

Prevalence, characterisation and management of
anthelmintic resistance in gastro-intestinal
nematodes of Scottish sheep

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Declaration

I declare that I have made significant contributions to the papers submitted for this thesis and I have highlighted the extent of the role I have played in the design and execution of work, as well as interpretation of results, at the end of each chapter. The work presented within this thesis is my own original work and conducted at the parasitology division of Moredun Research Institute unless otherwise stated. This work has not been submitted in full or in part for the award of another degree and permission to include the presented papers has been obtained from all joint authors.

David Jon Bartley
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June, 2008

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Abstract

The studies within this thesis have made a valuable contribution to our understanding of anthelmintic resistance in Scotland and in particular to the prevalence of benzimidazole (BZ) and ivermectin (IVM) resistance, the expression of multiple resistance and its management. Parasitic gastroenteritis (PGE) is a major welfare issue not only for Scottish, UK and European farmers but also for livestock producers throughout the world. Parasites such as *Haemonchus*, *Trichostrongylus* and *Teladorsagia* are estimated to cost the sheep industry hundreds of millions of dollars annually. To date control has largely been achieved using anthelmintics, but over reliance on anthelmintics has led to the development of multi class anthelmintic resistance (AR) and the realization that intensive chemoprophylaxis is not a sustainable approach for the control of nematodes.

The first two papers contributing to this thesis assessed the prevalence of benzimidazole (BZ) and ivermectin (IVM) resistance within ovine gastrointestinal nematode populations in Scotland. The prevalence of BZ resistance in selected Scottish lowland sheep farms was around 24% in 1991 but this had risen to over 80% by 2001. The first cases of ivermectin resistance in sheep were only detected in 2001 but a small scale survey in 2004 showed that 35% of the farms (6 from 17) surveyed had IVM resistance, with *Teladorsagia* and *Trichostrongylus* being identified as the resistant genera. The isolation of a triple class resistant *T. circumcincta* (MTci5) population has enabled research to focus on the important issue of the therapeutic and prophylactic management of this emerging problem. The third and fourth papers detail a series of controlled efficacy tests conducted on MTci5 that confirmed, in the short term at least, it should be possible to use a milbemycin (moxidectin; MOX) or combination treatments, with IVM and one other class of anthelmintic to control nematodes (>90% efficacy) caused by adult and/or immature worms. However the study examining larval susceptibility highlighted the important role that immature stages can play in the selection and transmission of resistance. Currently there are no tests that can detect the presence of these resistant larval stages.

The fifth paper outlines parasitological findings from the farm where MTci5 was isolated following the confirmation of multiple class resistance. Substantial

efforts were made to find solutions to maintain sustainability and profitability of the enterprise though ultimately the use of MOX selected for a, predominately *Teladorsagia*, population against which the persistent activity of the compound was only negligible with the reappearance of eggs in faeces occurring between 21 and 28 days post treatment.

Effective sustainable control of AR populations not only requires an understanding of the phenotypic and genotypic mechanisms that underpin resistance but also improved means of ensuring that our farmers are made aware of and utilize identified best practice approaches. The written and verbal responses of the farmers to questions relating to best practice advice (papers six and seven) would suggest that many of the recommendations for delaying the selection and transmission of AR (ACME, Moredun Foundation and sustainable control of parasites of sheep (SCOPS), DEFRA) are not being followed, recommendations such as the effective quarantine treatment of newly purchased animals and dosing animals at the manufacturers' recommended dose rate were followed by only 20% and 56% of farmers respectively.

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Abbreviations

ABZ	Albendazole
AVM	Avermectin
AR	Anthelmintic resistance
BZ	Benzimidazole
CET	Controlled efficacy test
CLOS	Closantel
COWP	Copper oxide wire particles
CP	Crude protein
CYP	Cytochrome P450
dH ₂ O	Distilled water
DLS	Dose limiting species
DOR	Doramectin
DM	Dry matter
dNTP	Deoxynucleotide triphosphates
EHT	Egg hatch test
EL ₄	Early fourth-stage larva
EPG	Eggs per gram of faeces
FEC	Faecal egg count
FECRT	Faecal egg count reduction test
<i>g</i>	Gram
GIN	Gastro intestinal nematode
IVM	Ivermectin
Kg	Kilogram
L	Litre
L ₁	First stage larvae
L ₂	Second stage larvae
L ₃	Third stage larvae
L ₄	Fourth stage larvae
L ₅	Fifth stage larvae
LDT	Larval development test
LEV	Levamisole
LFIT	Larval feeding inhibition test
LL ₄	Late fourth-stage larva
LMIT	Larval migration inhibition test
LP	Low protein
µg	Microgram
mg	Milligram

ml	Millilitre
µl	Microlitre
MOR	Morantel citrate
MOX	Moxidectin
ML	Macrocyclic lactone
mM	Millimolar
MRDR	Manufacturer recommended dose rate
NaCl	Sodium chloride
OXF	Oxfendazole
°C	Degrees Celsius
PCR	Polymerase chain reaction
PCV	Packed cell volume
PI	Post-infection
PPi	pyrophosphate
rpm	Revolutions per minute
SEM	Standard Error of the Mean
<i>TBZ</i>	Thiabendazole
TST	Targeted selective treatments
X g	Relative gravity

1. General Introduction

1.1 Gastro-intestinal nematodes

In excess of seventy parasite species have been isolated from small ruminants, with over thirty nematode species being isolated from the digestive system worldwide (Taylor et al., 2007). Nematodes of the family Trichostrongylidae form a major component of the parasites found and are responsible for much of the economic losses in ruminants world wide. The most common ovine nematode genera in the UK include *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus* species (Boag and Thomas, 1975).

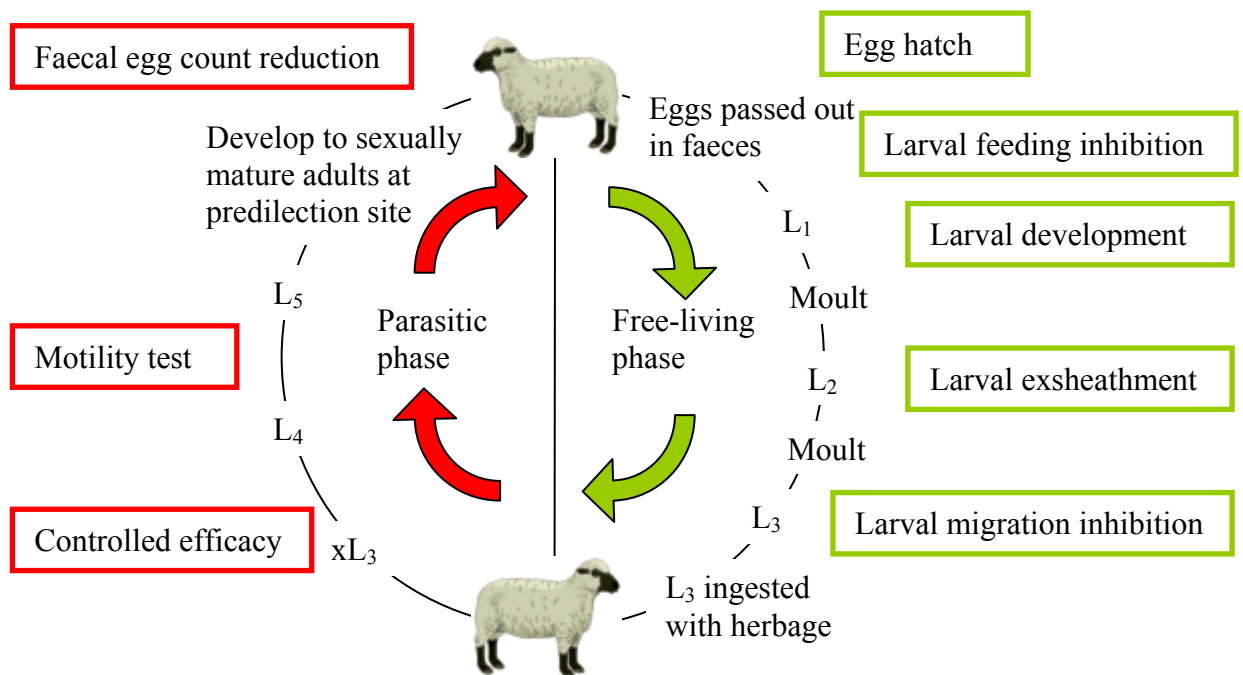


Figure 1.1 Simple direct nematode life-cycle and selected bioassays that are used to detect and characterise anthelmintic resistance, more details in Table 1-5.

Most gastro intestinal nematodes follow a simple direct lifecycle (Figure 1.1), in brief, eggs are passed in the faeces of infected hosts onto pasture, optimal embryonation, development and hatching of first stage (L₁) larvae occurs if temperature and humidity are between 22-26°C and 100% humidity respectively,

within 24 hours. First stage larvae feed on bacteria and protozoa within the faeces. Once they have fed they will enter lethargus prior to moulting to become a second larval stage (L₂). Prior to the development into the infective third stage (L₃) the process of feeding, lethargus and moulting occurs again. The L₃ do not feed, they are enclosed by a protective, impermeable sheath (the retained L₂ cuticle). Ensheathed L₃ survive by utilizing nutrient reserves stored in food granules in the intestinal cells. If these nutrient reserves become depleted before the L₃ can find a suitable host they will die. Infective larvae do not actively search for a host, but are swallowed with herbage. The larvae are negatively geotrophic and positively phototrophic to mild light enabling them to migrate up to the top of the herbage, increasing their chances of being ingested. Once ingested the larvae start the parasitic phase of their life-cycle (Soulsby, 1982).

Exsheathment sites of the larvae are species-specific for example *T. circumcincta* larvae exsheath within the rumen whereas *Cooperia curticei* exsheath within the abomasum of the host. The exsheathment sites are always proximal to the predilection site and the process may occur within 30 minutes of arrival at the appropriate site. Following exsheathment the L₃ migrate, within 2-5 days, to the preferred site in order to develop. Progression from L₄ to L₅ and to sexually mature adults can occur in as little as 14 days post infection. The pre-patent period of adult female trichostrongylids ranges from 14 – 42 days post infection but is species dependent (Soulsby, 1982).

1.2 Epidemiology

In order to assess the elements that affect the occurrence or absence of disease, it is essential to understand factors that may influence nematode populations. As seen in

Figure 1.2 numerous internal and external factors can influence parasite numbers, but it is the interactions between these factors which determine the extent of the problem. The outbreak of disease has been attributed to three basic reasons, which are classified and reviewed by Armour (1980), a) an increase in infecting mass, b) a

change in the susceptibility of stock or c) the movement of susceptible stock to an infected area.

Within temperate regions the pattern of nematode infection in sheep, with the exception of *Nematodirus* species which is a lamb to lamb infection, tends to follow a very similar pattern; eggs are laid down onto pasture, in the first instance by lactating ewes that suffer a temporary relaxation in immunity (Morgan and Sloan, 1947, cited in Armour, 1980), and develop into infective larvae which again infect the hosts, develop to become adults and shed further eggs onto pasture. Numbers of infective larvae build up over the summer to a peak in autumn, after which numbers decline. Lambs become exposed to infection on turn out and are susceptible to infection for 6 – 12 months (Waller and Thomas, 1978) thereafter they become refractory to infection unless under nutritional/reproductive stress or ill health.

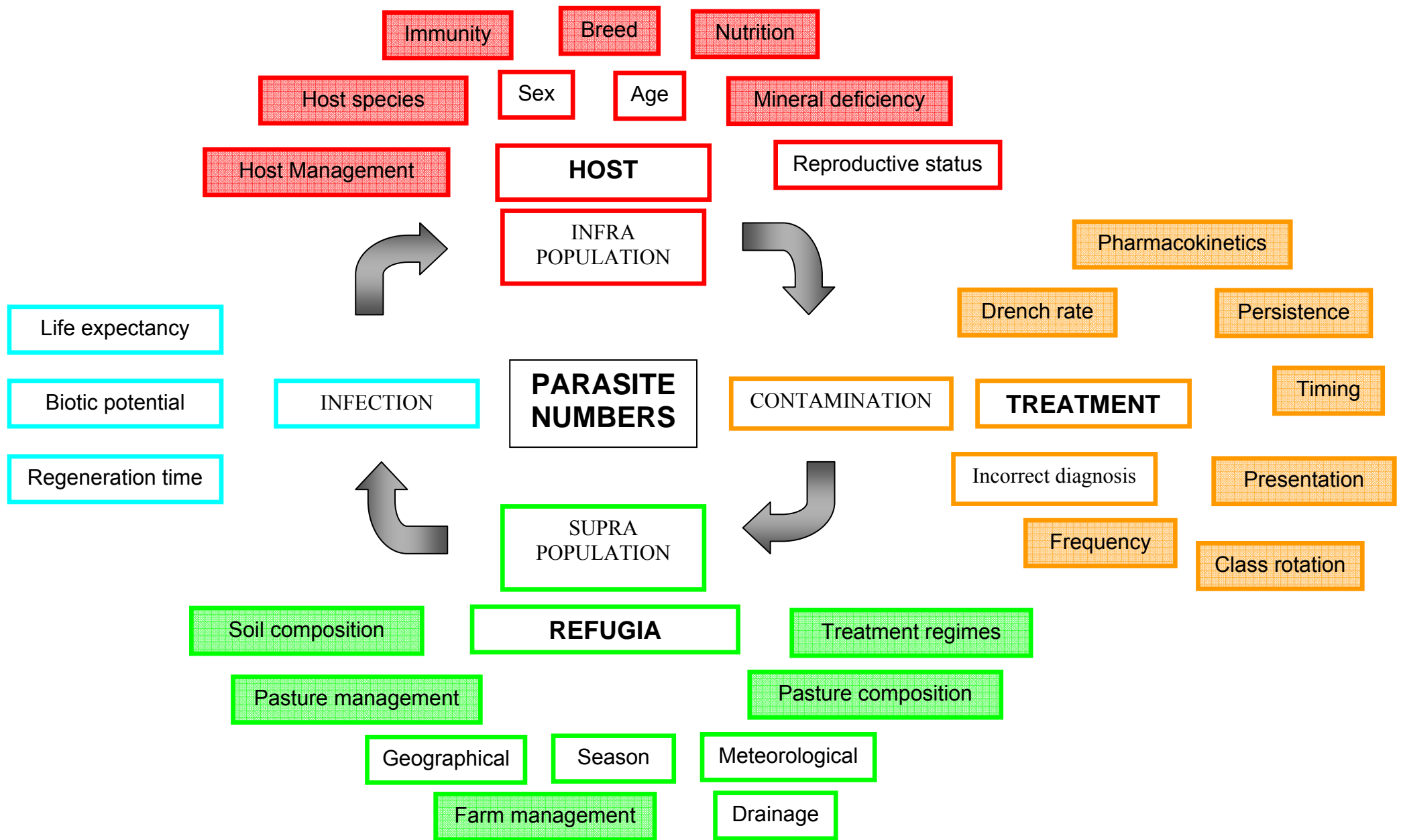


Figure 1.2 Diagrammatic representations of factors affecting nematode populations, shaded boxes identify areas that have been targeted for intervention strategies or identified as factors associated with the selection for anthelmintic resistance.

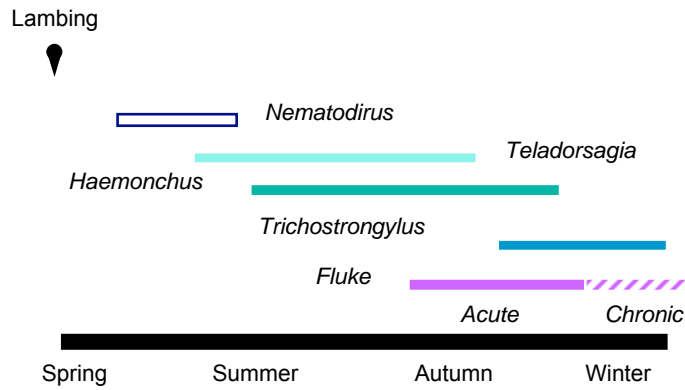


Figure 1.3 Theoretical progression of parasite species affecting sheep throughout the seasons adapted from data from Boag and Thomas, 1977

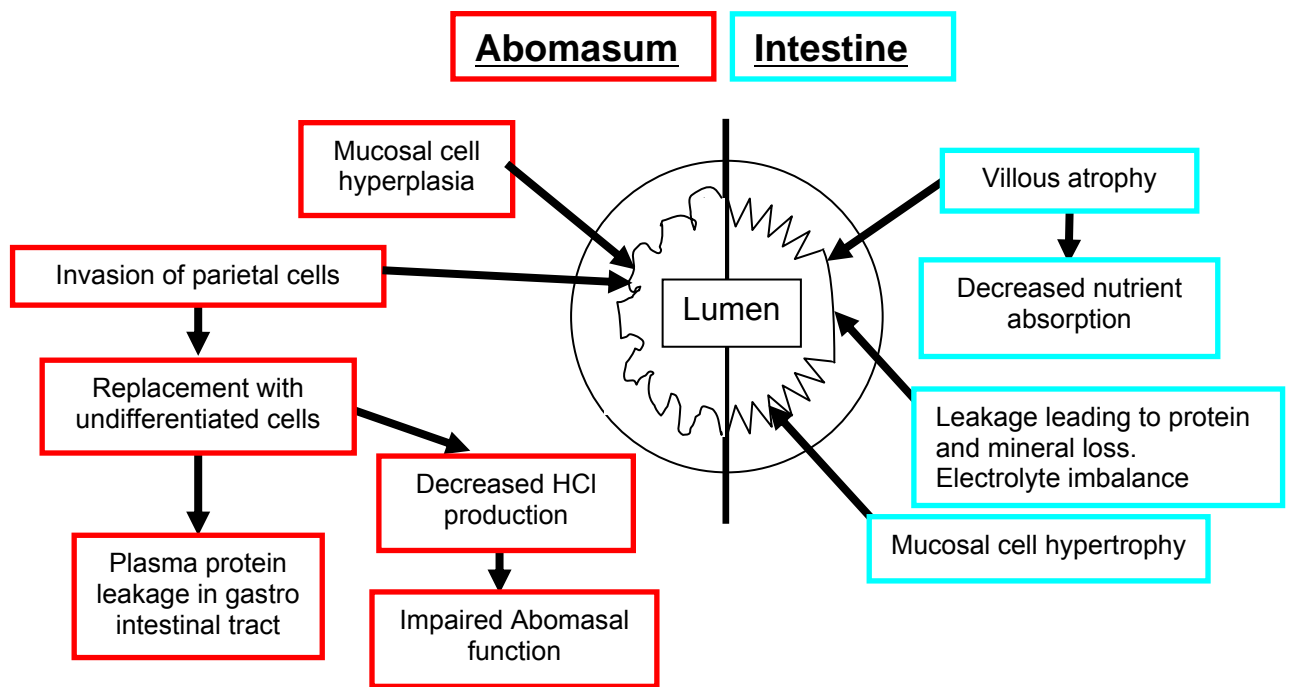
A further compounding factor is that different parasite species have different epidemiological patterns and preferred developmental conditions resulting in changes in distribution patterns throughout the year (Figure 1.3). For example *Nematodirus battus* eggs passed in the spring of one year slowly develop to infective larvae and overwinter to hatch *en masse* over a very short period of time the following spring causing disease in predominantly young lambs (Crofton and Thomas, 1951), whereas *T. colubriformis* requires a minimum threshold temperature of 10°C in order to develop to an infective larva (Leathwick et al., 1999 cited in Vlassoff et al., 2001). Studies have illustrated a succession of nematode species in grazing lambs under UK conditions (Crofton, 1955 and 1957; Boag and Thomas, 1977).

1.3 Pathogenesis

Gastro-intestinal parasites are responsible for a range of clinical signs in hosts, particularly young or nutritionally stressed animals or animals carrying concurrent infections. The degree of pathogenesis observed in an infected animal depends on the infective species and the predilection site (Figure 1.4). *T. circumcincta* invade the gastric gland in the abomasa, forming noticeable nodular lesions; during development the parasite causes damage to the hydrochloric producing parietal cells which are replaced with undifferentiated non-acid producing cells (Armour et al.,

1966). The increase in pH, plasma protein loss through the damaged abomasum and rapid cell division are thought to be partially responsible for the loss of appetite and weight loss observed in clinical infection. With *H. contortus* infections the pathology observed is due to the haematophagic behaviour of the parasites. Estimates suggest that each worm can remove 0.05 ml of blood per day, (Clark et al., 1962) leading to a fall in packed cell volume and anaemia which, if left untreated, can result in death. Under severe nutritional stress, chronic haemonchosis can become an issue where several hundred worms and the associated blood loss cause inappetence and weight loss rather than anaemia (Barger and Cox, 1984).

A hypothesis that has been explored in New Zealand (NZ) is that it is the host's own immunological response to *T. colubriformis* and *T. circumcincta* infections that is predominantly responsible for the pathological effects seen in animals, rather than mechanical damage by the parasite *per se* (Greer et al., 2005a and 2005b). In trials where animals were either infected with *T. colubriformis* and *T. circumcincta* (INF), INF and immunosuppressed with methylprednisolone (ISINF) or left parasite naïve. The productivity of the uninfected control and ISINF groups were significantly better than the INF group, though the ISINF was carrying a larger worm population. A theory to account for this effect is that sheep evolved with *T. colubriformis* and *T. circumcincta* as commensals rather than parasites and that immune responses directed against them have arisen due to the need to respond to other more pathogenic species such as *H. contortus* (Love, 2005).



Net Effect: Protein diverted from productive tissues such as skeleton, muscle & wool to intestinal, abomasal and plasma proteins

Figure 1.4 Schematic of potential causes of pathology in nematode infected ruminants

1.4 Immunology

The development of immunity tends to follow a biphasic pattern, Figure 1.5, firstly an acquisition phase, secondly an expression phase. In ewes around parturition or animals carrying concurrent infections there may be a relaxation in immunity, sometimes termed as the peri-parturient relaxation of immunity (PPRI) in ewes. The sequence of events that leads to the expression of immunity in continuously infected animals tends to be, firstly a rejection of incoming larvae (around 4 weeks with *Trichostrongylus*), secondly a depression of fecundity (around 10-12 weeks) and finally expulsion of adult worms (after 16 – 20 weeks; Seaton et al 1989a or 1989b; reviewed by McClure; 2000). The development to the expression of immunity, as detailed above, is dependent on host and parasite species and can occur in as little as seven weeks of continuous infection with *H. contortus* (Barger et al., 1985) or *T. colubriformis* (Dobson et al., 1990).

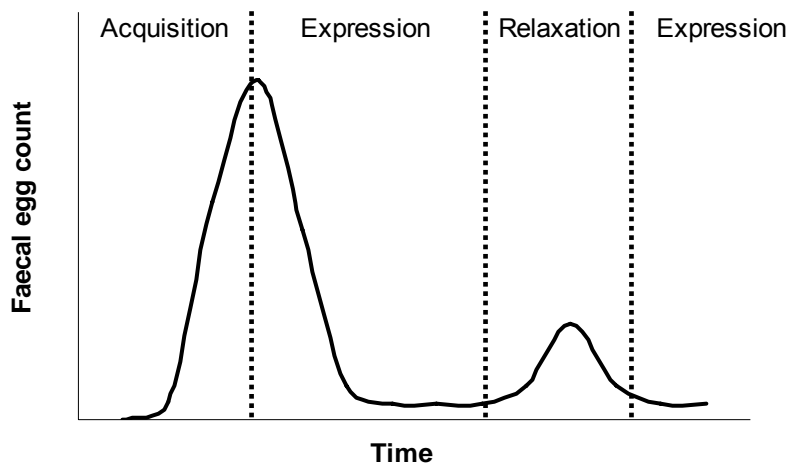


Figure 1.5 Typical pattern of immunity development in female sheep.

The mechanisms associated with the exclusion and expulsion of nematodes are poorly understood, but involve both innate and adaptive immune responses.

Innate immunity is the first line of defence and is mediated by cells and mechanisms that are not antigen-specific, do not confer any long lasting protection and may account for some of the host differences observed in primary susceptibility to infections. Nematode infections lead to increased goblet cell numbers and mucus production (McClure, 2000) as well as changing the composition and viscosity of the mucus produced (Douch et al., 1983; Jones et al., 1990; Kimambo and MacRae, 1988; Meeusens et al., 2005) which are thought to help entrap and prevent larvae from establishing. Host resistance has been associated with an increased leukotriene levels in intestinal contents (Jones and Emery, 1991) and mucus (Gray et al., 1992). The secretion of galactins (Gal-14; Dunphy et al., 2002 and Gal-15; Gray et al., 2004) and other inhibitory molecules (Meeusen et al., 2005; Balic et al., 2006) into the mucus have also been correlated with nematode killing and rejection. Physical mechanisms such as increased peristalsis (McClure, 2000), epithelial sloughing (McClure et al., 1992) and fluid and electrolyte movement into the lumen (Miller, 1996) have been associated with a hastening of parasites along the gastro-intestinal tract and reduced establishment.

Adaptive immunity is a highly specific system that is active and allows the host to mount a stronger more effective response with repeated exposure to a particular pathogen. Nematode rejection is the result of the culmination of several events, antigen recognition, induction of an appropriate immune response and activation of effector pathways and cells (Meeusen et al., 2005). The development of immunity is believed to be dose dependent (Dineen, 1963; Smith et al., 1984) and reliant on antigenic stimulation. The steps leading to the development of an effective adaptive immune response are poorly defined and the responses to infection that have been observed in the past have led researchers to wonder whether they are causal or casual (McClure, 2000). Observations of infections with gastro-intestinal nematodes have shown increased local cellular activity (mucosal mast cells, globular leucocytes, eosinophils; Huntley et al., 1987 and 1995) and systemic humoral responses (specific antibody production, IgA; Smith et al., 1984; IgE; Huntley et al., 1998, IgG; McClure et al., 1992) and are believed to be important in worm expulsion and exclusion. The generation of cytokines such as IL-4, IL-5 and IL-13 have also been correlated with nematode expulsion. The response in “older” immune animals can be extremely rapid with larvae being expelled between four (Jackson et al., 1988) and 24 hours (McClure et al., 1992) post challenge. The interactions between the various components of the innate and acquired immune systems are extremely complex and have been extensively reviewed for gastrointestinal nematode infections of sheep (McClure 2000, Schallig, 2000; Miller and Horohov, 2006) and cattle (Claerebout and Vercruysse, 2000).

1.5 The economic importance of gastro-intestinal helminth of ruminants

Livestock play an important role in the generation of stability and wealth of many communities around the developing and developed world. In the year 2007 there were in excess of 1.4 billion cattle, 1.1 billion sheep and 0.85 billion goats worldwide, the 27 European Union countries had 6.5%, 9.6% and 1.6% of these numbers respectively, (Food and Agriculture Organization of The United Nations, <http://faostat.fao.org/site/573/DesktopDefault.aspx?PageID=573#anchor> last accessed 10SEP08). Livestock are used not only a protein source but also provide a workforce,

raw materials and a source of income. In the examination of the cost implications of parasitism, attention needs to be focussed on the costs incurred in the treatment, such as labour, provision of facilities, time to plan and prepare for treatment as well as the pathology and production losses. Figure 1.6 shows the numbers of diagnosable samples submitted to the Scottish Veterinary Investigation centres for analysis, further details can also be obtained from Veterinary Laboratory Agency (VLA), (http://www.defra.gov.uk/vla/reports/docs/rep_vida_sheep99_06.pdf last accessed 10SEP08). The graph shows a steady increase in the numbers of diagnosable cases of PGE in sheep. Possible reasons for these increases are a) easier access to diagnostic services, b) Greater awareness by both producers and veterinarians in regards to the problems that gastro-intestinal parasites cause and therefore more samples being submitted for diagnosis, c) Changes in climatic conditions leading to prolonged parasite and disease seasons, d) Failure of treatments due to an increasing prevalence of anthelmintic resistance leading to more diseased animals.

In the year 2007 the global animal health market was worth \$17.9 billion, of which \$5.7 billion was spent in Western Europe. Products for ruminants form a large component of the overall worldwide expenditure (31%); \$4.8 billion was spent on cattle and \$830 million on sheep (IFAH annual report, 2007 – http://www.ifahsec.org/media_room/IFAH_annual_report_2007_final.pdf last accessed 10SEP08). The market includes expenditure on medicinal feed additives, biologicals such as vaccines, anti-infectives, parasiticides and other pharmaceuticals. Parasiticides accounted for \$5.2 billion of the global expenditure. In Australia the cost implication of parasitic disease was estimated to be around A\$337 million and is thought to account for around 90% of all production losses in sheep (Collins, 1992 cited by Hennessy, 1997).

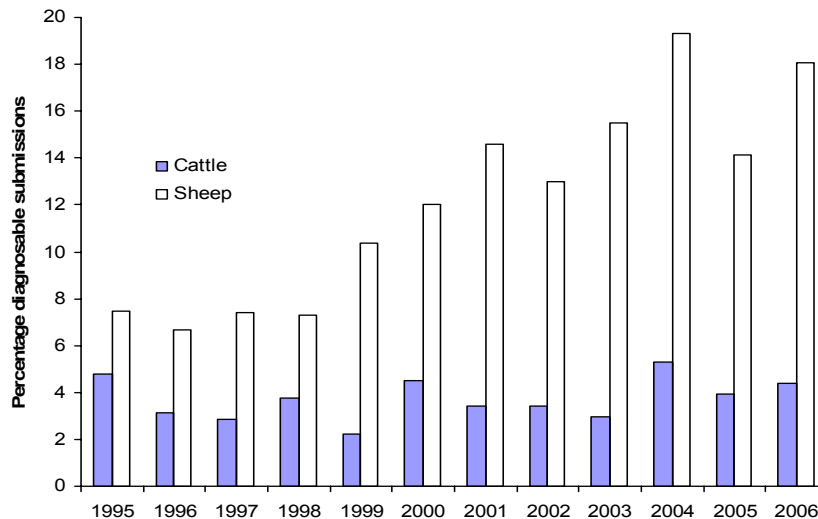


Figure 1.6 Parasitic gastro-enteritis cases in sheep and cattle expressed as a percentage of diagnosable samples received by Scottish veterinary investigation centres.

1.6 Chemical control strategies

The significant financial losses incurred by farmers due to parasitism of livestock, has led to research into chemical and non-chemical based methods of control.

Treatment of gastro-intestinal parasites has in past centuries relied on the use of medicines comprising metals, such as tin, pewter or iron filings or plant extracts that were poisonous to the worms, mechanically irritated the parasites from their predilection site or removed the mucous linings of the bowel making it difficult for the parasites to develop and maintain stasis (McKellar and Jackson, 2004). In the late 19th century parasitic treatments became chemically based with the use of compounds such as arsenic, copper sulphate, nicotine sulphate and carbon tetrachloride but these tended to be either ineffective or had a highly toxic effect on the host as well as the parasite (McKellar and Jackson, 2004). It was not until the introduction of phenothiazine in the late 1930s that treatments become more sophisticated with larger safety margins and few side effects. By the 1960s the first “safe” commonly available broad-spectrum drugs were available for the treatment of helminths of ruminants, Figure 1.7. These are detailed further in the following

sections. Although many treatments are registered for monogastrics and large ruminants, namely cattle, the following sections will focus on anthelmintics for use in small ruminants.

