A SURVEY OF ANTHELMINTIC RESISTANCE AND PARASITE MANAGEMENT PRACTICES ON SHEEP FARMS IN NEW ZEALAND

A DISSERTATION PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF VETERINARY STUDIES (VETERINARY PARASITOLOGY) AT MASSEY UNIVERSITY

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ABSTRACT

The occurrence of anthelmintic resistance in sheep nematodes in New Zealand was conducted by using DrenchRite and Inhouse larval development assays (LDA) on 25 randomly selected farms. Samples from 6 farms were put onto both DrenchRite and Inhouse LDA plates and were available for comparison. Both showed a similar LC_{50} and LC_{50} well for benzimidazoles but not for levamisole. Including results from both assay systems it is concluded that anthelmintic resistance in *Trichostrongylus* (either suspected or high level) was demonstrated in 60% of the farms (9/15) to benzimidazole (BZ), resistance to levamisole (LEV) in 66% of farms (10/15), combination drench (BZ+LEV) on 43% of farms (3/7) and avermectin on 1 of 8 farms. For those farms where *Trichostrongylus* was the predominant genus there was resistance to at least one anthelmintic on all tested farms (n=12). A survey of parasite control procedures over 2002/2003 was conducted on 38 farms. The principle findings were: about 58% farmers (n=37) performed quarantine drenching of brought-in sheep for which a majority of farmers (52%) used macrocyclic lactones alone or in combination with other anthelmintics; about 78% (n=37) of farmers followed a 5-6 drench programme to lambs/hoggets; the annual frequency of anthelmintic treatments (n=38) were 6.1 drenches to lambs/hoggets, 1.4 to two-tooths and 1.8 to mature ewes; about 71% farmers used macrocyclic lactones for lambs/hoggets on at least one occasion either alone or in combination with other anthelmintics; a majority (61%) of farmers followed a planned annual drench family rotation; about 76.5% (29/38) farmers had never carried out a test for drench resistance and about 57% (4/7) of those farmers who had tested did identify resistance on their farms to either the benzimidazole or combination (benzimidazole + levamisole) drench. Worms were considered "very important" by 55% farmers (n=38) for the cause of economic losses in their farm, whereas 42% farmer considered it "important". Drench resistance was considered as a "serious problem" today for the industry by 47% farmers (n=38), whereas, 34% farmers believe it as a problem but "not serious one today" for the industry.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The widespread use of anthelmintics for control of nematode infections in the grazing livestock has resulted in anthelmintic resistance that became a major problem in many countries. Resistance to one or more anthelmintic drug is a growing problem worldwide (Taylor *et al*., 2002) and has been reported in various countries such as South Africa (Berger, 1975; van Wyk and Malan, 1988; Van wyk *et al*.,1997; Van Wyk *et al*.,1989a; Van Schalkwyk and Schroder, 1989; Reinecke *et al*., 1991), Kenya (Njanja *et al*., 1987; Mwamachi *et al*., 1995,Maingi, 1991; Waruiru *et al*., 1998), Malaysia (Chandrawathani *et al*., 1999), Australia (Le Jambre *et al*., 1979(a); Green *et al*., 1981; Le Jambre, 1993(a); Beveridge *et al*., 1990; Martin *et al*.,1985; Besier and Wroth, 1993), New Zealand (Vlassoff and Kettle, 1980; McKenna, 1989; McKenna, 1989; Pomroy *et al*., 1992; Hughes, 1988, Middelberg, McKenna, 1983; Kettle *et al*.1982; McKenna and Seifert, 1985; Gopal *et al*., 1999), Argentina (Eddi *et al*., 1996) and Brazil (Charles *et al*., 1989; Echevarria *et al*., 1991).

Overall, there has beeen great concern about emergence of anthelmintic resistance in gastrointestinal nematodes of goats, sheep, horses and cattle. In the field it is usually suspected when anthelmintic treatments fail to provide expected efficacy.

It is particularly a problem for sheep and goats. Resistance has been recorded in every class of anthelmintics in sheep/goats (Conder and Campbell, 1995).

1.2. Incidence of anthelmintic resistance in sheep and goats in various countries of the world

There are various reports of resistance to benzimidazole, levamisole and macrocyclic lactones in sheep/goat gastrointestinal nematodes from various countries that are shown in the Table 1.1.

Table 1.1. Reports of anthelmintic resistance in sheep and goat nematodes from various continents/countries of the world.

