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F I N A L R E P O R T

Helminth Parasites in the New Zealand Meat & Wool Pastoral Industries : A Review of Current Issues

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CONTENTS

1.0 POPULAR SUMMARY

- Internal parasites have been the major animal health problem on NZ farms for over 100 years. The round worms are the most important.

- Twenty-nine species were inadvertently introduced to NZ and the most important are *Nematodirus, Trichostrongylus, Haemonchus, Ostertagia* and *Cooperia*.

- Internal parasites cost the sheep industry approximately $300m annually in lost production and drench use, while the cattle industries spend about $50m on drench per annum.

- Parasite resistance to drenches cost an estimated additional $20m per year and this is predicted to rise to $60m per year by 2022.

- A knowledge of some aspects of the internal parasites' life cycles and epidemiology is useful in devising control programmes.

- Over 90% of the population of various stages of roundworm parasites live outside the animal on the pasture and soil.

- Throughout NZ the roundworm parasite numbers appear to have a reasonably characteristic annual pattern of faecal egg counts, which generally parallels infective larvae numbers on pasture, reaching a major peak in autumn, and a variable or minor peak in spring. Some exceptions to the general pattern may be occurring.

- Drenching has been the major control method for roundworms for over 40 years.

- The main aim of the drenching programme is to prevent the autumn rise in pasture contamination.

- Parasite drench resistance has developed dramatically in the last 20 years.

- Alternative control procedures to drenching are used in planned or integrated control programmes.

- Each control programme is specific to each farm.

- It is now recommended that each farm should monitor the parasite status of stock classes and drench resistance status of the farm (i.e. which worm species are resistant to which drench types).

- FEC (faecal egg counts) are the most commonly used diagnostic aid to measure parasite status of animals. Variability has been a problem, but a new, accurate validated test is now available.

- Previously FECs from cattle were considered questionable value but they were of some use in diagnosing parasite status. A new more sophisticated technique with much greater accuracy are now available.

- Assays for drench resistance, are usually based on faecal egg count reduction (FECR) and larval culture is required to identify the resistant species of worms. "DrenchTest" and the less accurate "DrenchCheck" are routine in NZ.

- Drench resistance is encouraged by persistent anthelmintics or boluses as they give the resistant worms a much longer time to breed and contaminate pastures. The use of CRCs, especially with adult ewes, is no longer considered prudent. The problem is exacerbated when CRCs are used to create "safe" pasture.

- Rotation of drenches families was previously recommended to slow the development of drench resistance. This is no longer considered the case and combination drenches are now recommended, as the chances of worms having resistance to two or three of the drench families is quite remote, because of the different modes of killing action of the active ingredients.

- Animals develop immunity to the various roundworms, and need periodic or continual larval challenges to maintain this immunity.

- This develops in young stock by 8-10 months of age even under a preventative drenching programme.

- Adult ewes are generally immune to worms, but under stress this immunity can break down. This often occurs after lambing for a short period (four weeks approx.) and leads to a periparturient rise in FEC and pasture contamination until immunity is regained.

- The periparturient rise is due to the additional nutrient demand or stress in late pregnancy and early lactation and is most obvious in ewes with multiples or ewes in poor condition.

- Improved levels of nutrition, especially protein levels, have improved the immune response in young sheep and periparturient ewes.

- The detrimental effects seen in the animal from most non-blood sucking roundworm infections are due to the immune reaction and a subsequent sequence of events, rather than direct damage caused by the parasite.

- Loss of appetite and protein loss from the damaged gut are the main consequences. The gut needs to replace lost protein and this takes priority over liveweight gain, wool growth and lactation. This explains why additional protein is very important in reducing the damage and lost production caused by worms.

- Integrated and planned control programmes, in addition to preventative drenching, emphasise optimal feeding; the use of pasture species of forages (e.g. chicory, legumes) that reduce parasite survival or reduce larval migration up the plant; and

plants containing condensed tannins (e.g. *Lotus* or Sulla). These tannins protect dietary protein from rumen degradation and also may have a direct anthelmintic effect.

- Such programmes also emphasise the use of safe pasture from newly sown areas or hay/silage regrowth (although there can be some risk with the latter) , or forage crops.

- The use of a different species of ruminant (e.g. cattle in a sheep system or vice versa) to graze areas in preparation for susceptible young stock is a key element of these programmes, as worms rarely transmit from one species to the other and the alternative ruminant species can "vacuum" up infective larvae from the pasture.

- Immune adults of the same species can also be used for this purpose, but there is still some risk of contamination, especially if their immunity breaks down.

- Animals can be bred that have either a high immunity (resistant animals) or a high tolerance (resilient animals) to worms. The former prevent the worms establishing in the gut while the latter are productive even with a worm burden.

- Resistant animals may not be the most productive because of their very effective immune system so the animal selection policy should include productive characteristics as well as FEC. Selection for resistance alone has some undesirable negativity related characteristics such as increased dagginess, decreased fleece weight and lower live weight gains.

- The disadvantage of selecting for resilience is that pasture contamination can result in parasitism of the non-resilient younger animals in the flock. Resilient animals generally have reduced drench requirements.

- Future or novel control methods include vaccines, reducing the immune response, altering gene expression, use of natural control compounds, biological control and computer models.

2.0 INTRODUCTION

Parasites are organisms that live in (internal or endoparasites) or on (external or ectoparasites) a "host" animal. They can be found on virtually all-living things and in natural conditions generally existing in comparative harmony with their hosts, with outbreaks of clinical parasitism relatively rare (Brunsdon *et al*., 1975). Domesticated animals harbour a wide range of parasites, the most important of which are internal parasites. The main reason being that the ruminant domestic animals are run in large flocks or herds, concentrated in confined areas that favour the build up of parasite infections. In contrast to the wild state parasite infections in domestic animals, especially ruminants, result in subclinical (unseen) or clinical (recognisable) disease symptoms.

Helminth parasites are by far the most serious cause of production losses in farmed ruminants (Familton & McAnulty, 1997) and the nematodes are the most important of these. Internal parasitism is the major disease syndrome of pastoral ruminants simply because environments which favour pastoralism also favour the survival and development of the free-living stages of helminth parasites (Sykes, 1997). Nematode parasites have been a major factor limiting sheep production in NZ for more than 100 years (Vlassoff *et al*., 2001). Twenty-nine species of nematodes were unintentionally introduced with sheep into NZ, but it is principally species of *Haemonchus, Ostertagia, Trichostrongylus, Nematodirus* and *Cooperia* that are associated with production losses and clinical disease.

This review is confined to helminth parasites of sheep, cattle, goats and deer, run mainly under temperate pastoral grazing systems. Most of the published material in this area is on sheep, with much less on cattle and very little at all on goats and deer.

The effects of internal parasite infections vary between wide extremes. At one end, death and total economic loss can occur and at the other, effects may be undetectable and control measures unwarranted (Brunsdon *et al*., 1975; Pomroy, 1997a). Generally clinical and even subclinical internal parasite infections have some economic cost to the farming operation (Vlassoff, 1998; Pomroy, 1997a). Economically important consequences include increased mortality; and reductions in liveweight gain, wool growth or quality, fertility and milk production; rejection of carcasses or organs for human consumption; and predisposing to other diseases.

Generally the classification of the parasites importance is based on their disease-causing role (prevalence, intensity of infection and pathogenecity). However they also can have considerable importance from public health, meat hygiene and international trading perspectives (Pomroy, 1997a). Politics in this area in Europe, especially, is attempting to force trading partners to adopt EU practices (Mirams, 2003). Such an issue is selling anthelmintics as prescription only medicines, and the EU is trying to get the UK to have appropriate legislation in place by 2004 or 2005.

The cost of internal parasitism to the NZ meat and wool pastoral industries is considerable. There are a number of estimates. Sutherland (1998) states "It is estimated that gastrointestinal nematodes cost the NZ sheep industry at least $270m per annum. This is despite farmers spending nearly $30m every year on anthelmintics". Familton (1991) cites similar figures: "The effects of parasitism (both short and long term effects)

have been estimated to cost the NZ sheep industry approximately $270m a year with anthelmintics costing a further $27m annually". Leathwick *et al*. (1998) gave figures of $260m for production loss and $57m expenditure on anthelmintics, while Merial (2001) gave corresponding figures of $270m and $48m, respectively. Merial (2003) cited the total cost to sheep farmers, including the use of drenches is about $320m a year, obviously the total of their 2001 estimates. None of the authors cited a source of these estimates, but they are basically similar figures or at least in the same "ball park". These may be updates or derived from the estimates of Brunsdon (1988), that the NZ sheep industry spends more than $80m annually on drenches while losing more than $250m worth of productivity annually from parasite-affected animals. The estimates of Brunsdon (1988) are quite dated, being based on a completely different cost/price regime and a much larger sheep population in NZ than at present. These estimates were also based on a number of debatable experimental procedures and were prior to the recognition of drench resistance. Mirams (1999) cites the annual spend on anthelmintics as $25m in 1991 rising to $46.6m in 1998, despite a drop in sheep numbers wintered of 13.6m (55.2m to 41.6m).
During this time the percentage of the total spent on the more expensive endectocides rose from 30% in 1991 to 62% in 1998.
So the cost to the industry is considerable, and a revised estimate would seem warranted.

Estimates for cattle drench use are more up to date. In 2000, the cattle anthelmintic market in NZ was worth $47.9m, an increase of approximately 9% in 12 months (Familton, 2001). The cattle population was around 10 million, which would mean that approximately $4.00-$5.00/head was being spent on internal parasite control in cattle.

The introduction of the broad-spectrum drenches in the 1960s, especially the white drenches, heralded in a major advance in the control of gastrointestinal parasites (Mason & Niezen, 1998) and even some ectoparasites. Some even thought they had seen the end of ill thrift due to the control of subclinical internal parasitism. Indeed drenching became almost the sole method of control of internal parasites (Brunsdon *et al*., 1975). Drenching recommendations at that time included monthly treatment of young stock and production responses were common.

But alas good things came to an end with various gastrointestinal parasites developing a resistance to some of the drench families. By 1995, 65% of NZ sheep farms had already developed some form of anthelminthic resistance (Leathwick *et al*., 1998). This was a huge incentive to develop alternative gastrointestinal parasite control strategies and a considerable number of related research projects and approaches, many funded by Meat NZ, were conducted in the mid-to-late 1990s.

Currently considerable confusion reigns within the NZ meat and wool pastoral industries on the issues surrounding the control of internal parasites. Different parasitologists, researchers, vet, drench firms and farmers have expressed different views on the best practices and approaches (or combinations) for optimum control, and these have changed with time, especially in recent years.

This review was commissioned by MWI to summarise what was currently known and where there were still gaps in the knowledge or uncertainties, and hopefully to clarify some of the areas of confusion.

The report includes a review of the international literature, with an emphasis on pastoral conditions relevant to NZ, and incorporates existing reviews or booklets, such as the NZ Sheep Council as well as any relevant R&D reports held by MWI or the parent boards and company literature.

In addition, discussions were held with researchers at Massey and Lincoln Universities, AgResearch, key veterinary practitioners, drench companies and key farmers; highlighting areas of confusion or differences in philosophy. Unpublished information from these discussions are also recorded in the report.

3.0 REVIEW: CONTROL OF HELMINTH PARASITES IN THE NZ MEAT & WOOL PASTORAL INDUSTRIES

3.1 Introduction

Sheep in NZ pastoral systems are continually exposed to infection by nematode parasites. As eradication of the parasites is not practical, the aim of control measures is to maintain their populations at levels that are compatible with economic production (Brunsdon *et al*., 1975). Although farmers have been familiar with the clinical effects of parasitism since the introduction of sheep to NZ, a true appreciation of the magnitude of the production penalties associated with subclinical losses only became apparent following the introduction of the first of the modern broad-spectrum anthelmintics in the 1960s. Brunsdon (1970) reported lamb mortalities of between 10-45% in an intensively-reared flock that remained untreated in its first year of life. However, in many sheep flocks, the most important production penalties were significant reductions in liveweight gain, wool production and fecundity, often unaccompanied by obvious clinical symptoms (Brunsdon, 1963c, 1964a, 1966a; McLeod, 1963; Hight & Cairns, 1966; McLeod & Wolfe, 1968; Andrews, 1969). The effects of parasitism on productivity in sheep are now well documented (Brunsdon *et al*., 1975; Brunsdon, 1988) and estimates suggest that approximately one third of NZ's sheep production (approximately NZ$950m) may be dependent on the effective control of nematode infections by anthelmintics (Brunsdon, 1988; Vlassoff *et al*., 2001). In fact, despite the current level of control of nematode parasites, an estimated NZ$200m of production is still not being realised (Vlassoff *et al*., 2001). Brunsdon (1988) made a conservative estimate that one third of NZ's annual sheep production was reliant on anthelminthic drenches, and that without their use lambing percent would decline by 23% and wool production by 11%. No more recent estimates have been published and these values are still cited regularly (Vlassoff *et al*., 2001; Merial, 2001; Merial, 2003). Whatever the actual figure is currently it is substantial and has been further complicated by the onset of drench resistance. Recently Campbell (2003) estimated that if our the ability to control internal parasites accounts for 30% of farm production, on the average sheep farm (average of all classes) this was equivalent to 30% of gross revenue and 72% of EBIT: a very considerable figure

The control of internal parasites in NZ has historically been based almost exclusively on the use of anthelminthic drenches (Kettle *et al*., 1981; Kettle *et al*., 1982; Brunsdon *et al*., 1983; Leathwick *et al*., 1992). New Zealand sheep farms typically involve intensive, all year round grazing of pastures. Since climatic conditions are generally suitable for parasite development in the spring and autumn, and to a lesser extent during summer, there is considerable reliance by farmers on anthelminthic treatments to maintain animal growth throughout the year (Vlassoff & Brunsdon, 1981). Although grazing management was promoted as a means of improving parasite control in lambs almost 30 years ago (Brunsdon *et al*., 1975), a 1979/1980 survey of farmer drenching practices (Brunsdon *et al*., 1983) found that the annual number of drenches administered to lambs was 6.3 (range 0 to >12), to 1-2 year old animals was 1.8 (range 0-12) and to animals older than 2 years was 1.2 (range 0-10). Average drenching frequency has not declined in the last 20-25 years (Macchi *et al.,* 1999).

Over time with regular and frequent use of anthelmintics, gastrointestinal parasites have developed some resistance to drenches. The first case of resistance in NZ was to benzimidazoles and was reported in 1980 (Vlassoff & Kettle, 1980). This has become a considerable problem since the 1980s and although there have been no recent surveys conducted to assess accurately the extent of the problem, MAFQual Animal Health Lab reports suggested at least 60% of NZ farms have some level of drench resistance present (Hosking, 1998; Merial, 2001; Pomroy, 2000). The increasing dominance of the use of the newer endectocides on sheep from 1991 to 1998 (30% to 62% of total expenditure)
Adds further to the concern of developing resistance to these drygs also. Leathwick & Sutherland (2002) recently estimated the cost of drench resistance to the NZ sheep industry at $18m per year rising to a cost of $60m per year over the next 20 years.

With the rapid rise, in recent years, in the incidence of anthelminthic resistance (McKenna, 1989a, b; Mason, 1989; West *et al*., 1989; Pomroy, 2000) the need for a change from the strategy of regular drenching at short intervals is clearly warranted.

The epidemiology of the internal parasites is complicated by interactions between the effects of weather on the development, migration and survival of the free-living stages, the variety of mechanisms of host resistance to the parasitic stages, the numerous grazing management practices used by farmers and the number of nematode species involved (Leathwick *et al*., 1992). As many as eight nematode genera may be present in the host at one time (Brunsdon, 1970a; Douch *et al*., 1984). Control and prevention of the gastrointestinal parasitic infection in sheep as opposed to the treatment of the clinical parasitic disease requires an understanding of the development and transmission of the parasites under varying pasture management and climatic conditions (Familton & McAnulty, 1995). Internal parasite disease dynamics are therefore highly variable and difficult to predict, and it is unwise to generalise from short-term field experiments (Callinan, 1987).

The demands of parasite control on-farm has been continually changing over the years, especially with the development of drench resistance, and as an understanding of the many factors contributing to its onset has increased in recent years, management recommendations have been changing and evolving very very rapidly. This has led to considerable confusion among farmers, consultants, vets and extension specialists. Merial (2001) summarised this complex situation as follows:

- "The science of parasite control is complex, is a relatively new field, and there is no one answer to every situation.
- Farmers are regularly confronted with the results of new research which, on occasions, is contradictory to previously held positions and practices.
- Scientists themselves are aware of this and there is a consensus that it is unwise to generalise on the results of short-term field experiments.
- Some past predictions and recommendations made about parasite control have, as a result of new scientific findings, been withdrawn or amended in subsequent years as a consequence of greater knowledge."

Some of the areas of confusion are as follows: anthelmintic resistance and the factors that hasten its onset; the merits of drenching adult sheep and their contribution to pasture contamination; use of persistent anthelmintics to create safe pastures; the merits of rotating drench families; the use of the new ML drenches to delay the onset of drench resistance; the merits of using combined drenches; the accuracy of FEC faecal egg counts

as a diagnostic aid, especially for cattle; the use of FEC "trigger" levels to drench lambs; the merits of drenching lambs or calves prior to weaning; the importance of tapeworm in sheep; the "breakdown" of safe pasture preparation practices; and understanding the difference between breeding for parasite resistance or resilience in sheep.

What has added to the confusion is the fact that different research teams have challenged each other's views on some issues and that some commercial firms at times challenge or side some of the differing researchers' views (Merial, 2001).

The aim of this review is to examine current research findings on the various issues involved and clearly define what is known and accepted universally in terms of control procedures. Where differences in opinion or philosophy exist these are highlighted and where possible reconciled. The literature review does not attempt to completely cover all publications and reports on gastrointestinal parasitism, but rather cover the issues that are currently topical to the control of internal parasites in the NZ meat and wool pastoral industries. Some aspects of the parasites' biology are summarised as well, because they influence potential control measures.

Aspects of this literature review might appear to be unduly repetitious, but to avoid extensive cross referencing, I found it necessary to repeat various principles in a number of sections for completeness. In addition, most readers may only wish to read one particular section and could miss out on key points. A good example is in the peripartum rise in ewe faecal egg counts which is mentioned under epidemiology, the host immune response, drench use, drench resistance, integrated control and animal breeding.

3.2 Internal Helminth Parasites of NZ Ruminants

The internal parasites of concern fall into three groups: nematodes (roundworms); cestodes (tapeworms) and trematodes (flukes), with the nematodes being by far the most important. Sheep, cattle, goats and deer are hosts to large numbers of these parasites and various authors have compiled check lists (Brunsdon, 1960; Brunsdon *et al*., 1975; McKenna, 1976, 1998; Vlassoff, 1998; Vlassoff & McKenna, 1994; Bisset, 1994; Mason, 1994, 1997a, b; Charleston, 1997a, b; Pomroy, 1997a, b; FECPAK, 2001a). Discussion is limited to those where the adult form infects the ruminant. There are a host of such internal parasites and they vary in importance and in the host(s) they infect (Table 1).

The most important ones in sheep (Vlassoff, 1998) are *Haemonchus* (barbers pole worm, late summer and autumn), *Ostertagia sp.* (small brown stomach worm, spring and summer), and *Trichostrongylus axei* (black scour worm, late summer and autumn), *Nematodirus sp.* (thin-necked intestinal worm, early spring through summer) and to a lesser extent, *Cooperia sp.* (small intestinal worm, common in autumn but rarely important) all found in the small intestine.

Goats can be infected with many of the same worms as sheep, and they can transmit them to each other (Mason, 1997b; Pomroy, 1997a). However there is less information on goats from which to judge the relative importance of the different species (Pomroy, 1997a). The most important ones are *Ostertagia sp.* in the abomasum and *Trichostrongylus sp.* in the small intestine. *Haemonchus* can cause problems in goats in parts of the country where it does not cause problems in sheep. *Nematodirus* causes

more problems in the South Island. Goats seem more prone to parasitism because their natural resistance develops at a later stage than sheep (Mason, 1997b; Pearson, 1988; Pomroy *et al*., 1986). This inability of goats to develop the same degree of immunity to nematodes as adult sheep leads to some species, such as *Muellerius capillaries*, being important in goats but not in sheep. This species can cause extensive lung lesions in goats (Pomroy, 1997a).

Of the many species that infect cattle in NZ, the three most important are *Ostertagia ostertagi* and *Trichostrongylus axei* in the abomasum and *Cooperia oncophora* in the small intestine (Bisset, 1980, 1994; Brunsdon, 1964; Charleston, 1997a; Pomroy, 1997a). However other species can occasionally be important: other species of *Ostertagia*; intestinal *Trichostrongylus* species, *Nematodirus helvetianus* and *Oesophagostomum radiatum*. *Ostertagia* is the most significant of these, and mixed infections are most common. The only nematode found in the respiratory system is *Dictyocaulus viviparous* which causes parasitic bronchitis. Clinical cases are found occasionally, but very little is known about this parasite.

Although a number of internal parasites are found in farmed deer, the lungworm *Dictyocaulus viviparous* (also known as *D. eckerti*) was the most important and is found throughout the country (Mason, 1979, 1983, 1997a; Charleston, 1980; Gladden, 1981; Orr, 1991). Other species, especially abomasal parasites, are now causing problems in deer

The only nematode considered important in all four ruminants is *T. axei* and it has also been found in horses, pigs and rabbits (Pomroy, 1997a).

3.3 Life Cycles

To develop effective and sustainable control programmes against helminth parasites, it is important to have a good knowledge of their life cycle both within and outside the host animal (Familton & McAnulty, 1997; Vlassoff *et al*., 2001).

3.3.1 Nematodes

With the exception of *Nematodirus*, the gastrointestinal nematodes have a similar basic life cycle with no intermediate hosts (Vlassoff, 1982, 1998; Vlassoff *et al*., 2001; Brunsdon *et al*., 1975; Familton & McAnulty, 1997; Pomroy, 1997a). Adult females lay eggs which pass out in the faeces. A small first stage larva develops inside each egg then hatches and feeds on bacteria in the faeces. It then develops through two stages to become the infective third stage (L_3) larva. When the last molt is completed, the L_3 larvae have a protective cuticle around them. Consequently they cannot feed and rely on stored energy sources. Development ceases until the L_3 larva is ingested by its potential future host. These larvae are about 1 mm in length and many migrate out of the faeces and swim in moisture on to the herbage. This depends on the weather (temperature and moisture) and takes from one to ten weeks.
Eggs of *Haemonchus* require a relatively higher temperature to complete their development and this worm is, therefore, more common in the warmer northern areas of the country. Unlike those of other worms, larvae of *Nematodirus* complete their

development within the egg and larvae can readily survive over winter. Infections by this species are usually due to pasture infection from one year's lambs surviving to infect young lambs in the spring/early summer of the following year.

Table 1: Internal helminth parasites of ruminants in NZ: distribution in the primary and their importance (0, 1, 2, 3)*

Organ	*Type***	*Sheep*	*Goat*	*Cattle*	*Red Deer*
Reticulo-rumen					
Calicophoron calicophoron	T	1	0	1	0
Abomasum					
Apteragia quadrispiculata	N	0	0	0	0
Haemonchus contortus	N	3	3	1	1
Ostertagia circumcinta (including *O. trifurcata* and *O. pinnata*)	N	3	3	1	1
Ostertagia leptospicularis (=*O. crimensis;* including *O. kolchida*)	N	1	0	2	2
Ostertagia ostertagi (including *O. lyrata*)	N	1	1	3	0
Spiculopteragia asymmetrica	N	0	0	0	2
Spiculopteragia spiculoptera	N	0	1	0	2
Trichostrongylus axei	N	3	3	3	2
Small Intestine					
Bunostromum trigonocephalum	N	1	1	0	0
Bunostromum phlebotomum	N	0	0	1	0
Calicophoron calicophorum	T	1	0	1	0
Capillaria bovis	N	1	1	1	1
Cooperia curticei	N	2	2	1	0
Cooperia oncophora (including *Cooperia surnabada* =*C. mcmasteri*)	N	1	1	3	0
Cooperia pectinata	N	0	0	0	1
Cooperia punctata	N	1	0	2	0
Moniezia expansa	C	2	2	1	1
Nematodirus abnormalis	N	1	0	0	0
Nematodirus filicollis	N	3	3	1	0
Nematodirus helvetianus	N	1	0	1	0
Nematodirus spathiger	N	3	3	1	0
Strongyloides papillosus	N	1	0	0	0
Trichostrongylus capricola	N	1	2	0	0
Trichostrongylus colubriformis	N	3	3	2	0
Trichostrongylus longispicularis	N	0	0	2	0
Trichostrongylus vitrinus	N	3	3	2	0
Large Intestine					
Chabertia ovina	N	2	2	1	0
Oesophagostomum radiatum	N	0	0	2	0
Oesophagostomum venulosum	N	2	2	1	1
Skrjabinema ovis	N	0	1	0	0
Trichuris discolour	N	0	0	1	0
Trichuris globulosa	N	0	1	0	0
Trichuris ovis	N	2	2	1	1
Trichuris parvispiculum	N	0	1	0	0
Lungs					
Dictyocaulus filarial	N	2	2	0	0
Dictyocaulus viviparous	N	0	0	3	3+
Muellerius capillaries	N	1	3	0	0
Protostrongylus rufescens	N	1	0	0	0
Varestrongylus sagittatus	N	0	0	0	1
Liver					
Fasciola hepatica	T	2	2	2	2
Body Tissues					
Elaphostrongylus cervi	N	0	0	0	2

* Importance: 0 = not found; 1 = minor importance; 2 = intermediate importance; 3 = major importance

** Type: N = nematode; C = cestode; T = trematode

\+ In deer the species of *Dictyocaulus* is probably distinct and has been named as *D. eckerti* by some.

When the L_3 larvae are ingested with the herbage by a susceptible host, some develop into adult worms capable of laying eggs in about 21 days (2-4+ weeks). The L_3 larvae are carried in the ingesta to the specific part of the gastrointestinal tract that they normally inhabit (Table 1). They ex-sheath in response to CO_2 concentration, temperature and pH (Vlassoff *et al*., 2001). Their development within the tract is basically similar for all species. They enter the mucosal lining into the gland crypts and develop through two more stages and return to the lumen as adults. During this time, they grow from less than 1 mm to 7-30 mm or more in length depending on the species. Male and female adults mate and the female produces eggs, thereby completing the lifecycle. Most die a few weeks after reaching adulthood.

Some exceptions (in addition to *Nematodirus*) occur: *Bunostomon* (Hookworm) and *Strongyloidis* can also infect the host through the skin and reach the intestines via the bloodstream and lungs. Lungworms (*Dictyocaulus Sp.*) migrate from the gut to the lungs in lymph and blood. Lungworm eggs are carried up the trachea from the lungs; then swallowed into the intestine where they hatch and the larvae pass out in the faeces.

3.3.2 Trematodes

The life cycle of the flukes is quite different; involving an intermediate host (Brunsdon *et al*., 1975; Charleston, 1997b; Southey & Hosking, 1998). The trematodes are hermaphrodites, possessing both male and female sex organs. They lay their eggs in the bile duct or reticulo-rumen and the eggs pass out in the host's faeces.

Liver Fluke: Liver fluke (*Fasciola hepatica*) may cause loss of productivity of sheep, cattle and goats in NZ (Pomroy, 1997a). Liver flukes live up to three years and can produce 10,000 eggs per day. These eggs hatch into small larvae in water of the correct temperature. The life cycle depends on water and a temperature of 10°C or more: optimum rates of development (9-10 days) occur around 25-27°C (Charleston, 1997b). At 15°C development takes a month. The ciliated larvae are quite mobile in water and can only develop further inside a particular species of fresh water snail (family Lymnaeidae) found in pools, dams, drains, marshy areas and slow flowing streams. They must find the snail host and infect within 24 hours. The larvae bore into the snail over several weeks, passing through a number of larval stages undergoing multiplication or self-replication at the same time to produce up to 500 "cercariae". Development in the snail is temperature-dependent and takes a minimum of five weeks at 25-27°C - usually much longer (2-3 months or more) under field conditions. The level of multiplication in the snail depends on how well-fed and how heavily infected it is, but, theoretically, one miracidium can give rise to several hundred cercariae (Charleston, 1997b).

The later stage larvae exit the snail and attach themselves to herbage as an encysted form of larva (metacercaria). These are the infective stage and must be eaten with the herbage by the host and enter the gastrointestinal tract. The larvae then bore through the intestinal wall into the body cavity and migrate to the liver preferring the ventral (left) lobe. They then take several weeks (5-8) migrating through the liver parenchymal tissue before entering the bile duct where the trematode matures and produces eggs. The development process inside the host takes at least two months before eggs appear in the host's faeces, at which stage the flukes are not fully grown or fully productive (eggs).

Rumen Fluke: One rumen fluke, *Calicophoron calicophorum* (syn. *C. ijimai*) (Paramphistomatidae) has been isolated from both cattle and sheep in NZ (Charleston, 1997b). The life cycle, involving a different snail intermediate host, is similar to that of the liver fluke. The adult paramphistome is pear-shaped with a large posterior acetabulum with which it attaches to the lining of the reticulum and rumen. Eggs are passed out in the faeces and those that are in water develop. A miracidium develops (in 10 days at 27°C), hatches and invades the intermediate host, the flat-spiralled planorbid snail (*Gyraulus corinna*). This snail is widespread in NZ and is found in streams, ponds and swampy areas. Development proceeds through sporocyst and redial stages, and from the latter, cercariae are released. These encyst to become metacercariae which are infective to the host. After being ingested, the metacercariae excyst in the small intestine and then migrate up the intestine, through the abomasum and into the reticulo-rumen. This migration takes about six weeks, longer with heavier infections. It appears that they may first develop in the rumen before moving into the reticulum. The pre-patent period has not been established for this species.

3.3.3 Cestodes

There are two species of tapeworm: *Moniezia expanza* most common in sheep; *Moniezia benedeni* most common in cattle. Tapeworms of sheep, cattle and deer have a simple lifecycle that also involves an intermediate parasite (Brunsdon *et al*., 1975; FECPAK, 2001a; Southey & Hosking, 1998; Pomroy, 1997b). They live in the host's small intestine and eggs pass out in the faeces. The adult tapeworm consists of a head (scolex), with four suckers, followed by a body (strobila) which may reach 5 or 6m in length and 15mm in width, consisting of a ribbon of segments (proglottids). New segments continually form behind the scolex. These develop and mature, producing large numbers of microscopic eggs, and break off the hind end of the tapeworm. The segments may be seen singly or in pieces of strobila up to several centimeters in length passing out with the faeces. The eggs, each of which contains an embryo (oncosphere), may be expelled from the segment before or after it has passed. They only develop into larval stages when eaten by common species of mites (oribatid mites) in pasture, where it develops in 15-30 weeks in the body cavity to the larval or cysticercoid stage. The mites in turn are eaten by the ruminant and the adult cestodes subsequently develop in the gut growing to maturity in 5-6 weeks.

3.4 Epidemiology of the Helminths

Epidemiology is defined here as the study of the dynamic changes which occur both within the host and within the environment. Understanding epidemiology allows us to appreciate the complexity of developing control programmes which work (Familton & McAnulty, 1997; Vlassoff *et al*., 2001).

3.4.1 Nematodes

The development of appropriate procedures for control and prevention of helminth parasite infection (*versus* treatment with anthelmintics), requires a knowledge of the

factors that promote development and transmission of parasite under varying pasture management and climatic conditions (Familton & McAnulty, 1995). Over 90% (up to 99%) of the total parasite population exists outside the ruminant host (Familton & McAnulty, 1995, 1996, 1997; Vlassoff, 1998) as eggs or larvae in the faeces or in the soil. Even under arid Australian conditions, only 3% of the population in a haemonchosis outbreak lived within the host animal (Le Jambre, 1978). The proportion of the population that is on pasture, especially larvae, varies with the time of the year, generally being lower during drier summer months because of higher temperatures and desiccation. Whatever the summer conditions are, it is important to realise the significance and size of this pasture reservoir of infective larvae and its effect on the epidemiology of clinical parasitism and that drenches, however efficient they may be, will be acting only on a very small proportion of the total parasite population (Familton & McAnulty, 1997). Use of irrigation or constant summer rain will provide an optimal microclimate which will help to maintain the population size during summer.

The economically important nematode genera of sheep and cattle in NZ - *Trichostrongylus, Ostertagia, Haemonchus, Nematodirus* and *Cooperia* - are typical of cool temperate climatic zones around the world (Vlassoff & Bisset, 1991). Our moist moderate climate is particularly favourable for the development and survival of their larval stages. Generally these nematodes are found throughout the country and with two exceptions there appear to be few significant geographical differences in their prevalence or importance. The exceptions are *Haemonchus contortus* and the two species of *Nematodirus* that occur in sheep (i.e. *N. spathiger* and *N. filicollis*). *H. contortus* has a higher temperature requirement for the development of its larval stages and consequently is more prevalent in the North Island. Available data, from the 1970's, for the two species of *Nematodirus* important in sheep indicated that *N. spathiger* was more prevalent in the north of the country and *N. filicollis* in the south. More recent data indicate the *N. fillicollis* has decreased and that of *N. spathiger* increased in the south. This is probably the result of the intensive drenching of young lambs, as most of the cases of benzimidazole resistance for *Nematodirus* involved *N. spathiger*.

Many of the trials, on which we base our expected parasite development rates have been carried out using constant temperatures in a laboratory environment (Familton & McAnulty, 1995). Within the pasture environment, because diurnal temperature variation can be large, the rate of development of the various stages of both eggs and larvae is much slower. It is also possible that parasites can adapt to climatic variations (Jacobs & Rose, 1990).

3.4.1.1 *Environmental Factors*

Development of the free-living stages occurs over a restricted range of temperatures, if sufficient moisture is present (Vlassoff, 1973; Vlassoff & Bisset, 1991; Vlassoff *et al*., 2001; Familton & McAnulty, 1997).

The three most important factors influencing egg hatching, development and survival of nematode larvae are oxygen, moisture and temperature (Familton & McAnulty, 1997). The eggs and L_1 and L_2 larvae are basically aquatic (Gronvold, 1989). The conditions required by L_1 and L_2 larvae are different from those required by L_3 larvae. Only a small proportion of eggs in the faeces (1% to 17%) reach the infective L_3 stage, depending on

environmental conditions (Vlassoff, 1982; Familton & McAnulty, 1994). It is possible that a higher proportion of cattle parasites may develop because of the larger faecal mass (Familton & McAnulty, 1997).

Oxygen

Oxygen is necessary for the development of eggs and lack of oxygen inhibits hatching, subsequent larval development and larval activity (Familton & McAnulty, 1997). In cattle, dung eggs develop more rapidly closer to the surface, presumably because of lack of air penetration into the centre. As faeces break down, egg development proceeds progressively as a result of aeration of the dung (Gronvold, 1989).

Moisture

Water is essential for the development and maintenance of the free-living larvae. The presence of a thin water film with adequate levels of oxygen favours development of both eggs and larvae (Vlassoff, 1982). Optimal levels of moisture and oxygen probably occur at different times within the decaying faeces, and may account for the different time periods over which eggs hatch and larvae develop (Familton & McAnulty, 1997). In moist environments a larger proportion of the eggs will develop to the infective larval stage. In very dry summers, larvae may develop successfully to the infective stage in faeces, but do not emerge until moisture levels are optimal. Infected faeces continue to be passed by the host, so that when moisture is available, pasture contamination by larvae can rise very rapidly (Michel, 1982). In dry conditions, the surface of the faeces desiccates providing a protective crust which prevents drying out of the interior of the faecal mass and also prevents release of the infective larvae (Gronvold, 1989). This situation may continue until water breaks down the crust allowing larval release. Other influences such as irrigation (Bullick & Anderson, 1978; Gruner et al., 1989) can enhance the ability of larvae to migrate from the faeces and increase their survival time, as well as assisting their migration, either passively or actively throughout the soil (Al Saqur et al., 1989; Rose & Small, 1985). Cattle dung pats generally contain adequate moisture even during dry periods (Vlassoff & Bisset, 1991).

Temperature

Temperature has a big influence on egg hatching, larval development and the subsequent survival of the pre-parasitic stages (Vlassoff, 1982).

Development: The preferred developmental conditions of different nematode species vary and are reflected in their distribution and relative abundance from season to season and year to year (Vlassoff, 1982; Vlassoff et al., 2001). For example, *O. circumcinta* appears earlier in the season than *T. colubriformis*. For successful development of eggs and pre-infective stages into L_3 larvae in faeces, they must survive a range of climatic conditions (Vlassoff & Bisset, 1991; Vlassoff et al., 2001). Optimal development occurs between 15°C and 30°C, but development will take place at varying rates within the temperature range of 4°C to 35°C if moisture is present (Vlassoff, 1982). Eggs of *Haemonchus*, *Trichostrongylus, Ostertagia* and *Chabertia* develop to L_3 larvae most rapidly at mean monthly air temperatures of 15°C-24°C. Below 10°C, development is slow, and most eggs fail to hatch. Eggs and larvae of most species of gastrointestinal parasites of sheep (with the exception of *Haemonchus contortus*) tolerate cold temperatures but their metabolic rate is reduced (Familton & McAnulty, 1997; Pomroy, 1997a). Leathwick et al. (1999b) found 0.5% of *O. circumcinta* eggs developed to L_3 at 5°C but development of *T. colubriformis* eggs required a minimum threshold temperature closer to 10°C. Even at a

temperature of 0.6°C and a relative humidity of 76%, some parasites reach the L_3 stage in 65 days (Vlassoff, 1982).

The commonly held view was that development was generally restricted to those months of the year with a mean air temperature greater than 10°C (i.e. generally October to May) (Vlassoff & Bisset, 1991). The rate of development to the infective stage under optimum conditions is 5 to 7 days. However, under field conditions this varies substantially with the season and commonly takes 2 to 3 weeks or more. The percentage of eggs that develop to the infective stage is small - ranging from less than 1% in the dry mid-summer conditions to 13% (the maximum recorded at Wallaceville) in moist warm autumn conditions. Once the infective third stage is reached, larvae can survive for considerable periods as this stage is very resilient. In the summer, larvae may survive up to 10 to 12 weeks depending on the degree of exposure to sunlight or desiccation (Rose, 1963).

Reports of development success in the field over winter are conflicting. Familton & McAnulty (1994, 1995) reported significant development of both *O. circumcinta* and *T. colubriformis* in winter in Canterbury. Average air temperatures were between 5.8°C and 7.6°C and considerable egg hatching and larval development occurred. These findings were unexpected because temperatures were below the 10°C threshold found by Leathwick *et al*. (1999b) for *T. colubriformis* and at much greater levels than previously believed (Familton, 1996; Familton & McAnulty, 1995). These findings differ from studies in the Manawatu (Niezen, 1996; Leathwick *et al*., 1999b) which found development during winter to be negligible.

At higher temperatures (>30°C) particularly in summer, developmental success is also limited as eggs are subjected to temperature stress and desiccation (Vlassoff, 1982, Vlassoff *et al*., 2001). Temperatures in faeces often reach much higher levels than air temperatures (Familton & McAnulty, 1995).

One other important factor in maintaining an optimal larval environment in both the summer and winter is the mass of the dung. A soft mass of faeces provides a more protective environment than the small pellets (Familton & McAnulty, 1997). The importance of protected environmental conditions, on the development and survival of larvae, was demonstrated by Skip *et al*. (2000). Larval hatching was almost threefold greater (7000 *vs.* 2500 L_3 larvae) from dung buried under 25 mm of soil than from dung on the soil surface. This would be a combination of moisture and temperature control. The observations on the size of faecal mass lend credence to the often rejected practice of harrowing to break up the faeces and hasten desiccation of the smaller faecal particles.

Laboratory studies under controlled conditions such as below (Table 2) give an indication of the factors affecting development and the longevity of some of the stages, but they do not reflect fluctuations, such as diurnal temperature variation, that occur in the field. Development of pre-parasitic stages observed in the field is often much longer than predicted from laboratory experiments (Young *et al*., 1980), so models of the population biology on pasture should be based on field rather than laboratory studies (Smith *et al*., 1986).

Survival: Once development to the L_3 stage is complete, larvae are considerably more resistant to adverse conditions (Vlassoff *et al*., 2001). The period of time they survive varies, depending on the season of the year. Although a proportion can survive 12

months until the following summer, most do not survive more than 2-3 months (Vlassoff, 1982). The influence of temperature on egg survival in faeces and larval survival in water for *O. ostertagi* is shown in Table 2 for controlled laboratory conditions. These are survival temperatures and not those required for continuous development.

Table 2: Effect of a constant temperature on egg and larval survival of *Ostertagia ostertagi* (Pandey, 1972)

Temperature	*Egg Survival*	*L_3 Survival*
-10°C	6 weeks	6 weeks
1°C	46 weeks	>52 weeks
4°C	50 weeks	>52 weeks
20°C	nd	42 weeks
25°C	nd	30 weeks
30°C	nd	18 weeks
35°C	nd	10 weeks
40°C	24 hours	2 days
45°C	12 hours	8 hours
50°C	4 hours	1 hour

nd = not determined

Larval (L_3) movement is temperature-dependent and as it increases, their movement increases but so does the consumption of stored reserves (Pomroy, 1997a). At steady temperatures from 30-35°C larvae will only survive for a few weeks but at about 5-10°C larvae are inactive and will survive for months. Larvae are not necessarily killed by frost, but cannot tolerate repeated freezing/thawing conditions. Most require several degrees of frost before ice crystals form in them (Pomroy, 1997a). The arrival of the first frosts of winter do not kill the parasites, as is often thought; it only slows down the activity of the pre-parasitic stages (Familton & McAnulty, 1997). L_3 larvae, however, survive for a very long time in the pasture environment; up to 11 to 12 months or more under suitable climatic conditions (Vlassoff, 1998; Familton & McAnulty, 1997). Larval populations may exist in greater numbers and for longer periods than recognised from earlier work (Familton, 1996).

In summer months, larvae which had emerged from faeces, were destroyed in 2-4 months due to high air temperatures and low relative humidity (Holosova *et al*., 1988).

3.4.1.2 *Larvae Levels on Pasture*

The pasture larval population can increase by a few hundred or several thousand per hectare per day, depending on the season and rate of contamination (Vlassoff & Bisset, 1991). Faeces are a reservoir of infective larvae.

Season, year and district: The greatest numbers of larvae on pastures usually occur in late spring and autumn (Vlassoff, 1998). As discussed above, many developing eggs and larvae are killed by hot dry weather in summer and fewer eggs develop in the cold winter months because of the low temperatures. However, once larvae have reached the infective stage they are very hardy, many surviving for over 10 to 12 weeks with some able to survive on pasture for more than 12 months. Some larvae from the autumn peak

commonly survive 3 to 6 months (Vlassoff & Bisset, 1991) with smaller numbers up to 12 months and in the case of *Nematodirus* for over 18 months (McDonald, 1963). The practice of spelling pastures for 2 or 3 weeks generally has little effect on the larval population other than the dilution by grass growth. At some times of the year, particularly in late summer/autumn, larval numbers can often increase between grazings at such intervals. Once pastures are heavily contaminated it will take 2 to 3 months for larval numbers to decline significantly. In winter this may take even longer as contrary to common belief, larvae are not necessarily killed by frosts. Although few larvae develop over the winter months, cool conditions can actually enhance the potential for infective larvae to survive (Vlassoff & Bisset, 1991).

In the spring to late autumn period when conditions are normally favourable for larval development, the development and translocation of larvae onto the herbage may take from 2 to 10 weeks (Vlassoff & Bisset, 1991; Vlassoff *et al*., 2001). During wet periods in spring and autumn there is usually sufficient moisture for the immediate migration of the larvae onto the grass. The pattern of the succession of species in the host is also reflected in variations in the pasture populations of infective larvae (Tetley, 1959; Brunsdon, 1963a, b; Vlassoff, 1973, 1976). Although some larvae are found on the herbage at all times of the year, a distinct seasonal pattern of availability occurs each year (Vlassoff & Bisset, 1991; Vlassoff *et al*., 2001) sometimes (although not always) with a minor peak in late spring/early summer and generally a major peak in late summer/autumn (Vlassoff, 1973, 1976, 1982). The magnitude of these larval peaks can very markedly from year to year depending on the prevailing weather conditions.

The small peak in spring/early summer is comprised of larvae that have survived over winter as well as larvae derived from the post-partum egg rise of ewes, while the larger peak in late summer/autumn is from eggs deposited by lambs in summer/early autumn (Vlassoff, 1973, 1976). In the Manawatu, a spring rise in larval numbers on pasture from contamination by lactating ewes was only detectable in one of three years (Leathwick *et al*., 1998).

Variations in the magnitude of the larval peaks on pasture between years is undoubtedly related to differences in temperature and rainfall pattern as these factors regulate the development of the worm eggs deposited on pasture. Despite several seasons work to relate larval infestation levels to weather patterns, it was not possible to predict the severity of outbreaks of parasitism from climatic factors alone (Vlassoff & Bisset, 1992). This may have been due to confounding interactions between the weather, animal management and predators/pathogens of nematode eggs and larvae.

However in 1992 (Leathwick *et al*., 1992) developed a strategic model of mixed nematode infections in lambs in New Zealand. It successfully predicted known patterns of epidemiology as well as production losses. The model was most sensitive to factors affecting survival and migration of free-living stages and host resistance. Previous models had focused on single species (Ratcliffe *et al*., 1969; Paton *et al*., 1984; Grenfell *et al*., 1987; Barnes & Dobson, 1990). Callinan *et al*. (1982) presented parameter values for two species and Paton (1987) assumed that an *Ostertagia sp.* model could be applied to other species by varying egg output. However, processes other than egg output differ between species. In contrast, Leathwick *et al*. (1992) used a different approach and focused on a general model for mixed nematode infections with generalised NZ epidemiological patterns (Vlassoff, 1982; Brunsdon & Vlassoff, 1982).

The annual climatic pattern is basically similar for most of NZ with warm dry summers and cool wet winters. As a result, the pattern of larval availability, with some seasonal variations due to weather and farm management practices, tends to be similar over most of the country. Data on larvae availability on pastures grazed by lambs from Dargaville, Ruakura, Wallaceville, Winchmore and Woodlands over three years had a broadly similar pattern (Vlassoff & Bisset, 1992; Vlassoff *et al*., 2001). In this study the variations in larval numbers between locations within a year were no greater than the between year differences at a particular site. Although the magnitude of the larval populations differed between sites, the maximum numbers for each occurred at approximately the same time.

Concentration and pasture growth: Levels of infective larvae on pasture are expressed as a concentration, or as number of larvae per kilogram of pasture (Familton & McAnulty, 1997). This can range from 0 to 30,000 larvae per kilogram of pasture and intakes of over 2000 larvae per day can adversely affect sheep. The number of L_3 larvae on pasture is determined by their rate of release from the faeces relative to the growth of pasture. Rapid pasture growth will tend to reduce larval concentrations by dilution (Michel & Bere, 1982). In late autumn and winter, as pasture growth slows down, the rate of release of remaining larvae is increased as a result of faecal breakdown and greater moisture. Larvae can reach their highest levels in winter in the United Kingdom (Michel & Bere, 1982) and New Zealand for both sheep (Familton & McAnulty, 1996) and cattle (Bisset, 1995) pastures. A very high proportion of these larvae successfully over-winter and contribute substantially to the larval population recorded on pasture in the following spring (Familton & McAnulty, 1997).

Distribution of larval on pasture and larval migration: The distribution of the larvae on pasture is related to the feeding/defaecation pattern of the stock (Vlassoff *et al*., 2001). Larval distribution tends to be highly clumped around faeces, decreasing from the centre outwards horizontally and also vertically from the roots to the top of the sward (Gruner & Sauve, 1982; Rees, 1950; Rose, 1963, 1964; Skinner & Todd, 1980).

Migration of L_3 larvae from faeces can be either active or passive (Familton & McAnulty, 1997). Active larval migration is dependant on the available water film. This is dependent on rain, dew or irrigation. The movement of L_3 larvae from the faeces occurs in waves, coinciding with the presence of water. Rainfall of 25-50 mm is usually required for migration (Gronvold, 1989; Vlassoff, 1982). Larvae generally are not found any further than 30 cm away horizontally from the faeces and their concentration decreases as the distance from the faeces increases (Gronvold, 1989).

Vertical migration of L_3 larvae occurs up plant material but the majority of larvae (50%) are found in the first 2 cm of the plant or in the first 1 cm of the soil (Vlassoff, 1982; Vlassoff *et al*., 2001). Increasing numbers of larvae in the upper sward component occur in conditions of high humidity and temperature but this can vary with plant species (Gronvold, 1989; Vlassoff, 1982). L_3 larvae are also capable of downward movement. It has been suggested that sunlight may also play some part in the active migration of larva.

Passive transport of L_3 larvae away from the faecal mass has been caused by raindrops splashing and this may be an important factor in the movement of cattle *Cooperia* and *Ostertagia* larvae (Vlassoff *et al*., 2001). Larvae have been measured up to 90 cm horizontally and 30 cm vertically from the faecal mass after simulated rain (Gronvold,

1989). Some transmission may be performed by insects, earthworms, birds or fungi although the earthworms may be more important in the destruction of L_3 larvae (Gronvold, 1989; Skipp *et al*., 2000).

The distribution of larvae over a farm is largely dependent on the grazing management system employed. Some larvae will usually be found on all paddocks of a farm, but higher concentrations of infective larvae can be expected to occur wherever lambs are grazed for extended periods (Vlassoff & Bisset, 1991).

Removal of larvae: Larvae die when climatic conditions are unfavourable, as discussed above, and they can be removed from the pasture by grazing. One important factor is the rate of removal of the larvae from pasture by the various classes of livestock (Familton & McAnulty, 1997). If larvae are not specific to the animal species or the animal has a high degree of natural immunity, such animals may be used as "vacuum cleaners" to remove larvae from pastures.

Feed intakes of animals at different physiological stages vary considerably and influence the number of larvae removed from the pasture at any particular time and therefore the resultant size of the parasite challenge to susceptible animals. This is shown in Table 3. It can be seen that levels of between 800 and 2000 larvae/kg of fresh herbage can result in intakes well in excess of 2000 larvae per day. The grazing height can also have a huge bearing on the number consumed.

Table 3: Relationship between daily larval intake and light, moderate and heavy herbage contamination (larvae/kg fresh herbage) (assuming pasture 20% dry matter) (Familton & McAnulty, 1997).

Category	*Dry Matter Intake (kg/day)*	*Larval Contamination (larvae/kg fresh herbage)*		
		200 (low)	*800 (medium)*	*2000 (high)*
Lactating ewe	2.7	2700	10800	27000
12 month old sheep	1.6	1600	6400	16000
Unweaned lambs	0.8	800	3200	8000

Sources of contamination: It has been known for many years that both mature and immature animals can act as sources of contamination. The relationship between the worm population in the host, the faecal egg output of ewes and lambs, and the larval population on the pasture is shown in Figure 1 (page 27). There is a continuous intake of larvae from the pasture by stock and their origin has been clearly defined (Brunsdon, 1976; Skinner & Todd, 1980). Larvae on pasture in spring and early summer are made up of those that over-wintered as well as those from the periparturient contamination from ewes. The relative importance of the peri-parturient rise in faecal egg count of the ewe and over-wintered larvae as the initial source of infection to lambs varies from year to year depending on the weather (Brunsdon, 1976; Vlassoff, 1973, 1976; Leathwick *et al*., 1998). In some years the two sources of infection can be additive while in others the ewe contamination may not contribute significant numbers to the larval population. Larvae on pasture over the period from mid summer until the following spring are mainly derived from the increasing number of eggs passed in the faeces of lambs.

Lambs provide the main source of nematode eggs, and contribute the greatest proportion of the larval population on pasture (Vlassoff, 1976; Leathwick *et al*., 1998; Vlassoff *et al*.,

2001). Even with frequent drenching, lambs remain a significant source of pasture contamination because they become readily reinfected between drenches unless these are at intervals shorter than the development period of the parasites, or controlled-release capsules are used (Vlassoff *et al*., 2001).

In their first year at pasture, lambs ingest larvae from the moment they begin to eat grass and can develop variable worm burdens prior to weaning (Foster *et al*., 1991). These authors found the predominant species were *Ostertagia, Nematodirus* and *Haemonchus*. The peak period of challenge occurs in autumn (Vlassoff & Bisset, 1991). While there is a continual intake of larvae by lambs, there are basically only two generations of parasites annually. The first is derived from over-wintered larvae and those that have developed from the peri-parturient rise of the ewe, while the second is derived from larvae that develop from the lambs' own contamination over the summer/autumn period. *Nematodirus sp.* is an important exception as the transmission of these species only involves one generation each year and is basically from the lambs in one season to those of the next (Vlassoff, 1973).

The worm burden in lambs builds up over summer to a peak in autumn/early winter when self cure normally occurs (Brunsdon, 1970). The worm burden of adult sheep tends to remain relatively constant with the exception of breeding ewes. Parturition and lactation stress results in a temporary relaxation in the ewes' immunity allowing an increase in worm burden and in the fecundity of the worms already present. This leads to the peri-parturient rise in FECs, generally peaking between 6 to 8 weeks after lambing (Brunsdon, 1970; Brunsdon & Vlassoff, 1971a, b). European and UK (HISHA & SAC, 2000) work has identified a number of factors associated with FECs at this time. Underfeeding, in general, and in particular underfeeding of protein, reduces this immunity. Poor body condition (less than condition score 2.5) and multiple-rearing ewes excrete more eggs than good condition and single-rearing ewes. Two-tooths excrete more eggs than mature ewes. New Zealand findings are similar (Sykes *et al*., 2001; Vipond, 1998).

The general outline, given in Figure 1 (page 27) is the sequence of events in the parasite cycle both in the host and on pasture and can be applied to most of NZ (Vlassoff *et al*., 2001). There will however be some variation in the magnitude of the seasonal peaks of larvae on pasture- and consequently in the worm burdens acquired by stock - due to individual farm management practices and local climate. Figure 1 suggests that the timing of the seasonal peaks in the larval population on farms as a whole will be similar in a region and farms with the same/similar management and stocking rate and mix will be able to employ the same basic control procedures (Vlassoff & Bisset, 1991; Vlassoff *et al.,* 2001).

Because ewes usually have a high level of acquired immunity and low FECs, except at parturition or at times of nutritional and environmental stress (Brunsdon, 1971), they have not normally been regarded as a major contributor to the larval population on pasture (Vlassoff *et al*., 2001). In Europe and the UK (HISHA & SAC, 2000) extension recommendations distinguish clearly between two stages in adult ewes. They are considered solidly immune from weaning until mid-pregnancy and are useful for "vacuuming" larvae from the pasture without excreting any eggs in their faeces. However from mid-pregnancy and during lactation, when their immunity breaks down, they are considered a source of infection. However, several authors (Familton & McAnulty, 1996; Sykes, 1982; Milligan, 1982; West, 1998) emphasise the potential danger of under-

estimating the contribution of the ewe prior to periparturient egg rise, by ignoring the volume of faecal material (Table 4) and concentrating solely on FEC. A ewe with an egg count equal to only 30% of a weaned lamb's egg count will be an equivalent source of pasture contamination. Two thousand ewes producing 2 kg of faeces per day with an average worm egg count of 250 eggs per gram will pass 1 billion eggs onto the pasture each day (Familton & McAnulty, 1997). In the spring/summer period, a ewe with a faecal egg count of 338 epg is capable of producing 1 million eggs in a 24-hour period. If 6% of these eggs reach the L_3 larval stage, then there are potentially 60,000 infective larvae from each ewe in a 24-hour period. During spring and early summer, ewes produce three to five times as much faeces per day as lambs produce and there is a danger of being misled by their relatively low FECs. Although FECs in ewes may be lower compared to lambs, their contribution to pasture contamination could be greater. During spring and late summer to autumn, ewes (especially two-tooth ewes) may be an important source of pasture contamination on some farms (West, 1998).

Table 4: Faecal output (mean wet weight for each four month period, g/day) of sheep throughout a year (Familton & McAnulty, 1997).

		Period		
		Spring/Summer	***Summer/Autumn***	***Autumn/Winter***
Mean fresh				
Faeces	Lambs	870	2000	1900
Output	Hoggets/2 tooths	1700	1500	1700
(grams/day)	Ewes	2960	1750	1800

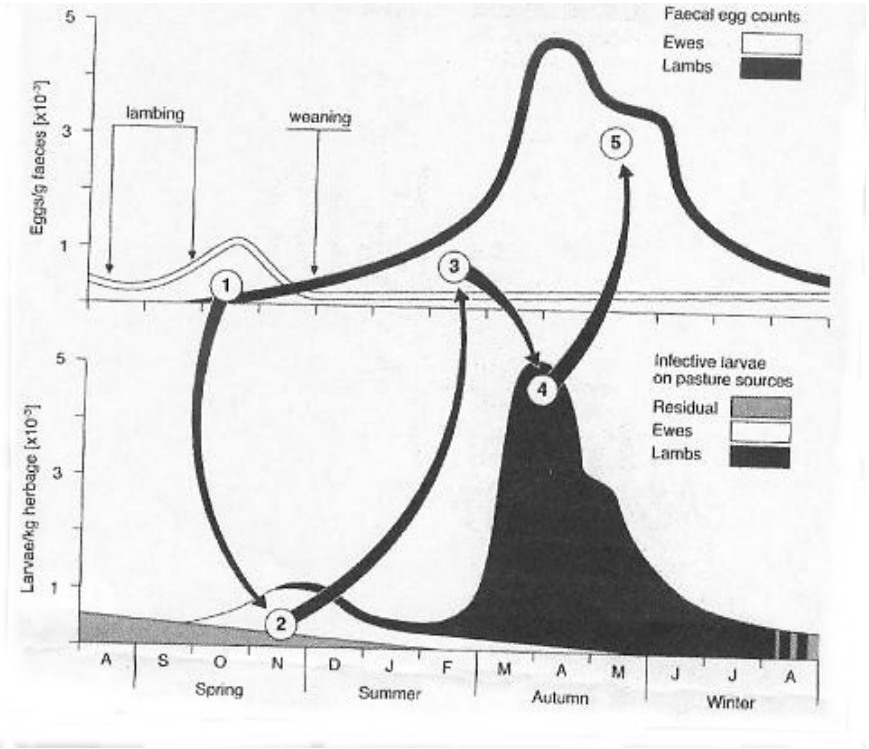
However the benefits of treating ewes and the relative contribution they make to larval populations on pasture compared with drenched lambs has been subjected to ongoing scrutiny and debate (Vlassoff *et al*., 2001). These authors remain unconvinced and state that although the faecal output of ewes is considerable, this association does not consider the very low percentage of eggs that actually develop, particularly over the winter, because recent studies have shown that ewes with a high level of acquired immunity shed low numbers of eggs and that the majority are not viable (Jorgensen *et al*., 1998). This indicates that FEC alone is not an accurate measure and will invariably overestimate ewes' contribution to pasture contamination. This difference in philosophy between researchers is one of the sources of confusion to farmers.

3.4.1.3 *Patterns of Worm Infection*

At most times of the year, the majority of worms are present as infective larvae on pasture but the numbers of eggs in sheep faeces are a good indicator of the size of worm burden in an animal (Vlassoff, 1998). The worm numbers present in untreated sheep at different times of the year and the resultant pasture infestation with larvae is shown by the generalised pattern of faecal egg counts (FEC) in Figure 1 (page 27). This pattern is a result of the various external epidemiological factors discussed earlier in this section. It is important to recognise that parasitic species may require different environmental conditions to achieve optimal development (Familton & McAnulty, 1995). It is well known that *Haemonchus contortus* requires higher temperatures than for example *Ostertagia (Telodorsagia) circumcinta* or *Trichostrongylus colubriformis* which can develop at lower temperatures. This knowledge explains the different geographical and seasonal distributions of these parasites (Vlassoff, 1982).

The seasonal pattern of gastrointestinal nematode infections in sheep tends to follow a relatively similar pattern from year to year (Brunsdon, 1970; Vlassoff, 1982; Vlassoff *et al*., 2001). In lambs and to a lesser degree in adult sheep, the pattern of faecal egg output (Figure 1) closely follows the level of infection (Brunsdon, 1970a; McKenna, 1981; Vlassoff *et al*., 2001). Generally, young sheep harbour mixed infections comprising species of the five principal genera referred to previously. There may, however, be some seasonal changes in the relative abundance of the various genera. Early infections in young lambs, for example, are usually dominated by populations of *Strongyloides; Nematodirus* tend to predominate in late spring, followed by *Ostertagia; H. contortus* and small intestinal *Trichostrongylus* in late summer/autumn. High numbers of *Cooperia sp.* and *T. axei* can also occur in autumn but infections with these genera usually extend well into winter and form a major part of the worm burden of animals in their second year (Brunsdon, 1970a; Vlassoff *et al*., 2001).

Figure 1: The interrelationship between the pasture contamination by untreated ewes and lambs and the availability of infective larvae on pasture (Vlassoff, 1982).



1. The peri-parturient rise in FEC of breeding ewes is the main source of contamination contributing to the spring peak of L_3 on pasture.
2. Over-wintered L_3 and those from the spring peak give rise to the first generation of worms which accumulate in lambs over summer.
3. Eggs deposited by lambs in summer/early autumn are the source of the autumn/winter population of larvae on pasture.
4. L_3 from the autumn peak produce the second generation of worms in lambs.
5. In late autumn/winter a decreasing proportion of eggs develop because of declining temperatures.

Young lambs have little innate ability to resist infection, but because of their initially low intake of herbage, worm numbers in their gut build up slowly during spring and early

summer, reaching a peak in autumn. A rapid decline in worm burden in winter is associated with the development of a significant immune capability. Following the elimination of the major part of their worm burdens when they are 10-12 months of age (Brunsdon, 1970) sheep tend to remain relatively resistant to serious re-infection (Vlassoff *et al*., 2001).

Once sheep have acquired full immunity, however, they require constant exposure to some level of infection to maintain their resistant status. Sheep's ability to maintain immunity at a high level can also fail at times of nutritional stress, illness at any age, and declines in breeding ewes around the time of lambing (Brunsdon, 1971). The latter effect results in the well-known periparturient rise in FEC in lactating ewes (Figure 1), in which their immune capability is compromised by the stresses imposed by pregnancy, lambing and lactation, often accentuated by sub-optimal feed levels. Some of the larvae ingested over the lambing period are therefore able to establish and develop to maturity. There may also be an associated maturation of inhibited larvae (Brunsdon, 1966b) and increased fecundity of existing adult worms. Soon after giving birth, however, the ewe's immunity returns, so that she is largely safe from larval establishment four weeks after parturition (Leathwick *et al*., 1999a). The worms which have already established remain in the ewe for several weeks longer, producing a characteristic peak in FEC, 6-8 weeks after parturition (Brunsdon, 1971), before the majority are expelled. Removal of these worms has been the basis of the strategic use of an anthelmintic treatment of ewes about four weeks after lambing, although more recently this has tended to be replaced by the pre-lamb administration of a long-acting anthelmintic or controlled release capsules (Vlassoff *et al*., 2001). This practice is currently questioned in control programmes (see later).

3.4.1.4 *Summary of Important Epidemiological Factors in Nematodes*

Familton & McAnulty (1997) summarise the main points as follows:

> "Any attempt to achieve sustainable parasite control must involve a good understanding of the epidemiology and life cycles of the parasites concerned. In the development of control programmes, we must take the following points into consideration:
>
> - Considerable development of eggs and larvae occur over the winter months (i.e. May, June and July) and these augment the larval reservoir already present on pasture which has over-wintered from the previous autumn.
> - Administration of anthelmintic has no effect on the parasite reservoir outside the host animal and that this is a very large component of the total parasite population.
> - The temperature within the faecal mass does not equate with the ambient air temperature. During winter months the faecal environment is much more conducive to parasite development than previously thought and considerable development occurs, albeit if it is slightly delayed and possibly occurring at lower percentage development rates.
> - During autumn and winter adult ewes on an all-grass grazing system make considerable contributions to the infectivity of pasture, particularly under intensive rotational grazing systems even when the faecal egg output is considered to be low.

- Under NZ conditions, the highest levels of infective larvae on pasture are consistently found during the winter months and larval challenge to ewes on an all-grass diet can be as high as 30,000 larvae per day.

Despite the vast array of knowledge of the pre-parasitic stages of gastrointestinal parasites of sheep, it would be a mistake to assume that we have all the answers to deal with outbreaks of infection, particularly when we are faced with control programme breakdowns. There is still a lot of basic research to be done if parasite control programmes in the future are to be based on sound epidemiological evidence. All of NZ may be regarded as a temperate climatic zone but it is essential to recognise that large differences in climatic factors which affect parasite development occur between districts and between the microclimatic zones which can occur within a farm. Parasite control programmes, therefore, must be based on sound understanding of the specific environmental conditions prevailing at each site".