1.7 Broad-spectrum anthelmintics

Three discrete classes of broad-spectrum anthelmintic are available for the treatment of ruminants. Class I anthelmintics include both the benzimidazoles (BZ) and the pro-benzimidazoles (PRO-BZ), class II include imidazothiazoles and tetrahydropyrimidines and class III include avermectins (AVM) and milbemycins (MIL),

Table 1-1. Each of the classes has a different chemical structure (Figure 1.7) and differing modes of action, sections 1.7.1 to 1.7.3

Table 1-1 Broad spectrum anthelmintic classes available for use in the UK sheep market (NOAH compendium, 2008)

Benzimidazole/ pro-benzimidazoles	Imidazothiazoles/ tetrahydropyrimidines	Macrocyclic lactones
Albendazole	Levamisole	Doramectin
Febantel	Morantel citrate	Ivermectin
Fenbendazole		Moxidectin
Mebendazole		
Netobamin		
Oxfendazole		
Ricobendazole		
Thiophanate		

1.7.1 Benzimidazoles/pro-benzimidazoles (Class I)

The BZ were the first broad-spectrum anthelmintics to be brought onto the market. Thiabendazole (TBZ) was introduced in the early 1960s and brought about a change in the treatment of helminths in ruminants, possessing both a wide spectrum of activity as well as a high therapeutic index. The actions of all the BZ/PRO-BZ are similar, but the mechanisms for their actions are different. For example, TBZ is an active compound which is metabolised to an inactive compound, netobimin is an inactive compound that is broken down into active moieties (albendazole, ABZ) and

finally ABZ when administered can be further metabolised to become an active metabolite, albendazole oxide (McKellar and Scott, 1990).

The BZ act by binding to the nematode tubulin, inhibiting the formation of microtubules within the intestinal cells. This binding interferes with nutrient absorption by the parasite and leads to starvation (Barragry, 1984a and 1984b). Binding to the cytoplasmic microtubules, which are involved in the transportation of secretory granules and the secretion of enzymes into the cell cytoplasm, can lead to prolonged storage of the secretory material resulting in disintegration of the cells (McKellar and Scott, 1990). The BZs have also been shown to disrupt metabolic processes. Interference of the enzyme fumarate reductase by TBZ inhibits energy generation thus leading to starvation of the parasite (Prichard, 1973). The BZ are active against both worms and eggs particularly against adult and immature nematodes, cestodes (*Monezia* species) as well as trematodes (*Fasciola* species) although the spectrum of individual drugs varies.

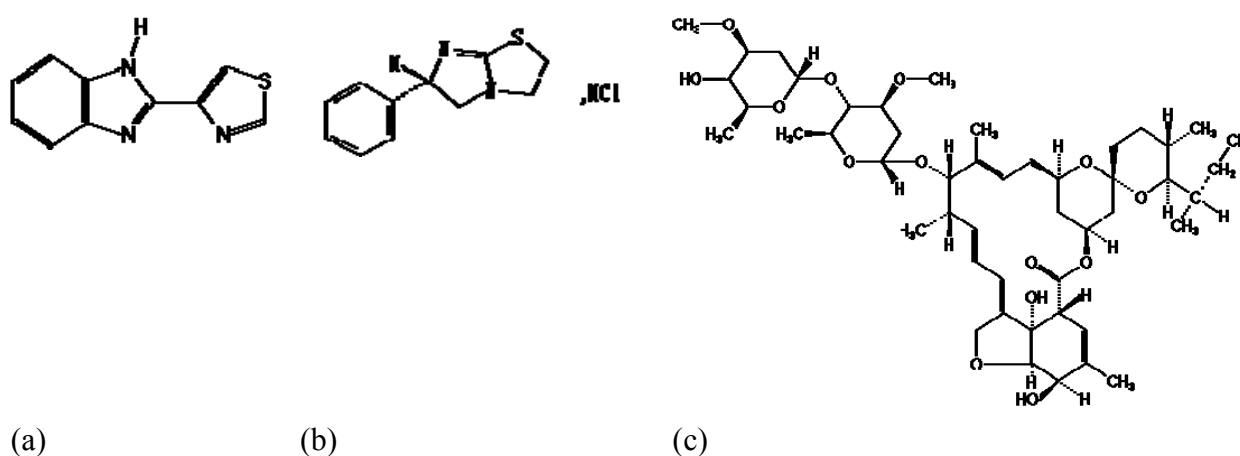


Figure 1.7 Basic chemical structure of compounds from the three broad spectrum anthelmintic families (a) thiabendazole, (b) levamisole hydrochloride and (c) ivermectin B_{1a}

1.7.2 Imidazothiazoles/tetrahydropyrimidines (Class II)

The imidazothiazoles namely LEV and tetramisole were introduced onto the market for use in ruminants in 1968 (McKellar and Jackson, 2004) and have a safety index of 6, i.e. adverse effects are induced at 6 times the manufacturers' recommended dose rate (MRDR) in the most susceptible animals (Anon, 2005). Tetramisole is a racemic mixture of two optical isomers, laevo (L) and dextro (D); the L-isomer is called levamisole and is the active component. Most oral levamisole preparations use hydrochloride salt, though injectable solutions use phosphate salt. The tetrahydropyrimidines include the compounds pyrantel and morantel citrate/tartrate (MOR). Both LEV and MOR act by stimulating cholinergic ganglia at the nicotinic neuromuscular junctions causing spastic paralysis of the nematodes. The anthelmintics open and then block the acetylcholine receptor-mediated cation channels (Robertson and Martin, 1993). The action of the drugs is wholly against the roundworm nervous system and shows no ovicidal activity.

1.7.3 Macrocyclic lactones (Class III)

The macrocyclic lactones (ML, avermectins/milbemycins) are chemical derivatives produced through fermentation by the actinomycetes of the soil micro-organism *Streptomyces avermitilis*. The milbemycins and avermectins were first identified in 1973 and 1975 respectively (Burg et al., 1979; Takiguchi et al., 1980). Ivermectin, doramectin (DOR) and moxidectin (MOX) are all members of the macrocyclic lactones that are commercially available for use in sheep in the UK. The ML have a high safety factor, around 20 times the MRDR, even in collies which are known to be sensitive to IVM (Shoop et al., 1995), with a low dose rate (0.2 mg/kg) and a high potency against endo- and ecto-parasites. The level of potency and length of persistence of activity differ between compounds, i.e. IVM<DOR<MOX. The difference in persistence is believed to relate to the lipophilicity and excretion rates of each the anthelmintics. All ML have a high affinity for lipids within the body with depletion half lives of unchanged drug ranging from 4.3 to 15 days for IVM and MOX respectively in cattle (McKellar and Benchaoui, 1996). The ML bind to the glutamate-gated chloride channel receptors (Glu-Cl) in nematodes causing them to

open, and once open, the channel allows an influx of chloride ions that cause flaccid paralysis (Cully et al., 1996; Martin, 1997). The MLs are also known to enhance the effect of the neurotransmitter gamma amino butyric acid (GABA) in the muscles of parasitic nematodes (Brownlee et al., 1997; Feng et al., 2002).

1.8 *Narrow-spectrum anthelmintics*

1.8.1 Salicylanalides and substituted phenols

The Salicylanalides (closantel, CLOS or oxyclozanide) and substituted phenols (nitroxynil) have limited use in the treatment of nematodes when administered as individual compounds and are generally used for the treatment of haematophagus parasites such as *H. contortus* and *Fasciola* (NOAH compendium, 2008). The anthelmintics have a very strong affinity for plasma protein (Mohamed Ali and Bogan, 1987) which may partially explain their activity against blood feeding parasites. The anthelmintic molecules, also known as proton ionophores, possess a detachable proton which may be able to shuttle across membranes (particularly mitochondrial and tegument membranes). The mode of action of these compounds is thought to involve disruptions in the normal biochemical and physiological processes of these membranes (Martin, 1997).

In order to increase their spectrum of activity the salicylanalides are often combined with a broad spectrum compound such as mebendazole (Mebadown Super[®]) or levamisole (Nilzan Super[®]).

1.9 *New potential products*

No new novel broad spectrum anthelmintic compound has been brought to the market in over thirty years, but announcements by two of the large pharmaceutical companies would suggest that this may change in the near future.

1.9.1 Cyclooctadepsipeptides

The cyclooctadepsipeptides, PF1022A and emodepside, were discovered in 1990 (Sasaki et al., 1992) and are thought to act on the nematode pre-synaptically by stimulating the release of inhibitory neuropeptides which cause muscle relaxation and inhibition of acetylcholine mediated muscle contraction (Willson et al., 2001 and 2003). The compounds, first registered for use in cats, have been shown to be highly effective at reducing both faecal egg counts and worm burdens of BZ, LEV and IVM resistant isolates of *H. contortus* in sheep when administered orally subcutaneously or intravenously, as well as *Cooperia oncophora* in cattle when administered orally (von Samson Himmelstjerna et al., 2000 and 2005).

1.9.2 Amino-Acetonitrile Derivatives (AAD)

The Amino-Acetonitrile Derivatives (AAD) were patented in 2006 and have been shown to have a broad spectrum of activity in both sheep and cattle with low toxicity. The compounds, AAD 450 and AAD 1470, are believed to act upon the nicotinic acetylcholine receptors and have been shown to be active against a wide range of economically important anthelmintic resistant and sensitive nematodes and trematodes at 20mg per kg (United States Patent application [7091371], 2006; Kaminsky et al., 2008; Prichard and Geary, 2008).

1.9.3 P-amino-phenethyl-m-trifluoromethylphenyl piperazine (PAPP)

The serotonergic agonist PAPP has been shown to have anti-parasitic activity in a gerbil model with >98% efficacy in the treatment of *T. circumcincta* and *H. contortus* infection at doses of 50-100 mg/kg but low efficacy against the intestinal parasite *T. colubriformis* (83%; White et al., 2007).

1.9.4 Paraherquamide

Paraherquamide is the oldest of the “new” compounds, and was originally isolated in 1981 (Yamazaki, 1981). The compound is a metabolite of *Penicillium parherquei* and has been shown to exhibit good anthelmintic activity at dose rates of >0.5 mg/kg against six common ovine parasites (Shoop et al., 1990).

1.10 Ill thrift

Before treatment failure or AR can be identified in a flock it is essential to ascertain the origin of the ill thrift/disease in the animals. Potential causes of ill thrift in small ruminants are wide ranging, covering areas such as infectious agents (viral, bacterial and non nematode parasites), physical damage to the animals and metabolic disorders (Table 1-2).

Table 1-2 Potential causes of ill thrift in sheep

Nutritional	Infectious agent	Miscellaneous
Nutrition (insufficient or poor quality diet)	Bacterial disease (e.g. pasteurella, Johne's disease)	Damage to mouth or throat, (faulty teeth, drench gun injury)
Cobalt/vitamin B12 deficiency	Viral disease (e.g. Border disease)	Tumours
Selenium deficiency	Parasitic disease (<i>Cryptosporidium</i> , <i>Fasciola</i>)	Insufficient trough space for feeding
Copper deficiency	Fungal infections in rumen	Bullying
Plant poisoning	Chronic infection (e.g. navel ill, pneumonia, foot rot)	

1.11 Treatment failure

As well as ensuring the correct diagnosis of gastro-intestinal parasitism, there are also other complex and compounding factors that influence; the degree of infection observed in animals (Figure 1.8), the effectiveness of administered treatments (Figure 1.2) and the rate of development of AR. These three areas are not mutually exclusive and many of the interactions between them play an important role in determining how effective a treatment will be at controlling GINs. The important factors that have been examined as parts of intervention studies (shaded boxes in Figure 1.2) will be discussed further in chapter 1.20.

1.11.1 Chemical

The quality of generic compounds has been shown to vary drastically between products and even between batches. A report by Monteiro et al., (1998) revealed that

the quantities of actual active ingredient in nine separate LEV based products ranged between 0 and 118% of the amount specified on the label. The variability between batches of the same product was also shown to be extremely high, with between 0 and 85% of the expected quantities being detected. Obviously with 0% of the active ingredient, the product should be considered fake but with the levels of variability observed, confidence in products would be low and effectiveness would be inconsistent and unpredictable at best.

Formulation and route of administration has been shown to influence the efficacy of MOX against an IVM resistant *T. colubriformis* population (Gopal et al., 2001) with faecal egg count reduction efficacies of 62%, 100% and 0% for oral IVM, oral MOX and injectable MOX respectively. The difference in efficacy being attributed to the peak plasma concentration of orally administered MOX being 3.4 times greater than that achieved with injectable MOX formulation (Alvinerie et al., 1998).

Interactions between compounds administered in combination have been observed in a range of hosts. Such interaction may be synergistic/additive (Anderson et al., 1988; McKenna, 1990) or antagonistic (McKenna, 1990). Antagonist interactions may be due to target site competition and/or the preferential elimination of a compound from the host when administered in combination, as is the case with DOR when administered with IVM or MOX (Barber et al., 2003).

1.11.2 **Physiological**

Pharmacokinetics and pharmacodynamics of anthelmintic treatments can also be affected by a range of different host and parasite factors, which can lead to overall efficacies lower than would be expected.

Breed differences have been shown to affect both susceptibility to infection and response to anthelmintic treatment. Sheep breeds such as Texels and Red Masai have been shown to be much more refractory to parasite infections than breeds such as Suffolk (Barger, 1989; Kloosterman et al., 1992; Good et al., 2006). A difference

in susceptibility to nematode infections also leads to a potential disparity in the numbers of treatments that a particular breed may need per annum.

In cattle, differences in the rate and degree of absorption and systemic availability of pour-on MOX have been observed between Aberdeen Angus and Holstein cattle (Sallovitz et al., 2002). The slower absorption and reduced plasma concentration seen in Aberdeen Angus cattle were attributed to smaller fat reserves than the milk producing Holsteins and therefore less reserve for the lipophilic compound to bind too. Differences in skin composition or physiology may also have affected the absorption of the compound. In sheep, the story is slightly less clear cut with differences in treatment efficacies being observed between different young merino and Border Leicester x Merino lambs (Sangster et al., 1979), but not in older sheep of the same breeds (Sangster et al., 1980). Differences within breeds, both in susceptibility to infection (Barger 1989) and in response to anthelmintic treatment are as variable as between breeds. An example of this is from the trial detailed in chapter 4 where five lambs were artificially infected with the same number of a field derived isolate of *T. circumcincta*. The individual treatment efficacies based on worm burden data ranged from 65% to 95% with an average of 82%.

Host species differences in pharmacokinetics of BZ, LEV, IVM and CLOS, particularly between sheep and goats, have been well documented when anthelmintics have been administered at the recommended dose rates for sheep (Galtier et al., 1981; Gillham and Obendorf, 1985; Bogan et al., 1987; Sangster et al., 1991; Hennessy et al., 1993a and 1993b). In a field trial where sheep and goats were grazed together on naturally infected pastures, treatment efficacies against *T. circumcincta* and *T. colubriformis* were around 20% lower in goats than sheep with LEV (8mg/kg body weight, BW) and MOR (10mg/kg BW) respectively (McKenna and Watson, 1987). Sangster et al., (1991) observed that differences in OXF efficacies between parasitized sheep and goats were due to decreased absorption and metabolism of the compound in goats because of increased activation of the oesophageal groove and therefore increased rumen bypass.

Sex/gender differences have also been shown to affect plasma concentration and half-life of IVM. Elimination was faster in male sheep compared to females (Ndong et al., 2007). Again the observed differences were thought to correlate to level of storage of the compound in fat deposits.

Quantity (Warner, 1981; Ali and Hennessy, 1993; 1995a; 1995b and 1996) and quality (Warner, 1981; Ali and Chick, 1992) of feed has been shown to influence the residence time of the digesta and associated BZ in the gastro-intestinal tract leading to a decrease in plasma concentrations. Further work showed that the pharmacokinetics of FBZ and IVM is significantly lower in sheep (Taylor et al., 1992), cattle and buffalo (Sanyal et al., 1995) at pasture or fed green herbage compared with animals fed on a hay and/or concentrate diet. Both the grazing sheep and cattle were shown to have increased digesta flow, lower anthelmintic absorption and possible changes in the rumen flora and physiology.

1.11.3 Parasitological

The absorption, storage, presentation and efficacy of anthelmintics have been shown to be much reduced in heavily parasitized animals compared with their parasite naïve counterparts. Parasites such as *T. circumcincta* and *Trichostrongylus* species can directly and indirectly change the physiology of the host, leading to changes in mucosal permeability, increased gut motility and increases in abomasal and intestinal pH. The mechanical damage to the hydrochloric acid-producing parietal cell leads to an increase in abomasal pH, which has been shown to affect the absorption and pharmacokinetics of orally administered benzimidazoles (Marriner et al., 1984).

In animals where fat reserves are depleted, either through parasitism or pregnancy and lactation, compounds such as the MLs and sulphonamides have been shown to be less effective (Van Gogh et al., 1990; Lespine et al., 2004; Perez et al., 2006 and 2007). The differences in the case of the MLs have been attributed to faster absorption rates and reductions in elimination half-life in parasitised animals which tend to have lower level of fat deposition and therefore fewer reserves for the MLs to bind to and increased gut motility compared to their non parasitised counterparts.

Secondly, differences are believed to be due to increased biotransformation (enzymatic breakdown) of the drug within the plasma of parasitised animals. In a separate experiment the loss of moxidectin's persistent efficacy against *T. circumcincta* infections was noted in animals with lower fat reserves (Rolf et al., 1995, cited in Perez et al., 2007).

Treatments with compounds that require secondary processing before they become pharmacologically active, such as the pro-benzimidazoles which require sulphonation by liver oxidases, are susceptible to infections that damage the site of enzyme production. Galtier et al., (1991) demonstrated a significant reduction in ABZ activity in lambs eight weeks after they had been infected with 150 *F. hepatica* metacercariae.

For all anthelmintic treatments efficacy can be affected by the parasite population it is targeting, irrespective of resistance status. Parasite sensitivity to anthelmintics can be extremely variable. In sheep treated with ABZ the dose limiting species (DLS), i.e. the species that requires the greatest concentration of drug in order to effectively control it, is *T. circumcincta* whereas in cattle the DLS for MOX and DOR is *Nematodirus helvetianus*.

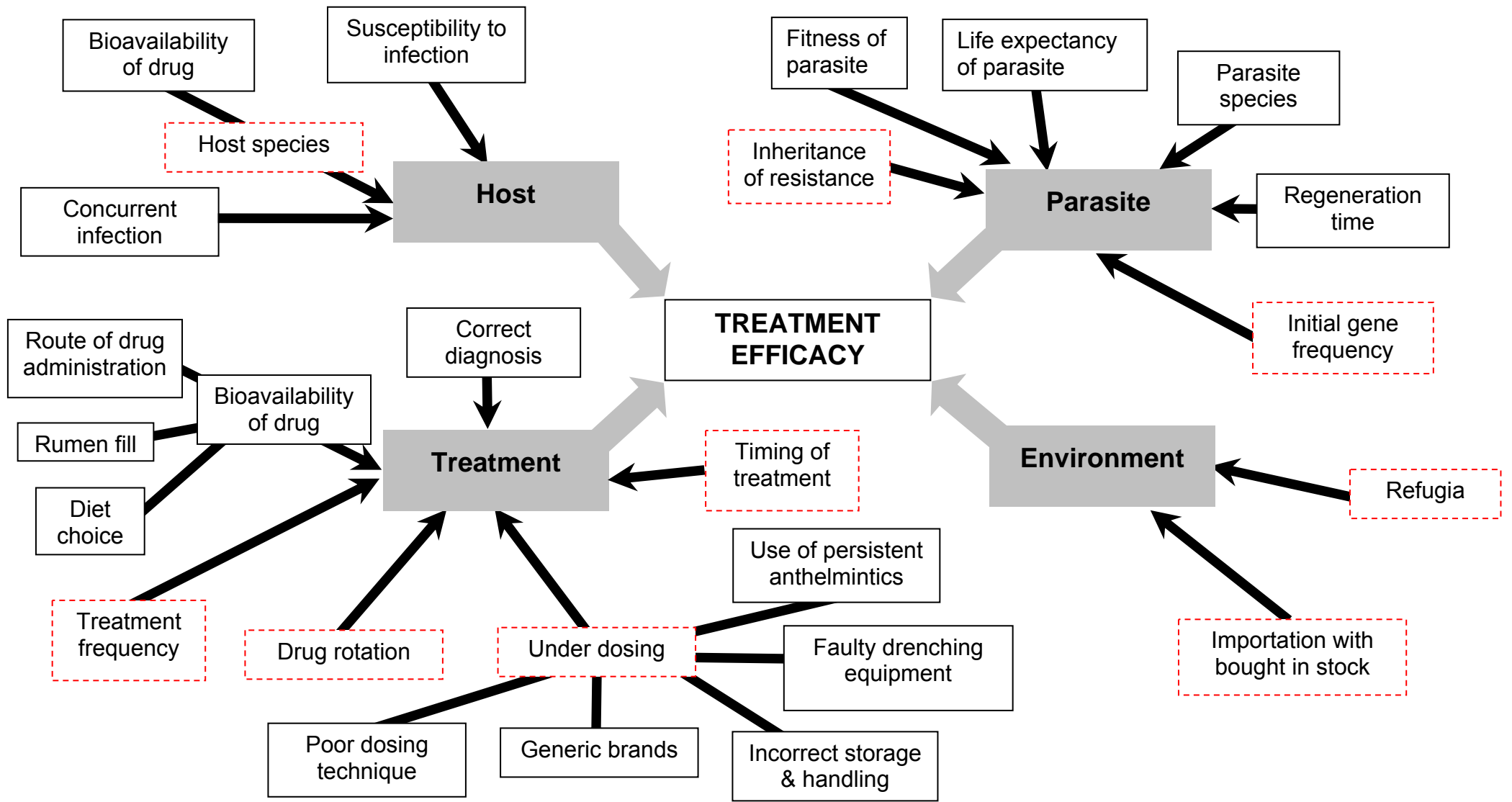


Figure 1.8 Diagrammatic representations of factors affecting drug efficacy and potentially the development of anthelmintic resistance in ruminants (red dashed boxes).

1.12 Anthelmintic resistance

Anthelmintic resistance (AR) has been described as “a heritable reduction in the sensitivity of a parasite population to the action of a drug” (Conder and Campbell, 1995). Anthelmintic resistance affects the nematodes of many host species, though primarily small ruminants, and all three classes of commercially available broad-spectrum anthelmintics (Chapter 1.7). Reports of resistance to at least one anthelmintic class and one parasite genus, Table 1-3, have been made in no less than 38 of the 172 sheep producing countries of the world (<http://faostat.fao.org> last accessed 10SEP08).

Table 1-3 Reported cases of anthelmintic resistance in small ruminants Worldwide, selected references.

Country	BZ	LEV	ML	Reference	Country	BZ	LEV	ML	Reference
Algeria	+	-	+	Bentousi et al., 2007	Mozambique	+	+	-	Atanasio et al., 2002
Argentina	+	+	+	Eddi et al., 1996	Netherlands	+	+	+	Borgsteede et al 1997
Australia	+	+	+	Overend et al., 1994	New Zealand	+	+	+	Waghorn et al., 2006a
Belgium	+	-	-	Vercruyse et al., 1989	Paraguay	+	+	+	Maciel et al., 1996
Brazil	+	+	+	Echervaria et al., 1996	Philippines	+	-	-	Ancheta et al., 2004
Cameroon	+	-	-	Ndamukong & Sewell, 1992	Slovakia	+	-	+	Cernanska et al., 2006
Denmark	+	+	+	Maingi et al., 1996	South Africa	+	+	+	Van Wyk et al., 1997
Ethiopia	+	+	+	Sissay et al., 2006	Spain	+	+	+	Alvarez-Sanchez et al., 2006
France	+	+	-	Chartier et al., 1998	Sweden	+	-	-	Rudby-Martin and Nilson, 1991
Germany	+	-	-	Bauer et al., 1987	Switzerland	+	-	+	Artho et al., 2007
Greece	+	-	-	Papadopoulos et al., 2001	Sri Lanka	+	-	-	Van Aken et al., 1989
India	+	+	-	Gill, 1996	Tanzania	+	-	-	Bjorn et al., 1990
Ireland	+	-	-	O'Brien et al., 1994	Thailand	+	-	-	Kochpakadee et al., 1995
Italy	+	+	+	Traversa et al., 2007	Turkey	-	+	+	Kose et al., 2007
Kenya	+	+	+	Waruiru et al., 1998	Uruguay	+	+	+	Nari et al., 1996
Malaysia	+	+	+	Chandrawathani et al., 2004	UK	+	+	+	Sargison et al., 2001
Martinique	+	-	-	Gruner et al., 1986	USA	+	+	+	Terrill et al., 2001
Mexico	+	-	-	Torres Acosta et al., 2003	Zambia	+	-	+	Gabriel et al., 2001
Morocco	+	-	-	Berrag, 2007	Zimbabwe	+	+	-	Boersema and Pandey, 1997

Initial reports of AR occurred within several years of the initial launch of all three broad spectrum anthelmintics, though this generally occurred faster in Southern hemisphere countries compared to the temperate Northern hemisphere countries (Figure 1.9). The environmental conditions in the southern hemisphere are either conducive for year round parasite development and therefore year round treatment

(section 1.20.2) or drought conditions commonly lead to increased selection pressure on parasite populations due to reduced parasite refugia (section 1.20.5).

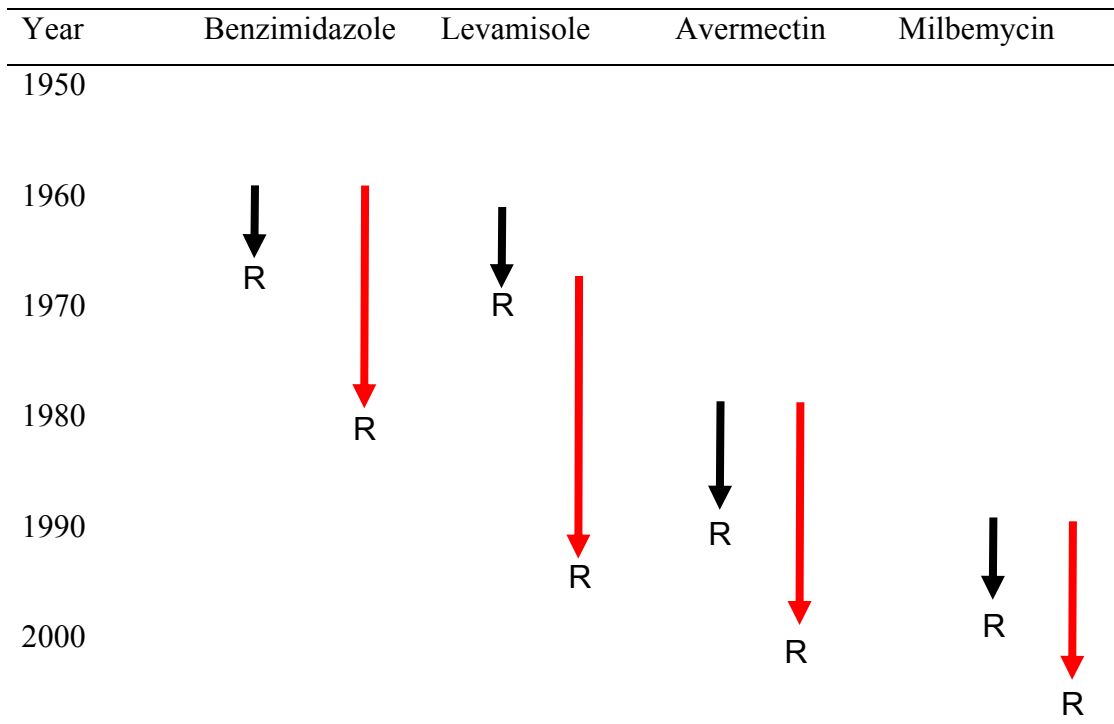


Figure 1.9 Year of commercial release of broad spectrum anthelmintics (black arrows, southern hemisphere; red arrows, United Kingdom) and the first report of resistance (R) in the target organisms (adapted from Waller 2006)

Resistance to multiple classes of compounds has been identified in all of the economically important nematodes of sheep and goats, in particular *H. contortus*, *T. circumcincta* and *Trichostrongylus colubriformis* (in depth reviews of AR nematodes can be found in Conder and Campbell et al., 1995; Jackson and Coop, 2000; Jabbar et al., 2006). Initial reports are generally restricted to single parasite species but incidents of multigenic resistance are commonplace (Chapter 4).

Anthelmintic resistance is not solely an issue for small ruminant farmers, resistance has been observed in horses (Craven et al., 1999; Kaplan et al., 2004), pigs (Roepstorff et al., 1987) and cattle (Borgsteede, 1991; Coles et al., 1998; Mason and McKay, 2006). The likelihood of the detection of anthelmintic resistant parasites within these hosts is generally ranked goats > sheep > horses > cattle (Mejia et al., 2003), though economically, treatment failures and production losses in cattle are

likely to have a significantly greater impact on global economies than any of the other hosts. Very few surveys have been conducted to examine the prevalence of AR in cattle. The general consensus was that cattle generally were extensively grazed and drenched infrequently due to the low pathogenicity of the predominant bovine parasite species, *Cooperia*. The expansion in the numbers of intensively farmed enterprises composed of monocultures of young stock has led to anthelmintics being heavily used in the control of parasitism (Waghorn et al., 2006a). A survey of 62 New Zealand beef cattle farms found that 94% of the enterprises had treatment efficacies of $\leq 95\%$ against one class of anthelmintic but 74% had resistance against 2 classes. Within Europe no large scale survey has been conducted but the situation appears to be less frightening, with the prevalence of ML resistance thought to be lower (Demeler and Höglund personal communications), but these results show that complacency can and has resulted in AR progressing to almost catastrophic levels without being realised.

1.13 Prevalence

The reports listed in Table 1-3 chart the progression of anthelmintic resistance, but do not provide a true picture of the prevalence of resistant nematodes in small ruminants. Due to the enormity of the challenge, the true nature of the problem is thought to be highly under-estimated (Sangster, 1999). In some of the large Southern hemisphere sheep producing areas of the world, large scale surveys have been conducted; Australia (Besier and Love, 2003), New Zealand (Waghorn et al., 2006b), South Africa (van Wyk et al., 1999), South America (Waller et al., 1996). The results of the surveys listed above highlight the problems that may face the temperate Northern countries including the UK in the future where anthelmintic resistance is less developed (Table 1-4). Total failure, or severely reduced efficacy of treatments have been reported in some sheep producing areas in South Africa (van Wyk et al., 1997 and 1999), Northern and Southern America (Terrill et al., 2001; Waller et al., 1996) and the UK (Sargison et al., 2007) leading to some producers finding it economically unviable to continue farming.

Table 1-4 Prevalence of anthelmintic resistance in the UK sheep and goat flocks.

Region	Sheep (S) / Goat (G)	Test used*	Number farms examined	Prevalence (%)			Reference
				BZ	LEV	ML	
S.E. England	S	FECRT/EHT	52	13	0	-	Cawthorne & Cheong 1984
S. England	S	EHT/LDT	209	36	0	-	Hong et al., 1992
S.W. England	S	EHT	54	15	-	-	Hong et al., 1996
N.E. England	S	EHT	84	44	-	-	Hong et al., 1996
England/Wales	S	LDT	151	-	1	-	Hong et al., 1996
England/Wales	G	LDT	70	-	7	-	Hong et al., 1996
England/Wales	G	EHT	63	-	65	-	Hong et al., 1996
Wales	S	LDT	122	77	36	-	Mitchell et al., 2006
Scotland	S	EHT	37	24	-	-	Mitchell et al., 1991
Scotland	G	FECRT/EHT	6	83	0	0	Jackson et al., 1992
Scotland	S	EHT/ LDT	90	64	0	0	Bartley et al., 2004
Scotland	S	FECRT	17	-	-	35	Bartley et al., 2006

* EHT, egg hatch test; FECRT, faecal egg count reduction test; LDT, larval development test; BZ, benzimidazole; LEV, levamisole; ML, macrocyclic lactone.

1.14 *Detection*

Methodologies for the examination and detection of anthelmintic susceptibility/resistance from the field and in the laboratory are well detailed. Standardisation of general parasitological methods used in the detection of AR has been reported for sheep and goats (Table 1-5, Coles et al., 1992; Wood et al., 1995; Taylor et al., 2002; Coles et al., 2006; von Samson-Himmelstjerna, 2006), whilst work is being conducted to standardise methods in cattle (<http://www.parasol-project.org>, last accessed 10SEP08). General parasitological based techniques such as controlled efficacy tests (CET, drench and slaughter), faecal egg count reduction tests (FECRT), egg hatch test (EHT) and larval development test (LDT) have been used in the initial reporting and subsequent surveying of BZ and IVM resistance throughout the world in ruminants (small ruminant surveys, Table 1-4) and monogastrics alike, data not shown.