Continents/ countries	GIT nematode spp.	Drug resistance	References
Africa			
Kenya	*H.contortus*	BZ/LV/ML	Njanja *et al.* (1998); Mwamachi *et al.* (1995)/Maingi (1991)/Waruiru *et al.* (1998)
	T. colubriformis	BZ/LV	Maingi(1991)/ Waruiru *et al.* (1998)
South Africa	*H. contortus*	BZ/LV/ ML	Berger(1975); VanWyk and Malan (1988)/Van wyk *et al.* (1997)/ Van Wyk *et al.* (1989a)
	Ostertagia spp.	LV/ML	Van Schalkwyk and Schroder (1989) / Reinecke *et al.* (1991)
	T. colubriformis	LV	Van Wyk *et al.* (1990)
Tanzania	*H.contortus*	BZ	Ngomuo *et al.* (1990)
Zimbabwe	*H.contortus*	BZ	Boersema and Pandey (1997)
	Cooperia spp.	BZ	Mukaratirwa *et al.* (1997)

Asia			
India	*H.contortus*	BZ/LEV	Uppal *et al.* (1992),Yadav (1990), Yadav *et al.* (1995), Swarnkar *et al.* (1999).
		LV	Yadav *et al.* (1993)
Malaysia	*H. contortus*	BZ & LV	Chandrawathani *et al.* (1999)
Sri Lanka	*H. contortus*	BZ	Aken *et al.* (1989)
Philippines	*Haemonchus* spp.	BZ	Ancheta *et al.* (2004)
	Trichostrongylus spp.	BZ	Ancheta *et al.* (2004)
Australia and Pacific			
Australia	*H. contortus*	BZ/ML	Le Jambre *et al.* (1979a); Green *et al.* (1981)/ Le Jambre (1993a), Le Jambre *et al.* (1995)/ Besier and Love (2003).
	Ostertagia spp.	BZ/ML/LV	Beveridge *et al.* (1990); Martin *et al.* (1985)/Besier and Wroth (1993), Besier and Love (2003).
	T. axei/T. vitrinus	BZ/LV	Beveridge *et al.* (1990)
	T. colubriformis	BZ/LV	Barton *et al.*(1985); Love *et al.* (1992)/ Sangster *et al.* (1979)
	Nematodirus spp.	BZ	Martin *et al.*(1985), Beveridge *et al.* (1990)
New Zealand	*H. contortus*	BZ	Vlassoff and Kettle (1980), Kemp and smith (1982), Pomroy *et al.* (1985), McKenna (1989)
	Ostertagia spp.	BZ/LV/ML	McKenna (1989); Badger and McKenna, 1990 / Pomroy *et al.* (1992)/ Leathwick *et al.* (2000), Sutherland *et al.* (2003).
	Cooperia spp.	BZ	Hughes (1988)
	Nematodirus spp.	BZ/LV	Middelberg and McKenna (1983); Pomroy *et al.* (1988), McKenna (1989)

	T. colubriformis	BZ/LV/ML	Kettle *et al.* (1982), Kent and Smith (1982), McKenna(1989)/ McKenna and Seifert (1985)/ Gopal *et al.* (1999)
	T. vitrinus	BZ	McKenna (1989)
North America			
USA	*Haemonchus* spp.	BZ/LV/ML	Conway (1964); Lyons *et al.* (1993)/ Miller *et al.* (1987)/ Craig and Miller (1990), Terril *et al.* (2001), Zajac and Gibson (2000)
Mexico	*H. contortus*	BZ	Torres-Acosta *et al.* (2003)
Cuba	*Haemonchus* spp.	LV	Arece *et al.* (2003)
	Trichostrongylus spp.	Tetramisole	Arece *et al.* (2003)
South America			
Argentina	*Haemonchus* spp.	BZ/LV/ML	Eddi *et al.* (1996)
	Ostertagia spp.	BZ/LV/ML	Eddi *et al.* (1996)
	Trichstongylus spp.	BZ/LV/ML	Eddi *et al.* (1996)
Brazil	*H. contortus*	BZ/LV/ML	Charles *et al.* (1989), Echevarria *et al.* (1991): Farias *et al.* (1997).
	Ostertagia spp	BZ	Echevarria *et al.* (1996)
	Trichostrongylus spp.	BZ/ML	Echevarria *et al.* (1996); Farias *et al.* (1997)
	Nematodirus spathiger	BZ	da Costa *et al.* (1985).
Paraguay	*Haemonchus* spp.	BZ/LV/ML	Maciel *et al.* (1996)
	Ostertagia spp.	BZ/LV/ML	Maciel *et al.* (1996)
	Trichostrongylus spp.*	BZ	Maciel *et al.* (1996)
Uruguay	*Haemonchus* spp.	BZ/LV/ML	Nari *et al.* (1996)
	Ostertagia spp.	BZ/LV	Nari *et al.* (1996)
	Trichostrongylus spp.	BZ/LV	Nari *et al.* (1996)