3.4.2 Trematodes

3.4.2.1 *Liver Fluke*

Temperature/season: The pattern of seasonal availability of infection is largely determined by temperature (Charleston, 1997b; Southey & Hosking, 1998). Except in the warmest areas of the country, average temperatures are in the region of 10°C or lower (in some areas much lower) for several months in winter and for all practical purposes the development of fluke stages ceases. The main period of infection is January to July/August. As temperatures rise in spring, the development of fluke stages can begin but is very slow, needing 3-4 months until the first cercariae are released. That is why little or no infection is likely to become available before January over much of the country. At the end of the season when temperatures fall below 10°C, further development of larvae and release of cercariae ceases. Metacercariae, if kept wet, can survive a month or two on pasture in the Manawatu. This explains why transmission ceases in July in this area.

District: There will be some regional differences in transmission period but these have not been studied (Charleston, 1997b). In colder areas of the South Island, one would expect the transmission period to start later and end earlier than in the Manawatu. Conversely, in Northland, where mean monthly temperatures exceed 10°C all year, some development to infection may occur throughout the year and there is some evidence to suggest that infection can be acquired year-round there, though the main infection period is still the late summer and autumn. Although no recent surveys have been conducted, earlier work indicated liver fluke was more common in the North Island than the South Island (7.5 *vs.* 1.1% in 1984/85) (Charleston, 1997b). Its distribution is uneven throughout NZ (Pomroy, 1997a).

Immediate host: The seasonal pattern of availability of infection is only one component of the epidemiology of infection. The risk of exposure of grazing animals to infection is greatly influenced by the proportion of the grazing area that is snail habitat (Charleston, 1997b). This varies widely between farms. Another important factor is the effect of weather conditions on the likelihood of animals grazing marshy areas that are snail habitat.

Liver fluke was first reported in NZ in 1896 and probably arrived with sheep from Australia. It established because of a suitable indigenous snail host (*Lymnaea tomentosa*). It has spread rapidly over the last 30-40 years with the introduction and spread of the exotic snail (*Lymnaea columella*).

Snail habitat/moisture: Although both *L. tomentosa* and *L. columella* will breed successfully in ponds and dams, it is marshy areas of pasture that are most important in the transmission of infection. Typically, these are poorly drained areas that are kept permanently wet by seepages and springs, or the margins of slow-moving streams or irrigation ditches. Observations in NZ indicate that snails are not found in habitats that dry out completely in summer.

The presence of snails in farm dams and ponds, and even water-troughs, is of little significance to transmission of infection unless metacercariae are encysted on vegetation at the margins of ponds which is then eaten. There is a slight possibility that if metacercariae have encysted on mud at the bottom of a pool or marsh and are stirred up, perhaps by animals walking in the water, animals drinking may ingest some metacercariae. It is unlikely that infection would be acquired this way.

Large numbers of snails can occur in irrigation channels and become infected by animals defaecating into them. Animals grazing these areas will be exposed to infection but, in addition, metacercariae can be distributed over the whole grazing area when it is flood-irrigated.

In Europe, there is a well-established relationship between the wetness of the summer and the severity of the fluke problem in that year and this is the basis of forecasting systems used there. Studies in NZ, however, have shown no positive relationship between the wetness of the summer and the acquisition of infection but rather suggest the reverse. Observations indicate that the level of infection of stock is likely to increase in dry summers as the animals selectively graze marshy areas or the margins of streams or irrigation ditches where green feed is still available. The situation in parts of Australia is similar.

The level of infection is not directly related to the numbers of snails present. A few, well-fed snails can produce more cercariae than large numbers that are poorly fed. Furthermore, high levels of transmission of fluke do not necessarily require a large proportion of snails to be infected. While infection levels in field populations of *L. tomentosa* of 1-5% or more have been recorded, particularly during transmission periods, this is not necessarily the case with *L. columella* with which the numbers infected can be extremely low (less than 0.2%). For this reason there is no point in submitting snails to a laboratory to see if they are infected. Although many infected *L. columella* die, those that survive produce almost twice as many cercariae as *L. tomentosa*.

3.4.2.2 *Rumen Fluke*

Season: The seasonal pattern of infection has not been studied (Charleston, 1997b) but it is likely that, as with *Fasciola*, most cercariae will be released from snails in the summer/autumn period. Circumstantial evidence indicates that clinical cases are often

preceded by flooding of pastures which carries released cercariae over the paddocks so that metacercariae are distributed over the grazing area. In addition, in dry weather, snail habitats can shrink leaving metacercariae available to grazing animals along their margins.

District: The parasite is probably widespread although no surveys have been published. Infections have been recorded in cattle in the Wairarapa, and clinical cases have been described from Westland, the Manawatu, Hawkes Bay and Coromandel. Unpublished reports indicate that infection is very common in cattle in the west and northwest of the South Island. Infections are apparently much more common in cattle than in sheep.

3.4.3 Cestodes (*Moniezia sp.)*

Intermediate hosts: The intermediate hosts of *Moniezia sp.* are free-living oribatid mites. Ingestion of eggs by infected mites is accidental as they are not coprophagous. Larvae (oncospheres) still within the egg are ingested and leave the egg and make their way into the body cavity of the mites where they develop into cysticercoids which are the infective stage for ruminants. Oribatid mites are a very diverse group with 127 species included in 27 families being implicated as intermediate hosts for *Moniezia* and related cestodes (Pomroy, 1997b). However, oribatid mites do vary in their susceptibility and not all species are suitable as intermediate hosts. Most infected mites have only one cysticercoid but up to 13 cysticercoids have been found in individuals of some species with the maximum number tending to be proportional to the size of the mite. The majority of oribatid mites are found in the soil humus layer with as many as 2.5×10^7/ha recorded on a permanent sheep pasture in the northeast USA with 3.9% of these being infected. One study in NZ on oribatid mites and *Moniezia* reported 2.6% infected mites.

Environmental factors: The number of mites on herbage has been found to correlate positively with temperature, relative humidity, rainfall and soil moisture on the day of collection (Pomroy, 1997b). The seasonality of mite numbers is not clear and probably varies with species. One US study found as many mites in frozen soil taken in the middle of a northern American winter as in summer. The development rate of cysticercoids in mites depends on temperature and host species and can be as short as 28 days. *Moniezia* eggs have been shown to survive up to four days exposure to direct sunlight, 20 days at about 0°C and at least seven months between 5-80°C. The longest report of infectivity being maintained on pasture appears to be 22 months.

The minimum pre-patent period (time after infection until eggs are produced) for *M. expansa* is about 35 days. The prevalence and intensity of infection will vary with season, host age and availability of the infected mites.

3.5 Host-Parasite Interactions

3.5.1 Parasite Damage to Hosts

3.5.1.1 ***Nematodes*** (Brunsdon *et al*., 1975; Vlassoff, 1998; Familton & McAnulty, 1997; Pomroy, 1997a; Sykes, 1977)

Gastrointestinal Worms

Nematode populations in animals are usually mixtures of species. The severity and nature of the changes produced in an animal chiefly depend on: the numbers of nematodes present relative to the size and physical condition of the host; the species of nematodes present and their relative proportions. The majority of these symptoms, especially infected by mucosal browsers, are largely the hosts' reaction to the infection, rather than a direct effect of the parasite (Heath *et al.*, 2000; Hein *et al*., 2001). The general state of health, age and level of nutrition are also very important as they influence the effectiveness of the host's immune response. The duration of exposure influences the development and maintenance of an effective immune response, which has a considerable energy cost (Sykes, 1997). Internal parasites have a number of effects on the metabolism of the host. The extent to which these effects are produced varies enormously. The effects may be so slight that no symptoms of infection are shown and production is not affected. At the other extreme, a severely affected animal may exhibit marked symptoms of diarrohea, dehydration, anaemia, reduced appetite, loss of body weight and may die. Between these extremes a wide range of effects can occur.

Subclinical parasitism, when the animals do not show any clinical symptoms of disease can still have quite large production costs (Sykes, 1997). Brunsdon & Vlassoff 91982) found reductions in liveweight gain and wool growth of 35% and 15% in weaned lambs on contaminated pasture (200 larvae/kg herbage). This was prior to them establishing immunity.

Young sheep display the most obvious and serious effects of parasitic disease but they acquire more immunity as they become older. This does not mean however (West, 1998) that older sheep are not affected by parasites. Ewes can suffer significant reductions in wool and lamb production from the effects of parasites, even if they ward off the infection and do not develop clinical disease. In addition, some ewe flocks may develop clinical parasitism with loss of body weight, scouring and even death in some instances. Such farms are usually highly stocked, have high sheep to cattle ratios, low feed availability, low ewe body weights and high FECs (West, 1998).

Clinical data indicates that 12% of two-tooth ewes and 8% of mixed-age ewes have mean FECs and above 500 epg, a level considered sufficiently high for some ewes to suffer clinical parasitism (West, 1998). Two-tooth ewes are more susceptible than older ewes to clinical parasitism and their parasite burden should be assessed separately. *Haemonchus contortus* (barber's pole worm) is one parasite that is important in adult sheep as deaths in ewes, as well as lambs, have been reported regularly from this parasite (West, 1998).

Losses in cattle are less well documented and they may be more resistant than sheep to gastrointestinal parasites (Sykes, 1997). Use of anthelmintics has led to improvements in liveweight gain of 20-65% (Somers *et al*., 1987; Enterocasso *et al*., 1986).

In addition to the effects specifically on the animal, there can be a considerable cost to the production system from failure to meet target slaughter or drafting weights or dates (Sykes, 1997).

A few nematodes such as *Haemonchus* and *Bunostomum* suck blood and most of their effects are due to this. However, the majority are mucosal browsers and do not suck

blood. They produce their effects through a complicated interaction between the parasites and their hosts. Although there are differences in the effects caused by various species, there is a basic pattern.

The general effects of gastrointestinal nematode infection is a partial or complete loss of appetite; interference with the production of digestive juices; damage to the lining of the alimentary tract so that materials such as proteins leak into the gut from the blood stream; diarrhoea which leads to dehydration; and possibly interference with digestion and the absorption of digested nutrients. Some of the mechanisms are not clearly understood (Fox, 2000), but they do interfere with the host's overall metabolism of protein, energy and minerals.

Intake: A common feature of the infection of a susceptible animal with nematode parasites is reduction in feed intake and there is evidence this is both centrally (brain) and hormonally mediated (Dynes *et al*., 1997; Fox, 2000). The reduction can range from 16-20% in chronic subclinical infections (Coop *et al*., 1982; Sykes & Coop, 1976; Sykes *et al*., 1988) to complete inappetence before resistance can develop. As a consequence, efficiency of use of metabolisable energy (ME) is reduced because a larger proportion of energy intake has to be used for maintenance.

Feed digestion and absorption: The evidence that feed digestion is impaired is less clear. Most studies have found no or only small (<2%) reductions in feed energy digestion (Sykes & Coop, 1976; Sykes *et al*., 1998) though another study (MacRae *et al*., 1982) did record larger effects.

Protein and energy metabolism: Infection induces protein deficiency, not because protein absorption is impaired (Poppi *et al*., 1986; Bown *et al*., 1991), but because damage to the alimentary tract increases losses of serum proteins (Holmes, 1993; MacRae, 1993), sloughing of epithelial cells and mucus secretion into the gut (Poppi *et al*., 1986). The alimentary tract, accounts for about 5% of total body protein and 25-45% of body protein synthesis, compared with muscle which, makes up 45% of body protein mass and accounts for about 20% of body protein synthesis (Attaix *et al*., 1987). The damage to the alimentary tract causes major stimulation of body protein synthesis and therefore ME requirement for maintenance (Sykes, 1997). Not only is lost protein reabsorbed with lowered efficiency (70-85% is reabsorbed (Poppi *et al*., 1986; Bown *et al*., 1991)) but the additional protein synthesis has an energetic cost (Sykes, 1983). The shift in protein synthesis away from the carcass towards the liver and alimentary tract, causes a reduction in efficiency of ME utilisation for growth (Sykes, 1997).

There is little information on changes in specific amino acid metabolism though changes in gastrointestinal secretions and the immune response are likely to increase requirement for sulphur-amino acids (MacRae, 1993; Sykes, 1997) (see later).

Mineral metabolism: Bone growth is reduced by nematode infection and prolonged infection can result in osteoporosis (Sykes & Coop, 1975, 1977). Matrix osteoporosis probably is due to diversion of amino acids away from bone and muscle to the alimentary tract (Sykes, 1997). In addition, absorption of phosphorus is impaired in infection of the proximal small intestine (Wilson & Field, 1983) resulting in hypophosphataemia, bone osteoporosis (Sykes & Coop, 1975) and slower skeletal growth. Infection with haematophagic parasites, such as *Haemonchus contortus* can result in anaemia

(Jennings, 1976). Evidence of trace element metabolism is less clear, though copper absorption is reduced in animals with elevated abomasal pH (Bang *et al*., 1990). Evidence of other effects on trace element metabolism or trace element status suspected on the basis of clinical experience is still mainly anecdotal (Sykes, 1997).

Gut Nematode Species

The species which are dominant vary, especially with season; species also differ in their ability to cause disease (Brunsdon *et al*., 1975). Generally, the severity of infections increases as the number of nematodes increases, but the relationship is not a simple one. Even within a single group of animals, a wide range of effects may be evident (Brunsdon *et al*., 1975, Vlassoff *et al*., 2001).

There are some instances where particular worms produce distinctive diseases:

- *Haemonchus* can be particularly damaging and, under certain conditions, is able to build up in numbers very quickly. It can cause outbreaks of disease in sheep which suffer a rapid and often fatal anaemia with little weight loss. This has no similarities to other worm infections.
- *Nematodirus* larvae have an exceptional ability to over-winter on pasture so that large infections may occur in young lambs before other species become numerous.
- *Ostertagia* can cause severe disease in adult cattle, associated with the accumulation of large numbers of immature nematodes whose development has been arrested within the animal.

Lungworms

The lungworms affect their hosts differently from the worms in the alimentary tract (Brunsdon *et al*., 1975; Pomroy, 1997a). The mature lungworms live in the airways (bronchi and bronchioles) in the lung. The young larvae reach the lung in the blood and break out into the air passages. This damages the lung and may cause some coughing. As the worms grow, fluid builds up in the lungs and blocks the small air passages so parts of the lung cannot function properly. Depending on how much of the lung is affected, animals show signs of bronchitis, coughing and difficulty in breathing; and in some cases pneumonia. The worst effects occur 2 to 3 weeks after infection; from about the 4th week, most animals start to recover and become strongly resistant to reinfection. Almost all cattle and sheep become infected with lungworms during their lives. Symptoms of infection are often seen in cattle, but rarely in sheep.

3.5.1.2 ***Trematodes*** (Brunsdon *et al*., 1975; Charleston, 1997b; Pomroy, 1997a; Southey & Hosking, 1998)

Liver Fluke

Pathogenesis of disease and its clinical consequences: The disease conditions caused by *Fasciola* can be considered under three headings: that caused directly by migrating juvenile flukes, that associated with adult flukes in the bile ducts, and the clostridial toxaemia secondary to fluke damage commonly referred to as Black Disease or infectious necrotic hepatitis (Charleston, 1997b).

Most experimental studies of the disease have been carried out on sheep but are generally applicable to cattle. He main differences are that in cattle the reactive fibrosis in the liver is more marked and affected bile ducts become calcified. The disease processes

have been described in detail in numerous papers and reviews (cited by Charleston, 1997b).

Acute and subacute fasciolosis: Large numbers of migrating flukes cause traumatic damage to the liver parenchyma as they migrate through it for a minimum of five weeks and often longer, especially in cattle. They feed on liver tissue, damaging blood vessels and liver parenchyma, and the damage increases as they grow. This phase of the life cycle is the cause of *acute fasciolosis* which results from the migration of large numbers of flukes, usually a few thousand, acquired over a short period of time.

The acute disease in sheep is sudden and often results in deaths before the flukes enter the bile duct. Affected animals are weak and lethargic with pale mucous membranes, signs of abdominal pain and possibly ascites. At necropsy, the liver is swollen with haemorrhagic tracks caused by the migrating fluke clearly visible. The ventral lobe is most affected but most of the liver can be involved. Subcapsular haemorrhages, fibrinous material attached to the liver capsule, and a blood-tinged exudates in he abdominal cavity are usually present. The liver is easily broken up in water, and if this is done, large numbers of immature flukes, about 3-10 mm long will be seen. Deaths usually occur 4-6 weeks after ingestion of the metacercariae, but sometimes earlier, in which case the flukes will be smaller. Acute disease has rarely been reported in NZ.

Acute fasciolosis is very rare in cattle although it has been produced experimentally and a field outbreak has been described overseas. It has not been recorded in NZ.

Subacute disease in sheep is also associated with ingestion of large numbers of metacercariae but less than in the acute disease. Deaths usually occur 6-10 weeks after infection so there is more time for animals to develop clinical signs. Affected animals lose condition rapidly; are severely anaemic and hypoalbuminaemic. The liver is enlarged and shows extensive migration tracks, large numbers of flukes in the parenchyma and bile ducts, and fibrinous tags on the liver capsule.

Little has been published on subacute disease in cattle.

Chronic fasciolosis: This is associated with adult flukes in the bile ducts and it is by far the most important in all classes of stock. Depending on the level of infection and its duration, it is characterised by poor growth or loss of condition, lowered productivity, anaemia, hypoalbuminaemia (low blood albumin) and, in some cases, diarrhoea. The pathogenesis is complex.

The adult flukes in the bile ducts feed on bile duct lining, ingesting and causing loss of blood and plasma proteins into the bile. Anaemia and hypoalbuminaemia develop over a period of weeks -the rate depending on the numbers of flukes present and the nutritional status of the animal. The anaemia is primarily caused by blood loss with secondary effects on the bone marrow due to induced iron deficiency and disruption of protein metabolism. Plasma albumin loss is of major importance and is caused by leakage through the bile duct epithelium in addition to that lost with blood. Levels of plasma globulins increase, probably reflecting antibody responses to infection.

In both sheep and cattle, fluke infections cause loss of appetite. Reduced protein intake also affects iron absorption. The severity of fasciolosis is increased markedly by poor nutrition.

Erosion and inflammation of the bile ducts leads to hyperplasia of the duct lining and fibrosis of the walls which, in cattle, is followed by varying degrees of calcification. Bile ducts, particularly those of the ventral lobe where most of the flukes are found, become enlarged and visible in the visceral surface of the liver. Fibrosis of the liver parenchyma, chiefly of the ventral lobe, results mainly from the physical damage and necrosis caused by migrating fluke.

At necropsy, the enlarged bile ducts and fibrosis of the ventral lobe of the liver are characteristic lesions. Poor carcase condition, and signs of anaemia and hypoalbuminaemia are other prominent features. Overall the liver is usually enlarged as although the ventral lobe is fibrosed and often reduced in size, there is hypertrophy of the remainder. However, in longstanding or very severe infections, the whole liver may be shrunken. The numbers of adult flukes present will vary with circumstances but, as a guide, it is considered that 100-300 adult flukes can kill an adult sheep in 3-5 months. In clinical fasciolosis in calves or yearlings, fluke numbers usually exceed 200.

Black disease: infectious necrotic hepatitis: This is a sporadic bacterial disease secondary to invasion of the liver by *Fasciola*. Necrosis of the liver caused by migrating fluke can provide anaerobic conditions suitable for the multiplication of clostridia (*Clostridium novyi* type B) present in the liver. The bacteria produce toxins which are rapidly fatal. Black disease is more commonly recorded in sheep than in cattle. The disease does not require the presence of large numbers of migrating fluke. It can be prevented by vaccination.

Many animals carry *Fasciola* infections which have no detectable effect on health and productivity; on the other hand, some sheep may die of the chronic disease, usually in winter. An important aspect of the disease is the condemnation of affected livers from slaughtered animals.

Rumen Fluke

The importance of the rumen fluke has not been fully documented, but is generally regarded as unimportant as clinical cases are rare (Bisset, 1994; Pomroy, 1997a).

Pathogenesis of disease and its clinical consequences: The adult flukes are generally considered to be of little or no significance. Very heavy infections may cause some low-grade rumeno-reticulitis. It is the migrating immature flukes that are the pathogenic stage of the life cycle. If several thousands of these are migrating simultaneously, they cause severe damage to the upper small intestine and abomasum. This results in severe diarrhoea, rapid loss of condition and dehydration, and in some cases, death.

Most reports of disease outbreaks are anecdotal and involve cattle. It is generally considered that cases occur more commonly in the West Coast regions of the South island than elsewhere. There is one report of probable clinical disease in sheep in the Manawatu (cited by Charleston, 1997b).

3.5.1.3 ***Cestodes*** (Brunsdon *et al*., 1975; Pomroy, 1997a, b; Southey & Hosking, 1998; FECPAK, 2001a).

Little is known of precisely how *Moniezia* affects its ruminant hosts. In spite of their size and the fact that large numbers of cestodes can be present in an animal, it was believed for years is that symptoms of infection or effects on productivity are rare (Brunsdon *et al*., 1975).

This is still the commonly held belief, but the effect of *Moniezia* on productivity has caused ongoing debate (Pomroy, 1996; Mason *et al*., 2002). Sales of tapeworm drenches increased significantly during the 1990s (Mirams, 1999). Tapeworm in lambs causes an emotional response from farmers because of the obvious expelled proglottids (Mason *et al*., 2002). Because they are so visible, large in size and numbers in lamb faeces, many farmers are convinced that tapeworm infection must be causing some to the animal and lowering production.

Over the years there have been many investigations in NZ, all but one have failed to show tapeworms cause production losses. Earlier trials using niclosamide (Elliott, 1984) found no benefits in terms of weight gain, faecal consistency, or dagginess between treated and untreated lambs. This supported the results of investigations carried out elsewhere and reviewed by Elliott (1986). The exception was Southworth *et al*. (1996), who reported the results of four controlled slaughter trials and one productivity trial, with the latter demonstrating a production response to praziquantel. In FECPAK (2001a) it is stated that "There does not appear to be any substantial evidence to support the belief held by many farmers and probably some veterinarians that tapeworms affect growth rates in young sheep and cause diarrohea". The confusion is largely caused by poor experimental design and reporting, and the use of inappropriate anthelmintics (Elliot, 1986; Mason *et al*., 2002). The one recent positive result (Southworth *et al*., 1996) should be balanced against all the other NZ trials that showed no difference (Elliott, 1984; Brunsdon, 1964; Mason, 1986; Mason *et al*., 2002; Pomroy, 1997b). The reason for the one positive response to anthelmintic could be due to a number of causes other than tapeworm (Southey & Hosking, 1998). Virtually no work has been done with *M. benedini* in cattle.

M. expansa has no alimentary tract but absorbs nutrients through the body surface and does not feed on the gut lining (Southey & Hosking, 1998). In contrast, roundworms feed on and burrow into the gut wall, causing considerable damage and inflammation which results in production loss.

Probably few parasites have been the subject of more misleading and unsubstantiated claims of importance than has *M. expansa*. Tapeworms in sheep have been blamed for causing digestive disturbances, intestinal blockage, anaemia, diarrhoea, constipation, stunting, emaciation, and depressed liveweight gain and wool production (Southey & Hosking, 1998). These effects could have been caused by other parasites or pathogens (e.g. roundworms or various microorganisms) or by trace element deficiencies. There have also been unsupported claims of increased susceptibility to pulpy kidney (enterotoxaemia), trace element and mineral deficiency, and of anal discharges (with or without scouring), which predispose lambs to flystrike, caused by tapeworm infection (FECPAK, 2001a; Southey & Hosking, 1998).

3.5.2 Host Responses to Helminth Parasites

In any discussion of host-parasite interaction, mention must be made of the concepts - resistance and resilience (Bisset *et al*., 2001; Sykes *et al*., 2001). Resistance has been used to describe the ability of the host to prevent or limit establishment or development of infection, and resilience - the ability to maintain a reasonable level of production when subjected to a parasitic challenge (van Houtert & Sykes, 1996). The latter concept can also be referred to as "tolerance" (Bisset *et al*., 2001). These two concepts cannot be independent (Sykes & Coop, 2001). Both are assessed using FECs, yet "resistance" can be defined in terms of the ability of an animal to limit or suppress larval establishment, and the development of the helminth infection. Thus, lambs described as resistant are those able to maintain a relatively low worm burden despite grazing pasture contaminated with roundworm larvae. Most research has focused on the use of FECs in lambs, following a standardised exposure to roundworm challenge, as the most practical indicator of resistance. Lambs whose FECs are below average in a flock left untreated for an appropriate period are considered to be of above average resistance.

"Resilience", on the other hand, can be defined as the ability of a host to withstand the pathogenic effects of worm challenge and/or infection and thus maintain acceptable health and productivity with minimal anthelmintic treatment. The pathogenic effects of roundworm challenge and/or infection can include depressed growth rates and wool production, diarrhoea and/or anaemia or even death, depending on the predominant species of parasite involved and the severity of challenge. In theory, resilience could be assessed in terms of any of these parameters, although strictly speaking this would require measurements to be made under both parasite-free and infective environments. In practice, however, this is not easy to achieve and traits such as growth rate are usually measured under infective environments (Bisset *et al*., 2001).

Interestingly, studies carried out under NZ conditions have indicated that lambs whose growth rates are above average despite prolonged roundworm challenge are, in many cases, not those which are most resistant to roundworm infection (Bisset *et al*., 2000). Lambs showing above average productivity under challenge, despite having above average susceptibility to worm infection, can be described as "tolerant".

In practice, increased resistance would be expected to lead to improved resilience or performance in the face of incoming larval challenge (Sykes & Coop, 2001). Yet NZ selection studies for improved resistance have generally not yielded increases in resilience (McEwan *et al*., 1995; Morris *et al*., 1997; Williamson *et al*., 1997).

Because of the possible confusion to the reader of the use of the term "resistance" to describe both host resistance to parasitism and parasite resistance to anthelmintics, the term host immunity or partial immunity will be largely used to refer to host resistance in this report.

A variety of factors are associated with the ability of sheep to either reject nematodes (resistance), or to serve as permissive hosts without showing evidence of severe pathological change (resilience) (Hein *et al*., 2001).

3.5.2.1 *The Immune Response*

Until comparatively recently, it was thought that ruminants could not develop natural immunity to gastrointestinal nematodes because they were effectively outside the body and could not affect or be affected by the immune system (McFarlane, 1997; Wakelin, 1984). This was found to be not so and the possibility of developing vaccines against internal parasites suggest an additional possible means of control (McFarlane, 1997).

The development of immunity is complex and not completely understood. McFarlane (1997) describes the processes involved. The response of the immune system to infectious agents can be either innate (non-specific) or acquired (specific). The innate immunity is important for initial exposure to parasites and is important in young lambs. However in a temperate climate such as NZ where ingestion of infective larvae occurs throughout the year, acquired immunity is most important (McFarlane, 1997).

In all vertebrate animals, the immune system is made up of two parts: the innate or non-specific immune response and the adaptive or specific immune response. The two immune responses are not entirely distinct. They depend on and interact closely with each other.

Innate immunity: From the immune system's perspective, the first goal is to block any pathogenic organism (i.e. bacteria, virus, or parasite) from entering the body and infecting the animal. This aspect is called the innate immune system and is made up of several barriers. The innate immune system is sometimes referred to as the "non-specific" immune system as it is a general response that does not recognise any pathogen specifically. It is important to know that the innate immune system is highly evolved in mammals as a general defence mechanism against infection. The goal is to ensure the integrity of the innate immune system and to lower immunological stress situations that can successfully breach its barriers and begin an infection.

The innate immune system is made up of four kinds of barriers. The first being the anatomic barrier which provides the physical barrier of the skin or hide as well as the mucous membranes we associate with the mouth, nose, and eyes as well as within the deeper tissue of the respiratory, gastro intestinal, and reproductive systems. The second is known as the physiologic barrier that relies on temperature, acidic conditions in the gut, and complex of non-specific soluble proteins that can kill pathogens. The third is the phagocytic barrier that relies on a group of specialised cells that ingests pathogenic organisms and break them down. The final barrier is the inflammatory barrier or response. This is the immune system's defensive mechanism that is triggered when tissue is damaged by injury or by the invading pathogen and creates conditions that can signal the phagocytic and physiologic defensives that an infection has taken place and then helps to wall-off the infected area so surrounding healthy tissue is not damaged.

If an infection breaks through the innate immune response mechanisms, the second part of the immune system, the adaptive or acquired immune response, is triggered through a complex interaction with the innate response.

These mechanisms are similar with repeated infections. The acquired immune response is dependent on aspects of innate immunity such as phagocytic activity (McFarlane, 1997).

Adaptive or acquired immunity: Adaptive immunity differs from the innate response in that it recognises a specific pathogen and develops a memory that can respond again if reinfection from the same pathogen occurs. This memory response is the basis of why we have "booster" shots for vaccines. The adaptive immune response is made up of two types of specific immunity discussed below. Acquired immunity involves the ability to specifically recognise and selectively eliminate parasites and has four attributes: antigenic specificity, diversity, memory and self/non-self recognition (McFarlane, 1997). This means in terms of gut parasite infections that immunity develops against a wide range of antigens from specific worm species, independently of each other. While immunological memory exists after the infection, it is neither as solid nor long-term as with other infective agents. In general, sheep need to graze the infective larvae, and therefore be exposed to the different developmental stages for up to 12 weeks to develop immunity (Coop *et al*., 1982).

This process was not always apparent in young lambs and it was believed they needed to be 6-8 months of age to allow the development of acquired immunity (Gibson & Parfitt, 1972; Kambara *et al*., 1973). However, Australian research (Kahn & Watson, 2001) recently demonstrated that housed neonatal lambs (i.e. birth-7 weeks old) also mount good protective immune responses to *T. colubriformis*, and moderate immunity to *H. contortus*, and there is evidence that this protection is better than that in older lambs. Immunity to *H. contortus* does not appear to develop as rapidly, possibly because of its greater pathogenicity. In pen trials, daily infection of neonates and weaned lambs with *T. colubriformis* resulted in an 80% reduction in worm burden in the neonates, but only a 28% reduction in weaners, compared with uninfected (control) sheep, following challenge infection, and worm burdens of the neonates were 80% lower than those of the weaned lambs. Lambs can however, develop worm burdens prior to weaning (Foster *et al*., 1991).

Acquired immunity is commonly divided into humoral (antibody or immunoglobulin-Ig based) or cellular mediated mechanisms. Both mechanisms are closely involved in parasitic infections (McFarlane, 1997).

Humoral immunity: Humoral immunity comes from specialised immunity cells (B cells) that produce specific antibodies known as immunoglobins against a pathogen and then produces memory cells capable of reproducing antibodies against reinfection. Antibodies do not generally kill pathogens on their own. Antibodies play a key role in first identifying a specific pathogen and then activating processes that can remove and kill the pathogen. Some antibodies primarily circulate in the bloodstream while other kinds of antibodies are secreted from the mucosal membrane.

There is still some uncertainty over the role of serum antibodies in the expulsion of gut parasites. Even though negative correlations of up to 0.63 (worm burdens) and 0.62 (faecal eggs) have been found between serum antibody levels (especially IgG) and host resistance (Douch *et al*., 1995) in older lambs, it is possible that the associations are indirect. Other factors may be more important. Strong evidence exists that levels of IgA from lymph draining the abomasum indicate protection against *Ostertagia circumcincta* (Smith *et al*., 1987) but this is not often correlated with blood levels of IgA. Levels of serum IgE have been associated with resistance to *Ostertagia ostertagi* in cattle (Baker *et al*., 1993) but not always in sheep infected with *T. colubriformis*. Humoral immunity, either

in the peripheral blood or locally, is important only after sufficient stimulation by parasite antigens and may be less important in young lambs (McFarlane, 1997).

Cell-mediated or cellular immunity: Cell-mediated immune responses occur when a cell is infected with a virus, bacteria, or parasite and signals the immune system cells (known as T cells) that it is infected. T cells then kill the infected cell together with the invading pathogen. As with humoral immunity, there is also a T cell memory response that can be called in at later time if reinfection from the same pathogen reoccurs.

The importance of mononuclear lymph cells (lymphocytes and monocytes) in mechanisms of immunity was initially shown in laboratory animals (Miller, 1984) but later also in sheep (Smith *et al*., 1984), when lymphocytes derived from sites infected with gut parasites conferred resistance when transferred to naïve lambs. It appears that lymphocytes have a major role in the amplification (multiplication of sensitized cells) and regulation (secretion of cytokines) of the immune response. In general, it is believed that a particular type of helper T (thymus derived) cell population is important for helminth infections of livestock (Mosman & Coffman, 1989). These cells secrete cell regulators (interleukins) which promote IgG, IgA and IgE production from plasma cells, mast cell hyperplasia and the increased production and release of eosinophils in the blood (Finkelman *et al*., 1991). The development of a systemic eosinophilia is associated with parasitic burdens such as *T. colubriformis*, but not *Haemonchus contortus* (Windon, 1996). Local hypersensitivity reactions in the gut are frequently associated with the immune expulsion of gut parasites (Stewart, 1995; Jones & Emery, 1991). Mucosal mast cells appear in animals made resistant by repeated infections, which upon being triggered by parasite antigen that activates bound IgE, release vasoactive substances (histamine, leukotrienes, prostaglandin E_2) that cause an inflammatory response allowing plasma antibodies into the but lumen as well as acting directly on the parasites (Douch *et al*., 1983). This response is similar to an immediate hypersensitivity response. Studies have shown that a number of these mechanisms act together and that if they are antagonized individually then overall host immunity is not greatly impaired. The helper T cells (CD4) appear to be critical in mature animals (Gill *et al*., 1993).

For the important gastrointestinal nematodes, the major components of the immune response must be effective locally in the alimentary tract (Sykes *et al*., 2001). Although circulating antibody responses can be measured, it is now accepted that a local cell-mediated response is crucial and that changes in mast-cell populations and their secretions have, increasingly, been correlated with change in immune competence (Dough *et al*., 1986; Coop *et al*., 1995; Huntley *et al*., 1995; Houdijk *et al*., 2000). These changes are undoubtedly directed by a multifactorial cell signaling system involving cytokines which is not yet fully understood (Sykes *et al*., 2001; McFarlane, 1997).

The resultant immune response may interfere with worm development by: rejection of incoming larvae, retardation of larval development, rejection of adult worms, and interference with worm fecundity (Miller, 1984). Parasite species differ in certain aspects of immunity (McFarlane, 1997). *Nematodirus battus* stimulates a particularly rapid response to primary infection leading to a high degree of protection. In general, blood sucking helminths such as *H. contortus* are more affected by systemic immunity as compared with mucosal browers such as *T. colubriformis* which are especially affected by local immunity. The rate of ingestion and development time (>7 days necessary for *T. colubriformis*) of infective larvae may influence the type of immune response and

subsequent protection level. Some particular immune responses may need a certain threshold of infection to trigger them and persistent exposure to parasites may be necessary to maintain a long term immune response.

The typical development of immunity is described by Sykes (1997) from the trial of Kimambo *et al*. (1988) using clean lambs that were infected daily for 34 weeks with 2500 larvae of *Trichostrongylus colubriformis*. The typical rapid rise in nematode eggs in faeces during the period 2-8 weeks after commencement of infection was observed indicating a period of establishment of a mature worm population. The subsequent rise in eosinophil count is indicative of developing immunity at the height of which numbers of nematode eggs in the faeces were falling rapidly. Loss of weight gain was most closely associated with the period of rise in eosinophil count. From about 14 weeks after infection commenced, numbers of eosinophils began to fall and growth rate returned to normal, despite continuing exposure to nematode larvae. These changes demonstrate two phases - one of active development of host immunity followed by the immune phase itself. The immunity will wear off, if continual or repeated parasitic challenges are not received by the host animal.

There is widespread variability in sexually reproducing populations of parasites and they have evolved systems to help them evade the host's immune system. For example, adult worms of *T. colubriformis, H. contortus* and *O. circumcincta* secrete enzymes (cysteine proteases) that can cleave and inactivate blood clotting factors and antibodies, and disable lymphocytes (Riffkin *et al*., 1996).

The Host Reaction to Infection

How well an animal responds to an infection depends directly on its general health, prior immune status against the disease, and how much stress it is exposed to. A healthy animal under low levels of stress that is either resistant to the infection or has built up an immunity through either previous exposure or vaccination will generally be able to generate a high level of immune resistance. If however the animal is in poor condition, has a low immune status against the disease pathogen, or is under some form of other stress, it will not respond well to fighting an infection.

The immune response involving local inflammatory reactions, epithelial cell secretions and antibody production (Sykes & Coop, 2001), has significant nutritional costs, as yet not quantified.

Many of the pathological changes in the gastrointestinal tract of parasitised sheep probably occur as an undesirable consequence of an exaggerated immune response against the parasites (Hein *et al*., 2001). In heavy infections, the parasites themselves undoubtedly inflict significant damage directly, particularly in the case of blood feeders such as *H. contortus*. However, in some animals, moderate nematode infections may give rise to clinical signs and pathological changes which seem out of proportion to the pathogen load. Evidence clearly suggests that these animals have a genetically-determined propensity to mount an exaggerated allergic-type response to nematode antigens, which then causes inflammation and other changes in the intestinal tract. An exaggerated hypersensitivity response in predisposed hosts then leads to the undesirable clinical outcomes often associated with nematode infections - dysregulation of gastrointestinal physiology, scouring and dags, retarded muscle and wool growth and a general decline in health and body condition. Heath *et al*. (2000) state that "Almost

without exception parasitic worms do not harm stock: it is the response of the stock to worms that causes harm". This will not be true for blood feeding parasites but is probably close to the truth for the mucosal browsers.

Sheep generally do not develop full resistance to parasites until 8-24 months of age (van Houtert, 1997). There is large inter-animal variation in the rate of development of resistance, both within the same breed and between breeds, which appears to have a strong genetic basis. In addition, there are strong age effects and, as will be examined later, nutritional effects. The acquired immune response is an important component of host resistance to parasites. However, acquired immunity is complex and poorly understood (McFarlane, 1997). Different components of an acquired immune response appear to target different phases of parasite development within the host, starting with the prevention of establishment of incoming infective larvae or an arrested development of these larvae and/or stunting as adults (McFarlane, 1997). Fecundity of female worms may also be reduced and the final manifestation of acquired immunity is the rejection of established worms. Most work on the influence of diet on the development of immunity or resistance has been done with young susceptible sheep. Studies with other young ruminants or with periparturient ruminants are less common.

Age & Genetic Factors

A feature of ruminant immunity to gut nematodes is the large between-animal variation in response (Bisset *et al*., 2001; McEwan *et al*., 1997; McFarlane, 1997). Resistance differences between sheep breeds or within a breed undoubtedly have significant genetic components (Windon, 1996). The former may be due to innate or acquired differences but the latter appears to be largely a difference in acquired immunity. Attempts to find linkage between resistance and certain genes have been variable. Resistance to the *Trichostrongylus* selection flocks in Australia and NZ and the *Haemonchus* selection flock in Australia have an immunological basis. High responder animals have greater parasite recognition (enhanced cellular and humoral responses) and effector responses (elevated MMC and eosinophil numbers and release of mediators) than low responder animals (Windon, 1996).

Physiological Factors

Physiological status has a marked influence on host immunity. Male sheep have been shown to be more susceptible than females to experimental infections with *T. colubriformis, H. contortus* and *O. columbianum* (Barger, 1993). The reasons are unknown. A temporary loss of acquired immunity to gut parasites around the time of parturition and during lactation has been described in ewes but also occurs in other species such as cattle. This leads to increased worm burdens and excretion of faecal eggs (Donaldson, 1997; Donaldson *et al*., 1995). Ewes may be particularly susceptible to *O. circumcincta* infections late in pregnancy (Donaldson, 1995) and lactating ewes are more susceptible to the establishment of *T. colubriformis* (O'Sullivan *et al*, 1973). Causes suggested include changes in hormonal status, stress, and under supply of nutrients such as protein (Donaldson, 1997; Sykes *et al*., 2001). The resistance of young growing lambs is dependent on adequate dietary protein (Kambara *et al*., 1995; van Houtert *et al*., 1997; Bown *et al*., 1991; Sykes *et al*., 2001) (see later).

Interactions Between Nematodes & Alternative Hosts

Interactions between gut parasites of sheep can occur as a result of cross immunity, or physiological factors such as competition between established nematode species. Thus,

existing infections with *O. circumcincta* interfere with the establishment of *H. contortus* in the same site (Dobson *et al*., 1995) and *T. vitrinus* situated downstream in the small intestine (Jackson *et al*., 1997). Clinically this protective effect may be more than offset by the increased pathogenicity of concurrent mixed infections. However, it could also account for some differences in the seasonal prevalence found in these species. Resistance to infection with heterologous nematodes (*H. contortus* or *T. colubriformis*) in flocks selected for genetic resistance was not as great as for the homologous infection against which the selection was based (Windon, 1996).

It has been frequently noted that goats are more susceptible to gut parasites than sheep are (McFarlane, 1997). This has been associated with a paucity of mucosal mast cells and lower concentrations of mast cell proteinase (Huntley *et al*., 1995).

As with sheep, acquired immunity to *Ostertagiasis* in cattle develops slowly after prolonged or repeated infections, and is more pronounced in degree and faster in adults. Sequential infections of *O. ostertagi* induce serum IGG, IgM and IgA antibody responses to infective larvae (L_3) antigens in calves, with secondary responses. It is believed that his parasite induces an immediate hypersensitivity response in the abomasal mucosa with increased production of IgE, leukotrienes and prostaglandins which are particularly elevated in Type I infections (Baker *et al*., 1993). Limited information is available on the nature of the antigens of *Ostertagi ostertagi*, many of which are cross-reactive with other gut parasites of cattle such as *Cooperia oncophora* (Klesius, 1993).

3.5.2.2 *The Immune Response & Dietary Protein*

The importance of nutrition, especially dietary protein, in the immune response should not be underestimated (Donaldson, 1997; Sykes 1983, 1997, 2000; Sykes *et al*., 2001; van Houtert, 1997). Recent work has shown that the development of host immunity responds dramatically to dietary protein in a number of situations and this has led to a rethink of the protein requirements and physiological priorities for nutrients (Sykes *et al*., 2001). The host immune response to internal parasitism appears to be huge and this is in addition to the substantial nutritional or metabolic cost of the damage *per se* caused by the parasites. The larval and adult stages of nematodes in the gut cause damage to and sloughing of the epithelial cell layers, leakage of plasma and extracellular fluids and an increase in mucus production. These have to be replaced at a cost and as they are proteinaceous, there is an increased cost of protein synthesis to maintain body integrity and function (Poppi *et al*., 1986; Bown *et al*., 1991). In subclinical infections, this can lead to a 50% reduction in growth at the same feed intake (Sykes & Coop, 1976). The combined effects of increased loss of host protein and increased requirement of the gastrointestinal tract for protein synthesis at the expense of muscle growth may reduce production by as much as 25-35% for weight gain, 10-25% for wool growth and 20-30% for milk production, even id subclinical infections (Kahn & Watson, 2001). However, the nutrition of the animal strongly influences the magnitude to which infection reduces production and also the extent to which the animal can develop resistance to infection.

Sheep have been well studied, but there is little information for cattle and goats (van Houtert, 1997) and the limited results are equivocal (van Houtert & Sykes, 1996). A large number of pen trials and a smaller number of field trials with sheep have demonstrated that animals supplemented with “bypass” protein have increased resistance and resilience

to gastrointestinal parasites (Donaldson, 1997; Kahn & Watson, 2001; van Houtert, 1997). Increase supply of digestible protein hastens the acquisition of resistance, which allows animals to more rapidly expel established worms.

Research in NZ has demonstrated that an increased supply of rumen-undegradable protein to young sheep and periparturient ewes reduces FEC and worm burdens, whereas increasing the supply of metabolisable energy (ME) is ineffective (Donaldson, 1997; Kahn & Watson, 2001; van Houtert, 1997).

When the supply of digestible protein to young sheep infected with *T. colubriformis* was increased by the addition of 60g crude protein (CP), worm burdens were reduced by 65%. When naïve, growing sheep were infected with *T. colubriformis* while being infused abomasally with casein they were able to reduce worm burdens and FECs to 50% of those in saline or glucose-infused controls after 12 weeks (Bown *et al*., 1991). As a result of the additional protein infused they were able to maintain normal rates of body growth, suggesting that if the nutritional costs of reacting to nematode exposure could be met and surplus nutrients could be utilised and to promote normal tissue growth. Similarly, casein infusion also accelerated the development of immunity by young sheep to *O. circumcincta* (Coop *et al*., 1975). However supplementation of young sheep on a hay diet with small amounts of fish meal had no effect on the number of *T. colubriformis* after 5 or 10 weeks of infection, but it enhanced the subsequent rate of worm expulsion. This was associated with elevated levels of circulating eosinophils and intestinal mast cell protease concentrations (van Houtert *et al*., 1995a). Young sheep (2-6 months) given a low protein diet (11% CP; based on lucerne hay and concentrate) showed lower resistance to parasites than sheep given a high protein diet (20% CP; with concentrate containing soyabean meal and meat/bone meal), but diet had no effect in older sheep (7-12 months; Kambava *et al*., 1993). Other animal studies have similarly shown increased ability to limit worm burdens or FECs in animals on improved dietary protein supply (van Houtert *et al*., 1995b; Wallace *et al*., 1995; Knox & Steal, 1996). More specifically, supplementation of sheep with sodium caseinate while they were exposed to *T. circumcincta* larvae resulted in a threefold greater mast-cell protease activity in abomasal tissue and lower worm establishment than in non-exposed sheep offered the supplements, when both were subsequently challenged with infection (Coop *et al*., 1995).

Australian work (Kahn & Watson, 2001) has also demonstrated that both increased protein and ME supply can increase production of infected sheep to levels equivalent to those from uninfected sheep not receiving the supplement. Of significance to the economics of using nutrition for worm control; are findings that beneficial effects of protein supplementation on resistance to infection, growth rate and wool growth lasted up to 15 months after cessation of supplementation. Currently further work is further investigating whether protein supplementation will be a useful strategy to avoid production losses while encouraging the acquisition of immunity in weaned lambs, and over what time period beneficial effects are expressed. Similar trials with weaned ewe lambs to determine the effects of short-term supplementation on immunity to infection, liveweight gain and subsequent reproductive performance are also underway.

Recent NZ work which has also shown that the typical relaxation of the established immunity of the breeding ewe in the peripartum period can also be prevented by feeding protein supplements (Donaldson *et al*., 1998; Houdijk *et al*., 2000, 2001; Donaldson *et al*., 2001), has opened the question of the nutritional cost of the immune response. Typical

results show when the supply of digestible protein was increased to periparturient ewes infected with *T. colubriformis* and *O. circumcincta*, worm burdens shortly after lambing were reduced by 87%. The most recent studies (Donaldson *et al*., 2001) have suggested that the nutritional cost of maintaining immunity in late pregnancy and early lactation under larval challenge may require metabolisable protein supplies 30% higher than conventional estimates. This may not be surprising since conventional estimates are largely based on trials using penned sheep in which nematode parasitic challenge is non-existent (Sykes *et al*., 2001).

Australian research (Kahn & Watson, 2001) has demonstrated that supplementation of periparturient ewes with cottonseed meal (CSM) for the six weeks immediately prior to lambing lowered FEC. Cottonseed meal typically contains 36% CP of which about 50% is rumen undegradable. Supplementation with 250 g/day CSM reduced FEC by about 50% in both years. Australian work has also looked at non-protein nitrogen (NPN) supplements (Kahn & Watson, 2001). When livestock are grazed on low quality roughage, the most critical nutritional deficiency is often nitrogen. Provision of NPN, such as urea, in the diet can compensate for this deficiency and stimulate feed intake, increase the supply of microbial protein and improve feed utilisation. A trial has demonstrated that NPN supplementation (urea as 3% of total intake) to infected animals on low-quality feed increased feed intake, liveweight gain and wool production with some benefits to worm resistance. NPN supplementation to infected animals on low quality roughage can result in similar levels of production to those from uninfected animals without NPN supplementation.

Whether the beneficial effects against internal parasites in ruminant livestock, achieved by feeding high protein diets and/or rumen bypass protein and/or forages containing condensed tannins (see later) (Bown *et al*., 1991; Donaldson *et al*., 1997a; Hoskin *et al*., 2000; Houdijk *et al*., 2001) is due to protein *per se* or specific amino acids is unclear. Supplementation with specific amino acids may elicit similar responses (Hoskin *et al*., 2002) and recent knowledge indicates that responses by animal defence mechanisms to infection involve the amino acids cysteine and glutamine (Malmezat *et al*., 1998; Lobley *et al*., 2001). Demand on muscle cysteine stores by the immune system is thought to arise from synthesis of acute-phase proteins, leucocytes plus the antioxidants glutathione and taurine (Breuille & Obled, 2000; Malmezat *et al*., 1998, 2000). Glutamine is a fuel and precursor for protein and nucleic acid synthesis (Gate *et al*., 1997). A high demand for glutamine comes from proliferating immune cells such as lymphocytes and the cells of the gastrointestinal tract. Glutamine supplementation has been shown to assist in the recovery of surgically-damaged gut tissue and hence may have a role in enhancing repair of parasite-damaged intestinal mucosa (Lobley *et al*., 2001). This suggests that specific amino acids may have a role in the immunity to internal parasites (Hoskin *et al*., 2000).

Daily abomasal supplements of cysteine and glutamine to *T. colubriformis* infected lambs increased nitrogen retention, reduced circulating eosinophils and changed the FECs, however did not influence final nematode counts (Hoskin *et al*., 2002). These responses were less than would be expected from a general protein supplement so the role of specific amino acids remains unclear.

These recent findings have led to a hypothesis as to where the immune response fits as a priority in the body's physiological processes, especially the nutrition - immunity interactions in parasitism (Coop & Kyriazkis, 1999). Improved nutrition increases host

resilience, but the mechanism and priorities appear to change with the stage of development of the immune system. Acquisition of immunity in the young lamb has a higher priority than growth but, in the adult, immunity will have lower priority than the demands of pregnancy and lactation. This fits with clinical observations of depression of growth in lambs during infection, but relaxation of immunity in ewes in the face of the competing nutrient demands of pregnancy and lactation. Both demands appear to be met if protein supply is adequate and this is in keeping with findings on protein supplementation (Bown *et al*., 1991; Donaldson *et al*., 1998; Houdijk *et al*., 2000; Donaldson *et al*., 2001). It also fits well with the findings of reduced immunity (and resilience) in ewes bearing or rearing multiple lambs (Donaldson, 1997, 1997a; Donaldson *et al*., 1995, 1997, 1998; Houdijk *et al*., 2000, 2001), reflecting their greater nutrient demand and in ewes in poor condition (HISHA & SAC, 2001).

The reason for loss of resistance parasites in ewes around lambing until recently was poorly understood (Donaldson, 1997). Poor nutrition, stress or lack of antigenic stimulation have been suggested (van Houtert, 1997). The most favoured hypothesis has until recently been an impairment of immune status triggered by immunosuppressive hormones such as prolactin, corticosteroids, progesterone or oestrogens, but the evidence is not compelling (Donaldson, 1997). The dietary protein story is much more plausible.

Sykes *et al*. (2001) questions whether the protein supply from pastures is adequate for a number of physiological states or to meet the increased demands of parasitism. This is a crucial question for the NZ pastoral industries because of their reliance on ryegrass-white clover systems and lack of use of costly supplements. There is now evidence that pasture does not provide the protein requirements for newly weaned lambs less than 30 kg or prolific ewes during late pregnancy and lactation (Robinson, 1990). The protein requirements will be even higher in parasitised animals. Sykes *et al*. (2001) pose the question “Can we use our increasing knowledge of the nutrient demand of the immune system to design more effective feeds to increase resilience through increased resistance”.

3.5.2.3 *Trace Elements & Minerals*

In addition to protein and energy, dietary deficiencies or variables inhibiting the utilisation of minerals can also limit the ability of the immune system to deal with parasites (Kahn & Watson, 2001). Trace elements are components of enzymes and therefore have key roles in biochemical reactions in animal physiology. Increased metabolic activity can induce clinical signs of minerals deficiency in animals with subclinical deficiency. Cells with a short half-life such as lymphocytes are particularly sensitive to trace element deficiency. Theoretically, therefore, deficiencies of most of the trace elements could affect the development of protective immunity to worms, but this is a relatively unexplored area.

There is increasing evidence that minerals may be important but their effects are less well established than for protein (Sykes *et al*., 2001). In one study, worm establishment was reduced by 90% when sheep were offered a diet containing 2.75 rather than 1.88 g P/kg DM (Coop & Field, 1983). This effect may be mediated via a rumen effect on microbial protein supply. It is unlikely that this will be important in NZ.

Other studies have indicated that Co deficiency may be associated with reduced immunity to nematode parasites (Clark, 1982; Kahn & Watson, 2001; Ferguson *et al*., 1989; Gruner, 2001). Again, no mechanism has been advanced. There may be a specific requirement, as yet undefined, of the immune response for vitamin B_{12} (Sykes *et al*., 2001). In an Australian trial (Kahn & Watson, 2001) there were no differences between Co bullets or monthly B_{12} injections.

Copper undoubtedly has direct anthelmintic properties, especially against abomasal parasites (Bang *et al*., 1990, 1990a; Kahn & Watson, 2001). While Cu therapy using Cu-oxide wire particles (COWP) reduced the establishment of incoming larvae of *Haemonchus contortus* and *Ostertagia circumcincta* by 95 and 65%, respectively, it had little effect against larvae of *T. colubriformis*. This is probably a direct anthelmintic effect rather than relieving Cu deficiency or an effect on the immune response.

There is also evidence that the outcome of a larval challenge may be influenced by dietary Mo concentration (Suttle *et al*., 1992a, b; McLure *et al*., 1999). In two of these trials (Suttle *et al*., 1992a, b), addition of Mo to the diet reduced worm populations of *T. vitrinus* and *H. contortus* by 23% and 78%, respectively. Molybdenum is a well known Cu antagonist in ruminants, but the effects could not be attributed to Cu depletion. More recent independent studies (McLure *et al*., 1999) have shown 90% lower FECs and worm burdens in lambs offered diets containing 6-10 mg Mo/kg DM compared with lambs offered diets containing Mo above or below this range. Correlated responses in intestinal antibody, jejunal mast cell and blood eosinophil numbers suggest that Mo has an important role in the cell-mediated immune response. It is possible that Mo enhances the inflammatory response by increasing superoxide radical concentration in the mucosa either directly, or by reducing the effectiveness of local trace-element-dependent anti-inflammatory enzymes (Suttle *et al*., 1992a, b). Field trials on supplementation with Mo have not yet been done (Kahn & Watson, 2001). Further studies may indicate that interactions with other minerals affect the ideal range of Mo concentrations, and caution will need to be exercised, as supplementation of a diet already adequate in Mo or deficient in Cu may result in toxicity.

3.5.2.4 *Development Periods of Host Immunity*

Nematodes

Animals infected with most parasites develop an immunity just as they do to other disease-producing agents such as bacteria. With most nematodes, the development of resistance requires a period of infection lasting several months (Brunsdon *et al*., 1975). However, the rate at which animals become immune to particular parasites varies considerably for a number of reasons:

- Parasite species differ in their ability to stimulate immunity.
- Individual host animals have inherited differences in their ability to develop immunity.
- Animals develop immunity more rapidly as they grow older.
- Infection rates are not constant throughout the year but have seasonal and short-term variations.
- Animals infected at too fast a rate or subjected to malnutrition or other diseases may be overwhelmed and are unable to develop and maintain a level of immunity sufficient to protect themselves.

Animals exposed to infection at low levels for sufficient time can develop a satisfactory level of immunity without ever showing signs of infection. Periodic reinfection helps to maintain this resistance (Brunsdon *et al*., 1975; Vlassoff *et al*., 2001).

Lambs and calves progressively develop immunity to the various nematodes they encounter over the first 10 to 15 months of life. Goats take considerably longer. Once immunity to a nematode is fully developed, infective stages taken in are unable to establish and mature except in extremely small numbers. With some nematode species there may be a phase during the development of immunity where larval stages taken in by the animal are inhibited or retarded in their development; these inhibited larvae may later be destroyed but, in certain circumstances, may resume development and cause disease.

Young lambs have little innate ability to resist infection, but because of their initially low intake of herbage, worm numbers in their gut build up slowly during spring and early summer, reaching a peak in autumn. Lambs can however develop small-moderate worm burdens pre-weaning, especially *Haemonchus* and *Nematodirus* (Foster *et al*., 1991). A rapid decline in worm burden in winter is associated with the development of a significant immune capability. Following the elimination of the major part of their worm burdens when they are 10-12 months of age (Brunsdon, 1970a) sheep tend to remain relatively resistant to serious re-infection (Vlassoff *et al*., 2001) if they are periodically challenged.

The level of immunity of animals can be lowered in some circumstances, not all of which are understood as discussed earlier. As discussed earlier in some normal physiological states, immunity may be temporarily relaxed; this occurs during lactation in sheep when increased outputs of worm eggs may occur (Brunsdon *et al*., 1975; Donaldson, 1997; Donaldson *et* al., 1995, 1977, 1997a; 1998, 2001; Vlassoff *et al*., 2001; Sykes & Coop, 2001).

Adult sheep can suffer serious effects from *Haemonchus* infection because resistance to this parasite appears particularly short-lived and easily broken down (Brunsdon *et al*., 1975).

Once sheep have acquired full immunity, however, they require constant exposure to some level of infection to maintain their resistant status. Sheep's ability to maintain immunity at a high level can also fail at times of nutritional stress, illness at any age, and declines in breeding ewes around the time of lambing (Brunsdon, 1971). The latter effect results in the well-known peri-parturient rise in FEC in lactating ewes in which their immune capability is compromised by the stresses imposed by pregnancy and lambing, often aggravated by sub-optimal feed intake and has been discussed in detail earlier in this review. Some of the larvae ingested over the lambing period are therefore able to establish and develop to maturity. There may also be an associated maturation of inhibited larvae (Brunsdon, 1966b) and increased fecundity of existing adult worms. Soon after giving birth, however, the ewe's ability to resist ingested larvae returns, so that she is largely refractory to larval establishment by four weeks after parturition (Leathwick *et al*., 1999a). The worms which have already established remain in the ewe for several weeks longer, producing a characteristic peak in FEC, 6-8 weeks after parturition (Brunsdon, 1971), before the majority are expelled. Removal of these worms has been the basis of the strategic use of an anthelmintic treatment of ewes about four weeks after lambing, although more recently this has tended to be replaced by the pre-lamb administration of a

long-acting anthelmintic or controlled release capsules (Vlassoff *et al*., 2001). The routine use of this practice is now questionable.

Cattle, at times, can accumulate inhibited larvae of *Ostertagia* in large numbers. This can occur even in adult animals. In circumstances which are not understood, these larvae can suddenly resume development and cause severe disease in these mature animals. If this occurs, it is normally some months after the infection was acquired (Brunsdon *et al*., 1975).

In general, however, animals develop a level of immunity that will protect them against any level of infection they encounter. They can ingest infective larvae without any adverse consequences. They also reputedly contribute relatively few nematode eggs to contaminate the pasture (Brunsdon *et al*., 1975; Vlassoff *et al*., 2001).

Trematodes

Sheep do not develop immunity to reinfection with *Fasciola*. As this parasite is also very long-lived, sheep may accumulate infection over several years (Brunsdon *et al*., 1975; Charleston, 1997b). Recent research indicates that cattle do develop a measure of resistance to *Fasciola* infections but little is known at present of the effectiveness of this under field conditions. With cattle, previous infection does lead to a substantial level of resistance to re-infection and animals may reject established flukes either spontaneously or in response to re-infection (Charleston, 1997b). There is some debate about whether this resistance is, in fact, the result of immunological responses *per se* or attributable to fibrosis of the liver and fibrosis and calcification of the bile ducts, interfering with the ability of flukes to develop and survive. The result is that young cattle are more susceptible to infection and older animals, following exposure to infection, become relatively highly resistant. This resistance does not always provide total immunity to the effects of fluke. Re-infection can cause further liver damage even if few or no flukes reach maturity; any that do mature are unlikely to survive. The development of resistance by cattle also has implications for the relative importance of cattle and sheep as sources of infection for snails.

Cestodes

Both sheep and cattle develop a strong immunity to *Moniezia* infections at 4 to 6 months of age (Brunsdon *et al*., 1975).

Lambs infected in spring become substantially immune after 4-5 months. A trial with lambs in NZ found an infection rate of 100% (19/19) in November falling to 9% (1/11) in June with the mean volume of tape-worms falling from 100 ml in November to 29 ml in January to <1 ml in June (Pomroy, 1997b).

3.6 Diagnostic Procedures to Assist Control Programmes

Successful parasite programmes are unique to each individual farm and they should be flexible to constantly changing situations (FECPAK, 2001b). The main tools to diagnose parasitism, without killing the host are: faecal egg counting, faecal larval cultures, pastoral larval counts, blood sample antibody measurement, weighing stock and body condition scoring. While faecal larval cultures (FLC), pastoral larval counts (PLC) and blood sample antibody measurement (BAS) are useful tests for farmers there are factors, which

currently limit their usefulness as part of a regular monitoring programme. BAS requires yarding and handling, and PLC are time consuming to collect and have other limitations. Samples for FLC are easy to collect but require about 10 days to allow for incubation and hatch before results can be calculated. All three diagnostics require lab time, technical proficiency and are relatively expensive. Cost, technical difficulty, time delays and skills effectively limit the regular use of these techniques by farmers. Regular weighing of stock (LWG) and assessment of body condition score (BSC) has also been suggested as a useful tool for identifying parasitism. LWG and BCS are both essential tools for gauging animal performance, however total reliance on them as a parasite diagnostic is extremely risky. While both measures are capable of showing a decrease/increase in performance and the magnitude of change, they do not show the cause of change. Changes in liveweight gain or BCS could be influenced by a host of issues other than internal parasites such as nutrition, mineral deficiencies, climate or stress.

If weighing and BCS are used in isolation from other diagnostic tests, it will be difficult for farmers to accurately pin point the cause of poor performance. Drenching is likely to be used early by farmers. This may lead to over treatment of animals and time delays in identifying the real source of the problem where something other than parasites has caused the production loss.

The physical demands of regularly weighing stock are also a limiting factor. Distance from facilities, number of animals, time and labour constraints means that for many farmers weighing stock more often than monthly would be extremely difficult. Failure to weigh regularly may mean that a considerable length of time may elapse before a problem is detected and treated.

Faecal egg count (FEC) is probably the most useful (FECPAK, 2001b) being easy, quick and cheap, so it is the most commonly used in a monitoring programme. Animals do need to be handled.

There are a number of other laboratory tests available to assist veterinary practitioners in the diagnosis of gastro-intestinal parasitism in ruminants. All have their limitations and none should be considered as capable of providing a definitive diagnosis on their own. Rather such tests should be looked on merely as aids to help in the achievement of parasite control (McKenna, 1997). Most of these techniques will be discussed in more detail.

3.6.1 Worm Counts

Postmortem worm counts are an obvious means of determining the numbers and identities of gastrointestinal worm burdens in grazing ruminants, but the procedure has obvious disadvantages because it is terminal and expensive. Because the level and composition of infection may vary considerably between individuals, worm counts need to be performed on several animals in order to obtain meaningful information on the parasite status of the flock or herd as a whole (Brunsdon, 1970). In sheep, it is a relatively simple procedure (Pomroy, 1995a) as most of the abomasal parasites in these hosts are easily recovered during washing, (McKenna, 1976). A similar situation is likely to apply to goats (McKenna, 1997; Pomroy, 1995a).

In cattle, and probably deer, (Connan, 1991, 1996, 1997) prolonged saline/water soaking or pepsin digestion of the abomasal mucosa is required (Herlich, 1956; Knox, 1984; Pomroy, 1995a) particularly in animals over one year of age. These procedures are not only required to provide an indication of the numbers of early fourth stage *Ostertagia* larvae present - important for the differential diagnosis of Type I and Type II ostertagiosis - but also to provide an accurate measure of burdens of *Trichostrongylus axei*, many of which remain adhering to the mucosa following washing (Brunsdon, 1969). In addition, differentiation of *Ostertagi* burdens into adult, late fourth and early fourth stage larvae may enable estimates of larval intake to be made while examination of the condition and degree of development of adult female worms may permit assessment of the host's acquired immunity to infection (Hong, 1989).