1.15 *In vivo* tests

In vivo tests are the cornerstone of AR detection in the field. Two tests are commonly cited in the literature, the faecal egg count reduction test (FECRT, section 1.15.1) and the controlled efficacy test (CET, section 1.15.2). The first technique is most commonly utilised by scientists, veterinarians and sheep advisors alike.

1.15.1 Faecal egg count reduction test

The test assesses the reduction in faecal egg counts of treated animals expressed as the percentage reduction compared to an untreated control group (Coles et al., 1992). Efficacies less than 95% are indicative of potential resistance. In brief the test involves taking rectal faecal samples from all animals to be examined and performing faecal egg counts (FEC) on them. Animals are allocated into groups, of at least 15 animals, for each treatment and an untreated control, ensuring minimal difference in group mean faecal egg count. Groups are randomly assigned an anthelmintic treatment or left untreated to act as controls. Each animal is dosed with its designated anthelmintic on the basis of bodyweight, ensuring that each animal receives its full dose. The group mean faecal egg counts are calculated for the pre-treatment samples. The optimal time for re-sampling of treated animals is 3–7 days, 8–10 days and 14–17 days post treatment for the LEV, BZ, and ML respectively, to avoid possible false positive/negative results (Coles et al., 2006). Levamisole has no label claim against juvenile worms and therefore re-sampling needs to be conducted before maturation of surviving immature stages occurs (Grimshaw et al., 1996) whereas suppression of egg production may occur for up to 10 and 14 days post treatment with BZ (Martin et al., 1985) and ML (Jackson 1993; Tyrell et al., 2002) respectively. The mean faecal egg counts of the groups are calculated for the post-treatment samples. The efficacy is estimated using one of a range of standard formulae, where C_1 and C_2 are the FEC of untreated control animals pre- and post treatment respectively and T_1 and T_2 are the FEC of animals pre- and post treatment respectively:

$(1 - [T_2/C_2]) \times 100$ using arithmetic means (Coles et al., 1992),

$(1 - [T_2/T_1][C_1/C_2]) \times 100$ using geometric means (Presidente, 1985),

$(1 - [T_2/T_1][C_1/C_2]) \times 100$ using arithmetic means (Dash et al., 1988),
 $(1 - [T_2/T_1]) \times 100$ using arithmetic means (McKenna, 1990; Kohapakdee, 1995),
 $(1 - [T_2/C_2]) \times 100$ using logarithm back-transformed estimated means within a generalised linear model (Mejia et al., 2003).

1.15.2 **Controlled efficacy test**

The test, also known as “drench and slaughter”, assesses treatment efficacy in infected animals compared to untreated control animals by estimation of total worm burdens at post mortem (Wood et al., 1995; Coles et al., 2006) and can be used with field infected or artificially challenged animals (see chapters 4, 5 and 6). The CET can be used to assess all stages of parasitic life cycle, from day one post-artificial infection to infections carried by naturally infected animals. To ensure that findings are both biologically and statistically relevant, groups should contain a minimum of five animals per drug compound plus a control group. Rectal faecal samples and faecal egg counts need to be conducted on all animals prior to treatment to allow allocation of animals into groups with minimal difference in group mean faecal egg count if examining adult egg laying populations. Treatment groups should be allocated randomly and animals need to be weighed and treated according to bodyweight. Notes should be made of any treatment errors or immediate adverse reaction to drug. After the allocated time the animals are euthanased and the gastrointestinal tract is removed and processed according to the appropriate protocol. Estimated total worm burden estimations allow calculation of the efficacy of the treatments using either arithmetic or geometric means in the formulae as detailed above (section 1.15.1). The test is generally only used in research laboratories to characterise new isolates of parasites or to assess novel treatments, due to the prohibitively expensive running costs.

1.16 ***In vitro* bioassays**

Most *in vitro* bioassays examine the response of a developmental stage(s) of the parasites to xenobiotic/anthelmintic treatment when administered in a dose-dependant fashion. From the dose response, it is possible to determine the concentration of anthelmintic required to inhibit a known percentage of the population from completing their normal development, e.g. ED₅₀ is the concentration

of compound required to inhibit 50% of eggs from developing and hatching within an egg hatch test. Table 1-5 lists some of the bioassays that have been examined.

Table 1-5 Bioassays used in the evaluation and/or characterisation of anthelmintic compounds against free living and parasitic nematode stages, selected references.

Test	Spectrum	Target	Authors
Egg hatch	BZ	Egg	Le Jambre and Whitlock 1976
	BZ	Egg	Hunt and Taylor 1989
EH (LP)	LEV	Egg	Dobson <i>et al</i> 1986
Larval development	BZ, ML	Egg to L ₃	Coles and Simpkin 1977
	BZ, LEV	Egg to L ₃	Taylor 1990
	BZ, LEV, ML	Egg to L ₃	Hubert and Kerboeuf 1992
	BZ, LEV	Egg to L ₃	Varady and Corba, 1999
Feeding (Larval)	ML	L ₁	Jackson and Coop 2000
	ML, LEV	L ₁	Alvarez-Sanchez et al., 2005
Larval paralysis/ Larval migration	BZ	L ₃	Sutherland and Lee 1990
	LEV	L ₃	Martin and Le Jambre 1979
	ML	L ₃	Gill <i>et al</i> 1991
	ML	L ₃	D'Assonville <i>et al</i> 1996
Exsheathment	-	L ₃	Brunet <i>et al.</i> , 2007
Video tracking	BZ, LEV, ML	L ₃	Glasswell et al., 2003
Feeding (Adult)	ML	Adult	Geary et al., 1993
Motility	ML	L ₄ to adult	Kotze et al., 2004
Conventional PCR	BZ	Adult	Roos et al., 1990
	BZ	-	Beech <i>et al</i> 1994
	BZ	-	Elard <i>et al</i> 1999
Real time PCR	BZ	All	Walsh et al., 2007
Pyrosequencing	BZ	-	v. Samson Himmelstjerna et al., 2007a

1.16.1 Egg hatch test (EHT)

The *in vitro* EHT (Le Jambre and Whitlock, 1976, Hunt and Taylor, 1989; Coles et al., 1992) assesses the ability of eggs to hatch in different concentrations of thiabendazole (TBZ). Approximately 100 strongyle eggs are incubated in final concentrations of TBZ of 0.05, 0.1 and 0.3 µg/ml for 48 h at 22 °C in 24 well cluster plates. Lugol's iodine is used to stop the test and prevent further hatching of eggs. The numbers of eggs and larvae are counted and the data are used to determine the ED₅₀ estimation. Estimates of greater than 0.1µg/ml are indicative of resistance (Le Jambre and Whitlock, 1976).

1.16.2 Larval development test (LDT)

The *in vitro* LDT (Coles and Simpkin 1977; Taylor, 1990; Hubert and Kerboeuf 1992; Varady and Corba, 1999) assesses the ability of eggs to hatch, develop and moult through two larval stages to become third stage larvae in the presence of increasing BZ and LEV concentrations. Data published in Australia showed that the LDT was unreliable at detecting ML resistance with field-derived material, particularly with *T. circumcincta* (Besier 1998; cited in Kotze et al., 2006).

Several different techniques for conducting the test exist, though the method used in the survey outlined in chapter 2 was conducted using the following methodology: Strongyle eggs were extracted and incubated overnight at 22 °C. Approximately 100 eggs/80 µl of water, containing 5 µg amphotericin B/ml, was combined with 20 µl nutritive medium (1 g yeast extract/90 ml 0.85% physiological saline) and 20 µl lyophilized *Escherichia coli* (150µg/ml in PBS) according to the method of Hubert and Kerboeuf (1992). One microlitre of anthelmintic was combined with 150 µl of 2% agarose in a 96 well microtitre plate as described by Amarante et al. (1997). Final concentrations of TBZ (0.05–0.3 µg/ml), LEV (0.05–2 µg/ml) and IVM (0.0004–0.13 µg/ml) were investigated. One hundred microlitres of the egg suspension was added to the surface of the agarose and the plates were covered and incubated at 22 °C for 7 days. Eggs, first and second stage larvae counts were combined and third stage larvae were counted separately, allowing an estimation of the percentage development.

1.16.3 Larval motility /migration inhibition test (LMI)

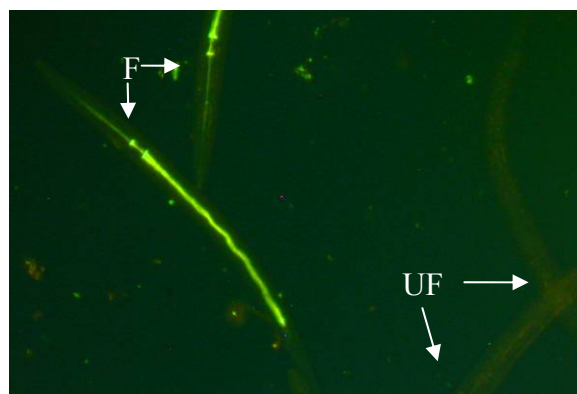
In 1991 Gill et al., detailed a motility test for characterising resistance in infective *H. contortus* larvae (L₃). The L₃ were incubated in the dark for 24 hours on IVM-impregnated agar beds and following incubation, the larvae were exposed to light and their motility, or lack of, was recorded. Gill reported that differential dose responses were optimal after three rounds of dark and light incubations. More recently the motility test has been used to compare and characterise the effects of the emodepsides to other commercially available anthelmintics in trichostrongylids (Schurmann et al., 2007).

The larval migration test, like the larval motility test, assesses effects on somatic musculature but avoids the need for potentially subjective assessment of activity by examining the ability of anthelmintic-treated L₃ to migrate through mesh filters. The test has been trialled with a range of parasitic nematode species from a range of hosts e.g. pigs, (Petersen et al., 2000), sheep (Kotze et al., 2006) and equids (Matthews unpublished data).

1.16.4 Larval feeding inhibition test (LFIT)

The larval feeding inhibition test is based on a procedure that examines the effect of ivermectin on the feeding behaviour in adult *H. contortus* worms (Geary et al., 1993). Modifications were made to allow the examination of feeding behaviour in first stage nematode larvae. Briefly, the procedure involves the extraction and incubation of eggs to produce first stage larvae, these larvae are incubated in the test substance suspensions at 22°C for two hours. The larvae are then given access to a fluorescein isothiocyanate-labelled *E. coli* suspension and incubated for a further 18h. Following incubation, the larvae are immobilised and examined under a fluorescence microscope. If the test substance has been effective at paralysing the pharyngeal musculature, then they are unable to feed and no fluorescence can be seen. If the substance has been ineffective then a clearly defined gut can be seen, Plate 1-1.

Plate 1-1 Picture of fed (F) and unfed (UF) *Haemonchus contortus* first stage larvae



1.17 Molecular assays

Single nucleotide polymorphisms (SNP) are changes in DNA sequence due to differences in a single nucleotide within a population. Polymorphisms can either be synonymous (silent mutations) or nonsynonymous (result in changes in the amino acid sequence) and can occur in both coding and non-coding regions of the gene. Single nucleotide polymorphisms have been associated with the presence of AR, particularly with BZ resistance Table 1-6.

Table 1-6 Examples of important single nucleotide polymorphisms associated with anthelmintic resistance in nematodes, selected references.

Nematode species	Codon	Gene of interest	Amino acid change		Reference
			From	To	
<i>Benzimidazole</i>					
Hc	167		Phenylalanine	Tyrosine*	Prichard 2001 Silvestre and Cabaret, 2002 Njue and Prichard 2003 Drogemuller et al., 2004
Tci					
Co					
Cyath					
Hc	198	β -tubulin	Glutamic acid	Alanine	Ghisi et al., 2007 Kwa et al., 1994
Hc					
Tco	200		Phenylalanine	Tyrosine	Grant and Mascord, 1996 Silvestre and Humbert, 2002 Njue and Prichard, 2003
Tci					
Co					
<i>Levamisole/Pyrantel</i>					
<i>Ce</i>	153	UNC-38	Glutamic acid	Glycine	Rayes et al., 2004
<i>Ce</i>	57	UNC-63	Glutamine	Glycine	Bartos et al., 2006
<i>Ivermectin</i>					
Co	256	GluCl α 3	Leucine	Phenylalanine	Njue <i>et al</i> , 2004
Hc	300	GluCl	Threonine	Serine	Jagannathan, 1999 [#]

* A substitution of Phenylalanine to histidine has also been identified. [#] SNP associated with changes in GluCl channel, though no confirmed involvement with resistance. *Ce* - *C. elegans*; *Co* - *C. oncophora*; *Cyath* - cyathostome species; *Hc* - *H. contortus*; *Tci* - *T. circumcincta*; *Tco* - *T. colubriformis*.

As described in Chapter 1.7.1 the principal effect of BZ on parasitic nematodes is the disruption of cellular function, in particular inhibition of microtubule formation, by its binding to the β -tubulin monomer (Dawson et al., 1984; Sangster et al., 1985; Lacey, 1988). Several genetic mechanisms have been associated with the presence

of BZ resistance in parasitic nematodes of sheep, loss of isotype 2 of the β -tubulin gene (Kwa et al., 1993) and single nucleotide point (SNP) mutations within isotype 1 of the β -tubulin gene. The point mutations in the β -tubulin gene are responsible for an amino acid transversion at each of the sites; phenylalanine to tyrosine at codon 200 (F200Y), phenylalanine to tyrosine or phenylalanine to histidine at 167 (F167Y) and adenine to cytosine at 198 (E198A). The presence of single nucleotide polymorphisms in isotype 1 is not straightforward, and not all of the mutations are found in all ovine parasitic nematode species. Recent work suggests that the presence of one SNP is not usually accompanied by a second (Silvestre and Humbert, 2002). Methodologies for identifying the presence/absence of these SNP are highlighted in Table 1-7.

Table 1-7 Methodologies for single nucleotide point identification associated with BZ resistance (Adapted from von Samson-Himmelstjerna et al., 2007).

Assay	SNP(s) investigated	Species	Reference
Allele-specific PCR	F200Y	<i>H. contortus</i>	Kwa et al., 1994
	F200Y	<i>T. circumcincta</i>	Elard & Humbert, 1999
	F167Y	Various	Silvestre & Cabaret, 2002
	F200Y	<i>C. oncophora</i>	Winterrowd et al., 2003
PCR-RFLP	A198E & F200Y	<i>H. contortus</i>	Ghisi et al., 2007
	F200Y	<i>T. circumcincta</i>	Shayan et al., 2007
	F200Y	<i>H. contortus</i>	Tiwari et al., 2006
Real time PCR	F200Y	Various	Alvarez-Sanchez et al., 2005a
	F200Y	<i>H. contortus</i>	Walsh et al., 2007
Pyrosequencing	F167Y , A198E & F200Y	Various	Skuce & Donnan unpublished
Sequencing	F200Y	<i>C. oncophora</i>	Njue and Prichard, 2003
	F167Y	Various	Silvestre & Cabaret, 2002

1.17.1 Conventional allele specific polymerase chain reaction

Original work by Kwa et al., (1994) resulted in a polymerase chain reaction (PCR) based assay that demonstrated that a SNP, TTC (phenylalanine) to TAC (tyrosine), at codon 200 of the β -tubulin isotype 1 gene encodes for BZ resistance in *H. contortus*

and *T. colubriformis*. The methodology was streamlined to enable the test to be performed as a single multiplex reaction by Elard *et al.*, (1999). The technique involves the potential amplification of up to three β -tubulin products of different lengths via the use of a set of four primers, a BZ-susceptible specific primer, a BZ-resistant specific primer and two non specific flanking primers. The procedure has been shown to be effective at determining the genotype of *H. contortus*, *T. circumcincta*, *T. colubriformis* and several cyathostomin species (von Samson-Himmelstjerna *et al.*, 2002).

The technique has been combined with a restriction fragment length polymorphism step to provide both genotyping and speciation information on *H. contortus*, *T. circumcincta* and *T. colubriformis* isolates (Silvestre and Humbert, 2000).

1.17.2 Real time polymerase chain reaction

A real time PCR technique has been developed (Alvarez-Sanchez *et al.*, 2005a) to allow the amplification and quantification of the wild type and resistant β -tubulin isotype 1 alleles in nematode DNA samples. Quantification of the amplified DNA is achieved by the use of non-specific intercalating dyes such as SYBR[®] green or SYBR[®] red which fluoresce when bound to double stranded DNA. Measuring the amount of fluorescence generated after each round of amplification by different isolates allows comparative quantification of allele frequencies to be conducted. Subsequent work has used other detection systems such as TaqMan[®] (Walsh *et al.*, 2007) or fluorescent labelled oligonucleotide probes.

1.17.3 Pyrosequencing

Pyrosequencing[™] is a high throughput technology that can facilitate sequence analysis, genotyping and allele specific SNP quantification. The technology works on the quantitative detection of light generated by an enzymatic cascade following the incorporation of deoxynucleotide triphosphates (dNTPs) onto a PCR amplified DNA template. The dNTPs are dispensed individually in a predetermined sequence and result in the release of pyrophosphate (PPi) if incorporated onto a PCR amplified DNA template. The quantity of PPi released is equimolar to the amount of dNTPs

incorporated. A cascade of enzymic reactions is set into motion resulting in the conversion of luciferin to oxyluciferin leading to the generation of visible light. The emitted light is detected by a charge coupled device camera and the intensity is proportional to the number of nucleotides incorporated. Further enzymic reactions degrade unincorporated dNTPs prior to the addition of the subsequent dNTPs to ensure accuracy of the sequencing data generated. Further details can be obtained from <http://www.pyrosequencing.com/>, last accessed 10SEP08.

1.17.4 Sequencing - chromatograms

Comparative SeqDoC analysis of PCR amplified products from pools of parasites, particularly phenotypically anthelmintic-sensitive and anthelmintic-resistant isolates, has been suggested as a method of rapidly scanning areas of interest for potential SNP markers (Blackhall, personal communication). The example in Figure 1.10 (Skuce, personal communication) shows the chromatograms of PCR products in the region of codon 200 of β tubulin isotype 1 from pools of BZ resistant and BZ sensitive *T. circumcincta*. Comparative SeqDoc analysis identifies regions where the tracers differ from each other, in this case at P200.

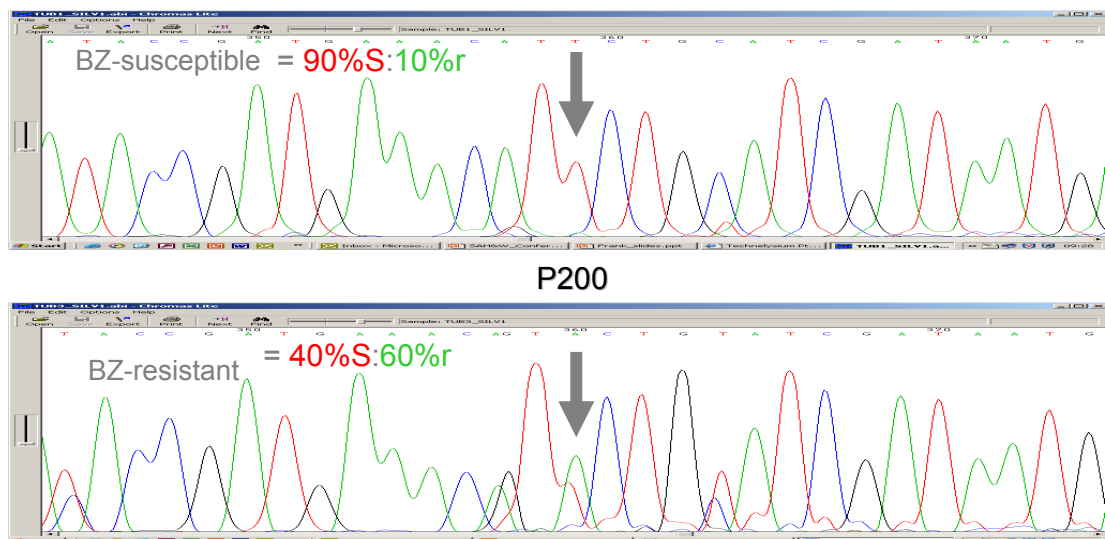


Figure 1.10 Chromatograms of PCR products in the region of codon 200 of β tubulin isotype 1 from two populations of *Teladorsagia circumcincta* (Skuce, personal communication).

1.17.5 **Models/alternative methods of analysing effects of parasitism and anthelmintic resistance**

In conjunction with traditional techniques for investigating mechanisms of resistance, much work on exploring alternative strategies has been conducted. Small animal models, primarily mice and gerbils, have used both rodent specific parasites such as *Nippostrongylus braziliensis* (Bottjer and Bone, 1985), *Heligomosomoides polygyrus* (Kerboeuf and Jolivet, 1980; Monroy and Enriquez, 1992; Njoroge et al., 1997, Su and Dobson, 1997; Kristan, 2002a and 2002b; Githiori et al., 2003; Iraqi et al., 2003) and *Hymenolepis nana* (Haugwitz et al., 1979) as well as ruminant specific parasites such as *T. circumcincta* (Court et al., 1988), *H. contortus* (Ellis et al., 1993; Molento and Prichard, 1999; Forrester et al., 2004), *T. colubriformis* (Ostlind and Cifelli, 1981; Lewis et al., 1982; Maclean et al., 1986; Ostlind et al., 1990) and *F. hepatica* (Shoop et al., 1995) to investigate areas such as parasite behaviour, chemotherapeutic/phytotherapeutic activity and efficacy, effects of parasitism on mammalian physiology and aspects of genetics and immunity to parasites. Translation of the results from these experimental models to large animal trials can be difficult due to differences in parasite lifecycles in different hosts, physiological differences in hosts resulting in retardation of parasite growth and establishment of parasites in atypical areas of the gastrointestinal tract (Court et al., 1988) and differences in the host immune mechanisms.

The use of the non parasitic nematode *Caenorhabditis elegans* (Kwa et al., 1995; Dent et al., 2000; Cheeseman et al., 2001; Liu et al., 2004) and specific cell lines such as PgP over expressing mammalian cells (Dupuy et al., 2006) have been extensively used to investigate and elucidate mechanisms of drug resistance.

1.18 **Genetic selection of anthelmintic resistance**

Prior to treatment of a naïve/susceptible population the alleles for resistance (R) are rare and the alleles for susceptibility (S) predominate, however, following treatment the prevalence of R alleles increases. Under continued anthelmintic selection pressure, and assuming that there are no deleterious effects or fitness costs associated with the genes for resistance, they will continue to increase over time in a population until they predominate (Prichard, 1990). The rate of selection for resistance does not

occur at a predetermined rate and is affected by a large number of variables e.g. starting frequencies of resistance genes, dominance/recessiveness of the genes and whether resistance is mono- or polygenic (Figure 1.8, red dashed boxes). There are three stages in the progression of anthelmintic resistance. Firstly establishment, during which resistance allele frequency is generally low. Secondly development, which occurs following selection pressure and can be influenced by treatment frequency. Frequent and inappropriate treatments can lead to a rapid selection in the alleles for resistance. The method of selection i.e. via under-dosing or via full therapeutic dose rates has been shown to influence the means by which an organism deals with anthelmintic exposure (Gill et al., 1998) The final stage, expression, is the point at which allele frequency is high and may be detected as clinical disease in animals.

It has been suggested that resistance is a pre-adaptive phenomenon, that is to say that a small percentage of the population has the capacity to survive at a concentration of the drug that would be expected to remove a sensitive population, even prior to any exposure to the compound (Roos et al., 1990). Consequently the initial frequency of resistance alleles present in a population can vary from region to region even before a single treatment has been administered in an area. Coles, (2005) reported that the dose rate to treat *Schistosoma mansoni* infections in Brazil is lower than those needed in East Africa.

Both between parasite species and anthelmintic classes differences occur in the ways that the genes for resistance are inherited -

Benzimidazole resistance in *H. contortus* was reported to be semi-dominant (Le Jambre et al., 1979) though subsequent work on different isolates has found it to be an incompletely recessive autosomal trait (Sanster et al., 1998) reliant on multiple genes (Le Jambre et al., 1979; Herlich, et al., 1981). In *T. colubriformis* TBZ resistance is inherited in a co-dominant fashion (Martin et al., 1988).

Levamisole resistance in *H. contortus* is inherited as a multigenic and incompletely

recessive trait (Sangster et al., 1998) whilst in *T. colubriformis* it is monogenic or polygenic on closely related genes, sex linked (Martin & McKenzie, 1990) and determined by a dominant gene (Dobson et al., 1987). The use of TBZ has been shown to select against LEV resistance (Dobson et al., 1987).

Ivermectin resistance is reported as being an autosomal, monogenic trait (Le Jambre, 1993; Dobson et al., 1996; Le Jambre et al., 2000) and a completely dominant trait in *H. contortus*, whilst the story with MOX resistance is more complex. If populations are pre-selected with abamectin (ABA) the resistance appears to be semi-dominant but if the parasite is selected with MOX then the trait is semi-recessive (Le Jambre et al., 2005). With *T. circumcincta*, IVM resistance is dominant whilst MOX resistance is thought to be recessive (Sutherland et al., 2002a)

1.19 Mechanisms of resistance

The mechanisms of expression of resistance are thought to differ between anthelmintic classes, nematode species and between various nematode isolates (Figure 1.8). Wolstenholme et al., (2004) characterised the mechanisms in four broad categories which will be discussed individually.

1.19.1 Changes in the molecular target rendering the drug ineffective

Benzimidazole

Initial studies correlated BZ resistance with a loss of high affinity BZ receptor binding sites (Lacey, 1988; Lacey and Gill, 1994). This loss of binding capability has been identified in *H. contortus*, *T. colubriformis*, *C. oncophora* and *T. circumcincta* and is associated with SNP at codon's 167, 198 or 200 in isotype 1 of the β -tubulin allele. Subsequent work has also implicated a similar β -tubulin SNP at codons 167 in isotype 1 and codon 200 in isotype-2 with BZ resistance in *H. contortus* (Prichard 2001). Mutations in *H. contortus* at either codon 167 or 200 appear only to occur in isolation i.e. a mutation at codon 167 has not been identified

in populations with mutations at codon 200 (Silvestre and Cabaret, 2002; Ghisi et al., 2007). In combination with the SNP mutations a loss of β -tubulin isotype 2 has also been correlated with BZ resistance in *H. contortus* (Kwa et al., 1993).

Levamisole

With levamisole resistance, the mechanisms are less well defined. Work conducted by Sangster et al., (1998) demonstrated that membrane preparations from resistant *H. contortus* and *T. colubriformis* appeared to have decreased LEV affinity at the nicotinic acetylcholine receptors. Further work conducted using *C. elegans* as a model has shown that LEV resistance is associated with a modification (Lewis et al., 1980) or reduction in the number (Richmond and Jorgenson, 1999; cited in Wolstenholme et al., 2004) of nicotinic acetylcholinesterase receptors (nAChR) and Fleming et al., (1997) initially identified three nAChR genes that were strongly associated with LEV resistance (*unc-29*, *unc-38* and *lev-1*). Polymorphisms associated with LEV or pyrantel resistance have not been found in parasitic nematodes, though they have been found in *C. elegans*. Two interesting candidate genes are *unc-38* and *unc-63* at codon 153 and codon 57 respectively (Rayes et al., 2004; Bartos et al., 2006).

Macrocyclic lactones

Macrocyclic lactones, as mentioned in chapter 1.7.3, act on ligand gated chloride channels (glutamate, GluCl and gamma aminobutyric acid, GABA). Comparisons between the GluCl- α 3 subunits of a susceptible and a resistant *C. oncophora* population showed that they differed at three amino acid positions, but only one (L256F) was correlated with decreased sensitivity to IVM (Njue *et al.*, 2004).

Work on *C. elegans* identified four genes, *avr-14*, *avr-15*, *glc-1* and *glc-3*, which encode GluCl channels activated by IVM. Null mutations for each of these genes individually do not confer resistance, but when examined in combination led to a greater than 4000 fold increase in tolerance of IVM (Dent et al., 2000).

As well as the association of ML resistance with the ligand gated chloride channels, a correlation has been made between the length and organisation of the anterior sensory structures, amphid sensilla and the presence of IVM tolerance/resistance in *C. elegans* (Dent et al., 2000) and *H. contortus* (Freeman et al., 2003; Guerrero and Freeman, 2004). The amphids have been shown to facilitate the binding and transportation of compounds like MLs and may play an important role in their entry into the parasite. Shortening, degeneration and/or loss of detail of these structures in nematodes is thought to reduce the efficacy of their ability to transport compounds.

1.19.2 **Amplification of target genes to overcome drug action**

Resistance in *H. contortus* or *T. circumcincta* to IVM is thought to be partially due to an increase in the numbers of low affinity L-glutamate binding sites, though no target site mutations have been identified (Hejmadi et al. 2000). Work by Blackhall et al., (1998a) showed that the frequency of alleles to a GluCl α -subunit increased in three ML selected *H. contortus* isolates.

Comparative single-strand conformation polymorphism (SSCP) analysis between drug sensitive and IVM and MOX resistant *H. contortus* strains showed a selection for alleles at a GABA receptor in the resistant nematodes (Blackhall et al., 2003).

1.19.3 **Changes in the metabolism that inactivate/remove/prevent activation**

The ability of both host (Tynes and Hodgson, 1983) and parasite species to utilise enzymatic mechanisms for handling xenobiotics has been acknowledged for many years. Distribution of the various systems between parasitic families is variable, with glyoxalase being shown to be important in nematodes (Brophy et al., 1990) whilst glutathione transferase is the main system in intestinal cestodes, digeneans and *Onchocerca gutturosa* (Pemberton and Barrett, 1989, Brophy et al., 1989). More recently research has focussed on the cytochrome oxidase pathways, particularly cytochrome P450 (CYP). Cytochrome P450's are haemoproteins, found in a wide range of hosts, capable of catalysing enzymatic reactions in both exogenous and endogenous compounds. Commonly, this is a monooxygenase reaction.

Anthelmintics, particularly BZ (Chiu and Lu, 1989 cited in Kotze et al., 2006) and ML (Gottschall et al., 1990; Alvinerie et al., 2001), are metabolised by CYP. Kotze, (1997) suggested that oxidative pathways are likely to be less important in adult worms compared to the free-living larval stage due to the reduced oxygen tensions in the intestinal tract, though work by Alvinerie et al., (2001) found that MOX was metabolised by adult worm homogenates indicating a role for CYP.

As with many enzymic reactions the CYP can be induced/enhanced or inhibited by various compounds and it was the inhibition of CYP by piperonyl butoxide (PBO; Benchaoui and McKellar, 1996) that was suggested as a mechanism for increasing the efficacy of FBZ in ruminant hosts. Work by Barrett (unpublished data) showed a marked increase in the efficacy of FBZ (5 mg/kg BW) when administered in combination with PBO (63mg/kg BW). More recently, work investigating the co-administration of triclabendazole and PBO to sheep showed an increased systemic availability of the anthelmintic (Virkel et al., 2007), whilst *in vitro* synergistic interactions between rotenone and PBO have been reported (Kotze et al., 2006). Decreased cuticular penetration in the house fly *Musca domestica* has also been shown to be important in abamectin resistance (Scott, 1989; 1991) though it is uncertain whether such a mechanism occurs in nematodes.

