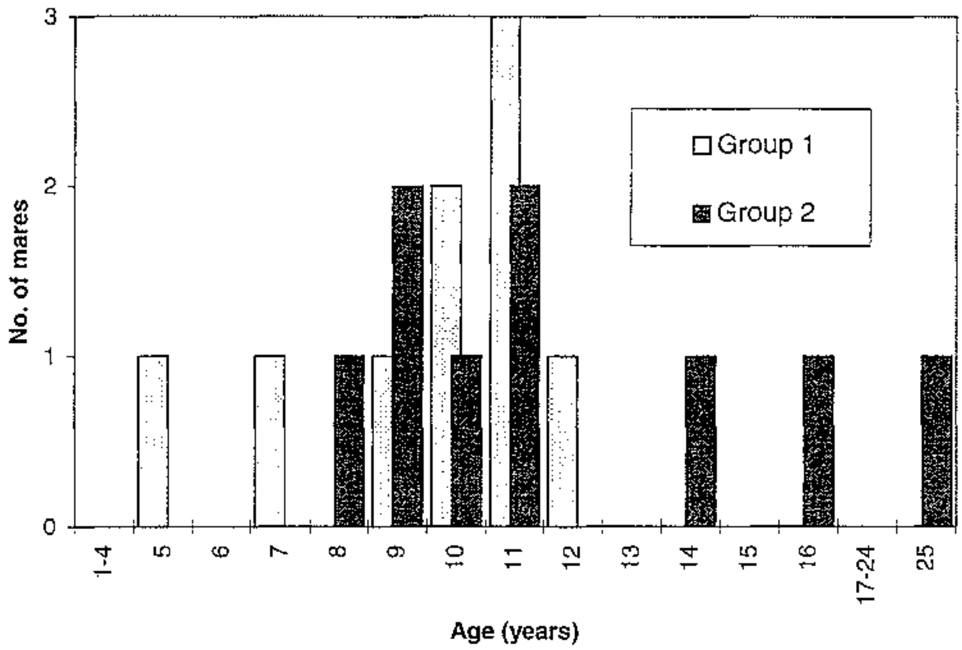

Figure 1. Age of mares by treatment group

Mares were matched for both age and due time of foaling. However, in the final sample mares from Group 1 appeared to be younger, on average, than mares from Group 2, and to foal at later dates (Figure 2).

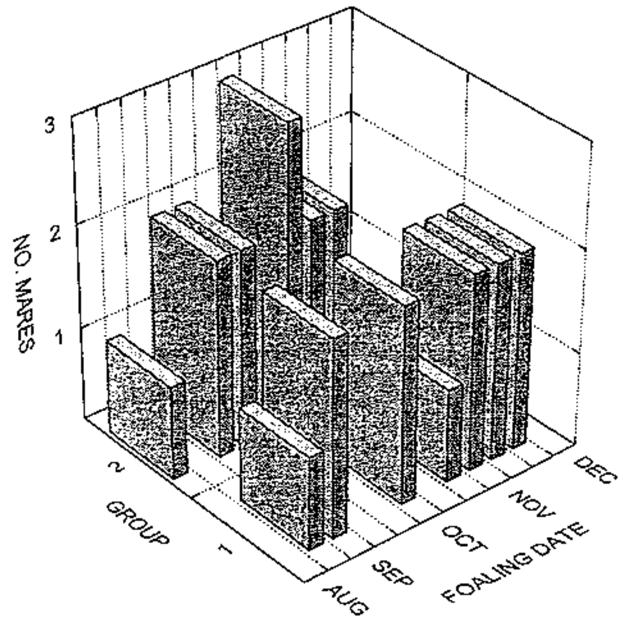

Figure 2. Distribution of foaling dates by treatment group

Two mares from Group 1 conceived twins, one of which was crushed between 16 and 19 days of pregnancy. One of the mares aborted the remaining embryo, whereas the other maintained the pregnancy and gave birth to a normal foal the following year. No twin pregnancies were observed in Group 2.