There have been several attempts to score the importance/pathogenicity of the common parasites of sheep (Pomroy, 1997a). The figures are debatable and do not take into account the prevalence or intensity of infection of different parasites. Gardiner and Craig (1961) reported a point system for scoring helminth infections of sheep which was later modified by Gordon (1973) and is as follows:

Chabertia	100 worms = 1
Bunostomum	300 worms = 1
Fasciola	300 worms = 1
Trichuris	300 worms = 1
Haemonchus	500 worms = 1
Ostertagia	3,000 worms = 1
Nematodirus	3,000 worms = 1
Trichostrongylus	4,000 worms = 1
Strongyloides	4,000 worms = 1
Immature Worms (all species)	4,000 worms = 1

Points are combined for a mixed infection. Any figure in excess of 2 was considered economically important for young sheep and for older sheep any figure in excess of 4. Examination of these recommendations in the 1990s would suggest that they should be more conservative (Pomroy, 1997a), but the scores do at least give one opinion of the relative pathogenicity of each parasite. A few of these scores are definitely unrealistic such as that for *Fasciola* where burdens from 50-100 adults would be likely to result in obvious production loss in young lambs (Hawke & Morris, 1978; Sykes *et al*., 1980). A similar but slightly different system for sheep as proposed by Lenghaus (1987) is:

Chabertia	100 worms = 1
Haemonchus	500 worms = 1
Trichuris	500 worms = 1
Ostertagia)	
Trichostrongylus)	4,000 worms = 1
Nematodirus)	
Immatures)	

Again numbers from a mixed infection are allocated fractions of a point and combined. It was suggested that any figure in excess of 2 units would affect production with 5 units being potentially fatal in lambs.

3.6.2 Faecal Egg Counts

The faecal egg count (FEC) technique is the most widely used diagnosis of gastrointestinal parasitism in ruminants (James & Southey, 1998; McKenna, 1997; FECPAK, 2001b). In many respects, faecal egg counting is an ideal procedure for determining the worm burdens of groups of animals. It is comparatively cheap and easily performed enabling a large number of samples to be processed quickly. As a result, it has the potential to provide a better parasite profile of groups of animals than do worm counts.

FEC can be used for differentiation between two major worm types: *Nematodirus* and other strongylids, and some other species outside the strongylids such as *Trichuris* (FECPAK, 2002b).

The main distinguishing features (Kingsbury, 1965) are as follows:

Nematodirus: All the eggs in this group share the following characteristics:

- Relatively large size (compared to the strongyle group).
- Thick shell which looks like a double shell.
- Large clusters (blastomeres or cells) within the egg (of which there are eight).

Strongyle: Most eggs in this group share the following characteristics:

- Relatively small size (compared to the *Nematodirus* group).
- Thinner shell.
- Small clusters within the egg.

Others: There is some work to suggest that egg counts in sheep of greater than 3000 epg suggest the presence of *Haemonchus* (barbers pole worm).

There are many variations in the basic FEC procedure (Pomroy, 1995b). The basic procedure requires that a set amount of faeces is mixed with a known volume of saturated salt solution (James & Southey, 1998; Pomroy, 1995b). From the resulting well-mixed suspension, a set volume is examined in a special “McMaster” slide under a microscope. By using known quantities the numbers of eggs per gram (epg) faeces can be calculated. This is the standard value by which FEC levels are referred. While faecal egg counting could be described as being only ”moderately” accurate, apart from post-mortem worm counts, it is recognised as the best test available for determining the size of worm burdens in sheep. The level of accuracy is lower with cattle and deer tests and more careful interpretation of FECs is required (see below).

FEC has been in use for a number of years and there are now several different methods and techniques of varying sensitivity used for calculating the Eggs Per Gram (epg) statistic. The standard egg detection levels (sensitivity) for sheep and cattle in NZ are 1 egg counted = 50 epg (1:50 epg), although 1:100 epg is still not uncommon. Internationally standard egg detection levels for some species (such as cattle) tend to be much lower (FECPAK, 2001b).

In FEC, worm eggs that can be reliably identified (James & Southey, 1998) are those from *Nematodirus, Strongyloides, Trichuris* and *Moniezia* (sheep tapeworm). The roundworm eggs (excluding *Nematodirus, Strongyloides* and *Trichuris*) all appear very similar under

the magnification used and culturing these eggs is the only way to determine the actual worm population composition. The diagnosis of liver fluke and lungworm both require different laboratory procedures.

Although the FEC technique is fairly simple to learn, the difficulty lies in the interpretation of results. To interpret FEC results correctly an understanding of factors such as seasonal worm patterns and parasite biology are required. Furthermore, to provide the best recommendations (James & Southey, 1998) the advising person will require background information on:

- The age and condition of the sheep.
- Why samples were submitted.
- Date the animals were last drenched.
- Drench used (including formulation, e.g. oral, injectable or CRC).
- Quality and quantity of feed available.

There is considerable variation of opinion over the accuracy of FEC (FECPAK, 2001b), but there is also variation between test methods - test sensitivity, sampling method, sample size, collection methods, test frequency and technical competence. The confidence interval of the method is important (FECPAK, 2001b). Test methods with lower accuracy have larger confidence intervals. Some methods used in NZ have confidence intervals of 200-400 epg, which in some circumstances could be a problem if production losses occur at relatively low FEC levels (<500 epg for sheep and <100 epg for cattle) or if results from the same mob are highly variable. FECPAK (2001b) have recently developed a technique with considerably higher accuracy than the traditional McMaster system. The McMaster system also may not be suitable for adult cattle.

Cattle: The rationale behind the use of FEC is based on the assumption that there is a good relationship between the number of worm eggs in faeces and the number of worms in the host. Although this has generally been shown to be true of mixed gastrointestinal nematode burdens of sheep and goat (Kingsbury, 1965; McKenna, 1981, 1985; McKenna & Simpson, 1987; FECPAK, 2002a), until recently, FECs were usually considered to be of less diagnostic value in cattle (Brunsdon, 1971; McSporron, 1986; FECPAK, 2002a). In *Ostertagia* infections in calves, for example, FECs generally follow a typical pattern due to the effects of host immunity on worm egg production. They were thought to have little diagnostic value except when they deviated from the expected pattern (Michel, 1968, 1969). While much has been made of the customary decrease in *Ostertagia* egg production and the limitations it imposes on the interpretation of FECs in cattle, some believe that its importance has been greatly overstated (Baker, 1988). *Ostertagia* infections rarely occurs on their own and egg production in *Cooperia* and *Trichostrongylus* may not be affected by host immunity to the same degree. Consequently, in mixed infections, FECs may still provide useful guidelines regarding herd parasite status even though they may not diagnose the primary genus involved. Some support for this was provided by trial data in NZ (Brunsdon, 1968; 1972) which indicated that, for their first 6-8 months of life, differences in the degree of parasite control achieved in groups of July/September born dairy calves may be reflected by their mean FECs. Undoubtedly FECs in older cattle were frequently unreliable with high worm burdens (of arrested stages) often being associated with low egg counts (Brunsdon, 1971a). Nevertheless, cattle FECs still had some diagnostic value (McKenna, 1997). High egg counts in older cattle, for example, indicated a breakdown in host immunity (Michel, 1968).

Cattle FEC were originally thought to be unreliable because FEC and animal performance did not seem to be closely related. Animals suffering from obvious clinical parasitism often had zero epg results when tested and other wormy animals showed large variations in FEC when tested repeatedly (FECPAK, 2002a). Recent advances in testing methods indicate that this was likely to have resulted from the use of older less sensitive, and more unreliable test methods rather than variation in egg output from the animals themselves (Mes *et al*., 2000).

From 1998 to 1999 FECPAK ran a project (Cattletech) in NZ to test the usefulness of a more accurate FEC test method for cattle under 18 months of age (FECPAK, 2002a). The Cattletech project found that FEC and performance were closely related and that cattle production appeared to be affected by relatively low epg levels (<100 epg). Older FEC methods that only test to 50 or 100 epg were found to be too "coarse" as a difference of only one egg counted during testing dramatically changed FEC results making them highly variable and unreliable.

The FEC test for cattle currently used by the majority of laboratories in NZ needs to be re-examined (FECPAK, 2002a). By the time egg levels are high enough to detect, severe production losses may well have occurred. This means, as insurance, farmers drench regularly to ensure parasite burdens are not affecting production, as a FEC using the traditional system may not identify a parasite burden until it was too late. Also, using the traditional system, any problem with the effectiveness of the anthelmintic used may not be identified until failure reaches a high level.

Deer: Even less is known about the relationship between FECs and worm counts in deer than cattle. Usually FECs in deer are low (Connan, 1991; Johnson *et al*., 1984) probably because gastrointestinal parasitism is rarely a problem in these hosts (Mason, 1994, 1997a).

Sheep and goats: In sheep and goats a good correlation between egg and worm counts, particularly in animals in their first year of life (McKenna, 1981, 1985) has led to their widespread adoption in NZ for a variety of diagnostic and other purposes, including monitoring the effectiveness of worm control programmes and as aids to drench decision-making. Faecal samples are usually collected from 10 to 15 members of a flock and egg counts performed on each individual (McKenna & Simpson, 1987).

Composite FECs: In cases where group mean counts only are required, a quicker and cheaper procedure is to utilise a composite faecal egg count technique (McKenna, 1987, 1996; FECPAK, 2001c; Baldock *et al*., 1990; Ward *et al*., 1997). Using this procedure, individual faecal samples from a group of animals are pooled and a single egg count carried out to provide a mean egg count for the group. Although composite counts have been little used in NZ, they are being used by some laboratories in Australia (Baldock *et al*., 1990; Nicholls & Obendorf, 1994).

The composite FEC technique may not be suitable for all of the purposes that traditional FECs are currently used for (McKenna, 1997; FECPAK, 2001c). Information regarding mean egg counts only is unlikely to be appropriate for use in faecal egg count reduction (FECR) tests where information about individual animal counts may be of considerable relevance to their correct interpretation. Group mean counts may not always be as good as individual counts in helping to identify the causes of scouring or illthrift either,

particularly if *Nematodir*us is involved. *Nematodirus* is a poor egg producer and information regarding mean egg count levels for this parasite may, therefore, be of less relevance than that relating to the numbers of proportions of animals passing these eggs (McKenna, 1996).

Where group mean counts, and accordingly composite FECs, may be more usefully employed are in the areas of parasite monitoring, in determining if parasite infections are of sufficient magnitude for anthelmintic resistance testing purposes and where FEC levels are being used as aids to drench decision-making. In the latter case, this is especially likely to be true in those instances where drenching is being based on a mean FEC "trigger-level" concept (McKenna, 1997; Vlassoff *et al.*, 2001) (see below).

Regardless of what FEC procedure is adopted, they provide little information on the identity of the worm genera represented. This problem can, of course, be readily overcome by the use of faecal larval cultures in conjunction with FECs. However, since treatment to remove mixed gastrointestinal nematode infections in grazing ruminants usually involves the use of broad-spectrum anthelmintics, the utilisation of larval cultures for routine diagnostic purposes may be of limited value (McKenna, 1997).

3.6.3 Faecal Egg Counts as a Guide for Drench Use

Some farmers monitor FEC levels in their flocks to help decide when or whether to drench their various classes of sheep (James & Southey, 1998; Vlassoff *et al*., 2001). With this evolved the concept of "trigger levels" on which drenching decisions are based (FECPAK, 2001a, 2002a; Vlassoff *et al*., 2001). One of the most common questions that parasitologists are now asked is "at what FEC level should sheep be drenched?" Unfortunately a simple answer is not possible (James & Southey, 1998) and under- or over-treatment could result (FECPAK, 2001a, 2002a). Each individual situation is different and numerous factors can contribute to different recommendations being given. The local veterinarian or animal health advisor is in the best position to interpret results.

Relatively few data are available to substantiate this particular use of FECs (Vlassoff *et al*., 2001). McKenna (1981) showed a reasonable correlation (r=0.74) between mean FECs and worm burdens in lambs using the following FEC classes: "Low" being <500 epg, "Moderate" being 600-2000 epg, and "High" being >2000 epg. In that study, these FEC classes were shown to be of considerable diagnostic value. However, these FEC classes are broad and subjective (Vlassoff *et al*., 2001) and their threshold values are higher than those deemed significant by many farmers, consequently, sheep are often treated when group mean FECs are considerably lower than 500 epg. Some farmers are effectively using FECs and associated trigger-levels as a basis for making all drenching decisions for their lambs. However, these tend to be farmers who have high cattle:sheep ratios and who are using integrated grazing of different stock classes to produce "safer" pastures. There appears to be little evidence to substantiate the view that sheep-only farmers are successfully using FEC trigger-levels alone as a basis for making all their drenching decisions (Vlassoff *et al*., 2001).

FECs alone are not a suitable criterion to indicate when drenching is required (Cook, 1997). FEC, stock class and condition, as well as the quantity and type of feed available are all factors likely to influence the benefits of, or need for, anthelmintic treatment.

Unfortunately, there are currently no clear guidelines in this area, but FECs together with close monitoring of liveweight gain and level of nutrition are valuable aids.

FECPAK (2002a) have taken this concept further. Because of the imprecise relationship between FEC and performance, an alternative to the traditional "trigger level" approach is the "Integrated" or "FECPAK Zone Approach" in which a large number of factors based on an understanding of the life cycle or epidemiology of the parasite are also used, in addition to FEC levels, in the decision whether to drench or not. The zone approach is based on three zones: Zone One - sheep 0-100 epg, cattle 0-50 epg; Zone Two - sheep 100-500 epg, cattle 50-100 epg; Zone Three - sheep 500+ epg, cattle 100+ epg. Interpretation is less sensitive to issues relating to FEC accuracy because FEC is only one component of the decision making process. The zone approach should be altered and adjusted in each farming situation to suit the season, stock and test method. FECPAK (2002a) give details of the factors affecting drenching decisions in each zone and they are quite complex.

3.6.4 Improved Faecal Diagnostic Techniques

FEC techniques used have not drastically improved over the 40 years since the development of the McMaster slide (Kahn & Watson, 2001), although there have been some modifications (FECPAK, 2002a).

Australian researchers have recently been examining a number of improved assays (Kahn & Watson, 2001). Their justification follows:

> "There are a number of disadvantages associated with FEC and larval differentiation, including considerable variation in FEC between faecal samples taken from the same sheep, and variation between nematode species in their development during culture. Furthermore, FEC are unable to indicate a worm burden until egg laying commences at three to four weeks after infection, by which time the worms are well established and the host may already be suffering adverse effects".

Other faecal diagnostic techniques have been examined, but most are not routinely available as yet. These include: flow cytometry and fluorescent staining to count and identify nematode eggs in the faeces; faecal antigen detection to identify a range of parasite-derived proteins, present with different worm infections; and DNA assays of nucleic acids from the nematodes. An advantage of this latter technique is that it is rapid, sensitive and specific and infections can be detected before eggs appear in the species. Immunodiagnostic assays of faecal antigens have been available for some time (Johnson *et al*., 1996; Ellis *et al*., 1993).

3.6.5 Biochemical & Other Aids

Perceived limitations in the reliability of FECs, particularly as an aid for the diagnosis of ostertagiosis in older cattle, has resulted in a search for alternative laboratory tests (McKenna, 1997). These include clinical biochemical procedures such as plasma pepsinogens, serum gastrin, serum albumin and serum fructosamine and measurements of abomasal pH (Hilderson *et al*., 1989; Thomas & Waller, 1975; Pitt *et al*., 1988; Fox *et*

al., 1988; Hilderson *et al*., 1992; Lawton *et al*., 1996; Heath & Connan, 1991). All of these tests are aimed at providing an indirect measure of parasitism but none have completely fulfilled their promise (Baker, 1988). Many diagnose mainly abomasal worm burdens and have greater applicability to cattle than other ruminants since the most important worm species are restricted to this organ in cattle (Brunsdon, 1964; McKenna, 1985). A possible exception is the use of serum fructosamine concentrations for the diagnosis of both abomasal and small intestinal parasitism in sheep (Heath & Connan, 1991).

The use of pepsinogen determinations to diagnose bovine ostertagiosis is well established. In NZ, a plasma pepsinogen level of about 2.6 iu/l in calves has been found to be indicative of total abomasal worm burdens of above 30,000 (Brunsdon, 1971, 1972). British findings (Armour, 1970) concluded that plasma pepsinogen levels in excess of 3 iu/l are usually associated with severe clinical ostertagiosis in calves and that levels of between 2 and 3 iu/l may be present in an outbreak or after recovery. Plasma pepsinogens should be interpreted in relation to age and clinical condition of the animals and the level of nutrition (Hilderson *et al*., 1989). The use of serum gastrin and serum albumin levels with plasma pepsinogens may enhance the diagnostic reliability of this procedure (Hilderson *et al*., 1992; Heath, 1991; McSporran, 1986).

3.6.6 Larval Counts

There has been recent interest in larval counts as a diagnostic aid in parasite control (FECPAK, 2002b; Kahn & Watson, 2001). Although FEC is acknowledged as one of the most useful methods, it is considered by many to be too imprecise for modern parasite management programmes (FECPAK, 2002b).

Interest in larval counts has arisen from the recognition that numbers and species of infective larvae on pasture may be a useful management tool for integrated parasite control. The only reliable way of correctly identifying the types of parasitic worms present is to either identify the infective larvae available on pasture, or incubate and grow the eggs present in faeces to the infective larval stage and then identify the larvae. Species identification is important in making informed anthelmintic selections and in diagnosing anthelmintic resistance (FECPAK, 2002b).

Pasture Larval Counts (PLC)

PLC have been successfully used as a research tool for decades (Vlassoff *et al*., 2001). Although it would be desirable to know the number of larvae on pasture so a parasite control programme could be adjusted, a commonly held view is that pasture larvae sampling is a tedious research tool which is time consuming and never likely to be used for routine diagnostic purposes (Pomroy, 2000). Even as a research method, however, there are serious concerns about their accuracy and validity of the pasture samples being representative of the herbage ingested and the reliability of recovery of larvae from pasture and accuracy of larval identification and counting (Kahn & Watson, 2001). There is little or no encouragement in the scientific literature to use PLC as a diagnostic or predictive tool on farms (Kahn & Watson, 2001). Pasture larval counts involve sampling of pasture by plucking in a designated pattern across a chosen area. Pooled samples are then subjected to extraction procedures, and nematode larvae recovered, identified and quantified (FECPAK, 2002b, Kahn & Watson, 2001).

FECPAK, 2002b are also cautious about the use of PLC on farms because the sample can be highly variable. For example, areas where the stock camp have a relatively denser distribution of faecal pacts. As a result, sampling from random areas may dramatically under- or over-estimate the true pasture larval level. To minimise this effect, complex-sampling procedures must be used to avoid sampling error. In addition, larval migration varies throughout the day so the time the sample is taken may affect results. For these reasons, PLCs are rarely used in NZ at present. Also the time required for collecting and assessing a sample means that this technique has considerable limitations. Recent work has been underway to try and make this technique more "user friendly" for farmers (FECPAK, 2002b).

Australian workers recently evaluated the practicality and cost-effectiveness of pasture larval counts (PLC) as a diagnostic and predictive tool for sheep nematode parasite burdens (Kahn & Watson, 2001) with some success. In spite of the concerns, the technique was used successfully to evaluate the viability of pivot irrigation systems for finishing lambs in South Australia, by helping to overcome the major constraint of heavy *Trichostrongylus vitrinus* infections that occur under irrigation.

Faecal Larval Culture

The purpose of faecal larval culture (FLC) is to incubate the parasitic worm eggs present in the host animal's faeces, to hatch the eggs, encourage the larval worms to develop through to the infective stage, and then identify them at the third larval stage (L_3) (FECPAK, 2001b). FLC are easy and quick to collect, and relatively inexpensive and show exactly which worms are in the animal and is perhaps currently the more commercially applicable than PLC. A number of laboratories perform the test (FECPAK, 2001b).

Like most diagnostic techniques, the FECR test has its limitations and its inability to detect reliably low levels of anthelmintic resistance in particular, is well known (McKenna, 1990, 1997a, 1997c; Martin *et al*., 1989). However the results of recent studies in sheep (McKenna 1997b, 1997c) indicate that larval cultures could considerably enhance the sensitivity and the diagnostic reliability and should always be performed on pre-treatment samples, thereby enabling the numbers and identities of worms to be determined.

Both tests (PLC and FLC) require some lab time and a certain level of skill in order to perform the test and correctly identify the worms.

3.6.7 The Detection of Anthelmintic Resistance

Worm counts from slaughtered animals also provide the most accurate data for determining the drench resistance status on a farm. Unfortunately it is also the most impractical method (James & Southey, 1998).

While the previously discussed laboratory aids are primarily concerned with determining whether or not there are parasite burdens that warrant anthelmintic treatment, tests for the detection of anthelmintic resistance are more for identifying which anthelmintics are still likely to be effective (McKenna, 1997).

There are a number of *in vitro* and *in vivo* procedures available for the detection of anthelmintic resistance (Condor & Campbell, 1995; Johnson, 1989). However, apart form the faecal egg count reduction (FECR) test and a recently introduced commercially available larval development assay (DrenchRite™), many of these procedures remain more appropriate for research rather than evaluating anthelmintic performance in the field (McKenna, 1997).

As cattle generally carry lower numbers of parasite eggs per gram of faeces than sheep, the ability of a FEC to identify a drench failure problem has been limited due to the lack of a suitable test to identify low counts, until recently. Therefore there had been no easy way to determine low level drench failure problems and the industry has not believed drench failure in cattle to be a problem to the extent of the widely accepted failure of sheep anthelmintics (Sanders *et al*., 2002).

Most tests are based on FEC and the two routine methods in NZ are DrenchCheck and DrenchTest (James & Southey, 1998).

DrenchCheck

These "checks" give an indication whether or not the chosen drench and/or drenching technique are satisfactory. A DrenchCheck can be understand at any time but should be completed at least once and preferably twice annually, or if drench failure is suspected. Recommended times are after weaning and again in the early autumn. Ten to 15 fresh faecal samples are usually collected about 7-10 days after drenching and submitted to a laboratory for analysis. The cost is minimal considering the potential to better utilise drenches, improve productivity and safeguard the effectiveness of the available drench groups.

Pre-drench FECs are not completed with a DrenchCheck and the drench effectiveness is based on the assumption that worms were present prior to drenching. A DrenchCheck result could therefore be misleading if no, or very few, worm eggs were present before treatment. If suspicious, positive FECs are obtained after a DrenchCheck then a complete, more accurate DrenchTest should be undertaken.

DrenchTest

A DrenchTest overcomes the problem of low worm burdens (pre-treatment) by collecting faecal samples before drenching and again after drenching. In a DrenchTest, a minimum of 10 animals, ideally lambs, are identified, faecal sampled, weighed and then dosed to their individual liveweight with the drench that is under test. Seven to 10 days later a second faecal sample is collected. It is important that faecal samples are collected from the same animals hence the need to be able to identify them. For more accurate results it is preferable to have a further group of lambs that remain untreated during the test period. Often representative drenches from all drench groups will be investigated during the DrenchTest. The mean pre-drench FEC needs to be greater than 200 epg.

The percentage reduction in worm eggs between the pre- and post-drench faecal samples is the measure of the efficiency of the target drench. If an untreated group of lambs is included in the DrenchTest their results are incorporated into the final calculation of resistance levels.

When a DrenchTest is undertaken it is important to identify which species of worms are present at the time of initiating the test and any that are subsequently determined to be resistant. This task, called "larval culturing" can be easily completed by most parasitology laboratories. Pre-drench faecal samples are pooled together into one "culture" and incubated at 27°C for seven days to allow the worm eggs to hatch and develop to the infective stage when they can be identified by microscopic examination. The same procedure is adopted for any treatment groups that return positive counts after drenching.

With this type of information it is possible to design a drenching programme that will most effectively control any resistance problem.

Important points to remember:

- Samples sent to the MAFQual Animal Health Laboratories need to be submitted through your veterinarian. This does not apply to some private laboratories.
- Sample collection:
    - At least 10 animals are needed to give a meaningful result for each drench type tested.
    - Avoid yarding sheep for too long a period prior to faecal sampling.
    - Only one faecal sample per container.
    - Faeces must be fresh when collected.
    - Keep samples in a cool place- store them in the fridge until they can be delivered to a laboratory but do not freeze them.

DrenchRite™ Larval Development Assay

The DrenchRite™ assay was developed at CSIRO's McMaster Laboratory (Australia) and has now been made available commercially by Horizon Technology Pty Limited (McKenna, 1997; James & Southey, 1998; FECPAK, 2001f). The advantage of this test over the FECR procedure is that it involves only a single collection of faecal samples an can be conducted on any occasion (providing the mean faecal egg count is greater than 100 eggs/g). It does not require the treatment of animals before testing, their sorting into treatment groups or the collection of both pre- and post-treatment samples. However, while the DrenchRite™ assay greatly reduces the time and complexity of the field component of the test, it vastly increases the laboratory time and expertise required and is unlikely to be any cheaper than the faecal egg count reduction (FECR) test.

A farmer collects faeces from sheep known to be infected with worms, and uses a specifically designed kit to submit them to the laboratory (James & Southey, 1998). The worm eggs from these samples are extracted and then exposed to various concentrations of drench in special laboratory plates for seven days in an incubator and determines the point where 50% of them are blocked from developing to the third larval stage (McKenna, 1997; FECPAK, 2001f). The level of resistance can be quantified for benzimidazole, levamisole and combination drenches (James & Southey, 1998; McKenna, 1997), but only detected for avermectin/milbemycin drenches (until further resistant strains are investigated). Drench resistant *Nematodirus* cannot be measured with this assay. Drench efficacies can be determined only for *Haemonchus contortus, Ostertagia (Teladorsagia) circumcincta* and *Trichostrongylus colubriformis* (Jones & Southey, 1998; McKenna, 1997; FECPAK, 2001f). The assay also cannot be used to detect resistance in other gastrointestinal parasites present in NZ sheep such as *Cooperia, Oesophagostomum/Chabertia, Nematodirus* or other species of *Trichostrongylus*, nor to quantify the efficacy of the milbemycin/avermectins against nematodes. A number of

these latter genera may be of minor significance, but *Nematodirus* is important and commonly associated with benzimidazole resistance in NZ sheep (McKenna, 1995). The presence of strongylid worm eggs other than *Haemonchus, Ostertagia* and *Trichostrongylus* in faecal samples in sheep in NZ is likely to complicate interpretation of the assay and the reliability of the test or these latter genera as well (McKenna, 1996).

Faecal Egg Count Reduction Test (FECR)

Like the larval development assay, the FECR test is primarily aimed at providing an indirect measure of anthelmintic efficacy against adult worms (McKenna, 1997). It is capable of detecting resistance to any anthelmintic type in all nematode genera in all grazing ruminants. In this test, the post-treatment egg counts of a group of anthelmintic-treated animals are compared with those that were recorded at the time of anthelmintic treatment or with those of an untreated control group. In this test, a less than 95% a reduction in FEC is usually taken as an indication of resistance (McKenna, 1994).

An important factor in the correct interpretation of the FECR test, and one that has been the subject of some confusion, is the appropriate interval between anthelmintic administration and sampling. In NZ, the current recommendation is for post-treatment samples to be taken 5-10 days after administration of anthelmintic (McKenna, 1990). Some authors (Cole *et al*., 1992, Dash *et al*., 1988; Vermunt *et al*., 1995) however, have suggested that a 10 to 14 or even a 21 day (Watson *et al*., 1996) post-treatment sampling interval may be more appropriate. This is because a temporary suppression of egg production for 10 or more days may occur in worms surviving anthelmintic treatment. This could mean that adoption of a 5-10 day post-treatment sampling interval might over-estimate FECR values with some cases of resistance being overlooked.

Evidence relating to anthelmintic treatment and suppression of egg production in gastrointestinal nematodes of sheep and cattle has been reviewed recently (McKenna, 1997b). This review suggests that if a temporary suppression of egg production does occur in sheep nematodes following anthelmintic treatment, then such effects are unlikely to be of much practical significance and that little benefit would be derived from extending the currently recommended 5-10 day post-treatment sampling interval in the FECR test to 10-14 days. Other evidence (Grimshaw *et al*., 1996) indicates that any extension is likely to produce false positive results, especially with anthelmintics with limited effectiveness against developing immature stages.

In cattle, the relationship between sampling interval and the reliability of the FECR test was less obvious than in sheep (McKenna, 1997). Almost all of the cattle FECR cases reviewed involved infections of *Cooperia* and the testing of milbemycin/avermectin-type anthelmintics with residual activities of over 99% for 7 to 14 days. Because resistance to persistent anthelmintics may be expressed either as a reduced ability to remove worms or reduced residual activity (Rolfe *et al*., 1990, 1996) the interval between drench administration and sampling is less critical for FECR testing purposes than may be required for non-persistent anthelmintics.

McKenna (1997b) reported the % FECR at 5-8 days exceeded that recorded at 11-14 days in 10 of 12 cattle cases. On average, the difference was 21.7% over all 12 cases. These results showed that resistance to milbemycin/avermectin-type anthelmintics would have been overlooked on three out of eight occasions if the shorter post-treatment interval had been used. Therefore, when testing for resistance to these types of anthelmintics in

cattle, adoption of the longer post-treatment interval is advisable. If this interval is adopted, it should only be used for anthelmintics with a known persistent effect against the species of gastrointestinal nematodes present. Even for these anthelmintics, the period of protection may vary with both the formulation and the parasite involved (Yazwinski *et al*., 1994). Because of this and differences in the gastrointestinal nematodes which contribute to faecal egg counts in cattle during FECR testing, the use of larval cultures for each individual genus is also advisable.

3.7 Anthelmintics & Their Use

3.7.1 The Anthelmintics

Anthelmintics come in four different formulations:

- Oral liquid
- Injectable
- Controlled release capsule (CRC)
- Pour-ons.

The commonest type of anthelmintic formulation continues to be the liquid oral form but the injectable, CRC and pour-on formulations are increasing in popularity, especially for the treatment of adult ewes. Pour-on anthelmintics have been routinely used for cattle and deer, but recently abamectin formulation was developed successfully for application to sheep (Loveridge, 2002). These formulations incorporate active ingredients from one or other of the anthelmintic groups (Table 5). It is the active ingredient that is important in the choice of anthelmintic not the brand or trade name (Hosking, 1998).

These anthelmintics are sold under a huge and confusing array of trade names. Table 6, for example, lists the anthelmintics that were available in 1997, along with other details such as administration method, active ingredient, target ruminant species and withholding periods, as well as the trade name and manufacturing company (Barrell, 1997).

A good simple description of the main types of anthelmintics is given by Hosking (1998). The following is a direct adaptation:

> "Anthelmintics are highly complex pharmaceuticals that have resulted from the investment of millions of research dollars and the ingenuity of scientists (Hosking, 1998).
>
> **What is the 'Ideal' Anthelmintic** (Hosking, 1998)
> An "ideal" drench should:
>
> - Exhibit a high level of toxicity to the target parasites, but not to the animal when used under a variety of conditions.
> - Have a wide margin of safety to animal and operator.
> - Have no adverse effect on the environment.
> - Be easy to administer.
> - Produce no severe pain reaction or stress to the animals it is administered to.

- Leave no residual substances that are harmful to humans who consume meat or milk products from the treated animal.
- Be cost effective.

Table 5: Anthelmintic groups and their active ingredients (Hosking, 1998)

Anthelmintic Group	*Currently Available Active Ingredients of Each Group*
Benzimidazoles	Albendazole Febantel* Fenbendazole Mebendazole Netobimin* Oxfendazole Ricobendazole
Levamisoles	Levamisole Morantel
Avermectin Milbemycin	Abamectin Doramectin* Eprinomectin& Ivermectin Moxidectin
Combinations	Albendazole + Levamisole Fenbendazole + Levamisole Netobimin + Levamisole Oxfendazole + Levamisole Ricobendazole + Levamisole Ivermectin + Albendazole + Levamisole**
Narrow Spectrum	Clorsulon* Closantel Niclosamide Nitroxynil Oxychlozanide Praziquantel Triclabendazole

* More accurately defined as pro-benzimidazoles which metabolise to benaimidazoles when administered to an animal.
* These products are registered for use in deer and/or cattle only.
** Merial, 2003; McPherson, 2003.

Table 6: Anthelmintics for use in ruminant animals in NZ, as at June 1997 (Barrell, 1997)

Trade Name	Group	Administration	Ingredient	Company	Species	Withholding Times	
						Meat	Milk
Adtape	O	Oral	Praziquantel	Ancare	S	7d	28d
Albendazole C	W	Oral	Albendazole	Ancare	CD	14d	
Albendazole Sheep	W	Oral	Albendazole	Ancare	CSG	7d	
Albezol Mineralised Cattle	W	Oral	Albendazole	Pfizer	C	7d	48h
Albezol Combo	W/C1	Oral	Albendazole & Levamisole	Pfizer	S	21d	
Albezol Sheep	W	Oral	Algendazole	Pfizer	S	7d	
All-Min Levamisole	C1	Oral	Levamisole	NuFarm	CS	10d	24h
Anthelpor	C1	Pour-on	Levamisole	Youngs	C	10d	24h
Arrest	W/C1	Oral	Albendazole & Levamisole	Ancare	S	10d	
Arrest C	W/C1	Oral	Albendazole & Levamisole	Ancare	C	14d	
Axilur 10	W	Oral	Fenbendazole	Bomac	CDSG	10d	4d
Bomatak C Mineralised	W	Oral	Oxfendazole	Bomac	CDSG	10d	3d
Bomatak S Mineralised	W	Oral	Oxfendazole	Bomac	CDSG	10d	3d
Bomatak White Strip	W	Pour-On	Oxfendazole	Bomac	C	21d	7d
Centor	C1	Oral	Levamisole	Mallinckrodt	CS	10d	24h
Citarin-L	C1	Oral	Levamisole	Bayer	CS	10d	24h
Closal	W/O	Oral	Albendazole Closantel	Bayer	S	28d	
Combitape	W/C1/O	Oral	Ricobendazole Levamisole Praziquantel	Youngs	S	10d	
Cydectin Oral	C2	Oral	Moxidectin	Cyanamid	S	10d	
Cydectin Injection	C2	Inject	Moxidectin	Cynamid	CS	35d (C) 28d (S)	
Cydectin Pour-on	C2	Pour-on	Moxidectin	Cynamid	CD	28d (C) 21d (D)	Nil
Dectomax	C2						
Double-Strength Ox-Fen	W	Oral	Oxfendazole	Ancare	CSDG	10d	5d
Duotin	C2	Inject	Abamectin	MSD Agvet	C	49d	
Dual-Atak	W/C1	Oral	Netobimin Levamisole	Bomac	S	21d	24h

Trade Name	Group	Administration	Ingredient	Company	Species	Withholding Times Meat	Withholding Times Milk
Endex Sheep	C1/O	Oral	Levamisole Triclabendazole	Novartis	S	28d	
Endozole	C1	Oral	Levamisole	Novartis	CS	10d	4d
Eradox	W	Oral	Oxfendazole	Youngs	CS	10d	3d
Eradox Low Dose	W	Oral	Oxfendazole	Youngs	CS	10d	3d
Exhelm-E	C1	Oral	Morantel	Pfizer	CS	7d	24h
Externder 100	W	Oral (CRC) 3 month	Albendazole	NuFarm	S	0	0
Extender Junior	W	Oral (CRC) 3 month	Albendazole	NuFarm	S	0	0
Fasinex 10	O/W	Oral	Triclabendazole	Novartis	CSG	28d	
Fenben	W	Oral	Fenbendazole	Ancare	CS	10d	
First Drench	W/C1/O	Oral	Albendazole Levamisole Praziquantel	Ancare	S	10d	
Genesis	C2	Oral	Abamectin	Ancare	S	14d	
Genesis Injection	C2	Inject	Abamectin	Ancare	C	49d	
Genesis Pour-on	C2	Pour-on	Abamectin	Ancare	C	35d	
Genesis Tape	C2/O	Oral	Abamectin Praziquantel	Ancare	S	14d	
Ivomec Eprinex Pour-on for Cattle & Deer	C2	Pour-on	Eprinomectin	MSD Agvet (nil for calves born to treated cows	CD	14d	Nil
Ivomec Injection for Cattle & Pigs	C2	Inject	Ivermectin	MSD Agvet	C	28d	
Ivomec Liquid	C2	Oral	Ivermectin	MSD Agvet	SG	28d (S) 28d (G)	
Ivomec Maximiser Adult	C2	Oral (CRC)	Ivermectin	MSD Agvet	S	126d	
Ivomec Maximiser Lamb	C2	Oral (CRC)	Ivermectin	MSD Agvet	S	126 d	

Trade Name	Group	Administration	Ingredient	Company	Species	Withholding Times	
						Meat	Milk
Ivomec Oral (Cattle)	C2	Oral	Ivermectin	MSD Agvet	C	14d	
Ivmoec Plus	C2/O	Oral	Ivermectin Clorsulon	MSD Agvet	C	28dq	
Ivomec Pour-on	C2	Pour-on	Ivermectin	MSD Agvet	CD	28d (C) 28d (D)	
Ivomec SR Bolus	C2	Oral (CRC)	Ivermectin	MSD Agvet	C	180d (dairy heifers)	
Leviben	W/C1	Oral	Ricobendazole Levamisole	Youngs	S	10d	
Levicare	C1	Oral	Levamisole	Ancare	CS	10d	24h
Levitape	C1/O	Oral	Levamisole Praziquantel	Ancare	S	10d	
Mansonil-M Powder	O	Oral	Niclosamide	Bayer	S	4d	
Nufarm Levamisole	C1	Oral	Levamisole	Nufarm	CS	10d	24h
Neguvon 98%	O	Oral	Trichlorphon	Bayer	S	24h	
Nemadet	W	Oral	Albendazole	Nufarm	SG	7d (S) 3d (G)	
Nilvax	C1	Inject	Levamisole	Mallinckrodt	S	14d	36h
Nilverm Gold	C1	Oral	Levamisole	Mallinckrodt	CS	10d	24h
Nilzan	C1/O	Oral	Levamisole Oxyclozanide	Mallinckrodt	CS	10d	24h
Oxfen	W	Oral	Oxfendazole	Ancare	CDSG	10d	5d
Oxfen C	W	Oral	Oxfendazole	Ancare	CD	10d	5d
Panacur	W	Oral	Fenbendazole	Novartis	S	10d	4d
Panacur 100	W	Oral	Fenbendazole	Novartis	CDS	10d	4d
Rycoben Cattle & Deer	W	Oral	Ricobendazole	Youngs	CD	4d (C) 14d (D) 36h (C)	
Rycoben Sheep & Lamb	W	Oral	Ricobendazole	Youngs	S	5d	
Rycomectin	C2	Oral	Abamectin	Youngs	S	21d	
Rocotape	C1/O	Oral	Levamisole Praziquantal	Youngs	S	10d	
Rycozole	C1	Oral	Levamisole	Youngs	CS	10d	24h
Scanda	W/C1	Oral	Oxfendazole Levamisole	Mallinckrodt	CDS	10d	3d (C) 6d (S)

Trade Name	Group	Administration	Ingredient	Company	Species	Withholding Times Meat	Withholding Times Milk
Span Controlled Release Capsule (40-80 kg)	C2	Oral (CRC) 100 day	Ivermectin	Nufarm	S	126d	
Span Controlled Release Capsule (20-40 kg)	C2	Oral (CRC) 100 day	Ivermectin	Nufarm	S	126d	
Syanthic Minidose	W	Oral	Oxfendazole	Mallinckrodt	CD	10d	3d (C)
Synanthic Oral	W	Oral	Oxfendazole	Mallinckrodt	CDSG	10d	3d (C) 6d (S)
Synanthic R1	W	Inject (rumen)	Oxfendazole	Mallinckrodt	C	10d	3d
Systamex Low Dose	W	Oral	Oxfendazole	Mallinckrodt	CD	10d	3d
Systamex Oral	W	Oral	Oxfendazole	Mallinckrodt	CDS	10d	3d (C) 6d (S)
Tandem	W/C1	Oral	Fenbendazole Levamisole	Novartis	S	10d	4d
Trodax	O	Inject	Nitroxynil	Rhone Merieux	CS	30d	15d
Valbazen Mineralised Cattle	W	Oral	Albendazole	Pfizer	C	7d	2d
Valbazen Sheep	W	Oral	Albendazole	Pfizer	CDSG	7d	2d (C) dnu (S) 3d (G)
Valbazen Combo	W/C1	Oral	Albendazole Levamisole	Pfizer	S	21d	
Vermatak	C1	Oral	Levamisole	Bomac	CS	10d	24h
Vermatak Mineralised	C1	Oral	Levamisole	Bomac	CS	10d	
Vetdectin Injection	C2	Inject	Moxidectin	Cyanamid	CS	49d (C) 28d (S)	
Vetdectin Oral	C2	Oral	Moxidectin	Cyanamid	S	10d	
Vetdectin Pour-on	C2	Pour-on	Moxidectin	Cyanamid	CD	28d (C) 21d (D)	Nil

Key:
W = white drenches
C1 = clear drenches (levamisole and morantel)
C2 = clear drenches (avermectins/milbemycins)
O = other drenches (mostly narrow spectrum against flukes, tapeworms or *Haemonchus*)
d = days

C = cattle
D = deer
S = sheep
G = goats
h = hours
Nil = no withholding time

Types of Anthelmintics

Drenches are first categorised as either broad or narrow spectrum, this depending on the number of parasite species they control.

1. *Broad Spectrum Anthelmintics*

Broad spectrum anthelmintics may be classified according to one of the following action groups:

- Benzimadole: these compounds (sometimes called the "white" drenches - although not all are white) work by interfering with the ability of the parasite to absorb nutrients and as a consequence the worms starve to death.
- Levamisole/morantel: Levamisole and morantel are often referred to as the "clear" drenches (although not all are clear) and they work by acting on the parasite's nervous system causing paralysis.
- Combination drenches: these are specially formulated mixtures of levamisole and benzimidazole products. It is important that farmers do not attempt to create their own combination drenches as the separate compounds may not be compatible nor form stable or evenly mixed suspensions.
- Avermectin/milbemycin: this is the newest class of drench. They are sometimes called endectocides or macrocyclic lactones. Members of this chemical group kill worms by paralyzing their nervous system (although in a different way to the levamisole/morantel-based drenches).

(**Author's Note:** A new combination drench now includes all three major drench families).

2. *Narrow Spectrum Anthelmintics*

These drenches control a limited number of parasites, and have been developed in response to a particular need, e.g. the control of liver fluke or tapeworm. The narrow spectrum products listed in Table 5 belong to various drench groups (most of them different from the broad spectrum groups).

Conclusion

It is essential that farmers are able to identify the action group to which each brand of drench belongs. Changing brands does not necessarily mean that the action family has been changed. Remember that brands belonging to the same action group share the same mode of action and hence side resistance will occur. The active ingredients of drenches currently available are listed in Table 5."

The above description is very much a simplification of a very complex subject. McKellar (1997) gives an indepth description of the various anthelmintics based on their chemical classification. He covers in detail for each main class, their mode of action, pharmacokinetics (which include their persistency of activity in the body) and for each the spectrum of activity (i.e. the species of parasite that are susceptible, partly susceptible or not susceptible). This latter subject is very complex with subtle differences existing within and between classes. The subject is far too complex to cover in detail here, but it is important to re-emphasise the need for farmers and other users to read the instructions carefully and identify the active ingredients and their effectiveness towards the parasites of concern.

3.7.2 How Anthelmintics Work in the Host

When anthelmintics are administered, the active ingredient is absorbed from the gut, skin or injection site, circulated in the bloodstream throughout the body and resecreted into the gut (Mason, 1998). Blood levels usually reflect the level of the active ingredient of the drench circulating in the body and presumably present in the gut. While levels of active ingredient are above a nominal "active" level, the drench will be effective. This is the interval known as the "Killing Zone". When blood levels drop below this "active" level, the drench becomes ineffective. The period of time when the drench is still present, but at ineffective levels if often known as the "tail".

The active level may vary however, depending on the active ingredient in the drench, the species of worm present in the host and their stages of development. Some active ingredients are also able to kill the worms through direct contact with the parasites in the gut (Mason, 1998).

3.7.3 Anthelmintic Performance

Anthelmintics administered according to the manufacturers' instructions should kill all the worms they claim to kill. If this is not happening, then there is either a problem occurring with administration or there are resistant worms present (Mason, 1998).

Doubling the dose of a liquid oral drench does little to improve its efficacy (Mason, 1998). Giving a second dose, 12 hours after the first can however improve the efficacy of short-acting drenches such as the benzimidazoles. Increased or repeat dosing with products containing levamisole is not recommended for animal safety reasons. Care should be taken to ensure overdosing of lambs does not occur when using low dose volume drenches containing levamisole as these can be toxic to sheep at three times the recommended dose rate (Mason, 1998).

4.7.4 Practices That Improve the Effectiveness of a Drench

Australian researchers developed strategies that improved the efficacy of benzimidazole (BZ) drenches (Ali & Hennessy, 1996; Kahn & Watson, 2001; Mason, 1998). These improved strategies are based on detailed knowledge of the whole-body pharmacokinetics of BZ drenches. The approach taken and incorporated into the improved drenching strategies has been to increase the duration of exposure of the nematode to the drug. The most effective means of increasing the duration of exposure are: following the correct drenching technique; and slowing the rate of movement of the drug through the animal. Ali & Hennessy (1996) demonstrated that with BZ drenches it is the length of exposure to the drug, and therefore the period of uptake, rather than the maximum drug concentration that is the key to effective drenching.

The anthelmintic efficacy of benzimidazole and avermectin/milbemycin drugs is enhanced when they are administered into the rumen, rather than bypassing the rumen and going straight into the abomasum (Ali & Hennessy, 1997; Kahn & Watson, 2001; Mason, 1998). Thus, any factors which stimulate closure of the oesophageal groove and rumen bypass may reduce the efficacy of these drenches (especially against resistant worms). When

suckling lambs are drenched with a conventional volume of drench, rumen bypass frequently occurs. This effect appears to persist for about two months after weaning (Mason, 1998).

The correct drenching technique involves placing the drench-gun tip over the tongue to direct the entire dose down the throat and into the rumen (Kahn & Watson, 2001; Mason, 1998). This also prevents sheep spitting out some of the dose. Placement of the drug into the rumen is important because it is the rate of flow of digesta from the rumen that defines the duration over which the drug is in contact with the parasite. Incorrect placement of the drench-gun may result in closure of the oesophageal groove allowing some or all of the drench to pass directly to the abomasum, greatly reducing the duration of the anthelmintic action. Field trials have demonstrated that as many as 60% of drenched sheep may not receive the entire dose into the rumen (Kahn & Watson, 2001).

Yarding of pasture-fed lambs or hoggets for 24 hours before dosing with a conventional volume drench, also appears to promote rumen bypass in a high proportion of animals (Mason, 1998). Recent NZ studies indicate that the effects of suckling and of 24 hour starvation on rumen bypass can be largely overcome if low volume drench formulations are used.

There appears to be a significant effect of food intake on duration of an effective concentration of some anthelmintics in the gastrointestinal tract (Mason, 1998). The second means of increasing the duration of exposure of the nematode to drenches is by slowing the rate at which digesta moves out of the rumen. A reduced rate of passage of digesta prolongs the time for drug absorption and recycling. Withholding feed for 24-36 hours prior to drenching with BZ, and, for maximum effect for 6 hours after, slows the movement of digesta through the gastrointestinal tract and increases the exposure time of parasites to the drug, resulting in a greater reduction in worm numbers (Ali & Hennessy, 1996; Mason, 1998). Trials with sheep infected with BZ resistant *H. contortus* and *T. colubriformis* have shown that withholding feed is more effective than doubling the dose rate in a single drench (Kahn & Watson, 2001). In a practical context, the effectiveness of BZ drenches against BZ resistant parasites can be greatly improved by withholding sheep from feed for one day prior to drenching.

This observation is the basis for the widely-published recommendation that “in field conditions, sheep could be held in a paddock or yard with little or no feed for 12-24 hours before and after drenching”. The data on which the recommendation is based however, may not be strictly applicable to NZ pasture-fed lambs (Mason, 1998).

3.7.5 Persistent Anthelmintics

Persistent activity is the ability of a formulation to control worms for a period of time after the administration of the product (Mason, 1998). There are two mechanisms:

- Persistent active ingredients (injectable and some oral or pour-on formulations).
- Controlled release capsules (CRCs).

Persistent anthelmintics involve those that, whether because of the nature of the active ingredient and/or because of their formulation, remain active for an extended period of time following administration of a single dose.

In the last 10 to 15 years, new formulations of some broad-spectrum anthelmintic classes have appeared on the Australian and NZ markets for use in farmed ruminants (Barger, 1997a; Leathwick *et al*., 2001; Venning, 1991). They maintain effective plasma concentrations for extended periods, which protects against reinfection after administration and was reputed to have enhanced efficacy against resistant worms. There are two broad types. Firstly, the Captec controlled-release device or a controlled release rumen capsule (CRCs) releases its anthelmintic at a constant rate over a period of 90 or 100 days (currently either albendazole or ivermectin), and secondly, by pharmacological variants of the macrocyclic lactone (ML) class of anthelmintics that have high initial activity that subsequently declines logarithmically, but with an extended biological half-life when compared with the original ivermectin molecule; particularly when administered as a subcutaneous injection (e.g. moxidectin and Closantel). Controlled-release and long-acting drugs are referred to as persistent anthelmintics.

Currently, broad-spectrum anthelmintics with this activity are all in the avermectin/milbemycin family and have different durations of persistence against different species of worms (Mason, 1998). They are active against all the worm species, for which they have a claim, for their life span. This includes the prevention of reinfection by newly ingested larvae. Early work showed key effects were:

- To remove existing worm burdens.
- To kill infective larvae as they are ingested with the pasture.
- To suppress egg output of any surviving worms.

This ovicidal, larvacidal and adulticidal activity was clearly seen in many trials and was considered when using the capsule for best effect (Barger, 1997a; Venning, 1991). The net effect was to eliminate existing worms, continuously protect against animals from reinfection and reduce the level of larvae on pasture. The latter was achieved by preventing recontamination with eggs (for 120 days or longer), and by the "vacuum cleaning" effect as sheep remove larvae while they graze (Venning, 1991). When the capsules were used in the preventive mode and when used at the epidemiologically appropriate times the capsule had the potential to have a long term helminthological and subsequent productivity effects on both target and non-target animals. Venning (1991) reviewing work to that date stated that ideally sheep should be treated at times of the year when few larvae are present on the pasture, just before conditions for larval survival improve or just prior to periods of increased nematode egg output. Apart from the period of total freedom from productivity loss in capsule treated animals, the "safe" pasture was available for future grazings (Venning, 1991).

There is no doubt that at the time persistent anthelmintics added a new dimension to on-farm worm control. Barger, 1997a in reviewing this subject stated:

> "As well as killing worms resident in the sheep at the time of administration, which they will do at least as well as the more traditional formulations, persistent anthelmintics provide protection from reinfection for periods ranging from one to four weeks (depending on parasite species) for injectable Moxidectin to around 14 weeks for Albendazole or Ivermectin capsules. This extended protection period has two major consequences. First, the treated sheep are allowed to express their full production potential without the constraint of parasitism for at least this period. Secondly, the epidemiological effect of this prevention of pasture contamination

with worm eggs during, and for three weeks after, the protection period can be profound. Following a traditional drench, egg counts fall to close to zero for about three weeks, which represents the time taken for larvae picked up from pasture after the drench to reach maturity and produce eggs. A persistent drench with a four-week protection period extends this "egg-free" time to seven weeks, while a 14-week capsule extends it to 17 weeks. The pasture is thus not being contaminated with worm eggs for periods that are long enough for significant mortality to occur among the existing free-living stages."

The impact of reinfection should not be underestimated (Sykes, 1997). The importance of limiting larval intake, as opposed to removing a mature worm population from a host was demonstrated by Coop *et al*. (1982) who found very frequent use of anthelmintic (every 17 days) which prevented re-establishment of mature egg-laying worms restored only 30% of the loss of growth caused by continuous exposure to larvae of *Ostertagia (Teladorsagia) circumcincta*. Even the early larval stages of *O. circumcincta* cause major damage to the host. This may not apply with other nematode parasites (L_3 larvae of *O. circumcincta* burrow into the gastric glands), but this has not been studied (Sykes, 1997).

At Lincoln (Familton, 1996, 1998) used controlled release albendazole capsules with ewes for a three year period over winter and autumn compared with controls which received one anthelmintic treatment three weeks after lambing. This resulted in heavier ewes (+5 kg), higher lamb birth weights, lamb wool weights (+3.5%) and lamb weaning weights (34.0%). Pasture larval levels were reduced. The author felt the CRCs were useful in parasite control programmes and resulted in reduced drench use in the first 12 months of life. Although some benzimidazole resistance was found on both farmlets, the author felt the CRC did not pose a greater risk of developing anthelmintic resistance than other control methods.

A subsequent experiment using albendazole capsules with hoggets (Sutherland, 2000), found evidence that capsules result in higher levels of drug resistant parasites not only during the 100 days of drug release, but also for several weeks after exhaustion. This had long term detrimental effects on production. The capsules were effective in reducing FECs; but some resistant larvae were still being shed and therefore "safe" pasture did not result. Capsules may temporarily reduce egg output of resistant parasites which can be misleading.

These apparent conflicting results have confused readers, especially when commercial interests sided with one research finding against the other (Merial, 2001). Differences in age of the experimental animals may have had a bearing on the rate of onset of drench resistance. However the early impressions on the efficacy of persistent anthelmintics were probably close to the truth because of the virtual absence or very low frequency of resistant species of worms (Leathwick *et al*., 2001), but their continued use brought on a changing scene as they encouraged selection for anthelmintic resistance.

In spite of the attributes of persistent anthelmintics, their development has led to a proliferation of claims and counter-claims by manufacturers regarding both the efficacy and sustainability of the use of such formulations (Barger, 1997a). Many sheep producers are understandably confused by this conflict, particularly as it impinges on selection for drench resistance. In an environment where the ML drenches are often the only fully effective broad-spectrum group available, and with increasing reports of resistance by

worms to this class of drench, producers are concerned that they do not exacerbate their resistance problems by inappropriate use of these persistent formulations.

The early very successful results with CRCs and persistent anthelmintics is now understandable given either the absence or very low frequency of resistant parasite genotypes. Simulation models led to a much better understanding of the situation (Leathwick *et al*., 1998). Results of the trials now show that while long-acting drenches almost completely prevent infection with susceptible parasites, they do not prevent resistant worms from infecting the sheep. Thus, when animals treated with a long-acting drench graze pasture which has some resistant parasites on it, they will become infected with worms, virtually all of which will be resistant. This gives a huge reproductive advantage to the resistant worms and indicates that persistent drugs will select more strongly for drench resistance. Models showed that the situation is actually more complicated than this. If, for example, the persistent drug is more effective than a non-persistent drug at killing resistant adult worms in the sheep, then this will tend to slow the development of anthelmintic resistance.

The models also showed the early work on using CRCs to produce "safe" pasture was also very risky. The long-acting drench prevents only susceptible parasites from contaminating the pasture (Leathwick *et al*., 1998). But these long-acting drenches do not effectively control drench resistant parasites, which can survive and produce eggs in these sheep. The end result is that nearly all the parasites which survive will be resistant. In this situation it does not matter how effective the persistent drug is against resistant worms unless it kills them. As long as some resistant worms survive they will contaminate the pasture and the larvae ingested by lambs will establish in the host as adult worms and have largely only other resistant worms to mate with to produce the next generation of resistant parasites. Therefore, using long-acting drugs to create "clean" pasture for lambs is likely to select strongly for drench resistance.

It is now quite widely acknowledged that longer acting anthelmintics have increased selection pressure for resistance because of the period over which they are acting and the survival advantage they give to any resistant worms and their viable eggs (Leathwick *et al*., 1998, 2001; Leathwick & Sutherland, 2002; Pomroy, 2000; Vlassoff *et al*., 2001).

3.7.6 The Changing Scene of Drench Recommendations in NZ

Protective vs preventative treatments: Basic control programmes have, for several decades, centred entirely on the use of anthelmintics. Options for different stock classes were reviewed by Brunsdon *et al*. (1975) and more recently by Brunsdon & Vlassoff (1982) and Bisset *et al*. (1986). The recommendations for control were regarded as conservative by many farmers and their animal health advisors, and were often disputed. Traditionally, most parasite control programmes consisted of protective drench treatments for lambs, given at times of greatest risk to serious infection, usually in late summer and autumn (Vlassoff *et al*., 2001). Subsequently, it was demonstrated that further production gains in lambs were possible by using a preventive approach to control parasitism (Vlassoff & Brunsdon, 1981). This reflected the fact that in the absence of drenching, lambs are by far the largest source of pasture contamination. Minimising their infection using a planned programme of drench treatments produced substantial reductions in pasture contamination and production gains. This approach was promoted as a

preventative programme comprising five drenches given at approximately monthly intervals, commencing in late November (Brunsdon & Vlassoff, 1982), the requirement for additional treatments (as in years exceptionally favourable for larval development and/or survival) being determined on the basis of FECs of the flock conducted at strategic times (Bisset *et al*., 1986).

Thus the drenching policies have evolved over the last 30 years. In 1975 the recommendation was for "Protective Control" (Brunsdon *et al*., 1975) which involved a weaning drench around early December followed by a further three drenches at 4 to 6 week intervals commencing in February/March. If *Haemonchus* was a problem it could necessitate an earlier treatment in summer. It was noted by the authors that this programme would "prevent mortalities and ensure adequate liveweight gain and wool production". In 1982, the "Preventive Control" approach was advocated (Vlassoff & Brunsdon, 1981; Brunsdon & Vlassoff, 1982) which was principally aimed at limiting pasture larval levels, particularly to void the autumn larval peak. This was achieved by preventing contamination earlier in the season. By this time the aim had changed to maximising productivity, not just achieving adequate control. The associated research used a four drench programme from weaning at 28 day intervals, but the resulting recommendation to farmers was a given drench programme with quite strict interval times of 21, 21, 28 and 28 days between treatments. A farmer survey in 1980 (Brunsdon *et al*., 1983) showed that the majority of farmers were already drenching regularly at about monthly intervals, so for them the main change to achieve Preventive Control was to be careful with the interval between drenches. In recent years this recommendation has eased to intervals being not more than 28 days.

At about the same time as the protective approach was proposed, an alternative was put forward. This was "Integrated Control" (Brunsdon *et al*., 1975; Brunsdon & Vlassoff, 1982) which involves drenching being co-ordinated with movement to clean pastures in late November/early December and again in late February/early March with or without an additional drench given a month after moving. This approach had a low uptake, probably because it involved long term planning and did not necessarily result in many fewer drenches being given (Pomroy, 2000) or because the system seemed to break down (Nicol & Everest, 1997). The advocated drenching and moving to clean pasture may have been a particularly effective way to select for anthelmintic resistance (Pomroy, 2000; Leathwick & Sutherland, 2002). With the increase in drench resistance, interest in integrated control programmes has now rekindled (Heath *et al*., 2000; Vlassoff *et al*., 2001).

Productivity trials in the 1980s showed additional drenches, on occasions, resulted in still further gains in productivity (Milligan, 1982; Beckett, 1993). As a consequence, the concept of the preventative programme outlined above was widely adopted, but with increased frequency of drenching, as indicated by a national survey on drench usage (Brunsdon *et al*., 1983) which showed farmers drenched adult sheep an average of 1-2 times and lambs seven times each year. Even at this level of treatment, some advisors and veterinarians were routinely recommending additional drenches for most age classes of sheep to maximise production (Familton, 1991a; Beckett, 1993), despite the occurrence of anthelmintic resistance (Vlassoff & Kettle, 1980). Even with increasing anthelmintic resistance, drenching practices have remained essentially unchanged over the last 15 years (Macchi *et al*., 1999). In fact, with the advent of persistent anthelmintics, although number of treatments has remained stable, parasite exposure to drenches may have

increased substantially in recent years (Leathwick *et al*., 1998, 2001; Leathwick & Sutherland, 2002; Pomroy, 2000; Vlassoff *et al*., 2001).

As a consequence, selection pressure for anthelmintic resistance on NZ sheep farms is likely to be as high today as it has ever been. Reducing the current reliance on anthelmintics without compromising productivity will require the implementation of management strategies to reduce the exposure of susceptible lambs to L_3 (Vlassoff *et al*., 2001). Currently, the only practical options for this are limited to the alternate grazing of pastures with different stock classes, pasture spelling and renovation, making hay or silage or the use of specialty forage crops. In many situations, however, these options will not, on their own, be sufficient for managing parasite populations within acceptable limits and anthelmintics will continue to remain the principal means of control.

The preventive approach was evaluated in the Hastings region and reported in 1991 by Beckett (1991). This involved three inter-related studies. In the first study, 15 farms using the preventive approach were compared to 15 others which followed their normal drenching routine. All preventive" farms" required extra drenches to prevent parasite build-up in April, May and June. This assessment was based on a small random sample of FEC in he flock. In another study it was concluded that a preventive approach followed by follow-up drenches based on FEC achieved reasonably good growth rates but these were still less than a group given regularly monthly drenches through until mid-August. They also concluded that the role of adult sheep in parasite control could not be ignored. Routine treatment of ewes did not apparently occur in any of these studies.

Drenching adult ewes: The pros and cons of drenching ewes were reviewed in 1998 (Pomroy, 1998). Early studies found no advantage in routinely drenching ewes around lambing or at docking (Brunsdon *et al*., 1975) but a study by Brunsdon & Vlassoff (1985) showed beneficial effects of a docking drench combined with an integrated approach to nematode control for weight gains and wool weights in lambs at weaning and the following autumn/winter. About that time that regular docking drenching of ewes were generally promoted although survey figures would suggest that many farmers were already drenching their ewes at least once annually (Pomroy, 2000).

The advent of slow release capsules and macrocyclic lactones (MLs) with persistent activity changed the way farmers view treatment of ewes around docking. There are no recent surveys to monitor their uptake other than industry data which shows that sales had increased by 2000 (Pomroy, 2000). Research on the subject showed positive results (Familton, 1996, 1998). Farmer reports suggested they were pleased with the responses but there were few well monitored trials (Pomroy, 1998). Regardless, the use of these longer acting anthelmintics has increased selection pressure for anthelmintic resistance if only because they have replaced short-acting anthelmintics so the period over which ewes are now protected by anthelmintics has increased (Pomroy, 2000; Leathwick *et al*., 2001; Leathwick & Sutherland, 2002).

Docking treatment of ewes, or a slow release anthelmintic over lactation, is a well targeted anthelmintic that will probably select more strongly for anthelmintic resistance than the same treatment given at some other time or even to lambs later in the season (Pomroy, 2000; Leathwick *et al*., 2001).

Despite the fact that adult sheep are effectively immune to gastrointestinal nematodes (except over lactation), clinical/subclinical parasitism in the ewe is not uncommon and is periodically reported (Pomroy, 2000; West, 1998). This emphasises the point that the immune response to nematodes is fragile and can be overcome by poor nutrition or other stress. Generalising can be dangerous as there are many exceptions to the rule. Routine treatment of ewes, however to cover for these occasions would impose severe selection pressure for anthelmintic resistance even if it can be justified in increased productivity and is now not advocated. As discussed earlier, the role of the we in pasture contamination has been the subject of debate (Familton, 1991; Familton & McAnulty, 1994; Familton & McAnulty, 1997). A healthy adult ewe also removes and kills a large number of infective larvae and it is the balance that is important. This balance can be influenced by grazing systems, i.e. "who follows who" in a rotation. Recent research has shown that the immune response may have an influence on the viability of nematode eggs so that eggs from immune ewes are less viable than those from lambs (Jorgensen *et al*., 1998), but how this influences the balance of eggs produced and resulting number of larvae; and those removed during grazing is still not known (Pomroy, 2000).

The changing scene on anthelmintic use over the years was justified but it is still a source of confusion to farmers, especially the changes in recent years on treating adult sheep and using persistent anthelmintics.

Recent drenching practices: The number of drenches given to sheep roughly doubled during the period of the 1970s so that by 1980 the drenching rates were 6.3 for lambs, 1.8 for 1-2 year olds and 1.2 for older sheep and was approximately the same in the North and South Islands (Brunsdon *et al*., 1983). Fifteen years later in 1995 Macchi *et al*. (1997) reported another survey of 300 southern North Island sheep farmers with about a 50% response rate. In this survey the annual drenching rates were remarkably similar to those in 1980 being 6.2 for lambs, 1.4 for 1-2 year olds and 1.3 for older ewes. This survey was completed just as the first albendazole slow release capsule came on to the market before moxidectin was promoted for use in ewes (Pomroy, 2000). The advent of these new products probably meant that overall anthelmintic coverage in "days per season" that an anthelmintic is active in an animal had increased and will give any resistant worms that survive and lay viable eggs a distinct survival advantage.

There were several other interesting results from the 1995 survey (Pomroy, 2000). Only 37% of farmers followed a standard 5-6 drench programme with 18% treating regularly throughout the year. Another 13% only treated during periods of risk and 11% only if animals were scouring or obviously showing signs of ill-thrift. Only 7 of 174 farmers relied exclusively on FEC to determine when to drench. There was only limited use of grazing management to avoid contaminated pasture. About half (54%) weaned onto clean pasture but most had lambs grazing pasture contaminated by lactating ewes within two months of weaning.

Rotation of drench families was advocated by the AGCARM Task Force in the mid 1990s, as a method of minimising the development of anthelmintic resistance, but current evidence does not support this (Pomroy, 2000; Merial, 2003; McPherson, 2002). The 1995 survey (Macchi *et al*., 1997) showed 33% followed the practice compared with 3% in 1980.