1.19.4 Changes in the distribution of the drug in the organism preventing access to site of action

Modulation of xenobiotics via efflux proteins (Xu et al., 1998) have been examined in both free-living and parasitic nematodes. The P-glycoprotein (Pgp) is an energy-dependant transporter, also known as an ATP binding cassette (ABC) transporter, which is involved in the transport/efflux of noxious compounds. P-glycoprotein molecules contain six trans-membrane domains attached to an ATP-binding site (Sangster 1994, Figure 1.11). P-glycoproteins have been implicated in multidrug resistance in tumour cells (Pouliot et al., 1997) as well as resistance to all three broad spectrum anthelmintics; the BZ (Beugnet et al., 1997; Kerboeuf et al., 2002), imidazothiazoles / tetrahydropyrimidines (Rothwell and Sangster, 1997) and ML (Xu

et al., 1998; Molento and Prichard, 2001) and have been described in *C. elegans* (Broeks et al., 1995) and *H. contortus* (Blackhall et al., 1998b; Le Jambre et al., 1999). P-glycoprotein genes are numerous in nematodes, unlike in mammals. *C. elegans* have 14 genes (Kerboeuf et al., 2003) and *H. contortus* have at least 7 (number identified at present, Skuce personal communication) and appear to play an important role in the protection of the parasite neurones against anthelmintic molecules (Prichard and Roulet, 2007).

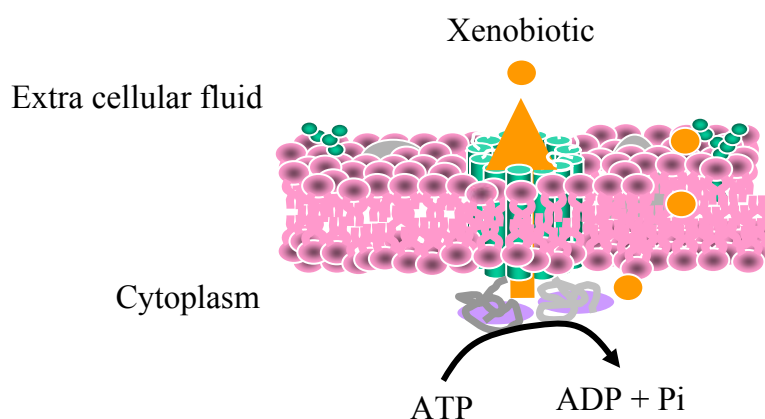


Figure 1.11 Diagrammatic representation of cell membrane structure illustrating the permeation of drug and export of the compound via P-glycoprotein (modified from Sangster, 1994).

Work investigating the association between Pgp and anthelmintic resistance / inefficacy has been conducted both *in vitro* and *in vivo*. Partial reversion of resistance to BZ (Beugnet et al., 1997; Kerboeuf et al., 2002; Stenhouse, 2007) and ML (Bingham et al., 2007; Bartley unpublished) in the free-living stages of nematodes has been demonstrated *in vitro* using verapamil hydrochloride (VER). In the field, the results are mixed, with significantly increased systemic availability of ivermectin being reported in sheep treated with VER (Molento et al., 2004a). In parasitized cattle treated with loperamide, a Pgp modulator, in combination with either IVM or MOX respectively (Lifschitz et al., 2007), the reductions in faecal egg counts were only 27% and 18% respectively.

1.20 Factors associated with resistance development

Figure 1.8 highlights factors that affect both the supra- and infra-populations and that may be associated with treatment failure and/or the selection of anthelmintic resistance. Some of the factors have been addressed earlier in section 1.11.

1.20.1 Dose frequency

Frequent treatment of stock has been repeatedly cited as a means of selecting rapidly for resistance, particularly when intervals between treatments are shorter than the pre-patent period of the parasite that is being targeted, meaning that only resistant worms are passed onto pasture. Early research on sheep (Barton, 1980 and 1983; Martin et al., 1982 and 1984), goats (Chartier et al., 1998) and horses (Round et al., 1974; Kelly et al., 1981) identified that treatment frequency strongly correlated with the presence of BZ resistance, though it may also correlate with other classes of resistance. It has also been suggested that one of the reasons that AR is more prevalent in areas of the world where climatic conditions are suitable for grazing all year round may be the frequency of dosing required to maintain parasite control (Conder and Campbell, 1995).

1.20.2 Under-dosing

Under-dosing is described as occurring when a host is administered “a weight-dependant dose that is less than that recommended by the manufacturer” (Smith et al., 1999). The practice allows heterozygote resistant individuals to survive treatment and contribute genes for resistance to the subsequent populations (Roush and McKenzie, 1987). Many reasons exist for the underdosing of animals, and these may occur inadvertently, Table 1-8.

Table 1-8 Possible reasons for animals being under dosed

Inadvertent	Intentional
Estimating weights incorrectly	Dosed at average weight of stock
Faulty equipment	Dosed at rates less than recommended to save money
Using sub-standard compounds	
Different dose rates required for sheep and goats	
Dosing at rate insufficiently high to kill all types of parasites harboured by host e.g. use of spot on treatment for ectoparasites not killing all endoparasites.	

Although under-dosing may lead to an increase in the number of heterozygote individuals in a predominantly susceptible population, thereby selecting for resistance, the reverse may occur in a predominately resistant population and under-dosing may result in preservation of treatment efficacy (Silvestre and Humbert 2002).

1.20.3 Non-alternation of drug classes

No long-term practical experiments have been conducted to assess the effect of annual rotation of anthelmintic classes. It has been suggested that in the early stages of the selection process for resistance, alternation between anthelmintic classes will reduce the selection on a parasite population and therefore prevent or slow the development of resistance (Prichard et al., 1980). Simulated mathematical modelling by Barnes et al. (1995) suggested that drug rotation, either annually or longer only preserved the life expectancy of a compound when the resistant gene frequency in a population was greater than 2%.

1.20.4 Dose and Move

The movement of freshly treated animals to clean pasture was extolled as a method for prolonging the usefulness of anthelmintic compound by reducing re-infection (Boag and Thomas, 1973) and was identified as a method for improving productivity, such as overall wool production (Morley and Donald, 1980) and lamb growth by 10-20% prior to weaning (Waller and Thomas, 1978). The practice has

subsequently been shown to select heavily for resistance with survivors of anthelmintic treatment rapidly contaminating clean pastures with eggs (Silvestre and Humbert, 2000).

1.20.5 **Lack of refugia/farm management**

Maintaining a proportion of a population *in refugia*, i.e. not exposed to drug treatment has long been acknowledged in entomology (Meyer et al., 1990) and helminthology (Martin et al., 1981; Michel et al., 1985) as an important system for sustaining genes for susceptibility in a population. The degree of refugia can be affected by a range of environmental and managerial factors. The advice has been brought back into vogue by the need to maintain productivity and sustainability in the livestock market in the face of ever increasing resistance. In the past, advice has centred on increasing productivity with little to no thought on sustainability.

Some of the advice provided to farmers in the past has been unfounded and resulted in an increase in the selection of resistance, as mentioned in section 1.20.4, for example dose and move. Other advice has included the treatment of ewes pre-lambing with long-acting products or post-lambing with a short-acting product in order to reduce pasture contamination and thereby infection level experienced by the lambs. Unfortunately, the practice has been identified as a factor associated with the presence of IVM (Lawrence et al., 2006) and ABZ resistance (Leathwick et al., 2006) in *Teladorsagia* and *Trichostrongylus* populations in New Zealand, although the practice is not thought to be selective in treatment directed against *Trichostrongylus* infections, administered to Merino sheep in Australia (Barnes and Dobson, 1990). The procedure is thought to heavily select for resistance in New Zealand because the rate of re-infection in Romney ewes, in which acquired anti parasite immunity may start to return two weeks post partum (Leathwick et al., 1999), is low and therefore dilution of resistant parasites on pasture i.e. refugia is less (Leathwick et al., 2006).

The “summer drenching program”, as advocated by the Australian State Departments of Agriculture in Western Australia (WA), has the same effect as dose and move, as described in section 1.20.4, and can effectively control parasites but

may actually contribute to selection for resistance. In WA, where the daytime temperatures average around 30°C there are virtually no worm larvae alive on pasture and therefore few *in refugia* (Besier, 2008).

Timings of treatments have also been questioned. Pre-tupping treatments are routinely administered in UK (Chapter 8) and French flocks (Silvestre and Humbert, 2002) in order to bring rams and ewes into condition but rarely coincide with high levels of infection.

1.21 *Reversion*

Previous work has shown that reversion to susceptibility will depend on a number of factors such as fitness costs associated with possessing particular genes for resistance, hybrid vigour, selection pressure and initial gene frequency for resistance (Martin et al., 1988; Maingi et al., 1990; Prichard, 1990). Initial reports of reversion towards susceptibility involved the use of LEV treatment against BZ resistant populations (Waller et al., 1983; Martin et al., 1988) and vice versa (Waller et al., 1985), but subsequent work would suggest that once the genes for resistance have been forced to a certain level and have re-associated with genes for general fitness, reversion will not occur naturally (Prichard, 1990).

An example of this is on the Moredun Research Institute farm where BZ resistance was detected in 1983; using a faecal egg count reduction test (FECRT) or controlled efficacy tests, the FBZ (5mg/kg body weight) efficacy was 44%. Following detection of resistance, BZ usage was suspended and an annual rotation of LEV and IVM was adopted. Efficacies in subsequent tests, over a 17 year period, decreased to 6% (Figure 1.12; Jackson unpublished data).

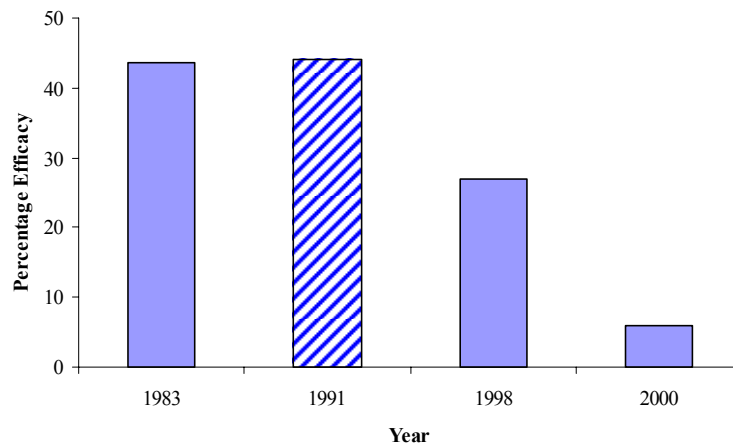


Figure 1.12 Fenbendazole efficacy in lambs grazing pastures at Moredun Research Institute farm treated at 5mg per kg body weight (BW; ■) and 10mg/kg BW (▨).

1.22 Targeted selective treatments

The need to maintain genes for susceptibility to anthelmintic treatments by utilising the untreated, and therefore unselected, parasite population *in refugia* was suggested (Prichard et al., 1980; Michel, 1985; Barnes et al., 1995), but it was not until recently that this advice has been heeded (van Wyk et al., 2001; Besier, 2001; Hoste et al., 2002a and 2002b). Targeted selective treatments (TST) have been used primarily to maintain refugia in GIN of small ruminants (see 1.22.1; 1.22.2; 1.22.3) but the principle has also been trialled against horse (Krecek and Guthrie, 1999) and dairy cattle (Höglund, 2006) parasites. At present three key TST approaches exist for identifying infected animals which would benefit from anthelmintic treatment. Firstly, strategies for those infected with haematophagous species; secondly, lactating animals infected with non-haematophagous species; thirdly non-lactating animals infected with non-haematophagous species.

1.22.1 Haematophagous species - FAMACHA[®]

FAMACHA[®] was devised in South Africa, named after its originator Dr. Francois Malan (**F**Affa **M**Alan **C**HArt; Bath et al., 1996) The technique involves the examination of small ruminant ocular mucous membranes to allow the rapid

identification of levels of anaemia. The chart, Figure 1.13, identifies when animals are anaemic and require anthelmintic treatment. The method uses a scale that ranges from one to five, with one representing a healthy animal and five an anaemic animal. Anthelmintic treatment is advised for animals with a score of four to five and should be considered for scores of three; those with scores less than three are considered optimal/acceptable and require no treatment. The technique allows the selective treatment within flocks, reducing the reliance of farmers on chemoprophylaxis and slowing down the selection of anthelmintic resistance by increasing the nematode population *in refugia*. Treatments can be reduced by between 40-50% in sheep and goat flocks (Vatta et al., 2001; Kaplan et al., 2004) with a corresponding reduction in flock mean faecal egg count of between 35% and 83%, depending on the criterion used apply treatment (Kaplan et al., 2004).

Treatment of animals is generally considered essential when the PCV of an animal is less than 15%. The technique has been shown to give false negatives in goats, i.e. scores of between one and three when the PCV is less than 15% in less than one percent of cases (Kaplan, 2004).



Figure 1.13 FAMACHA[®] eye charts for identification of anaemia in small ruminants (Livestock Health and Production Group of South African Veterinary Association, 2002, reproduced with the kind permission of Gareth Bath).

1.22.2 Non-lactating animals infected with non-haematophagous species

Work on non lactating animals infected with non-haematophagous species is more difficult, but a new European Framework 6 STREP project (PARASOL,

www.parasol-project.org/project/parasol.php) has been charged with the objective of creating low input and sustainable strategies for the control of GIN infections, particularly *T. circumcincta* and *T. colubriformis*, in small ruminants. Work in France, Great Britain, Greece, Italy, Morocco, Slovakia and South Africa has focused on the use of FEC or pathophysiological markers (weight gain, condition score, diarrhoea score, dag score and anaemia) as indicators for TST treatment. Preliminary work conducted at Moredun Research Institute in Scotland has explored the use of a production based decision support system to identify animals that would benefit from targeted anthelmintic treatment. The decision to treat or not is based on the difference between actual and expected weight gain as determined by the model. The model integrates estimates of the efficiency of energy utilization in growing lambs in the face of environmental factors such as herbage availability and quality, dam milk production and climate to calculate expected weight gains. Results from replicated field trials conducted in 2006 and 2007 would suggest that in blackface sheep under challenge from predominantly *T. circumcincta*, it is possible to reduce overall anthelmintic treatment by 20% and 50% using this decision support system compared to animals treated strategically or monthly respectively. The second, and potentially more important, finding from these trials is that treatment efficacy is maintained in the TST group whilst within the monthly treated group treatment efficacy has fallen drastically, from 98% reduction in FEC at the beginning of the trial in 2006 to less than 50% in 2007 (Kenyon et al., 2007; Jackson and Waller, 2008). “Strategic” treatments based on short interval weight gains have been trialled in Western Australia, where electronic tagging combined with automatic weighing and drafting systems have meant that the system can be applied on a large scale. Preliminary results suggested that productivity can be maintained whilst maintaining refugia on pasture (Besier, 2007a).

In South Africa, field trials are being conducted to assess a new “5 Point system”, Figure 1.14, for assessing internal parasites in sheep (Bath, personal communication). The system is a concept at present and will need refinement before it can be released for general use but can be used to gauge the overall picture of an individual animal’s well-being by checking for nasal discharge, body condition,

bottle jaw, dag score as well as FAMACHA[®]. Ultimately, it may become a universal system for assessing an individual animal's need of treatment.

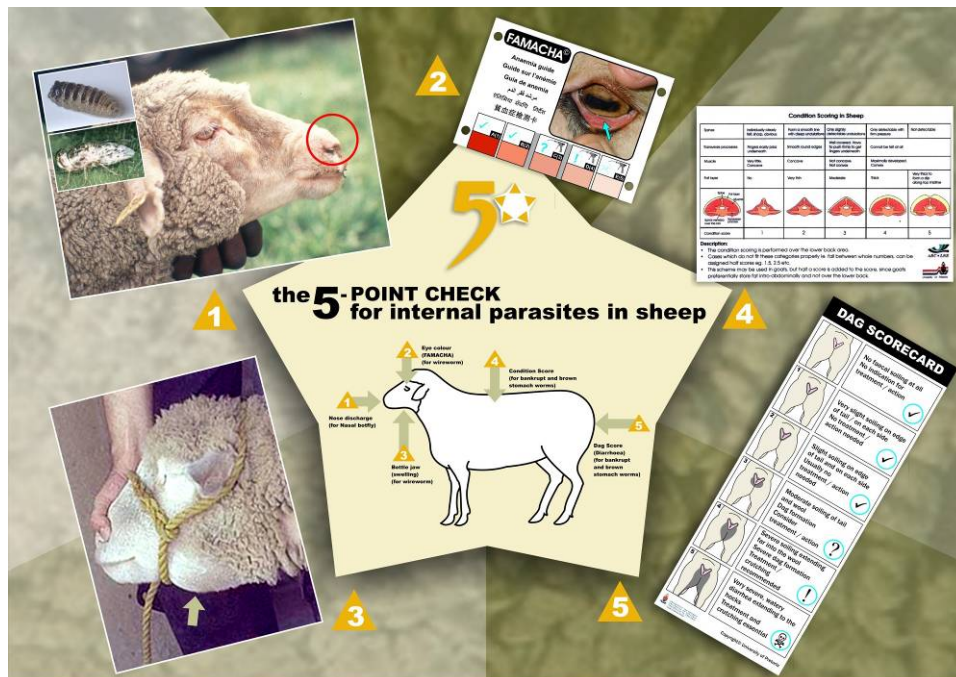


Figure 1.14 Five Point system for internal parasites of sheep (reproduced with the kind permission of Gareth Bath)

1.22.3 Lactating animals infected with non-haematophagous species

Targeted selective treatment (TST) strategies aimed at non-haematophagous species are more difficult to implement due to problems in identifying suitable indicators. Infections with *T. circumcincta* and *T. colubriformis* are commonly accompanied by diarrhoea and weight loss, but unfortunately these are not specific to these infections and can be attributable to other factors, Table 1-2. A strategy that has been employed with success has been targeted at dairy goats; high milk production has been positively correlated with susceptibility to parasitism (Chartier and Hoste, 1997). Subsequent research found that treatments targeted on the basis of milk production or kidding numbers was effective at controlling PGE whilst maintaining productivity. Anthelmintic usage was reduced by around 40% (Hoste et al., 2002a; 2002b; 2002c).

1.23 Non chemical control strategies

Non-chemotherapy based treatments have centred around reducing parasite host contact by judicious use of pasture management, improving host resistance/resilience to infection, nutritional manipulation, development of the hosts immune response via vaccination or by controlling the parasite directly by the use of biological controls.

1.24 Pasture/farm management

1.24.1 Rapid pasture rotation

Rapid grazing systems, where animals are grazed for only several days at a time and then moved are very effective at reducing infection or re-infection of animals at pasture particularly in the Southern hemisphere and tropics. The development of eggs to infective larvae in the warm humid conditions of the tropics can occur in as little as 3-7 days and they can deplete their energy reserves within 4-6 weeks (Barger et al., 1994; Niven et al., 2002). If pastures are separated into small paddocks or separate tethered sectors, as illustrated in Figure 1.15, grazed for several days and then spelled for a period of around a month, larval contamination on pasture is reduced and consequently so is the intake of parasites by grazing animals. This rotational method has been shown to reduce faecal egg counts in goats by half compared to set stocked animals in the same area meaning that less anthelmintic was required over the course of the year (Barger et al., 1994). In Australia rotational grazing incorporating anthelmintic treatment has been examined in a two year trial where paddocks were either set-stocked over a four to five month period (late October to February) or intensively grazed for one month then spelled for one to two months and re-stocked for a further two months with wethers. Parasitological and productivity parameters were examined. Both groups received anthelmintic treatment at the beginning of the trial and after three months. Tracer lambs were run over both pastures for six months (April to October) and those from the “rotated paddocks” had significantly lower worm burdens and faecal egg counts and improved productivity (body and fleece weights) compared to set-stocked tracers (Niven et al., 2002). Work is less conclusive in cooler temperate regions where

larval development and survival occurs over a longer period of time and as such this technique has not been readily incorporated into integrated management systems in these areas. (Healey and Walkden-Brown, 2005)

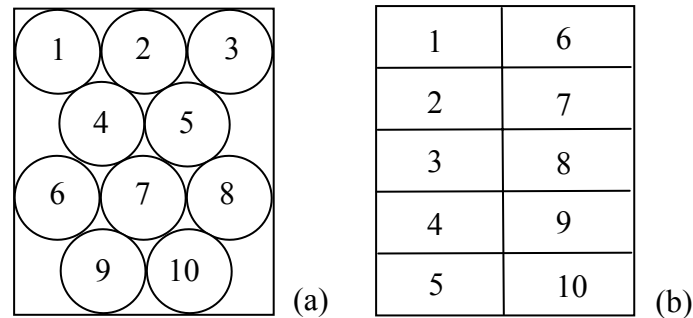


Figure 1.15 Illustrations of pastures separated into smaller sectors (numbers 1-10) for the rapid rotation of (a) tethered animals or (b) roaming animals.

1.24.2 Rotational/co-grazing

The use of “mower” animals to clean pastures has been suggested (Waller, 1993) as a viable method of worm control for both sheep and cattle, reducing the need for anthelmintic intervention over a grazing season. These “mowers” could be non-pregnant adult sheep or cattle and exploit the host specificity of many gastro-intestinal nematodes such as *Ostertagia ostertagi* and *T. circumcincta* which are generally less or non-pathogenic to alternative hosts. These adult animals are usually more immune to gastro-intestinal infections and are therefore able to withstand infections more readily, removing more parasites from pasture than they shed. Work conducted in Australia on pastures naturally infected with nematode parasites showed that paddocks alternately grazed by sheep and cattle resulted in reduced parasite populations, compared to pastures grazed solely by sheep (Barger and Southcott, 1975; Southcott and Barger, 1975). More recently, the application of a “cut and carry” policy has also been explored in Malaysia (Chandrawathani et al., 2008). Trials conducted in the UK have shown that rotational grazing and co-grazing permanent pastures with cattle and sheep reduced the faecal egg counts and improved weight gains of weaned lambs compared to paddocks where sheep have grazed solely (Forbes et al., 2005).

1.25 Improved host nutrition

The relationship between parasitism and nutrition is well documented. Improving the host plane of nutrition, in particular protein, leads to improved resistance and resilience to gastro-intestinal parasites. Reviews covering the topic are numerous (Coop and Holmes, 1996; Coop and Kyriazakis, 1999; Sykes and Coop, 2001; Coop and Kyriazakis, 2001; Knox et al, 2003). Protein availability and/or allocation appear to play a critical role in maintaining ewe responsiveness to infection during the periparturient period (Donaldson et al., 1998). Competition for available protein is greatest around parturition where there are demands from both the developing foetus as well as the ewe. For the ewe, production of colostrum and milk, as well as maintenance of the ewes own body condition all reduce the available resources needed to mount an effective expression of immunity, Figure 1.16.

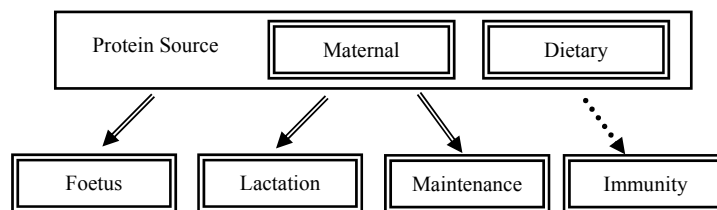


Figure 1.16 Schematic showing protein allocation in the peri-parturient ewe.

1.25.1 Non rumen degradable protein

Previous studies have examined the use of improved nutrition, in particular crude protein, to combat the loss of immunity in pregnant sheep especially during late pregnancy/early lactation. This weakening of the immune response is known as the peri-parturient relaxation of immunity and generally is accompanied by a corresponding increase in capability of parasites to develop and become patent within the host. Supplementations with non-rumen degradable proteins, such as; fishmeal (Donaldson et al., 1998), cotton-seed meal & urea (Datta et al., 1999) or soyabean (Dawson et al., 1999), have shown varying success in improving resistance

and resilience to both nematode and protozoan parasitism in small ruminants. Increasing concentrate feed prior to lambing has also shown promise (Donaldson et al., 1998; Kahn et al., 2000). Work on dietary supplementation carried out recently has been centred on growing kids (Knox and Steel, 1996; Katunguka-Rwakishaya et al., 1997) and dairy goats (Etter et al. 2000, Chartier et al., 2000). Hussein & Jordan reported in 1991 that fishmeal supplementation proved successful in non-ruminants but was less consistent than in ruminants. Galbraith (2000) observed no influence on cashmere yields from goats supplemented with white fishmeal.

1.25.2 Feed blocks (urea molasses blocks, UMB)

Multi-nutrient blocks are supplementary feedblocks that generally contain a mixture of agro-industrial by-products, non-protein nitrogen source (e.g. urea), binding agent (e.g. cement) and a preserver (e.g. salt). The blocks have the advantage of being cheap and simple to produce, easy to transport and allow the farmer to achieve improvement of performance and productivity in animals that are grown on poor quality pastures-forages. This is achieved by enhancing appetite and therefore feed intake and correcting any deficiencies that may arise, be these vitamins, minerals or other nutrients. Historically farmers in the Middle East have taken advantage of the benefits of incorporating left-overs such as cottonseed cake, sugar beet pulp, and wheat bran into animal diets. With help from organisations such as International Center for Agricultural Research in the Dry Areas (ICARDA) they have begun to incorporate other ingredients in animal feed such as tomato pulp, molasses, burghul derivatives, crude olive cake, sesame cake, citrus pulp, sunflower cake, and mulberry leaves, with promising results at a fraction of the cost of traditional feed stuffs (<http://www.icarda.cgiar.org/mmproject/feedblock.htm>, last accessed 10SEP08).

Field trials examining the production and parasitological advantages of UMB have shown a range of benefits in sheep (Knox and Steel, 1996 and 1999), goats (Waruiru et al., 2004; Vatta et al., 2005) and cattle (Waruiru, 2004). The supplementation of poor quality diets with UMB has led to increased body weights, haematocrit values and packed cell volumes and reduced nematode burdens and faecal egg output.

1.25.3 **Phytomedicines /nutraceuticals/ ethnomedicines**

The search for and use of compounds from plant resources for veterinary and medicinal purposes is not a new phenomenon. Estimates suggest that treatments for animals have been around for approximately seven thousand years (Riviere, 2007). Phytomedicines (plant based medicines), nutraceuticals (extracts of foods claimed to have a medicinal effect on health; <http://en.wikipedia.org/wiki/Nutraceuticals>, last accessed 10SEP08) and ethnomedicines (local or indigenous knowledge and methods for caring for, healing, and managing human lives and livestock; <http://www.africanethnomedicines.net/>, last accessed 10SEP08) using traditional inspirations for treating livestock have grown in acceptance in the western world but are still much associated with “quackery” (Githiori et al., 2005). In order to overturn this image, rigorous scientific validation needs to be conducted on potentially effective compounds (Hoste et al., 2008). Studies on a number of African plants and plant preparations have so far been unsuccessful at finding any with significant anthelmintic properties (Githiori et al., 2002; 2003a; 2003b; 2004).

1.25.4 **Bioactive forages**

Over the last 15 years, interest in bioactive forages has gained impetus due to a need for effective and accessible treatments for livestock in developing countries and an increased public concern regarding food and environmental residues by consumers in developed countries. Bioactive forages are plants that contain secondary compounds that are considered for their beneficial effects on health rather than for their direct nutritional value for animals (Waller, 2006). Plants produce a range of plant secondary metabolites (PSM), which are not directly involved in normal growth, development or reproduction but instead are thought to be waste or stress products or defence mechanisms against herbivores and insects (Harborne, 1999; Karban et al. 1999).

Interest started with temperate legume forages rich in condensed tannins (CT) such as chicory (*Cichorium intybus*), birdsfoot trefoil (*Lotus corniculatus*) and sulla (*Hedysarum coronarium*; Niezen et al., 1995; 1998; 2002a; 2002b; 2002c; Bernes et al., 2000; Athanasiadou et al., 2001; Marley et al., 2003; Hoste et al., 2005;

Tzamaloukas et al., 2005 and 2006) but has extended to derivatives from plants in tropical regions (Kahiya et al., 2003; Cenci et al., 2007; Minho et al., 2008). The most significant findings have related to work on plants containing polyphenols and condensed tannins (Waller, 2006) or proteases (Steppek et al., 2004). In addition a European Union funded grant REPLACE (replacing antibiotics in animal feed) is analysing a library of over 500 plant extracts for biocidal activity against a range of pathogens of livestock (cattle chickens, fish, pigs and sheep).

The PSM are thought to have both direct effects on the viability of larval stages, adult worms and/or fecundity of worms as well as indirect immunologically mediated effects. Condensed tannins have a high affinity for proteins protecting them from the digestive processes in the rumen. Disassociation of these CT/protein complexes occurs, under the correct pH conditions, at the small intestine and leads to improve protein availability (Hoste et al., 2006). Anthelmintic effects have been variable throughout the trials but have shown degrees of promise. Much more work is required to address some of the questions regarding intra- and inter-seasonal effects, bioavailability of active compounds, selective grazing behaviour, concerns about toxicity/anti-nutritional effects as well as concerns of Northern European farmers relating to longevity of forage paddocks, climate effects and cost of implementation.

1.25.5 **Copper oxide wire particles (COWP)**

The use of copper sulphate (CuSO_4) has long been advocated as a means of treating copper deficiency in grazing livestock. Hall and Foster, 1918, demonstrated the first evidence of CuSO_4 anthelmintic activity with efficacy against gastro-intestinal parasites in ewes and lambs. Work conducted over the following two decades confirmed this anthelmintic activity and elucidated some of the mechanisms of action of CuSO_4 . More recently work on the anthelmintic properties of copper has centered on the use of copper oxide wire particle (COWP) boluses. The efficaciousness of COWP has been demonstrated against abomasal nematodes, in particular *H.*

contortus and *T. circumcincta*, with little or no effect being noticed against intestinal parasites. Work has primarily been conducted under penned conditions, and has given efficacies of greater than 90% and 55% against adult *H. contortus* and *T. circumcincta* respectively (Bang *et al.*, 1990; Knox, 2002; Waller *et al.*, 2004; Burke *et al.*, 2004 & 2005). The results in the field have been more variable.

1.26 Biological control

1.26.1 *Bacillus thuringiensis*

Work into the use of the crystal (CRY) and cytolytic (CYT) proteins of the soil borne bacterium *Bacillus thuringiensis* has shown great success against a wide range of economically important plant-damaging and disease-carrying insects (reviewed by Bravo *et al.*, 2007). The bacteria secrete water soluble proteins, known as pore forming toxins, which create large crystalline formations in the infected insect host midgut (Bravo *et al.*, 2007). The effects of CRY and CYT proteins on nematodes have been less well investigated. Preliminary work has been conducted on free-living nematodes such as *C. elegans* and *Panagrellus redivivus* (Wei *et al.*, 2003) and parasitic nematodes such as *Ancylostoma ceylanicum* (Cappello *et al.*, 2006) and *N. brasiliensis* (Wei *et al.*, 2003). Work on ruminant GINs is also at an early stage with *in vitro* and/or *ex vivo* assays being conducted on parasitic and free-living stages of *T. colubriformis*, *T. circumcincta* and *H. contortus* (Hassanain *et al.*, 1999; Kotze *et al.*, 2005).

1.26.2 *Nematophagus fungi*

Nematophagus fungi were originally isolated from soil cultures (Cooke and Godfrey, 1964 cited in Larsen *et al.*, 1994) and patented in 1997 by Wolstrup *et al.*, (Patent number 5643568). The two main fungal isolates that have been investigated are *Duddingtonia flagrans* and *Arthrobotrys* species. These fungi form sticky nets or constricting rings that have been shown to be extremely effective at ensnaring nematode larvae *in vitro* and *in situ* in faeces. The fungi grow hyphae that enter the body of the trapped nematode larvae and facilitate digestion. The fungal chlamydospores are resistant to gastro-intestinal tract conditions, are able to pass

through the host unaffected (Larsen et al., 1992) and have previously been shown to be very effective (>80%) in reducing larval development in faeces deposited by small ruminants particularly in studies conducted in tropical and subtropical regions (Chandrawathani et al., 2004). However, work in Australia (Knox and Faedo, 2001) Denmark (Githigia et al., 1997), Sweden (Waller et al., 1994) and the UK (Jackson et al., 2005) has shown more variable results.

1.27 Immunological

Two approaches have been utilised to improve the host's immune response to gastrointestinal nematodes; vaccines and genetic selection of individuals for greater resistance/resilience to infections.