Table II shows the results of the cross-tabulation comparing pregnancy rates in mares from the two treatment groups. The differences did not reach statistical significance.

Table II. Comparison of pregnancy rates. Results of χ^2 analyses

	GROUP 1	GROUP 2	RR(CL)	p-value*
Number in group	12	14		
Pregnancy rate, % 1st served oestrus	58.33% (7/12)	71.43% (10/14)	0.74 (0.33, 1.67)	0.595
Pregnancy rate, % 2nd served oestrus	40% (2/5)	25% (1/4)	1.33 (0.43, 4.13)	0.386
Overall pregnancy rate, % 1st & 2nd served oestrus	75% (9/12)	78.57% (11/14)	0.95 (0.62, 1.46)	0.596

** = Fisher exact 1-tailed p-value comparing Group 1 service outcome with Group 2*

RR = relative risk of becoming pregnant to stated service for Group 1, compared with Group 2; CL = confidence limits

Figure 3 shows the survival curve for time from foaling to pregnancy for all mares. A survival analysis was not undertaken because it was judged inappropriate (see Discussion).

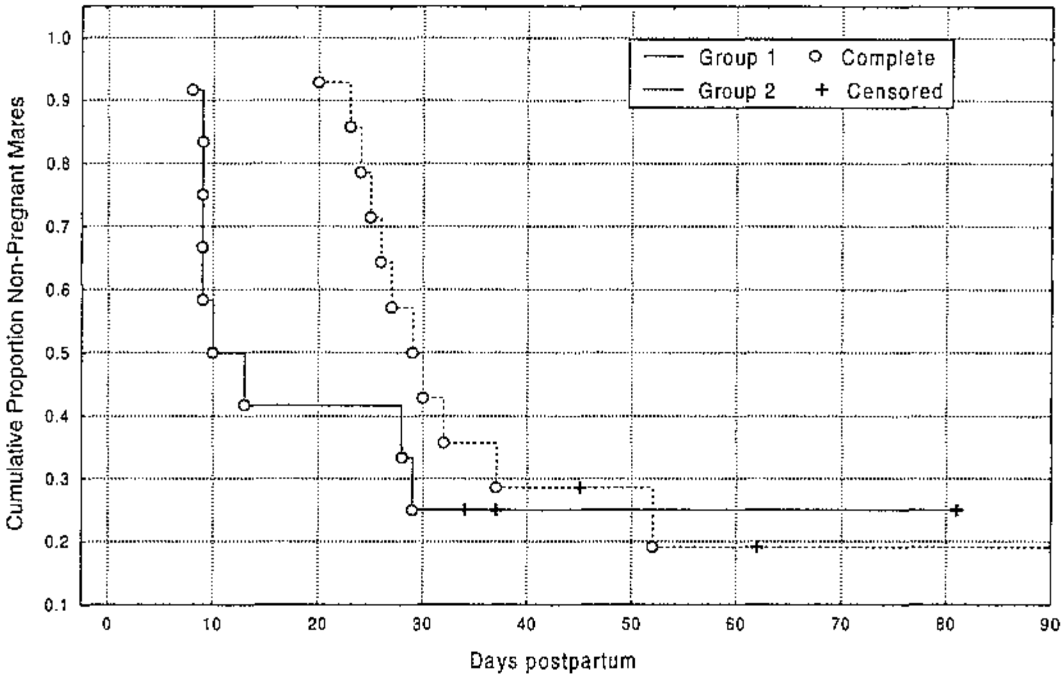

Figure 3. Kaplan-Meier survival function. Days from foaling to conception

Given the change in the original trial design, analyses were carried out to determine the power of the analyses performed on the data collected at the end of the trial, the sample size that would have been needed to detect the observed difference in pregnancy rates with a power of 0.80 and a probability of 0.95 of rejecting the null hypothesis of no difference between the groups (α=0.05), and the difference in pregnancy rates that would have been detected by the actual sample size of a total of 26 mares. The results are shown in Table III.