Another recommendation was quarantine drenching incoming stock with a combination of ivermectin-type drench (Pomroy, 2000). In the 1995 survey about half the respondents did so by some means. Since this concept did not exist in 1980, it showed many farmers were aware of the problem and taking preventive action (Pomroy, 2000).

Another recommendation of the Task Force was that "certain farm management practices can reduce the optimum number of drench treatments needed (and hence slow the development of drench resistance)" (Pomroy, 2000). Experiences in northern New South Wales, on reducing the number of treatments by a better understanding of nematode epidemiology and hence better targeting the fewer treatments given did not necessarily mean lower selection for anthelmintic resistance (Pomroy, 2000; Kahn & Watson, 2001; Leathwick *et al., 2001*). In NSW there are problems with multiple resistance in *Haemonchus* to all anthelmintics.

Most current advice given to sheep farmers for nematode control is a variation of the preventive approach (Pomroy, 2000; Heath *et al*., 2000). As such, it is prescriptive and does not allow for a change in the number of drenches given. Attempts to move back to a protective programme or base a programme solely on monitoring of FEC is also likely to lead to problems with parasitism. Most programmes now focus on how to minimise the development of anthelmintic resistance (Hosking, 1998b). The reality is that selection for anthelmintic resistance is strongly related to the degree and extent of control attempted by using anthelmintics compared to that achieved by other means such as mixed grazing, use of crops etc. (Vlassoff *et al*., 2001) and there has been a resurgence of interest in alternative control methods and integrated control programmes (Hosking, 1998a; Harrison *et al*, 1998; Heath *et al*., 2000; Vlassoff *et al*., 2000).

Control with anthelmintics will not eradicate internal parasites, as over 90% of the population can live outside the host, but assist in reducing populations to levels that interfere minimally with the economics of farm production. Historically most parasite control was based solely in regular anthelmintic treatment, but now this method is just one of a number of procedures and practices used in modern integrated control programmes (Heath *et al*., 2000). The aim in recent years has been to reduce the use of these chemicals (Cook, 1995; Barger, 1995a; Hosking, 1998a; Heath *et al*., 2000) so as to minimise the occurrence or development of anthelmintic resistance (Barger, 1995b). Previously drenching, especially regular drenching of young stock, usually resulted in production responses and, if animals were heavily infected, prevent mortalities. If animals were lightly infected, no appreciable production response was achieved - although subsequent pasture contamination may have been reduced. However, if animals were returned to heavily infected pasture after drenching much of the advantage of drenching was lost.

Clearly, there was considerable scope for improvement in both the effectiveness and the economy of anthelmintic usage. Regular drenching regimes are not effective in preventing the exposure of animals to high levels of pasture infection. They serve rather to limit the effects of infection after it had been acquired.

The effects of returning animals to contaminated pasture are probably more serious today with the increasing occurrence of drench resistant parasites (Hosking, 1998a). In the 1980s and 1990s the emphasis turned to preventive rather than protective drenching (Vlassoff & Brunsdon, 1981; Beckett, 1991; Barger, 1995a; Heath *et al*., 2000; HISA &

SAC, 2000) both in NZ and overseas. The main aim of the current preventive drenching programmes is not a protective one controlling the parasites in the host (Hosking, 1998). This takes into account epidemiological factors that the majority of the internal parasite population is on pasture not in the sheep and the aim is to reduce pasture contamination such that even when the weather is favourable for parasite development, larval numbers remain low and production losses in the grazing animal are minimised. If an animal does become heavily reinfected soon after drenching, it is clear that the treatment resulted in only a few "worm-free" days. A temporary improvement may occur, but the fully opportunity for a production response is never realised. An important misconception is that many consider a response to drenching should be obvious. In reality, a visible response to drenching would indicate that the drench was given too late and that a parasite burden had already had a significant effect on production (Hosking, 1998).

It is important to realise that if drenches are used to reduce pasture contamination they will only be effective against those worms which are susceptible to the particular drench group being used (Hosking, 1998b). Resistant worms can remain in the drenched animals after treatment and will continue to contaminate the pasture. This can have the undesirable effect of promoting a drench resistant population. Where possible, alternative means of preparing pastures with low worm levels should be employed (see later).

3.7.7 Dosing Procedures & The Use of Drenches

Calibrate and Check Equipment

Animals should be dosed to the weight of the heaviest sheep in the mob (Hosking, 1998a). Animals should be weighed where possible. If there is a wide range of weights, drafting into more even lines could be worthwhile, first as a cost saving measure, and second to avoid overdosing lambs if a product with a low safety margin is being used. Ensure that drenching equipment is well maintained and checked for accuracy of dose delivery before drenching commences and again at regular intervals while drenching.

To Rotate Drench Group or Not

It was earlier advocated (Hosking, 1998a; Pomroy, 2000) that the drench groups which remain effective on the farm can be rotated on an annual basis and the same drench group should be used in a all classes of stock within each year. Combination drenches could be used in these rotations. The changeover time was advocated as the first drench around lambing (if ewes are treated) or the weaning drench for lambs. However, rotation alone will not prevent the onset of resistance.

This practice was based on 1995 recommendations from an "Anthelmintic Task Force" set up in 1990 and convened by AGCARM (Pomroy, 2000). At that time the annual alternation of drenches was considered to be possibly useful, and certainly not harmful but it was not supported by any firm scientific evidence. It was believed that anthelmintic resistance in a population would slowly decline and revert to susceptibility but there is little evidence this occurs (Leathwick *et al*., 2001). Since then computer simulation models and a better understanding of the genetic basis of anthelmintic resistance has shown that such alternation probably achieves nothing in terms of delaying the onset of anthelmintic resistance (Pomroy, 2000; McPherson, 2002; Merial, 2003).

Quarantine Drenching
All stock, irrespective of age, being imported onto a farm should be quarantine drenched with an avermectin/milbemycin drench (Hosking, 1998a). Despite the logistical problems that can occur, it is strongly recommended that, after treatment, the treated animals should be held overnight in the yards, woolshed or in a quarantine paddock, with access to fresh water. This also applies to any rams being imported onto the farm.

Testing for Drench Resistance Should Be Regular
The effectiveness of drenches and drenching technique should be tested once, but preferably twice annually (Hosking, 1998a).

3.7.8 Current Sheep Recommendations

Hosking (1998a) summarises recent guidelines for sheep drenching programmes. These recommendations were prepared after consultation with parasitologists from AgResearch, MAF Quality Management, animal health companies, consultants and veterinarians from both private practice and universities. These guidelines may be amended to suit specific farming situations and environmental conditions. Different recommendations may depend on the balance between production benefits and action to delay the onset of resistance. It must be re-emphasised that drenching is only one tool in an integrated internal parasite control programme (Heath *et al*., 2000; Hosking, 1998a). Drenching is not a substitute for good feeding. A successful parasite programme combines the effective use of drenches, grazing management and FEC monitoring to prevent sheep being challenged by a significant parasite threat (Heath *et al*., 2000; Hosking, 1998a).

Lambs
For most farms, a preventative drenching programme is recommended (Hosking, 1998a; Heath *et al*., 2000). This generally comprises a series of five or six drenches commencing at weaning (November/December). The drenching interval should be no more than 28 days and certainly not less than 21 days. Heath *et al*. (2000) recommended the first three at three week intervals and the last two at four week intervals. Capsules can replace regular treatments but meat withholding periods for slaughter should be strictly observed. Drenching of lambs before weaning should only be necessary when veterinary advice confirms an early problem which can sometimes occur, especially with *Nematodirus* (Hosking, 1998a). Drenching for tapeworm control is not generally recommended as there is little proven economic advantage from doing so. Any additional drenching after the basic preventative programme should be based on factors such as FEC, feed availability and lamb growth rates.

The preventive drenching programme for lambs advocated by Vlassoff and Brunsdon (1981) was basically similar and involved drenching at weaning, twice at intervals of 21 days, then twice at 28 days. Subtle variations were tried (Beckett, 1991) and the modern guidelines represent one of these. This contrasts with the early regime advocated by Brunsdon *et al*. (1975) of no drench at weaning, then three drenches at four to six weekly intervals commencing in February; which was basically a protective policy (Hosking, 1998a; Heath *et al*., 2000).

The full benefit of a preventative treatment programme will only be obtained if the lambs are returned to the pastures prepared by this programme. Such a preventative

programme can reduce autumn pasture L_3 larvae levels by over 80% (Heath *et al*., 2000). Because the pasture larval contamination has been reduced the sheep are never seriously at risk from a major parasite burden. Trials have demonstrated that the preventative approach to roundworm control will result in greater liveweight advantages than a protective approach (Hosking, 1998a).

A protective drenching programme once at weaning and then three or four at monthly intervals in autumn (commencing in February), temporarily controls the worms in the lambs and reduces FECs, but pasture contamination levels remain high (Hosking *et al*., 1998a). In this situation the drench programme will have little positive effect on sheep productivity because the worm burden can be quickly replaced from the available pasture larvae which have peaked at high levels during the autumn.

An alternative to the preferred planned preventative programme is to monitor lamb FEC and drench the animals only when these counts start to increase (Hosking, 1998a). While such a "semi-protective" management system can be effective, intensive monitoring of FECs is required, particularly when the system is being introduced, otherwise it may fail. Similarly, failure is also probable if this type of management practice is attempted under conditions of medium-high pasture larval availability. It is desirable to initiate a suitable grazing management plan to support this type of control programme.

Replacement Two-Tooths

The general recommendation is that no drench should be given (Heath *et al*., 2000) unless symptoms and FEC suggest parasitism is or may become a problem (Hosking, 1998a). In areas prone to *Haemonchus*, regular checks on FEC during mid-summer and autumn are recommended.

Adult Ewes

Ewes are normally immune to worms and so regular drenching is not recommended unless symptoms and FEC suggest that parasitism is or may become a problem (Hosking, 1998a) or if feed is in short supply (Heath *et al*., 2000). Although a ewe drench pre-tupping or about lambing can have potential benefits, computer modeling studies suggest these treatments may increase the risk of selecting drench resistant worms (Hosking, 1998a; Harrison *et al*., 1998; West, 1998). The quality and quantity of feed available for the ewes, and body condition, as with other age classes of sheep, must also be considered in the decision making process (Hosking, 1998a; Heath *et al*., 2000; HISCA & SAC, 2000). Poor conditioned ewes may respond to drenching (HISHA & SAC, 2000; Vipond, 1998).

It is important not to generalise about parasitism in adult sheep (West, 1998). Every flock is different and farmers should assess the need to drench ewes on their own farm. This assessment is best done by monitoring FECs and taking into consideration ewe body weights, the feed available, evidence of scouring, sheep to cattle ratios and grazing management.

The indiscriminate drenching of ewes pre-tupping, about lambing time and on other occasions without due consideration of the above factors could be expensive and is likely to accelerate the development of anthelmintic resistance (Hosking, 1998a). On the other hand, ewes with evidence of clinical parasitism will show a considerable production benefit from drenching and controlling parasitism in ewes may have significant benefits for other,

more susceptible sheep on the farm such as lambs. In general, clinical parasitism in ewes is associated with mean FECs above 500 epg and except for the period around lambing, counts are usually below 200 epg (West, 1998). The dilemma is does one drench for short term economic gain at the risk long term detriment to the farm's drench resistance status.

Where parasitism in adult sheep is a regular problem, consideration should be given to modifying pasture management and increasing the cattle to sheep ratio to help reduce the larval challenge to ewes (Niezen, 1998; Heath *et al*., 2000).

The rise in FEC in ewes at lambing time can be a major source of pasture larval contamination. Reducing ewe worm burdens at this time can have significant production benefits for both the ewes and the lambs (West, 1998). Thus, drenching ewes 3-4 weeks after lambing should contribute to the development and maintenance of cleaner pastures and indirectly improve the performance of lambs. If there are no or few resistant species of worms then safer pastures should ensue, but if there are any species resistant to the drug, long acting anthelmintics exacerbate the onset of drench resistance. Unfortunately this is occurring on more and more farms. Computer simulation indicates that drenching ewes around lambing significantly increases the risk of drench resistance development (West, 1998; Harrison *et al*., 1998). This is because much of the subsequent summer and autumn contamination may come from the ewes in early spring, either directly or after cycling through the lambs. The dilemma is that the more useful a single treatment is at providing cleaner pasture, the greater the risk of it hastening the development of drench resistance (West, 1998). This topic is the subject of considerable and ongoing debate as can be seen in other parts of this review.

There are currently three schools of thought on drenching adult stock (FECPAK, 2001d). The first is no drench at all, because of the expense, time and likelihood of selection for drench resistant worms. The second is based on the contentious issue that adult stock may be a major cause of pasture contamination and that routine drenching helps create safe pastures. The third, advocated by FECPAK (2001d) is based on drenching the so-called "polluters" or the small number in the flock that have high FECs, which may be due to age, body condition, pregnancy status or breed. Drenching these animals only should help create "safe" pasture and increase their performance. Because this is only a proportion of the flock, it is relatively cheap, less time consuming, and less likely to lead to the development of drench resistance compared to the "drench-all" option, however FEC and condition score need regular monitoring and the ewes may need to be in separate mobs.

Use of Persistent Anthelmintics With Sheep

The dangers of generalising on anthelmintic recommendations have been discussed earlier in this review (Pomroy, 2000) and the same is the case for CRCs and other persistent anthelmintics. Because young breeding animals (ewe hoggets, two-tooth ewes) have not fully developed their immune systems, as a rule these types of anthelmintics are not recommended for them (FECPAK, 2001e). With adult ewes all the above considerations are important in considering whether the use of CRCs will give a return on investment (FECPAK, 2001d, 2001e); i.e. age, resistance, status, multiple *vs*. single rearing, body condition, FEC, lambing paddocks etc. There has been a movement away from "whole flock" treatment. Other methods of providing clean pasture should be adopted where possible. Species of parasite also has a bearing. *Nematodirus* is basically

transmitted from lamb to lamb so CRC treatment of ewes will have no impact on this pathogen and even drenching of lambs pre-weaning may be necessary (FECPAK, 2001e) if FECs indicate a potential problem.

Adult Rams

For rams, no firm recommendation is made (Hosking, 1998a). As most farms carry few adult rams in relation to adult ewes and lambs, any drenching of rams will have an insignificant effect on the selection of drench resistant worms.

Liver Fluke

Unlike the nematodes, there are no specific recommendations for liver fluke, but anthelmintics that are effective against it were summarised by Southey & Hosking (1998). They had this to say:

> "Albendazole and ricobendazole are effective against adult liver flukes that are 12 weeks of age or older. Closantel is very effective against adult flukes but only aids in the control of immature flukes more than six weeks of age. Nitroxynil has good activity against flukes eight weeks of age and older. Oxyclozanide is effective against adult fluke but has poor efficacy against the immature stages. This drug is the only one effective against rumen flukes. Triclabendazole is more than 95% effective against liver flukes from only week of age. This compound has a major advantage over all of the other products as it is the most efficacious drench against liver fluke. In NZ, there are no known cases of drench resistance by liver fluke to any of these compounds."

Tapeworm

Although treatment for *Monezia* is not recommended, there are a number of anthelmintics that are effective against it. These were reviewed by Pomroy (1997b). At that time the most commonly used benzimidazole anthelmintics were highly effective at recommended dose rates except perhaps fenbendazole which appeared to have an efficacy of about 95%. In NZ there had been two cases reported where it appeared there may have been anthelmintic resistance to albendazole. Other anecdotal reports of inefficacy of benzimidazoles may also have been cases of resistance. Praziquantel, which has been recently formulated for use against *Moniezia* in NZ, also had a high efficacy at 3.75 mg/kg. Niclosamide appeared to leave some scolices behind at the recommended dose rate although at 100 mg/kg it was 95-100% effective.

3.7.9 Recommendations For Cattle

Brunsdon *et al*. (1975) provided detailed recommendations for different classes of cattle, however because that was before the emphasis on preventive drenching programmes and before anthelmintic resistance, they are now out of date. There has been a lot less research and written guidelines on cattle drenching programmes. Some of the most recent available are those of Heath *et al*. (2000) who provide guidelines for both dairy and beef cattle of different classes and ages.

Dairy and Bull Beef Calves

Do not drench pre-weaning. A preventative programme post weaning of the first drench in early November, followed by five more at four weekly intervals until early April.

Traditional Beef Calves
Do not drench pre-weaning. The first drench or pour-on is given at weaning (late March/early April), followed by another late in winter (July/August). If safe pasture is not available, extra drenches may be required if there are signs of parasitism.

Yearlings & Older Cattle
There are no firm recommendations. Routine drenching is unlikely to be economic but Type II *ostertagiasis* does require anthelmintic - drench or pour-on.

3.7.10 Goats

In the absence of recent drenching guidelines for drenching goats, recommendations should be similar to the sheep preventative and protection schemes. However dose rates are higher (approximately double) (Mason, 1997b). Anthelmintic resistance is a big problem in goats (Mason 1997b), so all alternative control measures should be included in the control programme.

3.7.11 Deer

I have not been able to find any up-to-date drenching recommendations for deer. Early recommendations for deer were not to drench for gastrointestinal parasites, but they needed anthelmintic to control lung worm (Pomroy, 1997a; Mason, 1997a). With ML pour-ons, lungworm is now largely under control (Mason, 1997a; Familton pers. com), but recently gastrointestinal nematode has become a problem, especially the *Ostertagia*-type abomasal worms: *Apteragia; Spiculopteragia; Rinada* and *Skrjabinagia*. FECs and plasma assays do not seem to be much use in deer (Mason, 1997a). In 1998 (NZ Farmer, 1998) the standard drenching regime was 12 drenches annually, but with chicory grazing this was reduced to two. Currently recommendations would be less than 12 per year. Mason (1997a) gave a summary of the changing drench requirements in deer as follows:

> "Earlier work on the efficacy of anthelmintics concentrated on the efficacy against lung worm in red deer. More recently, there has been a shift towards looking more closely at efficacy against gastrointestinal worms and incorporating wapiti and wapiti x red hybrids. This has happened because of the improved availability of wapiti type stock and because of the health problems that have occurred in some of these animals.
>
> The summary of lung worm drenching studies in red deer in the mid 1980s put anthelmintics into three categories:
> - Diethylcarbamazine, levamisole and cambendazole had low activity;
> - Mebendazole, albendazole, oxfendazole, fenbendazole and febantel had moderate to good activity; and
> - Oral ivermectin (200 µg/kg) had very good activity.
>
> After further work using injectable (200 µg/kg) and pour-on ivermectin (500 µg/kg) at cattle dose rates, the guidelines for where the risk of reinfection with lung worm was high were:

- Use second generation benzimidazoles at 21 day intervals;
- Use oral ivermectin (200 µg/kg) at four weekly intervals;
- Use injectable ivermectin (200 µg/kg) at five weekly intervals; and
- Use pour-on ivermectin (500 µg/kg) at seven weekly intervals. Subsequently, moxidectin pour-on (500 µg/kg) and eprinomectin pour-on have demonstrated similar or better activity against lung worm.

These guidelines are still applicable, but on most farms drenching need not be as frequent. Note however, that oral and injectable ivermectin are not licensed for use in deer".

Triclabendazole is effective against liver fluke.

3.8 Internal Parasite Resistance to Anthelmintics

Internationally there was a massive proliferation of drench resistance in the trichostrongylid nematodes of sheep and goats in the 1980s, to the extent that it now occurs in all countries (Barger, 1997b) and threatens the sustainability of small ruminant production in some properties, including parts of Australia, South Africa and South America (van Wyk *et al*., 1997; Rolf *et al*., 1997; Waller *et al*., 1995). Some farms in NZ may be approaching a similar situation (Ridley, 2002).

Since 1980, the prevalence of anthelmintic resistance in NZ has increased to the point where it is now common and occurs for all of the currently available broad-spectrum anthelmintic classes (Hosking, 1998b; Leathwick *et al*., 2001). Despite this, the frequency of anthelmintic treatments applied by NZ sheep farmers has remained essentially unchanged (Brunsdon *et al*., 1983; Macchi *et al*., 1999). Given the routine use of persistent anthelmintics, it is likely that parasite exposure to anthelmintics is greater now than it ever was (Hosking, 1998b; Leathwick *et al*., 2001). It seems unlikely that this will change in the immediate future since most farmers appear to be making little effort to reduce drench usage (Macchi *et al*., 1999). It is clear that current patterns of anthelmintic use are applying significant selection pressure for resistance, and in the absence of any major change, it is inevitable that resistance levels will continue to increase. Unless new chemical classes of anthelmintics become available, current chemical-based parasite control practices will be unsustainable in the long term.

Anthelmintic resistance has been reviewed numerous times over the years from a number of perspectives (Barger, 1997a; Leathwick *et al*., 2001; Hosking, 1998b; Mason, 2001; Pomroy, 2000; Pomroy *et al*., 2002; Waller *et al*., 1988, 1995; Waller, 1997a, 1997b; Sangster, 1999). This review focuses on the pastoral situation in NZ and on issues and aspects relevant to NZ. Where data are conflicting or recommendations have been made for another country, an attempt is made to interpret these from a NZ perspective. The situation on anthelmintic resistance has been continually and rapidly changing over the years and updated or new information has been confusing to farmers. A lot has been published on the subject and more is appearing all the time. There is no doubt that computer models have led to the recent improved knowledge in the area, especially on the roles of persistent anthelmintics (Leathwick & Sutherland, 2002) and combined drenches (McPherson, 2002).

Broad spectrum benzimidazole (BZ) drenches were first released in 1961 (Brown *et al*., 1961) and other anthelmintic groups followed over the next 35 years, the most recent addition being that of moxidectin in 1995 and the re-release of naphthalophos in the same year (Pomroy, 2000). The release of new anthelmintic groups has been followed by the development in worms of resistance to these anthelmintics. Resistance to BZ drenches now exists over 60% of NZ sheep farms (Hosking, 1998b; Merial, 2001; Pomroy, 2000) and in Australia on 100% of farms in West Australia and 90% in N.S.W. (Kahn & Watson, 2001). For the foreseeable future there is little prospect of novel anthelmintic compounds with unique modes of action becoming commercially available (Animal Pharm,2003; Pomroy, 2000). Therefore it is imperative that existing drenches are used in ways that preserve their efficacy for as long as possible.

Drench resistance is already present on many sheep farms and its rate of development is influenced by a number of management decisions (e.g. grazing strategies, frequency and timing of drench treatments), the genetic basis of the resistance and climatic conditions (Hosking, 1998c). The most effective way to reduce the potential for resistance developing whilst still achieving the productivity benefits required, is to use drenches correctly in a planned management programme.

Anthelmintic resistance was first detected in NZ in 1979 (Vlassoff & Kettle, 1980) and warnings of possible consequences followed. Despite these warnings, farmers have, for the most part, continued to successfully control parasites using anthelmintics. Continued use of anthelmintics to which resistance has developed has been conservatively estimated to result in reduced liveweight gains of lambs by 0.5-2.0 kg over 80-200 day periods (Mulvaney, 1995; Macchi *et al*., 2001), but if the presence of resistance continues to be ignored, greater losses are likely. Once resistance has been detected on a property, it has generally been possible to use an alternative drench from a different chemical class that is still fully effective. However, the accuracy of the original warnings has been demonstrated on some goat farms in NZ and sheep farms overseas, where resistance has now developed to all of the available broad-spectrum anthelmintic classes (benzimidazoles, levamisole, and the macrocyclic lactones), with serious consequences, particularly where more than one parasite species is involved (Leathwick *et al*., 2001). There is little doubt that farming practices need to change subsequently as the level of anthelmintic resistance increases (Leathwick *et al*., 2001).

3.8.1 What is Anthelmintic Resistance?

The accepted definition of anthelmintic resistance in NZ, and that of the World Association for the Advancement for Veterinary Parasitology, is a failure to reduce faecal nematode egg counts (FECs) by at least 95% (Coles *et al*., 1992; Hosking, 1998b; McKenna, 1994). A prevalent attitude is that "if the drug is killing over 95% of the worms - what is the problem?", as up to 95% efficacy can still be a useful level of control (Pomroy, 2000).

Technically a more accurate definition is that resistance is a genetic decline in the efficacy of an anthelmintic to a specific parasite that is generally susceptible to that drug (Sangster & Gill, 1999). The difference between the two definitions has led to confusion. Anthelmintics are marketed at dose rates determined to be effective against one or more particular parasite species, but that rate may be many times greater than required to kill 95% of more susceptible species (Leathwick *et al*., 2001). Also, resistant parasites are

initially present in a population at low frequencies and only increase after continued selection pressure. Resistance can develop and anthelmintic efficacy decline on a property, and not be clinically apparent. For all practical purposes, the anthelmintic is effective. Experimental work in Australia showed that benzimidazole resistance in both *Trichostrongylus colubriformis* and *Ostertagia circumcincta* could not be definitely detected using a FEC-reduction test until the frequency of resistant genotypes in the population approached 50% (Martin *et al*., 1989). It is erroneous to assume that resistance is not present on a property simply because it has not been detected using the FECR test (Leathwick *et al*., 2001; Leathwick & Sutherland, 2002). McKenna (1997c) found 15 (36%) of 42 mixed gastrointestinal nematode infections in sheep, identified as drench-susceptible by the undifferentiated faecal egg count reduction test, were found to actually include anthelmintic-resistant worms, when analysed on the basis of changes in the egg counts of individual nematode genera. Most of these cases involved resistance in a single nematode genus, with *Ostertagia* and *Trichostrongylus* being implicated most frequently. Larval culture results help to reduce the chances of the faecal egg count reduction test producing these and similar types of errors. There are other limitations to the 95% FECR test guideline that most veterinarians and animal health advisors will be aware of, and should account for, when reporting results of resistance tests (Hosking, 1998b). An example is the presence of a worm species that the drench would not normally control adequately anyway.

A simplified outline of the selection process for drug resistance is that anthelmintics remove most but not all of the parasite population in host animals (Leathwick *et al*., 2001). Worms remaining generally possess some genetic ability which makes them less susceptible to the anthelmintic. After treatment, it takes approximately three weeks (the pre-patent period) for new infections to establish and develop to potency. During this time, the resistant surviving worms are the only contributors to pasture contamination from eggs output in host faeces; thus, they have a reproductive advantage over susceptible worm genotypes for the duration of the pre-patent period. In this way, each treatment increases the frequency of resistant genotypes in the overall parasite population.

Unless large numbers of resistant worms are introduced with newly acquired sheep, the worm population does not suddenly change from being drench susceptible to drench resistant (Hosking, 1998b). On many properties resistant worms are not apparent until a drench is failing outright and the worms are out of control. If such resistance had been identified earlier, the production losses associated with resistant worms could have been avoided and the useful life of the drench prolonged.

3.8.2 Types of Resistance (Hosking, 1998b)

The parasite population on a farm is made up of many different worm types. When resistance occurs it is highly likely that each of these types will be affected to a varying degree This can lead to quite complex resistance patterns within the overall parasite population. Knowing which resistance type (if any) is present on a farm is therefore essential to planning an effective drenching programme (Hosking, 1998b; FECPAK, 2001d, 2001e). The types of resistance are:

Single Resistance
Where the farm has worms that are resistant to a single drench group only. If more than one worm type is involved in the resistance the term "multi-generic" resistance is often used.

Dual Resistance
This occurs when the farm has some types of worm that are resistant to one drench group and another worm type resistant to another drench group. To date in NZ, the benzimidazole and levamisole drench groups are the only ones known to be implicated on sheep farms. Combination drenches will remain effective in these situations.

Multiple Resistance
This is where a farm has one or more types of worm that are simultaneously resistant to more than one drench group. On farms with multiple resistance, the specific nature of the resistance will determine whether the combination drenches are effective or not. Only drench testing can determine this.

Side Resistance
Side resistance is when use of a particular member of a drench family results in drench resistance to all members of that drench family. Side resistance occurs when resistant worms are not killed by any member of a particular drench group. For example, when a strain of worm is resistant to albendazole it will also be resistant to all other benzimidazole drenches.

3.8.3 Drench Families & Anthelmintic Resistance

Anthelmintic resistance is now common but there are few reports of animal mortality occurring despite regular drenching with a product to which resistance has occurred (Pomroy, 2000). It is interesting to note that BZ drenches still hold a reasonable (but declining) share of the anthelmintic market despite the high level of resistance to BZs. On many farms a moderate degree of control must be achieved with BZs, and is presumably acceptable (Pomroy, 2000).

Once drench resistance has developed to one member of a drench family, it is only a matter of time until the parasite is resistant to all members of that family. It is unwise to use another drench from the same action family to delay resistance to that family (Leathwick & Sutherland, 2002; Merial, 2003). If, for example, there is ivermectin resistance in a flock, do not use moxidectin or abamectin. Because the compounds have very similar chemistry, the benefits will be temporary and the detrimental effects will be permanent. The eventual outcome will be a higher proportion of avermectin/milbemycin resistant worms in the population.

Drench Rotation & Combination Drenches
To slow down the development of resistance it was previously recommended that farmers change drenches annually (Hosking, 1998; Stephens *et al*., 1998; Pomroy, 2000), by rotating amongst the drench groups that are effective on the farm; using a different drench group each season, and for the whole season. Farmers were advised that it was essential that to identify the action group to which each brand of drench belongs because changing brands does not necessarily mean that the action family has been changed. Brands

belonging to the same action group share the same mode of action and hence side resistance will occur. Drench rotation was however only one very minor component of the strategy to delay the onset of resistance.

In recent years recommendations on drench rotation have been revised (Barnes *et al*., 1995; Pomroy, 2000). Merial (2003) state "It has become apparent there is now no need to alternate (rotate) the use of drenches from different families on an annual basis. While a rotation strategy can be used, it neither speeds up, nor slows down, the onset of resistance".

Combination drenches have recently been introduced. Because they are more effective against a wide range of worms than a single drench, it is claimed (Dobson *et al*., 2001; McPherson, 2002; Merial, 2003) that combination drenches are the best strategy for delaying the onset of resistance and optimising production. This is true especially if they are used before drench resistance is present (Merial, 2003).

When the combination of broad spectrum levamisole and benzimidazole anthelmintics was originally introduced, support for their use to slow the development of drug resistance was limited (McPherson, 2002). The original combination products comprised formulations containing two anthelmintics only and were not considered to be of significant value if resistance to either member of the combination was already present; however they were considered beneficial as a means of delaying the onset of resistance to both anthelmintics prior to establishment of resistance (McKenna, 1990; McPherson, 2002; Ridley, 2002). Such combination drenches are likely to be most effective if the resistant gene frequency is low (Ridley *et al*., 2002).

Since their introduction, the use of dual combination anthelmintic products has steadily increased as evidenced by market share statistics through the decade 1990 to 2000 which show market share of these products had risen to 8% from an initial share of 2%. Market share during bimonthly periods of peak use is now 16-18% (McPherson, 2002).

Merial has recently marketed Triton™, a triple combination drench, to slow the onset of resistance (McPherson, 2002; Merial, 2003). The introduction of this triple combination, containing ivermectin, levamisole and albendazole has focused attention on the use of modeling to demonstrate the effectiveness of combinations of anthelmintics in slowing the development of anthelmintic resistance (McPherson, 2002). Initially suggestions that combinations of anthelmintics administered by concurrent use of different action families were considered theoretical only. Modelling studies have supported the use of combinations of anthelmintics to slow the development of anthelmintic resistance and show that these combinations are most effective prior to the development of resistant genotypes in the parasite population (McPherson, 2002). Simple logic also suggested that the alternative anthelmintic in a combination was effective against susceptible genotypes as well as against genotypes resistant to a different action family. If anthelmintic resistance has not developed, the probability of an individual with resistance genes for all three anthelmintics present is extremely remote. If resistant genotypes are already present then one or other of the alternative anthelmintics should be effective. Use of a triple combination should slow the development of anthelmintic resistance. Modelling has enabled these different scenarios to be investigated and be explained in terms of changes in gene frequency (McPherson, 2002). Modelling has also indicated that the use of combination products is better than anthelmintic rotation (Dobson *et al*., 2000).

Resistance To All Drenches Has Now Occurred In New Zealand

Resistance to levamisole and benzimidazole drenches is now common place on NZ sheep farms. Moreover, cases of resistance to combination drenches are being diagnosed. Worms resistant to ivermectin and moxidectin-based drenches have been reported in goats in NZ and will readily transmit into sheep if given the opportunity (Hosking, 1998b).

Worms resistant to benzimidazole, ivermectin, moxidectin and doramectin-based drenches have been diagnosed on cattle properties in NZ (Hosking, 1998b). These reports have been on properties where high numbers of young cattle have been managed and emphasises the need to plan parasite control programmes for cattle to slow the development of resistance in the cattle industry.

ML Drenches

These newer drenches are worth a special mention. There use has increased greatly on the last 10-15 years (Mirams, 1999). While resistance to ivermectin has been around for some time and avermectin for a lesser period, the first record of ML (moxidectin) resistance with *H. contortus* in sheep was found by Vickers *et al*. (2001). Although the efficacy of moxidectin was high against resistant *H. contortus* and *O. circumcincta* there was evidence of reducing effectiveness.

Within the ML drenches there are acknowledged differences in efficacy (Leathwick *et al*., 2000; Merial, 2003). With ivermectin resistant *O. circumcincta* efficacies measured by worm count were 42, 96 and 99.9% (Leathwick *et al*., 2000) and 54, 89 and 97% (Woodgate *et al*., 2001) for ivermectin, abamectin and moxidectin. Australian workers have found similar differences with various oral, capsule or injectable ML anthelmintics with *H. contortus* (Kahn & Watson, 2001).

Abamectin and moxidectin are more effective against ivermectin resistant worms and computer models indicate that their use (instead of ivermectin) will delay the onset of resistance to the avermectin/milbemycin family (Leathwick & Sutherland, 2002). Abamectin is substantially better than the other drugs because of its lower persistence. Although moxidectin has even greater efficacy than abamectin against resistant types, it has a persistent "tail" which has low efficacy against infective resistant L_3 larvae (see later). Pomroy *et al*. (2002) and Ridler *et al*. (2002) reported that although moxidectin remained highly effective against ivermectin resistant *O. circumcincta* and *H. contortus* its persistent activity was greatly reduced or lost. This offsets its advantage against the adult worm. The use of these alternative ML drenches to ivermectin will offer a short-term advantage, and their continued use will result in increased resistance and eventually failure of these drenches also (Leathwick *et al*., 2000; Merial, 2003).

Merial (2003) disputed that the use of these alternative family products delay the onset of resistance. Such conflicting statements in the literature adds to the state of confusion in the minds of farmers and other readers.

3.8.4 History of Resistance in NZ

Although no formal surveys have been conducted for over 20 years, there is little doubt that anthelmintic resistance is now common in nematodes of sheep and goats in NZ.

Moreover, the presence of benzimidazole and macrocyclic-lactone resistance in nematodes of cattle in NZ, especially in *Coopera oncophora*, is well documented (West *et al*., 1994; Vermunt *et al*., 1995; Hosking *et al*., 1996; McKenna, 1996; Pomroy *et al*., 2002). Resistance to benzimidazoles in cyathostomes in horses has also been recorded in NZ and there is suspicion of resistance to macrocyclic lactones in this species as well (Islam *et al*., 2001).

Anthelmintic resistance was first found in NZ in 1979 and reported in 1980 (Vlassoff & Kettle, 1980). This was relatively late by comparison with overseas. Since that time there has been a steady increase in prevalence (Pomroy, 2000; Leathwick *et al*., 2001). Some notable milestones recorded by Pomroy (2000) include the following:

"1980-81: A survey of 32 South Island sheep farms (Kettle *et al*., 1982) found benzimidazole (BZ) resistance on one farm (91% reduction in a faecal egg count reduction test (FECRT) and resistance to levamisole (LEV) on three farms (78-92% reduction in a FECRT).

1980-81: A survey of 54 randomly selected North Island sheep farms (Kettle *et al*., 1981) found BZ resistance on four farms (87-93% reduction in a FECRT) and resistance to LEV on three farms (72-89% reduction in a FECRT).

1987: First case of BZ resistance in cattle reported (Jackson *et al*., 1987).

1986-88: MAF laboratory data (McKenna, 1989) showed resistance was common in the lower North Island with a prevalence of 21-45% in those cases submitted for a FECRT. These were mostly to the BZs and included most common *Trichostrongylid* genera.

1990-92: First cases of ivermectin resistance in *Ostertagia circumcincta* in goats confirmed.

1989-90: MAF laboratory data for the 12 month period showed resistance was increasingly common in sheep throughout NZ, mostly involved BZs (95%) with only a few involving LEV (5%) but included seven cases of ivermectin (IVM) resistance in goats (Bailey, 1991).

1986-92: MAF laboratory data (national) for this period showed that 63% of requests for faecal egg count reduction tests in sheep showed that resistance was present (McKenna, 1994). Of those that were positive, 74% involved BZ, 23% LEV and 30% combinations of BZ+LEV.

1993: MAF laboratory data (national) for this year showed BZ resistance on 61 and 72%, LEV resistance in 29 and 29% and combination resistance in 11 and 22% of North and South Island cases submitted for FECRT respectively (McKenna, 1995a).

1992-1994: Inefficacy of ivermectin against *Cooperia oncophora* in cattle was reported (West *et al*., 1994; Vermunt *et al*., 1995; McKenna, 1995b) which for at least one isolate was subsequently confirmed by FECRT and slaughter studies to be resistance to all MLs.

1996: MAF laboratory data (McKenna, 1996) shows a total of 16 cases of BZ resistance in cattle, principally involving *Cooperia* but with a larval cultures showing some *Trichostrongylus* and *Ostertagia* present post-treatment as well.

1996-97: MAF laboratory data (national) for this period showed 68% of requests for FECRT demonstrated BZ resistance, 42% LEV resistance and 39% to the combination BZ+LEV (McKenna, 1998).

1999: First case of ivermectin resistance in *Ostertagia circumcincta* in sheep confirmed (Mason, 1999).

1999: First case of ivermectin resistance in *Trichostrongylus colubriformis* in goats confirmed which is also resistant to BZs and possibly to LEV (Gopal *et al*., 1999)."

Since Pomroy's (2000) review, some of the notable findings have been additional cases of resistance to the ML anthelmintics in sheep. In 1999/2000 the first cases of resistance to the macrocyclic-lactone anthelmintic, ivermectin, in *O. circumcincta* in sheep were detected (Leathwick *et al*., 2000; Mason, 2001; Mason *et al*., 2001a), although ivermectin resistance in this nematode had been detected in goats a decade earlier.

The first documented therapeutic failure of the macrocyclic-lactone anthelmintic, moxidectin, also occurred earlier in *O. circumcincta* from goats in NZ (Leathwick, 1995; Watson *et al*., 1996). Ivermectin resistance in *O. circumcincta* from goats is often associated with resistance to all three chemical classes of anthelmintics and, consequently, control of nematodes on affected farms is difficult. Cross infection of multiple-drug resistant strains from goats to sheep has long been considered a significant risk to sheep farmers (Watson, 1994), and at least one such occurrence has been documented (Leathwick *et al*., 2001).

Ivermectin resistance has also now been confirmed in *T. colubriformis* from goats (Gopal *et al*., 1999) and moxidectin resistance in *O. circumcincta* and *Haemonchus contortus* from sheep (Vickers *et al*., 2001).

3.8.5 Prevalence

New Zealand: Since first confirmed in sheep in NZ in 1979 (Vlassoff & Kettle, 1980), the prevalence of anthelmintic resistance on NZ farms has steadily increased. Many early cases involved benzimidazole resistance in *Nematodirus spathiger* but resistance is now found to both benzimidazoles and levamisole in all common sheep trichostrongylids (McKenna, 1995). Amongst parasitological submissions to animal health laboratories in 1993, benzimidazole resistance was evident in 66%, levamisole resistance in 29%, and resistance to combinations of benzimidazole + levamisole was diagnosed in 16% of cases (McKenna *et al*., 1995). Although based on fewer submissions, prevalence appeared to have increased to 68%, 42% and 39%, respectively, by 1996/97 (McKenna, 1998).

Australia: Although on the increase in NZ, the levels of anthelmintic resistance is still considerably less than in Australia (Kahn & Watson, 2001). In Australia, the future of sheep farming in some areas was threatened, similar to what has occurred in South Africa (Rolfe, 1997; van Wyk *et al*., 1997). This was due to the severe clinical effect of one of the species present (*Haemonchus*), resistance to existing anthelmintics; including in some areas the latest group the avermectins and the extreme susceptibility of the Merino to worms. The widespread use of broad spectrum drenches during the 1980s and 1990s, whether they were required or not, when sheep burdens were low and pasture levels were very low, intensified the selection pressure for drench resistance in Australia (Hall, 2002). In NSW approximately 90% of farms have resistance to the "white" drenches (BZs); 80% to clear drenches (LEV), 60% to combinations (BZ+LEV), and 38% to macrocyclic lactone

(ML) drenches (Northern NSW). Rare incidents of resistance to naphthalophos, closantel (*Haemonchus* liver fluke) and triclabendazole (liver fluke) have been reported.

It was commonly believed that drench resistance was only a problem of the higher rainfall sheep raising areas of Australia (Kahn & Watson, 2001). More frequent drenching was required in these areas and this, combined with occasional under-dosing, would produce greater selection for resistance in worm populations. However, there now is evidence that drench resistance in western NSW may be more prevalent than previously realised, especially given the Western Australian experience suggesting that selection for resistance is stronger in dry environments (Kahn & Watson, 2001).

Resistance to anthelmintics affects virtually all Western Australian sheep farms, and is believed to reflect the heavy selection pressure favouring resistant worms, which survive in sheep after summer drenching. In environments where no or very few larvae survive on pastures over summer, any resistant worms remaining in sheep after summer drenching are the main source of future worm populations. Although the number of resistant worms is initially very low, they can increase very rapidly once pasture growth resumes in winter, if they are not diluted with non-selected susceptible worms (Hall, 2001; Kahn & Watson, 2001). In the dry areas, one summer drench per year is typical, and resistance to MLs has evolved on some farms after four to six years. In the higher rainfall areas, there are farmers who have used MLs for eight years in a row, two to four times per year, with no evidence of resistance developing (Kahn & Watson, 2001).

Resistance to benzimidazoles and levamisole in the major parasite genera, *Trichostrongylus* and *Ostertagia*, is present on almost every Western Australian sheep farm. These drench groups are now of minimal value when used individually; test figures indicate their effectiveness to be less than 60% on 85% (BZs) and 63% (LEV) of farms. Combination BZ-LEV anthelmintics are more than 95% effective in about 25% of cases, but remain useful as a tactical drench on most farms.

The use of naphthalophos with a BZ, LEV or combination BZ-LEV drenches has increased, as other drenches fail. However, considerable variability in efficacy has been shown in tests, and poor results can occur. It is recommended that naphthalophos be used only in combination (except against *Haemonchus*), with an efficacy test conducted soon after the time of use.

The number of confirmed cases of macrocyclic lactone (ML) resistance in *Ostertagia* has increased rapidly in Western Australia in recent years. The recommended test format differentiates ML efficacy (>95% effective at a full recommended dose rate) from resistance (effectiveness of ivermectin at a half dose rate). On this basis, figures from 1999 showed ML resistance in approximately 38% of tests, and reduced efficacy at the full dose on 19% of farms. ML resistance has not been detected in *Haemonchus* or *Trichostrongylus*. Closantel resistance in *Haemonchus* has also not been detected in Western Australia.

The extent of the problem in South Australia is now known and is being evaluated (Kahn & Watson, 2001) although some cases of ivermectin and moxidectin resistance have been found.

3.8.6 Genetics of Resistance

The genetics of anthelmintic resistance is complex (Leathwick *et al*., 2001), as the number of genes involved and whether they are dominant or recessive, affect the rate at which resistance develops (Leathwick *et al*., 2001). Gene dominance refers to the ability of the heterozygotes to survive a particular drug. If a drug kills the same high percentage of heterozygotes (RS) as the susceptible homozygotes (SS), the R gene is recessive. If the RS genotype has equivalent ability to survive as the resistant homozygote (RR), the R gene is dominant. At low gene frequencies, most R genes are present as RS genotypes. If they are recessive, R-gene survival is poor, and resistance is slow to develop. However, if the R gene is dominant, then most RS genotypes will survive and resistance will develop rapidly.

Different anthelmintics and different species of parasites develop different genetic forms of resistance (Kahn & Watson, 2001; Leathwick & Sutherland, 2002).

Benzimidazole resistance in *H. contortus, T. colubriformis* and other strongylids appears to involve selection for two or more independent genes and is a partly recessive trait (Dobson *et al*., 1996; Roos, 1997). With *T. colubriformis*, levamisole resistance is inherited as a sex-linked recessive trait, controlled either by a single gene or a cluster of tightly linked genes (Martin & McKenzie, 1990). Despite being a recessive trait, and because male nematodes have only one X chromosome (whereas females have two), resistance is dominant in males. In contrast, levamisole resistance in *H. contortus* is inherited as a recessive trait that is not sex-linked (Dobson *et al*., 1996). This difference probably accounts for the observation that levamisole resistance which is relatively common in *T. colubriformis* is rare in *H. contortus* (McKenna, 1995; Sangster, 1999).

Relatively few genetic studies have been published on macrocyclic-lactone resistance (Leathwick *et al*., 2001). In *H. contortus*, resistance to ivermectin appears to be inherited as a completely dominant trait, but its expression is influenced by sex in that efficacy against RS females is lower than against RS males (Dobson *et al*., 1996; Le Jambre *et al*., 2000). Ivermectin resistance in *O. circumcincta* may also be inherited as a completely dominant trait (Leathwick *et al*, 2001), while ivermectin resistance in *T. colubriformis* may be inherited as a partially dominant trait, not under the control of a single gene (Gill & Lacey, 1998; Gopal, 2000). Because ivermectin resistance is inherited as a dominant or partially dominant trait once the gene is present in a population, resistance will develop quite rapidly when ivermectin is used. This emphasises the importance of quarantine drenching and use of appropriate drugs, when transferring stock, to prevent the spread of this gene.

The above discussion on macrocyclic-lactone resistance is simplistic and it is in fact much more complex (Leathwick *et al*., 2001). Gill & Lacey (1998) categorised macrocyclic-lactone-resistant isolates into five types based on results of *in vitro* assays which may reflect five different genetic changes in macrocyclic-lactone resistance. Studies in *Caenorhabditis elegans*, a free-living nematode (Kohler, 2001) support this hypothesis.

3.8.7 Reversion

"Reversion" refers to a population drift back to drug susceptibility after the parasite population had become resistant to that particular drug (Leathwick *et al*., 2001). There are few reports in which this phenomenon has been studied in any detail and most involve benzimidazole resistance in *H. contortus, O. circumcincta* or *T. colubriformis*. There may be two processes in the field (Leathwick *et al*., 2001). One is when a survival disadvantage is associated with the resistant genotype and the other is "counter-selection", which occurs when a different selection pressures are applied by the use of alternative anthelmintics. The genetic changes associated with resistance differ between nematode species and reversion may also differ both between species resistant to the same anthelmintic and within a species that is resistant to different anthelmintics.

Early laboratory studies showed little or no reversion to susceptibility. Hall *et al*. (1982) reported no reversion in benzimidazole-resistant *H. contortus* and *T. colubriformis* over 12 generations. Martin (1987) found no reversion after six generations in a highly benzimidazole-resistant strain of *O. circumcincta*. Simplin & Coles (1978), however, reported a reduction in benzimidazole resistance if resistant strains were cycled without further exposure to benzimidazoles.

Field studies are more relevant as they also include survival or fitness characteristics of the free-living larval stages and usually include more than one anthelmintic which could lead to an additional drench resistance problem. Regular use of levamisole to control benzimidazole-resistant trichostrongylids led to a reduction in the level of benzimidazole resistance in *O. circumcincta* after four years (Martin, 1987; Waller *et al*., 1988) and *T. colubriformis* after eight years (Waller *et al*., 1989). However full susceptibility was not reached and reintroduction of benzimidazoles led to the rapid re-emergence of resistance to former levels in all cases. Other studies did not find that the use of levamisole for extended periods resulted in reversion to benzimidazole susceptibility, after 15 years with *O. circumcincta*(Jackson & Coop, 2000); and in *H. contortus* after six years (Borgesteede & Duyn, 1989) or eight years (Waller *et al*., 1989). Others found survival characteristics increased with level of resistance, thus influencing the potential for reversion. With *H. contortus*, an increasing level of benzimidazole resistance was associated with increased larval establishment, egg production and longevity (Maingi *et al*., 1990). Another study found no difference in establishment rate, egg production or development rate from eggs to L_3 larvae between benzimidazole-resistant and -susceptible genotypes of *O. circumcincta* (Elard & Sauve, 1998). There may be differences in nematode species in their ability to revert to susceptibility, and there are a number of conflicting findings in the above studies. Overall, the findings indicate that some reversion may occur in some cases, but the redevelopment of resistance is likely to be rapid following the re-use of benzimidazoles. For all practical purposes, therefore, once a substantial benzimidazole resistance is established it is effectively permanent (Leathwick *et al*., 2001).

There have been few studies of reversion to levamisole or macrocyclic-lactone anthelmintics (Leathwick *et al*., 2001). Over five years, the use of thiabendazole was found to select against levamisole resistance in *T. colubriformis* to a greater extent than reversion in the absence of anthelmintic treatments; however, the level of reversion in both cases was small and full susceptibility was not reattained (Waller *et al*., 1989). Ivermectin resistance in *O. circumcincta* rapidly appeared in goats after a five year period during which macrocyclic-lactone anthelmintics were not used (Pomroy *et al*., 1998). In another

study, there was no difference in establishment rate, egg production, larval development or larval survival between a goat-derived ivermectin-resistant strain and two susceptible strains of *T. colubriformis* (Gopal, 2000), suggesting that all three strains had similar survival characteristics. These early indications suggest that reversion of resistance to ivermectin is likely to be slow if it occurs at all (Leathwick *et al*., 2001).

For all the anthelmintic action families it would be reasonably safe to assume reversion is non-existent or minimal and the drug resistance is therefore permanent.

3.8.8 Drenching Frequency

Drenching frequency is considered very important in the selection for anthelmintic resistance and drug resistance management programme usually aim to reduce drenching frequency as a major aim (Familton *et al*., 1995; Leathwick *et al*., 2001). While this is usually a good strategy, drenches are not equal in their ability to select for resistance and development of resistance is not necessarily proportional to drenching frequency (Leathwick *et al*., 2001). Epidemiological factors also play a key role. For example, drenching lambs in winter is less likely to select for resistance than drenching lambs in late spring or summer because fewer eggs after the winter treatment are likely to survive on pasture to contribute to subsequent generations of worms. The main aim in managing anthelmintic resistance is not so much to reduce drenching frequency *per se* but to reduce selection pressure for resistance. Unfortunately, the latter is much more difficult to achieve (Leathwick *et al*., 2001).

In NZ, the main aim of nematode control has for many years been "preventative" with the focus on creating "safe pastures" to minimise numbers of larvae to which grazing animals are exposed (Brunsdon & Vlassoff, 1982). Selection pressure for anthelmintic resistance is related to the extent to which the "safe pastures" are achieved by anthelmintic use, compared with other methods such as pasture spelling, grazing with an alternative ruminant species and use of forage crops. Achieving low levels of infective larvae on pastures by relying almost exclusively on anthelmintic treatment of stock can result in high selection pressure for resistance. The issue is complicated by the fact that when numbers of larvae on pasture are low, anthelmintic treatment of animals results in higher selection pressures than when levels of pasture contamination are high. This has been clearly demonstrated in the hot dry summer areas of Australia (Kahn & Watson, 2001; Ball, 2002) where pasture contamination is lower than in temperate NZ, and effective parasite control can be achieved using only 2-3 drenches annually (Barger, 1995). Despite a much lower drenching frequency, anthelmintic resistance has developed to higher levels faster in Australia than in NZ. An analogous situation occurs in NZ when drenched lambs are moved to "clean" pasture and eggs passed by worms that survive drenching become the major source of subsequent pasture contamination. This illustrates the danger of relying solely on reducing drenching frequency rather than reducing selection pressure for resistance (Leathwick *et al*., 2001).

3.8.9 Persistent Anthelmintics & Resistance

The relationship between treatment frequency and/or frequency or duration of exposure to sublethal doses of drug and selection in nematodes for anthelmintic resistance is

complicated by the use of anthelmintics that have persistent activity over weeks or months (Leathwick *et al*., 2001). Use of such products result in fewer treatments being given but this does not result in reduced selection for resistance; generally the reverse. Any anthelmintic treatment has the potential to select for resistance for as many days as it is effective against susceptible worms plus the prepatent period of newly established larvae (Leathwick *et al*., 2001).

For decades, broad-spectrum anthelmintics were only available as oral formulations with little persistent activity. As discussed earlier in this review, the last 10-15 years has seen an increasing array of anthelmintics registered, and purchased, that have persistent activity (Mirams, 1999). Some of these products have substantial capability to suppress parasites but less is known about their ability to select for resistance (Leathwick *et al*., 2001), although our understanding of this aspect is increasing all the time (Leathwick & Sutherland, 2002).

Other types of pesticides, such as insecticides and herbicides, when long-acting are more likely to select for resistance than short-acting products (Hughes & McKenzie, 1987). Data relating to anthelmintics in grazing ruminants are much more limited and other factors such as treatment frequency require careful consideration. The acceptance by the marketplace of persistent anthelmintics has preceded the knowledge of there impact on the development of anthelmintic resistance. As stated earlier, this has not prevented considerable debate and numerous contradictory claims about which product(s) will most rapidly select for resistance (Barger, 1997a; Kieran, 1994; Leathwick *et al*., 2001; Rothwell & Rolfe, 1994). Given the complexity of the issues, it is not surprising that some confusion exists. In recent years modeling has been instrumental in helping to clarify these issues (Leathwick & Sutherland, 2002).

Anthelmintic Type; Oral; Capsule or Injectable

Most of the information and debate on persistent *vs.* short-acting drenches for sheep is about oral formulations of ivermectin and moxidectin, and to a lesser extent, moxidectin-injection (Leathwick *et al*., 2001). However, the basic principles apply equally to all persistent anthelmintics. The only difference between CRCs and persistent oral or injectable drug formulations is the duration of activity and efficacy against established worms and incoming resistant larvae.

Early NZ models suggested CRCs need not increase selection pressure for resistance, provided the continuous action of the capsule made the RS genotype recessive, when this did not happen with the same drug given as a single oral dose, and/or where there were susceptible genotypes on pasture or in untreated animals which were able to reproduce without exposure to anthelmintic (Leathwick *et al*., 1997). There is evidence that the first of these criteria may be met by the albendazole CRC, which has shown greater efficacy against resistant worms than oral formulations of the same drug (Barger, 1993), particularly against resistant infective third-stage larvae. In NZ there is no clear indication that using an albendazole CRC to replace the standard five-drench preventative programme in lambs (Barger, 1993; Vlassoff *et al*., 2001) will delay the onset of resistance.

Studies with ivermectin CRCs indicate that efficacy against resistant adults of *H. contortus* and *O. circumcincta* is no greater than that of the oral formulation (Barnes *et al*., 2001; Leathwick *et al*., 2001) indicating that the CRC is unlikely to make the RS genotype

recessive. For *H. contortus*, this also applies to ingested L_3 larvae for which the efficacy of ivermectin CRCs against RS genotypes was similar to that against RR genotypes (Barnes *et al*., 2001). In contrast, for *O. circumcincta*, establishment of RS L_3 was significantly lower than RR L_3 in lambs treated with ivermectin CRCs (Leathwick *et al*., 2001). Currently evidence suggests that the development of resistance will be accelerated by the use of ivermectin CRCs.

Oral moxidectin is highly effective against ivermectin-resistant adults of *H. contortus* (Barnes *et al*., 2001), *O. circumcincta* (Leathwick *et al*., 2001) and *T. colubriformis*. The injectable form was highly effective against the first two species, but it performed very poorly against ivermectin-resistant *T. colubriformis*(Gopal *et al*., 2001). The ivermectin CRC, however, is not more effective than oral ivermectin (see above). Whereas resistance appears to be dominant in the presence of the moxidectin "tail" for both *H. contortus* and *O. circumcincta.* In the latter species it appears to be incompletely-recessive in the presence of an ivermectin CRC. These different persistent drug formulations vary in their efficacy against ivermectin-resistant genotypes of different parasite species, so no general conclusions can be drawn as to which products are likely to result in the most rapid development of resistance (Leathwick *et al*., 2001).

Selection Pressure

Many factors influence the intensity of selection pressure for drug resistance. A simplified outline of the selection process for short- and long-acting anthelmintics follows (Leathwick *et al*., 2001). Treatment with short-acting drenches, such as oral benzimidazole and levamisole products, remove most but not all of the parasite population in the host animals. Worms remaining generally possess some genetic resistance to the anthelmintic. After treatment, it takes approximately three weeks (the pre-patent period), in lambs, for new infections to establish and develop to potency. During this time, the resistant surviving worms are the only contributors to pasture contamination, thus, they have a reproductive advantage over susceptible genotypes for the duration of the pre-patent period. In this way, each treatment increases the frequency of resistant genotypes in the overall parasite population. The selective removal of worms present in the host at the time of each treatment occurs with all drenches and is referred to as "head" selection (Le Jambre *et al*., 1999; Leathwick & Sutherland, 2002).

A source of susceptible larvae slows the onset of resistance: In the absence of a source of susceptible genotypes, modeling studies indicate that assumptions or uncertainties about gene dominance and drench efficacy become irrelevant. When there is no source of susceptible genotypes, such as when drenches are used to produce "safe" pasture, the rapid development of resistance is inevitable. In NZ, reduction in pasture contamination was seen as the major benefit of using persistent anthelmintics in adult sheep (Gogolewski *et al*., 1997; Familton, 1996). Generating pasture with very low numbers of susceptible larvae gives a huge advantage to the resistant parasites that survive the anthelmintic and so it is not surprising that using anthelmintics in this way will result in the rapid development of resistance (Leathwick *et al*., 2001). This is analogous to the Australian findings of more rapid development of resistance in arid situations than in higher rainfall conditions (Ball, 2002; Kahn & Watson, 2001).

The use of persistent anthelmintics has two additional consequences (Leathwick *et al*., 2001). Firstly, the period of reproductive advantage enjoyed by resistant worms that survive the anthelmintic treatment is much longer than that following the use of short-

acting anthelmintics. For example, if a persistent drug provides four weeks of protection against the establishment of ingested larvae and the pre-patent period is a further three weeks, then the reproductive advantage to the survivors of the initial treatment will be seven weeks. The longer resistant worms are able to pass eggs onto pasture in the absence of susceptible genotypes, the more they will contribute to the overall pool of infective larvae and subsequent generations of worms (Dobson *et al*., 1996). Secondly, during the period of persistent activity, larvae of susceptible genotypes cannot establish and develop, but larvae of resistant genotypes can. The drug acts not only on worms present at the time of initial treatment, but continues to screen the parasite population for the total period of persistent activity, allowing only resistant worms to survive and develop, which can only mate with other resistant worms during that period. Jointly, these two processes are referred to as "tail" selection (Dobson *et al*., 1996; Leathwick & Sutherland, 2002). These processes interact to determine the overall selection pressure for resistance.

Australia Conclusions on the "Heads" *vs.* "Tails" Debate

Dobson *et al* (1996) published the first recommendations about the use of anthelmintics that have persistent activity and their effects on the development of anthelmintic resistance (Barger, 1997a; Sangster, 1999). This concept was based on the "heads" *vs*. "tails" selection scenarios (Leathwick & Sutherland, 2002). "Head" selection results from therapeutic activity at the time of drenching and "tail" selection from the persistent activity of the anthelmintic. The persistent ("tail") activity prevents susceptible worms establishing.

Different parasites may have different mechanisms controlling population dynamics in the hosts (A. Sykes, pers. comm.). In some the resident adult population may play a key role and in others the incoming L_3 larvae have a key role. This may lead to two different resistance scenarios.

For *T. colubriformis*, Dobson *et al*. (1996) modeled the effects of a persistent drug, varying drug efficacy and duration of persistence against susceptible and resistant genotypes under conditions simulating those of the Australian "Wormkill" parasite management programme. The results showed that greater persistency of anthelmintic effect ("tail " effect) was associated with greater selection pressure for resistance. However, if the frequency of treatment with a persistent anthelmintic was reduced and the persistent drug had a high level of efficacy against resident adult worms at the initial treatment ("head" effect), then selection for resistance was not increased, and could even be reduced. They concluded from this simulation that "head" selection, the efficacy of the drench against established worms, was the most important factor in selection for resistance. The persistent "tail" activity was secondary in importance, because it determined the reproductive advantage of resistant survivors, and establishment of resistant larvae during the "tail" period was less important.

However, a subsequent comparison of the development with resistant *H. contortus* using ivermectin and moxidectin indicated that effects on the establishment of resistant worms during the "tail" phase was more important than the selection of resident adult resistant worms at the time of treatment, i.e. "head" selection (Le Jambre *et al*., 1999). The proportion of the worm population subsequently present in animals that was resistant to anthelmintic was greater following the use of the persistent drug (moxidectin) than following the use of the non-persistent drug (ivermectin). Simulations by these authors using the *T. colubriformis* model previously described by Dobson *et al*. (1996) indicated

that, despite these empirical findings, moxidectin could select for resistance more slowly than ivermectin under some conditions. It appears, then, that the relative importance of "head" *vs.* "tail" selection processes varies with management, epidemiological factors (such as the number of treatments and the use of "safe" pasture) and possibly the different population control characteristics of the parasite in question.

Some of the factors which influence selection for resistance between long-acting *vs.* short-acting drugs are discussed using two macrocyclic-lactone anthelmintics, ivermectin and moxidectin as examples. In general, at the manufacturer's recommended dose rates, moxidectin has greater efficacy against gastrointestinal nematodes than ivermectin (Sangster, 1995) and has consequently been shown to have greater efficacy against both heterozygotes (RS) and homozygous (RR) ivermectin-resistant genotypes of both *H. contortus* (Barnes *et al*., 2001) and *O. circumcincta* (Leathwick *et al*., 2001). Therefore, following treatment with moxidectin, fewer resistant worms remain in the host than following treatment with ivermectin and this will delay the development of resistance in the population (Barnes *et al*., 1995). Moxidectin, however, also has persistent activity which affords a reproductive advantage to those resistant worms which do survive the drench (Dobson *et al*., 1996). In addition, for most of its duration, moxidectin does not prevent establishment of resistant worms form ingested L3 larvae (Rolfe & Fitzgibbon, 1996; Sutherland *et al*., 1997; Le Jambre *et al*., 1999), whether they be RS or RR ivermectin-resistant genotypes (Barnes *et al*., 2001; Leathwick *et al*., 2001). These factors are likely to result in increased selection pressure for resistance (Dobson *et al*., 1996). Results of the modeling work of Dobson *et al*. (1996) and Le Jambre *et al*. (1999) suggest that under the conditions of the Australian "Wormkill" programme, the selection pressure for resistance that results from the high initial efficacy and persistent activity of moxidectin *vs*. the lower initial efficacy and non-persistence of ivermectin, is approximately the same. There was no clear or consistent benefit to selection for resistance from using one drug in preference to the other. However, differences in selection pressure between these two drugs can occur under some conditions. For example, computer modeling (Dobson *et al*., 1996) showed that selection for resistance occurred more slowly if lambs were drenched three times with a non-persistent drug (e.g. ivermectin) compared with twice using a drug with a four-week "tail" (e.g. moxidectin). In contrast, if the duration of persistent activity was only two weeks, the reverse occurred. In further work, Dobson & Barnes (1999) estimated that a drug with a "tail" 20-35 days long which allows all RS and RR, but no homozygous susceptible (SS) larvae to establish (as is the case with moxidectin) will select for resistance at approximately the same rate as a non-persistent drug which has no efficacy against established RS and RR worms (such as ivermectin). This also supports the view that there is no consistent advantage to the use of one of these drugs over the other.

Abamectin, a third member of the macrocyclic-lactone anthelmintics, might exert less selection pressure for resistance than either ivermectin or moxidectin. Leathwick *et al*. (2000) and Woodgate *et al*. (2001) both demonstrated that abamectin had greater efficacy against ivermectin-resistant isolates of *O. circumcincta* than did ivermectin. Although, not as effective as moxidectin against these isolates, abamectin, like ivermectin, has little persistent activity (Leathwick & Sutherland, 2001). By combining high efficacy against resistant genotypes with low persistence, abamectin may exert less overall selection pressure for resistance than either ivermectin or moxidectin, thus delaying the development of resistance to this important class of anthelmintics. More work in this area is needed to fully understand the problem.