1.27.1 Vaccines

Research toward developing a vaccine for veterinary helminths of ruminants has been long running with only two notable successes on the commercial front, an attenuated *Dictylocaulus viviparous* vaccine in cattle (Dictol; Peacock and Poynter, 1980) and a recombinant *Taenia* vaccine in sheep (Lightowlers et al., 2000).

Work on ovine nematode vaccines has had a roller coaster ride, with early successes at identifying prospective candidates but ultimately frustration at generating protective recombinant antigens (Smith and Zarlenga, 2006). Early work focussed on killed vaccines (Clegg and Smith, 1978), attenuated whole parasite vaccines (Urquhart et al., 1966; Smith and Angus, 1980) and excretory/secretory (E/S) products from *T. circumcincta* (Rose, 1976 and 1978; Redmond et al., 2006) and *H. contortus* (Vervelde et al., 2002). These approaches showed limited success.

More recently, research has focused on the “hidden” antigen approach which involves immunization of animals with antigens derived from the gut lining of larval (Tavernor et al., 1992; Turnbull et al., 1992) and adult *H. contortus* worms (reviews by Smith and Zarlenga, 2006; Vercryse et al., 2007; Smith, 2008). The two most well defined, protective native antigens are from the intestinal brush border of *H. contortus*, H11 and H-gal-GP. Both native antigens have shown, individually and in

combination, substantial protection (>85% reduction in worm numbers) against naturally acquired *Haemonchus* infection (Munn et al., 1993; Smith and Smith, 1993; Smith et al., 2001). Unfortunately the high degree of protection afforded by the native antigens are lost with the recombinant proteins (Newton and Meeusen, 2003; Smith et al., 2003). Potential explanations for this lack of protectiveness of recombinant versions have included antigen related issues such as inaccurate configuration, incorrect post translational modifications, inefficient administration protocols, inhibition of activity due to contamination with other proteins and poor product stability (Smith and Zarlenga, 2006). Further suggestions for lack of activity include lack of conservation of antigens associated with protection between and within species and isolates (Maizels and Kurniawan-Atmadja, 2002) as well as the inherent variability observed between host immunological responsiveness to any vaccine (Smith and Zarlenga, 2006).

1.27.2 **Selective breeding**

Selective breeding programmes against a range of small ruminant diseases have been implemented with varying degrees of success throughout the world. Some such as the national breeding programs selecting for resistance against scrapie were driven by concerns regarding consumer health, and led to the initiation of compulsory programmes within the European Union member states in 2005 (Roden et al., 2006). Others programs, such the selection of resistant/resilient animals against nematode infections, aim to enable the maintenance of acceptable animal health and improved productivity. Breeding programs have been successfully implemented in New Zealand (NZ; Albers *et al.*, 1987; Baker, 1990; Bisset et al., 1991 and 2001) and Australia (Woolaston et al., 1992). Recent reports have described a flock of Rylington merinos in Western Australia that has been successfully bred to be resistant to GINs to such a degree that only 5% of the flock require treatment within the grazing season (Karlsson, 2008 cited in Van Wyk electronic debate, 2008), but this has taken in excess of 15 years to achieve.

1.28 Aims

The prevalence of resistance to the three commercially available broad spectrum anthelmintics has risen dramatically within many of the major sheep rearing countries of the world, particularly South America, South Africa and Australia. The resultant situation has left many farmers with difficult questions relating to strategies of worm control in their flocks. The main aims of this thesis are to gauge the prevalence of AR in Scottish sheep flocks, assess tools and strategies for investigating the selection and dissemination of AR gastro-intestinal nematodes and examine potential managerial factors that may be associated with the development of AR.

2 A survey of anthelmintic resistant nematode parasites in Scottish sheep flocks

David J. Bartley, Elizabeth Jackson, Kelly Johnston, Robert L. Coop, George B. B. Mitchell, Jill Sales and Frank Jackson. (2003). *Veterinary Parasitology*, **117** (1-2): 61-71

As mentioned in section 1.7 three classes of broad spectrum anthelmintic are available for the treatment of GIN of small ruminants in the UK. Reports of resistance to the oldest of these classes, the benzimidazoles (BZ), has been well documented in the gastrointestinal parasites of a host of livestock (section 1.12) and companion animal species (Boersema et al., 1991; Lyons et al., 2007; Cirak et al., 2004) throughout the world. The prevalence of resistance is highest in small ruminants, particularly sheep and goats. Within the United Kingdom, previous work estimated the prevalence of BZ resistance in sheep flocks from England and/or Wales to range from between 14 and 47% (Cawthorne and Cheong, 1984; Hong et al., 1992). A survey of 37 Scottish lowland sheep flocks conducted in 1991 identified BZ resistance in nine (24%) of the flocks using an *in vitro* egg hatch test (EHT; Mitchell et al., 1991).

The aim of this paper was to assess the prevalence of anthelmintic resistance (AR), in particular BZ resistance, in a cross section of Scottish sheep flocks. The non-random survey relied on responses from members of the Moredun Foundation, which is a charitable organisation with a remit to provide information on issues effecting ruminant health and productivity. One thousand members were mailed with details of the survey and invited to take part, 227 farmers responded favourably and received kits and instructions for collection and submission of faecal samples. Pooled mob faecal samples were sent under anaerobic conditions by 98 of the 227 respondents from across all geographical regions of Scotland. As in the previous Scottish survey by Mitchell et al., (1991) the EHT was used to examine submitted samples for thiabendazole (TBZ) resistance. Where possible a LDT was also

performed on the faecal material seeking evidence of levamisole (LEV) and ivermectin (IVM) resistance.

The EHT and LDT examine the effects of anthelmintics at inhibiting the “normal” behaviour of the parasite life stage, the EHT examines the effect of TBZ at inhibiting egg development and hatching and has been used to detect BZ resistance in the *Trichostrongylidae* e.g. *Ostertagiinae*, *Haemonchinae*, *Cooperinae*, *Trichostrongylinae* (Hunt and Taylor, 1989; Coles et al., 1992) and *Molineidae* e.g. *Nematodirinae* (Obendorf et al., 1986) families of nematodes and to detect LEV resistance in *trichostrongylid* nematodes (Dobson et al., 1986). The LDT assesses the inhibitory effects of BZ and LEV on the development of eggs or first stage larvae through to third stage larvae (L₃). Resistance is confirmed in the EHT if the ED₅₀ estimation, i.e. the concentration of TBZ at which 50% of the eggs do not hatch, is greater than 0.1 µg/ml (Whitlock et al., 1980; Kelly et al., 1981). The assessment of ED₅₀ estimations were performed using a logit model that makes allowance for natural mortality. The data analysis for the manuscripts was conducted using Genstat for Windows, 6th edition. A similar type of analysis was performed for the LDT: LD₅₀ estimates i.e. the concentration of anthelmintic at which 50% of the eggs did not develop to L₃.

Results were evaluated in a variety of ways to examine the impact of regional and geographical location i.e. lowland, upland or hill and enterprise type i.e. commercial or pedigree on the prevalence of AR. Overall TBZ resistance was detected on 64% of the farms examined, with prevalence's of resistance of 81%, 61% and 55% on lowland, upland and hill flocks respectively with no discernable differences between the prevalence rates of commercial and pedigree flocks, 64% and 60% respectively. Further analysis of these data highlighted regional differences of TBZ resistance prevalence but no appreciable difference between enterprise types. Regional and geographical difference may be due to a variety of reasons, differences in the initial starting frequency of genes for resistance in the initial population, selection pressures on the resident populations, the degree of refugia that remains on paddock following treatment, the amount of movement within and between farms, difference in the

length of grazing time, increased reliance on anthelmintics, stocking rates and/or climate (Hong et al., 1996; van Wyk., 2001; Coles 2005).

Breed differences may also play a part in the selection pressures exerted on a parasite population, breeds such as Texels and red Masai have been shown to be much more refractory to parasite infections than breeds such as Suffolk's this disparity in susceptibility may influence the requirement for treatments needed per grazing season (Barger, 1989; Kloosterman et al., 1992; Good et al., 2006). In addition to breed differences in susceptibility to parasites there are also differences in treatment efficacies due to decreased drug absorption, and presentation in sheep (Sangster et al., 1979) and cattle (Sallovitz et al., 2002) potentially due to differences in skin composition, physiology or fat deposition.

These Scottish BZ prevalence findings are very similar to those seen in more recent surveys conducted in temperate regions with prevalence of BZ resistance at 41% and 77% in New Zealand and Wales respectively (Waghorn et al., 2006a; Mitchell et al., 2006). Resistance to imidazothiazoles or avermectins was not detected in any of the small number of samples examined using the LDT in the survey. Data published more recently from work conducted in Australia showed that the LDT was unreliable at detecting ML resistance with field derived material particularly with *T. circumcincta* (Besier 1998; cited in Kotze et al., 2006). With the predominant species on both TBZ resistant and sensitive farms, in the Scottish sheep survey, being identified as *T. circumcincta*, the LDT results have to be viewed with some caution. The finding of monogenic resistance in the Scottish flocks is encouraging and may be in part attributable to the incompletely recessive (Sangster et al., 1998) or semi-dominant/co-dominant (Martin et al., 1988) nature with which the resistance is thought to be inherited. In other drug classes such as the avermectins where the gene(s) for resistance are thought to be dominant (Le Jambre et al., 2000), the detection of multigenic resistance is more readily and rapidly observed (Chapter 3) leading to fewer options for the control of parasitic gastro-enteritis (Chapter 6).

The survey detailed in the manuscript characterised the parasite populations phenotypically and highlighted an increase in ability to tolerate 2-3 times higher

concentrations of TBZ compared to those reported by Cawthorne and Cheong (1984). Subsequent work on *in vitro* bioassays, such as the larval feeding inhibition test (LFIT, Alvarez-Sanchez et al., 2005b; Bartley unpublished data) and larval migration inhibition tests (Kotze et al., 2006), have provided methodologies for investigating alternative phenotypic markers of resistance by examining the behaviour of first and third stage larvae respectively. The LFIT can characterise the feeding behaviour of resistant and susceptible nematodes populations to the macrocyclic lactone (ML) and levamisole (LEV) classes of anthelmintics and facilitates investigations into non-specific mechanisms of resistance such as the P-glycoproteins (PgP) and the cytochrome P450s (CYP) *in vitro* (general discussion).

Several problems exist with the EHT, firstly as with many other bioassays, its sensitivity is poor when the frequency of genes for resistance are low (Martin, 1989), therefore even though the prevalence data suggests an extremely high prevalence rate, this is potentially an under estimation of the problem. Secondly, as the test stands, it provides no data on the species composition of the sample, consequently it is possible to miss the early signs of resistance in species that do not form the bulk of the sample, for example *Trichostongylus* species are not found in numbers until around late summer early autumn. The survey was also unable to assess/investigate the material genotypically and therefore provided no information regarding the diversity (Silvestre and Humbert, 2002) or frequency (Stenhouse unpublished data) of BZ resistance alleles within the populations or to determine any information on the population genetics of the parasites (Gilleard and Beech, 2007). Since the publication of this manuscript other studies have been published detailing reliable, accurate, sensitive and repeatable ways of detecting potential markers of BZ resistance, Table 1-7.

The future

In the last decade new or novel acting broadspectrum anthelmintic compounds have been described or re-assessed, the cyclooctadepsipeptides, amino-acetonitrile derivatives, p-amino-phenethyl-m-trifluoromethylphenyl piperazine and paraherquamide (sections 1.9.1 to 1.9.4). The compounds have all been shown to

have a degree of activity against nematodes either *in vitro* and/or *in vivo* but still have many hurdles to jump before they will reach the market. Lessons need to be learnt from the use and marketing of current anthelmintics to ensure that these new compounds are used wisely and to prolong their usefulness and reduce the pressure of selecting resistance to them.

In conclusion, the work here provides an update into the prevalence of BZ resistance on lowland sheep farms and supplies a snapshot of the regional and geographical prevalence of resistance in Scotland. The findings suggest that the genes for BZ resistance are well established in Scottish sheep flocks and that the likelihood of reversion to susceptibility via the use of alternate drug classes (Waller et al., 1983 and 1985; Martin et al., 1988) is slight.

The questionnaire data provided an insight into the farming practices and management in relation to their anthelmintic resistance status. The work has been presented at both international and national meetings and conferences and has been cited in advisory group publications such as “Sustainable Control of Parasites in Sheep” and “Scottish Animal Health and Welfare Advisory Group”. These guidelines have been extensively distributed amongst the farming and veterinary communities in an effort to maintain the sustainability and competitiveness of the UK sheep industry. The published body of work has been instrumental in promoting the importance of anthelmintic resistance to the farming community and has also provided leverage for obtaining funding for anthelmintic resistance research at Moredun Research Institute.

Contribution to the work

All steps of experimental design from contacting potential participants, providing instructions for returning material, designing the questionnaire, processing and analysing the samples, assessing and streamlining the LDT, collating, preliminary analysis and interpreting the data. Involved in returning results to participants via mail and telephone and presentation of data at national and international conferences and meetings.

3 A small scale survey of ivermectin resistance in sheep nematodes using the faecal egg count reduction test on samples collected from Scottish sheep

Bartley, D. J., Donnan, A. A., Jackson, E., Sargison, N., Mitchell, G.B.B., Jackson, F. (2006) *Veterinary Parasitology* 137 112-118.

Detailed surveys investigating BZ resistance in GINs of sheep flocks had been conducted in the UK (Chapters 1 and 2), but to date no effort had been directed on determining the prevalence of IVM resistance. The aim of this study was to gain an understanding of the prevalence of IVM resistance on the selected Scottish farms by using a small cohort, predominantly from the Lothian and Borders regions. The criteria for these selections were made on both a scientific and practical basis. Farms from the Lothian and Borders regions were easily accessible for provision of survey materials and information such as anthelmintic usage and had a very high prevalence of TBZ resistance, 80% and 92% respectively (Bartley et al., 2003). The high prevalence of BZ resistance in these areas provided us with background information that they may be good areas to assess for the prevalence of IVM resistance. Also recently published research has suggested that resistance to one class of anthelmintic may predispose a population to developing resistance to another class of anthelmintics (Eng et al., 2006; Hughes et al 2007).

As mentioned in chapter 2, AR surveys have historically relied on *in vitro* tests, namely the EHT and LDT, because of their ease of use, cheap running costs and perceived reliability. In 1996 the Drenchrite® LDT was launched commercially in Australia for detection of multiple class resistance, including AVM resistance, in mixed species populations of ovine nematodes. Subsequent work showed it to be unreliable at detecting IVM resistance, particularly in field derived material that included *T. circumcincta* (Besier 1998; cited in Kotze et al., 2006). The company have since stopped using the test for avermectin (AVM) resistance detection. This poses problems for surveying IVM resistance in areas of the world where the predominant species is *T. circumcincta*, such as is the case in the UK.

Alternative *in vitro* tests have exploited the paralysing effects of the macrocyclic lactones (ML) on the somatic and pharyngeal musculature to characterise (ML) resistance but have not been used in AR surveys for assessing prevalence. One reason for this is that the appropriate test may again depend on the species being tested. Differences in sensitivity to IVM observed by Gill and Lacey 1998 for example, suggested that effects on motility are important for the expulsion of *H. contortus* and *T. colubriformis* but effects on the pharyngeal musculature are probably more important in *T. circumcincta*. In 1991 Gill et al., detailed a motility test for characterising AVM resistance in *H. contortus* L₃, a subsequent study has used the motility test to compare and characterise the effects of the novel anthelmintic compounds, the emodepsides, to commercially available anthelmintics in trichostrongylids (Schurmann et al., 2007). The larval migration test, like the larval motility test, assesses effects on somatic musculature but avoids the need for potentially subjective assessment of activity by examining the ability of anthelmintic treated L₃ to migrate through mesh filters. The test has been trialled with a range of parasitic nematode species from a range of hosts e.g. pigs (Petersen et al., 1996), sheep (Kotze et al., 2006) and horses (Matthews, 2008).

Tests characterising the feeding responses of adult worms (Geary et al., 1993) and first stage larvae (L₁; Alvarez et al., 2005b) following exposure to ML have been useful at investigating the importance of the pharynx as a site of action for ML compounds. To date none of these *in vitro* tests are routinely used tests for detection and diagnosis of ML resistance, and we are still reliant on *in vivo* tests. The controlled efficacy test (CET) is the gold standard test for detecting AR, it is an extremely versatile test that allows the precise determination of anthelmintic activity against all stages and ages of parasitic nematodes (Wood et al., 1995, more details in chapter 1.15.2) but is prohibitively expensive so the decision was made to use the faecal egg count reduction test (FECRT) for the survey.

The FECRT has been used world-wide to characterise and assess anthelmintic efficacy and/or survey for the presence/absence of AR in sheep (Hughes et al., 2007),

goats (Ram et al., 2007), cattle (Suarez and Cristel, 2007), horses (Kaplan et al., 2004), pigs (Dangolla et al., 1997), dogs (Kopp et al., 2007) and chickens (Sharma et al., 1990). The official World Association for the Advancement of Veterinary Parasitology (WAAVP) has described appropriate FECRT methodology for small ruminants to assess the efficacy of anthelmintic treatments. The test uses reductions in the faecal egg count (FEC) of treated animals compared to untreated control animals, with samples being collected after a specific period of time depending upon the drug class being investigated. Resistance is inferred if the reduction in FEC is less than 95% with lower confidence intervals of less than 90% (Coles et al., 1992, chapter 1.15.1).

As with the survey detailed in chapter 2, Moredun Foundation members were approached to take part in the survey, as well as farmers registered with the large animal practice of Edinburgh (Dick Vet) University. The respondents all received IVM oral drench (Oramec®, 0.08%; Merial Animal Health, Holland), 10 and 20 ml syringes, Banquet® Supaseal™ re-sealable bags for returning individual faecal samples, freepost-envelopes and detailed instructions for dosing the stock and taking the faecal samples. Since all previous UK reports of ovine derived IVM resistance had involved a single species, *T. circumcincta* (Sargison et al., 2001, 2004; Yue et al., 2003; Bartley et al., 2004), it seemed reasonable to assume that if cases of IVM resistance were to be found in numbers it would be in this species. In order to maximize the likelihood of assessing the IVM resistance status of predominately *T. circumcincta* populations, participants were requested to collect 30 pre-treatment faecal samples, ideally but not essentially, from peri-parturient ewes. The relaxation of immunity at and around parturition makes the ewe more susceptible to parasite infection at pasture, and to infections caused by the emergence of over wintering inhibited larvae that the animal may be carrying (Armour et al., 1966; Waller and Thomas, 1978). In the UK hypobiosis of *T. circumcincta* is variable, but commonly observed in ewes (Stear et al., 1995). The test animals were not to have received anthelmintic treatment, in the previous 4 weeks where non-persistent anthelmintics were being used or 8 weeks in the case of persistent anthelmintics and should have grazed ‘contaminated’ pasture. All of the sampled sheep were administered oral IVM

(Oramec®) via a syringe at the manufacturers recommended dose rate (MRDR; 0.2 mg/kg body weight) or at the dose rate appropriate for the weight of the heaviest animal in the group by the farmer. Drench efficacy was determined using one of two formulae, $(1 - [T_2/C_2]) \times 100$ (Coles et al., 1992) or $(1 - [T_2/T_1]) \times 100$ (McKenna, 1990; Kohapakdee, 1995), where C_2 is the arithmetic mean FEC of untreated control animals post treatment and T_1 and T_2 are the arithmetic mean FEC of animals pre- and post treatment respectively:

During January and July of 2004, 38 of the 50 farms that were approached to take part in the survey returned samples. Of these farms, 17 contained sufficient parasite material to be assessed with confidence (i.e. FEC \geq 150 eggs per gram (EPG) pre treatment) and eight samples needed to be viewed with caution due to the low numbers of eggs observed in their pre treatment samples (FEC $>50 < 150$ EPG). Results showed a much higher than expected prevalence of IVM resistance in the farms that were examined, with 6 of 17 farms (35%) with confirmed cases of IVM resistance and one suspected case. The efficacies on the resistant farms ranged from 66% to 92% whilst the efficacies from the sensitive farms were almost wholly 100%.

Three flocks (flocks 23, 24 and 25) were tested against all three broad spectrum anthelmintic classes and the results identified three cases of multiple class resistance, two with triple class resistance and one with double class resistance (FBZ and LEV).

As mentioned previously much debate has been made concerning the most appropriate way of analyzing the data generated from a FECRT including basic questions such as whether to use arithmetic or geometric means (McKenna, 1990), individual or pooled counts (Cabaret and Berrag, 2004; Morgan et al., 2005; McKenna, 2006a and 2006b; 2007) and what is the appropriate form of analyses e.g. Bootstrapping (Cabaret and Berrag, 2004), maximum likelihood mathematical techniques (Torgerson et al., 2005) or linear mixed model, followed by a Tukey's sequential trend test (Kaplan et al., 2007). Plate 3-1 illustrates a screen dump from a program devised and kindly supplied by Jacques Cabaret that estimates some of

these calculations, as well as providing boot strap re-sampling calculations. Bootstrapping is an approach that allows the operator to make statistical inference by estimating properties of an estimator such as treatment efficacy, by drawing many samples from a population. One standard procedure is the generation of a number of resamples, generally greater than 1000, from the observed dataset (and of equal size to the observed dataset), each of which is obtained by random sampling with replacement from the original dataset (<http://en.wikipedia.org/wiki/Bootstrapping>, last accessed 10SEP08). Previous reports have used various formulae to determine efficacy, some of these are detailed below, where T_1 and T_2 are the FEC of treated animals pre- and post-treatment respectively and C_1 and C_2 are FEC of the untreated control animals at the appropriate pre- and post days.

Method 1 - $1 - [T_2/C_2] \times 100$ using arithmetic means (Coles et al., 1992).

Method 2 - $(1 - [T_2/T_1]) \times 100$ using arithmetic means (McKenna, 1990).

Method 3 - $(1 - [T_2/T_1][C_1/C_2]) \times 100$ using arithmetic means (Dash et al., 1988).

Method 4 - $(1 - [T_2/T_1][C_1/C_2]) \times 100$ using geometric means (GM; Presidente, 1985).

Method 5 - $(1/n) \times \Sigma ((1 - [T_2/T_1]) \times 100)$ using individual counts (Cabaret and Berrag, 2004).

Method 6 - $(1/n) \times \Sigma ((1 - [T_2/T_1][C_1/C_2]) \times 100)$ using individual counts (Cabaret and Berrag, 2004).

Table 3-1 illustrates the differences in treatment efficacy estimations for flock 24 from the survey, derived by using the various formulae detailed above.

Table 3-1 Percentage efficacy estimates for sheep flock 24 treated with ivermectin (0.2mg/kg body weight) using 6 different calculation methods.

Sample	Faecal egg count (eggs per gram)				Individual efficacies	
	C1	C2	T1	T2	Method 5	Method 6
1	135	414	729	18	98	99
2	1764	1089	297	18	94	90
3	324	396	15	0	100	100
4	117	54	171	42	75	47
5	36	441	378	18	95	100
6	162	693	306	2	99	100
7	621	594	90	1	99	99
8	288	117	441	0	100	100
9	72	486	504	0	100	100
10	153	369	180	4	98	99
11	135	171	54	6	89	91
12	135	5	9	12	0	0
13	180	216	261	18	93	94
14	108	261	117	0	100	100
15	189	288	126	0	100	100
16	12	288	459	*	-	-
17	783	693	*	*	-	-
AM	285	412	275	8	91	89
GM [#]	134	280	136	4		

Method	% Efficacy	Method	% Efficacy
1	98	4	99
2	97	5	91
3	98	6	89

GM[#] - Based on egg count (n+1), * No sample

Efficacies for flock 24 ranged from 89%-99% with four of the method classifying the parasite populations as being IVM sensitive, and two methods, 5 and 6, suggested the worms were resistant (efficacies of 89% and 91% respectively). As a general rule, formulae using only treated animal results provide lower efficacies than those that include control animals (Miller et al., 2006) and hence diagnose resistance more readily. Though analysis of 210 previously published FECRTs by Mckenna (2006a) which compared four different methods of calculating percentage efficacy showed no

difference in the detection of anthelmintic resistance irrespective of whether they included untreated control data or not. The potentially small risk of over estimating resistance on farms due to only using treated animal calculations must be balanced with the practicalities of sample collection. In borderline cases where a false positive result might occur, further investigation may be required. Miller et al., (2006) expressed the need for a degree of caution when interpreting resistance when FECRT efficacies range between 90-95% because of the possibility of reporting either false positive or false negative results and McKenna (1990) suggested that a “suspected” resistance category be used for results between this range.

Plate 3-1 Screen dump of the “Bootstreat” program showing treatment efficacy estimates and associated bootstrapped re-sampling values for flock 24 following IVM treatment. (Program kindly supplied by J. Cabaret from INRA, France).

	C1	C2	T1	T2
Host 1	135	414	135	1
Host 2	1764	1089	729	18
Host 3	324	459	297	18
Host 4	117	396	954	1
Host 5	36	54	15	1
Host 6	162	441	171	42
Host 7	621	693	378	18
Host 8	288	594	306	2
Host 9	72	117	90	1
Host 10	153	486	441	1
Host 11	135	369	504	1
Host 12	135	171	54	4
Host 13	180	5	261	6
Host 14	108	216	117	12
Host 15	189	261	126	18
Host 16	12	288	459	1
Host 17	783	693	1	1

Initialization
 Erase
 Remove All
 Other treatment
 Calculate

Bootstrap
 Pulling numbers (between 100 and 2000): 2000 Bootstrap

Results
 Fecr1 (Coles) : 97.836 % : 98% (96% - 100%)
 Fecr3 (Kochapakdee) : 97.102 % : 97% (94% - 100%)
 Fecr4 (Dash) : 97.76 % : 98% (94% - 100%)
 Fecr5 (Presidente) : 98.537 % : 98% (94% - 101%)

The efficacies generated in this survey are from treatments at the MRDR. Work in Australia (Palmer et al., 2000) suggested that the inclusion of an IVM group that was treated at half of the MRDR (0.1mg/kg) would provide a significantly more sensitive technique for detecting resistance in the earlier stages of selection, particularly for species such as *T. circumcincta*. The reason for this increased sensitivity is due to

the differences between the MRDR i.e. 0.2mg/kg for IVM and the minimal effective dose rate for the dose limiting species. For example adult *Cooperia*, *Nematodirus* and inhibited *Teladorsagia* (Reid et al., 1976; Armour, 1980; Armour et al., 1982; Grimshaw et al., 1996) are “naturally” more refractory to IVM treatment than species such as *Haemonchus*. Surveys examining AR in sheep have used ½ IVM MRDR as one of the FECRT treatments in Australia (Rendell et al., 2006) and New Zealand (Waghorn et al., 2006a) to great success and may have provided a clearer/truer picture of the degree of resistance that was present in the flocks that were examined.

Practical and analytical adaptations have been made to the methodology to make it more acceptable to farmers for use in field prevalence surveys and, as mentioned earlier, statisticians for data analysis and interpretation. One practical alteration is the use of treated animals only, and using pre and post egg counts for the determination of efficacy (McKenna 1990; Kohapakdee et al., 1995). One area where the usefulness of the test can be improved is in determining the species composition of samples pre and post treatment (McKenna, 2007). Traditionally speciation has involved the coproculture of pooled faecal material to generate L₃ for identification using morphological traits. Recent advances in molecular biology techniques have presented new opportunities for development of high through-put assays that could potentially provide rapid, sensitive and accurate assays for the speciation of material based on specific markers such as internal transcribed spacers regions (ITS; Gasser and Hoste, 1995; Silvestre and Humbert, 2000; Wimmer et al., 2004).

As with previous reports of IVM resistance in the UK (Sargison et al., 2001, 2004; Yue et al., 2003; Bartley et al., 2004), the predominant species involved in most of the cases identified in the survey were, *T. circumcincta*, though surprisingly two cases of resistant *Trichostrongylus* were also identified. This finding of multigeneric IVM resistance in sheep was the first in of its kind in Europe, though cases have been reported in South Africa (Carmichael et al., 1987), Brazil (Echevarria and Trinidad, 1989) and New Zealand (Badger and McKenna, 1990). The finding of multigeneric IVM resistance may suggest that the selection process may be well advanced,

possibly more advanced than with BZ resistance (Chapter 2), within some UK sheep flocks. Possible reasons for the differences in findings between the BZ and IVM resistance surveys are discussed further in the general discussion but may include differences due to the; genetics of selection, mechanisms of resistance, selection pressures, persistency's of each drug class and length of time on market. The findings of high prevalence of BZ resistance and multigeneric IVM resistance in conjunction with the reports of multiple class resistance (Chapters 4, 5, 6 and 8) has serious implications for the sustainability of British sheep farming (General discussion).

The Future

One of the areas of weakness with the FECRT is the inability to detect resistance in the early stages of development. Stage specific differences in susceptibility to anthelmintic treatment have been observed in previous studies with *H. contortus* (Echevarria et al., 1992; Taylor et al., 2002) or *T. colubriformis* (Giordano et al., 1988; Shoop et al., 1990) and may be attributed to differences in the feeding behaviour of various developmental stages, drug bioavailability/presentation, stage specific differences in drug receptors/binding specificity and/or metabolic resistance mechanisms (Chapter 5; Marriner et al., 1985; Forrester et al., 1999; Paiement et al., 1999). The identification of stage specific differences in susceptibility to treatments highlights the need for genetic markers or other bio-markers that will enable a rapid and early identification of AR sensitivity and permit prompt early intervention. The early detection of AR is essential if producers are to stand any of maintaining a high degree of productivity in their stock.

As with the BZ survey the samples were only analysed phenotypically and no attempt was made to assess the material at a genetic level. The mechanisms for macrocyclic lactone resistance are not as well defined as with the BZs and are thought to possibly involve the glutamate (GluCl; Culley et al., 1994) and/or gamma-aminobutyric acid (GABA; Holden-Dye and Walker, 1990) gated chloride channels or ATP binding cassette (ABC) transporters such as PgP (Kerboeuf et al., 1999). A better understanding of the mechanisms of resistance will hopefully provide useful

molecular markers or *in vitro* tests for resistance detection and characterisation. Until such time as there are reliable and affordable *in vitro* methods for ML resistance detection, the FECRT will remain as the mainstay of detection in the field.

The increasing prevalence of both BZ and IVM resistance will make the decision making of some farmers more complex when it comes to controlling nematodes on their farms. As mentioned previously in chapter 2 new drugs will help alleviate the pressure on farmers with multiple resistances in the short term but are unlikely to remain wholly effective if used in the same fashion as the three broad-spectrum drugs that are on the market at present. Where applicable the routine use of pasture management and integrated management strategies will play an important role in the economic viability of some enterprises. Strategies such as the use of parasite replacement, combination treatments of compounds, including any new active compound that may come onto the market, are discussed in the general discussion. Loss in productivity, due to decreased carcass weight and wool production, is estimated to be around 10% on farms where moderately inefficient treatments are administered to stock (Barrett et al., 1998; Besier, 2007a). As treatment inefficiency increases and involves more species, losses will increase markedly.

In conclusion, the survey has provided the first clear evidence that the scale of ivermectin resistance in Scotland and possibly the UK is potentially higher than originally predicted, though caution must be observed due to the small non random cohort used for the survey. The identification of multigeneric resistance on two separate farms adds weight to this argument and further work on determining a clearer picture on the true prevalence of IVM resistance needs to be conducted urgently.

Again, as with the data generated from the BZ resistance survey, the work has been presented at both international and national meetings and conferences and has been cited in advisory group publications such as “Sustainable Control of Parasites in Sheep” and “Scottish Animal Health and Welfare Advisory Group” to highlight the need to administer effective quarantine treatments to newly purchased or in coming

animals, adopting best practice with regards to anthelmintic usage and to routinely monitor anthelmintic treatment efficacies on individual farms. Work published by Morgan et al., 2005 and McKenna in 2007 showed that treatment efficacies based on composite samples could be as accurate as those generated by individual samples. These findings could have a profound effect on the cost implications of conducting a FECRT and therefore the uptake by the farming community, leading to a better understanding of the scale of the problem in UK production animals.