Table III. Results of power analysis on comparison of pregnancy rates between treatment groups.

	Cumulative pregnancy rates	Pregnancy rates at first served oestrus
Power of current analysis *(Ha: P1<P2; α=0.05)*	0.077	0.170
Sample size (mares/group) that would have been needed to achieve desired power given the current findings *(Ha: P1<P2; α=0.05; Power: 0.80)*	1699	167
Proportion of mares becoming pregnant in Group 1 that would have been detected as significant given the current sample size and pregnancy rates for Group 2 *(Ha: P1<P2; α=0.05; Power: 0.80)*	0.320	0.24

Discussion

The study was initially designed for a total sample size of 90 mares. Unfortunately, most mares had to be withdrawn from the trial. This prevented us from performing more analyses and from reaching significant results. Mares were withdrawn for one of two reasons. One major source of research funding supporting the breeding programme at Flock House was scheduled to end in mid-1996. As a result, a decision was made to reduce the number of mares bred to a number which could be maintained by alternative funding sources. This decision was only made after the start of the 1995-96 breeding season. In addition, the owners of some horses decided not to breed their mares during the 1995-96 season. Thus the number of mares actually bred and available for the trial was reduced to 26 animals only.

The analysis of foaling to conception intervals presents some problems. First of all, the biology of the reproductive cycle does not allow the data to be treated as continuous. In fact, the probability of conceiving is not constant over the whole study period, as it is dependent upon the stage in the reproductive cycle. Therefore, the two treatment groups could not be compared by means of the commonly used statistical tests (such as the non-parametric Mann-Whitney U test). These tests have the additional disadvantage of making use of non-censored information only, i.e., data from mares which had conceived before the end of the trial. The latter problem could be overcome by the use of survival analysis. However, time from foaling to conception is subject to bias because it is influenced by foaling date, which is dependent on previous year's breeding management. In our study, foaling dates covered an extended period of time, ranging from August to December (see Figure 2). Mares were matched by expected foaling dates when assigned to groups. However, the presumed day of foaling may not be a very reliable estimate of the actual foaling date. This finding may indicate that there are differences in gestation length of mares, possibly associated with time of conception. Figure 2 shows that, on average, mares from Group 1 foaled later than mares from Group 2. This, however, could be partly due to the marked reduction in sample size that we had to face during the trial and not necessarily to inappropriate matching. Figure 3 illustrates conception patterns in the two treatment groups, which are noticeably different. The difference was significant ($p<0.001$) when analysed by means of Cox's F test. However, Kaplan-Meier survival analysis in this situation was judged appropriate as a descriptive analysis tool only. In fact, the two groups can only be compared after day 30, as mares from Group 2 did not have any chance to conceive before the second oestrous cycle. Moreover, when assessing the survival curves in Figure 3 it should be kept in mind that survival time in this situation is composed of two components, foaling to first oestrus and interval from first oestrus to conception.

Overall pregnancy rates appeared to be lower in mares from Group 1. The difference was especially marked at the first served oestrus. However, the differences were not statistically significant. The inadequacy of the sample size was underlined by power analysis, as shown in Table III, which indicates the study had very low statistical power. Using the pregnancy rates observed in the current study, group sizes of 167 and 1700 mares would be required to identify the observed differences at first served oestrus and in cumulative pregnancy rates respectively as being significant. This suggests that it would be more likely to achieve the desired group sizes in a study aimed at detecting differences in pregnancy rates at first served oestrus.

The results of this study suggest that on farms with high mare:stallion ratios, the practice of breeding at foal heat should be avoided in order to minimise the use of the stallion(s), due to the risk of lowered pregnancy rates at this oestrus.

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