Extrapolating the "heads" vs "tails" theory to NZ conditions

The above "heads"/"tails" scenario explained anthelmintic resistance findings under the Australian "Wormkill" programme but it was not clear whether they covered the NZ situation. Leathwick *et al*. (2001) proposed the following hypothesis for NZ situations. In the "Wormkill" programme, both adult ewes and their lambs are drenched at weaning and the lambs moved to previously prepared "safe" pasture and receive two further broad-spectrum drenches during the year (Dobson *et al*., 1996). Some of these drenches are given under conditions of low larval challenge, which would reduce "tail" selection. In temperate NZ, where conditions are more suitable for development and survival of larvae on pasture (Barger, 1995), use of "safe" pasture is more limited (Macchi *et al*., 1999). Larval challenge is probably higher and hence "tail" selection more significant than in the Australian models. Persistent anthelmintics are commonly used in NZ as a pre-lamb treatment for ewes, so it is not possible to reduce treatment frequency to the ewes. Reducing drenching frequency in the Australian studies limited the rate of development of resistance (Dobson *et al*., 1996).

In addition, the population dynamics of parasites around lambing differ markedly from those around weaning. The relaxation of immunity in ewes around lambing is well documented (Sykes & Coop, 2001; Vlassoff *et al*., 2001) and anthelmintic treatment of ewes 2-3 weeks pre-lambing would coincide with the start of this period of relaxed immunity. At this time, ewe worm burdens tend to be low (Brunsdon, 1970), coming at the end of a period of high immunity, and so the importance of "head" selection would be reduced. In contrast, 4-6 weeks following drenching, ewes' immunity is lowered and more ingested larvae are able to establish. Thus, the opportunity for "tail" selection (i.e. establishment of only resistant larvae) is increased. Once the ewes' immunity returns to full strength, 2-4 weeks after lambing, further establishment of larvae is minimal (Leathwick *et al*., 1999), and therefore further persistent anthelmintic activity is unimportant. As long as a drench is persistent enough to cover the relaxed immunity period in ewes, any additional duration of persistence would have little additional selection pressure for resistance. This is supported by results from modeling under NZ conditions (Leathwick *et al*., 1995; Leathwick *et al*., 2001).

The above is a hypothesis, but it may have more validity than extrapolating the Australian findings to NZ conditions (Leathwick *et al*., 2001). However, the findings from the Australian work indicates that, under some NZ conditions (e.g. the pre-lamb treatment of ewes), persistent anthelmintics may select more strongly for resistance than reported in the original studies (Leathwick *et al*., 2001).

In a recent modeling study, Leathwick & Sutherland (2002) concluded that the ideal drug to delay the development of resistance was likely to have the highest therapeutic activity against the resistant adult genotypes ("head") and the shortest persistence, and that the longer the "tail" or more persistent the drug, the greater the rate of onset of resistance. These conclusions were from modeling studies with *O. circumcincta*. Findings in laboratory studies in Australia with *H. contortus* support these conclusions (Kahn & Watson, 2001).

3.8.10 Effects of Host Immunity

Drenching adult, fully immune, sheep results in greater selection pressure for resistance than drenching lambs in which immunity has not yet fully developed (Dash *et al*., 1985; Leathwick *et al*., 1995; Le Jambre *et al*., 1999; Smith *et al*., 1999). Selection pressure for resistance is minimised when treatment is followed by the ingestion and establishment of larvae that are close to the initial mixed population of susceptible and resistant genotypes at the time of treatment (Smith *et al*., 1999). The reproductive advantage of the drench-resistant survivors is minimised. An immune host, such as an adult ewe, prevents all but a few larvae establishing (Leathwick *et al*., 1999), so the diluting effect of incoming larvae is reduced and selection pressure for resistance is increased. In a similar manner, an anthelmintic that had persistent activity that kills all incoming susceptible larvae, regardless of genotype, for a period, is highly selective for resistance (Dobson *et al*., 1996). Australian work with ewes is contrary to the NZ findings (Barger, 1997b). Using the Australian *T. colubriformis* model (Barnes & Dobson, 1990) showed drenching ewes soon after lambing did not select strongly for resistance. Barger (1997b) noted that there was a significant difference between the two models and the level of immunity in the ewes. This was the likely cause of the different results. The Australian model, based on Merino sheep, assumes a complete loss of immunity over lactation. This means Merino ewes behave similarly to lambs while the NZ model, based on Romney sheep, assumes a shorter and lesser decrease in immunity. Results from both models are consistent with findings in their respective countries and with the breeds of sheep used (Leathwick *et al*., 1999).

A similar phenomenon may occur with strains of sheep that are bred for increased immunity. Two independent simulation studies indicated selection for anthelmintic resistance would be intensified if immune or genetically resistant hosts were drenched at similar frequencies to susceptible hosts (Barger, 1989, 1995a; Leathwick *et al*., 1995). There is no current evidence that this is occurring, however in many practical situations with parasite resistant or resilient sheep there has been a decline in drench use.

More recent work has indicated another interesting interaction between host immunity and anthelmintic use, affecting selection for resistance (Sutherland, 2000; Sutherland *et al*., 2000). Level of resistance of parasite eggs passed by sheep that had been either untreated or treated with CRCs at seven months of age differed. While the capsules were active and the animals were challenged with a mix of resistant and susceptible parasites, only resistant genotypes established and produced eggs that also showed a high level of resistance (approximately 100x higher) in the CRC treated animals than those from the non-treated animals. After the capsules expired (day 100) challenge was switched to susceptible larvae only, it was expected that once parasites were again able to establish in the hosts the susceptible parasites would rapidly dilute out the resistant worms which had established while the capsules were active. This did not happen, as the sheep had developed a substantial level of immunity and virtually none of the susceptible larvae survived. For 5-7 weeks after the capsules had expired, the level of resistance of eggs passed from the CRC-treated animals remained as high as when the capsules were active. These results show that CRCs can combine with developing host immunity to produce an extremely long period (>149 days) of reproductive advantage for highly resistant worms that survive the initial treatment or subsequently establish during the period of CRC activity.

3.8.11 Key Points on Anthelmintic Resistance

- Management of anthelmintic resistance is difficult; much is unknown, but knowledge is increasing.
- Widespread resistance now occurs to all existing broad-spectrum anthelmintic chemical classes and is a result of current drenching practices.
- Once resistance has developed, reversion to susceptibility is unlikely and resistance will reappear if a failed drug is reintroduced.
- Number of anthelmintic treatments is not a reliable indicator of selection pressure and should not be the only factor considered in strategies for minimising the development of resistance.
- Extrapolation of recommendations for the management of resistance from Australia to NZ is unwise because of differences in climate, parasite ecology, farming practices and main breeds of sheep used (Merino *vs.* British breeds).
- The potential of persistent anthelmintics to select for resistance varies and generalisations about their effect should be interpreted with caution, but their use in the appropriate circumstances does encourage resistance. Recent work suggest the more persistent the "tail" activity the greater the onset of drug resistance.
- Using drenching only to generate "safe" pasture for lambs is likely to result in the rapid development of resistance.
- The onset of resistance can be slowed down if there is a reservoir of susceptible larvae on the pasture. This occurs by dilution of resistant larvae on the pasture and dilution of resistance of resistant adults in the host, but more importantly by providing susceptible adults for the resistant worms to mate with. This prevents the next generation of worms becoming homozygous for drug resistance
- Anthelmintic treatments applied to animals with a high level of immunity, or which become immune while the anthelmintic is active, are likely to select for resistance faster than treatments applied to non-immune stock.

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3.9 Integrated & Planned Control Programmes for the Management of Internal Parasites

Since the introduction of broad spectrum drenches, these became for 20 years or more, a relatively cheap, highly effective control measure (Bisset *et al*., 1991). Drench was an easy option and reduced the need for detailed planning by the farmer. The long term repercussions were probably unknown or ignored, especially when serious drench resistance started to emerge.

Since 1980, the prevalence of anthelmintic resistance in NZ has increased to the point where it is now common and occurs for all of the currently available broad-spectrum anthelmintic classes. Despite this, the frequency of anthelmintic treatments applied by NZ sheep farmers has remained essentially unchanged (Brunsdon *et al*., 1983; Macchi *et al*., 1999). Given the routine use in recent times of persistent anthelmintics, it is likely that

parasite exposure to anthelmintics is greater now than it ever was and it seems unlikely that this will change in the immediate future since most farmers appear to be making little effort to reduce drench usage (Macchi *et al*., 1999). Current patterns of anthelmintic use are applying significant selection pressure for resistance, and in the absence of any major change, it is inevitable that resistance levels will continue to increase. Unless new chemical classes of anthelmintics become available, current chemical parasite control practices will be unsustainable in the long term (Leathwick *et al*., 2001).

Pomroy (2000) had the following to say about potential new anthelmintics for the future:

> "Don't hold your breath! Whilst there are hints of new classes of actives becoming available, none have yet passed sufficient "hurdles' in development to have reached the point of being discussed publicly or being field trialled. Thus we need to plan to farm for the next decade at least, with the existing armory of anthelmintics. For some farmers (and their veterinarians) this will present interesting challenges. It is possible different delivery systems/approaches may extend the usefulness of the existing anthelmintics but some of these are difficult and tedious. For example, holding animals off feed and multiple dosing. Such approaches will create challenges with issues such as withholding periods (and pneumonia)".

There have been no new drench families on the market for the past 20 years and there is little likelihood of any within the next 10 years (Merial, 2001) or at least that is what we are being told. Animal health companies are notoriously tight-lipped about their R&D pipelines. Pharmaprojects is a leading worldwide source of intelligence on all drug R&D and new product development. They report that most of the work on anthelmintics has ceased, and there appear to be no new products on the horizon (Animal Pharm, 2003).

Farmers need to use the whole range of control methods available to them in an integrated control programme against internal parasites to manage anthelmintic resistance. Modern control programmes still largely rely on a preventive drenching approach as outlined earlier in this review, however only as part of the overall biological and chemical control strategy (Heath *et al*., 2000; Nicol & Everest, 1997; FECPAK, 2001a).

Based on opportunities in the epidemiology and life cycle of helminth parasites of farmed ruminants, a number of workers (Brunsdon *et al*., 1975; Brunsdon, 1980, Jagger, 1982; Nicol & Thompson, 1982) advocated more than 20 years ago the use of grazing management to assist in reducing the exposure of susceptible stock to L_3 larvae.

The severity of parasitism on any particular farm is influenced not only by the seasonal conditions, but also by many other factors, potentially controllable by the farmer, such as stocking rates, ratio of stock classes, levels of nutrition, grazing and pasture management practices, use of alternative forages or crops, timing of parturition, duration of retention of young stock and obviously drenching (Bisset *et al*., 1991; Niezen, 1998; Hein *et al*., 2001). These factors can be manipulated to minimise parasitism through two main strategies: controlling contamination of pastures to the extent that buildup of dangerous levels of infective larvae is avoided; and/or anticipating periods of buildup of pasture infestation and removing susceptible animals from affected areas in order to protect them from infection ("larvae avoidance").

Difficulties in achieving success from this type of approach have been put down to the general lack of understanding by farmers of relevant aspects of basic worm biology/epidemiology or poor planning/record keeping (Bisset *et al*., 1991; Michel, 1982; Nicol & Everest, 1997; Waller, 1993), but that is not the complete story.

Nicol & Everest (1997) point out that many farmers have tried very conscientiously to prepare and use safe pastures with mixed success and that many have become disillusioned with the concept when the so-called "safe" pasture failed for one or a number of reasons, which are now known: spelling intervals that were too short, use of unsuitable classes of stock to prepare the pasture, and insufficient area of prepared "safe" pasture. These will be discussed in this section. Nevertheless there are numerous reports of improved gain (50-100 *vs.* 80-160 g/day), especially of lambs, when grazing pastures with low levels of contamination (McAnulty *et al*., 1982; Nicol & Everest, 1997; Nicol & Thompson, 1982; Thompson *et al*., 1982). The previously advocated use of CRCs to create safe pasture (Venning, 1991; Barger, 1997a) has merely added to the confusion.

Heath *et al*. (2000) outline the main steps in a modern planned integrated control programme as follows:

1. "The first step is to assess your farm and its stock mix, the scope for manipulating this mix, and for improving pasture management and incorporating arable land if required.
2. The second step is to decide, in consultation with your veterinarian, the internal parasite status of your farm in terms of, at very least, its severity and drench resistance status.
3. The third step is to look for ways to produce "safer" pasture for stock and especially young stock, so as to reduce their intake of infective (L_3) larvae.
4. Finally, decide on a drenching strategy that can be combined with a pasture use and management strategy."

In relation to Step 2, drench resistance does not always result in clinical symptoms (FECPAK, 2001a). Very few farmers check the effectiveness of their drenching programmes (FECPACK, 2001f) but in NZ it is now essential to know the resistance status of a farm when designing worm control programmes (Pomroy, 2000; FECPAC, 2001f, 2001a).

One of the big problems with drench resistance is that it is very difficult to work with and assess in the field (Leathwick *et al*., 1998). Because resistant worms look and behave exactly like susceptible worms, they only way to tell them apart is whether a drench will kill them. At present the most practical way to do this is by carrying out a "DrenchCheck" or "DrenchTest". The fact that, initially at least, there are very few resistant worms on any farm, and resistance can only be detected when the level is relatively high, makes it extremely difficult to measure the buildup of resistant worms on farms. Consequently there have very few field trials in NZ to assess the effect of different farm management practices on the development of drench resistance.

FECPAK (2001f, 2001) recommend the "Faecal Egg Count Drench Check", often referred to as "DrenchCheck", and outline precautions to prevent the occurrence of false positives. This is the first and easiest step to check for drench resistance. To determine what parasite species survive treatment, larval cultures are necessary (FECPAK, 2001f). The

DrenchRite™ assay is suitable for this purpose, but has some limitations, as outlined earlier.

Step 3 is key to most of these programmes as the fewer infective larvae on the pasture, the lower the infection rates. While most advocates of integrated control programmes refer to the preparation and use of "clean" or "safe" pastures (Bisset *et al*., 1991; Brunsdon *et al*., 1975, Brunsdon 1980; Waller, 1993; Nicol & Thompson, 1982; Brunsdon & Vlassoff, 1982; Michel, 1982) some authors prefer to use the term "safer" or "less contaminated" pastures (Heath *et al*., 2000; Nicol & Everest, 1997) because of the risk or uncertainty in producing pastures with larval populations sufficiently low as to not to impair production of susceptible animals let alone prevent reinfection.

The main reason for implementing a mixed animal (integrated) grazing system is to reduce the need to drench (Niezen, 1998). It is therefore important that, at the same time, a regular FEC monitoring programme be set up to determine when to drench. This reduces the time and costs of drenching, and minimises selection for drench resistance. Integrated grazing without a reduction in drenching may still increase selection pressure for drench resistance as discussed in the previous section (Leathwick *et al*., 2001).

While key components of integrated control programmes (Heath *et al*., 2000) involve knowing the parasite and drench resistance status of the farm and using a preventive drenching programme (steps 2 and 4 above), this section will concentrate on pasture, grazing and animal management issues on the farm.

These control programmes are specific to each farm, but the basic principle and practices are similar. While no single grazing system applies to all sheep farms; there are several key rules (Niezen, 1998):

- The longer the interval between grazings by sheep, the cleaner the pastures become.
- Greater numbers of cattle and larger areas of cropping or conserved feed (hay and silage) will make it easier to maintain cleaner pasture.
- The greater the proportion of white clover or herbs (such as chicory) in pastures, and the greater use of specialist forages containing condensed tannins, the better lambs will perform and be able to tolerate the effects of roundworms.
- Regular monitoring of FECs to determine when to drench will reduce costs and help minimise selection for drench resistance.

3.9.1 Ratio of Stock Classes

Young or susceptible animals are generally responsible for the vast majority of pasture contamination on a farm (Vlassoff *et al*., 2001). Therefore contamination rates and parasitic disease may be reduced simply by reducing the proportion of young or susceptible stock on a farm (Bisset *et al*, 1991). This can be assisted by selling or removing young stock earlier, saving fewer replacements or changing the principle product of the operation, e.g. from lamb to beef. Obviously these sorts of decisions will be dictated largely by economic considerations.

In a sheep finishing situation, the main aim is to minimise the larval challenge to the most vulnerable and economically sensitive class of stock, the naïve lamb pre- and post-weaning (Nicol & Everest, 1997). Any reduction in lamb growth rate due to internal

parasites reduces carcass weight and/or extends the time period from weaning to slaughter which in turn decreases lamb value; increases competition between finishing lambs and ewes (pre-joining) for late-summer pasture; and increases the total pasture consumption of lambs to a given carcass weight.

In the case of goat farms, because all classes of animals tend to remain relatively susceptible to infection, reducing the proportion of susceptible stock will normally mean replacing a proportion of goat stock units with cattle (or less preferably adult sheep). Long term intensive farming of goats by themselves is unlikely to be viable due to difficulties in achieving adequate parasite control (Bisset *et al*., 1991).

3.9.2 Level of Feeding

Optimal levels of nutrition are essential in combating parasitism and achieving good levels of production in its presence for all classes of stock. Level of nutrition, especially protein nutrition, allows the animals to tolerate internal parasite infections and develop a good immune response (Sykes, 1997; Sykes & Coop, 2001). Heath *et al*. (2000) state that "Drenching is not a substitute for good feeding" and "There is no better anthelmintic than good quality green grass". To optimise feeding levels, a knowledge of feed requirements and optimum pasture covers for susceptible classes of stock is essential. Grazing management decisions should aim at providing these, or if unachievable, high quality supplements should be fed.

Good levels of feeding of pregnant and early lactating ewes, in particular multiple bearing ewes and poor conditioned ewes, will help prevent the temporary breakdown in their immunity and the periparturient rise in faecal egg counts (Sykes, 1997; HISHA & SAC, 2000; Heath *et al*., 2000). This will result in lower levels of pasture contamination than otherwise would have been the case.

3.9.3 Provision of "Safer" Pasture

The main methods of potentially achieving this are (Bisset *et al*., 1991; Nicol & Everest, 1997; Niezen, 1998):

- Grazing hay or silage regrowth.
- Cultivation and establishment of new pasture or forage crops for grazing with susceptible stock.
- Using areas previously grazed by a different ruminant species or a non-infective/immune stock class of the same ruminant species.

Hay or silage aftermath: paddocks are usually closed for 40-60 days before hay or silage is cut and removed, and then it is several more weeks before the regrowth is grazed. This time interval combined with the harvesting removes a large proportion of the larvae (Bisset *et al*., 1991; Niezen, 1998; Nicol & Everest, 1997). If the cutting height is above 5 cm, fewer larvae are likely to be removed than if the pasture is cut lower. Most of the larvae that remain on hay stubble should be killed by ultraviolet radiation and desiccation. Some contamination can remain, especially if the areas were previously grazed by contaminating stock or if the spell is not long enough (Nicol & Everest, 1997). Generally the area of such

prepared pasture on most farms is too small to provide sufficient safe grazing for susceptible animals, and sooner or later they will have to graze contaminated pasture.

New pasture and summer forage crops: These are generally considered to be free of internal parasite larvae (Bisset *et al*., 1991; Brunsdon, 1980; Nicol & Everest, 1997; Niezen, 1998). Newly established pasture areas have not had any contamination for a long period of time and cultivation should have ensured that very few, if any, larvae survive. Generally the area of new pasture is limited. With specialist crops there is generally a long interval between the last grazing of pasture and the establishment and grazing of the crop. This interval and the cultivation should ensure few larvae are present. The physical structure of many fodder crops may preclude the migration of any larvae present into the grazing zone, although grass margins may remain a potential source of larvae. In many situations, such crops may be impractical (hill country) or not economic. In some situations where serious drench resistance has arisen, such as on goat units, taking non-forage crops such as potatoes for two to three years has cleaned the area up.

Areas grazed by non-infective animals (Bisset *et al*., 1991; Brunsdon, 1980; Nicol & Everest, 1997; Niezen, 1998)*:* Pastures from which all infective sheep or goats have been excluded for at least 2-3 months but which have been grazed by cattle during that time can provide safe pasture for sheep or goats and vice versa. This is because they share very few of the same species of worm parasites (Bisset & Vlassoff, 1991) and cross-contamination of pasture by the alternate ruminant species is likely to have minimal infectivity for the principal species. This does not imply that absolutely no cross transmission can take place, but that a high proportion of ingested larvae do not establish in the heterologous host (Bisset *et al*., 1991). Those that do, often have a limited, if any, period of patency (egg production) as adults. Morley and Donald (1980) distinguished three levels of cross transmission between susceptible sheep and cattle: very low cross-infectivity without reproduction (e.g. *Ostertagia, Oesophagostoum, Nematodirus*); reduced cross-infectivity with limited or only short term patency (e.g. *Cooperia, Trichostrongylus sp*); minor differences in infectivity (e.g. *Trichostrongylus axei*). In Australia, *H. contortus* is considered to fall into the third category (Morley & Donald, 1980), but in NZ, cross-transmission of this species from sheep or goats to cattle is very low (Bisset *et al*., 1991). On the other hand, *Ostertagia leptospicularis* (= *O. crimensis*), which was introduced into NZ as a parasite of deer is often found as a minor component in cattle *Ostertagia* burdens, and seems capable of a moderate cross-transmission to goats though not to sheep (Bisset, 1980).

One ruminant species can essentially clean up pasture contaminated by the other (Niezen, 1998; Nicol & Everest, 1997; Bisset *et al*., 1991). Cattle are an appropriate alternate species to sheep. In the case of cattle, preparing pasture for lambs, once lambs have grazed and contaminated a paddock with worm eggs, subsequent grazing by cattle will help remove a proportion of any larvae that develop in the following ways:

- Cattle act as vacuum cleaners. As they graze, they ingest larvae and those of sheep origin do not establish in cattle and hence die. (Likewise, sheep will help to remove some of the larvae that originate from cattle).
- Cattle grazing opens up the sward, exposing the larvae to desiccation and ultraviolet radiation. In addition, cattle grazing can increase the white clover content of swards. This can reduce the production losses due to parasitism as well as boosting lamb performance.

- Because of the extended interval before sheep return to the paddock, in some seasons there will also be considerable reduction in larval contamination through natural larval mortality.

There are good examples in the literature where interchange of cattle and sheep has led to effective parasite control, particularly when appropriate spelling intervals have been adopted (Barger, 1978; Niezen *et al*., 1991). This practice of interchanging ruminant species has been used as the basic grazing management tool in organic systems (Mackay & Betteridge, 1998). Cattle have used on organic or low chemical systems in an attempt to create "safe" pasture for lambs, along with other measures, with some degree of success (MacKay *et al.,* 1998; 2001). This practice, while generally successful, has been found on occasion, such as under irrigation, not to reduce the parasite burden of lambs subsequently grazing such prepared pasture. Moss *et al.* (1998), found with irrigated pastures, that grazing contaminated pasture with cattle reduced pasture larval contamination by about 80%, yet when subsequently grazed by sheep there was no reduction in their FECs or worm burdens.

Warnings have been issued (Donald & Waller, 1982) that alternate grazing of species is likely to increase the importance of species which can cross-infect and perhaps reduce the host-specificity of others. To date there seems to be little evidence for this occurring (Nicol & Everest, 1997; Bisset *et al*., 1991).

Integrated control programmes make use of sheep-cattle interchange where possible (Nicol & Everest, 1997; Niezen, 1998). For example, areas grazed by cows and calves or finishing cattle from calving to lamb weaning, are often grazed by lambs after weaning. Provision of sufficient pasture from cattle grazing for lambs after weaning requires 30-50% of stock units as cattle (Nicol & Thompson, 1982; Morley & McDonald, 1991). At least 30-50% cattle stock units are needed to consistently generate cleaner pasture for lambs (Nicol & Thompson, 1982; Nicol & Everest, 1997; Morley & Donald, 1991; Niezen, 1998). If other methods of creating cleaner pasture are used, a 65:35 or 70:30 sheep:cattle ratio may suffice. Ideally, the number of lamb stock units at weaning should be less than or equal to the number of cattle stock units to balance feed requirements. With this stocking rate ratio, it should be possible to keep generating clean pasture ahead of the lambs, beginning at weaning. Few farms have sufficient cattle stock units to prepare sufficient safe pasture to graze all the lambs on without the need to return to contaminated areas.
No intensive finishing NZ livestock farms contain this proportion of cattle (NZMBES, 1996) although on many North Island hill country farms cattle make up over 40% of the stock units. Because of low cattle numbers, many integrated control programmes only make use of the "dilution" effect of cattle in mixed grazing (Nicol & Everest, 1997), because faeces returned by cattle will not contain infective sheep nematode eggs, and will lower the average pasture contamination. This mixed grazing of sheep and cattle may involve rotational grazing, set stocking or set stocking of sheep with cattle rotationally grazed through the mobs of sheep. Where cattle are used in this "dilution" role, simultaneous rotational grazing induces less competition between sheep and cattle and enhances cattle liveweight gain (Kitessa & Nicol, 1995). Another way of using a limited number of cattle is to graze them for shorter periods of time on areas known to have a high level of contamination with infective larvae (Nicol & Everest, 1997).

A lamb-ewe-cattle rotation, where lambs do not return to graze the same pasture for at least 60 days should reduce drenching requirements. In this type of management system, the lambs stay in each paddock for 7-10 days, with an adequate feed allowance; ewes graze it for the next 28 days; followed by cattle which graze the area for as long as possible. In this situation, ewes are used to initially reduce pasture larval contamination and the cattle continue the cleaning up process.

Non-lactating ewes and hoggets are considered net removers of larvae from contaminated pasture, but because they will still pass low numbers of parasite eggs in their faeces they may not adequately clean up pastures for lambs, hence the benefit of integrating cattle into the grazing programme. Another option for generating "cleaner" pasture for lambs is a lamb-cattle rotation (without ewes) which was used successfully in an experimental organic unit (Niezen, 1998). Lambs grazed paddocks for seven days, again with an adequate pasture allowance; the paddocks were spelled to allow larvae to develop and then were grazed by cattle. This rotation worked well on white clover dominant pastures maintaining high levels of lamb growth, and virtually eliminated the need for drenching. The longer the grazing interval between lamb grazings, the better the result.

Transmission of internal parasite species from deer to sheep is also limited so deer have been suggested as an alternative to cattle in producing safe pasture for sheep (Bisset *et al*., 1991; Nicol & Everest, 1997). However, grazing for alternate species (sheep) within a deer unit is normally only available in late spring, when the hinds have low feed demands, whereas the need for lower-contamination pasture for lambs occurs in summer/autumn when most deer units are fully stocked. There potentially may be other health problems (e.g. malignant catarrhal fever) with integrating deer and sheep (Nicol & Everest, 1997).

In the case of goat farms, it is more difficult to provide adequate safe pasture because goats of all ages tend to remain relatively susceptible to worm infection (Bisset *et al*., 1991). The most practical means of providing adequate safe pasture (apart from hay aftermaths, fodder crops etc.) is to incorporate cattle into the grazing system. Much the same management strategies as above can then be used. The higher the proportion of cattle to goats, the easier it will be to achieve adequate control of nematodes because of the greater area of safe pasture which can be created.

Other benefits from using different classes or species of stock in this way are more efficient pasture utilisation and improved pasture quality (Bisset *et al*., 1991). Generally, in combination, areas grazed by cattle plus new pasture and hay silage regrowth only provide sufficient lamb grazing for 6-8 weeks post-weaning (Nicol & Thompson, 1982). Inevitably sooner or later lambs will have to return to areas previously grazed by lactating ewes (Nicol & Everest, 1997).

Sheep only operations: When a single species of ruminant is farmed preparation of "safe" pastures can be difficult (Nicol & Everest, 1997). In sheep only systems, non-lactating ewes and ewe hoggets have been advocated as suitable for producing safe pasture for lambs and indeed several successful demonstrations of safe pasture have involved these classes of stock (McAnulty *et al*., 1982; Thompson *et al*., 1982; Nicol & Thompson, 1982). Over two years at Wallaceville (Brunsdon, 1980) grazing two-tooth wethers or adult non-lactating ewes over the summer on pasture contaminated during the spring by ewes and their lambs, reduced pasture infectivity by up to 93% and 98% respectively, relative to similar pastures grazed over that time by lambs. Much of the dissatisfaction with safe

pasture probably occurs because ewes and hoggets may not be as immune to parasites or non-contaminating as originally thought (Nicol & Everest, 1997). Ewe lambs can develop significant infections and high levels of egg production (700-900 epg) during April to June and later (Familton *et al*., 1996). Although faecal egg output in pregnant adult ewes in winter may be low (low feed intake and low FEC/g), their contribution to pasture contamination under winter rotational grazing can be high by virtue of the high stocking rate (1500-2000 ewes/ha/day). With higher than expected winter survival of larvae, significant spring pasture larval levels have been found on areas grazed by adult ewes (Familton *et al*., 1995).

Consequently, recommendations are now much less dogmatic about the potential of various classes of sheep to produce lower-contamination pasture for lambs unless their parasite status is known (Nicol & Everest, 1997).

Areas grazed exclusively by ewe hoggets are still used for lambs after weaning. Low larval concentration and prevention of contamination by hoggets can be ensured by monitoring the development of parasitic burdens by FEC and controlling them (Nicol & Everest, 1997). However the risks of using anthelmintics to produce safe pasture are now recognized (Leathwick *et al.,* 2001). The benefits of good parasite control, however, can result in increased hogget fleece weight and two-tooth mating weight (Kempthorne *et al*., 1996).

Control of the periparturient faecal egg count in the ewe: In integrated control programmes this is an important consideration (Nicol & Everest, 1997). Control of the periparturient rise has two advantages. It reduces exposure of the lambs to pre-weaning larval challenge and lowers contamination of areas subsequently grazed by lambs post-weaning.

Control of the periparturient rise is achieved by minimising the extent of the breakdown in ewe immunity by maintaining good body condition and correcting major dietary deficiencies. Recent evidence with adult ewes suggests the immunity of ewes is improved by both higher liveweight and condition score, and higher levels of nutrition especially protein intake (as discussed earlier in this review). Previously control of the periparturient rise was by strategic pre- and/or post-lambing anthelmintic treatment or through slow release anthelmintic administered pre-lambing. This control procedure has been shown to reduce faecal egg output very markedly in ewes. Ewes in poorer condition may require anthelmintic protection before lambing whereas, a drench at tailing may be adequate control for ewes in better condition. This use of anthelmintics is no longer routinely recommended as part of prudent integrated control programmes, especially the use of persistent anthelmintics or CRCs, because of their role in hastening the development of drench resistant parasites.

3.9.4 Rotational Grazing

Rotational grazing by itself is not an effective means of internal parasite control (Bisset *et al*., 1991), but it does have some inherent advantages. The main one is that it also allows for “safer’ pasture to be prepared, especially if the spelling interval is adequate.

Grazing interval: As discussed in the section on epidemiology, the infective larvae on pasture are non-feeding and actually survive on stored metabolites. In cooler temperatures, some of these larvae can live for up to 6-8 months (even longer under certain conditions), while in warmer temperatures survival may be only 2-3 months. Because of the variable climatic conditions in NZ, hard and fast rules are not possible but in general, the longer lambs are kept off pasture previously grazed by sheep, the lower the larval contamination should be (Niezen, 1998).

Rotational grazing utilises the concept of pasture spelling. It has been widely practiced in NZ for many years largely in the interests of more efficient pasture use, pasture rationing and higher levels of pasture productivity (Bisset *et al*., 1991).

As a means of parasite control, rotational grazing of young stock in its simplest form is generally considered to contribute little of value. Early advocacy was based on the belief that all nematode eggs develop and move onto pasture quickly and then die after a relatively short time (Michel, 1969). However, from our knowledge of nematode epidemiology we now know that this is not the case. Depending on conditions, it may take some weeks for larvae to develop and move out of the faeces. Once pasture has been contaminated, larvae can remain viable and available for some months, provided they remain in the relatively stable (and shaded) microclimate within the lower strata of the pasture. Brunsdon (1980) suggested "safe" pasture would require spelling for an interval of three months or more, while efficient pasture utilisation in a rotation would normally require an interval 6-8 weeks or less between grazings. Grazing susceptible stock on pasture at such an interval (6-8 weeks) may subject them to peak larval levels. This does not discredit rotating or breakfeeding of susceptible animals on "safe" pasture between weaning and sale, particularly if a single rotation can be achieved.

Pasture mass and height: One indisputable advantage of rotational grazing over a set stocked situation is that animals will be offered a "bank" of fed of relatively long pasture and high pasture mass. This has the double advantage of diluting the infective larvae by the greater amount of feed on offer and the taller pasture will offer a grazing horizon above the greatest concentration of larvae near the base of the pasture and the bottom 5 cm of the sward. Finishing stock utilise only 20-30% of the pasture on offer, so larval intake should be low. Higher grazing pressures and lower grazing height would lead to a greater larval intake.

Very high grazing pressures by the ewe mob, grazing to low residuals (500-700 kg DM/ha) would result in the ingestion of considerable number of larvae but if the ewes are in a solid immune state (e.g. good condition, early pregnancy) worm burdens should be minimal and some "cleaning" of the pasture should result.

Increasing stocking rate does not necessarily increase the exposure to infective larvae, if it is accompanied by an increase in feed supply from, for instance, increased capital phosphate fertiliser dressing (Bisset *et al*., 1991). Increases in infective larvae per unit area are offset by a decrease in larvae per kg herbage. It is not surprising that there is no clear relationship between stocking rate and parasite status (Morely & Donald, 1980). On the other hand increasing stocking rates, not accompanied by increased pasture production, is likely to increase larval intake, especially of nematode species, such as *Ostertagia* and *Nematodirus*, that are least vulnerable to the effects of low plant cover

(Morely & Donald, 1980). An interaction also occurs in that lower quantities of pasture available results in low intakes of infected pasture per animal (Nicol & Everest, 1997).

Grazing behaviour: Goats, due to their particular grazing behaviour, may well get more advantage than sheep or cattle from rotational grazing (Bisset *et al*., 1991). It is well known that goats tend to graze the pasture "from the top down" more than sheep and cattle. Because the highest concentrations of nematode larvae are found in the lowest strata of the pasture, it has been suggested that a well planned grazing rotation which makes it possible to keep a bank of fresh pasture ahead of goats may reduce their rate of intake of worm larvae.

Level of subdivision: Increased subdivision of a farm will improve the potential for creating cleaner pasture as stock can be controlled to a far greater degree (Niezen, 1998), and a long grazing rotation to be used.

3.9.5 Spelling Intervals to Produce Safe Pasture

Theoretically if the interval between grazing by stock which have contaminated pasture and the subsequent grazing covers the period of infective larval development and death, the pasture will decontaminate. Alternatively, the spelling interval between grazing with infective and resistant classes of stock needs to synchronise the time period for maximum development of infective larvae on the pasture to coincide with grazing by the immune stock class (Nicol & Everest, 1997). There are many literature reports concerning appropriate spelling intervals and their success in achieving these objectives but the period required for decontamination is very variable.

Generally a spelling interval of 2-6 months (a huge range in terms of farm management implications) should produce safe pasture (Bisset *et al*., 1991; Donald & Waller, 1982). However, there are examples where extensive spelling intervals have not resulted in safe pasture. Grazing irrigated pasture in Canterbury by cattle for six months before use by lambs after weaning was not effective in producing safe pasture due to survival of larvae from contamination by ewes and lambs the previous season (Familton *et al*., 1996). Extending the period of cattle grazing to nine months (from April to November) gave lower worm burdens in tracer lambs and higher lamb liveweight gain (McAnulty *et al*., 1992). In Scotland, alternating calves and sheep annually was ineffective (Bairden *et al*., 1995). *Ostertagia* survived for 18 months until favourable conditions led to levels of infection in calves as high as in the control groups. There are also many farmer observations of failure to produce safe pasture using supposedly appropriate spelling intervals (Nicol & Everest, 1997).

Under hot wet, tropical conditions where hatching and development of eggs is rapid and continuous and larval survival time is relatively short (3-7 weeks), a 35 day spelling interval in a 10 paddock rotational grazing system was very effective in reducing the need for anthelmintic treatment in goats (Barger *et al*., 1994).

On areas not grazed by lactating ewes prior to weaning, larvae deposited in the previous autumn had dropped to minimum levels by weaning (Bisset *et al*., 1991).

In Taranaki, on areas not grazed by ewes from lambing to weaning, a 70 day interval between the first and second lamb grazing maintained levels of pasture infectivity below 200 larvae per kg pasture (Niezen *et al*., 1991) although even this level has caused production losses (Nicol & Everest, 1997).

It is currently not possible to predict accurately the length of time pastures need to be spelled to decontaminate or reach minimum levels of infectivity under the wide range of climatic conditions that exist in NZ (Nicol & Everest, 1997). This is critical information and this inability to define effective spelling intervals is one of the major reasons why the safe pasture concept has led to confusion and disenchantment amongst farmers (Nicol & Everest, 1997).

3.9.6 The Role of Pasture Species & Specialised Forages

There has been considerable NZ work on the effects of different pasture species on parasitism in lambs (Niezen, 1995; Niezen *et al*., 1995, 1996; Sykes & Coop, 2001; Hein *et al.,* 2001; Nicol & Everest, 1997; Knight *et al.,* 1996), and in the extent to which pasture or forage morphology (height, density etc.) affect the survival/availability of larvae and/or the likelihood of an infection developing in animals. Pastures modify the microclimate which may directly affect larval development and survival, affect egg and larval predators and pathogens, and/or alter the rate of faecal decomposition. Rate and extent of larval migration may be affected by pasture plants differing in morphology and composition.

Some species appear to retard the migration of L_3 larvae on plants and thereby minimise the subsequent infection by grazing ruminants (Moss & Vlassoff, 1993; Niezen *et al*, 1998). Some plant species contain chemicals which either help animals resist infection or make them more resilient to an infection (Nicol & Everest, 1997; Niezen, 1995, 1996; Niezen *et al*., 1995, 1996). A number of NZ studies have found quite large differences in liveweight gain and FEC in lambs grazing different forages (Robertson *et al*., 1995; Seales *et al*., 1994).

Grasses: Grasses promote moderate liveweight gains in non-parasitised lambs, but low gains in parasitised lambs with high faecal egg counts. Larval survival is high, but distribution is mainly in the lower levels of the sward. Of the grasses, brown top supports lower growth rate, and higher faecal egg counts than average, while Yorkshire fog supports relatively low faecal egg counts and better growth rates in parasitised lambs (Niezen, 1998; Nicol & Everest, 1997). Perennial ryegrass is about average. Removing browntop is a tactic worth considering (Niezen, 1998). In a Flock House trial with pure swards, FECs were highest on browntop and tall fescue, and lowest on ryegrass and fog (Niezen, 19998a). The Winchmore organic farmlet used alternate grass species to advantage (Moss, 2002).

Legumes and chicory: Legumes and chicory are well known for producing high liveweight gain in lambs. Legumes such as lucerne, clover and herbs such as chicory, inhibit the vertical migration of larvae in comparison to grasses but with lucerne the larvae are carried higher in the sward than with grasses (Nicol & Everest, 1997). If larvae can be prevented from reaching the regions of the sward that are the most consistently grazed (26-125 mm above soil level), then ingestion of L_3 larvae by livestock is substantially

reduced (Moss & Vlassoff, 1993). Another major advantage to legumes is that larval survival is lower. Larval survival on chicory is also lower than on grasses.

In addition liveweight gain of lambs grazing chicory is not so severely reduced by parasitism. Chicory has a number of positive factors going for it. Levels of internal parasite infection and faecal egg count has been less in lambs and deer on chicory than on resident pasture (Scales *et al*., 1995; Barry, 1998; Barry *et al*., 1998). Chicory contains 0.2% condensed tannins and 0.4% sesquiterpine lactones or lactusin (Barry, 1998), both of which are anti-parasitic (similar to tannins in sulla and trefoil as discussed below). The grazing height (above the parasite larval zone) and good levels of nutrition on this herb contribute to the lower worm burdens and FEC. The Winchmore organic farmlet (Moss, 2002) used a variety of legumes (red and white clover, lucerne) and herbs (chicory and plantain).

Plants containing condensed tannins (CTs): Beneficial effects, on the ability of ruminants to withstand internal parasite infection has been found with tannin-containing plants (Hoskin *et al.,* 1999, 2000; Niezen *et al*., 1995, 1996; Rattray, 2001; Sykes & Coop, 2001). Condensed tannins are well ecognised for their ability to complex with soluble proteins at normal rumen pH. This restricts the normal breakdown of protein to ammonia in the rumen and allows more dietary protein to pass unchanged to the small intestine, acting as "bypass" protein. In most situations this increases protein supply to the animal. A number of workers have shown that high levels of nutrition or protein intake can increase resistance of sheep to infection and disease, including gut nematode parasites (Molan *et al*., 1999; Kahn & Diaz-Hernandez, 2000; van Houtert & Sykes, 1996; Douglas, 2002).

As discussed earlier, this "anthelmintic" activity may be through increased protein supply and amino acid supply in the intestine leading to an enhanced immune response in parasitised sheep (Houdijk *et al*., 2000, 2001; Sykes *et al*., 1992; Sykes & Coop, 2001; van Houtert & Sykes, 1996; van Houtert *et al*., 1995). However the immune response takes longer to develop than the duration of many of the reported trials (Kahn and Diaz-Hernandez 2000; Niezen *et al*., 1998a; van Houtert *et al*., 1995) so there may be a number of direct and indirect effects operating simultaneously.

Recent studies have suggested there may be a direct anthelmintic effect of the tannins (Athanasiadou *et al*., 2000; Butter *et al*., 2000; Sykes & Coop, 2001). In sheep fed CT-containing forages faecal egg counts (FEC) has decreased by 25-50% and worm burdens decreased by up to a 2.5 fold (Butter *et al.,* 2000; Niezen *et al.*, 1995: Robertson *et al.*, 1995). This phenomenon has led to CTs or CT-containing forages to be considered seriously for parasite control in a drug-free or organic production sysrem (Kahn & Diaz-Hernandez, 2000; Niezen *et al.*, 1996; Robertson *et al.*, 1995).

A range of forages which contain CTs, sulla (*Hedysarium coronarium*), Maku Lotus (*Lotus pedunculatus*), and Goldie Lotus (*Lotus corniculatus*) have been shown to substantially increase parasitised lamb performance, reduce dagginess, and variously reduce faecal egg counts (FEC) and/or worm burdens (Niezen *et al*., 1993; 1995; 1996; 1998a; b; Leathwick and Atkinson, 1995; Robertson *et al*., 1995).

Not all species of parasite respond to CTs in the same way, and different CT containing plants do not have the same effect on the same species of parasite.

Niezen *et al.* (1998a) reported that *Lotus pedunculatus* lowered worm burdens and FEC of *Ostertagia circumcincta* in parasitised lambs but did not affect the numbers of *Trichostrongylus colubriformis*; while Niezen *et al.* (1998b) reported that sulla reduced worm counts and FEC but *L. pedunculatus* resulted in improved lamb performance despite high worm burdens and FEC. Robertson *et al.* (1995) found both sulla and *L. pedunculatus* sustained high levels of production from lambs despite high worm burdens.
In red deer calves (Hoskin *et al.,* 1999, 2000) infected with a variety of gastro-intestinal nematode larvae (*Ostertagia; Trichostrongylus, Cooperia* and *Oesophagostomum*) and lung worm larvae (*Dictyocaulus* sp.) that were fed either lucerne (*Medicago sativa*, 0.1% CT), birdsfoot trefoil (*L. corniculatus,* 1.9% CT) or sulla (*H. coronarium*, 3.5% CT) for 5 weeks, there was a significant negative linear relationship between dietary CT level and abomasal worm burdens, but no significant differences in FEC, lungworm burdens or feed intake. Sulla-fed deer had higher liveweight gain, carcass weight, dressing-out percentage; higher serum total protein and albumin levels, and lower serum gastrin concentration and faecal lungworm larval count, compared with lucerne-fed deer. They concluded that sulla reduced the impact of internal parasites and/or reduced the dependence on anthelminthics.

Molan *et al.* (1999) extracted CTs from *L. pedunculatus, L. corniculatus,* sulla and sainfoin and demonstrated *in vitro* that, at concentrations of CTs similar to that in the digestive tract of sheep fed these forages, reduced development of eggs, first and third stage larvae, reduced proportion of eggs hatching and decreased mobility of Stage 3 larvae for the nematode *T. colubriformis. In vitro* the CT from sulla was more paralyzing on *T. strongylus* larvae than the CT from birdsfoot trefoil (Molan *et al.,* 2000). These *in vitro* results suggest CT is able to disrupt the life cycle of some nematodes.

More recently quite a large number of CTs and phenolic fractions extracted from both *Lotus* species, sainfoin, sulla and pine bark were effective in bioassays at inhibiting a number of aspects of parasite development (egg hatching, larval development and larval motility in *T. colubriformis*) (McNabb & Molan, 2001a; b).

Recent Australian work (Kahn & Watson, 2001) has also shown condensed tannin extracts may also be directly toxic to gastrointestinal parasites. CT extracts from a number of woody plants were shown to reduce nematode viability by up to 60% and lower the proportion of eggs which developed into infective larvae by 40-70%. Further research is being conducted to understand the role of CT in parasite control and to screen CT-containing plants that may have potential for use in worm control.

Anthanasiadou *et al.* (2000) found CT from Quebracho added to the diet of parasitised sheep improved gains and feed conversion accompanied by reduced FEC and worm burdens of *T. colubriformis* and suggest a direct long-term anthelminthic effect.

The mechanisms involved in parasitised animals appear to differ and are not well understood but could be due to any of the following or a combination of them: improved protein nutrition and supply; enhanced immune response; altered gastro-intestinal physiology or a direct anthelminthic effect (Niezen *et al.,* 1998 a; b; Hoskin *et al.*, 2000; Molan *et al.*, 1999-2000; Anthanasiadou *et al.*, 2000). A number of the above studies, especially the *in vitro* bioassays suggest a direct anthelminthic effect but this may not be the whole story *in vivo*.

Excessive intake of tannins can lead to over-protection of protein, so that it passes undigested through the alimentary tract (Rattray, 2001). This leads to reduced rather than increased protein supply which, if the mechanism is via enhanced protein supply, could present problems. Irrespective of the mechanism, such plants offer opportunities for manipulating parasite infection via nutrition.

Currently the plants available are considered by some, to be agronomically inept and generally not capable of the levels of dry matter (DM) production of the conventional pastures or lucerne (Sykes & Coop, 2001).

All of these plants must be grown as pure swards as the current cultivars do not compete well with conventional pasture species (Niezen, 1998). Care must be taken in seed bed preparation, sowing and grazing management. Douglas (2002) gives a good account of the practices to follow. The plants are legumes and will persist for 2-4 years if properly managed. The *Lotus* species are best suited to lower fertility sites while sulla prefers heavy soils where it can yield up to 25 tonnes DM/ha. Because they are grown as pure swards, farmers can use these plants either as a specialist lamb finishing paddock or graze them intermittently.

These plants have shown the potential of pasture species as a tool for farmers to manage parasitism by means other than drenches. The current cultivars represent the first wave of pasture plants that are available. Breeders are developing cultivars of *L. corniculatus* which creep like white clover and it is hoped that such cultivars can compete as a component in mixed pasture (Niezen, 1998; Rattray, 2001).

The adoption of new pasture species can be one of the first steps which farmers can take to reduce drench usage. It can be done within one season and in addition will boost lamb growth rates as well.

3.10 Breeding for Host Resistance or Resilience

Breeding animals that are less reliant on anthelmintics for maintaining health and productivity is one option to manage the growing anthelmintic resistance problem in NZ, and to meet consumer demands for less drug usage in livestock (Morris, 2002).

Prior to the introduction of modern anthelmintics, selection policies on most farms would have favoured animals that had the greatest ability to withstand internal parasite challenge and infection (Bisset *et al*., 2001). The intensive anthelmintic treatment programmes practiced over the last 2-3 decades have greatly reduced the influence of nematodes on animal performance and has encouraged keeping replacement animals that otherwise would have been culled. Campbell (1986) pointed out breeding from such animals perpetuates the need for more intensive anthelmintic treatment, as such animals often determine the frequency of drench use in an entire flock. The more frequently anthelmintic have been used to control roundworms in livestock, the more dependent on their use we have probably become (Bisset *et al*., 2001).

For many years it has been known that genetic factors contribute to the ability of sheep to cope with roundworm challenge (Whitlock, 1958). Serious consideration of breeding for such factors in NZ only became clear after recognition that our heavy reliance on

anthelmintic use for worm control in livestock could not be sustained much longer, due to the growing anthelmintic-resistance problem and consumer demands to reduce chemical residues in meat. Most of the genetics research in this area is with sheep (Morris, 2002); some beef and dairy cattle studies have been undertaken, but there are very few studies with goats.

There is no doubt that breeding for parasite resistance can be successful. Recently with sheep, Eady *et al*. (2003) compared a genetic and a number of non-genetic strategies, and found the genetic strategy to be the most successful at reducing parasitism. Genetic selection for resistance to *Haemonchus contortus*, an experimental vaccine and protein supplementation were compared with a strategic drenching programme in a factorial grazing experiment to determine their effect on nematode faecal egg count (FEC) of young Merino sheep. Averaged over a 224 day period, FECs were reduced 69% by genetic selection ($P<0.001$), 35% by protein supplementation ($P<0.001$), 28% by drenching ($P<0.01$) and were unaffected by vaccination. FECs in undrenched selected sheep were lower than strategically drenched unselected sheep. Liveweight gain was decreased by vaccination (13%, $P<0.05$) and increased by supplementation (44%, $P<0.001$).

3.10.1 Sheep Breed Differences

Exploitation of parasite resistance genes in the host population can be achieved by both selecting resistant breeds or the most resistant animals within breeds (Vipond, 1998). It has been recognised for decades that breeds of sheep differ in their resistance to nematode infection (Stewart *et al*., 1937). Tropical breeds such as Red Maasai, St Croix, Florida Native and Barbados Blackbelly have been shown to be much more resistant to *Haemonchus contortus* than European breeds run under similar conditions (Preston & Allonby, 1979; Courtney *et al*., 1985). However such breeds are less productive than European breeds in temperate countries such as NZ (Woolaston & Baker, 1996) and many have other undesirable characteristics (such as coloured wool) that make them unattractive (Bisset *et al*., 2001). Generally Merinos appear to have lower resistance than other breeds (Kahn & Watson, 2001; Vipond, 1998). The Scottish Blackface is considered relatively resistant within UK breeds and resistant individuals control *Ostertagia circumcincta* egg output by mechanisms that reduce worm length and fecundity but not the number of worms carried (Stear *et al*., 1995).

In NZ, differences between the predominant breeds, which are mainly of European origin, is less marked. Perendales may be slightly more resistant to roundworm infection than Romneys and Coopworths (Watson *et al*., 1992; McEwan *et al*, 1994a), or Dorsets (McSporran & Andrewes, 1988), and Texel x Romney lambs have lower FECs than pure Romneys (McEwan *et al*., 1994b). In other studies, no differences in FEC were found between Wiltshires, feral Merinos, farmed Merinos and Romneys, but under roundworm challenge, Wiltshires had significantly less dags than the other breeds (Litherland *et al*., 1992). Similarly, Jopson *et al*. (2000) found little difference in FEC between East Friesian x Coopworths and pure Coopworths, but there were significantly less dags in the crossbreds. Breed substitution has been constrained in NZ, where the importation of breeds is a long drawn out procedure involving years of quarantine to avoid introducing scrapie and other diseases, but of the recent importations, the Texel is most likely to have resistance to worms (McEwan *et al*, 1997a; Vipond, 1998).

Breed choice by farmers is determined by many factors and it is considered that genetic selection for reduced drench requirements within breeds is likely to have a greater impact on the national flock than breed substitution in the medium term (McEwan *et al*., 1997a).

3.10.2 Sheep Breeding Objectives: "Resistance" *vs.* "Resilience"

Progress towards breeding sheep that are not affected by worms is much more advanced in NZ and Australia than in other countries (Vipond, 1998). This reflects the faith, particularly among NZ farmers in genetic improvements as permanent remedies to production problems. Major selection programmes for genetic immunity to internal nematode parasite have been undertaken in Australia and NZ, in the Merino and dual-purpose breeds respectively. New Zealand and Australian research workers are involved with a relatively small but committed group of stud breeders (Vipond, 1998; Pomroy, 2000; McEwan *et al*., 1997a; Kahn & Watson, 2001).

Lambs develop immunity as a result of parasitic challenge. Selection aims to improve both the rate of early acquisition of immunity and the final expression of that immunity (McEwan *et al*., 1997a; Vipond, 1998). The most important aspect of acquired immunity is that it reduces the establishment of ingested L_3 larvae from 50% in susceptible animals to less than 1% in resistant animals (McEwan *et al*., 1997a). In addition worms in immune animals produce less eggs than worms in susceptible animals (Morris pers. comm.; Shear *et al.,* 1996).

Breeding objectives in the selection programmes can be either for resistance to worms or resilience. As discussed earlier in this review, the definitions of resistance and resilience differ. Resistance to nematode parasites is related to host immunity, and is the ability of a host animal to prevent or reduce the level of nematode infection. In animal breeding studies, selection for resistance/susceptibility have focused on faecal worm egg count (FEC) as an indicator of levels of infection (Morris, 2002). Resilience or tolerance, on the other hand, is the ability of animals to maintain acceptable health and productivity under parasitic challenge, and is measured in terms of drench requirements, production levels and dag scores. There is some confusion among farmers over these two approaches and there has been considerable debate amongst academics on the merits of the two approaches.

The concept of "resilience" is confusing for the farmer. It is in fact "tolerance" to infection (Bisset *et al.,* 2001) and is the growth advantage under challenge compared with growth rate not under challenge (Vipond, 1998). The disadvantage of selection for resilience is that there is no overall effect on FECs, and individuals with lower resilience can receive a high challenge from contaminated pasture. In contrast selection for low FEC protects the individuals with lower resistance because the larval challenge is lower (Vipond, 1998; Sykes & Coop, 2001).

Albers *et al*. (1984) questioned whether "it might be more profitable to breed for low production losses due to infection (i.e. resilience), rather than high resistance to worms *per se*", and Campbell (1986) suggested a selection strategy that "encompasses high liveweight gain in the undrenched animal but does not exclude other criteria".

The first investigation of these issues was reported by Albers *et al*, (1987) in Australia for *H. contortus* in Merinos and indicated a moderately strong positive genetic correlation (r = 0.56) between resistance, measured in terms of FEC following challenge, and resilience, measured in terms of depression of growth rate. They suggested that selection for either trait would ultimately achieve a similar end point, and as the heritability of FEC was substantially higher than that of depression of growth rate, breeding for resistance to infection would be more rewarding than breeding for resilience.

Little further scientific attention was given to resilience until several years later (Bisset *et al.,* 1994; 1996a), when experience on breeding for increased worm resistance in dual-purpose sheep in NZ suggested that the relationship between the two traits was less straightforward than in the Australian study.

These two concepts cannot be independent (Sykes & Coop, 2001) and the increased understanding of the immune response and its interaction with level of nutrition may help clarify this aspect. Both are conventionally assessed in relation to FECs, yet resistance can only really be defined in terms of the ability of an animal to limit larval establishment, and resilience defined as tolerance against adult worm burdens. In practice, increased resistance would be expected to lead to improved resilience or performance in the face of incoming larval challenge.

Similar to the study of the genetics of *H. contortus* in Merino sheep in Australia where there was a moderate positive genetic association between resistance and resilience (Albers *et al*., 1987), Bisset *et al*. (2001a) reported similar relationships between resistance and various indicators of resilience in Merino sheep against *H. contortus* infections in South Africa. Others, working in Scotland, have reported a strong negative genetic correlation between FEC and liveweight in Scottish Blackface lambs exposed to challenge by *O. circumcincta*, suggesting there may also be a correlation between host resistance and resilience (Bishop *et al*., 1996).

The relationship between resistance to roundworm infection and productivity in Romney sheep challenged with *Ostertagia sp.* and *Trichostrongylus sp.* in NZ is not simple (Bisset *et al*., 2001) and NZ section studies for improved resistance have not resulted in increased resilience (McEwan *et al*., 1995; Morris *et al*., 1997; Williamson *et al*., 1997).

The key question is, “will more resistant sheep necessarily be more productive sheep?” (Sykes & Coop, 2001). Highly resistant animals have a very effective immune system, which results in significant nutritional demand for the immune response, which takes priority over other productive functions (Sykes & Coop, 2001). The most resistant (immune) animals may not be the most resilient and most productive. This interaction will be influenced by the breed of animal, the effects being more pronounced in highly productive breeds (Sykes & Coop, 2001). Breeding for nematode resistance and resilience in lambs is very complex (Bisset, 1999). Lambs with low FECs do not always perform better than lambs with high FECs and lambs which perform well when undrenched do not always have low FECs. The production benefit from selection of animals for improved resistance in the next generation may be due to reduced egg output by the current host population, reducing the larval challenge to the next generation (Bishop & Stear, 1997). Pasture contamination under susceptible flocks have been found to be threefold higher than under resistant flocks (Heath, 2000).

Over the last decade, considerable advances have been made in our understanding of the feasibility and implications of breeding strategies that might be used to reduce anthelmintic usage in sheep without seriously compromising productivity (Baker *et al.,* 1997; Morris *et al.,* 1995; Bisset & Morris 1996; McEwan *et al*., 1997a).

Selection Progress & Potential Anthelmintic Use

Early studies suggested that if selection strategies were used in conjunction with anthelmintic control, it would be important that anthelmintics be administered less frequently. Two independent simulation studies indicated selection for anthelmintic resistance is intensified if immune or genetically resistant hosts are drenched at similar frequencies to susceptible hosts (Barger, 1989, 1995a; Leathwick *et al*., 1995). However drenching frequency is basically unchanged in the last 20 years, which is understandable as genetic change is a slow process.

Significant research into breeding resistant and resilient sheep has been undertaken in NZ by AgResearch for two decades and some research flocks exist which are able to mount an early and reasonably effective response against gastrointestinal nematodes and to develop a more limited post-partum increase in egg count in ewes (Morris, 2002; Pomroy, 2000). No long-term farmlet studies have yet been conducted to assess the usefulness of this approach, but shorter-term trials have shown some promise. Pomroy (2000) stated "commercially any improvements in the relative immunity of sheep by standard breeding approaches is slow and unless biotechnology can assist, will not be the sole answer to the problem of nematode control". It is estimated that it will take about 20 years in a participating flock to reduce FEC by 50% (McEwan *et al*., 1997). In 1997 in the NZ ram breeding sector about 10% of dual-purpose ram lambs were evaluated by WormFEC (McEwan *et al*., 1997) so participation by the industry at that time was relatively small and hence overall progress liable to be very slow (Pomroy, 2000; Leathwick *et al*., 1998).

Vipond (1998) reviewed the situation in NZ while on a Stapledon Fellowship and was much less pessimistic than Pomroy (2000) about the influence of breeding programmes in reducing drench usage. He stated, "Breeders employing the selection programme use less drench and as the programme starts to work the risk of severe challenge recedes". However he did not cite any data or statistics on drenching frequency.

CSIRO has also had a long term (over 25 years) breeding programme on nematode resistance in Merinos (Kahn & Watson, 2001; Vipond, 1998). This is the Nemesis Programme. From this work Australian modeling studies (Kahn & Watson, 2001) come up with less pessimistic predictions on future drench usage with parasite resistant sheep than the NZ commentators. These modeling predictions indicate that it would take 10-12 years selection for worm resistance to be able to eliminate one drench from the annual programme (Kahn & Watson, 2001). Further reductions in drenches should follow quickly and predictions are that after about 15 years drenching could cease. These predictions are supported by results from a CSIRO experimental flock that has been selected for worm resistance for about 20 years (Kahn & Watson, 2001).

Breakdown in Immunity

Other concerns over genetically immune sheep relate to conflicting research reports over their response to a parasite challenge and/or loss of the immunity around lambing (Sykes, 1997). The effect of continuing larval intake in the immune-competent sheep has not been well researched and information that does exist is conflicting. Barger & Southcott (1975)

reported that “resistant” sheep, as judged by lack of egg count when infected with *T. colubriformis*, still suffered reduction in wool growth by 11 to 18% and liveweight gain. On the other hand Kimambo *et al*. (1988) were able to detect no effect of infection with *T. colubriformis* on nutrient utilisation or performance in “resistant” lambs.

Similar findings - lack of effect on FEC, feed intake, weight gain, wool production or lamb birth weight - have been observed during mid-pregnancy in a “resistant” sheep (Leyva *et al*., 1982) challenged with 3000 larvae/day *O. circumcincta*. However, resistance was lost during late pregnancy and early lactation, and early lactational body weight loss increased from 1-2 kg to as much as 5-6 kg, lactation yield was reduced by 20%, and wool growth and staple strength was reduced by 20% by typical rates of infection. This “window of susceptibility” lasts for about six weeks around parturition before resistance is again established (McAnulty), 1990).

These early reports were either from relatively early studies where selection progress would have been minimal or from the UK, where there has been little emphasis on breeding for resistance or resilience. Vipond (1988) states, “progress towards breeding sheep that are not affected by worms is much more advanced in NZ and Australia than in the UK”. One area for concern over the application of selection on FECs in the UK is the ethical dilemma of having to expose an animal to parasitism in order to quantify its response (Vipond, 1998). There has been some NZ work on the subject (Howse *et al*., 1992; Bisset *et al*., 1997; Leathwick *et al*., 1998). Howse *et al*. (1992) found little difference in production losses between lines of sheep differing in genetically susceptibility to internal parasites, however there was a trend towards greater losses from more susceptible sheep when exposed to internal parasites. Bisset *et al*. (1997), with weaned lambs, suggested the major reduction in pasture contamination would translate into increased production and expected additional production benefits to accrue from the resistant adult ewes, especially from the lack of a periparturient rise.

Leathwick *et al*. (1998) compared two lines of sheep (Hi FEC *vs.* Lo FEC) which differed in their genetic resistance to internal parasites as indicated by an initial 4.5-fold difference in FEC. They were grazed on separate farmlets with lambs drenched 3-5 times in the first year of life. Geometric mean pasture larval contamination levels differed by 3.6-fold (203 *vs.* 56 larvae/kg DM); geometric mean ewe FECs differed by 8-fold (49 epg *vs.* 6 epg); geometric mean lamb FECs differed by 13-fold (463 epg *vs.* 34 epg); egg viability was also depressed in genetically resistant ewes and lambs. Despite consistently large divergence in FEC this was not reflected in an equivalent divergence in pasture larval infestations. There was also little evidence that periparturient ewe contamination contributed significantly to pasture larval levels in most years and there was little evidence that differences in larval contamination carried over from one year to the next. Under these conditions, the production benefit of the reduced pasture larval contamination was estimated as 1.7 kg in lamb liveweight and a 5% and 8% increase in fleece weights of hoggets and ewes respectively. There was no evidence for major differences in dagginess between the lines of animals with the majority of comparisons favouring the Lo-FEC lines. Both the pattern and absolute level of pasture larval counts, faecal egg counts and production losses were consistent with a computer model of nematode epidemiology. A separate one-year experiment provided no evidence that production losses due to exposure to parasitic nematode infection differed between susceptible and resistant Romney flocks. Based on these results, the computer model was used to estimate the production benefits of breeding for host resistance to internal parasites. A 3.0 kg gain in

liveweight and a 0.31 kg increase in fleece weight, at 12 months of age were estimated to result from a 50% reduction in faecal egg count. When host resistance was included along with production traits in a dual-purpose selection index, the economic value of the genetic gain increased by more than 20%.

A number of NZ studies have shown the periparturient FECs are reduced in resistant ewes (Leathwick *et al*., 1998; Morris *et al*., 1993a, 1998; Watson *et al*., 1995) (see later). Susceptible ewes have had tenfold higher periparturient FECs than resistant ewes (Morris *et al*., 2002).

While the actual findings on the immune animals themselves appear mixed and confusing, the general consensus is that larval challenges should reduce in time conceding a definite economic advantage to the resistant animals.

3.10.3 Breeding Nematode Resistant Sheep

Worm resistant animals can be found in any flock, whether they are in a wormy, high rainfall environment or a dry area. Selecting for worm resistant sheep is a gradual but permanent approach to the sustainable control of internal parasites (Kahn & Watson, 2001).

Resistance to internal parasites varies considerably between animals and 20-30% of this variation is due to genetic differences between animals (Kahn & Watson, 2001). Presently the largest source of genetic variation in resistance is between sheep within a flock, rather than between different flocks. Therefore, it is unlikely that any particular ram source (which has not been selected for resistance for at least five to six years) will be more resistant than any other. The two major groups breeding for nematode resistance are AgResearch NZ with dual purpose sheep breeds and CSIRO Australia with Merinos.

Changing resistance by genetically selecting for lower FEC can be justified as a method of reducing worm counts because there is a reasonably close correlation between FEC and post mortem worm counts (Bisset *et al*., 1996a) for most economically important gastrointestinal nematode species. Reduced FEC and worm counts also lead to reduced pasture contamination. Heath (2000) found susceptible lamb flocks had three times greater pasture contamination than resistant flocks.