Contribution to the work

The candidate was involved in all steps of experimental design from contacting potential participants, providing instructions for returning material, processing and analysing the samples, collating, analysis and interpreting the data. Involved in returning results to participants via mail and presentation of data at national and international conferences and meetings.

4 Characterisation of two triple resistant field isolates of *Teladorsagia* from Scottish lowland sheep farms

Bartley, D. J., Jackson, F., Jackson, E., Sargison, N. (2004). *Veterinary Parasitology* 123 189-199.

In chapters 2 and 3 the prevalence of BZ and IVM resistance in Scottish sheep flocks was detailed. These studies provided an indication of the extent of single drench class resistance in Scotland but gave no indication of the relationship between different class resistances in multiple resistant populations and more importantly how these multiple class resistant parasites could be treated. In 2001, Sargison et al., reported on a naturally infected flock of Suffolk lambs (Farm A) that showed continued signs of ill-thrift following treatment with ivermectin at the MRDR. A FECRT was subsequently conducted on the flock to examine the efficacy of oxfendazole (OFZ; Systemex®), levamisole (LEV; Levacide®) and ivermectin (IVM; Oramec®). These investigations showed that the flock were still shedding large numbers of *T. circumcincta* eggs following treatment, indicating a total failure i.e. 0% efficacy at 12 days post treatment. This finding, though not unique, was the first of its kind to be reported in sheep in Europe, multiple resistant *T. circumcincta* had been reported in goats in Scotland almost a decade earlier (Jackson et al., 1992). At around the same time, a second case of ill thrift was being investigated by the parasitology department at Moredun Research Institute. This suspected case involved a second flock (farm B) that was discrete from the first farm but in the same geographical area. The principal aim of this paper was to examine the efficacy of anthelmintic treatment, at the MRDR, against each of the isolates and to confirm the findings from the field, under strict laboratory conditions. The second aim was to investigate potential treatment regimes using the farm A isolate that might be effective at treating sheep with multiply resistant *T. circumcincta* and to identify possible quarantine treatments for bought in and relocated stock.

Faecal material from lambs from each of the two suspect resistant flocks was brought into the laboratory and cultured at 22°C for 10 days using the techniques as described by Coop et al., 1995. The resultant infective larvae (L₃) from each isolate were

passed through parasite naïve sheep to provide sufficient material for further examination. Faecal egg count reduction tests (Coles et al., 1992; farms A & B), controlled efficacy tests (CET, Wood et al., 1995; farms A & B) and EHT (Hunt and Taylor, 1989; farm B) were conducted on the resultant material. The material from farm B was only tested with LEV (Levacide[®] 7.5mgs/kg bodyweight (BW)), and IVM (Oramec[®] 0.2mg/kg BW) to fully ascertain their efficacy on adult worm populations, the EHT having already confirmed the TBZ resistance status. The material from farm A was subjected to a more extensive range of treatments, fenbendazole (FBZ; Panacur[®], 5mg/kg BW), LEV (as above), IVM (as above) and moxidectin (MOX; Cydectin[®], 0.2mg/kg BW) singly or with combinations of FBZ+LEV, FBZ+IVM or FBZ+LEV+IVM.

The CET results confirmed that both isolates, A and B, were resistant to all three broad spectrum anthelmintic classes i.e. reduction in total worm burdens of less than 95% compared to untreated controls (Coles et al., 1992 and 2006). Fenbendazole, LEV and IVM treatments were 59%, 88% and 60% respectively against the farm A isolate and N/A, 51% and 72% effective respectively against the farm B isolate. The efficacy results were, as would be expected, higher under strict laboratory conditions than that seen in the field. Factors such as feed quantity (Warner, 1981; Taylor et al., 1992; Ali and Hennessy, 1995a, 1995b and 1996) and quality (Warner 1981; Ali and Chick, 1992) and body condition of the animals (Van Gogh et al., 1990; Lespine et al., 2004; Perez et al., 2006 and 2007) can affect the bioavailability, absorption and presentation of the anthelmintic compounds. Other factors that can affect treatment efficacy in the field include developing immunity and concurrent infections within the animals. Even under strict laboratory conditions the CET can be affected by those factors identified above but these can be managed more efficiently.

The combination treatments had efficacies of 94%, 93% and 92% for FBZ+IVM, FBZ+LEV and FBZ+LEV+IVM respectively and showed improvement over the singly administered drenches but were ineffective at removing all of the worms, the only treatment with $\geq 95\%$ efficacy was with MOX. Moxidectin and IVM are both from the ML class of anthelmintics (Chapter 1.7.3) but MOX has been shown to be

highly effective at removing IVM resistant parasite in the field (review conducted by Kieran, 1994). The mechanism(s) for resistance (Chapter 1.19) are generally thought to be the same for both compounds with the difference in efficacy being attributed to the increased potency and/or persistency of MOX (Conder et al., 1993; Shoop et al., 1993; Sutherland et al., 1999). Resistance to MOX has been detected in sheep flocks from around the world (Sutherland et al., 1999; Love et al., 2003; Le Jambre et al., 2005; Wilson and Sargison, 2007) and for *T. circumcincta* and *H. contortus* it is generally preceded by a loss of persistency against the establishment of incoming larvae (Sutherland et al., 1997; Sutherland et al., 1999; Barnes et al., 2001; Sargison et al., 2005 – see chapter 6; von Samson-Himmelstjerna et al., 2007b). In the UK the label claim of persistence for Cydectin 0.1% oral drench for sheep against re-infection with *H. contortus* and *T. circumcincta* is 35 days (www.noahcompendium.co.uk). Although there may be differences in the susceptibility of IVM resistant parasites to MOX it is accepted that an increase in the prevalence of IVM resistance in UK sheep flocks (Chapter 3) would, following further selection, ultimately lead to a decline in MOX efficacy. Since the publication of this manuscript the first case of MOX resistance has been identified in Europe (Wilson and Sargison, 2007).

The results from the FECRT showed a good correlation with those from the CET; overall the reductions in faecal egg counts were higher than the reductions in worm burdens with the combinations and MOX alone but lower with the other three singly administered anthelmintics. An in-depth analysis by McKenna (2006a and 2006b) of 61 previously published cases of AR, where CET and FECRT data were available showed this positive relationship between the two assays to be common. The high FECRT efficacies observed with the combination and MOX alone treatments suggests that the test might be less sensitive at determining multiple class and MOX resistance than with resistance to BZ, LEV or IVM alone. Post-treatment FECRT samples were collected when the animals were euthanased 7 days post-treatment for the CET. The optimal time for re-sampling of treated animals is 3-7 days, 8–10 days and 14–17 days post treatment for the LEV, BZ, and ML respectively to avoid possible false positive/negative results (Coles et al., 2006). Since the time period

used in this study was shorter than that recommended by the WAAVP guidelines for detection of combination treatment resistance, it is possible that the efficacies may have been higher than expected. The different re-sampling times are related to the different modes of action and potencies of the various compounds. Levamisole has been shown to be less effective against the immature stages of some worms and therefore re-sampling needs to be conducted before maturation of surviving immature stages occurs (Grimshaw et al., 1996). Suppression of egg production may occur for up to 10 and 14 days post treatment with BZ (Martin et al., 1985) and ML treatments (Jackson 1993; Tyrell et al., 2002) respectively. If suppression of egg laying were an issue, then under the most extreme circumstances one might expect under-diagnosis of resistance. Examination of the egg count data from day seven shows little or no evidence of high levels of suppression of egg production following IVM and BZ treatments since the efficacies for the two drugs were 56% and 44% respectively.

The CET results showed that the use of combination therapy treatments improved the efficacy by between 4% and 35% compared to singly administered treatments (except MOX), but did not wholly remove the population. The variation in improvements would suggest that the isolate is not a homogeneous population where all of the parasites carry the genes for triple class resistance but are individuals that are at varying stages along the AR continuum. The interactions of the FBZ + IVM and FBZ + LEV were examined using the formula as described by Anderson et al., (1991) and found to be synergistic and additive respectively. Improvement in efficacies in previously reported trials have ranged from 0-99%. Differences have been attributed to the individual isolate and species variation and to the initial frequency of resistance alleles. The use of combinations have been advocated as a method of slowing down the selection of resistance (Barnes et al., 1995) where the initial frequency of resistance alleles was 0.01%, but where the initial frequency was $\geq 1\%$ these effects were greatly reduced.

In addition to animal welfare issues there are financial implications to the farmer regarding the presence of multi class AR. There are many ways of assessing the

financial implications of AR, firstly there are the purely practical losses incurred with the increased costs of buying and administering treatments, for example one treatment of BZ, LEV, IVM or MOX for a 50kg ewe costs around 15p, 14p, 25p and 43p respectively (prices obtained from www.wessexanimalhealth.co.uk on 11NOV07). If combination treatments are required, the costs increase accordingly. Additional, less easily defined, costs include time spent planning alternative strategies, investigating and sourcing alternative compounds, purchasing anthelmintics, gathering and returning stock, constructing or maintaining handling facilities and treating animals. Secondly there are the indirect financial costs incurred with the loss in productivity associated with chronic gastrointestinal infections e.g. ill thrift, slower weight gain, decreased carcass quality, decreased fleece weight and quality (Brunsdon and Vlassoff, 1982; Sykes et al., 1997). Again additional costs may include veterinary intervention, the purchase and provision of additional food stuff to ensure fattening of animals at allotted times, reduced market prices and potentially additional time spent dagging affected animals to prevent fly strike. Finally there is the ultimate cost associated with total failure of all available treatments, in two reported cases in the UK farms have been totally destocked with flocks being culled and the affected fields ploughed up and reseeded, plate 4.1 (Sargison et al., 2005 – chapter 6; Blake and Coles 2007).

The drastic decision taken in both these cases followed concerted efforts at maintaining productivity by lowering stocking rates, providing additional feed for lambs and treating of stock with MOX. The issue of the correlation between ill thrift and anthelmintic resistance is poorly defined and further research in this area is required urgently.

Plate 4-1 Images of farm A pre (2001) and post (2003-2006) identification of multiple anthelmintic resistance.

(Photographs kindly supplied by N. Sargison)



The results from this trial highlighted the fact that in the short term, the use of MOX alone and/or combination may provide a useful tool as a quarantine treatment in sheep. Ultimately the sustainability of sheep farming will rely on the implementation and maintenance of good farming practices including alternative control strategies, good farm and pasture management as well as responsible anthelmintic usage.

In conclusion, the trial which was conducted under rigorous laboratory conditions provided the first confirmed findings of triple class resistant *T. circumcincta* in sheep in Europe. The situation elsewhere in other European countries is unknown due to the small numbers of surveys that have been conducted. Although, subsequent reports have identified multiple class and/or genera resistance in; England (Blake and Coles, 2007), the Netherlands (Borgsteede et al., 2007), Slovakia (Cernanska et al., 2006), Spain (Alvarez-Sanchez et al., 2006) and Wales (Mitchell et al., 2006), which would suggest that multiple resistance is an increasing phenomenon throughout Europe to a greater or lesser degree.

The studies established that the farm A population was comprised of various subsets of worms, some that exhibited either solely single drench class resistance and others that carried the genes for multiple class resistance. The end result is a population that could be treated, though not eradicated, with MOX or combinations of anthelmintics

classes. The research has provided support for the recommendations currently being made for strategies that incorporate quarantine treatments to slow the spread of AR through its importation with new or returning stock.

Again, as with the data generated from the previous two chapters the work has been presented at both international and national meetings and conferences and has been cited in advisory group publications such as Moredun Foundations ACME message, “Sustainable Control of Parasites in Sheep; SCOPS” and “Scottish Animal Health and Welfare Advisory Group”.

The unique nature of the isolate has led to it being given an isolate designation (Moredun *T. circumcincta* isolate 5 or MTci5) and for further *in vivo* characterisation to be conducted assessing the treatment efficacies directed against larval stages, chapter 5.

Contributions to work

The candidate was involved in all steps of experimental design from submitting experimental committee forms, processing and analysing samples, collating, analysing and interpreting the data and presentation of data at national and international conferences as well as scientific and farming meetings.

5 Further characterisation of a triple resistant field isolate of *Teladorsagia* from a Scottish lowland sheep farm

Bartley, D. J., Jackson, E., Sargison, N., Jackson, F. (2005). *Veterinary Parasitology* 134 261-266.

The phenotypic characterisation of anthelmintic treatments directed against MTci5 using FECRT and CET, as detailed in chapter 4, provided valuable information about the treatment of an established, predominately adult, population of a triple class resistant isolate of *T. circumcincta*. A secondary finding from the trial was that survivors of MOX and combination treatments, were mostly immature, accounting for 59%, 83% and 100% of the surviving populations for FBZ+LEV, FBZ+LEV+IVM and MOX respectively. Very little work has been conducted into the effect of treatments administered to early developmental stages of infections or the possibility of differential stage specific selection for AR.

The aim of this experiment was to ascertain if there were stage specific differences in the expression of single class and multiple class anthelmintic resistance in the field isolate, designated MTci5, using a similar CET experimental protocol as outlined in chapter 4. The protocols differed in the following ways; all of the treatments were administered on day eight rather than day 28 P.I. and the lambs were slaughtered on day 22 not day 35 P.I.

The CET results confirmed that the immature stages of *T. circumcincta* expressed resistance against single drench families (AVM, BZ and imidazothiazoles) and combinations of these drenches. The only anthelmintic which provided a greater than 95% efficacy was MOX. The variations in efficacies against MTci5 between treatments administered on day 28 P.I. and day eight P.I. ranged from between -50% to +22% (Table 5.1.)

Table 5-1 Percentage efficacies and associated variations of treatments administered against the MTci5 isolate of *T. circumcincta*, 8 and 28 days post infection.

Treatment	Percentage efficacy		Percentage difference day 8 v day 28
	Day 8	Day 28	
Fenbendazole (FBZ)	36	59	-23
Levamisole (LEV)	38	88	-50
Ivermectin (IVM)	82	60	+22
Moxidectin	97	98	-1
FBZ + IVM	86	94	-8
FBZ + LEV	60	93	-33
FBZ + LEV + IVM	88	92	-4

The efficacy of treatment against larval and adult stages can vary between different parasites and also by the specific anthelmintic being used. Echervarria et al., (1992) reported that IVM administered at the MRDR, was less effective against the fourth-stage larvae (day 6 P.I.) of an anthelmintic sensitive isolate of *H. contortus* compared to treatments given on day 21 P.I., with efficacies of 96% and 98.9% respectively. By way of contrast studies using the pig parasite *Oesphagostomum dentatum* showed an increase in IVM efficacy (0.3mg/kg) when directed against larval stages in comparison to adult stages, 91% and 69% effective respectively (Petersen et al., 1996). Taylor et al., (2002) proposed that thiabendazole (TBZ) treatment efficacies were lower against pressurised populations of a BZ resistant *H. contortus* when they were treated at 21 days P.I. compared to if they were treated at 5 days P.I. The parasites were pressurised with TBZ, at the full MRDR, for five generations. Surviving larvae, post treatment, from one generation were used to infect another animal for the next round of treatments i.e. both the adult and juvenile populations were ultimately exposed to five rounds of TBZ treatments.

The results are unequivocal and confirmed that stage specific variations in susceptibility to broad spectrum anthelmintics can occur within *T. circumcincta* populations. There are a number of potential reasons for these differences; a) differences in drug receptors and/or binding specificity (Paiement et al., 1999). b) Non specific anthelmintic handling mechanisms such as the ATP binding cassette transporters e.g. PgP or the oxidative enzymes e.g. cytochrome P450 (CYP) being

upregulated or differentially expressed at different developmental stages - see general discussion. c) Reduced drug bioavailability and/or presentation to larval stages. The parasite may be “buffered” from the deleterious effect of anthelmintic treatments in the gastric glands. When the concentration of anthelmintic has decreased sufficiently, the parasite can recover and resume development. d) Distinct feeding behaviour of the various developmental stages leading to different drug exposure. e) Physiological effects at mucosal epithelium with larvae developing into adults between day 8 and 16 (Scott et al., 1998) affecting anthelmintic availability and/or presentation (Marriner et al., 1985). f) Rise in gastric pH due early parasitic damage, reducing solubility and absorption of anthelmintics with a faster rate of excretion (Prichard 1985).

To conclude, the findings of significantly lower treatment efficacies in the current trial potentially have serious implications for the detection and treatment of multiple resistant *T. circumcincta* populations in the field. Resistance selected within or exhibited by larval stages obviously can not be detected via the most widely applied resistance test the FECRT. Since at present we have no means of detecting the presence of, or size of, larval populations in the live animal it follows that it is only when these immature stages finally mature that we will be able determine their sensitivity to anthelmintics. Given the ability of many endoparasitic nematodes to inhibit at an early stage of development for many months, the potential risk posed by resistant larval stages is one that needs consideration when designing biosecurity measures. However as the results from this study show, even combination treatments may not be wholly effective in eliminating immature resistant stages. For these reasons it is important that when returning post quarantine treated animals to pasture it is important to utilise the natural refugia found on contaminated pasture rather than using clean grazing.

Future

Due to the unique nature of the isolate, further *in vitro* characterisation of the MTci5 isolate has been conducted in the laboratory. Real Time PCR and pyrosequencing methodologies have been developed for detecting SNPs within the β -tubulin isotype

1 gene associated with BZ resistance, F167Y, A198E & F200Y. Preliminary work would suggest that the point mutations at codons 198 and 167 are not present in MTci5 and that the mutation at codon 200 does not wholly explain the BZ resistance story. Genotyping based on adult and larval populations pre and post FBZ treatment showed that both SS and RS survived treatment/exposure. Pre-treatment populations of L₃ and adult worms contained on average 9%, 48% and 43% SS, RS, and RR individuals, respectively, whereas post-treatment there were 2%, 21% and 77% SS, RS, and RR individuals (Stenhouse, 2007).

In tandem with the molecular work extensive effort has been placed into investigating the role of non specific mechanisms of resistance such as the oxidative enzymes (cytochrome P450; CYP) and the membrane proteins (P-glycoproteins; PgP). Further work into these areas has been conducted as part of a European Union funded project entitled PARASOL (www.parasol-project.org/) and is detailed further in the general discussion.

At present, the treatment of multiple resistant isolates in the field relies heavily on the use of milbemycins, though this is likely only to be a short term solution as was highlighted in conclusion of chapter 4 and by the recent emergence of MOX resistance in the UK (Wilson and Sargison, 2007). Two areas need to be investigated in the light of multiple class resistance, firstly there is an urgent need to investigate the specific and non-specific mechanisms that might be involved in single class and multiple class resistance and determine if these mechanisms will have a detrimental impact on any new compounds that may be brought onto the market. Secondly there is a need to assess alternative or integrated management systems that may be useful for maintaining productivity and economical viability in the face of a potentially ever decreasing arsenal of effective broad spectrum anthelmintics.

Contributions to work

The candidate was involved in all steps of experimental design from submitting experimental committee forms, processing and analysing samples, collating,

analysing and interpreting the data and presentation of data at national and international conferences as well as scientific and farming meetings.

6 Failure of moxidectin to control benzimidazole, levamisole and ivermectin resistant *Teladorsagia circumcincta* in a sheep flock

N. D. Sargison, F. Jackson, D. J. Bartley, and A. C. P. Moir. *Veterinary Record* 156 (4):105-109, 2005.

The MLs have been used extensively in the treatment of both endo- and ectoparasites of ruminants (Shoop et al. 1995) for over 30 years. Moxidectin, as described previously is a second generation ML from the milbemycin family, with a greater potency and persistency than the first generation parent compounds (Steel, 1993). As with the BZ and LEV classes of anthelmintics the MLs have not been immune to the development of anthelmintic resistance within ovine and caprine gastro-intestinal nematodes. The prevalence of avermectin (IVM) resistance in the Lothian and Borders region of Scotland, as detailed in chapter 3, has increased at an alarming rate. Fortunately resistance to the milbemycins (MOX) is still uncommon world-wide (Sutherland et al., 1999; Love et al., 2003; Le Jambre et al., 2005; Wilson and Sargison, 2007). Results by Ranjan et al., (2002) suggest that ML resistance is slower to develop than the other classes of broad-spectrum anthelmintics and that the development of resistance to MOX is slower than that seen with IVM. The difference in the speed of development to resistance can be attributed to a host of factors, chapter 1.12, but include initial resistant gene frequency, dominance/recessive nature of inheritance or whether resistance is mono- or polygenic. The loss of persistency, as mentioned previously, is generally thought to be a precursor to full blown resistance with MOX (Ridler et al., 2002), although it is thought that side resistance occurs between the avermectins and milbemycins (Shoop et al., 1993).

The aim of this trial was to investigate the effectiveness of moxidectin (Cydectin®, Fort Dodge), which has a higher therapeutic efficacy against *T. circumcincta* than IVM (Sutherland et al., 1999), in controlling nematodes on a farm where resistance to BZ, LEV and IVM had been identified and confirmed in the laboratory (Sargison et al., 2001; Bartley et al., 2004). Faecal material from animals grazing on pasture

on the farm had been collected, cultured and characterised previously (MTCi5; chapters 4 & 5). The farm was set stocked and intensively farmed with a heavy reliance on anthelmintic intervention to maintain productivity; the farm had solely used IVM for the previous two years prior to this investigation. Faecal egg counts (FEC) were conducted on ewes following MOX treatments around parturition/turn out and at housing, and on lambs throughout the grazing season. The trial ewes and lambs were turned out onto the “contaminated” pasture, where MTCi5 had been isolated, in April 2002. In June 2002 the pasture was subdivided into six one hectare paddocks and the weaned lambs were separated and set stocked in groups of 21 – 22 animals. Each of the lambs from the six trial paddocks received five MOX treatments throughout the grazing season. Parasite naïve tracer lambs were grazed on the six trial paddocks at the beginning (April), middle (June) and end (August) of the grazing season and euthanased four weeks later to estimate the size and composition of worm populations on the pastures.

The initial results shown in figure 6.1 (MOX 2, MOX 3 and MOX 4) confirmed the effectiveness of MOX, at the MRDR, in treating a BZ, LEV and IVM resistant parasite population under field conditions. This finding of high MOX efficacy against IVM resistant nematodes is in agreement with others such reports from around the world (review by Kieran, 1994).

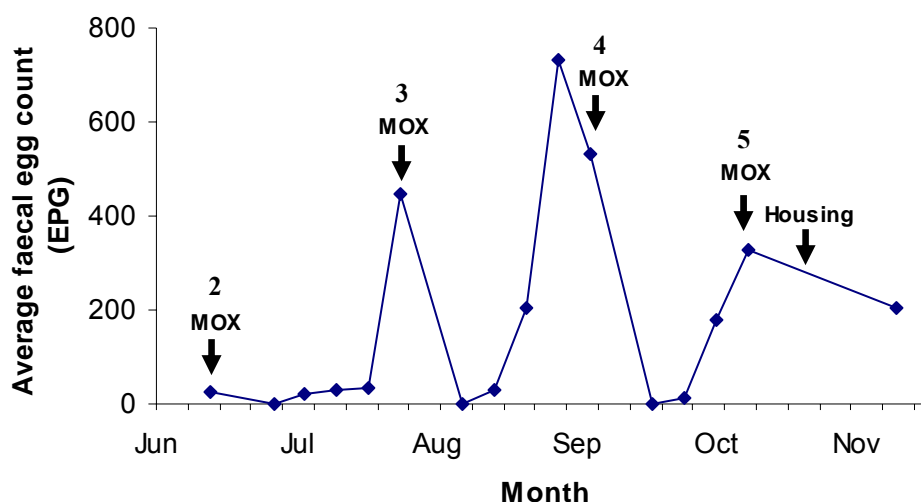


Figure 6.1 Arithmetic mean *trichostrongyle* faecal egg counts of lamb's pre and post moxidectin treatment on trial paddocks. MOX 1 (not shown) was administered in May to control *Nematodirus*.

Overall the reappearance of eggs, as determined by FEC, was quicker to return and of a greater magnitude compared to that seen following the initial round of treatments (MOX 1, data not shown). The reappearance of trichostrongyle eggs in faeces, table 6.1, occurred on average around 35 post treatment (P.T.) following the weaning treatment in June (MOX 2) and around 25 and 28 P.T. in the subsequent treatments, July (MOX 3) and September (MOX 4) respectively. In the UK the label claim for persistence with Cydectin® 0.1% oral drench for sheep against reinfection with *T. circumcincta* is 35 days (<http://www.noahcompendium.co.uk>, last accessed 10SEP08) meaning that if the population is monogeneric i.e. only one genera, egg counts should not be seen for around 50-55 days, much longer than observed here. The finding of reduced persistency of oral MOX and IVM and albendazole controlled release capsules against resistant populations of *T. circumcincta* have been observed in previous trials (Sutherland et al., 1997, 1999; Vickers et al., 2001).

Table 6-1 Time interval before reappearance of *trichostrongyle* eggs in egg counts of lambs grazing six trial paddocks following moxidectin treatments in June (MOX2), July (MOX 3) and September (MOX 4)

Paddock	Egg reappearance (days post moxidectin treatment)		
	MOX 2	MOX 3	MOX 4
A	35	21	35
B	21	21	21
C	35	21	28
D	42	28	28
E	>42	21	28
F	28	35	28
Average	32	25	28
Median	35	21	28

The apparent loss of persistency may be attributable to a number of factors, a) whilst high levels of MOX are maintained in the host only highly resistant individuals are able to reproduce and in so doing, rapidly amplify numbers of resistant larvae on pasture (Herd, 1984). b) Removal of susceptible individuals may improve the conditions in the host for resistant survivors and consequently increase the burdens and thereby increase pasture contamination (Sutherland et al., 2002b). c) One of the primary effects of MLs on IVM resistant female worms at an early stage of selection is a suppression of ova production and expulsion, as resistance progresses this effect become lesser and egg production occurs earlier and so egg are detected sooner (Jackson et al., 1993; Tyrell et al., 2002).

The increased potency and persistency of second generation MLs has rapidly made them the anthelmintic of choice for many farmers in the treatment of both ecto- and endoparasites. The indiscriminate and often ill timed use of these compounds may select heavily for ML resistance in parasite populations. Treatments administered to animals at times where the parasite burden in the host, or on pasture, is low means that pastures are reseeded with only resistant parasites (Besier and Love, 2003). The treatment of ewes around parturition is also commonplace (70% of surveyed farmers treated either pre or post lambing, unpublished data) with a view to reducing pasture contamination and thus reducing the parasite exposure to their offspring. The practices of treating ewes pre-lambing with long acting products or post lambing with a short acting product have been identified as risk factors associated with the presence of IVM (Lawrence et al., 2006) and albendazole resistance (Leathwick et

al., 2006). Further analysis of the works suggests that the practice is less selective for resistance in Merino ewes, than in Romney ewes, because the rate of re-infection in Romney ewes is low due to them re-acquiring their anti parasite immunity rapidly, around two weeks post partum (Leathwick et al., 1999). Therefore dilution of resistant parasites on pasture i.e. *in refugia* is less (Leathwick et al., 2006). Ewes on the farm in this current trial were treated immediately post lambing and this may have inadvertently applied a greater selection pressure for ML resistance, though the peri-parturient relaxation in immunity in Suffolk ewes is thought to be similar to that reported in Merino sheep by Barnes and Dobson (1990). Larval establishment increases from, the normal level, around 1% to 65% in the two week period prior to parturition and remains high until the end of lactation (Barger 1997).

The confirmation of *T. circumcincta* as the predominant species post treatment was expected. Previous work conducted on the farm and in previous Scottish anthelmintic resistance surveys has consistently linked this parasite species with reports of resistance.

So in conclusion, the results of this trial have highlighted the value of MOX treatments in the control of multiple resistant nematodes, in particular *T. circumcincta* and have confirmed its usefulness as a potential quarantine treatment. But the results have also highlighted the problems that can be encountered with a total reliance on one compound. The failure to effectively quarantine treat infected animals has been identified as one the most important factors in the increased prevalence of anthelmintic resistance seen in the UK (Coles, 1997) and has been implicated in the intercontinental spread of anthelmintic resistance (Himonas and Papadopoulos, 1994; Corba et al., 2002). In a questionnaire survey conducted in 2004, over 10% of respondents failed to quarantine treat newly purchased and transient stock with any anthelmintic and of those administering quarantine treatment 75% administered only a BZ or an IVM. The advice for quarantine treatments at present remains as the use of a triple class combination, preferably using a MOX containing compound in combination with a BZ and LEV and the grazing of treated stock on contaminated pasture after drenching (Dobson et al., 2001).

The use of MOX in the treatment of gastro-intestinal nematodes needs to be implemented wisely in order to maintain its usefulness in the future. The confirmation of the first UK case of MOX resistant *T. circumcineta* (Wilson and Sargison, 2007) highlights this point, and again raises the question of potential strategies for prolonging the usefulness of the compounds we have.

Contributions to work

The work was initiated by Neil Sargison as part of an ongoing investigation into ill thrift in the affected commercial sheep flock. The candidate was involved in the collation, analysis and interpretation of the data and preparation of the manuscript. The data has been presented nationally and internationally at scientific conferences and incorporated into advice given to farmer and veterinarians.

7 Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland

N. D. Sargison, F. Jackson, D. J. Bartley, D. J. Wilson, L. J. Stenhouse and C. D. Penny. *Veterinary Parasitology* 145 (1-2):65-76, 2007.

Efforts to investigate potential risk factors associated with the presence or absence of AR, particularly resistance to the ML class, have focussed heavily on statistical interpretation of questionnaire data obtained from farm holdings where details of treatment efficacies are also available. Much of this work has been conducted in areas where the prevalence of multiple class resistance is high, such as New Zealand and Australia (Suter et al., 2004, and 2005; Lawrence et al., 2006 and 2007; Larsen et al., 2006; Leathwick et al., 2006; Hughes et al., 2007).

The aim of this paper was to investigate and discuss the possible management risk factors that might have lead to the emergence of multiple class resistance in four discrete lowland sheep flocks in the south-east of Scotland.

Each of the four farms was either served by the Edinburgh University (Dick) Veterinary large animal practice or the Moredun Research Institute. The presence of multiple class resistant *T. circumcincta* had been confirmed on all four of the farms between the years 2001 and 2004, FECRT results shown in figure 7.1. The sheep flocks on each of the four farms differed in size, with holdings of 70, 60, 700 and 210 breeding ewes on 11, 20, 100 and 28 hectares for farms, 1, 2, 3 and 4 respectively. The length of ownership for each of the properties ranged from between 10 and in excess of 30 years.

An overview of the management practices adopted by the owners for each of the flocks can be seen in Table 7.1 the results are expressed as the farm either implementing (✓) or ignoring (✗) particular recommended practices.

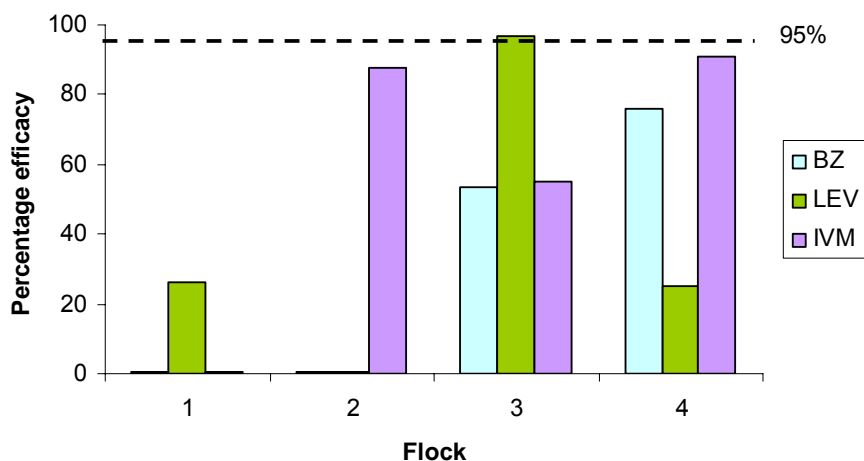


Figure 7.1 Percentage reduction of *Teladorsagia circumcincta* faecal egg counts, based on larval differentiation, following benzimidazole (BZ; 5 mg/kg body weight (BW)), levamisole (LEV; 7.5 mg/kg BW) or ivermectin (IVM; 0.2 mg/kg BW) treatment in flocks from four farms in south east Scotland.

The results in Table 7.1 demonstrated that, as with the respondents from the surveys in chapter 8, there was a variable uptake on the implementation of recommendations for decreasing the selection pressures on parasite populations. The practice of annual rotation of anthelmintic drug classes was universally adopted and rotational grazing with cattle was adopted where feasible but the uptake of long standing recommendations such as treating animals at the full MRDR, reducing drench frequency and quarantine treating imported animals on arrival were not.

A crude assessment of Figure 7.1 and Table 7-1 would suggest that a greater degree of resistance was present on the farms where fewer recommendations were implemented, though caution must be observed because no account is taken into the external non management factors that might influence the selection of resistance such as initial starting gene frequency, length of ownership or breed differences (other factors are detailed in chapter 8).

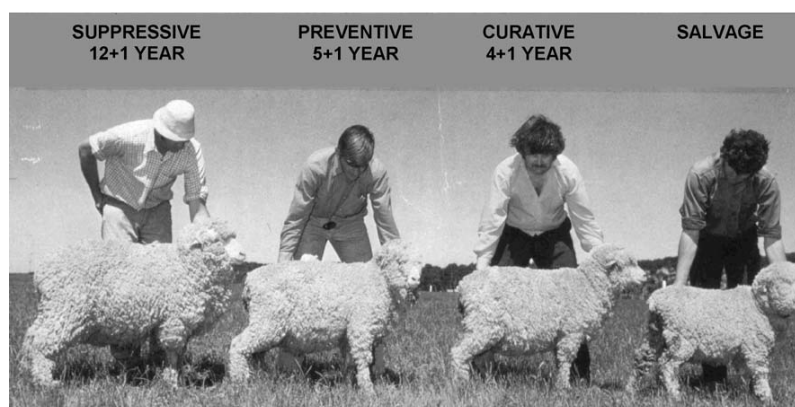
Table 7-1 Uptakes of recommendations for reducing selection pressures on parasite populations by owners of flocks 1, 2, 3 and 4. Shaded responses are recommended practices.

Managerial practice	Flock			
	1	2	3	4
Dose & move to “contaminated” pasture	✘	✘	✓	✓
Annual drench class rotation	✓	✓	✓	✓
Rotational/co-graze sheep and cattle	✘	✓	✓	✘
Quarantine drench imported stock	-	✓	✓	✘
Dose animals at full therapeutic dose rate	✓	✘	✓	-
Drench frequency (>28 day intervals at pasture)	✘	✘	✓	✓
Weaning <50% of lambs onto “clean“ pasture	✓	-	-	✓
Use of non persistent drugs pre/post lambing	✘	✘	✘	-
Total “non-recommended” practices adopted	4	4	1	2

So why are recommendations/advice not being implemented? The reasons are numerous and include the following; a) Recommendations unknown by farmer, unlikely within these farms due to their close involvement with academic/teaching facilities and a readily available source of information. b) Recommendations acknowledged, but farmer unable to implement advice due to practical issues, such as not having land or resources to rotationally or co-graze sheep and cattle. c) Recommendations ignored because they are not believed to be viable options for the farmer, conflicts with actual or perceived notion of acceptable management practices for maintaining productivity in the flock. For example it may suit the farmer to move newly treated animals onto clean grazing because they accept that the risk of increasing the likelihood of selecting AR is offset by the financial gains achieved by getting animals to saleable size quicker and therefore off pastures sooner. d) Recommendations disregarded because of the belief that there is no reason to follow advice, why fix what is not broken? Good productivity is being achieved, so why change the practices that have worked on the farm for generations? For example, the administration of a drench to ewes pre-tupping is widely thought to improve the condition of ewes and thereby improve fertility, this practice was performed by over 70% of farmers questioned by Sargison and Scott (2003) and Bartley et al., (Chapter 8) though the positive benefit has never been scientifically substantiated. A review of work conducted in New Zealand found that on a purely financial level the cost of treatment could not be recovered in over 30% of farms examined (Brunsdon et al.,

1983). e) Recommendations disregarded because of conflicting information from different parties or because of a change of attitudes, such as the advice provide by pharmaceutical companies that suppressive treatment of stock will lead to an increased return for the outlay costs, see plate 7.1. Though increased treatment frequency has been shown to heavily select for AR which may eventually result in decreased productivity (Barrett et al., 1998).

Plate 7-1 Promotional material issued by Merck, Sharpe and Dohme in the 1960-1980s to highlight the profitability of using Thibenzole® to suppressively treat Merino sheep in Australia Published in Waller (2006), reproduced with kind permission of Elsevier Limited.



f) Recommendations overlooked because of lack of facilities or assistance/labour to implement strategies. g) Recommendations not applied because of a lack of understanding due poor communication of information to veterinarians, farmers or farm advisors. The implementation of effective quarantine treatment on some farms has been hampered by the good uptake of annual drug class rotations advice. Farmers may administer the drug that is being used for that years rotation even though this may be ineffective as a quarantine treatment. Another example relating to anthelmintic rotation relates to widespread confusion about the classification of drug groups (Sangster, 1999). There are only three main classes of broad-spectrum anthelmintics available for use in ruminants but, in 2007 in the UK, there were at least 42 products being marketed by nine different pharmaceutical companies for use in sheep alone (www.noahcompendium.co.uk/). h) Recommendations may be ignored because it is cheaper, more convenient and simpler to administer an anthelmintic drench treatment rather than to use valuable time and resources to investigate/research alternative strategies. There is also a strong belief that problems can be rectified when they occur. Reports from Australia would suggest a changing

trend from testing for the presence of anthelmintic resistance in a flock to buying the most potent single or combination available (Besier, 2007b).

The influence that each recommendation has, individually or in combination, on the selection of resistance is unknown. Ineffective treatment of stock either due to resistance or improper use of anthelmintics can, though not always, result in slower growth rates (Barrett et al., 1998), decreased wool and carcass values, decreased fertility, increased mortality and ultimately increased costs for administering effective remedial action. Converse to this, research assessing the productivity and profitability of six merino flocks in South Eastern Australia over a 8-14 years period found that both parameters could be maintained or increased in self replacing flocks via the use of integrated parasite management (IPM) programs even in the face of BZ and LEV resistance (Larsen et al., 2006). The ultimate findings from their work was that AR was only one part of the whole farm management and needed to be kept in perspective and that IPM programs such as monitoring FEC to assess appropriate time for drenching, monitoring drench efficacy, genetic selection of animals for resistance to worms and the incorporation of pasture management strategies could be used to adequately control the selection of AR.

Larsen et al., (2006) also noted that many tactics for worm control were specific to particular climates and geographical regions, one example being the use of refugia based strategies to provide a pool of unselected parasite populations on pasture to dilute out resistant populations. These strategies are effective in areas with Mediterranean type climates (dry hot summers and drought) but were less suited for areas with consistent summer rainfall. Refugia based strategies resulted in lower productivity and an increased chance of PGE because sufficient parasites can survive on pasture through-out the grazing season with-out the need for leaving animals untreated.

The Future

As mentioned above and in chapter 8 there is a need to adopt strategies on farms that allow for the maximum productivity whilst maintaining sustainability and cost

efficiency. Waller (2006) stated that in order for livestock producers to implement IPM strategies in favour of the blanket use of chemical approaches, they would need to be reassured that reliable economic/management benefits would be achieved by the correct use of a particular method.

There is a need to provide good solid empirical data to support recommendations made by scientists and pharmaceutical companies to ensure that the information that is supplied to farmers, veterinarians and livestock advisors is as accurate, precise and trustworthy as possible. The back lash from misinformation or inappropriate information could lead to livestock owners ignoring advice in the future with potentially devastating consequences.

Contributions to work

The work was initiated by Neil Sargison as part of an ongoing interest in the relationship between anthelmintic resistance, ill thrift and potential risk factors adopted on farms. The candidate was involved in the collation, analysis and interpretation of data and preparation of the manuscript.

8 Questionnaire survey on the gastro-intestinal parasite control practices used on Scottish sheep farms

Bartley, D. J., Jackson, E., Coop, R. L., Sales, J., Mitchell, G. B. B., Jackson, F.

The incidence and severity of disease associated with gastro-intestinal parasites in ruminants is governed by an extremely complex set of factors with a number of major contributing elements, Figure 1.2; both the infra- and supra-populations of the parasites are susceptible to intervention and have been targeted in the past (shaded boxes, Figure 1.2). Routine strategies for the controlling PGE fall into two broad categories, pasture/host management or chemotherapy based, though in order to achieve sustainable parasite control or a combination of both is often desirable. Decisions made to adopt particular strategies are rarely considered in isolation from other parts of the farm management because they ultimately compete for resources such as labour, land, finances and time (Morley and Donald, 1980).

Pasture/host management strategies rely on reducing host parasite interaction by utilising; stocking rates, timings of parturition and weaning, availability and use of pasture rotation and spelling, appropriate use of reseeded or “clean” pastures, availability and feasibility of grazing pastures with non susceptible hosts or alternate host species and the incorporation of bioactive forages such as sainfoin and sulla into the grazing systems. Chemotherapy has been the mainstay of parasite control in livestock for centuries, but the practice was revolutionised in the 1960s with the introduction of thiabendazole (TBZ). Treatments prior to the 1960s tended to have had high dose rates, low safety margins and/or narrow spectrum of specificity but TBZ was shown to an extremely safe anthelmintic with efficacy against a range of helminths (nematodes and cestodes) at an effective dose rate of less than 100 mg per kilogram body weight (BW). In the following years, anthelmintic efficacy was refined to such an extent that it became possible to control parasites with dose rates as low as 0.2 mg/kg BW.

The heavy reliance on chemical intervention for parasite control has led to farmers, veterinarians and livestock advisors being bombarded with information and recommendations for the responsible and effective use of anthelmintics and measures for slowing the rate of development and transmission of AR. For many years recommendations focussed on either minimising host parasite interaction and/or reducing selection pressures for AR in parasite populations, these included a) the movement of freshly treated animals to clean pasture (Boag and Thomas, 1973). This practice was identified as a method for improving productivity, such as overall wool production (Morley and Donald, 1980) and lamb growth by 10-20% prior to weaning (Waller and Thomas, 1978) and prolonging the usefulness of anthelmintic compounds. The practice has subsequently been shown to select heavily for resistance with survivors of treatment reseeding clean pastures rapidly (Coles, 2001). b) Alternate grazing schemes with hosts of the same species (Michel, 1969). This practice is generally reliant on the use of older stock of the same species, which are less susceptible to establishment and development of ingested infective larvae, mopping up parasite contamination on the pasture to allow more naïve animals to graze safely. c) The use of cattle in an alternate grazing scheme to reduce pasture contamination (Southcott and Barger; 1975). An alternate or co-grazing pasture scheme is very dependant on the structure of the resident parasite populations on a farm and the degree of cross transmission that can occur. Morley and Donald (1980) classified this into three levels, firstly, very little cross infectivity between species and no reproduction in parasites e.g. *Ostertagia/Teladorsagia*, *Oesophagostomum*, *Nematodirus* and *Bunostomum*. Secondly, moderate cross infectivity but with reduced fecundity and patency of infection e.g. *Cooperia* and intestinal *Trichostrongylus* and finally little inhibition of infectivity between species which may disappear after a few generations e.g. *Trichostrongylus axei* and *Haemonchus*. Though generally this classification is still thought to hold true, subsequent work has shown that nematodes such as *Nematodirus battus* and *C. oncophora* can be transmitted well by both young calves and sheep (Coop et al., 1984; 1988; 1991; Bairden and Armour, 1987; Bairden et al., 1995). Concern has also been raised that the use of cattle may lead to increased selection for resistance due to fewer parasites *in refugia* (Good et al., 2006). d) Optimise stocking rates in order to provide best

possible grazing efficiency and therefore nutritional status of flock (Morley and Donald, 1980). Increased numbers of animals on pasture can lead to increased pasture contamination and decreased pasture availability, though increased grazing can lead to less favourable conditions for parasite development and survival, this is particularly relevant for less hardy parasite species such as *Haemonchus* (Southcott et al., 1967) but is less of a problem for *Teladorsagia* and *Nematodirus* (Downey, 1968). e) Annual rotation of anthelmintics classes (Kettle et al., 1982). Rotation was originally proposed as a means of reducing the selection pressure on any single drug class and possibly promote reversion to susceptibility (Prichard et al., 1980) thereby extending the effectiveness of all of the classes. However simulated mathematical models conducted by Barnes et al., (1995) found substantial development of resistance with annual, five year and ten year rotations. f) Avoid introducing resistant worms with newly purchased stock or sheep which return home from tack grazing on other farms or from common grazing (Abbott et al., 2004). All animals should be treated with full-dose combinations (Prichard et al., 1980; Anderson et al., 1988) and/or moxidectin (Dobson et al., 2001), yarded for 24 – 48 hours post treatment and turned out on to dirty/contaminated pasture. g) Identifying and using the most appropriate drench at the most suitable time (Abbott et al., 2004). Following this recommendation allows the farmer to target specific parasite species and avoid off-target and potentially ineffective combination products such as combination fluke and roundworm treatments or injectable MLs for ecto- and endo-parasite treatments. Treating ewe's pre parturition with MOX or ABA has been shown to select for ML resistance under New Zealand conditions (Lawrence et al., 2006; Leathwick et al., 2006). h) Reduction of drench frequency (Prichard et al., 1980; West & Probert, 1989) particularly in adult sheep/goats (Kettle et al., 1981, 1982, 1983) is again considered to be beneficial at decreasing the rate of selection by reducing the selection pressure on populations. i) Restricting feed for 24 hours prior to drenching has been shown to increase bioavailability and consequently efficacy of FBZ by around 40% compared to conventionally treated animals (Hennessey et al., 1991; Hennessey and Ali, 1997; Barrett et al., 1998). j) Follow the manufactures guidelines for anthelmintic usage i.e. dose at the MRDR for the heaviest animal in the group, ensure good dosing technique i.e. over the tongue not into the mouth and administer

correctly stored anthelmintics accurately with well maintained equipment. k) Routine testing of anthelmintics for the presence of lack of efficacy (Coles and Roush, 1992). The current sustainable control of nematode parasites in sheep (SCOPS) guidelines for reducing the selection pressure for anthelmintic resistance in the UK can be found at -

http://www.defra.gov.uk/animalh/diseases/control/wormcontrol_BW.pdf

(Last accessed 10SEP08; Abbott et al., 2004).

In more recent times emphasis has been placed on the maintenance of susceptibility to anthelmintic treatments by utilising the untreated, and therefore unselected, parasite population *in refugia* (Prichard et al., 1980; Barnes et al., 1995; van Wyk, 2001). Kettle et al., (1982) estimated that autumn treatments in New Zealand would target around 5% of the nematode population with around 95% of the population being on the pasture as larvae or eggs and therefore unselected.

The aim of this paper was to examine how advice on parasite control had been implemented on farms and identify areas where management practices may be being poorly adopted by selected Scottish sheep farmers. The questionnaires (Plate 8-1 and Plate 8-2) were designed to assess if there was a relationship between the results and the presence or absence of anthelmintic resistance. The paper is a combination of two questionnaires that were sent out and completed as parts of the BZ resistance survey conducted in the year 2000 (Chapter 2) and the IVM resistance survey conducted in 2004 (Chapter 3). The participants in the survey were mostly members of a charity called the Moredun Foundation, one of its roles is the dissemination of information regarding infectious diseases of small ruminants especially sheep. Members would potentially, over the preceding 10 years, have received three newsletters on the control of gastrointestinal nematodes and improving drench techniques to minimise the risk of selecting or importing anthelmintic resistance (Jackson and Coop 1994; 1999 and 2003) and been given the opportunity to attend talks on the subjects.

In the 2000 survey one thousand Scottish members of the Moredun Foundation (MF) were approached to participate in a survey to examine the prevalence of anthelmintic resistance as well as farming practices. The questionnaire consisted of 29 questions, covering; farm demographics and characteristics, pasture management procedures, anthelmintic usage and animal health information sources, Plate 8-1. At the time, as supplementary information for the farmers, a list detailing the class of all UK registered anthelmintics for use in small ruminants was sent out. The definitions of anthelmintic classes can be found in Chapter 1.7. In 2004, the questionnaire was an abridged version of the 2000 survey containing 14 questions relating to anthelmintic usage, farm demographics and characteristics and pasture management procedures, Plate 8-2.

The surveys were conducted in the spring of 2000 and 2004. On receipt of completed questionnaires the information was entered in duplicate onto the database by two separate operators and then cross referenced against each other to ensure accurate input of the data.

The questionnaire responses and resistance status details from the 2000 survey were used to investigate potential risk factors that might be the presence of resistance to anthelmintics. An analysis of variance of ED₅₀ estimates were conducted in Genstat for Windows 6th edition by fitting a general linear model with the AUNBALANCED directive. The standard errors of ED₅₀ were very variable and in order to ensure that assumptions about the residuals in the model were satisfactory, the estimated ED₅₀'s were ranked in size and the ranked values of ED₅₀ were used as the outcome variable in all the fitted models. There was no strong evidence from this survey that any of the management practices examined greatly affected TBZ resistance, but increased stocking rates, geographical location and farm type all tended to be positively associated with the presence of TBZ resistance.

Anthelmintic Resistance Questionnaire

ALL DATA WILL BE TREATED AS CONFIDENTIAL

Please tick the appropriate box or boxes unless otherwise stated

1. Farm name?			
2. Post code?			
3. Total area of pasture?	Acres orHectares	
4. Type of enterprise?		Lowland predominantly? <input type="checkbox"/> Hill or upland predominantly? <input type="checkbox"/> Crop or conservation land predominantly? <input type="checkbox"/> How much is permanent pasture?	
5. Annual numbers of sheep?		Ewes:	Lambs: Tups:
6. When are your lambing times? State which months:			
7. Annual numbers of goats?		Does:	Kids: Billies:
8. Do you co-graze, rotationally graze or graze your animals separately?		Graze separately: <input type="checkbox"/> Rotationally graze: Sheep & Cattle <input type="checkbox"/> Sheep & Goats <input type="checkbox"/> Co-graze: Sheep & Cattle <input type="checkbox"/> Sheep & Goats <input type="checkbox"/>	
9. Do you treat your animals with anthelmintic? If not what do you do instead?		Yes: <input type="checkbox"/> No: Organic <input type="checkbox"/> Grazing management <input type="checkbox"/> Other (please state) <input type="checkbox"/>	
10. How often do you drench - numbers of times and which months of the year? Circle the appropriate month(s).		Number of times per annum: Ewes: 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5 <input type="checkbox"/> 6 <input type="checkbox"/> 6+ <input type="checkbox"/> Lambs: 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5 <input type="checkbox"/> 6 <input type="checkbox"/> 6+ <input type="checkbox"/> Months - Circle the appropriate month(s). Ewes: J F M A M J J A S O N D Lambs: J F M A M J J A S O N D Rams: J F M A M J J A S O N D Does: J F M A M J J A S O N D Kids: J F M A M J J A S O N D Billies: J F M A M J J A S O N D	
11. How do you drench your animals? (Tick all that apply)		Ewes Lambs Follow a set drench program <input type="checkbox"/> <input type="checkbox"/> At sign of disease <input type="checkbox"/> <input type="checkbox"/> At housing <input type="checkbox"/> <input type="checkbox"/> When docking/h hoof trimming <input type="checkbox"/> <input type="checkbox"/> Weaning <input type="checkbox"/> <input type="checkbox"/> At turning out <input type="checkbox"/> <input type="checkbox"/> Pre tugging <input type="checkbox"/> <input type="checkbox"/> Pre/post lambing <input type="checkbox"/> <input type="checkbox"/>	
12. What class of anthelmintic(s) did you use this year?		Class I <input type="checkbox"/> Class II <input type="checkbox"/> Class III <input type="checkbox"/> (Benzimidazoles) (Levamisole/Morantel) (Macrocyclic lactones)	
13. How long have you been using present drug?		1 Year 2 Yrs 3 Yrs 4 Yrs 5 Yrs 5+ Yrs Ewes: <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Lambs: <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	

14. What anthelmintics have you used in the past 5 years?	Ewes				Lambs					
	'00	'99	'98	'97	'96	'00	'99	'98	'97	'96
	Class I	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	Class II	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Class III	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
15. How often do you change your anthelmintics?	Never: <input type="checkbox"/>				Each time you use drench <input type="checkbox"/> Every 3 months <input type="checkbox"/> Every 6 months <input type="checkbox"/> Annually <input type="checkbox"/> Longer <input type="checkbox"/>					
16. Do you drench all of your ewes/lambs/tups or do you drench selectively?	Drench selectively: <input type="checkbox"/>				Yes, all: Ewes <input type="checkbox"/> Lambs <input type="checkbox"/> Tups <input type="checkbox"/>					
17. Do you drench animals brought onto the farm?	No: <input type="checkbox"/>				Yes, with: Class I <input type="checkbox"/> Class II <input type="checkbox"/> Class III <input type="checkbox"/>					
18. How do you determine amount of drench to use?	Estimate weight <input type="checkbox"/> Dose to average weight of flock <input type="checkbox"/> Weigh & dose to heaviest animal <input type="checkbox"/> Weigh individual animals and dose accordingly <input type="checkbox"/>									
19. Do you ever withhold food before drenching?	No: <input type="checkbox"/>				Yes (How long before?): <input type="checkbox"/>					
20. What form of anthelmintic do you use to treat you sheep/goats? (Tick all that apply)	Drenches: <input type="checkbox"/>		Injectables: <input type="checkbox"/>		In feed: <input type="checkbox"/>					
21. Do you use anthelmintics with additional Cobalt/Selenium?	No: <input type="checkbox"/>				Yes: <input type="checkbox"/>					
22. Where do you find information about anthelmintics & deworming program? (Tick all that apply)	Journals/magazines <input type="checkbox"/> Advertisements <input type="checkbox"/> Meetings <input type="checkbox"/> Veterinarians <input type="checkbox"/> Other farmers <input type="checkbox"/> MAFF / SAC VIS <input type="checkbox"/> Pharmaceutical Reps <input type="checkbox"/> Other (Please specify) <input type="checkbox"/>				Rank _____ _____ _____					
23. Rank the three most importance sources, with 1 as the most important and 3 the least.										
24. Do you believe the efficiency of drenches are more, less or unaltered compared to previous years?	More: <input type="checkbox"/>		Less: <input type="checkbox"/>		Unaltered: <input type="checkbox"/>					
25. Do you have any confirmed anthelmintic resistance?	No: <input type="checkbox"/>				Yes, to which family: Class I <input type="checkbox"/> Class II <input type="checkbox"/> Class III <input type="checkbox"/>					
26. Do you move your animals to clean pasture after treatment?	No: <input type="checkbox"/>				Yes: <input type="checkbox"/>					
27. Do you have a problem with any other parasites?	No: <input type="checkbox"/>				Yes: Liver fluke <input type="checkbox"/> Tapeworm <input type="checkbox"/> Scab/Lice <input type="checkbox"/> Blowfly <input type="checkbox"/>					
28. Do you use ectoparasitic treatments?	No: <input type="checkbox"/>				Yes: Dips <input type="checkbox"/> Pour-ons <input type="checkbox"/> Macrocyclic Lactones <input type="checkbox"/>					
29. When do you use ectoparasitic treatments?	Spring: <input type="checkbox"/>		Summer: <input type="checkbox"/>		Autumn: <input type="checkbox"/>		Winter: <input type="checkbox"/>			

Thank you for taking the time to complete the questionnaire.

Plate 8-2 Copy of the anthelmintic resistance questionnaire used in the survey conducted in the year 2004

Anthelmintic Resistance Questionnaire -2004

ALL DATA WILL BE TREATED AS CONFIDENTIAL

Please tick the appropriate box or boxes unless otherwise stated

1. Farm name?													
2. Total area of pasture?Acres orHectares												
3. What percentage is used solely for use of grazing?													
4. Type of enterprise?	Lowland predominantly?			<input type="checkbox"/>									
	Hill or upland predominantly?			<input type="checkbox"/>									
	Crop or conservation land predominantly?			<input type="checkbox"/>									
5. Annual numbers of sheep?	Ewes:			Lambs:			Tups:						
6. When are your lambing times?	State which months:												
7. Do you co-graze, rotationally graze or graze your animals separately?	Graze sheep		Rotationally graze:			Co-graze:							
	Separately: <input type="checkbox"/>		Sheep & Cattle <input type="checkbox"/>			Sheep & Cattle <input type="checkbox"/>							
	Sheep only: <input type="checkbox"/>		Sheep & Goats <input type="checkbox"/>			Sheep & Goats <input type="checkbox"/>							
8. How often do you drench - numbers of times and which months of the year?	Number of times per annum:												
	Ewes: 1 <input type="checkbox"/>		2 <input type="checkbox"/>		3 <input type="checkbox"/>		4 <input type="checkbox"/>		5 <input type="checkbox"/>		6 <input type="checkbox"/>		6+ <input type="checkbox"/>
9. How do you drench your animals? (Tick all that apply)				Ewes			Lambs						
	Follow a set drench program			<input type="checkbox"/>			<input type="checkbox"/>						
	At sign of disease			<input type="checkbox"/>			<input type="checkbox"/>						
	At housing			<input type="checkbox"/>			<input type="checkbox"/>						
	When docking/hoof trimming			<input type="checkbox"/>			<input type="checkbox"/>						
	Weaning			<input type="checkbox"/>			<input type="checkbox"/>						
	At turning out			<input type="checkbox"/>			<input type="checkbox"/>						
	Pre tugging			<input type="checkbox"/>			<input type="checkbox"/>						
Pre/post lambing			<input type="checkbox"/>			<input type="checkbox"/>							
10. What class of anthelmintic(s) did you use this year?	Class I <input type="checkbox"/> (Benzimidazoles)			Class II <input type="checkbox"/> (Levamisole/Morantel)			Class III <input type="checkbox"/> (Avermectins / Macrocylic lactones)						
11. What anthelmintics have you used in the past 5 years?			Ewes					Lambs					
			'04	'03	'02	'01	'00	'04	'03	02	'01	'00	
	Class I		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
	Class II		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Class III		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
12. Do you drench animals brought onto the farm?	No: <input type="checkbox"/>			Yes, with:			Class I <input type="checkbox"/>						
							Class II <input type="checkbox"/>						
							Class III <input type="checkbox"/>						
13. How do you determine amount of drench to use?	Estimate weight			<input type="checkbox"/>									
	Dose to average weight of flock			<input type="checkbox"/>									
	Weigh & dose to heaviest animal			<input type="checkbox"/>									
	Weigh individual animals and dose accordingly			<input type="checkbox"/>									
14. Do you move your animals to clean pasture after treatment?	No: <input type="checkbox"/>			Yes: <input type="checkbox"/>									

Thank you for taking the time to complete the questionnaire.

A total of 97 (43%) completed questionnaires were returned from the initial 227 farms that noted an interest in participating in the 2000 survey and 33 (66%) of the initial 50 respondents from the 2004 survey. Questionnaires were returned from large farms (>1000 animals) and smallholdings (>30< 50 animals) alike and from all geographical regions of Scotland in 2000 and predominantly from the Lothian and Borders regions in 2004. Responses were predominantly received from hill or upland farmers. Farms ranged in size from 5 to 2500 hectares with holdings of between 30 and 4970 sheep.

Anthelmintic usage was extremely high on participating farms, with around 98% of farmers treating both adult sheep and lambs throughout the year. Annual treatment frequencies in ewes and lambs were variable, (Range: 0-7 times per annum for both classes of animal) with a median of 2 and 3 treatments per annum (TPA) respectively. Ninety nine percent (93 from 94) of respondents used oral drenches, 33% (31 from 94) used injectable anthelmintics and 1% (1 from 94) reported the use of in feed anthelmintics.

It is apparent from the results that farmers place a great importance on worm control and anthelmintic resistance with over two thirds of the respondents seeking advice from veterinarians. Suter et al., (2005) found that farmers in Australia who used veterinarians as their primary source of advice were half as likely to have IVM resistance develop on their farms compared to farms that relied on other sources of information.

Some of the advice and recommendations provided to farmers regarding the treatment and control of gastro-intestinal nematodes, as identified in the SCOPS guidelines, such as not treating and moving animals to clean pasture are being reasonably well adopted (over 60% take up). However in some areas such as the administration of quarantine treatments attitudes did not appear to have changed over the five years period with less than around 80% of respondents administering a single drench (Table 8-1) but only 20% of respondents administering a dual or triple class combination.

Table 8-1 Percentage uptakes of recommendations for reducing selection pressures on parasite populations by respondents of the 2000 and 2004 surveys. Shaded responses are recommended practices.

Recommendation	2000 (%)		2004 (%)	
	Yes	No	Yes	No
Dose & move	46	54	39	61
Drench rotation	84	16	90	10
Rotational/co-graze	66	34	56	44
Quarantine drench	85	15	88	12
Restrict food	79	21	-	-
Dose at MRDR	51	49	56	44

The results from both the 2000 and 2004 surveys have to be viewed with caution because of the biased non random nature of the selection of respondents, with all participants either being members of the Moredun Foundation or the large animal practice of the Edinburgh University (Dick) Veterinary School, the small size of the dataset and the potential for misleading results to be collated with respondents giving “expected” rather than “actual” responses. Results from areas that require a degree of long term recall such as drench class used over the last five years may also be subject to a degree of error (Maingi et al., 1996). Overall the results highlighted that the uptake and implementation of advice relating to recommendations for slowing the selection and spread of anthelmintic resistance was variable.

In light of the ever increased prevalence of anthelmintic resistance in Australia and New Zealand, much work has been conducted examining potential risk factors associated with the presence of ML resistance (Suter et al., 2004, and 2005; Lawrence et al., 2006 and 2007; Larsen et al., 2006; Leathwick et al., 2006; Hughes et al., 2007). Factors that were positively associated with the presence of IVM resistance included; using visual signs to assess the worminess of stock, weaning greater than 50% of lambs on paddocks not grazed by lambing ewes for three months or more, the presence of resistance to either BZ or LEV but not to both, breed of sheep and their requirements for treatment, the use of long acting anthelmintic formulations in ewes pre-lambing, importing resistant parasites with purchased stock

and the failure to ensure accurate administration of drench, by not testing drench gun prior to use. The research also identified one factor that was negatively associated with IVM resistance; returning lambs back to the same paddock after drenching.

Over half of the risk factors identified in the Australian and New Zealand studies support the hypothesis, put forward by Prichard et al., (1980) and van Wyk (2001), that the most important factor in slowing the development of IVM resistance is maintaining a susceptible parasite population *in refugia*. In response to the findings there has been much debate regarding the suitability, sustainability and appropriateness of many of the “best practice” recommendations that have been promoted in the past.

The Future

Unfortunately the questions posed in the 2000 and 2004 questionnaires were not constructed in a way that would allow exploitation of refugia to be investigated under Scottish conditions. Future questionnaires must incorporate questions regarding refugia to ensure that the recommendations given out to our farmers are as pertinent and relevant as possible.

In conclusion strategies need to be implemented to slow selection, development and spread of AR but in order for any strategy to succeed it must ultimately manage parasitism and anthelmintic resistance whilst maintaining acceptable levels of productivity. There are no “blue print” recommendations that can be used for all farmers and that it is important that individual farmers, their veterinarians and advisors assess the situation at the individual farm level in order to develop and implement appropriate treatment strategies.

Contributions to work

The candidate was involved in the construction and development of the questionnaire as well as the collation, analysis and interpretation of the data for preparation of the

manuscript. The data has been presented nationally and internationally at scientific conferences and incorporated into advice given to farmer and veterinarians.