The first step to incorporate resistance into a breeding programme is to carry out faecal sampling of young rams to determine faecal egg counts (FEC) (Kahn & Watson, 2001). The second step is to have the FEC analysed to produce FEC Estimated Breeding Values (EBV). This is an important step because this analysis removes variations in FEC that may occur from year to year, and means that sires tested in different years can be genuinely compared. Breeders can then determine what emphasis they want to place on breeding for resistance.

In NZ workers have concentrated on selecting for a low faecal count following a typical sequence of estimating heritability for the trait (0.23), its correlation with production traits (negative with fleece weight and dag score) and the development of an overall production and disease resistance index and its use by the industry (Vipond, 1998). FEC is a repeatable and heritable trait in both post-weaning lambs and periparturient ewes. The

repeatability estimates are 0.42 and 0.45 for lambs and ewes, respectively. This indicates that FEC data from individual animals at two sampling times will generally be ranked in a similar order, although there will be some exceptions. The heritabilities of FEC in lambs and ewes, averaged over various NZ trials, are 0.23 and 0.33, respectively (Morris *et al*, 1995, 2000; Watson *et al*., 1995). With heritabilities of this size, ram breeders who apply FEC selection among potential sires and dams will achieve selection responses as discussed below (Morris, 2002).

AgResearch (NZ) Resistance Flocks

AgResearch has two flocks in which lines have been selectively bred for high FEC (susceptible line) or low FEC (resistant line) : the Romneys since 1979 at Wallaceville, with a control unselected line added later (Morris *et al*., 2000) and the Perendales since 1986 (Morris *et al*., 1997)). Selection procedures for the Romney lines are as follows: each year, lambs are drenched with an effective anthelmintic at weaning, and then exposed to challenge by grazing on pastures contaminated by natural mixed-species nematode populations. Lambs from the resistant, control and susceptible lines are grazed together. When the mean FEC in a small monitor group reaches about 1000-1500 eggs/g, generally in January/February, faecal samples are taken from all animals in the group. Animals are then drenched and the cycle is repeated, with a second sample generally taken in March/April.

By 2000, the susceptible line had a mean FEC 39 times higher than that in the resistant line. The lines are still diverging and this year reached a 60 fold difference (Morris pers. Comm.).The genetic correlation between periparturient ewe FEC and their FEC as lambs is 0.6 to 0.7 (Morris *et al*., 1998); susceptible-line ewes have higher periparturient FECs than resistant-line ewes by a factor of at least ten-fold.

A small increase in net reproductive rate has been recorded in females of the resistant over the susceptible Romney line as a correlated response to selection (Morris *et al*., 2000). Anti-parasite antibody levels rise earlier and are higher in the resistant line at all times (measured up to 17 months of age) than in the susceptible line (Green *et al*., 1999), and *Trichostrongylus colubriformis*-specific Immunoglobulin E levels are also higher in the resistant line (Shaw *et al*., 1999). There are other indicators that the immune response differs between the two lines, such as eosinophil count and mucosal mast cell proteinases (Bisset *et al*., 1996a, 2001).

Unfavourable responses or side effects have been recorded in the resistant line compared with the susceptible line, for dag scores, fleece weight (in lambs, hoggets and ewes) and post-weaning weight gains (Bisset *et al*., 2001) (see next section). It is probable that these differences reflect the cost to the host of resisting parasitic infection. The side effects can be counteracted by using index selection simultaneously for high production and low FEC (McEwan *et al*., 1997; Heath, 2000; McEwan, 1999; Amer *et al*., 1999). This should ensure that production levels increase while FECs decrease. Heath (2000) found a low FEC/high production flock produced hoggets that were 3 kg heavier at 12 months than a low FEC flock.

These negative side effects reflect the experimental situation where animals from the resistant and susceptible lines were grazed together.

The CSIRO Nemesis Project (Kahn & Watson, 2001)
In November 2000 there were approximately 40 Merino breeders across Australia who have incorporated selection for worm resistance in their breeding programmes. The average length of time for which these breeders have been selecting for resistance is three to five years, although some started their selection programmes as early as 1991. The average selection pressure on worm resistance is in the range of 25-50% of the maximum achievable if FEC were the only selection trait. This is less than that used in modeling predictions, but any reduction in FEC is going to play a significant role in reducing pasture contamination and will be a permanent step toward long term worm control.

As part of the SCIPS programme, AWI is providing support for a project to increase the adoption of *Nemesis* by the Australian wool industry. The objectives of the project are to standardise testing and analysis procedures for FEC EBV, to quantify the relative economic value of worm resistance for different environments, and to prepare and deliver support material for breeders and ram buyers on applying *Nemesis* technology.

A separate but complementary SCIPs project is working with leading commercial woolgrowers and stud breeders to increase the availability of rams sold with FEC EBV in Victoria. An initial survey of 1062 wool growers has identified 28 key studs with the greatest influence on Victorian Merino genetics, and these are the target audience for the project.

3.10.4 Resistance in Dual Purpose Sheep Breeds

In contrast to most Australian studies which have involved Merino sheep selected on their response to artificial challenges using single species of roundworms (generally *Haemonchus*), NZ studies have mainly used Romneys, Perendale or Coopworth sheep exposed to continuous natural mixed-species challenge while grazing under conditions similar to commercial farming (Bisset *et al*., 2001). The predominant nematode genera present in the NZ studies have generally been *Trichostrongylus* and *Ostertagia*, significant numbers of *Nematodirus* also being present in studies undertaken in southern regions of the South Island.

Early investigations of postmortem worm burdens in 8- to 9- month old progeny of rams from the low- and high-FEC breeding lines confirmed that selective breeding for low FEC had led to lambs which possessed significantly lower burdens of the majority of economically important roundworm species, viz *H. contortus, Ostertagia circumcincta, Trichostrongylus colubriformis, T. vitrinus, Cooperia curticei* and *Nematodirus sp*., than their high-FEC counterparts (Bisset *et al*., 1991; Bisset *et al*., 1996b). However, the extent of the differences in FEC between the low- and high-FEC groups (six-fold) did not directly reflect the extent of the differences in trichostrongyle worm burdens (three-fold), suggesting that the low-FEC lambs were suppressing worm fecundity as well as establishment.

Data from the experimental breeding lines and associated progeny-test flocks, as well as from other sources including the nucleus flock of a large group breeding-scheme at Wairunga Romneys in Hawkes Bay (Bisset *et al*, 1992) and the AgResearch production

selection flocks in Southland (McEwan *et al*., 1992), have been used to estimate genetic parameters for FECs and associated traits.

Heritability of Resistance to Roundworm Infection

FEC in lambs exposed to natural roundworm challenge under NZ conditions appears to be moderately heritable, h^2 values for log-transformed FEC data averaging 0.23 in 6-7 month old lambs (Morris *et al*., 1995, 2002). In other words, about 23% of the variation in FEC data is due to genetic factors. This is not substantially different from h^2 estimates for other important productivity traits in sheep. When based on the average of two FEC measurements (representing two separate challenge periods), the h^2 estimate is considerably higher (approximately 0.35; Morris *et al*., 1995, 2002). The use of the average of at least two FECs for each animal tested in a selective breeding programme is therefore likely to result in faster genetic progress and is recommended (Morris, 2002).

Environment x Genotype Interaction

A common concern is that animals ranked highly for resistance on one farm or in one year may not maintain their ranking on another farm or in another year (Bisset *et al*., 2001). New Zealand studies have indicated that environmental factors are minor compared to the genetic factors (Morris *et al*., 1993a; McEwan *et al*., 1997b). Sheep farmers can, therefore, be reasonably confident that rams ranked highly for resistance to roundworm infection in a breeder's flock will be ranked similarly for resistance in other environments (Bisset *et al*., 2001).

Genetic Correlations & Correlated Responses

Growth Rates & Wool Growth Under Roundworm Challenge

Despite having substantially lower FECs, genetically resistant lambs in AgResearch's Romney and Perendale selection lines have similar growth rates to their susceptible counterparts when grazed together, even when the level of larval challenge is substantially higher and drench treatment less frequent than would be expected on commercial sheep farms. Most NZ studies have indicated a slightly unfavourable genetic correlation between resistance and growth rate under challenge in lambs (Bisset *et al*., 2001; McEwan *et al*., 1992; McEwan *et al*., 1995; Morris *et al*., 2000, 2002; Heath, 2000).

Resistant genotypes in the selection lines also produce lower yearling and ewe fleece weights than their susceptible counterparts when grazed together (Morris *et al*., 1997; Morris *et al*., 2000). This agrees with other NZ studies which have indicated a genetic antagonism between resistance (determined using FECs) and wool production (Bisset *et al*., 2001; Howse *et al*., 1992; McEwan *et al*., 1992; McEwan *et al*., 1995; Williamson *et al*., 1995). Merinos selected for worm resistance also produce less wool than unselected sheep. A *Haemonchus* resistant strain produced 9% less wool over a 224-day period of challenge (Eady *et al.,* 2003).

McEwan *et al*. (1997a) pointed out that it is important to view these results in context. Firstly, despite negative correlations between resistance and productivity, it should still be possible to make genetic gains in both traits simultaneously by the use of an appropriate selection index. Secondly, it should be noted that the correlations reflect a situation in which resistant and susceptible lambs were grazed together and thus faced the same level of roundworm challenge. As pointed out by Garrick *et al*. (1992) and Morris (2002), they do not take account of the significant benefits likely to result from reduced pasture contamination over time that can be expected to occur in resistant flocks when they are

not sharing pastures with susceptible or unselected animals. The benefits of grazing resistant (low FEC) genotype lambs by themselves have been demonstrated by Bisset *et al*. (1997). When lambs from the Romney selection lines at AgResearch were grazed on separate farmlets under identical management, substantially lower levels of roundworm infestation developed on pasture grazed by the resistant genotypes. This improved growth rates and fleece weights in the resistant lambs and counteracted their lower performance when grazed together with susceptible counterparts. Heath (2000) found susceptible lamb flocks had three times greater pasture contamination than resistant flocks.

Dags & Cockle

Sheep farmers in NZ often associate the presence of dags (faecal soiling of the breech area) on individual animals within flocks, with high levels of roundworm infection (Bisset *et al*., 2001). However, in the case of dual-purpose breeds exposed to challenge by *Ostertagia sp.* and *Trichostrongylus sp.,* most evidence indicates a positive (unfavourable) genetic relationship between degree of resistance and dags (Watson *et al*., 1986; Baker *et al*., 1991; Douch *et al*., 1995; Shaw *et al*., 1999; Morris *et al*., 2000; Bisset *et al*., 2001), although this is not consistent across all studies (Bisset *et al*., 1992). Increased dags has been evident in the low-FEC Romney selection line for some years, leading Bisset *et al*. (1991) to speculate that there may be a more acute inflammatory response to roundworm challenge in the gut of resistant-genotype lambs than normally occurs in their susceptible counterparts. Subsequent immunological studies have supported this. Greater genetic resistance to roundworm infection in lambs was associated with elevated concentrations of globule leucocytes, mast cells and eosinophils in the intestinal mucosa (Bisset *et al*., 1996b), as well as higher numbers of circulating eosinophils (Buddle *et al*., 1992), higher levels of total antibody, IgG_1 and IgM to the larval stages of *T. colubriformis* (Douch *et al*., 1995), and higher levels of total and *T. colubriformis*-specific IgE (Shaw *et al*., 1999). As pointed out by Shaw *et al*. (1999), these changes are consistent with evidence from mice that host immunity against multi-cellular parasites such as roundworms depends primarily on the induction of inflammatory responses (Finkelman *et al*., 1997). Evidence that inflammatory responses to roundworm antigens may be associated with scouring has also been provided by Larsen *et al*. (1994) in southern Australia, who showed that diarrhoea and dags in Merino ewes following natural challenge with infective larvae of *Ostertagia sp.* and *Trichostrongylus sp.*, were associated with elevated numbers of eosinophils in the gastrointestinal mucosa.

Although it might be expected that the lower pasture contamination for resistant lambs when grazed separately from susceptible counterparts would counteract the increased dags this was not the case (Bisset *et al*, 1997; McEwan *et al*., 1997a). Despite the substantially lower roundworm challenge, the resistant lambs still developed more dags than their susceptible counterparts for short periods in both years of the study. This is similar to the findings of Larsen *et al*. (1995), where there was no relationship between larval challenge and the severity of diarrhoea in Merino sheep. Alternatively, it is possible that selection for low FEC has led to an increased sensitivity to a range of different antigens encountered by grazing sheep. Recent evidence has suggested that the elevated inflammatory responses evident in the low-FEC Romney selection line are not directed at roundworm antigens. Preliminary results indicate a higher incidence of cockle (an inflammatory reaction in the skin to louse antigens) in the low-FEC lambs than in high-

FEC counterparts (Bisset *et al*, 2001). Australian workers (Kahn & Watson, 2001) have found a phenotypic correlation between resistance to lice and *T. vitrinus* (see later).
Further research is needed to improve our understanding of immune responses to multi-cellular parasites in sheep.

In the meantime, however, there has been little evidence to suggest that marked changes in degree of dagginess in lambs will occur, provided selection for reduced FEC is undertaken with a selection index that includes low dag score orhigh productivity and prolificacy under challenge (Morris, 2002; Greeff & Karlsson, 1999; Woolaston & Ward, 1999). In this way, any positive correlation between resistance and dags would be counteracted by the negative correlation between productivity and dags. Economic indices and breeding value estimation procedures for dags are currently being developed and these will eventually be available to commercial breeders for inclusion in a selection index (Bisset *et al*., 2001).

Lamb " Survivability"
Although the evidence suggests there may be production costs associated with mounting an immune response to roundworm challenge in resistant-genotype lambs, a recent field study indicated that these costs are likely to be outweighed by increased ability of resistant lambs to survive in the face of prolonged heavy roundworm challenge without drenching (Bisset *et al*., 2001). Low-FEC, control and high-FEC ewe-lambs, grazing as a single flock, were "salvage drenched" when their weight decreased by 15% relative to a starting weight. In both years of the study, substantially more low-FEC lambs remained untreated (i.e. "survived") compared with the control or high-FEC counterparts. It is clear that few NZ farmers will be satisfied with lambs that can survive prolonged periods of high roundworm challenge but tend to have lower productivity than more susceptible lambs at other times (Bisset *et al*., 2001). It is therefore important that an appropriate balance is maintained when selecting for resistance and productivity in sheep.

Periparturient Faecal Egg Counts & Reproduction Rates in Ewes
Periparturient FECs are reduced in ewes bred for reduced FECs in 4-7 month old lambs (Morris *et al*., 1993b; Watson *et al*., 1995; Morris *et al*., 1998), with FECs of ewes in the AgResearch low- and high-FEC Romney breeding lines diverging by 70%. Leathwick *et al*. (1998) found with resistant ewes an elevated periparturient FEC in only one of three years. As ewes can be the principal source of infection for lambs in the spring in some years (Vlassoff, 1973; Vlassoff *et al*., 2001), this result has important epidemiological implications.

There also a negative (favourable) genetic association between FEC and reproductive rates in ewes. Low-FEC genotype Romney ewes consistently weaned more lambs per ewe mated than high-FEC ewes (1.01 *vs.* 0.91, respectively; $p<0.01$) (Morris *et al*., 1997; Morris *et al*., 2000). There were significant advantages for ewes lambing/ewes mated ($P<0.01$). This is a positive change in the reproductive rate of the low-FEC ewes, and if a similar gain occurred under commercial farming conditions it could be economically significant.

Parasite Adaptation to Host Resistance Mechanisms
There is concern that removal of susceptible sheep by breeding for resistance to internal parasites may lead to roundworms adapting to overcome host resistance (Bisset *et al*., 2001).

Several related studies have been undertaken. With *H. contortus*, three studies found no evidence of adaptation of worms to genetically resistant sheep (Adams, 1988; Albers & Burgess, 1988; Woolaston *et al*., 1992). However *T. colubriformis* derived from resistant lambs had a significantly slightly higher fecundity than a similar strain from susceptible lambs, when administered to non-selected lambs (Windon, 1991). A more recent study of *T. colubriformis, H. contortus* and *O. circumcincta* with resistant Romneys (Bisset *et al*., 2001) did not support the results of Windon (1991) for *T. colubriformis*, but did show some evidence of adaptation in the other two nematode species. Postmortem worm counts for *H. contortus* indicated some divergence in infectivity between two populations passaged for 13 generations through resistant or susceptible lambs, and *in-utero* egg counts in similarly passaged populations of *O. circumcincta* indicated divergence in the reproductivity of these strains, although there was no evidence that this was accompanied by changes in infectivity. Further work is needed in this area (Bisset *et al*., 2001), but in the meantime breeding for parasite resistance is unlikely to result in roundworms that will counter the effects of host breeding programmes.

3.10.5 Breeding Nematode "Resilient" Sheep

Some farmers in NZ were attempting to selectively breed from sheep which were left undrenched as lambs. This prompted AgResearch to reconsider resilience challenge as a breeding option (Bisset *et al*., 2001). The genetics of resilience to roundworm challenge has been under investigation in NZ since 1991. Initial studies involved the progeny of 213 rams (some 14,000 lambs in total) from five commercial Romney ram breeding flocks in the Eastern Romney Breeders Group, as well as AgResearch experimental flocks. Genetic parameters were derived from these studies (Bisset *et al*., 1994, 1996a). A small experimental breeding line of Romney sheep, selected for resilience has been established (Morris *et al*., 2001).

In addition to host genetics affecting the extent of parasitic infection, they may also affect the way in which the host responds during an infection, and this has been studied for over ten years (Bisset & Morris, 1996; Bisset *et al*., 2001) Estimate the heritability for the following traits: total drenches required (during the first four months after weaning), age at first drench, dag score, and post-weaning gain during the parasitic challenge, calculated from the records of the 200+ sire-progeny groups of sheep exposed to mixed-species infections after weaning are 0.19, 0.14, 0.32 and 0.32, respectively (Morris *et al*., 2001). The heritabilities for drench traits are lower than the heritability for FEC.

Since 1994 selection increased resilience at AgResearch's Wallaceville and Ballantrae Stations has achieved a 25% reduction in the number of drenches required in lambs under challenge, and a 27% increase in post-weaning gain and 0.48 unit reduction in dags, in the elite resilience selection line (Morris *et al*., 2001). In addition, 51% of the elite resilient lambs came through the test period which included the autumn peak larval period without requiring a drench compared with 16% of the controls. The genetic correlation between FEC and resilience as measured by total drenches is low (-0.17) and not significantly different from zero (Bisset *et al*., 1996b). Thus if a breeder wishes to reduce total drenches and FEC at the same time, it is necessary to select against both at the same time. It should be noted that this is in contrast to the situation overseas, with Merinos in Australia and South Africa, where *Haemonchus* is the dominant nematode parasite.

Either because of the Merino breed, or the *Haemonchus* species or the different climate, compared with Romneys/Coopworths/Perendales carrying predominantly *Trichostrongylus* and *Ostertagia* in NZ, there is a good correlation between FEC and resilience in Merinos (low FEC is associated with high gain) (Morris *et al*., 2002).

Problems Associated With Assessing Resilience

The method used by Albers *et al*. (1987) to assess resilience, i.e. measurement of growth rate in lambs while subjected to roundworm challenge, relative to their growth rate while not subjected to challenge, is generally considered to be impractical to implement under field conditions. In principle, an indication of resilience could be determined by exposing lambs to severe roundworm challenge for prolonged periods and identifying those least affected (Campbell, 1986), but in practice, this option is unacceptable on animal welfare and economic grounds in commercial flocks. Reducing the duration and/or severity of worm challenge is likely to introduce another problem - that of confounding between an animal's genetic potential for growth *per se* and its ability to withstand worm challenge.

In an attempt to overcome these problems, Bisset *et al*. (1994, 1996a) in NZ studies on the genetics or resilience, used a "drench-on-demand" procedure, administering anthelmintic to selected individuals only as deemed necessary on the basis of body weight change or visual assessment of body condition. This approach allowed the most resilient lambs to be subjected to prolonged periods of moderate to high challenge without treatment, but avoided seriously jeopardizing the health of the least resilient lambs. Furthermore, it allowed the resilience trait(s) to be expressed in terms of "drench requirements under challenge", which was considered useful in view of the ultimate breeding objective of reducing anthelmintic usage.

Characteristics of sire progeny groups that were considered to indicate greater resilience were: greater average age at first drench; fewer lambs drenched within a defined period and; lower average number of drench treatments administered by the end of the test period. However, this selective drenching approach also has some major drawbacks from a practical point of view; frequent flock inspections are required to avoid the risk of serious nematodiasis in some animals and the fact that some animals receive more drench treatments than others can lead to difficulties in ranking animals for other production and/or parasite-related traits (Bisset *et al*., 2001).

Practical Approaches to Breeding Sheep for Reduced Anthelmintic Usage in Commercial Flocks in New Zealand

The ability of sheep to limit or tolerate the effects of roundworms, resulting in lower drench requirements, potentially involves both resistance and resilience (Bisset *et al*., 2001; Sykes & Coop, 2001). The main benefits derived from breeding for resistance (i.e. low FEC) in dual-purpose sheep are expected to be gained indirectly as a result of reduced pasture contamination, while the main benefits derived from breeding for resilience are likely to be gained through the improved ability of individuals to maintain health and productivity under challenge (Bisset *et al*., 2001). Currently, it is generally agreed that the best breeding strategy to reduce anthelmintic usage under NZ conditions is to select for sheep that show an appropriate combination of both these traits.

While resilience appears to be a desirable trait, selective drenching is not currently recommended as a means of assessing it on commercial farms, due largely to the management complexities involved (Bisset *et al*., 2001). Until genetic markers for

resilience become available, the most sensible solution is for breeders to use a "selection index" which combines measures of FEC and production under limited roundworm challenge. Animals showing with low FECs but excessive dags should be avoided (Bisset *et al*., 2001).

Assessing the genetic merit of sheep in a breeding programme usually involves the calculation of "breeding values" for particular traits (Bisset *et al*., 2001). Breeding values take into account the performance relatives as well as the individual's own performance.. Various scales are used to express breeding values but commonly they are expressed in measurement units above or below the flock mean value for a given trait (e.g. kg liveweight gain) or, alternatively, as a percentage above or below the flock mean value (e.g. as is used for log-transformed FEC data). The simplest and most economical way for most farmers wanting to incorporate genes for resistance and high productivity under challenge into their flocks is to buy rams from breeders who have appropriate performance recording systems in place and can demonstrate genetic improvements in these traits (Bisset *et al*., 2001).

WormFEC™

In NZ such a service has been established (McEwan, 1999; McEwan *et al*., 1997a) to help ram breeders identify animals that are both resistant to roundworm infection and highly productive under challenge (WormFEC™, AgResearch Invermay). This service provides advice to participating breeders, a faecal egg counting service and calculation of breeding values for several different resistance traits (including serum antibody levels). Protocols recommended for testing lambs for resistance to roundworm infection are described in detail by McEwan (1994). Estimation of breeding values is undertaken using a multiple trait, repeated measures, animal model "best linear unbiased prediction" (BLUP) analysis. The use of BLUP allows across-farm, across-sex and across-year comparisons of breeding values, making it easy for breeders to assess long term genetic trends. Where groups of breeders have genetic links between their flocks, across-flock analyses can also be provided (Bisset *et al*., 2001; Vipond, 1998).

Despite the negative correlations with production traits such as hogget fleece weight and dagginess over 50 farmers were actively using WormFEC selection indices in NZ by 1997 (McEwan *et al*., 1997a; Vipond, 1998). FECs are done by independent assessors with built in quality assurance by repeat testing of the same samples at different labs. An antibody test (see later) is done by AgResearch and is considered a useful additional test but not worth selecting for on its own (normally two FECs or the antibody test are used in the AgResearch's WormFEC service).

In 1997 results were favourable (McEwan *et al*., 1997). Those farmers operating the selection process for the longest (ten years) demonstrated significant reductions in worm egg output from ewes and lambs, improved wool production and higher growth rates. Genetic links were made by rotating rams between farms. Thirty flocks were involved in three separate sire referencing schemes, as well as some 20 unlinked flocks. In 1997 more than 7000 two-tooth and older rams retained for sale or used within the reference flocks were ranked on WormFEC's desired gains index in these three schemes. No evidence of genotype x environment interactions were seen.

Recently, breeding values for parasite resistance have been incorporated, as a breeder selectable module, into the genetic engine used by Sheep Improvement Limited (SIL) who

administer a national database for sheep performance recording in NZ (Newman *et al*., 2000).

Morris (2002) summarized conclusions and suggestions for ram breeders as follows:

> "For breeders using the Sheep Improvement Ltd (SIL) performance-recording scheme, it is suggested that an index is used to combine selection for high production and low FEC (Amer, 2000; Geenty *et al*., 2001). AgResearch offers a service (WormFEC™) to assist ram breeders in combining FEC and production data collected on individual animals.
>
> The heritability of the mean of two FECs is higher than for a single FEC. Using the mean of two FECs in the selection index therefore gives faster genetic progress than using single records, but it involves more work and greater costs of sample collection and analysis.
>
> Selecting for an increase in anti-parasite antibody level along with increased production levels in a SIL index is another option. Selecting on increased antibody level is predicted to result in slower response than selecting on FEC, but it has more flexibility for sampling date and fewer farm management implications".

Optimising likely financial returns from genetic selection requires knowledge of the relative economic values of the traits included in a selection index and these must be weighted appropriately. The benefits of low FECs to production are mainly indirect and come from reduced levels of pasture contamination and challenge (Bisset *et al*., 2001; Vipond, 1998). This makes economic evaluation difficult because to test it would mean separate flocks with confounding influences of pastures and sire breeding values for production. Nevertheless both modeling worm and onfarm experience indicates major benefits of including FEC in a selection index and that ignoring it leads to higher worm burdens. Around 10% of recorded sires were sold in 1995 with information on their resistance to worms. Availability of this information is seen as valuable to commercial farmers buying rams. The reason for the high uptake of the work done at Invermay has been the close contact between researchers and farmers in the development of the selection programme (Vipond, 1998).

Two studies, that used separate epidemiological models of a farm system including moderate anthelmintic usage, have estimated the economic value of reduced FECs under NZ conditions (Amer *et al*., 1999; McEwan & Amer, 2001). Both produced similar estimates of the benefits ranging from 5-7 cents/animal/year for each percentage unit reduction in FEC. An examination of the sensitivity of these estimates to changes in stocking rate, drenching frequency, lambing percentage, lambing date and magnitude of the periparturient rise in ewe FEC (McEwan & Amer, 2001) indicated that that they were robust under a wide variety of conditions. In addition, errors in the estimation of the economic value of resistance of up to 40% reduced the economic gain by less than 4%. An updated estimate of 7.3 cents/animal/year for each percentage unit reduction in FEC (1.8 cents for each of two FEC measurements in lambs and 3.7 cents for ewes and other adult sheep) has been derived for use in the SIL selection indices (Amer, 2000).

While there has been reasonable uptake of such technology by the ram breeding industry in NZ, major genetic advances will take considerable time to be achieved in the national sheep flock using this approach. McEwan *et al*. (1997a) estimated that it would take about

20 years for the average genetic susceptibility of the national flock to be halved. However, it is anticipated that marker assisted selection, a technology which should be available in the near future, will reduce this timeframe considerably (Bisset *et al*., 2001; Morris *et al*., 2002, Kahn & Watson, 2001).

3.10.6 Resilience to Nematodes in Dual-Purpose Sheep Breeds

Heritability of Resilience

Overall, heritabilities for resilience traits based on the above drench requirement criteria were relatively low, h^2 values ranging from 0.10-0.19 (Bisset & Morris, 1996). Heritability for resilience is low-moderate (Bisset, 1996) and lower than that for resistance. However, estimates depend to some extent on the severity of challenge experienced; considerably higher values (h^2 = 0.24-0.53) were derived from one particular flock which had been subjected to much higher levels of cumulative challenge over three breeding seasons than the other flocks (Bisset *et al*., 2001). In breeding for disease resistance traits, greater genetic progress is likely to be achieved in years (or in flocks) where challenge is high (Campbell, 1986). However this will also result in a lower productivity and/or survival of the animals (Bisset *et al*., 2001).

It should also be recognised that most of the variation in the ability of individual lambs to cope with nematode challenge in the field is due to so-called fixed effects (non-genetic factors), such as birth date (earlier-born lambs tending to cope better under challenge than later-born lambs), birth rank (single-born lambs tending to cope better than lambs born as twins or triplets) and age of dam (lambs born to 3-4 year old ewes tending to cope better than lambs born to 2 year old or $\geq$5 year old ewes) (Bisset *et al*., 1994). These factors exerted their effect largely via their influence on weaning weight. For this reason, simply selecting lambs that "do well" when left undrenched (i.e. phenotypic selection) could cause a bias towards lambs that are merely well grown at weaning. This would tend to select against twins and late-born lambs, regardless of their genetic potential to cope with roundworm challenge (Bisset *et al*., 2001).

Genetic Correlations Between Resilience, Productivity, Dags & Resistance to Infection

To overcome some of the difficulties of assessing production and/or other parasite-related traits under a selective drenching regime, Bisset *et al*. (1994, 1996a) restricted its use to ram lambs and measured productivity, dags, and resistance (FEC) characteristics in their paternal half sisters, which were maintained under a standardised minimal drenching regime. This allowed genetic correlations between these traits to be calculated across the sexes. Sires whose male progeny required the fewest drench treatments to maintain acceptable body condition under roundworm challenge (i.e. were more resilient), had female progeny that had above average growth rates and below average dag-scores under challenge. Drench-requirements in male progeny showed no significant correlations with parasite resistance (based on FECs) in their paternal half-sisters, showing that breeding for increased resilience in Romney ram lambs exposed to a mixed nematode challenge in the field resulted in little or no genetic change in their FECs. This is in contrast to the results of Albers *et al*. (1987) for *H. contortus* infections in Merino sheep. Consequently, while selection for resilience using the above approach may result in improved productivity and reduced dags in lambs under roundworm challenge, it would

probably not result in any benefits from reduced pasture contamination (Bisset *et al*., 2001).

Selection Pressure for Anthelmintic Resistance

The selective drenching strategy used in the above studies was undertaken to identify resilient lambs without jeopardizing the health of their less resilient flock-mates (Bisset *et al*., 2001). A useful side effect of such a drenching strategy is reduced selection pressure for anthelmintic resistance in the worm populations. Leaving even a small proportion of animals in a flock undrenched ensures that the small number of eggs from resistant worms that continue to be shed onto pasture by drenched lambs are greatly out-numbered by eggs from susceptible worms shed by the untreated lambs. Population modeling has shown that such a selective drenching strategy could significantly delay the development of anthelmintic resistance (Barnes *et al*., 1995), although there is the potential risk where haemonchosis is of major concern.

3.10.7 Blood Antibody Levels - The Use of the Host Resistance Test

New Zealand use: An alternative to measuring FEC as an indicator of host resistance is provided by a test for antibodies to roundworm antigens in lamb serum (Serakit, AgVax Developments Ltd, Upper Hutt, NZ) (Bisset *et al*., 2001). The use of this test for selection purposes relies on a positive genetic correlation between resistance and antibody levels (Douch *et al*., 1995). It has proven attractive to some breeders because it does not involve a long challenge period for lambs without drench treatment. In addition, only a single sample per animal is normally required because the test is highly repeatable compared with FECs. However, the main benefits of breeding for resistance to roundworm infection in dual-purpose sheep in NZ are derived from reductions in pasture contamination. As the relationship between serum antibody levels and FEC in lambs is not perfect, slower genetic progress occurs if ram selection is based on antibody levels only rather than on FECs. Douch *et al*. (1995) estimated this 51-67% of the genetic gain achieved by selecting on FECs.

However, measurement of antibody levels in blood has had widespread support as an alternative method to FEC to assess the level of host resistance to internal parasites and to provide the basis for selection programmes (Kahn & Watson, 2001; Morris, 2002; McEwan *et al*., 1997a; Bisset *et al*., 2001). The Host Resistance Test (HRT), distributed through AgVax Developments Ltd, NZ, is a commercial kit for determining blood antibody levels to *T. colubriformis* and *H. contortus*. Information provided with the HRT suggests that the most appropriate time to assess the antibody level of animals is from six to eight months of age and that only one sample per animal's lifetime is required as the repeatability of subsequent tests is very high (Kahn & Watson, 2001). This does not always appear to be the case.

Australian trial: Recently Australian workers have serially sampled 1200 young rams from four Merino studs for blood and FECs under a Producer Initiated Research and Development project funded through AWI (Kahn & Watson, 2001). The aim of the project was to determine the correlation between FEC and antibody levels and the repeatability of these traits, under Australian conditions. FEC and species differentiation were performed using routine procedures, and antibody levels in blood to *T. colubriformis* and *H. contortus* were determined with the HRT.

Analysis of the data showed this test to be unsatisfactory under the conditions of the Australian trial, and indicated the following. The repeatability of antibody level between samples from the same sheep was negligible at 0.02, indicating that one measurement on its own provided almost no information on subsequent samples. The repeatability of FEC between samples from the same sheep was also low at 0.2, which is in agreement with published estimates but substantially greater than for antibody level. The phenotypic correlation between FEC and antibody level was very low at 0.07, which indicates that the two traits are not closely related. The phenotypic correlation between antibody levels to *T. colubriformis* and to *H. contortus* was generally high at 0.70, suggesting that both tests may be measuring the same common trichostrongylid antigen or epitopes.

3.10.8 Genetic Markers & Marker Assisted Selection

Selection for worm resistance currently requires exposure of sheep to parasites either as a result of natural infection from pasture or by artificial challenge (Kahn & Watson, 2001). One way to avoid the need for parasite exposure is to use genetic markers which allow detection of specific regions of DNA which are associated with worm resistance. The usefulness of genetic markers depends initially on the genetic control of the resistance trait and on the proximity of the marker(s) on the chromosome to the gene of interest.

If the trait is due to the combined action of many genes of small effect (polygenic) it is unlikely that genetic markers will be useful in identifying animals of very superior resistance status (Kahn & Watson, 2001). However, if resistance is due to the effect of a single gene, or the actions of one or a few genes of moderate or large effect (i.e. Quantitative Trait Loci or QTL) the probability of identifying useful markers is much greater.

While the identification of genetic markers close to the genes contributing to parasite resistance would assist in the selection of resistant animals, it is not without problems (Kahn & Watson, 2001). The major issue is the need to confirm the association between the genetic markers and the resistance characteristic, FEC, in different populations and generations. This is because recombination events can disrupt the linkage between the resistance QTL and the informative genetic marker. This limitation can be overcome by fine mapping of the actual gene(s) responsible, which would result in a DNA test that would work across populations without the need to reconfirm links with the resistance characteristic.

The most effective way in the future for farmers to identify sheep that are both resistant to roundworm infection and able to withstand the impact of roundworm challenge on productivity will undoubtedly be to use marker assisted selection (MAS) techniques (Bisset *et al*., 2001). MAS is already being used for improving sheep reproductive performance (Galloway *et al*., 1999), resistance to scrapie (Yuzbasiyan-Gurkan *et al*., 1999) and carcass traits (Jopson *et al*., 2001). However, the technology is not yet commercially available for improving sheep resistance or resilience to roundworm challenge. How soon it will become available depends on the success of research currently underway to identify the genes affecting these traits. Once these are known, it should be possible to develop genetic markers to classify potential breeding animals by using a simple DNA test (Bisset *et al*., 2001; Morris *et al*., 2002). Ideally, such genotyping will be based on the actual

mutations responsible for the traits (i.e. direct gene tests), but closely linked markers may also be used in some circumstances.

MAS will have several advantages over traditional selection procedures for breeding animals for low drench requirements (Bisset *et al*., 2001). First, there will be no need to subject animals to prolonged roundworm challenge and so production costs associated with the test procedure will be avoided, as will costly and messy sampling procedures. Furthermore, it will be possible to determine an animal's genetic status at an early age. If the mode of inheritance is known, MAS will enable the problems associated with recessive or over-dominant genes under traditional selection procedures to be overcome. Finally, MAS will be valuable in overcoming the antagonistic association between low FEC and some production traits, as it will enable only those genes with favourable or neutral effects on production to be selected for (e.g. genes for resistance that have no unfavourable effects on the incidence of dags). Unfortunately, the current costs of the technology make it likely that, at least initially, it will only be used by ram breeders selecting stud flock replacements.

When MAS does eventually become widely available, it will be included into existing performance recording systems such as that administered by SIL (Bisset *et al.*, 2001). The only difference from the point of view of breeders will be that the animals involved will have to be sampled for DNA, using either a wool follicle sample or collection of a few droplets of blood. Breeding values and selection indices may then be modified on the basis of genotype results.

New Zealand research: In order to streamline breeding for resistance/susceptibility, AgResearch is undertaking a search for DNA markers linked to this trait. Progress in identifying genetic markers for various traits in sheep in NZ was recently reviewed by Crawford (2001). Established experimental selection lines are key resources for this work. Preliminary results indicate that there may be several genes that have substantial effects on host resistance. An area of the sheep genome containing one of these genes has already been patented as a marker (Crawford & McEwan, 1999), and work is continuing on the other genes involved. Development of the AgResearch selection line for resilience is not yet far enough advanced to be useful for gene mapping studies.

The genetically selected high and low FEC lines, described above, are prime candidates to evaluate this technology (Morris *et al*., 2002) and have contributed to these studies searching for "FEC genes" whose alleles differ in frequency. The more divergent the FEC lines, the easier that it will be, potentially, to isolate such genes (Morris *et al*., 2002). One such DNA marker closely linked to host resistance has been discussed by Paterson *et al*. (2001), but the authors stressed that they had found a marker near, but not within, the relevant gene. To use this marker at present, family studies would need to be carried out, and the marker could only be used within families. Ultimately, when the actual gene(s) controlling host differences are identified, selection will not be restricted within families.

With this ongoing research to develop phenotypic markers and genetic markers, new computer programmes like Findgene are being used to see if major genes for resistance are segregating (Vipond, 1998). The WormFEC database will be invaluable here. Other genetic tools include candidate genes and a genomic scan.

Australian research: There has also been considerable Australian interest in the use of markers (Kahn & Watson, 2001). Until recently the genetic control of resistance to internal parasites of sheep was thought to be mainly polygenic but recent mathematical analysis of data from the "Golden Ram" *H. contortus* resistant flock at the University of New England has indicated the presence of a QTL for resistance. The "Golden Ram" flock was established in the early 1980s after the progeny of about 60 Merino sires were screened for resistance to *H. contortus*. The progeny of one sire were found to be extremely resistant to *H. contortus* with FEC up to 4000 epg less than the mean FEC for the other sire progeny groups. This ram was subsequently referred to as the "Golden Ram" and was used as the founder ram for the "Golden Ram" flock.

Recent analysis of nearly 15 years of pedigree data, consisting of about 4400 Merino sheep with 2500 records for FEC, indicated that approximately one third of the genetic resistance to *H. contortus* in these animals is attributable to QTL (Kahn & Watson, 2001). This analysis indicated that when animals homozygous for the desirable QTL allele(s) were compared to those without the QTL, homozygous animals would have a FEC of approximately 5000 epg lower (46% reduction) at days 28-35 following challenge with 11,000 infective L_3 *H. contortus*.

Because host resistance in the "Golden Ram" flock has been identified as being partly controlled by a QTL the prospects for finding and eventually using genetic markers for worm resistance are good (Kahn & Watson, 2001). During the last ten years, a very good map of the sheep genome has been developed, which now consists of well over 1000 genetic markers. Recently Dr Ken Beh, CSIRO Livestock Industries, has completed the first stage of the search for genetic markers for parasite resistance. These studies revealed six chromosome regions of interest for further analysis as the most likely genome regions to carry parasite resistance genes. The final step to developing useful markers as selection aids is to correlate genetic differences in worm resistance to FEC. Ultimately, a simple test using blood or wool follicle samples will be developed to enable Australian breeders to select sheep based on their genetic make-up.

3.10.9 Relationship Between Resistance to Worms & Lice in Sheep

As with worms, control of sheep lice relies almost exclusively on chemicals. The continued use of external parasiticides is threatened by resistance (especially in the case of the synthetic pyrethroids) but more immediately, by rising concerns over occupational health and safety and the environmental impact of residues on wool. Australian researchers have recently demonstrated significant phenotypic correlation between resistance to lice and resistance to both natural and artificial challenge with *T. vitrinus* (Kahn & Watson, 2001). In addition, the immunological mechanisms that mediate protection against arthropods and helminth parasites appear to be similar. If resistance to helminths and lice are genetically related, by selecting for resistance to internal parasites growers may also increase resistance to lice, and in the longer term, reduce the need for chemical treatments. Further studies are underway to assess the genetic associations between resistance to lice and resistance to internal parasites.

3.10.10 Genetic Research With Other Farmed Ruminants

By far the greatest majority of research in this area is with sheep (Morris *et al*., 2002). There has been some limited work with cattle in Australia (Pomroy, 2000) and recently AgResearch in NZ has started some work with cattle (Morris *et al*., 2002). FEC and immunology data from NZ beef and dairy cattle of known pedigree have been collected (Morris *et al*., 2002). For beef cattle, the heritability of post-weaning FEC in calves appears to be higher than in lambs; the repeatability is similar to that in lambs (Morris *et al*., 2003). A similar finding occurs with dairy cattle (Morris *et al*., 2003b). There are significant sire effects in the NZ dairy herd on antiparasite antibody levels (Morris *et al*., 2002). There are good repeatabilities across herds of 0.29-0.39, and sire effects on antibodies to two different nematode species were highly correlated (r^2=0.47 for larval antibodies and 0.86 for adult antibodies). There is a negative (i.e. favourable) genetic correlation between FEC and anti-parasite antibody, as in lambs (Morris *et al.,* 2002). Antibody levels are positively correlated to each other when measured in post-weaning calves, yearling heifers and periparturient cows (at two and three years of age), for cohorts grazing together and managed the same. Some genetic evaluations have also been tested in dairy cattle, and have been found to be quite similar to those for beef cattle.

Relevant work with goats is very scarce, however Meat NZ (2002) has recently funded work on selection for nematode resistance in Cashmere goats.

3.11 Potential Future/Novel Control Methods

There are a number of research groups in NZ and overseas investigating alternatives to anthelmintics that may play a future role in internal parasite control programmes (Harrison *et al*., 1998; Hein *et al*., 2001; Pomroy, 2000). This is mainly to reduce the frequency or inappropriate use of anthelmintics that encourage the development of resistance to these chemicals. However another factor which may be equally important is the increasing consumer-led pressure for livestock producers to adopt chemical-free farming practices and the increasing probability that chemical residues could be used as non-tariff trade barriers (Mirams, 1999; Hein *et al*., 2001).

Several alternative technological solutions to nematode control are currently being investigated and developed (Harris *et al*., 1998; Hein *et al*., 2001). Some of them could be applied now at the farm level, while in other cases, further research is needed before safe and reliable products become available. These include vaccines, immunomodulants, biological control to destroy nematode larvae, biological anthelmintics, silencing of genes controlling nematode development and computer models.

3.11.1 Vaccines

The induction immunity from vaccines is a very cost-effective control method to protect ruminants from many important bacterial, viral and protozoal diseases (Lewis, 2000). Attempts to control diseases caused by helminth parasites using vaccines have met with more limited success. Lungworms (*Dictyocaulus filarial*) can be controlled effectively in sheep using an attenuated-L_3 vaccine (Jovanovic *et al*., 1965; Sharma *et al*., 1988).

Successful vaccines have also been developed to control the larval stages of the dog tapeworms *Taenia ovis* and *Echinococcus granulosus* (Heine *et al*., 2001).

These latter achievements demonstrate that vaccines based on recombinant antigens could effectively control and prevent disease internal parasitism in ruminants (Hein *et al*., 2001; Lightowlers *et al*., 2000). However, despite many years of research, this has not yet been achieved. Pomroy (2000) states, "Many promises have been made but no successful vaccines are available yet". However, in the 1990s efforts increased and there were several experimental anti-parasite vaccines under development (Harrison, 1998).

Over the past decade, several research groups, usually funded within commercial collaborations, have trialled both native antigens (harvested from worms) and recombinant antigens (synthesised e.g. by bacteria) from *Haemonchus, Trichostrongylus* and *Ostertagia sp.* as vaccines (Kahn & Watson, 2001). These antigens are of two types: concealed antigens, which are not normally "seen"; and conventional or excretory-secretory antigens that are "seen" by the host immune system during natural infection.

Research in this field has been disappointing because control of internal parasites with vaccines is not as high as the level of control afforded by anthelmintics. Unlike broad spectrum anthelmintics, vaccines are likely to be effective against only one species of parasite, so that the ultimate goal, a multivalent worm vaccine, is most likely to be achieved by combining several individual vaccines - a prolonged process. There are presently no commercial vaccines for any gastrointestinal worm parasite species of any host (Kahn & Watson, 2001).

However, vaccines would not have to match the control level of anthelmintics to provide substantial benefits. The benefits of lower FEC would continue to accrue over generations as larval numbers on pasture declined (Kahn & Watson, 2001). Moreover, some level of infection in vaccinated animals would be advantageous in allowing the development of natural immunity. Modelling indicates that a conventional vaccine producing a 60% reduction in worm numbers in 80% of the flock would provide better worm control than standard drenching programmes. The equivalent level of control needed from a concealed antigen vaccine is estimated to be higher (80% reduction in 80% of the flock) because natural exposure would not boost the vaccine-induced immunity.

Until the early 1980s, the prospect of using vaccines to protect against helminth infections seemed very remote (McFarlane, 1997). Early attempts to develop vaccines against gut parasites in ruminants utilised the approach successful in developing an irradiated larvae vaccine against *Dictyocaulis viviparous,* the lungworm of cattle, and *Ancylostoma caninum*, a hookworm of dogs. Although some reasonable protection was afforded against *O. circumcincta* (Smith *et al*., 1981) with high doses of vaccine, the logistics of generating such large numbers of larvae that were needed for the procedure and the requirement to distribute a refrigerated product discouraged further development. Modern methods of molecular fractionation, and more recently recombinant DNA methods, have allowed the production of highly specific vaccine preparations (McFarlane, 1997; Hein *et al*., 2001).

To make a vaccine against a gut parasite, it is necessary to identify the components of the worm (antigens) that are critical to stimulate a protective immune response in the host (Harrison, 1998). Because the worms are composed of hundreds of different proteins and

carbohydrates, it is not a trivial task to identify the necessary molecules. This is one of the reasons why vaccine development has been slow. In the last few years, however, a number of potentially useful antigens have been discovered for each of the main worm species of sheep. Some of these antigens have given high levels of protection in experimental trials and will be tested in field trials over the next few years.

A number of structural and excreted/secreted products from L_3 larvae or adult parasite stages have been used as vaccines (McFarlane, 1997). A major challenge remains as to the best way to apply them to the immune system so as to mimic natural infection and without inducing immune tolerance. This may necessitate a particulate oral formulation, possibly with enteric vectors and/or adjuvants. Vaccine delivery has therefore been another area for practical research (Harrison, 1998). Conventional immunisation using injections may not be appropriate for gut parasites and there is much interest in the development of delivery systems for direct immunisation of the gut using orally administered vaccines. These could take the form of slow release micro-particles or genetically modified organisms which secrete parasite antigens into the animal's intestine, thus stimulating immunity at the desired site.

In the early 1990s there were a number of nematode vaccines under study and some showed some degree of success (McFarlane, 1997). These are summarised in Table 6.

Table 6: Experimental nematode vaccines tested in the early 1990s

Nematode	Animal Species	Antigen Component	Source of Antigen	Recombinant Antigen	Reduction (%) In Worms	Reduction (%) In FEC
T. colubriformis	Guinea pigs	Tropomyosin	L_3 larvae	+	43-51	-
	Guinea pigs	Exsheathing fluid	L_3 larvae	-	<50	-
	Sheep	Excretory/secretory fluid	Adult	+	<50	-
	Sheep	Secretory fluid	Adult	-	31	0
H. contortus	Sheep	Exsheathing fluid	L_3 larvae	-	60	46-66
	Sheep	Tropomyosin	L_3 larvae	-	54	46
	Sheep	Gut H11	Adult	+	78-95	78-99
	Sheep	Intestine H-gal-GP	Adult	+	54-93	58-97
	Sheep	Surface	L_3 + L_4 larvae	-	-	-
	Sheep	Somatic	Adult	-	69	21
O. circumcincta	Sheep	Excretory/secretory	L_3 larvae	-	58	<50
O. radiatum	Calves	Excretory/secretory	L_3 larvae	-	99	75

Experimental vaccines have been made from structural or excreted/secreted products of the adult parasite or infective larvae (McFarlane, 1997). Immunogenic fractions have been identified by reacting fragments of the parasite with immune serum from resistant animals.

As mentioned briefly above, the two broad groups of antigens are the so-called "hidden" or "concealed" antigens and "conventional" or "natural" antigens. Both have been tested as potential nematode vaccines with variable levels of protection being achieved (McFarlane, 1997; Hein *et al*., 2001).

Hidden antigens have been so named because they do not appear to be recognised by or to stimulate a detectable immune response after natural infection (Munn, 1997). Although expressed by nematodes, the hidden antigen may be sequestered at internal sites within the nematode, making them unavailable for uptake by the host (Hein *et al*., 2001). The

mammalian gut is a complex immune environment and other factors might contribute to the "hidden" nature of antigens, such as an inability to be absorbed across the epithelial barrier of the intestine or specialised processing and presentation by the regional immune system, leading to outcomes not detectable with conventional assays. Although there have been great advances in our knowledge of immunity against roundworms, the precise mechanisms effective at the gut level are still being unraveled, particularly in the sheep (Harrison, 1998). There is a need to understand the mechanism of immunity in the ruminant host so that the vaccine can be designed to stimulate the most effective type of immune response.

Other formidable obstacles will also need to be overcome, including devising protein expression systems which allow recombinant proteins to retain their native antigenic efficacy and finding ways to formulate and deliver the proteins in vaccines so as to stimulate protective levels of immunity (Knox, 2000). This can be done by including enhancing substances known as adjuvants, and chemical messengers which can boost the immune response in a particular way. Whatever the explanation, delivery of some of these antigens together with appropriate adjuvants via parenteral vaccination routes can induce protective immunity (Hein *et al*., 2001).

Concealed Antigen Vaccines

Concealed antigens are based on proteins that have been isolated from the surface of the worm intestine. When they are injected into a sheep, its immune response is stimulated to make antibodies, which circulate in the blood (Kahn & Watson, 2001; Smith, 1997; McFarlane, 1997). These antibodies are then ingested by the parasite and adhere to the gut of the worm, blocking normal digestive processes so that the parasite is weakened, lays far fewer eggs and dies.

It is hopeful that at least an anti-*Haemonchus* vaccine will be commercially available within the next decade, probably from developmental work in the UK. Research in the UK has found good experimental protection against blood sucking worms (e.g. *Haemonchus*) using concealed antigens, but not so far against the non-blood feeding worms (e.g. *Ostertagia*) (Kahn & Watson, 2001; Smith, 1997; McFarlane, 1997). One antigen has been shown to be highly effective in very young lambs; able to prevent the periparturient egg rise in pregnant ewes; and capable of protecting against multiple drug resistant and geographically distant strains of *Haemonchus* in a variety of sheep breeds (Kahn & Watson, 2001).

The most promising candidate antigens have been discovered for this worm; and because this worm feeds on blood, host antibodies generated by vaccination can directly attack the worm's gut causing damage or death (Harrison, 1998). These vaccines for *H. contortus* have been of the hidden or concealed variety, derived from the parasite gut (Jasmer & McGuire, 1991; Tavernor *et al*., 1992; Smith, 1993; McFarlane, 1997; Newton & Munn, 1999; Hein *et al*., 2001). This is the best example of a hidden or concealed antigen (Hein *et al*., 2001).

The earlier workers identified the antigen as a peptidase enzyme but Newton & Munn (1999) identified it as H11, a protein that is expressed within epithelial cells lining the gut of *Haemonchus contortus*. It seems that localised expression of H11 within the intestinal tract of the parasite precludes the host from being exposed to the antigen during natural infection (Hein *et al*., 2001), however specific immune response can be detected if highly

sensitive assays are employed, suggesting that in this case, "hidden' may equate with exposure to very low doses of antigen. Good levels of immunity have been achieved in many trials after vaccination with native H11, presumably due to antibodies against H11 that bind to the parasite gut interfere with the nutrition of the parasite (Hein *et al*., 2001).

The major problem that has so far prevented commercialisation of a vaccine based on H1 is the inability to obtain adequate levels of protective immunity after vaccination with recombinant versions of the H11 protein (Newton & Munn, 1999). Unfortunately it is not cost-effective to harvest these candidate vaccine proteins from *Haemonchus* for use in a commercial product. Attempts to synthesise recombinant antigens by means of biotechnology have suffered problems, mainly involving inability to induce expression of the recombinant protein in the correct configuration to generate an effective protective response (Kahn & Watson, 2001). However, once the protein is folded correctly, the technology is already well placed for both the formulation and injection of vaccine to produce high and sustained levels of protective antibody as in the "Tickguard" vaccine sold for controlling the cattle tick (Kahn & Watson, 2001).

Dr Sue Newton's group at the Victorian Institute of Animal Science (VIAS), Agriculture Victoria, is collaborating with Dr Munn in the UK with the support of Novartis Animal Health in the development of recombinant H11 vaccines (Kahn & Watson, 2001). VIAS and Novartis scientists have been engaged in a five year joint research programme recently renewed for a further three years to develop novel recombinant worm vaccines. This $2m per annum project is the largest in the world on antiparasite vaccines for farm animals.

Unfortunately current indications reveal no sufficiently promising alternative approaches for use of concealed antigen vaccines against the non-blood feeding species, although this is a subject of active research (Kahn & Watson, 2001).

Conventional Antigen Vaccines

To get adequate all round protection for our livestock, it will be necessary to develop vaccines that are effective against other abomasal and intestinal worms (Harrison, 1995). Parasites such as *T. colubriformis* and *O. circumcincta* which browse on the mucous membrane are not so affected by concealed antigen vaccines, although it has been noted that these worms ingest small amounts of host antibody (McFarlane, 1997). Cross protection has not been found between parasite species, for example against *Nematodirus battus* or *Ostertagia circumcincta* following *H. contortus* vaccination (Smith, 1993). How well other vaccines will work against the mucosal browsers is still not clear (Pomroy, 2000). Experimental work on mucosal browsers has been underway for about as long as with *Haemonchus* but less progress has been made. There are no potential vaccines being discussed publicly for nematodes of cattle (Pomroy, 2000).

Generally, vaccines comprising subunit conventional antigens have not been able to generate the high levels of protective responses that are acquired following natural infection (Kahn & Watson, 2001). The high levels of protective immunity following natural infection in adult sheep are associated with an allergic-type response (distinct from "scouring hypersensitivity"). Recent research at CSIRO Livestock Industries with neonatal lambs has indicated that trickle and "truncated" infections more effectively immunize suckling lambs than weaned lambs against *Trichostrongylus* and *Haemonchus*. This effect may be immunological (as a carryover of maternal immunity) or nutritional. Current research in this area is attempting to produce cost-effective strategies for adoption by

industry, whether these involve nutritional or immunological means. Nevertheless, at present, levels of protection achieved by natural vaccines in pen trials are not sufficiently high to justify their commercial release.

Natural antigens are so named because the immune responses which they stimulate are readily detectable after naturally acquired infection (Meeusen, 1996). Several natural antigens from a number of nematode species have been identified and tested for vaccine efficacy (Hein *et al*., 2001). Natural antigens have usually been identified by using immune serum or other biological fluids to probe mixtures of nematode proteins and then searching for correlations between the apparent strength of the immune response to a particular antigen and the level of parasitism in the host. Antigens which are consistently labeled strongly by serum taken from animals with few parasites are then tested for their protective efficacy. In contrast to the situation with hidden antigens, immunity to natural antigens will be boosted by field exposure to the parasite, and this may enhance the practical usefulness of natural antigens as vaccine candidates. Variable levels of protection have been achieved using this approach (Knox, 2000).

Research at the Centre for Animal Biotechnology, University of Melbourne, has identified and isolated some of the antigens involved in natural protection against gastrointestinal nematodes and has shown significant levels of protection can be achieved after vaccinating sheep with a single purified antigen from *Haemonchus contortus* (Kahn & Watson, 2001). Dr Wayne Hein and his group at AgResearch, Wallaceville, NZ, are concentrating on developing natural vaccines specifically for *Trichostrongylus colubriformis*.

The impact of several recent developments should allow progress towards effective natural anti-nematode vaccines to accelerate in future. Current knowledge is greater than ever before about the genetic makeup of parasites. The sequence of the genome of the free-living nematode *Caenorhabditis elegans* has been elaborated fully and the genomes of several parasitic species, including gastrointestinal nematodes of sheep, are being characterised (Hoekstra *et al*., 2000). In combination with technical advances in protein analysis, this knowledge will allow nematode proteins to be identified more readily than at any time in the past, aiding the selection of candidate protective natural antigens

Irradiated larval vaccine

CSIRO scientists postulated that by boosting immunity prior to parturition by vaccination with an irradiated larval vaccine (ILV), FEC could be lowered to a safe level during the periparturient period (Kahn & Watson, 2001). Irradiated larvae have been exposed to a prescribed level of radiation that effectively renders them sterile and incapable of complete development within the sheep. While this treatment removes the potential for severe production losses, the host is nevertheless exposed to a large dose of worms in order to initiate an effective immune response.

Pregnant ewes at pasture on three collaborating farms were vaccinated with both 10,000 *H. contortus* and 20,000 *T. colubriformis* irradiated larvae seven and three weeks before lambing. Two weeks after the completion of lambing and again three months after lambing, at weaning, FEC and parasite-specific IgG antibody levels were determined. Compared with ewes that had not been vaccinated, FEC in vaccinated ewes tended to be lower, with 35% and 15% reductions in FEC two weeks after lambing and at weaning, respectively. There was a negative correlation between FEC and circulating antibody

levels at both sampling periods after lambing, with antibody levels accounting for 35% of the variation in FEC. The higher antibody levels were associated with lower FEC. These results indicate that pregnant ewes on pasture can respond to ILV but the level of protection was not sufficient to prevent disease.

The Future

Recent advances in immunology and molecular biology have led to the development of experimental vaccines against gut parasites and with continued effort, this progress will lead to practical products to aid parasite control. However, these products are not likely to be available within a short timeframe (Harrison, 1998). The exciting development of vaccines against ruminant nematodes led to some field trials getting underway (McFarlane, 1997), but results were disappointing (Kahn & Watson, 2001). Recent testing of a promising vaccine with lambs (Eady *et al.,* 2003), showed that productivity (live weight gain) may be depressed. This may present a whole new range of problems that need solving if vaccines are to live up to their promise.

Refinement will hopefully lead to vaccines effective against several of the economically important worm species in NZ and which can be integrated into the current control measures.

3.11.2 Modulation of the Host Immune Reactions

Many of the pathological changes in the gastrointestinal tract of parasitised ruminants occur as an undesirable consequence of the immune response (Heath *et al*., 2000; Hein *et al*., 2001). In heavy infections, the parasites themselves undoubtedly inflict some damage, particularly the blood feeders such as *H. contortus*. However, in some animals, medium or light nematode infections may give rise to clinical signs and pathological changes which seem out of proportion to the pathogen load. Accumulating evidence clearly suggests that these animals have a genetically-determined propensity to mount an exaggerated allergic-type response to nematode antigens, which then causes inflammation and other changes in the intestinal tract. An exaggerated hypersensitivity response leads to the undesirable clinical outcomes often associated with nematode infections - dysregulation of gastrointestinal physiology, inappetence, scouring and dags, retarded muscle and wool growth and a general decline in health and body condition. Heath *et al*. (2000) goes as far as saying “Almost without exception, parasitic worms do not harm stock; it is the response of stock to the worms that causes harm”.

An alternative strategy for hypersensitive animals would be to ameliorate the damaging effects of nematodes by reducing or “down regulating” the immune response which occurs (Hein *et al*., 2000). This is similar to various approaches used in human medicine, where potentially dangerous allergic responses are either modified or dampened, and may disappear altogether (Stirling & Chung, 2000). This is usually achieved by giving graded doses of an allergen so the immune response to a natural challenge is altered and no longer leads to severe pathological change. Rodent models are identifying individual molecules, such as cytokines (Akdis *et al*., 2001; Finkelman & Urban, 2001; Tokura *et al*, 2001) and reactive mediators of enteropathy (Lawrence *et al*., 2000), which play important roles in the development of the adverse immune reaction sequence. By adapting methods similar to those used to treat human allergies, or by targeting the important molecular intermediates, it may be feasible in the future to develop therapies which achieve similar effects in grazing animals (Hein *et al*., 2001). This technique is currently being used

experimentally at Lincoln University (Sykes, pers. comm.) and in lambs, worm burdens of 300,000 have not led to loss of appetite or other detrimental effects.

3.11.3 Gene Silencing

Another new technology that could impact on development of anthelmintics is double-stranded RNA (dsRNA) inhibition (Hein *et al*., 2001). An increasing number of organisms are capable of taking in dsRNA from the environment and transporting it to most or all of their cells. If the dsRNA is closely homologous to an RNA type within the cell, that RNA will be targeted for specific degradation (Zamore *et al*., 2000) and, therefore, substantially reduced expression of translation products encoded by the RNA or gene silencing. This approach has been used to systematically silence virtually every gene present on chromosome 1 of *C. elegans* (Fire *et al*., 1998; Fraser *et al*, 2000). Inhibition of these genes can have profound effects on embryo development, fecundity and/or larval development. This technology has enormous potential to identify useful gene targets for anthelmintics (Hein *et al*., 2001).

3.11.4 Biological Anthelmintics

There have been anecdotal claims of biological or organic anthelmintics, such as garlic or cider vinegar, giving positive results (Betteridge *et al*., 1996; Poletti, 2001). A garlic-based tonic was evaluated by Betteridge *et al*. (1996) and Robinson (1998). Garlic was unsuccessful as an anthelmintic: there was no reduction in FECs and gains were lower than in sheep treatment with a combination drench.

Another approach is to examine the role of simpler molecules. Through improved understanding of nematode parasites and the diseases they cause, new opportunities are being created to develop anthelmintic therapies that use natural molecules to interfere with natural processes (Hein *et al*., 2001). These biological anthelmintics will likely be small peptides, enzymes, antibodies or other natural molecules that specifically interfere with normal worm metabolism, larval development, egg production or the mechanisms used by the worms to resist chemical anthelmintics. Such agents might be delivered orally like current drenches, or by genetically modified forage plants or bacteria resident in the gastrointestinal tract (Hein *et al*., 2001).

These approaches are being used in the search for new biological anthelmintics. The first involves high-throughput screening of natural products (Hein *et al*., 2001). While based on traditional methods, automation of screening has greatly increased the number of biological extracts which can be screened, potentially leading to more rapid identification of promising compounds.

Other opportunities to develop novel biological anthelmintics come from research on parasitic nematodes (Hein *et al*., 2001). One important example arises from gastrointestinal nematode parasites being closely related to the free-living nematode, *C. elegans,* which is a widely studied model (Wood, 1998). *C. elegans* is an extremely useful research model because can be easily grown and maintained in the laboratory and is translucent at all stages of development. The adult forms are either hermaphroditic or male, which makes it simple to produce both homozygous animals and genetic crosses. The recent development of technologies to introduce foreign genes into the *C. elegans*

germ line, or to “knock-out” existing genes, has added substantially to the utility of the model. Furthermore, the sequence of the entire *C. elegans* genome has been published recently and made available with unrestricted access to all investigators (Plasterk, 1999). This extremely detailed knowledge of *C. elegans* biology will substantially increase the rate and scope of future research into parasitic nematodes.

A variety of other technical breakthroughs have enhanced the ability of researchers to develop biological anthelmintics (Hein *et al.*, 2001). These include powerful “phage display” strategies to screen a huge variety of peptides or recombinant antibodies for those capable of binding to specific targets (Cwirla *et al*., 1990; Barbas *et al*., 2001). Mixtures of bacteriophage displaying different peptides on their surface are exposed to a biological target and phage that bind are selectively enriched by affinity. Since the phage contain the DNA that encodes the binding peptide, this peptide can be characterised simply by sequencing the DNA. If desired, the peptide or antibody can be re-expressed in a different recombinant protein expression system. Through the identification of consensus binding sequences and a variety of iterative improvement steps, it is possible to discover peptides or antibodies of increasing affinity to the target, often approaching affinities of natural ligands for receptors (Wrightson *et al*., 1996). Such peptides could be used to identify exposed proteins, or available binding sites, present on complex biological targets such as a worm surface. Alternatively, the peptides could become biological anthelmintics as targeting sequences for bioactive molecules such as protein toxins or as receptor binding agonists or antagonists (Hein *et al*., 2001).