Questionnaire survey on the gastro-intestinal parasite control practices used on Scottish sheep farms

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The prevalence of anthelmintic resistant nematode populations has increased in the United Kingdom. Surveys conducted on sheep farms provide prevalence data that range from 23 – 64% for benzimidazole (BZ) resistance (Hong and others 1996; Bartley and others 2003) and potentially up to 35% for ivermectin (IVM) resistance (Bartley and others 2006). Control of gastro-intestinal parasites in the UK is heavily reliant on chemotherapy. Recognition of the crucial role played by chemoprophylaxis and the need to maintain high levels of efficacy of the current broad spectrum anthelmintics has led to the farmers being bombarded with advice on methods for slowing the rate of development of resistance and limiting its transmission through animal movement. For many years recommendations for reducing the selection and dissemination of anthelmintic resistance have included an annual rotation of anthelmintics; drenching animals at the manufacturers recommended dose rate; the reduction of drench frequency in adult sheep; checking for anthelmintic resistance; the discontinuation of drug classes which are not effective and the treatment of new stock on the farm with full-dose combinations of a macrocyclic lactone

and a imidazothiazole (Coles 1997; Dobson and others 2001; Sargison and others 2003; Abbott and others 2004). This short communication outlines the results of two separate questionnaires that were designed to enable the examination of how this advice had been implemented on selected Scottish sheep farms where some information on the anthelmintic resistance status of the farm was known. Participants in the surveys were mostly members of the Moredun Foundation, the surveys were collected as part two anthelmintic resistance surveys examining the prevalence of BZ resistance throughout Scotland using egg hatch assays in 2000 (Bartley and others 2003) and the prevalence of IVM resistance in the Lothian and borders region using faecal egg count reduction tests in 2004 (Bartley and others 2006).

A total of 97 completed questionnaires were returned from an initial 227 farms in 2000 and 33 from 50 in 2004. Responses were predominantly received from hill or upland farmers. Farms ranged in size from 5 to 2500 hectares with holdings of between 30 and 4970 sheep. A summary of the results from the two surveys are shown in table 1.

Information regarding anthelmintic usage and the implementation of perceived “best farming practices” is readily available to farmers from a variety of sources, this questionnaire highlighted the fact that the advice published within historical and current guidelines is being adopted at variable levels between farms. Within our cohort, veterinarians were cited by almost two thirds of respondents as the commonest source of information regarding gastro-intestinal helminths and their treatment. These figures differed from those detailed previously (Coles, 1997) who reported that veterinarians were rated as the most influential source of information by only 24% of respondents. This indicates either possible national differences in the emphasis that farmers place on parasitism and its management within their flocks, or the way that these farmers utilise their veterinarians for advice.

Within the current survey we investigated the possible relationships between farming practices and the presence or absence of anthelmintic resistance, though no significant associations could be found, possibly due to the small

sample size but areas of interest that were identified from the survey are discussed further.

Table 1 Questions and responses from surveys into management practices of sheep farmers in 2000 and 2004.

Questions	Variables	Responses (n)	
		2000	2004
Type of enterprise?	Lowland	19	11
	Hill/upland	75	19
	Other	2	0
Farm size (median)	Hectares	131	180
Annual numbers of sheep? (median)	Ewes	472	646
	Lambs	639	730
	Tups	15	20
Do you co-graze, rotationally graze or graze your animals separately?	Separately	36	4
	Sheep only	N/A	11
	Rotate sheep & cattle	20	8
	Co-graze sheep & cattle	49	11
Do you believe that efficacy of your anthelmintic(s) has changed over time?	More effective	19	N/A
	Unaltered	65	N/A
	Less effective	9	N/A
How do you determine amount of drench to use?	Estimate weights	33	12
	Average weight	15	3
	Heaviest animal	48	17
	Individually	1	2
Do you ever withhold food before drenching?	No	77	N/A
	Yes	20	N/A
Do you move your animals to clean pasture after treatment?	No	52	19
	Yes	44	12
Do you have a problem with any other parasites?	None	28	N/A
	Fluke	31	N/A
	Tapeworm	17	N/A
	Scab	16	N/A
	Blowfly	46	N/A

N/A - not asked

Anthelmintics were widely used, 98% of farmers used chemotherapy and/or chemoprophylaxis in both adult sheep and lambs. Annual treatment frequencies in ewes and lambs were variable, (Range: 0-7 times per annum for both classes of animal) with a median of 2 and 3 treatments per annum (TPA) respectively in both years of surveying. Ewe treatments pre-tupping and around parturition were commonplace almost three-quarters of the farms examined in 2000 (71% and 72% respectively) used these occasions to treat animals. This is probably based on the common perception that the pre-

tupping treatment promotes better condition in ewes for servicing and increases conception rates, though this is an area of debate. The treatment of the ewes pre/post lambing is based on the premise that it will reduce parasite contamination laid down on pasture and therefore reduce the infection levels to which lambs are exposed. Interestingly there appeared to be no correlation between timings of ewe treatment and a reduction in the frequency of lamb treatments. Lambs received treatments predominantly at weaning and at signs of disease.

There appears to be an association between the use of “mowers” to clean up pastures, either through rotational grazing or co-grazing between cattle and sheep, and a reduction in the use of anthelmintics usage in lambs. The median numbers of TPA for lambs on farms that graze their animals separately or only had sheep was 3.5, compared to 2, 3 and 2 TPA on farms where rotational grazing, co-grazing or combination of rotational and co-grazing with cattle occurred respectively. These findings differ slightly from those reported earlier by Gettinby and others (1987), where farms that alternated grazing between sheep and cattle used more anthelmintic annually compared to the average respondent in their survey. The reasons for these differences are not clear but might include changes in the meteorological or geographical climate, management practices adopted on the farms or attitudes of respondents, brought about through an increased awareness of the benefits of co-grazing.

In 2000 and 2004, 41% and 33% respectively, of respondents administered class I anthelmintics as the sole worm control treatment to their ewes compared to 22% and 33% respectively who administered class III anthelmintics solely. Considering the high prevalence of TBZ and IVM resistance detected, these treatments could be considered to be sub-optimal and lead to an increased risk of disease and/or reduced production in the lambs and extended finishing times (Barrett and others 1998; Macchi and others 2001). This pattern of high reliance of BZ anthelmintics is historical with over 10% of the respondents in 2000 never using any other drug family for helminth treatment in ewes or lambs. The belief by some of the farmers that tapeworms are a problem on their farms (18%) may be one reason for a high reliance on BZ but these

figures do not fully explain the treatment patterns. The pattern of drug class rotation over the preceding four years prior to the survey was markedly different over the two surveys; in 2000 around 16% of the farmers used a single drench class exclusively but this had fallen to 10% by 2004 whilst those respondent that continually rotated their anthelmintic classes annually had fallen from 32% to 17% respectively. Silvestre and others reported in 2002 that the exclusive treatment of lambs with an ineffective treatment increased the frequency of BZ resistant worms from 25% to 80% within two years compared to an increase to 50% in lambs alternately treated with BZ and LEV.

As with other reports in the UK (Coles, 1997; Sargison and Scott, 2003) approximately fifty percent of respondents determined the quantity of drench administered on estimated and average body weights, these practices theoretically could result in the sub-optimal dosing of some animals. For example, when farmers are asked to estimate the weight of ewes and/or lambs the weights are often under-estimated. Eighty six percent of estimates, for groups of 10-20 sheep, by Australian farmers (n=237) were below the actual weight (Besier and others 1988). Similar results were observed in Scotland where 71% of farmer's estimates (n=125) of weights for a ewe and a lamb were below the actual weights (Jackson, personal communication). This is of relevance to the selection of resistance because, when the frequency of anthelmintic resistant alleles is low, under-dosing positively selects for resistance, conversely if resistant allele frequencies are high, selection pressure exerted on heterozygote parasites is less and therefore there is the possibility of the conservation of susceptible alleles (Silvestre and others 2001).

Interestingly advice regarding the improvement in drug bioavailability by with-holding food prior to administration of anthelmintic has been embraced by a relatively large proportion of farmers (Barger, 1993). The average period of starvation stated was approximately 6 hours which may be insufficient to facilitate emptying of the rumen (Ali and Hennessey, 1995).

Treat and move to clean pasture was, in the past, extolled as a effective method of parasite control (Boag and others 1973) but has recently been condemned as a practice that heavily selects for anthelmintic resistance (van

Wyk, 2001). The practice is still commonly conducted; over a third of the respondents in 2004 treated their animals and moved them to clean pasture, though this was a drop of 15% in the numbers of farmers conducting this practice in 2000. These results suggest that some farmers have not been exposed to this advice or feel that the production benefits of grazing treated animals on clean grazing outweighs any perceived risk increased selection for anthelmintic resistance.

Quarantine drenching of “new stock” was more commonly adopted in the present 2 surveys, figure 1, compared to reports published in a previous UK survey, over 85% compared to 17% (Coles, 1997). In the 2004 study 12% of the respondents still failed to administer any treatment to new animals, more worryingly is the fact that of those administering quarantine treatment 75% administered only a BZ or an IVM. The importation of resistance alleles with new stock is probably a major contributor to the spread of anthelmintic resistance (Coles, 1997). The administration of potentially ineffective quarantine treatments may be due to the apparent perceived high efficacy of anthelmintic treatments *per se*, the belief from the majority of the respondents in 2000 was that the anthelmintics used are at least as effective as those used in the past and this figure was borne out by the number that had had their flocks examined for the presence of anthelmintic resistance. Only two percent of the respondents in the 2000 survey were aware of the presence of any anthelmintic resistance on their farms. This level of awareness is lower than in other reported in other UK surveys, (Coles, 1997; Sargison and Scott, 2003).

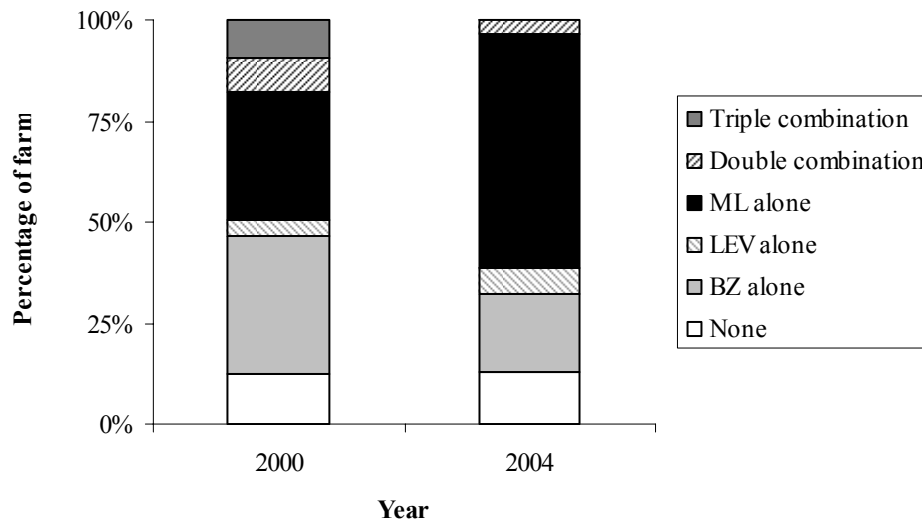


Figure 1: Quarantine treatments administered to “bought in” stock by survey farmers in the years 2000 (n= 73) and 2004 (n= 31); no treatment □, benzimidazole alone □, imidazothiazole or tetrahydropyrimidine alone ▨, macrocyclic lactone alone ■, combination of 2 ▩ or 3 ■ different anthelmintic classes.

The results from the questionnaire survey suggest a clear need to improve the awareness of farmers towards aspects of anthelmintic resistance. More farmers need to be encouraged to test for anthelmintic resistance, using mob sampling i.e. taking representative faecal samples from a number of animals and pooling them together to be examined as a single sample, after treatment is a straight forward and cost effective method of identifying anthelmintic inefficacy. Assuming that similar sized faecal samples are taken from a number of animals, mob samples can also provide a good indication of flock egg counts and the extent of contamination being laid down.

As can be seen, within these surveys, some of the advice and recommendations provided to farmers regarding the treatment and control of gastro-intestinal nematodes are being adopted but on areas such as administration of quarantine treatments, determination of dose rates of anthelmintic treatment and annual drug rotation, attitudes have not changed over the past five years. It is evident that we need to improve knowledge transfer to farmers, veterinarians and advisors in order to slow the rate of

development of resistance and minimise the development of multiple resistance which has become commonplace worldwide.

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9 General Discussion

The research within this thesis has provided much needed baseline data with regard to the prevalence of benzimidazole (BZ, chapter 2) and ivermectin (IVM, chapter 3) resistance in Scottish sheep flocks. The studies have also provided some insight into the potential reasons for the large increases in prevalence that have been observed (Chapters 7 and 8) and provided useful advice on controlling multiple resistant isolates of gastro-intestinal nematodes (Chapters 4 and 5). The findings in this thesis clearly support the consensus view that anthelmintic resistance (AR), particularly multiple AR, poses an additional threat to the producer and thus to the sustainability of some modern production systems. The aim of this discussion is firstly to consider the threats that AR pose to the small ruminant livestock industry and secondly, in the context of our current understanding of AR, how these threats can be minimised.

9.1 *The need to maintain productivity*

Estimates suggest that the world's population will increase by anything up to 2.5 billion people by the year 2020 with an expected increase in demand for meat growing by 100% in developing countries (Waller, 2006). Bearing these figures in mind it is evident that we will need to maintain, or more probably, to improve productivity within the livestock sector. In the last 40 years anthelmintics have been used extensively to control nematodes which are arguably the major health constraint on ruminant production. However since any gains in productivity must be sustainable it is important that livestock producers and advisors give serious consideration to the impact of different chemical control strategies whilst there are still opportunities to affect the outcome.

9.2 *Threats to productivity/sustainability*

Parasitic gastroenteritis (PGE) is a major welfare issue for livestock producers throughout the world with parasites such as *Haemonchus*, *Trichostrongylus* and *Teladorsagia* estimated to cost the sheep industry hundreds of millions of dollars annually in lost productivity and treatment. The 'costs' of AR mostly derive from

morbid rather than mortal effects. Field trials in the UK and Australia found that the use of “ineffective” anthelmintic treatments could lead to slight production losses (up to 10%) compared to programmes where fully effective treatments were administered (Barrett et al., 1998; Besier, 2007a). Within the UK, as with other regions of the world, the pattern of disease is changing. Global land temperatures have increased by 0.7 °C since the end of the 19th century (www.metoffice.gov.uk/), with continued increases it is estimated that the UK growing season could be increased by between 30 and 90 days by 2080 (www.nfuonline.com). In temperate regions the potential benefits of longer growing seasons and grazing periods may be offset to some extent by changing patterns of endemic and exotic disease. Changes in the prevalence of endemic endoparasitic diseases such as fasciolosis, haemonchosis and nematodiosis have been noted from the submissions made to veterinary investigation centres throughout the UK (van Dijk and Morgan, 2008; Scottish Agricultural College (SAC) Veterinary services, 2008). With extended grazing seasons it seems inevitable that more treatments will be administered to stock, thus increasing the selection pressure for the development of resistance (Besier, 2008). The year long grazing available to sheep producers in the southern hemisphere has led to more treatments and has contributed to the rapid emergence of resistance in South America (Waller et al., 1996) and countries such as Australia and South Africa (van Wyk et al., 1997). In the UK annual data relating to parasitoses, treatment failures and meteorological parameters are collected by various individual agencies none of which has the prime responsibility for collating this data to provide an idea of trends/changes in patterns of disease.

9.3 *The extent of the AR problem*

As previously discussed (Chapter 8) it seems inevitable that chemotherapy and chemoprophylaxis will continue as the principal means of alleviating disease caused by gastrointestinal parasites not only in production animals but also in man and his companion animals. The apparent rapid increase in the prevalence of BZ and IVM resistance observed in Scottish sheep flocks is a worrying find that may have a serious impact in reducing the effectiveness of parasite control strategies used by UK producers. The pattern of emerging resistance in ovine parasites in the UK mirrors

that seen abroad, where an increase in prevalence of single drench family resistance usually BZ and IVM resistance has been closely followed by the emergence of triple class resistance (Chapters 4 and 5; Sargison et al., 2001; Yue et al., 2003; Wilson and Sargison, 2007) and multigenic resistance. The adaptive capacity of multiple resistant populations to deal with potent xenobiotics such as moxidectin is illustrated in Chapter 6 where the initial evidence of resistance, a reduction in persistent efficacy, was rapidly followed by complete therapeutic failure. Recent findings would suggest that resistance to one class of anthelmintic may predispose some *Haemonchus* and *Onchocerca volvulus* populations to developing resistance to another class of anthelmintics (Eng et al., 2006; Hughes et al 2007). If these findings also apply to other worm populations and species, they might not only be a key factor in explaining the rapid increase in IVM resistance seen in these studies in Scotland but more worryingly may have far reaching implications for the sustainability of chemical control strategies.

9.4 Mechanisms of resistance

The effective management of AR centres on the ability to conserve the efficacy of anthelmintics and requires an understanding of all of the mechanisms that contribute to resistance (Wolstenholme et al., 2004). Although modifications to the target sites of an anthelmintic are known to contribute to resistance for some compounds it is unfortunate that, as a general rule, their mode of action and key target sites are usually only identified some time after they first appear on the market. In an effort to identify potential markers of AR, researchers have utilised a range of techniques within the disciplines of biochemistry, genetics, genomics, stereo structural modelling, proteomics and molecular biology. Improvements in the accessibility and implementation of these techniques have made comparing discrete populations/isolates possible. Two main genetic approaches for the identification of markers exist; the whole genome approach, where techniques such as haplotyping and gene mapping of quantitative trait loci or the more focussed candidate gene approach (von Samson Himmelstjerna and Blackhall, 2005). The first approach is being aided by the commitment of the Wellcome Trust Sanger Institute to sequence the genomes of at least two of the most economically important veterinary parasites,

H. contortus and *T. circumcincta* (<http://www.sanger.ac.uk/Projects/Helminths/>). The second approach has been used extensively to identify single nucleotide polymorphisms (SNP) utilising new technologies such as Real Time PCR and pyrosequencing (Alvarez-Sanchez et al., 2005a; Von Samson-Himmelstjerna et al., 2007a; Walsh et al., 2007). These techniques are extremely useful and powerful, but even though particular mutations have been demonstrated to be involved in resistance in to particular compounds in the laboratory these findings can be isolate and species specific which causes problems for studies involving field populations. For example in the case of BZ resistance, none of the SNPs at codons 167, 198 or 200 on β -tubulin isotype 1 are fully and solely responsible for the loss of activity of the compound (Stenhouse, 2007).

In conjunction with work on target site mutations (specific mechanisms of resistance), efforts are being directed into elucidating the role that non specific mechanisms may play in the development of AR. Mechanisms include improved/enhanced expression levels of ATP binding cassette (ABC) transporters such as P-glycoproteins (PgP) or metabolic/detoxification enzymes such as cytochrome P450 (CYP; Kerboeuf et al., 2003). The P-glycoproteins transporters have been associated with multidrug resistance in tumour cells (Pouliot et al., 1997) and as well as resistance to all three broad spectrum anthelmintics; the benzimidazoles (Beugnet et al., 1997 ; Kerboeuf et al., 2002), imidazothiazoles / tetrahydropyrimidines (Rothwell and Sangster, 1997) and macrocyclic lactones (ML; Xu et al., 1998). *In vitro* studies at Moredun, examining the effects of ML's on the feeding behaviour of IVM sensitive and resistant *T. circumcincta* and *H. contortus* isolates have shown that by using ABC transporter interfering compounds such as verapamil hydrochloride (VER) and ketaconazole (KET) it is possible to increase the susceptibility to MLs in both IVM sensitive and resistant isolates (Bartley et al., 2006; Bingham et al., 2007, Lespine et al., 2007). The *in vitro* results suggest that, for these two parasites species at least, drug efflux by non specific mechanisms may play a key role in resistance against IVM. *In vivo* work in non-parasitised sheep has demonstrated significantly higher plasma ML concentrations can be achieved if the drug was co-administered with; loperamide (Lifschitz et al., 2002), quercetin (Dupuy et al., 2003), VER (Molento et al., 2004a) or itraconazole (Ballent et al., 2007)

compared to individuals where the ML was administered alone. In parasitized animals the story is potentially much more complex for four main reasons. Firstly, because of the possibility that utilisation of non specific mechanisms may be stage specific (Kotze, 1997) and secondly, different mechanisms of drug handling may be involved at different life cycle stages (Huang and Prichard, 1999; Kotze et al., 2002). Thirdly, inheritance and/or expression of genes for these non specific mechanisms, particularly PgP, may be sex linked in the parasite (Van Zeveren, personal communication) and finally the effects of ABC transporter interfering agents may be confounded by host sex differences (Lifschitz et al., 2004; Bartley, unpublished data). The hope remains that by elucidating the role played by these mechanisms in ivermectin resistance we may also gain a better understanding of their importance in xenobiotic resistance as a whole and that we can exploit that knowledge to develop novel, sensitive tests for resistance.

9.5 *Detection of resistance*

Sensitive tests, that can identify the presence of resistance at an early stage of development, are essential for assessing the impact that implementation of particular control strategies have on parasite populations (Colditz, 2008). It is vital that this sort of information, when supplied to farmers, is based on sound empirical data to ensure that stakeholders are aware of the consequences of particular treatment and management regimes. The extremely high prevalence of BZ resistance worldwide and the lack of genetic markers for LEV or ML resistance at present, mean that any markers that are identified in the near future are likely to be only useful as tools for monitoring the effects of treatment regimes, or as models for other classes of resistance, rather than as a useful diagnostic tools upon which to base management decisions. The establishment of a new global consortium interested in looking for SNPS associated with AR (Consortium on Anthelmintic Resistance Single nucleotide polymorphism markers; CARS) in both veterinary and medical parasites will hopefully facilitate the sharing of raw parasite material, knowledge and expertise to provide novel markers for detecting and monitoring AR.

9.6 Management of resistance

One long term retrospective study that examined the productivity and profitability of six Merino flocks in south eastern Australia, over almost 20 years, found that the presence of multiple class AR did not impede progress within an enterprise (Larsen et al., 2006). The study reported the need for at least one effective anthelmintic class or combination to allow the integrated management systems (IMS) to work efficiently. The maintenance of at least one effective treatment is essential because the loss of efficacies in all broad spectrum anthelmintic classes has been a contributing factor in sheep flocks being culled (van Wyk et al., 1997; Sargison et al., 2005; Blake and Coles, 2007).

9.6.1 New compounds

One question that is frequently asked by livestock producers is whether a new anthelmintic will be commercialised in the near future? Ongoing research since the introduction of the ML compounds over 25 years ago has offered a few promising candidates but, for various reasons usually relating to cost and safety, none have come onto the market (McKellar and Jackson, 2004). Recently two potentially new, novel mode of action compounds, the cyclooctadepsipeptides and the Amino-Acetonitrile Derivatives (AAD, Kaminsky et al., 2008; Hosking et al., 2008) have been described. In the case of the AAD monepantel, it is already known that resistance can be selected at least in the laboratory (Kaminsky et al., 2008).

9.6.2 Combinations

One strategy that has been debated is whether any novel actives that are marketed should be used individually or in combination with other actives with different modes of action (van Wyk, 2008, electronic debate). The debate centres on the degree of selection exerted on a population by the use of multi-class combination treatments compared to the use of singly administered treatments either solely until they are no longer efficacious or in an annual rotation. Combinations have frequently been shown to be extremely effective at controlling resistant populations (Chapters 4 and 5; Anderson et al., 1988 and 1991). Simulated mathematical models

conducted by Barnes et al. (1995) suggest that the use of combinations will slow down the selection of resistance where the initial frequency of resistance alleles is $\leq 0.01\%$ (Barnes et al., 1995), but where the initial frequency was $\geq 1\%$ these effects are greatly reduced. Dogma would suggest that if the genes for resistance are carried on two separate loci at low frequencies, then the likelihood of a single individual carrying both is small and this is reduced further if three compounds are used (Roush, 1993). The counter argument is that if parasites are exposed to two or more compounds simultaneously, then the selection pressures will force them into developing solid multiple resistance or face extinction, and history would suggest that the latter is unlikely to happen (Van Wyk, 2008, electronic debate). Another argument against the use of combinations, particularly in the face of an existing background of high levels of resistance, is that rather than being synergistic, mixtures may actually be antagonist for example workers in Brazil have reported treatment efficacies of 0%, 61% and 29% with MOX, nitroxylin (NIT) and MOX+NIT respectively against a predominantly *H. contortus* population (Molento, 2008 cited in electronic debate). The use of combinations may also mask the development of resistance until it is too late to intervene but, by way of contrast, if annual rotations or sequential programmes are adopted the development of resistance can occur over an extended period of time thereby giving farmers an early warning of imminent problems in sufficient time to alter their management practices (van Wyk, 2008, electronic debate).

9.6.3 Maintaining refugia and reducing treatment frequency

The requirements of the modern farmer to maintain sustainable productivity in the growing face of AR has led producers in some areas to actively considering the use of targeted selected treatments (TST). Although the necessity to maintain genes for susceptibility to anthelmintics by utilising the untreated, and therefore unselected, parasite population *in refugia* had been recognized many years ago (Prichard et al., 1980; Michel, 1985; Barnes et al., 1995), it is only recently that this advice has been heeded (van Wyk et al., 2001; Besier, 2001; Hoste et al., 2002a). Continued advice to treat all animals at a time when the numbers of infective larvae on pasture are low

is believed to be one of the major factors associated with the rapid and widespread rise of AR in Australia (Besier, 2001; Besier and Love, 2003).

The most widely used TST approach is FAMACHA[®] (Chapter 1.22.1; Bath et al., 1996). The system has been used to great success in South Africa (Van Wyk and Bath, 2002), Brazil (Molento et al., 2004b) and North America (Kaplan et al., 2004). Unfortunately the system has limited scope for use in the temperate regions of the world, such as the UK, where *H. contortus* is presently a sporadic rather than persistent problem. Identifying suitable indicators for use in TST strategies aimed at controlling non haematophagous species is difficult, though work using faecal egg counts, production parameters and pathophysiological markers have shown promising results (Chapter 1.22.2). More recently, the original strategies have been adapted in order to identify areas where economies in time and resources can be made. For example the examination of small cohorts of animals with FAMACHA[®] is considered acceptable in “*Haemonchus* seasons” whilst whole flock inspections are conducted at times of increased danger or when examining susceptible flocks (Besier, 2008). The use of individual FEC is acceptable for small groups of animals or animals with high market value such as pedigree animals, horses (Krecek and Guthrie, 1999) or dairy cattle (Höglund, 2006) but in larger flocks/herds it is more practical to use pooled FEC as an indicator for treatment (Besier, 2008). Systems that use weight gain as an indicator have been improved by the implementation of electronic ear tags and automatic weighing and drafting systems (Besier, 2007a).

The commonest criticisms aimed at TST strategies are that will be costly in time, labour and money and thus be of little benefit to the producer. Mathematical modelling suggests that a five fold increase in refugia would lead to only a two fold delay in selection for resistance (Dobson and Besier, 2007). If these findings are correct in the field the question arises as to whether increasing numbers of parasites *in refugia* will be sufficient to save anthelmintic efficacy whilst maintaining commercially viable levels of productivity and high levels of animal welfare. Recent studies, as mentioned above, would suggest that under the correct conditions the answer is yes, but caution must be observed in some geographical regions where

leaving animals untreated might compromise the health of the flock as a whole, such as South East Australia (Larsen et al., 2006).

9.6.4 Integrated Management Systems

There has been an increased acceptance within the farming community of the need for maintained productivity and/or sustainable nematode treatment and a drive for “greener” food in the UK. This change in emphasis has been in part driven by increasing consumer concerns regarding potential chemical residues in meat products and environmental contamination. Producers have looked to improve the resistance and/or resilience of their animals to better withstand parasitism whilst maintaining productivity via the use of Integrated Management Systems (IMS). Strategies include; improved nutrition/bioactive forages (Chapter 1.25), pasture management and/or selective breeding (Chapter 1.27.2). These strategies can play an important role in reducing reliance on chemotherapeutics but in the present economic climate may have a limited appeal for UK sheep producers due to their demands with regards to time, land, resources and environmental restrictions.

9.6.5 Quarantine treatment and monitoring

One of the key findings from this research is that, with the increased prevalence of AR, it is essential to administer effective quarantine treatments to newly purchased and returning animals in order to maintain effective biosecurity. Only around 20% of the respondents to the 2000 and 2004 questionnaires (Chapter 8) followed best practice advice which would suggest that resistance may have been spread throughout the country through animal movement. The administration of MOX alone or a dual/triple combination of BZ/LEV and/or IVM has been shown to be more effective at treating an immature and adult multiple resistant *T. circumcincta* isolate compared to non persistent anthelmintics administered alone (Chapters 4 and 5).

Large amounts of data are generated on individual farms on an annual basis for example; pasture condition, animal condition, treatment frequencies, dates of administration, live weights through out the grazing season, numbers of animal

losses and finishing times. The information generated can, with a small amount of additional information such as targeted faecal egg counts (FEC) throughout the grazing season, assist producers to evaluate their flock's treatment requirements and tailor their strategies accordingly. The cost of generating these data is low in comparison to the costs of an ineffective drug treatment. Studies throughout the world have confirmed that the data generated from pooled/composite samples, if generated accurately, was as precise in determining treatment efficacies as those generated from individual samples (Cabaret and Berrag, 2004; Morgan et al., 2005; McKenna, 2007). The use of composite samples is an area where costs can be cut, possibly facilitating improved adoption by farmers, whilst maintaining the integrity of the test. Another area where improved monitoring could prove invaluable is with post drench efficacy checks (PDEC), only 2% of questionnaire respondent were aware of the resistance status on their farms (Chapter 8). The service is now commercially available in the UK (Scottish Agricultural College veterinary investigation centres) and could assist in making substantial financial savings. Development of high throughput technologies such as pyrosequencing may, in the future, provide useful additional information on species composition from pooled faecal material (Donnan and Skuce personal communication).

If we convince producers to embrace this new era of routine monitoring, there needs to be clear guidance in regards to the interpretation/significance of those findings. It is clear that slowing the selection, development and spread of AR within livestock is a complex and multifaceted problem and sadly, there are no simple blueprint solutions. However it may be possible to simplify the FEC and PDEC systems by introducing a three tier system such as the traffic light system (Mirams personal communication). In this system a green classification (low egg counts/high drench efficacy) requires no action, an amber classification (moderate egg counts/moderate drench efficacy) raises some concerns and a red classification (high egg counts/low drench efficacy) requires urgent action. This type of system is not without its problems, primarily in being too broad in its recommendations, but has been shown to work well with FAMACHA[®].

9.7 Summary

In this ever changing world there is a need for producers, advisors and scientists to adapt to new and potentially challenging situations. New disease patterns and multiple class resistance are likely to continue to make the control of gastro-intestinal parasites more complicated. Information regarding the “*control of parasites of sheep*” is readily available; scientific and popular press articles, newsletters and websites (1.4 million hits on Google™, 42,400 hits on Google scholar™) help distribute information generated by governmental agencies, research centers, interested parties such as the Wool growers associations in South Africa and Australia. Though information is readily available, there is little consensus on the best practice worm control advice. Advice such as the “ACME advice” (Jackson and Coop, Moredun Foundation Newsletter 2007) regarding the **A**doption of an effective quarantine strategy to minimise the risk of importing resistance, **C**hecking the efficacy of the anthelmintics you are using routinely, approximately every couple of years, **M**onitoring of flocks to decide when to treat and what to treat against and **E**nsuring that best practice advice is followed, remain important. If farmers are to follow advice on best drenching practice and potential IMS strategies there needs to be sound empirical research to reassure them of the immediate and long term benefits and the nature and scale of the risks involved in implementation. The increasing prevalence of multiple class resistance highlights the fact that further research is urgently required to elucidate the importance of the various mechanisms involved in BZ, LEV and ML resistance and to try to identify useful phenotypic and/or genotypic markers. It is only when these markers become available that we will be able to determine how our pre-existing resistance mechanisms will impact upon the longevity of new compounds that may be brought to the market.

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