3.11.5 Biological Control of Nematode Larvae

Biological control agents rarely eliminate the target organism, but reduce the numbers to acceptable levels and maintains a balance between the pathogen and the antagonist (Waller, 1997c). In contrast to chemical control of nematode parasites which is directed entirely at the parasitic stage within the host, biological control focuses on the free-living stages on pasture. Within this environment, the pre-parasitic stages of nematodes are subject to a variety of both abiotic and biotic factors that can profoundly influence their development and survival. The most important abiotic factors are temperature, oxygen and humidity - extremes in these can be lethal on these free-living stages. With regards to biotic factors, there exist a vast number of organisms that can affect the success of worm eggs developing to infective larvae. Some of these are candidates for biological control.

Natural Biological Control

Natural biological control is control produced by native natural enemies in the environment (Waller, 1997c). Although such organisms certainly exist against worm parasites, under most livestock grazing enterprises they are likely to have little impact, otherwise there would not be a problem with worm parasites in the first instance. It has been argued that the major ecological disturbances that followed the intensification of livestock grazing systems, have tipped the balance in favour of parasites by providing an abundance of susceptible hosts and favourable pasture micro-environments for the free-living stages.

Applied Biological Control

Applied biological control is control produced by human intervention (Waller, 1997c). This is further divided into classical biological control, which is effected by the introduction of exotic natural enemies, or augmented biological control which is brought about by the

enhancement of natural enemies already in the environment. Regulatory authorities in many countries insist on thorough environmental assessments to be conducted before they sanction field release of introduced organisms (Waller, 1997c) therefore control of nematode parasites of livestock is likely to be by the augmentative approach, either by manipulation of the environment or of the existing natural enemies of parasites.

Biological Control of Parasites By Manipulation of the Environment

There are good examples of environmental manipulation, or management, for the biological control of insects (Waller, 1997c). These include changes in land use, habitat provision, reducing natural enemies of beneficial species, and improved pesticide utilisation - particularly more selective use. It may be possible to lessen the effects of worm infections in livestock by similar environmental manipulation. There is evidence that organic farming practices increase the abundance and variety of dung-dwelling microorganisms, particularly fungi, which may include nematophagous species (Bell, 1983). These findings may partly account for the good levels of parasite control in organically reared lambs in NZ (Mackay *et al., 2001;* Niezen *et al*., 1996). There is also some evidence that the type of plant species used in pastures can influence the species and type of fungi that colonise the dung of livestock that graze on the pastures (Hay & Niezen, 1995).

The practice of "green manuring" of land, by the ploughing-in of various crops, as a replacement for synthetic fertilisers, is now being strongly advocated in Western Europe. This is not only more ecologically responsible, but another "spin-off" benefit is that it encourages the proliferation of earthworms which can have an important influence on the free-living stages of parasites, as described in more detail below.

Biological Control of Parasites By Manipulation of the Organisms

Direct manipulation of natural enemies of parasite larvae consists of mass production and field release of individuals of a given species of organism (Waller, 1997). There are two types of release, namely inoculative and inundative. Inoculative release refers to the release of relatively small number of individuals where the expectation is that the progeny of these individuals will provide long-term pest suppression. In contrast, inundative release is the release of massive number of individuals with the aim of providing immediate pest suppression.

Candidates for Biological Control of Nematode Parasites

Dung Removers: Dung beetles are found throughout the world and these are often capable of rapid and often complete dung removal and thus are indirectly responsible for the significant reductions in the number of free-living stages of parasites (Waller & Faedo, 1998). However, such dung dispersal activity is very dependent on ideal weather conditions, therefore little opportunity exists to exploit these organisms in attempts to achieve cost-effective and reliable biological control of nematode parasites.

Earthworms take over the role of dung beetles in the cool, moist regions of the world. In northern Europe for example, the earthworms play an important and often dominating role in removal of cattle dung from pastures and can be responsible for significant reduction of infective larvae on pasture (Gronvold, 1987).

These two classes of invertebrates reduce L_3 intake by stock by removing dung or the larvae from dung so that there are fewer available to ascend herbage. This has been

shown to be possible using both dung beetles (Scarabeidae) and earthworms (Fincher, 1973, 1975; Gronvold, 1987; Skip *et al*., 2000). Both of these invertebrates break up dung pats and carry some of the faecal material below ground The individual effects of these activities are, first, to expose some nematode eggs and larvae to dehydration and, secondly, to remove others of them from the immediate vicinity of pasture. Climate will determine the effectiveness of dung beetles in a particular ecosystem because, despite the lethal dehydrating effects introduced by the burrowing of the beetles in dung, the frequency and amount of rainfall can have an ameliorating effect, as can aeration, and some larvae will survive (Reinecke, 1960; Durie, 1961; Bryan, 1973). There is also a risk that dung beetle activity could protect eggs form the lethal effects of ultraviolet radiation (Bryan, 1973). Dung burial may not always lead to a reduction in pasture larval burdens. Earthworm activity leads to broadly similar outcomes (Gronvold, 1987).

Two species of dung beetles that occur naturally in the Waikato reduced the weight of dung and larval survival (significant only in one of three trials) and one species reduced number of larvae in herbage (Skip *et al*., 2000).

Earthworms generally reduced larval numbers on the surface, but when they buried dung larval numbers increased (Skip *et al*., 2000). Persistence of soil moisture in the vicinity of faecal pats may cancel out the reductions achieved in surface larval numbers and actually encourage later larval migration onto herbage (Bryan, 1973).

The potential of these invertebrates as serious options for controlling parasite larvae numbers appears slim and have not been persevered with as effective options in NZ (Skip *et al.,* 2000).

Parasite antagonists: A number of organisms have been identified that exploit the free-living stages of parasites as a food source. These include microarthropods, protozoa, predacious nematodes, viruses, bacteria and fungi (Waller & Faedo, 1996). Although all are of some interest, it is from the latter two groups that biological control methods are likely to emerge.

Bacteria: Many species of bacteria are associated with the cuticle, body cavity and gut of nematodes and some of these are pathogenic (Waller, 1997c). *Bacillus penetrans* is a promising candidate for the control of parasitic nematodes of plants. It produces highly resistant spores, which attach to the cuticle and then invade the nematode host. This bacterium is highly host-specific, which is both good and bad. It is good that only the target nematode pest will be affected, but also bad as the search for the specific *B. penetrans* pathogen for each of the whole range of nematode pests would be most laborious, expensive and fruitless in many cases. Another factor that is hampering the exploitation of this organism is the difficulty in culturing large quantities of *B. penetrans*, which is absolute pre-requisite for commercialisation.

Many bacteria and closely related organisms, the Actinomycetes, produce important secondary metabolites, which include antibiotics, insecticides and anthelmintics. As such they should be regarded as microbial control agents, rather than true biological control agents.

Fungi: Fungi that exhibit anti-nematode properties have been known for a long time. They consist of a great variety of species which include nematode-trapping (predacious)

fungi, endoparasitic fungi, fungi that invade nematode eggs and fungi that produce metabolites that are toxic to nematodes (Barren, 1977). The most important groups of nematophagous fungi are:

- Nematode - trapping fungi: these fungi produce specialised hyphal trapping devices, such as adhesive networks, knobs, and constricting or non-constricting rings. Fungi in this class may also produce nematode chemoattractant and/or chemotoxic substances (Waller & Faedo, 1993). Within a short period of time following capture of the nematode, the fungus penetrates the worm and destroys it.
- Endoparasitic fungi: these fungi invade the nematode from adhesive spores on the cuticle, from spores that are ingested by the nematode, or from motile spores in water (Waller, 1997c).

Fungi from these two classes are found in all environments throughout the world, but are particularly abundant in rich agricultural soils. Under laboratory conditions, where fungi are grown as a monoculture on standardised, generally nutrient-poor media and are provided with a nematode prey that cannot escape, results can be spectacularly successful. Total capture and destruction of nematodes can occur within a matter of hours. However this type of work provides little relevant information as to how these fungi would perform as practical biological control agents against animal parasitic nematodes. Testing needs to be done to determine the limitations and opportunities for parasite control associated with the livestock production systems being considered.

Research has shown that many species of nematophagous fungi can move from the soil into faeces soon after it is deposited on the pasture (Hay, 1998). Furthermore, many of these fungi are able to reduce the number of worms which develop to the infective stage when added to fresh faeces containing worm eggs. Thus, naturally-occurring nematophagous fungi in pasture soil are probably already contributing to reduced worm contamination on pasture.). While there are many (>100) species of nematode-destroying fungi, those whose spores can survive passage through the gastrointestinal tract would be most useful in livestock parasite control programmes (Kahn & Watson, 2001).

To date, the only biological control agents with any real prospect of success against nematode larvae are predaceous microfungi of the genus *Duddingtonia*, a heterogeneous group which utilise nematodes as their main source of nutrients or as a supplement to a saprophytic diet (Hein *et al*., 2001). The fungi are ingested by grazing livestock, survive transit through the alimentary tract and then quickly colonise fresh faeces after excretion. *Duddingtonia flagrans* has a chlamydospore which is sufficiently robust that it can survive passage through the ruminant gastrointestinal tract, especially the rumen, and pass safely though the gut to the faeces (Hein *et al*., 2000; Pomroy, 2000). Once in the faeces it develops, produces a three-dimensional sticky network of ring traps with its hyphae. These constrict when any nematode larvae pass through and the fungus then invades the larvae utilising it as a food source. If sufficient chlamydospores are given on a continuous basis there is a significant effect on the number of infective larvae in faeces and hence on pasture. A number of field studies around the world have now been completed with ruminants, horses and pigs which have shown significant effects on pasture larvae numbers and some productivity parameters such as growth rates (Pomroy, 2000).

In NZ, nematophagous fungi were surveyed by Fowler (1970) and later studied to determine the rate at which they infected dung (Hay *et al*., 1997), three predaceous and

one endoparasitic species being commonly recovered. Two of the observed genera (*Arthrobotrys* and *Monocrosporium*) although less suitable than *D. flagrans* because either their mycelia and conidia did not survive passage through the ruminant gut (*Arthrobotrys*) or their response to other prey was too variable *(Monacrosporium)* (Mendoza de Gives & Vaszuez-Prats, 1994; Larsen, 2000). *D. flagrans* has not been reported officially as present in NZ, although it is known to occur here (Anon, 2000), and is found in Australia (Larsen *et al.,* 1994). This species has been shown to work well in reducing trichostrongyle infections in lambs (Githigia *et al*., 1997). The fungus is also strikingly capable of reducing the transmission of infective *Ostertagia ostertagi* larvae from faeces to herbage (Fernandez *et al*., 1999).

Research by CSIRO Livestock Industries has involved the utilisation of several strains of nematode-destroying fungi, which have been shown to be very effective in reducing populations of infective stages of nematode parasites in contaminated faecal material (Kahn & Watson, 2001). Research in Denmark and Australia has identified the fungus *Duddingtonia flagrans* as the most promising candidate for biological control (Kahn & Watson, 2001; Hein *et al*., 2001; Hay, 1998; Larsen *et al*., 1993

European field trials have demonstrated that pastures grazed by animals given fungal spores may have up to 80% less infective larvae on pasture and this can reduce by 80% the worm burdens in sheep that subsequently graze these pastures (Kahn & Watson, 2001). When the fungus was fed to calves at regular intervals, so that it is passed into the faeces along with eggs of gastrointestinal roundworms, it reduced subsequent worm contamination on pastures by up to 85% (Hay, 1998). CSIRO field trials have also established that fungal treatment of young weaned lambs in autumn resulted in lower faecal egg counts and greater liveweight gains over the following winter and spring (Kahn & Watson, 2001).

Computer modeling indicates that reductions in worm burdens of at least 75% over a minimum of 60 days will provide a level of worm control equivalent to that obtained with programmes of strategic drenching with effective drenches (Kahn & Watson, 2001). Environmental studies conducted to determine the effects of deployment of these fungi in faecal material on improved pastures recorded no detectable detrimental effects on soil nematode populations or other microfauna (Kahn & Watson, 2001).

Delivery systems: Although predaceous fungi have the potential to be very useful components of integrated parasite management systems (Thamsborg *et al*., 1999), further work is needed to optimise the delivery of these agents to ruminants so as to encourage their establishment in useful numbers on faeces. Environmental conditions will influence the prolificacy of *D. flagrans*, as it is a relatively slow growing fungus that is highly sensitive to temperature (Larsen, 2000) and thus may not be uniformly effective throughout all climatic regions of NZ. There also remains the problem of how to integrate predaceous fungi into farming systems, whether by feed blocks or in conjunction with a drench (Larsen, 2000), and when in the farming year these applications will suit both husbandry requirements and climatic impediments.

The challenge remains to increase the effectiveness of these fungi still further, and to devise practical methods of utilising them on the farm (Hay, 1998). Potential methods of application include direct application; supplementary feeding, feed blocks are controlled release devices (Waller, 1997c). In Australia scientists in Sydney carried out field trials in

which feed blocks containing the fungus were made available to grazing ewes and lambs (Hay, 1998). This strategy proved as effective for the control of roundworms as an integrated control programme recommended for the region. Researchers are hopeful that a commercial product incorporating the fungus *D. flagrans* can be developed within the next five years which will give farmers an additional tool for parasite control to further reduce reliance upon anthelmintic treatments.

Current research at CSIRO is progressing in conjunction with a commercial partner to scale up production of fungal material to commercial levels and to develop delivery systems of fungal spores for Australia's sheep, beef, dairy, goat and horse industries (Kahn & Watson, 2001). It is possible that fungal spores may be delivered to animals through onfarm addition to grain supplements, incorporation into lick blocks or in a controlled-release device. These delivery systems are most effective when spores are kept dry prior to ingestion, since spores that have already germinated have diminished survival through the gastrointestinal tract. The ultimate test for the effectiveness of this method of biological control may be if nematophagous fungi can control larval emergence of *H. contortus*, which can occur in very large numbers. Comparison of the efficacy of this method of biological control against current best practice strategic chemotherapy under controlled pasture conditions at Armidale will then lead to larger scale onfarm field testing on a national scale as part of registration requirements.

Confidential R&D, by both AgResearch and Ancare, on a commercial slow release device has been underway in NZ for sometime and a commercial product is nearly ready for release (C. Shoemaker, B .Hawkins & C. Harvey pers. comm..)

3.11.6 Use of Models for Internal Parasite Control

The role of modeling rose to prominence during the decade 1990-2000 especially in examining strategies such as sequential, rotational or simultaneous use of anthelmintics (McPherson, 2002) and in examining anthelmintic resistance (Leathwick *et al*., 2001; Leathwick & Sutherland, 2002).

Models are very common in many sciences and other disciplines to explain complex situations (Barger, 1997b; Leathwick *et al*., 1998). Models are merely a mathematical representation of a real situation or hypothetical and a series of equations are put together to mimic the scenario (Leathwick, 1991; Leathwick *et al*., 1998).

The population dynamics of the internal parasites of grazing sheep and their interactions with weather, management, immunity and drenching is a good example of a system that was simply too complex to model in its entirety before the advent of the computer (Barger, 1997b). Nor was it really considered necessary, except by a few computer enthusiasts, until the late 1980s because new drenches were thought to be the answer even after the first few case of drench resistance (Barger, 1997b).

The first models were quite simple (Michel, 1970; Barger, 1997; Leathwick, *et al*., 1992). Much more complex computer models have been developed by research groups from the Universities of Strathclyde and Glasgow (Donnelly *et al*., 1972), from CSIRO in Australia and from AgResearch in NZ (Leathwick *et al*., 1992, 1996, 2001, 2002) that stimulate effects of weather, livestock management and drenching strategies on parasite

populations in sheep and on pasture, and on their consequences for drench resistance. A simpler, but more generally applicable model of the evolution of anthelmintic resistance in nematode parasites has been developed at the University of Pennsylvania (Smith, 1990). Although the details, structure and uses of these four models differ widely, when asked the same questions they produce substantially the same answers, with the exception discussed earlier on anthelmintic resistance differences between the Australian and NZ scenes.

Early models based on climatic conditions alone were not very useful at predicting internal parasite outbreaks (Leathwick *et al*., 1992) and in the NZ modeling work, it became evident that host immunity and anthelmintic resistance factors had to be included in the model (Leathwick, 1991; Leathwick *et al*., 1992). The structure of the model (Leathwick, 1991) contains all the stages of the nematode life-cycle. The model works by shifting individuals from one stage to the next, adjusting numbers at each step due to the influence of parameters such as development, survival and migration rates. Values for these parameters were taken from the literature or calculated from unpublished data.

Nowadays any evaluation of nematode control strategies must include a consideration of the implications with respect to drench resistance. In this area modeling has become a very powerful tool given that drench resistance is so difficult to study in the field. The original nematode model incorporated different worm genotypes representing a single gene mechanism for drench resistance (Leathwick, 1991). Models later expanded to include two and three gene mechanisms of resistance. Predicting exactly when and where resistance will occur has become possible using these models (Leathwick *et al*., 2001; Leathwick & Sutherland, 2002). We can now compare different control strategies for their "selection pressure" on drench resistance, particularly as many of the factors influencing resistance development are ecological rather than genetic.

The NZ models (Leathwick *et al*., 1992, 1995) have been modified to incorporate such factors as the persistent action of various drugs (Leathwick & Sutherland, 2002) and its role in the development of anthelmintic resistance. Software models of the epidemiology of internal parasites and the development of resistance to anthelmintics in NZ have now become very sophisticated and make very realistic simulations (Leathwick, 1991; Leathwick *et al*., 1998, 2001; Leathwick & Sutherland, 2002; Pomroy, 2000; Merial, 2003; Kahn & Watson, 2001). Much of what is now understood about the processes involved in selection for anthelmintic resistance in NZ and overseas is based on the development and analysis of these mathematical models (Leathwick *et al*., 1992, 1995; Leathwick & Sutherland, 2002; Barnes *et al*, 1995; Dobson *et al*., 1996). This largely reflects the complexity of the contributing factors and timeframes and costs involved running controlled field experiments (Leathwick & Sutherland, 2002). Field trials to evaluate new or modified methods for the control of internal parasites of sheep are extremely time-consuming, expensive and virtually impossible to replicate in a wide range of environments (Kahn & Watson, 2001). One of the great advantages of models is that it is possible to systematically change each variable in turn, leaving all else unchanged, and hence observe the effect each variable has on the output of interest (Leathwick & Sutherland, 2002).

Australian workers at CRISO have also developed a simulation model of the population dynamics that included the genetics of worms (Barnes *et al*., 1995, 2001; Dobson *et al*., 1996; Dobson & Barnes, 1999). The development and survival of the free-living infective

stages of the parasite on pasture are controlled by climatic data such as rainfall, temperature and evaporation. This information is made available to the model on a daily basis from weather files for the locality in which it is desired to test the control programme (Kahn & Watson, 2001).

The model can be used to explore the potential effectiveness of control technologies still under development, such as genetically resistant sheep, worm vaccines or biological control, as well as modifications of current strategies, including drenching programmes, nutrition, grazing management, stocking rates, and lambing and weaning times (Kahn & Watson, 2001). The model could be used, for example, to predict the efficacy of worm control and the development of drench resistance over a 20-year period under a one or two summer drench worm control programme.

Currently, there are three separate models, for each of *Trichostrongylus colubriformis* (Black Scour Worm), *Ostertagia sp.* (Small Brown Stomach Worm), and *Haemonchus contortus*(Barbers Pole Worm) (Kahn & Watson, 2001). A new project under the SCIPS programme will see the three species combined into a single model. Two year field trials further validate the model are underway. After validation testing it is likely the predictive model will be made available to users as a module in CSIRO's Farmwi$e software (Kahn & Watson, 2001). When used together, Farmwi$e will provide Wormworld with data on feed intake and herbage density and the worm model will provide Farmwi$e with data on inappetence and deaths attributed to worms. In this way the software will provide better estimates of production benefits under different grazing management systems.

Because they are predictions, many people have questioned the validity of models' simulations (Leathwick *et al*., 1998) because of the assumptions involved (Sykes, pers. comm., Shoemaker pers. comm..). However despite any limitations they have supported a number of findings both in NZ and overseas (Leathwick *et al*, 2001). A good example is an insight into the development of anthelmintic resistance and different anthelmintic treatments (Barger, 1997b; Leathwick *et al*., 1998) e.g. drenching adult ewes, the use of CRCs and persistent drugs and their role in the development of resistance and the mis-use of CRCs in ewes to create "clean" or "safe" pastures for lambs.

With the continued use of these models, the understanding of many issues relating to internal parasite control and drench resistance is improving all the time (Leathwick *et al*., 1998). Recommendations can only be based on the state of current knowledge which, is always changing.

Models were originally designed as research tools (Leathwick, 1991) and are still mainly used in the realms of research (McPherson, 2002), but in future they could become increasingly important as one of the main extension or educational tools in internal parasite control programmes available to wider range of practitioners.

4.0 DISCUSSIONS WITH INDUSTRY PEOPLE

4.1 Farmers

John Reeves, Te Akau

- John had been involved in producing the 1998 NZ Sheep Council/Merial publication "Parasite Notes". He felt some parts of it were now out-of-date and required rewriting.
- He felt that areas of most confusion to farmers at present are:
  - The use of boluses.
  - Breeding for resistance *versus* breeding for resilience.
- The definitive work on the use of boluses; their advantages and disadvantages had now been done at Flock House by AgResearch. This was one topic in "Parasite Notes" which needed rewriting. This work showed they led to resistance and were detrimental long term.
- John has never used boluses on his farm. At the time of writing Parasite Notes he became concerned about them. He is convinced now that use of boluses leads to long term drench resistance. In the short term there may be an economic response, but this short term gain would be at the expense of the long term benefits on the farm.
- Much of the debate and confusion has been caused by drug companies who have a product to push and a dollar to make.
- Breeding for resistance or resilience is confusing. They are totally different and he is not sure which direction to take in a breeding policy. He was going to go down the resistance route, but others in the industry swear by the tolerance approach. This is currently his biggest worry. He is doubtful about the tolerance approach because of the egg output from the stock and risk to other classes or mobs of stock. The resistance approach would combat the worm burden problem, but the sheep's whole immune system is affected and total production may be down.
- Gordon Levit at Wellsford seems to have a good selection programme going. His stock are "Worm Guard" registered and he can then pick rams on ratings for liveweight and wool.
- John has settled for an FE selection programme. He initially was going to select against FE and internal parasites, but questioned the economics. He buys "Worm Guard" rams and wonders whether he currently should be into a more comprehensive selection policy against worms.
- Adult sheep: John believes the definitive research on this has been done. This is in the worm tolerance area. Ewes should not be drenched, as their lambs will not be challenged and will not build up an early immunity to worms. If such lambs are challenged later they have no immunity and can get huge worm burdens and a drastic drop in production.
- He believes the spring periparturient rise in eggs from ewes contributes very little to the pasture burden in autumn.
- Some farmers do drench their ewes and in the short term this appears economic, but how sustainable is it in the long term? While it might appear to "pay" today, will they still be farming successfully in 15 years?
- John does not feel there are any real R&D gaps and thinks the biggest opportunities are in extension. The challenge is to educate farmers, especially about the new

research findings from the last 5-6 years research, and get them to accept the findings.

- Recently John sent blood samples from 16 rams to Lincoln to have them tested for resistance to footrot. Interestingly the top three, and one by quite a margin, most resistant were the only "Worm Guard" rams in the group. This may be another example of a general improved immunity, such as seen in the correlated genetic responses between internal parasite resistance/immunity and improved resistance/immunity to facial eczema, ryegrass staggers and lice infestation."

Kerry Dunlop, Southland

- Kerry's interest was in breeding for resilience rather than breeding for resistance. He was a member of the Romney Development Group.
- He became disenchanted with breeding for resistance only and then in even breeding using a combined FEC/productivity index.
- The faecal sampling was very laborious and the negative aspects outweighed any positive productivity gains. He had been involved in the "FEC plus gains" index for three years and then moved to blood testing for antibodies as a substitute for FECs for another three to four years. Breeding Values were not in place at the time, but Kerry thought it was not useful enough or reliable enough with individual animals to continue with. He was also influenced by his ram buying clients. There was no big demand for parasite resistant rams - one client only.
- For this reason Kerry shifted emphasis to a minimum drenching programme and started breeding for resilience.
- He knows farmers who have contrasting views and are both making very good progress selecting for low FECs.
- Kerry decided to follow the resilience pathway. Kerry was also influenced by the results of the divergent selection lines for resistance (R) and susceptibility. The R line suffered a decline in productivity (liveweight and wool) and were daggy - they were unproductive sheep. John MacEwan tried to counter this in his index where selection was weighted 80% for productivity traits and 20% for FECs.
- The labour involved in the FEC was huge and Kerry used contract people to take the faecal samples for about 10 years.
- He was also sure new science such as vaccines would come along and overtake the FEC approach.
- Because resistance was a single trait (low FEC) he was also concerned that parasites might mutate to survive and overthrow 20 years of good selection.
- Another major concern Kerry had with FEC was the one or two samples taken per year and the time of the year they were sampled. What parasite was present at that time of the year? It seemed too simplistic and he was concerned he could select for resistance to a single species depending on the time of year the FECs were taken.
- Kerry has moved to a composite breed, using the natural resistance genes of the East Friesian and Texel rather than the low FEC (R) genes.
- He now has adopted a minimum drenching strategy. There are a number of parasite control alternatives he includes in his management, along with some drench. There are no cattle on the property to create safe pasture. They use whole crop silage to serve the same purpose, so has a lot of safe new pasture for his lambs. This is a very important part of his control programme.
- He avoids the use of CRCs or Extender with his sheep, except in ewe hoggets only.

Garth Shaw, Ram Breeder, Balclutha

- Garth has been breeding for parasite resistance in his Coopworth flock for over 10 years, but he says he is not into it "boots and all".
- He also has Texels and a Polled Dorset/Texel composite, but does not select for resistance with either of these. He restricts this to the Coopworth because they are the main maternal line.
- He is pretty positive it is working, but it is very hard to measure, as there are big climatic and feed variations from year to year. He is convinced by the SIL genetic trends graph that he is achieving a positive response.
- His Coopworth ram lambs are all challenged as they are undrenched. They take two faecal samples and these are averaged to give a SIL sub index for disease".
- He finds a big variation and culls the really bad ones. He sells rams using the index - those with an average or above index. Buyers really take notice of the index.
- Initially he did not list the resistance index in his selling material, because he thought there were too many figures already and that it would not make much difference. About four years ago he started to include the BVs, and when he first did he was very surprised at how interested the buyers were in the values.
- Garth can't buy resistant sires himself because not many of the Coopworth ram breeders are breeding for resistance, and in fact many (like Kerry Dunlop) challenge the researchers on the subject or don't think it is worth it. He sources the best productive trait genetics he can - generally Apex Sire Reference rams.
- He believes there is a lot of drench resistance about, but is not sure. He says you have only to ask the vets.

Allan Richardson, Ram Breeder, Otago

- Allan has a Perendale stud and has been breeding for parasite resistance for 13 years.
- He also has a registered organic unit. It started as a 35 ha block five years ago and has now increased to 310 ha.
- The hardest problem to overcome on this unit was the internal parasite challenge, but they now take the young stock through, generally without drenching them. Some young stock get one drench on the organic unit if FEC levels or condition score indicate a problem. They get good levels of production: the lambs this year had carcass weights of 14.8 kg on the organic unit *versus* 15.9 kg on the conventional unit, where they are drenched up to five times if necessary (tail end lambs). Stocking rates are the same.
- Allan is very excited about the potential for use of genetically resistant animals, and is familiar with all the resistance *versus* resilience arguments.
- He feels many people see organic farming as a threat when it is not. It should be seen as an opportunity to cut chemical costs. There is a huge opportunity for people to learn from some of the organic practices, and they could pick up things that would be beneficial on "main stream" conventional farms. Organic units offer a great opportunity for farmers to learn new practices. Many of the sheep industry bodies, such as the NZ Sheep Council, are very conservative and won't explore some of these learning opportunities and are not supportive of organic farming.
- There seem to be some other very interesting relationships or correlations between resistance against internal parasites and increased resistance against other pests and diseases, such as ticks (as also found in Australia), facial eczema (FE) and ryegrass staggers (RGS). The parasite resistant animals seem to have multiple resistance

against other diseases and there is NZ data relating to FE and RGS. This offers a massive potential to the NZ sheep industry by proliferating these resistant genes, especially in the North Island where FE and RGS are more prevalent. Allan progeny tested 15 ram hoggets this year for multiple resistance.

- They are currently in a FITT programme and applied unsuccessfully to the Sustainable Farming Fund to examine the link between internal parasite resistance and lice resistance. On Allan's organic unit, lice have minimal impact in perhaps 10% of the flock.
- Allan believes there is a very good R&D opportunity to examine this multiple resistance. There are already some good leads - there is some scientific data suggesting a link as well as a lot of farmer observations.
- Allan reiterated the huge production opportunity that breeding for internal parasite resistance had to offer NZ but said that 95% of stud flocks can't do it. This means people who do not use resistant rams have to resort to other control measures. The real test of these is whether they can stand up in an organic system - this is the ultimate challenge for any of these practices.
- Of major concern to the sheep pastoral industry is FRST's recent announcement of the huge reduction in R&D funding on internal parasites from $6m to $800k. This could lead to real problems in the sustainability of sheep farming in NZ.

Robin Campbell, Ram Breeder, Southland

- Robin has a big interest in internal parasitism from a number of aspects. The recent reduction in FRST funding of $3m is of concern to Robin in a number of roles. Initially it is a concern to him as a farmer and ram breeder, but he is also on the boards of AgResearch and Ovita.
- In a recent paper that Robin prepared for the AgResearch Board (Campbell, 2003) as on the potential consequences of the funding outcome, Robin was astounded at some of the figures he came up with. He emphasises that the paper is a farmer's perspective on a farmer's problem.
- A cost of $300m is generally quoted as the cost of internal parasites to the sheep industry in an environment where the anthelmintics generally work. In addition it is often quoted that 30% of sheep production is directly due to the use of these drugs. His calculations indicate that if these drugs were not used or become ineffective and gross revenue fell by 30% ($86.6k) this was equivalent to 72% of EBIT (earnings before interest and tax). This is a frightening figure and illustrates that the viability of the NZ sheep industry is dependent on our ability to control parasites.
- Robin says the search for new solutions is based on what he calls three "R's" - resilience, resistance and remedies. Amongst the remedies Robin includes such alternatives as nematophagous fungi and anthelmintic plants (plants containing condensed tannins [CTs]).
- The reduction of $3m in FRST funding is of huge concern to farmers - and may mean they cannot win the "war" against parasites, and may have to look at other strategies to "live with the enemy".
- The new tax of 9 cents/sheep to fund green house gas R&D may represent such an opportunity. If some of the money was directed into work on condensed tannin plants this would represent a "double whammy" in that it could provide a partial solution to both the methane production and the internal parasite problem.
- Robin has often urged AgResearch's CDMU Group (Cultivar Development Unit) to intensify their efforts to breed a cool climate condensed tannin plant other than docks.

- The other big issue relates to the breeding for parasite resistance *versus* resilience debate. Robin has backed the resistance approach. He feels that in the cool damp Southland climate that there are very high levels of survival of parasite eggs and larvae, especially *Nematodirus*. They routinely have to drench for *Nematodirus* pre-weaning. Other worm species do not seem to be a problem with lambs while they are on milk, but *Nematodirus* can cause a serious problem in lambs on a milk diet. Robin feels that breeding for resilience would lead to high levels of L_3 larvae on pasture and that the young lambs would not have any protection or immunity to the *Nematodirus* in particular.
- He feels that resistance and resilience are mutually exclusive. He selects on a breeding index with the resistance trait as 20% of the index and productive traits 80%. He feels that breeding for resistance on-farm will have a bigger impact because he wants to lower the L_3 larvae on pasture, and 90-95% of the total parasite population is on the pasture and not in the animal. This may not be the case in summer dry climates where the percentage of the population on pasture may be much lower. For these reasons, he believes using resistant sheep which don't shed many eggs is a key way to keep pasture levels low in the Southland high survival environment.
- Robin uses the Wallaceville blood test approach rather than FECs. It is much easier because of his off-farm commitments. He can get the samples taken on time, without weather delays etc. Timeliness is the essence of good farm management and when the stock are being challenged with the parasites his off-farm activities could result in a one to two week delay in taking FECs. He can set a date for the blood sampling and meet the commitments regardless of the weather. He is well aware of the debate that the blood antibody test may result in half the genetic progress of the FEC approach. Some people, however, say it is a better method than FECs.

Murray Rohloff, Ram Breeder, Southland

- Murray was involved with John McEwan and the WormFEC programme right from the start.
- Murray uses the FEC approach and is very enthusiast about it. He initially did FECs on ewe lambs as well as ram lambs, but now only does the ram lambs. With this greater background information, he had much greater accuracy than most other breeders on the BLUP analyses. He completely dominated the group for some time, with the top rams ranked on index.
- When Murray first included FEC in the breeding index, he dropped out NLB for a period of two years. In the space of two years he successfully introduced internal parasite resistance into his flock, and maintains that others could also do this just as rapidly. FEC is now about 20% of the index. He is currently considering repeating the process.
- Once he reintroduced NLB back into the breeding index, apart from a small dip in the genetic trend graph the NLB trend recontinued at the same rate of 2% gain per annum. He is currently scanning just over 3. Because the NLB aspect in his flock is so strong, he feels it is time to re-emphasize the low FEC part of the index.
- He culls severely against dags and scouring and does not believe inclusion of FEC in the index compromises production in any way, so is probably selecting for both parasite resistance and resilience.
- He has found that his drench usage has declined over the years and now uses only 3 drenches in the lifetime of the sheep, and has done so for several years now.
- Murray's ram buying clients are absolutely reliant on him including FEC in the breeding index and if he discontinued at this stage he feels he would lose most of

his clients. Many of Murray's clients, who include some organic farmers, have reduced their drench usage also to 3 or 4 times in the lifetime of their sheep. Many have completely eliminated use of anthelmintics with adult sheep.

- His clients have absolute confidence in his approach and are kept well informed and up-to-date with a twice-yearly newsletter. Good communication is considered to be very important.
- Murray feels there is huge potential in the sheep industry to use this approach much more widely than it is currently being used. Additional benefits over and above the productivity gains are lower use of chemicals and implied residue problems.

Holmes Warren, Ram Breeder, Wairarapa

- Holmes' son, Michael, has been managing 'Turanganui' for nearly 10 years now. Holmes is still involved. They had been involved with the Wallaceville resilience work over 10 years ago for a two-year period. This involved a total of 5 recorded ram breeding flocks, with about 10,000 ewes in total. At that time Chris Morris estimated an h^2 of about 0.1 for the trait.
- Currently they are working directly with Chris Morris, AgResearch, Ruakura. This initiative involves the Wairarapa Romney Improvement Group, with 9 ram breeding flocks , and the Auckland Romney Development group with about 6 flocks.
- One sex group is used, either the ram or ewe lambs. They are dosed at weaning then left until a 'tail end' is starting to show from parasitism. They are drafted off, numbers taken and the whole lot weighed. More drafts are taken until late March or so. Dung samples are taken. The sires with the most progeny, which have resisted parasites the best and have gained the most weight are identified.
- They have just completed their second year but won't know the h^2 values until October. The first year's data gave an improved h^2 value of 0.17.
- Holmes was surprised the h^2 had improved and was surprised a positive response was possible. Their impression from 10 years ago was that with a heritability of 0.1 there was not a lot of hope of making real worthwhile progress. They did however extend the length of time between drenches, culling the most affected lambs.
- A number of things could have contributed, other than just extending the interval between drenches.
- One interesting observation is that the top sires on growth rate, weaning weight etc that are selected for normal production improvements are also at the top end for resilience. This is evidence that these traits are linked and run together. Holmes feels there was no real selection for resilience, from just delaying drenching, because it is very hard to do, let alone measure. It is just part of a very complex picture, with those that do best under less frequent drenching, are those that are partly tolerant to parasites becoming obvious and not being culled.
- The Wairarapa Romney Improvement Group selects 3 or 4 sires to link the flocks using AI. These sires are proven for weaning weight, growth rate, wool weight and NLB. When the 10 most likely sires were assembled to select the 3 or 4 from, about half turned out to also be showing up well in resilience. It is reasonable to assume that resilience has been quietly improving over the last 10 years using the breeding values and index previously in Animal Plan and now SIL.
- With the ewe lambs, last year, they received a drench at weaning and very few showed any signs of parasitism. The last of the mob, about a third, was drenched in late March. The rest had come off at earlier stages. The drought year with the very long dry summer/autumn may have been a contributing factor.

- The group has just had its annual meeting and were brought up-to-date by Chris Morris. Everyone was encouraged by the progress so far and felt that three years is really too short a time to fully indicate the progress that could be made. It was decided to explore ways to extend the scheme for a further two years. In this time they may be able to establish a breeding value for resilience and low FEC, which would be part of the SIL system. The funding for the project for the three years planned initially was $240,000, of which the breeders contributed a third.
- Holmes emphasized that it was very much a group scheme and said the chairman John Le Grove would be a better spokesman than Holmes himself.
- There have been no problems with young lambs developing clinical parasitism to any parasites, such as *Nematodirus*, from high levels of pasture contamination. Holmes is not sure what the pasture larval loads are. He thinks the dry East Coast summer may be a factor in reducing pasture contamination compared to higher rainfall areas.
- Drench resistance. Holmes had not heard of any cases in his area, and believes it may not be a big threat in the dry East Coast summer.
- He believes farmers should use all the alternatives they have available to "assist nature" in parasite control.
- The breeding for resilience has been very encouraging but there is still a long way to go. He is looking forward to the day when gene markers will help. The challenge then will be which genes to concentrate on. They will probably need to prioritise which are the most economic or profitable for the farmers. An additional problem will be the requirement to test a very large number of sheep.

4.2 Researchers

Prof. Andrew Sykes, Lincoln University

- Lincoln's current research programme on the subject centres around the nutrition response with adult ewes. They get very good reliable improvements in immunity from feeding protein supplements around lambing.
- The original work was in pen feeding trials but now they get very reliable responses in the field.
- Early work showed major responses to fishmeal supplements, but recent work has now shown good responses also from barley based supplements. This suggests a possible stimulation of microbial protein synthesis in pasture fed animals and is an area where more R&D is warranted.
- They are currently attempting to quantify the amino acids requirements of ewes on spring pasture.
- Andrew questions whether enough is known about the immune response. At Loncoln they are immuno-suppressing sheep and getting massive increases in worm burdens. In one trial they obtained worm burdens in excess of 300,000 yet there was no effect on animal performance. The depression of appetite is a component of the immune response and immuno-suppression results in no decreased appetite.
- Andrew questions whether sheep should be selected for parasite resistance (or greater immunity) because it appears to be negatively related to animal performance.

- An area of confusion and conflict between Lincoln and AgResearch is on the merits of drenching ewes. Benefits are obvious but there are arguments that drenching ewes (which are kept on the farm) will speed up the onset of resistance much more than drenching lambs (many of which are sold). Lincoln still advocates drenching ewes but Andrew is unsure which is the right school of thought. The AgResearch models suggesting it is detrimental are only as good as the assumptions used in the models. Theoretical (and modeling) arguments suggest that because the ewes stay on the property, if there are any resistant worms in the gut, drenching will perpetuate the problem.
- On the merits of models, it could make a huge difference which parasite life cycle is used. The Leathwick model focuses on *T. colubriformis* which is common in the North Island. The literature suggests that the adult worms have a major regulatory role on the population and establishment of the incoming larvae. The half life of the adult of this species is quite long. In the South Island the most important parasite in the periparturient rise is *O. circumcincta*, which has completely different population regulatory mechanism. The population is regulated by the number of incoming larvae and the adults have a relatively short life span. These two different models could give very different answers depending on the assumptions built in to the model. This is an area warranting R&D - on the immune response to these two different mechanisms.
- There are some differences in research findings on the effects of protein supplementation on the immune response. Lincoln work suggests the protein effect is in preventing the establishment of L_3 larvae, while Australian work suggests the protein effect is in enhancing the ejection of adult worms.
- Lincoln is initiating new studies on the protective effect of milk, as parasites are seldom a problem in suckling lambs.

Bill Pomroy, Massey University

- Current internal parasite research at Massey centers around 12 small projects with students on topics such as *Cooperia* resistance to ML anthelmintics, the epidemiology of GIT helminthes and apicomplexan parasites..
- Currently there is considerable confusion amongst farmers on most aspects of parasite control, especially in relation to drench usage and anthelmintic resistance.
- Massey (in conjunction with Schering Plough Ltd) is conducting a survey of 100 plus farmers on current practices. This will give a clearer picture of what farmers are currently doing in terms of drench usage and track changes from the last survey 10 years ago. A smaller number of farmers are being surveyed for anthelmintic resistance, which will give a limited idea of the status of anthelmintic resistance to benzimidazoles and levamisole, but a larger survey is warranted.
- There is a big problem looming on bull beef properties. Of all the sheep and beef practices in NZ, this is the most heavily reliant on anthelmintics. It is a monoculture of young animals, with no adult cattle to keep clean pastures as in sheep systems. Adult cattle are similar to ewes in that if they are well fed and in good condition they are basically immune to infection. Cattle, like all ruminants slowly develop an immune response to worms and this does not fully develop until they are about 18 months of age - and it is around this stage that the majority are slaughtered. For this reason bull beef operations are almost totally reliant on anthelmintics and cannot utilize older animals as a means to prepare safer pasture.
- Currently there is drench resistance in *Cooperia oncophora* to both the MLs and BZs. The extent of this is not known but the ML resistance at least is believed to be

widespread. This is not regarded as too serious because *Cooperia* is not particularly pathogenic, but heavy burdens will restrict growth rates.

- If drench resistance develops in *Ostertagia* it will cause very serious problems. Bill feels it is only a matter of time until it does occur if it follows a similar pattern to other worm species. Resistant *Ostertagia* (syn *Telodorsagia*) has been in goats for decades and recently drench resistant *Ostertagia* have also been found in sheep. It is logical that it is only a matter of time until the cattle genera of *Ostertagia* develop drench resistance. This is likely to occur initially on bull beef properties or perhaps where dairy heifers are raised intensively.
- Bill believes that the time is approaching when standard dose rates for sheep will not be effective on some farms and this will create challenges for those farmers as they seek to manipulate both anthelmintics and their system to control worms without loss of productivity. With options such as increased dose rates or repeated doses, will come questions about appropriate withholding periods. We are already seeing this with some goat farmers who are facing failure of standard dose rates against several different worm species, and have resorted to multiple doses at increased dose rates to gain some level of control Most drench resistance appears first in goats and then in sheep 5-10 years later, but as they share species, a farmer with both goats and sheep will get resistance in both at the same time. Once widespread resistance to all the drenches families occurs it will be a very tricky decision as to what drench to use. We are not at this stage yet, but Australia, Brazil, southeastern USA and South Africa are already there in some circumstances. To date the main source of control failure is with *Haemonchus*., although *Trichostronglus colubriformis* is also appearing in some cases.
- Currently farmers in NZ are very confused over the use of persistent anthelmintics and capsules. They are bombarded with commercial information on their merits. Dave Leathwick's research is a move towards a better understanding of the issue, but one trial (current Flock House study) can't answer all the questions. Dave has done considerable research with these products over many years and the results generally point to slow release capsules being a potentially effective way to rapidly select for anthelmintic resistance.
- There are some new anthelmintic chemicals being evaluated by some companies, but none are being field trailed, so NZ farmers should plan to use existing the existing products for the next 5-10 years.
- Bill feels there will be no simple answer or "golden bullet" out of the research into sheep genitics.
- Vaccines do not seem to hold a lot of promise at present either. The only successful vaccine to date is the live attenuated lungworm vaccine. In Australia a huge amount of money has been spent for 20 years or so researching subunit vaccines with little success. There has also been a lot of work in the UK, with a vaccine to *Haemonchus* showing the most promise, if they can solve the protein configuration problems. Such a vaccine for *Haemonchus* would not have a lot of use in NZ because of the prevalence of other nematode parasites and the high price could also be a deterrent to its use. Sales of Closantel, which is used mainly for *Haemonchus* in the northern North Island are not high and are a reflection of the potential market for a Haemonchus-only vaccine.
- AgResearch also has a programme at Wallaceville, but it is largely kept under wraps and is very long term in nature. We will have to work out how to utilize vaccines as it is unlikely to be as simple as regular vaccinations. They seem unlikely to offer the high level of efficacy we currently see with anthelmintics. We do know, however, that sheep

do develop immunity, so research in this area should continue, as it should ultimately provide some solutions.

- One area that we still do not understand properly is the genetics of anthelmintic resistance, which is fundamental to manipulating selection for it.
- An interesting thing is occurring in the dairy industry. Currently, based on drug company warehouse sales data, 25% of the adult dairy cows are being drenched. Even if there is a 10% error in the figure, this is a huge amount of drench. There are several trials which show production advantages can be had from drenching lactating cows, but the downside is the additional selection pressure for anthelmintic resistance.
- Bill did not think that differences of opinion between different researchers was a very big issue as the ultimate goal is the same.

David Leathwick, AgResearch, Palmerston North

- The current research is a culmination of 15 years work on anthelmintic resistance, and Dave's team is the only group in NZ working extensively on the problem. The only other group working in the area are at Massey, and Dave's group have collaborated with them (Bill Pomroy) on various aspects, using his access to students and veterinary expertise. Bill has very limited funding for research.
- They now know much more than in the past and much more than any other NZ group on resistance.
- This programme received no FRST funding for the next year - which is about 90% of the group's funding.
- Their computer model has shed a lot of light on anthelmintic resistance and has made huge steps forwards in the last three or four years.
- The current trial (at Flock House) is being used to validate the model and the model has "stacked up" very well indeed so far, and things are continuing to look good.
- There has been a huge amount of debate on drug resistance, with numerous comments from people who have not done the R&D. Many have vested interests, because of the products they have to sell and selective use of data is not uncommon. Very little work has been peer reviewed and some trial designs have been inappropriate.
- Dave believes he now has a good understanding of the anthelmintic resistance/capsules/adult sheep scene, but it has taken 10 years of R&D and modeling to get there.
- The best approach to using drenches and minimsing resistance is to use combination drenches rather than rotating drench families.
- The Flock House work has led to very good progress on the adult sheep story.
- In the Flock House trial there are mobs of adult ewes that have had no drench for four years and they cannot be told apart from the drenched ones. Dave often defies experts and farmers to distinguish which are which.
- The problem is many farmers seem to be locked into drenching ewes. They have been drenching for so long they can't stop.
- Many believe they are getting $5 or $6 profit per ewe from the use of CRCs. Dave says that while this may be so in some cases, there is no good research data to support this kind of return.
- An early review by Brunsdon and Adam reviewed trials in which ewes received an oral drench at docking and these showed some positive and some negative responses, but no consistent benefit. Dave believes this is still true.

- In the Flock House trial, with oral drenching at docking (as opposed to CRCs), there has been no significant production response. The industry mentor group is aware of this.
- Usually an oral drench is assumed to give less of a response than CRCs or other persistent anthelmintics. Dave still believes returns are only marginal. The CRCs cost $3.00 so a return of $3.50 or $4.00 would be needed. If the return was one year in four, the economics just are not there and it is a costly exercise with say 5,000 ewes @ $3.00/head or $15,000 total.
- This is only on the economics without considering the resistance implications.
- Dave did not subscribe to Andrew Sykes' theory, that selection for resistance will vary depending on the "dominant parasite" in the ewe at drenching. While it is true that different parasites have different population control dynamics (adult *vs*. larval control), there is no known link between the relative abundance of different worm species and resistance development.
- The other problem with this theory is in practice - what should a farmer do? In pre-lamb treating with CRCs , or other long-acting drenches, a farmer has to make a decision before he knows or can monitor which parasites are dominant in the flock. In addition, up to six other parasites can occur at this time of the year and in some years at Flock House *Cooperia* was found to dominate. Many farmers do not do FECs and are less likely to do FECs and larval counts to help with any drenching decision. If they assume a similar parasite picture to the previous year, this could be misleading also.
- Some of the biggest opportunities are in extension, but it all depended on which groups would be involved. Lincoln and AgResearch would give very different recommendations. Lincoln would promote drenching ewes and not drenching lambs. Dave feels this is totally wrong and high risk, and not supported by the science.
- One of the biggest issues for the future is how we can easily modify farm management to integrate the range of control tools on the farm that give good parasite control, maintained production and minimised the development of resistance. Even on a sheep and cattle (and/or deer) operation, integrating stock classes is difficult.
- One very major compounding problem is how to measure resistance on a farm.
- For many years it has been advocated to reduce the frequency of drench use and these different management regimes would allow this. It assumes a high correlation between the number of drenches and the onset of resistance, but this is not necessarily the case as has been shown by strong evidence from both NZ and Australian research. Most current extension programmes to farmers (and this includes some Meat NZ monitor farms) advocate reducing the number of drenches, when it should really focus on the selection pressure of the drenching regime for drug resistance.
- The trial at Flock House is pointing to a change in future drenching recommendations. This is "selective drenching" where a small percentage of lambs are left undrenched. A triple combination drench is the best if there is no resistance as this gets a good kill. The trial at Flock House uses a preventative approach of six drenches. At each of these the heaviest lambs (15%) are left undrenched. The theory is that this leaves a pool of susceptible (S) parasites which ultimately breed with the resistant (R) types to produce heterozygous RS types, which along with other susceptible types dilute out the resistant RR genotypes and result in a very

high kill rate when a highly potent drug is used. The Flock House trial is proving this to be true and Australian work is doing the same.

- The recommendation to farmers is more likely to be to leave 5-10% lambs undrenched - say 50 "fat" lambs in 1000, which is very easy to do. In trials to date the practice has led to no production losses and only slightly higher pasture larval challenges (based on leaving 15%). These new recommendations are soundly epidemiologically based to let through enough susceptible worms to reduce the onset of resistance without affecting productivity.
- The FECPAK system is going back towards a semi "protective" rather than a "preventive" approach.
- The original Wallaceville five drench preventative programme was based on sound epidemiology to prevent the later problem of the autumn peak. The FECPAK move to leave out some of the drenches is a move back to the protective 1960s approach.
- Dave is currently sorting out where to find funding to finish the Flock House trial which still has a couple of years to go. The trial is massive and very expensive, costing somewhere in the region of $250k/year to run. They have had some preliminary discussions with Meat NZ about funding. AgResearch is currently looking at some king of "safety net" for Dave, but how much this involves or whether it allows the trial to continue is not yet known.
- In answer to a direct question of mine, Dave said he would be interested in participating in a national series of extension workshops if he "was still around at the time".

Robin McAnulty, Lincoln University

- Robin thought the big problems in the R&D area were the impending closure of Wallaceville and lack of funding for any production R&D on parasitism.
- The current main programme at Lincoln was based on using the periparturient ewe as a model in examining the effect of protein nutrition on the immune response and the immune response itself, as a very important and metabolically costly function.
- Epidemiology work at Lincoln has ceased due to lack of funding.
- They had been doing some related work with the fungus *Duddingtonia* on the epidemiology of free living stages and when the best time was to try and use the fungus. They had completed some field trials and had another to come. They found a 50-60% reduction in pasture larvae, but they did select a good time for its use. The reduction in pasture levels was roughly paralleled by a reduction in worm burdens of somewhat lesser magnitude but still over 50%, so the pasture effects on epidemiology do appear to follow through. They have not done a long term trial on how long to use the fungus or the best time of year for its use. They need to know for NZ conditions how well the fungus survives so there is still a lot of epidemiological work to do with the fungus and the various climatic conditions in NZ.
- AgResearch were currently working down the same track. Robin was not sure what progress they had made, but thought they were still running controlled experiments with animals and had no large scale field trials in progress.
- They would like to look at pasture larval technology at Lincoln. The current pasture larvae methods were tedious and costly. Robin was aware that Tom Fraser's AgResearch group was looking at a more rapid measurement technique for pasture larvae measurement.

(**Author's Note:** This is in the organic programme and is partly a spin off from the national ill thrift project run by MWI and AgResearch, with Annette Litherland's group having a similar role in the North Island).

As yet they were not sure how applicable the new method was or how much it costs. Robin anticipated there could be problems and that there will be a need for a lot of on-farm work.

- Robin was convinced there was a need to do a lot more basic epidemiology in various regions of NZ. The accepted gospel in the area from the Wallaceville Group is based on a centralized model, using an atypical set-stocked ewe and lamb system, which has limitations and may not represent what happens in practice. The original work was one trial conducted in the 1970s at Wallaceville, with several other sites around NZ (Ruakura, Dargaville, Winchmore etc.). While it did give some good basic information, there were some differences between sites and the 2-3 year duration of the trial was too short.
- Lincoln would like to measure larval hatching rates in various regions in the North and South of NZ and on the East and West costs where radiation hours differed significantly.
- In an initial study, they had screened faecal samples from about six farms in four geographically different areas of NZ (24 farms total and 1000 samples). They took samples from single and twin bearing ewes and lambing and non-lambing hoggets. They did FECs and looked at the parasite species. They appeared to be regional differences. *Haemonchus* increased from South to North; *Ostertagia* increased from North to South; *Nematodirus* did not vary much and *Cooperia* seem to peak in the North and the South, probably in response to rainfall. They are very interested in what egg species are shed during lactation and the pasture larval contamination levels. This work was funded by Meat NZ in one of the FITT projects.
- They now know the predominant larval challenges in different areas of NZ. For example, in Southland *Ostertagia* and *Nematodirus* are dominant but there are also levels of *Cooperia*. As resistance of various nematode species increases, this will be very important information, especially in relation to the lactating ewe which is the major source of contamination.
- Robin has major concerns over the NZ model of David Leathwick's. Part of this is due to the different predictions made by the Australian and NZ models for some similar situations. Models are very dependent on the assumptions on the genetics of resistance and some may not be valid. Data sometimes matches the predictions and sometimes doesn't. One example is that the New Zealand model predicts that the use of abamectin will delay onset of resistance by up to 15-fold while the Australian model predicts abamectin is less effective than moxidexin, and predicts the delay in the onset of resistance is only 3.5-fold.
- Another example relates to the worm species. Robin subscribes to the theory of different population dynamics control characteristics (adult *vs*. larval) of the different parasite species. Robin said the Leathwick model is a single species model looking at say *Trichostrongylus* or *Ostertagia*, while the Australian model could simulate two and three different species simultaneously. Different species do differ in the establishment rates. In the NZ model, *Trichostrongylus* has a very long half life. It may in fact be much less than this. This problem should be easy enough to sort out in the future.
- Robin has other queries re the NZ model. It fits the Wallaceville data very well, but he would like to see it fit data from other NZ regions, but there is no AgResearch data to validate it with. When the Lincoln data is used in the Australian model, it mirrors their situation, however they have not tried the Lincoln data in the NZ model.

- There has been little discussion or collaboration between the various research teams, due mainly to the severe competition for funds. Lincoln has no FRST funding and have previously done a lot of work for drug companies and some for MRDC/Wool*Pro*, but this appeared to be "drying up". They have been told it is a waste of time applying for long term 2-3 year trials because they are so expensive and the money is just not available.
- Robin felt there were still a number of R&D opportunities on quite a number of issues.
- There is a need to do epidemiological observations nationwide on 6-8 sites for a period of three years minimum to look at regional differences. This work should be run under commercial conditions and not an "artificial" system just based on ewes and lambs.
- It would be useful to know the effect of the great daily temperature variation, that occurs in winter in NZ, on larval hatching. In winter, daily temperature variations at Lincoln can be from -6 to +12ºC and at Wallaceville -4 to +15ºC. This contrasts to overseas reports on epidemiology where temperature variations, especially in continental climate situations, are much less, e.g. -2 to +2ºC.
- There is a need to know where the larvae are on the pasture relative to the grazing height. Goats graze to a certainly level and this was thought to be a parasite evasion strategy. Robin questions the findings from Wallaceville that 75% of the larvae are at the bottom of the sward. Winchmore had found contrasting data. Robin is not suggesting the original work is wrong, but that there may be regional differences.
- It was also very important to find out to quantify how fast the larvae hatch and develop in different situations. This could be critical in a rotational grazing system with lambs. It would be important to know whether a grazing interval of 21 or 28 days was detrimental or not, i.e. did grazing coincide with peak larval levels or not. No one has sequentially followed grazing at different intervals to measure larval challenge. The early Wallaceville work on which recommendations are based was in a set-stocked situation and they extrapolated to the rotational grazing scene. The patterns under set-stocking and rotational grazing may differ.
- If we knew how long it took the larvae to hatch and where they were located in the sward, we could manipulate the rotation length or grazing pressure beneficially to minimise the intake of L_3 larval. It will never be possible to avoid contamination and infection completely. The aim of the control programmes should be "larval avoidance" to reduce intake of L_3 by young stock. In the integrated control programmes all factors, such as this, should be considered, so farmers do not just rely on drenching.
- When farmers are asked why the drench, most mention the "preventative" strategy. However, the importance of "protective" drenching should not be discounted completely. In a trial at Lincoln where they had two suites of pasture, one with low and one with high larval contamination, they compared drenching at three, six and nine week intervals. In terms of lamb liveweight gain, the "low" pastures were 30% better than the "high" pastures, and three weekly drenching was better than six weekly, which was better than nine weekly (by approximately 30% in each comparison). Even with three weekly drenching, they did not recoup all the liveweight loss. This is similar to the work of Coop and Sykes where continually infecting with larval in spite of frequent drenching still resulted in production losses. Drenching is good, but it does not prevent production losses because it does not necessarily reduce larval intake.
- At Winchmore an AgMardt funded trial was run to integrate sheep and cattle in a farmlet study to get "safe pasture". The results appeared to be good but could not be interpreted properly because of a poorly planned trial design with confounded

treatments. Large differences in larval numbers were obtained, but they could not distinguish between cattle and sheep larvae.

- At Templeton, "safe pasture" is now a "dirty" word. It took three years to get really safe pastures and less than 12 months to re-contaminate them. They were just starting to make real progress when funding ceased. Hoggets could not produce clean pasture. Drenched ewes could produce clean pastures, but the practice is now a "no no" because of drench resistance. Cattle could produce clean pastures, but generally a higher ratio of cattle was required than most properties carried.
- In the integrated control system used, extending drenching intervals to six weeks still resulted good lamb gains. Where they had very low levels of pasture contamination they extending the drenching interval out further to nine weeks and still resulted in good lamb gains. However FECs started to rise and reached 700 epg and pasture larval counts started to increase. This emphasises how each system can be very different.
- Robin would like to study larval survival rates in very dry arid regions of NZ. In Canterbury pasture levels are very low in mid-summer, but by mid-April the numbers are substantial. They don't know if the reservoir is in the ground or in dung pellets. Work with Alex Familton measured temperatures over 50ºC in summer in dung pellets which should be fatal, so it would seem very little of the contamination increase would be from faeces. Robin asks "how important is the soil reservoir?" It would seem the April peak in larvae come from either the soil or new contamination from grazing lambs in February or March.
- Anthelmintic resistance is the big problem facing NZ and it will get worse. NZ is comparatively lucky however as it is in a much better situation than Australia.
- A reserve of susceptible larvae in the pasture slows the onset of resistance and in NZ a very large proportion of the population is on pasture. If there were lower pasture levels, this could increase the selection pressure for resistance. In this case, the two models (Australian and NZ) do agree. In the Australian scenario, the epidemiological knowledge makes sense. The drenches are not as effective as in NZ and this increases the selection pressure for resistance because the small percentage of survivors are 100% resistant and these produce the next generation of worms. In NZ selection pressure is not as high because contamination from undrenched ewes and lambs ensures a reservoir of susceptible larvae. Robin would like to know if there was a threshold level of susceptible larval that slowed or even prevented selection for resistance. The model should be able to predict this. We need to know more about the pasture larval levels on commercial farms. Almost all the data on this comes from research stations at this stage, with very little from farms.
- Long acting or persistent drenches for ewes: This practice is now not routinely recommended, but many farmers are still doing it. Even on one of the Lincoln farms the farm manager still uses "Ewe Guard" on the ewes in spite of being advised against it. He feels if he stops he will have to dag all his ewes and lamb gains will be lower.
- There is still a need to work on pre- and post-lamb drenching to quantify carryover effects on pasture larval contamination.
- A lot of work has been done on pre-lamb drenching with 53% of studies showing a positive response and 47% showing no response. There may not be an effect on the ewes but it there may have been a response in the lambs.
- It was unfortunate that in one of the early MRDC funded trials at Lincoln they used two capsules. This was driven by the need to get clean pastures, as one capsule did not achieve this. It was not done to give a production response in ewes, but farmers took

the ewe responses as the main result of the trial and many took up the practice. The trial was never planned with this intent.

- Robin feels it would be good to quantify the effect of capsules on pre- and post-tupping epidemiology. This has never been tried and in the current climate (re attitudes to capsules), he would not suggest it.
- So there are still quite a number of areas in epidemiology and drenching practices that still need to be researched. This is in spite of people like Peter Kettle saying well over 10 years ago that we knew it all and it would be better to spend the dollars elsewhere.

David West, Massey University

- The real difficulty is that people do not understand anthelmintic resistance. Resistance is not the problem, internal parasites are, resistance just makes them more difficult to control. They are still the number one sheep health problem in NZ. We still have some very good anthelmintics in NZ and they still give very good levels of control. Farmers get over concerned if say an anthelmintic gave 90% control against one species of parasite, but this is still a very good level of control compared to the 60-70% that is achieved by vaccines for other sheep diseases (bacterial etc.). Dave feels we should be cautious about being over critical about anthelmintic efficacy. Scientists sometimes talk "doom and gloom". We must learn to deal with the problems as they arise - that is work smarter and develop the tools required.
- The biggest problem is parasitism and resistance is just one aspect of it.
- The major problem faced by industry is how to manage parasitism.
- There is an incorrect perception that only poor farmers develop anthelmintic resistance. We need to get the right message out. Anthelmintic resistance arises from the correct use of the drugs - using approved products according to the manufacturers' instructions.
- It is often 'good' farmers who have anthelmintic resistance.
- Resistance is inevitable, but it is not something to really fear. It will emerge but we must get it in perspective and work smarter and know what is happening on each farm. Farmers must find out what the resistance problem is on the farm and how sustainable the overall management system is.
- Dairy farms are sustainable and they are virtually a monoculture of adult animals. Most of the calves are removed at birth and only 25% replacements kept - these can be spread out and "diluted" with adult cows to minimise effects of parasitism and reduce or eliminate the requirement to drench.
- Adult cattle are relatively "inert" and shed very few eggs - in fact they probably ingest more than they shed.
- Dairy farms can meet the requirements to be certified as "organic" because the calves could be spread out to minimise parasitism and drenching is not required.
- Dave was aware that about 25% of adult dairy cows are drenched regularly to get production responses. He said it could probably be justified on production grounds to drench every animal in the country once a month - but this would have a long term downside, especially in the sheep industry, in terms of drug resistance.
- Dairy farms are very sustainable systems, but bull beef and heifer rearing operations were much more risky from a parasite point of view and farmers had to be very aware.

- The traditional beef cow and calf were also okay as the beef calves generally get only two to three drenches in their life time - one at weaning and two more later on.
- The sheep system is very different, with the lambs being on the ewes for some time (not removed at birth cf. dairy calves) and they get infected.
- Ewes can contaminate pastures and can get clinical parasitism. Up to two-tooth stage they remain very susceptible.
- A 100% sheep system is probably not sustainable in the long term. We know that 100% goat systems are definitely not sustainable and this is a good indicator of what will happen to 100% sheep systems in the future. For dairy goat operations that are 100%, the only really viable option is indoor feeding.
- In the 1980s sheep farms commonly had over 95% of the stock units as sheep and this put a lot of pressure on parasite control.
- Sheep numbers are down now and there are more cattle on sheep farms, which should make other management control measures easier.
- In addition, scanning to identify twins and triplets can result in targeted anthelmintic treatment of these more vulnerable ewes.
- On most sheep farms, it is impossible to save enough safe pasture to graze the lambs on after weaning for long periods and sooner or later they must return to contaminated pastures. This is the main reason people drench ewes - attempting to use a strategic drenching programme to create more safe pasture.
- It is a fallacy that reducing drenching frequency will always reduce the onset of resistance. A single drench can select very heavily for drench resistance, in certain conditions.
- Dave is aware of a dairy farm that was taken over as a potentially "safe" sheep unit using a quarantine approach. Within six months serious drench resistance had developed. The quarantine drenching programme had heavily selected for resistance - the only survivors of the drench were a resistant strain of worms. There were no susceptible larval reservoir to dilute and interbreed with the resistant strain. People must understand the genetics of resistance and the role of the sex life of the worms. They mate within the host and the proportion of eggs with resistance genes influences the rate of onset of anthelmintic resistance.
- TV advertisements over the last 18 months or so advocating particular drenches to slow down the rate of selection for resistance have been misleading and very confusing. It was "a message people wanted to hear" and many people "latched on to it". For a marketing advantage, this concept was pushed, but it is a very short advantage as the delay of onset of resistance to that drench family is only temporary.
- Dave has some doubts about combination drenches being the answer. Modelling suggests they are okay, but in the meantime it is an "act of faith". It would be too simple to think that if 100% of animals were treated with a triple combination anthelmintic, worms would be eliminated.
- Each farm should be considered on a case by case basis to identify what management changes are required. Some farmers will not make the changes required anyway and prefer to just spend more on drench.
- Many bull beef farms use more than one action family in a season with some using all three during the year - individually, not always as combinations. Often only one worm is involved and it depends how important or pathogenic the species is. In cattle, *Cooperia* have developed resistance to the ML anthelmintics, however this species is not the most important one. *Ostertagia* is the most important worm in

cattle and the MLs are very effective against it. Levamisole is less effective for *Ostertagia* but okay for *Cooperia*.

- Dave said there were a number of "behind the farm gate" R&D candidates for possible MWI funding.
- Dave Leathwick's trial should be kept going. There has been a big investment to date, but one trial cannot provide all the answers. The work on different control procedures was very good work to continue.
- We need ways to accurately judge when pastures were safe or unsafe. The type of work that Annette Litherland has initiated in attempting to find a more accurate means of measuring or predicting pasture larval cultures was a good example.
- We need easier methods to identify resistant worms than the current double FEC and pre- and post-drench larval counts. These involved up to five groups of 10-15 animals to test the main drench families (BZs, MLs, LEV and half doses) and double sample, all of which is tedious and expensive ($600) and can put off a lot of farmers.
- As part of a parasite control programme (NOT identification of resistance) we also need better methods to tell the farmer when to drench animals. Individual FECs are too tedious and costly, and pooled samples have been tried. Some farmers are attempting to do their own. Another good reason is that with FECs the adult worms have already established and are shedding eggs so the farmer is "behind the eight-ball". The development of a more accurate pasture larval count method, mentioned above, has a real role here too. It is predictive rather than retrospective.
- With cattle because of the lower FEC and the need for two post treatment samples (one at two weeks and one at three weeks) it would be great to develop a more simple system to test for anthelminitic resistance.
- An accurate faecal larval development assay would be very useful. If the hatching of eggs one faecal sample could be used to tell if resistant or susceptible worms were present and what the gene frequency was, this would be very helpful to the farmer in knowing what drench to use.
- We need to establish the benefits in terms of resistance development and the consequences in terms of production of leaving the heaviest lambs undrenched.
- Dave had some reservations about how this MWI report and review would be used. There had been many reviews and booklets completed at huge cost and none had got it right.

Heather Simpson, Massey University

- Their related research is a fundamental programme on parasite biology examining the interaction with the host gut, whether the worms excrete anything to modify the gut environment and what nutrients they feed on.
- It is a study of the biochemistry, metabolism (both nitrogen and energy) of the various development stages in the life cycle of abomasal parasites. They are attempting to find the actual causes of the pathophysiology in the host.
- The aim is to first identify a target for disruption of the parasite, that could form the basis of a new anthelmintic or vaccine. It is important that this target differs from the ruminant host and gut microorganisms.
- Parasitology is an area where it is very difficult to get research funding in NZ. This appears to be a worldwide trend, especially with funding from Governments. The R&D funding is going into human and companion animal pharmaceuticals

internationally. Even the drug companies which sell livestock drugs are following the trend to fund human related R&D.

- Parasite R&D is not considered "sexy" or exciting enough. Parasitism is a "way of life". The hosts and the parasites have evolved together and basically do minimal harm to each other. It is not in the interest of the parasite to kill its host.
- In spite of the worldwide decline in funding for parasite R&D, it is becoming an increasing problem with the development of drug resistance. In parts of some countries, such as parts of South America, South Africa and parts of Australia, the pastoral industry is fighting a losing battle against some internal parasites.
- In NZ there are few (only three) parasitology groups and generally their programmes do not overlap. They could all potentially make individual contributions to controlling parasitism. Massey is focusing on basic nematode biology - biochemical and molecular biology. Lincoln's main thrust is on nutritional aspects. AgResearch has a number of areas – host immunology, with a molecular biology approach (different from Massey); control of resistance, genetics and biological control fungi. There is very little conflict. All the groups are producing something useful and the combination of things could help future control.
- Funding is the big problem and the decisions to terminate funding are not made by scientists, they are made by bureaucrats. This lack of funding will have disastrous long term effects. These areas of expertise will be lost to NZ and there will definitely not be a new generation of parasitologists coming along because of the attitude towards and funding science. There will be no programmes in which to train them.
- The Massey programme is funded by MWI (Meat NZ), MLA (through MWI) and AgMardt. There is no Government funding. They are getting $500k this year, for which they are really grateful. They have received Meat NZ funding for seven years now. Because it is annual funding, the downside is the uncertainty over continuity of funding, which makes planning difficult, especially in the recruitment and retention of good staff.
- AgResearch have been the recipients of virtually all the FRST funding in the area. Other groups have been unsuccessful in getting FRST funds. There has been a large decline in FRST funding of some AgResearch programmes this year and this could be detrimental.
- This competitive funding environment has led to poor cooperation and collaboration. Groups have become secretive and competitive. Because of potential IP there has been no sharing of knowledge or ideas. There is even suspected plagiarism of research ideas. Use of milk as a possible control measure is one possible example. Massey has worked in the area for some time and have published work in the area, and there are rumours of both Lincoln and AgResearch starting or wanting to start work in the area, after they have had discussions with Heather. No one else has published in the area, but they are all very interested in the subject.
- The milk R&D programme is small. It was unsuccessful for FRST funding (for economic not scientific reasons), but did receive $20k from the Alma Baker Trust this year. It has now been submitted to MWI for possible funding.
- Milk is detrimental to worm health and affects the worm's mobility. Heather's group has done work with the milk-fed lamb and *in vitro* and are about to publish it. They are interested in examining whether milk products, such as whey, which is regularly used on pasture as a fertiliser substitute can help reduce pasture contamination of the free living stages. In glasshouse studies, the effect of milk products on larvae looks promising and they are keen to examine the situation on a wider scale in the field.
- The biggest issue for farmers is anthelmintic resistance and how to deal with it.

- Breeding sheep for parasite resistance is one approach to minimising the effects of parasitism. This has resulted in scouring and reduced production. The animals seem to have exaggerated immune response - which over-reacts to the parasite challenge. The big question is "Do you want a good immune response or not?" in sheep. This confuses farmers.
- The effect of improved nutrition in helping control parasitism is a very good approach and should be utilised as widely as possible by farmers.
- The nematophagous fungi may also appear to offer good potential as a control measure.

Stewart Bisset, AgResearch Wallaceville

- Things are very disrupted at the moment with the FRST funding decisions on the Internal Parasite Programme and the pending closure of Wallaceville. FRST has decided to keep funding only a small programme on vaccine development – a risky decision for New Zealand farming considering the lack of success in this area over many years. No other work directed at sustainable worm control in livestock in the face of drench resistance was funded despite the huge potential economic impact to New Zealand. Some of the work had been "ring fenced" by Ovita.
- The reasons for the huge cut in funding were not made clear. Possibly they thought that the primary industry sector should fund the work but it should be recognized that many sectors in NZ benefit from primary exports so why should the rural sector carry the entire burden – especially in relation to the longer term, more basic work. It seems reasonable, however that the primary industry sector funds the very applied work.
- The goat industry's economics were very dependent on controlling internal parasites. When this proved difficult because of drench resistance, many people were forced to bail out of goats. Without the development of new approaches to worm control, in the long term the same situation may well develop in other livestock species.
- New Zealand already has some roundworm populations which are resistant to all drench types available. Stewart's research programme is looking at 2 aspects of managing the drench resistance problem - parasite genes involved in drench resistance and the genetics of breeding sheep for low drench requirements in the field.
- One aim of the work on drench resistance genes is to develop better tests to give early diagnostic warning of impending resistance and these will involve DNA/Polymerase chain reaction (PCR) assay. The FEC Reduction tests and other diagnostic tests for drug resistance currently used are too imprecise and retrospective, and the problem can already be quite serious by the time it is first detected.
- Work done here and overseas with white drench (BZ) resistance indicates the same gene is responsible in all of the most economically important worm types (Trichostrongylus, Ostertagia and Haemonchus). Different genes are probably involved in resistance to the other 2 drench families.
- PCR based diagnostic tests would be very useful for R&D as well as for on-farm diagnosis, and one such test to detect white drench resistance, which has already been optimized at Wallaceville, will be used in analyzing the results of field trials

being undertaken by Dave Leathwick's group at Grasslands to provide information on the risks and benefits of ewe drenching.

- Once the drench resistance genes have been identified it may be possible to specifically target them - either by modifying an existing drench or by developing a new form of drug (resistance antagonist). A resistance antagonist could have an advantage over simply developing yet another new class of anthelmintic which would almost certainly lead to another form of drug resistance.
- Funding the continuation of this work should have been appropriate for FRST but may be an opportunity for MWI.
- In relation to work on the genetics of breeding sheep for low drench requirements, the nematode resistant and susceptible Romney lines which were developed at Wallaceville have underpinned a range of basic immunological and genetics research including the work on vaccine development at Wallaceville, and the linkage mapping work being undertaken at Otago to develop DNA markers for the trait.
- Unfortunately however, our genetics analyses have shown that low faecal egg count (i.e. resistance) has some antagonisitic relationships with productivity. (including liveweight gain, wool growth and dags). This genetic antagonism has also been found in a Perendale selection line developed originally at Ruakura and several a productivity selection lines including a wool growth selection line maintained at Massey University. It is thought to be due to the inflammatory immune response that resistant sheep tend to mount in the gut against internal parasites. The inflammatory response probably leads to increased gut wall permeability so that proteins can leak into the gut and gut enzymes can leak into the blood. This is also the basis of the blood pepsinogen diagnostic test used commonly in cattle.
- There are indications that several separate genes (QTLs) may be involved in host resistance to worm infection and it is possible that only one of these is involved in the negative productivity response.
- Furthermore, when resistant animals are grazed by themselves the low FECs result in reduced pasture larval contamination which should help to counteract this negative response.
- However, ideally we need to incorporate both resistance and resilience in sheep flocks to achieve the maximum reduction in drench requirements (resilient sheep are those that are those which remain productive despite worm challenge), and therefore we are developing a small experimental line of resilient sheep which we hope to use to identify genetic markers for this trait. Resilient lambs do not necessarily have higher than normal worm burdens and in fact some resilient sheep family groups can be quite resistant. Unfortunately resilience is not an easy trait to select for within normal ethical and economic constraints and so we are not currently advocating that farmers attempt to select for it. Due to the problems with measuring the trait under normal farming conditions, identifying a genetic marker is even more important. Wallaceville has been supplying progeny-tested resilient animals to the Ballantrae Organic Farmlets study.
- However, these lines are also unfortunately a casualty of FRST funding decisions. It seems strange that FRST will fund programmes such as the vaccine, organic and genomic programmes without funding the animal resources which underpin them.

Chris Morris, AgResearch Ruakura

- The R&S selection lines of Romneys (selected for approximately 24 years) have continued to diverge and in 2002 the FECs differed by at least 40-fold with the R line having 100 epg and the S line 4000 epg. The control FEC (C) remained relatively steady.
- From a literature review in 1995 the heritabilities of FEC were 0.23 from a single measurement and 0.35 from a mean of two measurements.
- The R line, as well as having lower worm burdens, have the added advantage that the worms themselves in the low FEC line (R) have lower egg production than those in the high FEC lambs (S), because of the suppression applied by the host.
- When these three lines of lambs were grazed together from 1993-1997, the FEC were 339, 1255 and 3838 epg; weaning weights were 19.1, 19.3 and 18.9 kg; autumn liveweight gain was 9.0, 8.7 and 9.9 kg; autumn dag scores were 2.2, 1.7 and 1.4; yearling fleece weights were 2.4, 2.7 and 2.9 kg; and ewe fleece weights were 3.0, 3.3 and 3.7 kg for the R, C and S lines respectively. This demonstrates the lower productivity of the R line and the unfavourable relationship between productivity and worm resistance (low FEC). This shows the R line would be using protein or amino acids for the immune response rather than for wool growth (in particular) and body growth.
- When lines of ewes and lambs were grazed separately in replicated farmlet studies (carried out over 3 years with High and Low FEC lines of Perendales at Flock House), the production penalties were reduced or negated, because of the lower pasture larval challenge then experienced by the Low FEC line.
- Blood antibody levels to several *Trichostrongylus* species and *Haemonchus* were significantly lower in the susceptible animals all year.
- Untreated R lambs had greater "survivability" (lack of a need for a salvage drench) than C and S lambs, when all grazed together (100 *vs.* 87 *vs.* 37% remaining undrenched in March, and 83 *vs.* 63 *vs.* 23% in April). Of those lambs remaining undrenched in March, mean FEC levels were 768, 3976 and 9039 epg, respectively which would lead to major differences in pasture larval contamination. Of those lambs remaining undrenched in May, mean FEC levels were 55, 93 and 8163 epg, respectively, indicating that the R and C lines had reached immune status by that time, whilst the S line had not.
- Research on resilience: MRDC funded work on five farms from 1991 to 1993 on heritability of resistance. In 1994 resilient lines were established at Wallaceville, and in 1997, 360 resilient ewes were bred for running on the conventional *vs.* non-chemical farmlets (four replicates) at Ballantrae. In 1999 a new elite resilient line with higher productivity was established at Wallaceville. Elite rams are supplied to Ballantrae each year.
- Resilience or the ability to withstand infection is what natural selection would have done, however ethically and economically this is not a practical approach on commercial farms. A combined low-FEC/high-productivity index is the best approach on farms.
- On the research stations at Kaitoke (Wallaceville) and Ballantrae they used "selective drenching" to assess the genetic merit of animals in terms of a drench requirement index. In eight years they have made 25% progress, i.e. reduced drench requirements by 25% from two per year for the control group. Post weaning gains were also about 25% (2 kg) higher from weaning until April.
- Progeny testing (half breds) showed the resilient lambs, compared with the R and S lambs, to have intermediate FECs (1620 *vs.* 997 and 2222), lower dag scores (1.8 *vs.*

2.1 and 2.0), higher post weaning gain (7.6 *vs.* 6.7 and 6.9 kg), higher seven month weights (26.7 *vs.* 25.4 and 25.6 kg), higher 12 month weight (35.4 *vs.* 33.1 and 33.3 kg) and intermediate hogget fleece weights (2.53 *vs.* 2.40 and 2.55 kg).

- Additional benefits of increased parasite resistance are less pasture contamination, greater ewe reproductive performance and improved survivability if untreated.
- Resilience is a desirable trait but less strongly inherited than resistance. It is also difficult to measure on farm. It is not recommended that farmers breed solely for resilience, because there would be no change in pasture contamination with that strategy.
- Chris believes there is general confusion about the difference between resistance and resilience.
- The R and S flocks are in jeopardy as a result of the FRST funding decisions. AgResearch will support them for the next two years only. Other programmes also really need them. Chris made a plea that MWI look very seriously at assisting with keeping them going if funding falls through in two years time.
- Multiple resistance: They had found a small positive correlation (r=0.22) between FEC and Facial Eczema (FE) resistance in the parasite R and S lines (published in 1996 NZAPS Proceedings Volume 56: pages 84-86). In another resistance flock intentionally challenged with FE another small favourable response was seen also(r=0.15. the mean of the two estimates was 0.19. There also is a link between FE resistance and Rye Grass Staggers resistance (r=0.31).
- Cattle work: Basically the cattle work on internal parasite resistance has shown similar trends to the sheep work: similar heritabilities (0.22 to 0.32) for FEC and blood antibody levels, and reasonable repeatabilities (0.35-0.48) for blood antibody levels, and negative genetic relationships (r= -0.48) between the two.
- Beef cattle also show a periparturient rise in FEC due to a breakdown in immunity. This is a general mammalian response to prevent abortion, i.e. the foetus takes priority over the immune response at this time.
- Back crossing with Angus heifers shows for one selection line a normal bell shape distribution curve for FECs, but for the other a bimodal distribution of FECs suggests possible segregation of genes for resistance. They have the DNA samples, plus the FEC rankings and blood antibody levels and plan to search for DNA markers with these samples. Funding from MeatNZ has been confirmed for one year (2003/4) at this stage.
- Goat research: Chris has previously worked with a Saanen breeder with 800 milking does. Heritability for resistance appeared low, but they did have a small resistant group that could still graze outside while all the others were fed inside. They have all since been moved inside.
- Chris was aware of a CSIRO study with Cashmere goats where they found the heritability for resistance to be lower (<0.2) than in sheep and cattle.
- With lactating goats there is not just a brief periparturient rise in FECs. They remain elevated until well into December after a July/August kidding, i.e. 16-20 weeks *vs.* 4 weeks in sheep. They can't be drenched because of withholding regulations.

Chuck Shoemaker, AgResearch Wallaceville

- Currently the parasitology programme is facing an unsettling time with the shortfall in FRST funding and the pending closure of Wallaceville. Ovita and MWI are expected to provide additional funds to make up most of the shortfall.

- The largest current programme is in the area of immunology but there are others in molecular biology and field ecology.
- The AgResearch programme on predacious fungi was commercially sensitive, but there was a lot going on. Because of confidentiality agreements, Chuck could not divulge details to me. Celentis might be prepared to tell MWI some of what was going on. The best contact was Bridget Hawkins, Business Manager, Wallaceville. **(Author's note:** See section 5.3. It is all confidential)
- The saving grace in the applied parasitology area had been their earlier move into commercial aspects and this should help keep a critical mass of key staff together.
- Dave Leathwick was currently really delivering the goods. It had been a very long term programme and he has now validated the models and they are really getting there now. Dave does not publish much in the area of anthelmintic resistance until he has validated his models. Unlike a lot of modelers who publish only their simulations or predictions, he did not publish until he had tested them in the field. He is a "pure scientist" and not just after publicity. David did not trust the model's predictions until they were tested. Because he has not jumped on the bandwagon like many other scientists who have published prematurely, he has not had to reverse any of his claims.
- The current field trial is one of the best ever. (**Author's Note:** And one of the only ones ever on drench resistance). Results from this site are really answering a lot of farmer R&D questions.
- In their immunology programme they are now world leaders in the gastrointestinal mechanisms of immune rejection of parasites. They know at least a couple of the mechanisms rather than just have correlated responses like many overseas R&D groups. This work has the potential to lead to new anthelmintics and vaccines. There have been some big hurdles on the way, but now they have some very pleasing results.
- The Worm Biotechnology Group cultures a variety of worms on a large scale for experiments. They were the first in the world to produce a stable, transgenic animal worm parasite, albeit for possums, but there are very valuable spin-offs to other parasitology work.
- The possum model is unique, and offers potential biocontrol and other control methods. In a world first they are in a position to discover the genes required for parasitism in this possum parasite and hope to develop mutants that can't parasitise. They can maintain the mutant worms in a free living stage and identify the genes required for parasitisim.
- Another potentially important area that they are working on is RNA inhibition (RNAi) or "gene silencing". Warwick Grant was recruited especially for this work, and the parasite work and is a key researcher in the team. They have identified a number of specific genes as anthelmintic drug targets. These are being validated in variety of worm parasites. This work has received Meat NZ funding for some years.
- They have also been screening a large number of natural compounds as anthelmintics to try and discover new effective drugs for parasite control.
- They have not published much in these areas yet, but now that they have protected their IP in most of these areas with patents, there are many scientific papers on the subjects due to come out in the next year or so.
- They recently were interviewed by Penny Wardle of Meat NZ on much of this work on parasitology for an article due to come out soon in "The Producer".

Mirek Stankiewicz, Lincoln University

- Vaccines are the key to the future for internal parasite control, however no one has yet produced a vaccine that immunizes without significant changes to the metabolism of immunized animal leading to productivity loss He has been working on a method that will lead to immunisation that would not be detrimental to productivity.
- He has done some work in this area with gut parasites for Meat NZ. Mark Aspin is aware of it. The animals became immune without losing weight. MRDC have a patent in this area already.
- The world leaders in this field have so far not been able to do this. The latest article in the field (Eady *et al*., 2003) demonstrates that they still cannot get productive immunity in lambs. Liveweight gain was reduced significantly ($P<0.003$) by 13% by a vaccine.
- Everybody, especially vets, believe in immunisation against diseases caused by viruses and bacteria. Even in vaccination against common clostridial bacteria, although the animals become immune, they lose weight. The vaccines for parasites, so far, are similar. Although they become immune and get rid of the internal parasites, there is a decrease in productivity. This is similar to the productivity decline of the Low-FEC selection line of sheep that are bred for resistance to parasites.
- This is the key to the approach Mirek is taking - that is to develop a vaccination procedure that leads to immunity without the animals losing weight.
- Funding is the problem and Mirek has or is planning to discuss funding with Mark Aspin.
- Mirek told me that his approach is a very "leading edge". The biggest problem is lack of funding. The programme is not hugely long term in nature, probably 2-3 years.
- If the immunisation is developed for a one small intestinal nematode, but the host becomes immune to all small intestinal nematodes Likewise if they are immunized against one abomasal nematode species, they become immune to all abomasal worms.
- The next very important issue in internal parasitism is nutrition, especially protein nutrition. These parasites are very "clever" in that they affect the immune system to their physiological advantage - they cause a breakdown in immunity. They cause a massive leakage of protein into the gut that is lost to the infected animal. The net result is that they deprive the animal host of protein and it cannot mount an effective immune response.
- In late pregnancy ewes can often get into a negative energy or negative protein balance, and if supplements are not provided, their immunity breaks down.
- Protein supplements such as fish meal have been used. Such protein supplements in NZ are very expensive so they have been attempting to find a more viable affordable option for NZ farmers. There may be a few (one or two) specific amino acids that could lead to the improvement of the immune response.
- There is potential to do this very economically from fish processing waste products, and convert such waste into productivity. This is another area they could not get funding for. Research funding is a major area of concern. The NZ government does not subsidize the NZ farmer and yet does not want to fund R&D for the farmers, to provide tools that would allow them to produce cheaper products, and give them an advantage against their heavily subsidized competitors (EU or US

farmers) on the international market. Mirek believes this is a very "short sighted" view.

Andy Bray, AgResearch Lincoln

- R&D Programme: In response to the ageing and retiring nature of NZ parasitologists and, in particular, the decline in the associated applied R&D applicable to farmers (as the remaining parasitologists focus on molecular and physiological process), AgResearch, Lincoln are developing a R&D capability with FRST support. This is in the FRST "Research for Industry" category in the Organic Products Programme (niche products). They are also very keen to get industry support (such as MWI). The projects are as follows:
  - **Pasture Sampling Procedures** (*Dean Carson, doing a PhD with Andrew Sykes):* Procedures to improve the accuracy of determining the L_3 larval load on pasture: the scientific formulation of the sampling technique will lead to improved accuracy of the current highly variable L_3 measurement technique which was postulated over 60 yeas ago. Provision of a national standard will allow comparability of scientific data and farmer measurements. The aim is to develop a "true" or "best" method to measure pasture contamination.
  - **Lamb Diet Effects on Parasite Infections Before Weaning** *(Mark Hyslop initially; now taken over by Andy Bray):* This work is looking at different preweaning feeding levels on the impact of parasites. The protective role of milk was one of the aspects they were going to examine but this has not gone ahead. This work will be extended to determine if protein is the key
  - **Interaction Between Pasture Ectofungi and Internal Parasites** *(Tom Fraser):* This project is looking at the interaction between fungal toxins that depress animal performance and parasitism. The first results showed no interaction at all. This should not be confused with the work on nematophagous fungi. The AgResearch programme on this is very confidential as they are looking at a delivery mechanism for the fungus. This work is in competition with a number of other groups around the world.
  - **Impact of Sward Conditions on Development and Survival of Infective Larvae** *(Andy Bray and Ray Moss):* Treatments include the effects of sward removal and cultivation; sward density and size of faecal deposits on L_3 numbers/kg DM of pasture.
  - **Ability of Forages to Reduce the Impact of Parasites in Natural and Organic Farming Situations** *(Andy Bray and Ray Moss):* Treatments include *Lotus corniculatus vs.* low endophyte ryegrass *vs.* high endophyte ryegrass fed to infected or drenched lambs. Measurements include: liveweight gain, FEC, carcass weight, adult nematode numbers and nematode antibodies.
- These latter areas of R&D are focusing on the survival of the free living stages (eggs and larvae) of the parasites. It is linked indirectly with the national "Ill Thrift" programme of Meat NZ and AgResearch. They regularly have discussions with Greg Lambert and Annette Litherland on the programmes. They are related, but do not overlap.
- Annette's own project is attempting to measure parasite larvae in rumen fluid as a measure of larvae actually ingested from pasture and possibly as an indirect estimate

of pasture larval levels. This is a difficult task and Andy believes separation procedures could be very difficult.

- Lincoln is putting in a joint application to the Sustainable Farming Fund along with Greg Mirains of FECPAK. This is related to the pasture contamination work of the other programmes. Greg is a very good operator who seems to get very good adoption rates with the farmers he works with. The project is on "contamination mapping" using Greg's kits. This approach aims to identify which paddocks are heavily contaminated as this could influence grazing management decisions as to what class of stock to graze in a particular paddock. This could be very useful in integrated control programmes.
- Andy has a new slant on pasture contamination. He thinks rate of infection of animals may not be very closely related to pasture larval loading. He cites the work of Moss *et al*. (1998) where grazing pastures with cattle reduced pasture larvae by 80%, but made no difference to FEC in lambs as an example of this. The number of adult worms that establish may not be related to the number ingested above a certain level, because of adult worm population density control mechanisms. This could be a significant issue. Whether clinical parasitism results would depend on a number of factors such as level of animal resistance and level of feeding. In well fed mature ewes, low numbers may establish compared to the situation in naïve lambs.
- This also has a bearing on selecting animals for parasite resistance. Resistant animals while not necessarily more productive, sheep could lead to use of less drenches (which is less costly) and less eggs being shed to challenge subsequent grazers. Andy does not think the level of infection of these subsequent grazers is highly related to pasture contamination levels and will use the Sustainable Farming Fund project (if successful) to test this hypothesis.
- In regard to major areas of confusion, Andy believes everyone now knows about drench resistance, but they don't know how important it is or how to cope with it on the farm. We need to know how to prolong the useful life of a drench before parasites develop resistance to it and farmers need to know more about alternatives to chemicals - i.e. all the other approaches that can reduce larval intake or decrease egg survival and development. This latter aspect was key to the setting up of most of the Lincoln projects.

Annette Litherland, AgResearch Palmerston North

- Annette's parasite work is part of the national ill thrift project. They use Q-Graze to estimate potential lamb or hogget growth rate, and then have a battery of tests to find why animals are performing below potential. Of those performing below potential, parasites are the cause in 60% of the cases. Many farmers are regularly losing a lot of productive potential through subclinical parasitism.
- Annette feels there are no decent tests to estimate the level of parasite challenge.
- FECs have strong limitations. Apart from logistic problems etc., they are very variable. They are not corrected for faecal DM% or for the dry matter intake of the animal. They also vary markedly with the time of day and the parasite species involved. Another problem is that it is a "hindsight" test rather than a predictive one.
- In spite of the deficiencies of FECs, they have been using 500 epg in ewe hoggets as a "trigger point" for affecting live weight gain. They have been monitoring 13 farms for two years and in seven of the eight cases where FECs were above 500 epg animals were not growing to potential. They are currently getting together quite a useful database. Trevor Cook is a collaborator in this work.

- As a predictive test they have concentrated on an L_3 pasture larval assay. This is a complex area. There is not much known about the ecology of the larvae in pasture. their interaction with pasture. There appears to be a lot of unpublished material, and access to this would be useful. John Niezen started some work in the area but found it so fraught with sampling problems etc., gave up. The ecology has not been adequately studied because it is difficult and drenches were an easy option for control.
- There is evidence that a significant proportion of L_3 larvae are not in the grass and a some of them spend a lot of time in the soil. Some amazing things happen with L_3 levels. For example this year they made pasture larval measurements during the drought and found levels of virtually zero. Within three weeks after the drought broke they found 30,000/kg DM.
- They have been attempting to develop a commercial predictive test that farmers can use. Annette feel the pasture larvae technique may not fill the bill. It is very variable, with variation in a paddock ranging from 0 to 500,000 larvae/kg DM. It is the most variable biological observation that Annette has ever encountered. Plus it may not represent what an animal eats, and it is well known that animals avoid faeces or dung pats when they are grazing. In addition, the time taken to adequately pluck the number of samples per paddocks is about 30 minutes and this would put most farmers off the practice.
- In spite of these limitations, Trevor Cook finds it useful for indicating the species of parasites involve, even if it is not currently useful for quantifying the number.
- For the above reasons, Annette's group have been looking for more direct methods of estimating larval intake. Sampling the rumen fluid is one of these. They can get a sample by tube quite simply but because the parasites end up in a "green soup" traditional identification is difficult. They are going to attempt some of the procedures used with faeces - such as getting the larvae to swim through tissue paper.
- They also have no idea what happens when the larvae enter the rumen - whether they swim straight through in the liquid phase or whether they accumulate somewhere to "exsheath". Dogma has it that larvae exsheath in the organ ahead of where the adult is going to inhabit, i.e. abomasal parasites exsheath in the rumen and small intestinal parasites exsheath in the abomasum.
- Once they exsheath they are very difficult to separate and identify.
- Also the numbers found are critical. A count of 20/sample represents a large potential burden for the animal. So one or two can make a very large difference to the estimation of how serious the problem is.
- Annette thinks she has only a 20% chance of successfully developing this technique for onfarm use.
- An Australian lab is currently DNA typing the parasites. This would solve the problem in NZ to identify the species but it may not be any good for quantifying the numbers.
- In Australia the situation is different and easier in identifying L_3 larvae. They have fewer species that differ markedly in size. In their tests they kill the larvae, set up saline concentration gradients and spin them out. This approach was tried in NZ but had to be abandoned because in NZ most parasite larvae are similar in size and they need to be identified by their skin characteristics.
- The aim of the NZ work is to get a simple extraction test that can be run in any vet lab. Annette feels progress has been very disappointing to date - one step forward and two back.
- Currently L_3 pasture samples in NZ are sent to AgriQuality for extraction and identification at $50/sample.

- There are a number of other internal parasite projects in the AgResearch Systems Group. Andy Bray is looking at sward conditions and forage types and the influence they have on L_3 survival in the sward and adult survival in the animals. Tom Fraser has a Masters student looking at L_3 extraction procedures. Chris Broom is looking at the distribution of cattle dung pats in the field and how far larvae move away from them.
- Annette feels there is confusion amongst farmers about anthelmintic resistance. Drench companies have been "spouting the gospel" to sell drenches and this has misled many farmers. They do not want to get resistance and we need to put together an agreed strategy to slow the onset or prevent the development of resistance. This does not need to be a perfect strategy, but an agreed and consistent one.
- Annette believes there is a huge opportunity for an adult learning package on the subject. It would take a huge effort to generate change. A good example is the national package on pasture quality. Over 2000 people have been exposed to this programme over two years and only now are a few really coming to grips with the principles. The key thing is to keep hammering home the message and getting more people talking the same language. (**Author's note:** I told Annette I had similar thoughts and this represented a big extension opportunity for MWI.)
- Annette feels there are a number of farmers who have a chronic parasitism problem despite routine monthly drenching. In many cases this is probably either lack of knowledge on species and recontamination of pastures or drench resistance. One farm she had experience with this year had such a problem. They were using a long acting drench and a "safe pasture" approach. They were successfully wiping out three out of four parasite species. The fourth was not affected by the drench family being used, and became very virulent. It soon built up to high levels on the pasture and in the animals as a parasite "monoculture".

Alex Vlassoff, AgResearch Wallaceville

- For research a major concern is the move away from farmer useful R&D. FRST won't fund the work and AgResearch is moving into product development and away from R&D that provides technical information. There is a perception that technical information has no market value. Farmers will have to rely on existing information, as nothing new will be published.
- A good example of new technical information currently becoming available is from the Flock House trial of Dave Leathwick; being overseen by a mentor group comprising farmers, farm advisory, veterinary and parasitology specialists. It is reaching a very interesting stage and very useful information is just starting to emerge now. It is in its third year and it was planned to run it for four years. It will be closed down shortly if funding can't be found for the final year. The ewes have been mated but it could be wound up at the end of the winter. The total cost with the number of FTEs involved is $250-$300k per year.
- For farmers a big area of confusion arises from the conflicting arguments between the AgResearch and Lincoln researchers, on drenching adult ewes, in particular and on aspects of epidemiology. It is quite contentious and Alex believes the only way to resolve it would be for an independent person to asses the information and conflicting evidence, because of perceived biases of the individual research groups.
- Anthelmintic resistance is one of the big problems facing the industry. Trends in Australia and the UK indicate with some certainty that it will increase in NZ. Alex does not believe it will not be as radical as in South Africa or Western Australia, where drugs can no longer control parasites and very serious clinical outbreaks occur. In NZ

it will probably be more insidious. Alex believes interspecies competition and species succession will have an effect on host immunity and parasite epidemiology of resistant worm species so that very serious cases of resistance may not occur. This succession of species is *Ostertagia* in spring, *Haemonchus* in summer and *Trichostrongylus* species in autumn and winter. This will lead to a subliminal effect on farm production rather than the severe clinical disease outbreaks seen overseas. Alex would like to research this area but feels he would never get the funding from FRST. The only other potential source is from the primary industries.

- Drug company efforts are mainly in sales and marketing. Any R&D is usually restricted to product development. Massey and Lincoln have done such work under contract and there has been little independent work on products. Drug companies tend to down-play the incidence of resistance to their products. Paul Mason is an independent consultant and Phil McKenna, who is approaching retirement has been a very good independent source of diagnostic information and new statistics on parasitism, such as new instances of resistance. Nobody wants to fund work to find out the true incidence of resistance in NZ.
- There are becoming fewer and fewer workers in the field. Alex recently has trained some AgResearch people, such as Chris Boom at Whatawhata on some of the measurement techniques. So the AgResearch future capability in the area is very uncertain especially with the recent changes in FRST funding. Other sources of funding are uncertain.

Keith Thompson, Massey University

- Keith has no R&D on internal parasites.
- He is an advisor to the goat industry. One of the big concerns in that industry is concern over the use of drugs "off licence". Most of the drenches are not tested for goats and farmers tend to use them as for sheep and add a bit. It may officially reach a stage where there is legislation that will not allow drench use with goats.
- Mohair producers are sufficiently concerned that they may consider funding R&D to test for anthelmintic residues in goats - it is more the residue issue that will disallow useage rather than efficacy. We can easily check the latter using faecal egg count depression tests.
- Lots of goat operations are small block holders and rely on drenches more and more.
- Farming of goats (and probably sheep for that matter) as a monoculture on small or large properties is not sustainable, unless of course the stocking rate is very low. There are still a lot of small-holders doing just that and wondering why they are having problems.
- Bigger operations are combining them with cattle and they are complimentary and clean up weeds. They definitely have a place in mixed livestock systems.
- Many people unfairly blame goats as the source of resistance and feel if they have goats the resistant parasites will spread to sheep. Goats are <u>not</u> to blame, it is the people who use the drenches. Australia developed very bad resistance problems in sheep without goats being involved.
- And that's all he says he knows about internal parasites.

4.3 Commercial

Ovita Ltd, Otago

- Ovita's interest is in selecting commercial lines of sheep, or developing products that control internal parasites.
- The philosophical debate between the merits of selecting for resistance or resilience was confusing to farmers and others, and the various science groups are not in agreement.
- Ovita had interests in two research groups at AgResearch, Otago (Invermay and Otago University) and Wallaceville.
- They were attempting to develop markers for parasite resistance. They were not interested in resilience at this stage. Two projects have now merged into one and the two flocks - resistant and susceptible lines are being used for this work.
- They are also using the "micro array" approach which is a relatively new research technique to find the gene(s) responsible for resistance. DNA from a large number of genes is contained on a slide and this is used to tell what genes are involved. Gut tissue RNA (the message) is used to "probe" the micro array and get different signals or different patterns for the resistant or susceptible lines RNA.
- Eventually they hope to have an extension programme on QTLs or a marker for the resistant genotype. This can be used in "Marker Assisted Selection" to breed a resistant flock.
- Ovita is not doing much in the resilience area. They have access to a flock with resilient and non-resilient members, but are currently doing nothing. This is a potential research opportunity.
- The other programme Ovita have an interest in is the Wallaceville programme of Chuck Shoemaker and Wayne Hein. This is a totally different approach to internal parasite control based on host immunology. This group is working at the molecular level and have identified molecules on the surface of the worm. They are generating antibodies against these molecules that bind to the surface of the worms. These will be administered to parasitised animals and the worms will agglutinate and be "spat out" with the faeces.
- This approach shows potential for the development of new anthelmintics or vaccines. A similar approach has been used in Australia to develop a tick antibody, which is very much simpler and easier with a tick on the skin as opposed to a parasite in the gut.
- This is potentially a very exciting area and received FRST funding this year.
- Two compounds have been identified and their structure is being studied. One is an immunological product produced by immune sheep and not by susceptible sheep.
- The other is a natural non-toxic compound and a very good target for a new anthelmintic.
- Another worth consideration for funding was in the gene silencing area using RNAi to knock out a gene. This could potentially develop a new anthelmintic and other groups around the world are looking at it. It is a very long term approach and an anthelmintic will be a long way off. Ovita are not interested in it for this reason, but some work in this area was warranted and Warwick Grant has the skills. It might be worth MWI consideration for funding, but it would be very long term and would need big dollars, not just "dabbling".
- Ovita is aware that David Leathwick's AgResearch programme did not receive FRST funding this year. He is considered a major talent, and a very good communicator/extension person. He is very good with farmers but has come into

conflict with vets and commercial people trying to sell certain products. His current thrust will help to sustain the use of current drenches in the face of drench resistance. It is very much public good R&D, and it may be a major funding opportunity for MWI.

Trevor Cook, Veterinarian, Manawatu

- Trevor is very concerned about some of the new people at Lincoln (AgResearch) who have got funding to work in parasitism of sheep that have no understanding of worm problems and still think drenching is the answer.
- There are two major but separate issues facing the industry at present.
- The first is finding a predictive assay, such as pasture larval counting (PLC) that will anticipate a larval challenge. This is a real limitation. PLCs under NZ conditions are very variable and unreliable compared to the promise they show in Australia where very realistic predictions are obtained, probably due to the flat land and much lower variation. They give a very good indication in such conditions.
- This inability to predict makes it very difficult to set up and give confidence in grazing plans to minimise larval challenge. Everything stems from the inability to measure the L_3 challenge to get some idea of the potential impact of parasitism.
- FECs are of some use as a diagnostic tool, but they are very inaccurate. It is alarming how samples from groups of animals in the same flock can vary from 0 to 1000 epg. FEC use on the farm is very different from the experimental use.
- The other major issue that faces the industry is that farmers get 99% of their advice from drench manufacturers and sellers, and there is very little independent advice on parasitism. Often when Trevor talks to farmers on the subject, he is surprised they have never heard of some of the simple facts and principles that would help their understanding of the problems.
- Trevor feels there is a lack of "honest brokers" in the area, but one who stands out is a young vet, John Moffat, with Coopers. He was previously a vet in private practice.
- Trevor recently came across a farm near Taumaranui which had developed a very bad BZ resistance problem. He advised them on how to reduce selection pressure for drench resistance. Previous advice had all been from firms and based on "spurious" data and had no consideration of long term implications.
- Resistance is a real issue and farmers are unaware of factors that speed up the onset of resistance.
- Trevor has proved that "preventive grazing" planned with minimum use of drenching can be designed and that they do work, but farmers need to be very disciplined.
- The three monitor farms Trevor has been involved with are testimony to this and represent a very big achievement in reducing drench usage by 50% in three years. This has involved a combination of grazing management, better feeding and monitoring, using live weight gains and FECs. They set up grazing systems with very low L_3 challenges.
- It is very difficult to get farmers to reduce drench use. They generally place to performance ahead of sustainability. It is difficult to get them to reduce drench inputs because they are frightened of production losses as a consequence, even if the logic is that there will be a low worm challenge.
- FECs, in spite of their limitations, can be used as a guide, but you can still get "caught out". There is a need for a more reliable predictive tool.
- Many people are loathe to give advice because they do not have the confidence in the advice if it is to reduce drench inputs – because they can get caught out.
- Sustainability should be put ahead of top performance.

- "Best practice" approaches by reducing L_3 challenge can result in better production and no loss of potential. The message that is seldom promoted is: that the actions necessary to enable reduced drench inputs also result in improved animal performance because there is reduced worm challenge.
- Trevor believes MWI should look at funding some of the basic larval ecology work on pastures, similar to what AgResearch Lincoln are doing in their organic programme - looking at larval migration etc. This approach would give better measurement of production costs to parasitism. The production costs to animals grazing high levels of pasture contamination, regardless of drenching, are poorly defined. It is therefore difficult to use this to promote the principles and advantages of setting up grazing plans to reduce the exposure of animals to such high levels of worm challenge.
- The early ecology work of Brunsdon *et al*., at Wallaceville does not seem to fit all situations. Too many things happen that do not fit the original model. We need to know the L_3 differences to help predict pending problems.
- There is value in promoting alternative approaches too, such as nematophagous fungi, use of tannin containing plants and breeding animals for resilience.
- Vaccines that stimulate the immune response without a production cost would be a great breakthrough.
- The DNA/PCR test for resistant worm species was considered a very good initiative and of particular interest to Trevor, although he had not heard of the work until I mentioned it to him.
- David Leathwick's trial at Flock House, while it had some limitations (as all trials do) was very useful. Trevor believed funding was being put in place to keep it going. It would be a worthy recipient of MWI funding if no other funding was available or if there was a shortfall.
- Parasitism is a very confusing subject and it is very dangerous to generalise. The same advice and/or information may be wrong and right in different circumstances.

Colin Harvey, Ancare Auckland

- There are a lot of internal parasite problems in the industry, but Ancare are working on a number of solutions.
- Although drench resistance in sheep is there all the time, Colin feels the single biggest problem from their perspective is *Cooperia* resistance in young cattle. *Trichostrongylus* resistance is also on the increase but *Cooperia* is the biggest first up problem. The current pour-on endecticides sold by the industry give very inadequate control of *Cooperia*. The problem is contained somewhat by the good natural immunity that older animals develop and that under good feed conditions even calves can carry burdens with little clinical affect. Sub-clinical losses are real however, and a common indication of these are an increase in the number of poor doers and/or tail enders.
- An oral combination drench of BZ and levamisole works well. However Ancare are developing a combination pour-on for release in the New Year. This will be followed by an injectable.
- On the sheep drench resistance front there has been a considerable increase in ivermectin resistance in the last two years. There is growing concern in the farming industry about this, but abamectin and moxidectin with their greater potency are still working well. Ancare are working on solutions to this problem and the first option will be a new triple abamectin combination Ancare product similar to the Merial Triton ivermectin product. Again this is regarded as a short term option and longer term other solutions will be needed.

- In a slightly longer timeframe Ancare are working with the predaceous fungus, *Duddingtonia flagrans*. They are well down the track and the first product for release will be a pre-mix feed additive for goats and horses. In the longer term they are looking at a slow release bolus for sheep. AgResearch are also working on a bolus and there may be an opportunity to collaborate.
- They are also in the very early days of attempting to develop some new biological approaches.
- Colin does not hold hope that the genome R&D work and the animal breeding option offer realistic control in the medium term. He feels it is far too long term and cites the lack of progress over the last 20 years as evidence of this. He does not feel the animal breeding option is currently any threat to their business.
- Ancare are doing quite a lot of background research work on combination slow release boluses and long acting ML anthelmintics.
- Colin does not hold any hope for a new "active" in terms of an anthelmintic - he prefers to use the term new "entity" for completely different compounds that may be vaccines, antibodies or natural compounds.
- There will be no new chemicals being released in the next 10 years. Colin cites a recent article in the authoritative "Animal Pharm" which regularly reviews animal health developments internationally (Animal Pharm, 2003). They have a regular column of "new molecule development" and recently reviewed the anthelmintic area. They cite three large multi national drug companies that have recently stopped work in this area - and state categorically that there are none in the pipeline. This trend for animal drugs is similar to what has been happening with the development of new human drugs. In both human and animal pharmaceuticals, the production of all new therapeutics has been declining markedly every decade since the 1950s and 1960s.
- People always feel there will be a new compound coming on to the scene to rescue the situation. This attitude is especially prevalent in the anthelmintic field but in reality there are zero new products to be expected.
- We do need new approaches. Ancare has links with a company that is developing antibodies against AIDS and SARS, similar in the old pulpy kidney disease and other antisera. These are, however, very much more sophisticated and scientific, and involve "designer genes". Such an approach (antibodies) could be the first line of defence in the future.
- Integrated control programmes take the pressure off drench resistance. It means that farmers don't have to completely rely on anthelmintics, but also they cannot do without them.
- The 100% sheep properties will almost inevitably develop drench resistance similar to what occurred on goat properties over a decade ago and then this resistance will spread.
- Farmers listen to all the advice they are given, but don't necessarily practice it all. While they believe it, they find excuses not to follow it or take short cuts; such as in quarantine drenching (e.g. using ivermectin rather than the recommended combination) and preparation and use of safe pastures.

Andrew Roe, Veterinarian, Southland

- Drench resistance is a big issue facing farmers.
- There is an urgent need to develop alternative methods to do FECs. Currently we don't know how accurate FECs are and farmers are now relying very heavily on them in deciding when to drench. FECs seem to have limitations, as farmers are very

reliant on them, but are becoming quite disenchanted. In spite of doing FECs quite frequently "by the book", they are often still running into internal parasite problems especially in autumn.

- The problem as Andrew sees it, is that with farmers basing their whole lamb drenching program solely on FECs, they often stretch out the inter-drench interval in the spring, which, because young lambs can shed large numbers of eggs, allows a buid-up of egges on the pasture, which then manifests itself as high worm burdens in autumn, and can even have a detrimental effect on adult sheep. The problem is made worse if there is a dry summer. The eggs released in the spring lie dormant due to lack of moisture, and the farmers find that they can further extend their drench interval, so naturally they do FECs less often. Then when the autumn rain arrives they can get caught out by a sudden (and very large) rise in worm infection. Andrew's vet clinic has seen this scenario a few times now, so Andrew encourages farmers to only use FECs in the spring to determine when they need to start drenching, and then use 2 or 3 drenches about 4 weeks apart regardless of what the FEC may say. By drenching more regularly in the spring they may be able to avert problems in the autumn.
- Do FECs give a good indication of true worm burdens? What are the alternatives available to farmers? Farmers previously used their intuition on a number of factors in deciding when to drench, such as body condition, scouring, feeding level, etc., and even then probably ended up drenching too frequently in some circumstances. It has now been impressed on them that FEC is the best method and they have forgotten about the usefulness of all these other factors.
- This really is a big issue for farmers in determining when to drench. They must draw the line and strike a balance between immediate production gains and prolonging the useful life of a particular drench.
- Pasture larval counting is another area of interest. It would be marvelous if there was an easy, accurate measure of what was on the pasture, rather than a "hindsight" indication from FEC on level of infection. If there was a "do-it-yourself" method or a cost effective, quick service to measure samples, this would be a great tool for farmers.
- A method to quickly identify the early onset of resistance would be very useful for the industry.

Greg Mirams, Consultant, FECPAK

- Greg has 11 years experience in the internal parasite area and set up FECPAK to develop more accurate, quicker, diagnostic FEC tests because he was frustrated with what was available at the time and the lack of support to farmers.
- As with any other business, better decision making tools lead to faster, better business decisions and this is true in farming also.
- FECPAK was set up with this in mind because of frustrations with the FEC system at the time. Time delays, testing costs, courier costs and deterioration of samples was common. FEC was regarded as a useful diagnostic test, but many farmers stopped using it because of the frustrations and delays.
- In addition, the FEC system has not really changed since it was first devised 50 years ago. There are no industry standards, and lack of agreement between labs and between technicians was/is common. A standard was urgently needed because FECs were used routinely in drenching trials and for drug registration and this was very important indeed. The biggest issue was that there was no agreed standard.

- It is important for a farmer to build an egg count picture of the different mobs on his farm. This is a whole new data set that he can use in his management decisions. For example he may have three different mobs of lambs that were previously drenched at the same time, but have completely different worm burdens and larval counts because of the level of contaminated pasture they have been grazing e.g. new grass, or crop *vs*. cattle grazed pasture *vs.* ewe grazed pasture. The latter group may be due for a drench, while the other two mobs may be okay. A farmer can't just work from the calendar.
- He now needs a method that can do a number of mobs quickly and easy. He can't rely on remote labs with a three to four day delay in getting the results. He would send 10 samples per mob and the cost was $50-$60. This is the reason FECPAK was set up.
- The next thing is what to do with the information when he gets it back as it is very important for making management decisions. FECs are a very good guide or management aid, as parasites do not fit the calendar.
- This is why Greg has spent 11 years developing the FECPAK system. It is a much more accurate test than the old FEC tests and it is validated internationally.
- FECPAK now have the most accurate cattle test, which goes down to an accuracy level of 10 epg. Cattle have much lower FECs than sheep and levels as low as 100 epg indicate problem with parasites.
- A major frustration of Greg's, is that the "rest of the world" are using the FECPAK technique - Ireland, UK, Australia etc. Overseas press has hailed the system as "world leaders", and they have a number of international partners now. Yet in NZ Greg has not been able to get the funds either directly or via farmer groups to have it evaluated by the NZ pastoral industry.
- FECPAK is used quite widely throughout NZ. For instance, it is used right through AgResearch and Landcorp on all their farms, yet strangely enough it was never mentioned in a recent AgResearch report on internal parasitism.
- FEC is not a "fringe player" any more but is now a key tool in industry.
- Extension: FECPAK is not just a "slick box", they are involved in a lot of extension work for farmers. For example they have been commissioned by the NZ Beef Council to run a series of workshops on internal parasites for farmers.
- They have no tie to any drug company, which is a huge advantage. In NZ 40% of sheep drenches and 55% of cattle drenches are sold through vet practices. This makes vets a huge player in the extension area. They largely control the education side and knowledge on parasites, but they are <u>not</u> independent, because they are inherently tied to a drug company.
- It is also almost impossible for many vets to keep up-to-date. The problem is "too big and too fast". Internal parasitism is a huge topic with a very wide range of issues and things have been changing so incredibly quickly in recent years that in general vets are just not up-to-date.
- Greg feels education of farmers about worms is a "massive untapped tool", but nobody in NZ funds this type of teaching.
- FECPAK attempts to do this, then empowers them with a useful tool. They teach farmers about the life cycle of the worms, then with the FEC technique they can measure numbers and potential worm burdens, and make the appropriate management changes to avert disasters. The whole approach is "an empowerment model". Farmers are "hungry for knowledge" on the subject so the whole thing is "self driven".

- Drench trends: In 1991, NZ sheep drench sales totaled $25m per annum with the "mectins" being 25% of total sales. By 1998 sheep drench sales totaled $47m despite a drop in sheep numbers of about 13.6m, and the "mectins" were 65% of the total (Mirams, 1999). This gives an idea of the increase in drench use in NZ over the 1990s and the increasing dominance of the "mectins".
- The updated drench trends show a continuation of the pattern. The sheep drench market is now a valued at $62.2m and cattle $55m ($117.2m)
- Drench rotation: The industry advocated rotating drench families annually and it was very successful in that every drug company benefit financially, but it did not slow the onset of drug resistance. Farmers assumed rotation would take care of any resistance problems and tended to ignore all the other management issues that would have helped prevent it. Now there has been a change of focus on the farm and quite a change in the trend of use of drugs.
- The future: While drug resistance is a huge issue it will not bring about changes in drug use as much as future demand from customers and politics. Customers overseas are starting to demand product that was not exposed to any prophylactic drench usage. Such market signals will bypass all trade and tariff barriers internationally. International supermarkets are now coming up with rules on specification and production protocols, but are paying a 25% premium for the product. Everyone in the chain benefits from such quality control. Things like this will change drench usage much more than the onset of resistance. These trends are escalating in Europe and the UK. Greg recently returned from Europe and some of the changes were a revelation to him. In Europe farmers now can't buy drench without a prescription to show proof of a disease situation. The EU is forcing the UK to comply with this regulation by 2004 or 2005.
- Greg is concerned that the recent reduction in FRST funding for onfarm R&D will cause long term detrimental repercussions for the industry. While he thinks the decision is ill informed, FECPAK will probably pick up some of that business. Internal parasites are still the No.1 animal health problem in the NZ industry. The industry has been reliant on drenches for the last 35+ years. With the onset of drug resistance the industry is now under severe threat. Farmers always assumed, and still do, that a new drench will always come along and save the day. This is no longer true - there are no new drenches on the horizon. One of the main "drivers" of this is that internationally customers do not want drenches because of residues etc., and can put in place all sorts of barriers against products produced using drugs. Partly for this reason drug firms are cutting out R&D on animal drugs and diverting their efforts into new human drugs.
- Another problem Greg sees in NZ is that we do not know the size of the problem accurately. We have no monitoring/logging system in NZ to record the statistics. Other countries such as Australia and Europe do, so they have a much more accurate assessment of the seriousness of the problem. Everyday Greg sees examples of failure or breakdown of parasite control measures in the field. Vets are usually not involved until the problem is quite serious. This is disturbing as the use of FECs in NZ is declining, when in fact it should be used more intensively.
- The FECPAK web site is very useful. It is relatively new and contains a lot of useful information and reference documents.
- A really big concern is the big reduction in applied R&D that will now occur. The molecular biology/gene technology/genetic approaches have made slow progress to date and Greg believes these approaches will not provide any "magic bullet".

- Eventually the farmers will have to do their own applied R&D, but where will the funding come from and who will fund the urgently needed extension work in this area? (**Author's note:** The latter is a big opportunity for MWI).

Alex Familton, Consultant, Christchurch

- A major area of confusion is whether to treat adult ewes or not. The Lincoln group favours the procedure, while AgResearch (Dave Leathwick) advises against it.
- Another major area of confusion is over the use of capsules. The two types, either BZ or ML are very different products. It would appear that the rate of the development of resistance to MLs is occurring much faster than with the BZs (possibly due to a single gene involvement rather a multiple gene effect such as is thought to occur with the BZs). Merial and Captec are the only manufacturers of capsules. Use of ML capsules should be monitored closely and should only be used in young growing animals when necessary.
- BZ anthelmintics, especially in adult sheep, are still giving very good results even in the face of drench resistance.
- Lincoln University has reported very low levels of anthelmintic resistance in the last 12 months despite the intensive field trials conducted on these units. The introduction of NI goats at one point did introduce a problem but it does not appear that this has been carried over into sheep on the property.
- Alex recently came across a very interesting case study. A stud sheep farm near Lincoln has been using BZ drenches since about 1960 with no sign of resistance. He is unsure what their trick is but they only treat young animals twice - at weaning and again at January shearing. They seem to have very little pasture contamination. There have been very few new sheep bought on the property. The key might be the reduced drench usage with young animals.
- Another area that has caused a lot of argument is in the development rate of larvae at low temperatures over winter. At Lincoln they found an 8% hatch rate of L_3 larvae of *Ostertagia* and *Trichostrongylus* over winter. While they have published some work in this area, they have a lot more unpublished data on the subject. Work has not been done with *Haemonchus* but it is unlikely that this species will develop at low temperatures.
- Alex believes that part of the problem is that the different research groups (Lincoln and Wallaceville) have failed to have discussions on the issue. This reluctance to talk has been caused by competition for funding. As a consequence, there has been a lot of duplication, especially of larval cultures. There is no sharing and AgResearch, Massey and Lincoln have separate collections. One good point from this though is that no particular "lab" strain, with atypical characteristics has been perpetuated. In general there has been a lack of collaboration between groups in the internal parasite area.
- There is an urgent need for research in cattle, especially with *Cooperia*. There is a very extensive use of MLs with cattle, in fact 70% of the expenditure on MLs is for cattle use. Alex has been working with Novartis developing a combination LEV+ML pour-on that is very effective against *Cooperia*. LEV does have a very limited life for activity - about 24 hours or may be 36 hours in the pour-on.
- *Cooperia* is generally regarded as having low pathogenicity, but Alex feels this is incorrect. The majority of the damage is done by L_3 and L_4 larvae during development in the intestine. Most of this damage is done in the first 21 days or so. Removal of

these L_3 and L_4 larvae has led to increases in cattle growth rates of 140g/day in some overseas trials.

- There has not been a lot of research into internal parasites in cattle. There has been a lot of speculation, especially after DrenchCheck assays and larval culture.
- Alex has been trying to get Pfizer to bring a ParatechFlex bolus into NZ. Pfizer do not seem interested though. This contains morantel and has been on the market overseas since the early 1980s. In this time there have been very few reported cases of morantel resistance. A big question is why has resistance developed more rapidly with other anthelmintics. There may be a need to re-evaluate the BZs, levamisole and morantel in NZ. This could be an opportunity for MWI funding.
- Another research opportunity is with deer. Bill Pomroy has recently found evidence of moxidectin resistant parasites in deer. In the deer industry there is very widespread use of MOX pour-on because of the ease of the process. There may be a need to switch back to white drenches.
- Alex feels the old recommendations regarding drenching of deer only for lung worm and not for gastrointestinal parasites is "crap". With more intensive management and high stocking of deer, *Ostertagia* species build up. In cases where drenching has stopped for some time, clinical parasitism to this species has occurred in deer. They lose condition very easily - but this is hard to assess visually. Some have even developed symptoms of "battle jaw" similar to that seen in serious cases of *Haemonchus* in sheep. Recent Lab reports measured strongyle FEC of up to 1550epg in mature Wap/Elk cows (NB strongyles not lungworm).
- Abomasal parasites are common in deer and there are not many small or large intestine parasite problems. *Ostertagia* (three or four types) are the main offenders and have resulted in bad cases of permanent damage to the abomasum. Haemonchus contortus has recently been identified in Elk in the North Island together with some SI parasites.
- Not much lung worm is seen in deer these days, illustrating how very effective the ML pour-on has been.
- Colin McIntosh conducted some work on the use of capsules in deer at Invermay about 15 years ago with reasonable results. He was studying the "fading Elk syndrome" and internal parasitism was found to be a contributing factor.
- Cattle FECs. Alex has recently done some work with FECPAK in developing a new accurate cattle FEC assay with some greater degree of sensitivity. This has some value in assessing FECs in cattle between 5 and 15 months of age. In older cattle the significance is doubtful as a negative result does not signify a negative parasite problem but is probably due to the ability of the cattle beast to initiate the immune response in suppressing the development to full maturity of the parasite. Parasite egg-laying is not achieved but development to the L3 and L4 could and possibly does still take place.

Bridgit Hawkins, Celentis, Wallaceville

- Bridgit confirmed that AgResearch was working on *Duddungtonia* fungi as a possible control measure against internal parasites, but details were confidential.

4.4 Other

Fraser Broome, Programme Manager, FRST

- FRST does not collect much in the way of technical reports, and generally can provide only project titles and the dollars involved. The FRST reports are mainly milestone reports which are not very meaningful on their own.
- In the last funding round, work on internal parasites did not fare very well.
- AgResearch are the only contracted R&D providers in this area at present: Alec McKay's low chemical work and Chuck Shoemaker's immunology work.
- The links to the reports for July 2002 (latest), for these two programmes are:
  http://www.frst.govt.nz/database/CD02/html/reports/pdfs/C10X0025.pdf
  http://www.frst.govt.nz/database/CD02/html/reports/pdfs/C10X0016.pdf
- The following material was summarized or quoted from the websites.
- Parasite biotechnology (contract C10X0025) received $4.11m in 2001/2002 and the Research Leader is Dr Charles Shoemaker. This contract covers a wide range of areas. A progress summary follows.
  "Numerous strategies to reduce dependence on chemical drugs are under development including: vaccination to induce immune protection, vaccination to reduce Immunopathology, alternative biological control treatments, DNA based tests to accelerate breeding of sheep in which productivity is minimally affected by parasites, and incorporating grazing and field management schemes that reduce the impact of parasites. The results of these studies during the past two years have substantially advanced all strategies and, in some cases, initiated a path to commercialisation. Specific major achievements for the past year include: the identification, gene cloning and recombinant expression of all major allergen protein families in a parasitic nematode; the world first demonstration of an effector mechanism for the immune rejection of parasitic worms; chemical characterization of the antigen recognised by the effector antibodies that cause immune rejection of parasitic worms; world first use afferent lymphatic collection techniques to permit real time characterization of immune responses during parasitic worm rejection.
  The programme's unique, leading edge expertise with nematode parasites and ectoparasites is also being successfully leveraged through applications of new genomics and proteomics technologies to create additional intellectual property value. In particular, we are seeking to discover novel parasite genes that are ideal targets for new anti-parasitical drugs. In addition, parasites are being developed as novel vehicles to deliver biopharmaceuticals that could be beneficial to either animal or human health, or could offer new strategies to control pest animals such as possums. Using this technology, we have identified a class of proteins in worms that appear to be particularly strong candidates as targets for the development of new anthelmintics."
- Low chemical systems and associated branded products (contract C10X0016) received $1.1m in 2001/2002. Endoparasites is just part of this contract, but one of major focus.
- There is also an archive of research reports going back to 1995 on the FRST website. In the database: http://www.frst.govt.nz/database/index.cfm you can search out abstracts, earlier reports etc. Some of the objectives in the programme funded in 2002 were:
  Discovery of genes in sheep that reduce the impact of parasitism (b)
  Mr Stewart Bisset, AgResearch Limited, $247k.
  Develop new strategies to reduce the impact of parasites in deer
  Dr Colin Mackintosh, AgResearch Limited, $226k.

Comparing sheep immune rejection of *Ostertagia circumcincta* to those established for *Trichostrongylus colubriformis*.

Dr Ian Sutherland, AgResearch Limited, $206k.

Identify the epidemiological targets most amenable to effective control of ovine nematodiasis and myiasis

Dr Dave Leathwick, AgResearch Limited, $550k.

- Technology reports are also available on the website. One in particular (November, 2001) refers to biological control with the nematophagous fungus *Duddingtonia flagrans*. David Wright at Lincoln had discovered the first NZ strains of the fungus. Funding from FRST through the Technology NZ scheme, assisted the research, carried out for local animal health company, Ancare NZ Ltd. This work follows on from early work by researchers in Copenhagen and Sydney, who first isolated *Duddingtonia flagrans*.

Sam McIvor, Programme Leader, MWI

- There has been an urgent need for some time in the beef industry to clarify a number of issues.
- The sustainability of intensive beef systems with regard to internal parasites and young animals is a major priority for the beef finishing industry.
- Sam questions whether the information generated through sheep parasite research is directly applicable to the beef industry
- Ideally Sam would like a list (e.g. 70%) of irrefutable principles for extension methods and a list (e.g. 30%) where the answers are not clear cut. This could be used to develop workshops for farmers similar to the pasture quality workshops. Secondly it would identify issues for potential research which may or may not be worth investigating.
- A website (expert systems) while seemingly ideal would need to be complemented by other methods of delivering knowledge, namely interactive workshops. Farmers access to and utilizing the internet as an important business tool is probably 3-5 years away.
- There have been a lot of opinions expressed on parasite issues and some of the confusion relates to marketing of certain products.
- There could be marketing issues related to the use of drenches and their residues in future with the likes of EU formulating animal health/welfare principles in relation to suppliers.

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6.0 CONTACTS FOR FURTHER INFORMATION

Person	Location	Area of Expertise
Researchers		
Dave West	Massey University	Diagnosis, clinical findings
Bill Pomeroy	Massey University	Epidemiology
Heather Simpson	Massey University	Basic parasite physiology
Keith Thompson	Massey University	Goats
Andrew Sykes	Lincoln University	Immune response, periparturient rise
Robin MacAnulty	Lincoln University	Epidemiology
Mirek Stankiewicz	Lincoln University	Novel Vaccines
Dave Leathwick	AgResearch	Modelling, resistance
Chuck Shoemaker	AgResearch Wallaceville	Immunology
Alex Vlassoff	AgResearch Wallaceville	Epidemiology
Stuart Bisset	AgResearch Wallaceville	Cattle parasites, breeding for resilience
John McEwan	AgResearch Invermay	Breeding values
Allan Crawford	AgResearch Otago	
Chris Morris	AgResearch Ruakura	Gene markers, breeding for resistance
Company Representatives		
Colin Harvey	Ancare	Commercial issues, new products
Greg Mirams	FECPAK	Accurate FECs, extension
Robert Bower	Ovita	Gene markers, future anthelmintics
Veterinarians		
Trevor Cook	Fielding	Integrated control, clinical findings
Andrew Roe	Winton	On farm use of FECs
Farmers		
Murray Rohlof	Southland	Farmer attitudes, breeding for resistance
Robin Campbell	Southland	R&D funding, resistance, politics
Kerry Dunlop	Southland	Farmer attitudes, resilience
Holmes Warren	Wairarapa	Farmer attitudes, resilience
John Reeves	Waikato	Farmer attitudes, NZ Sheep council views
Allan Richardson	Southland	Organic farming, breeding for resistance
Other		
Fraser Broome	FRST	FRST Parasite R&D Programmes
Alex Familton	Consultant, Christchurch	Epidemiology, drench use and resistance