Europe/Scandinavia			
Belgium	*H. contortus*	BZ	Greets *et al.* (1990)
Czechoslovakia	*Trichostrongylus* spp.	BZ/LV/ML	Varady *et al.* (1993)
	Ostertagia spp.	BZ/LV/ML	Varady *et al.* (1993)
Denmark	*Trichostrongylus* spp.	BZ/LV/ML	Bjorn *et al.* (1991); Maingi *et al.* (1996)
	Ostertagia spp.	BZ/LV/ML	Bjorn *et al.* (1991); Maingi *et al.* (1996)
France	*H. contortus*	BZ/LEV	Kerboeuf *et al.* (1988); Hubert *et al.* (1991)/ Chartier et al. (1998).
	Ostertagia spp.	BZ/LEV	Kerboeuf *et al.* (1988); Hubert *et al.* (1991)/Chartier *et al.* (1998).
	Trichostongylus spp.	BZ/LEV	Kerboeuf *et al.* (1988); Hubert *et al.* (1991)/Chartier *et al.* (1998)
	Nematodirus spp.	BZ	Kerboeuf *et al.* (1988)
	Cooperia spp.	BZ/LEV	Hubert *et al.* (1991)/ Chartier *et al.* (1998).
Germany	*H. contortus*	BZ	Bauer *et al.* (1987); Duwel (1991)
	Ostertagia spp.	BZ	Duwel (1991)
	Trichostrongylus spp.	BZ	Duwel (1991)
Netherlands	*H. contortus*	BZ	Boersema *et al.* (1987), Boergsteede *et al.* (1996)
	Ostertagia spp.	BZ	Boersema *et al.* (1987)
	Trichostrongylus spp.	BZ	Boersema *et al.* (1987), Boergsteede *et al.* (1996)
	Cooperia spp.	BZ	Boergsteede *et al.* (1991), Boergsteede *et al.* (1996)
Spain	*O. circumcincta*	BZ	Requejo-Fernandez *et al.* (1997).

Switzerland	*H. contortus*	BZ	Hetzberg *et al.* (2000).
UK	*H. contortus*	BZ	Taylor and Hunt (1988); Scott *et al.* (1989), Hong *et al.* (1996).
	Ostertagia spp.	BZ/ML/ML	Coles *et al.* (1991)/ Britt (1986)/ Jackson *et al.* (1992), Hong *et al*, (1996), Bartley *et al.*, (2003).
	Trichostrongylus spp.	BZ/ML	Scott *et al.* (1990b)/ Britt (1986)
	Cooperia spp.	BZ	Hunt *et al.* (1992)

Note:- BZ = benzimidazoles, LV = levamisole and ML = macrocyclic lactones.

Despite the occurrence of anthelmintic resistance it is generally accepted that, anthelmintic chemotherapy will remain the mainstay of helminth control programmes and it is vital to maintain the efficacy of the currently available broad spectrum anthelmintics.

Reliable and appropriate methods for monitoring effectiveness of these drugs in the field are essential (Taylor *et al.*, 2002).

The investigations that are reported in this thesis focus on two themes. Firstly, a survey of parasite management practices on sheep farms in New Zealand. Secondly, a survey of anthelmintic resistance on New Zealand sheep farms using a larval development assay.

1.3. ANTHELMINTICS:-

Anthelmintics are drugs that are used against parasitic infections in animals or in humans. These drugs can be used for therapeutic or prophylactic purposes. Anthelmintics can be classified into various groups depending upon their chemical structure, mode of action or activity range against different parasites. Prichard *et al.* (1980), Bogan and Armour (1986) and McKellar and Scott (1990) have given summaries of anthelmintics that are used in animals.

A short summary of commonly available anthelmintics and their range of activity of in sheep and goats are given in the Table 1.2. (adapted from McKellar and Scott, 1990).

Table 1.2: Anthelmintic groups (from Mckellar and Scott 1990)

Drug group	Spectrum of activity	Mode of action	Drugs	Doserate mg/kg	GIT nematode	Cestode	Lung worm	Liver fluke	Ectoparasites
Benzimidazole	Broad	Tubulin binding	Thiophenate	5	yes	No	No	No	No
			Thiabendazole	44	yes	No	No	No	No
			Fenbendazole	5	yes	Yes	Yes	No	No
			Mebendazole	15	yes	Yes	Yes	No	No
			Albendazole	5	yes	Yes	Yes	Yes**	No
			Oxibendazole	10	yes	No	No	No	No
			Oxfendazole	5	yes	Yes	Yes	No	No
			Febentel	5	yes	No	Yes	No	No
			Triclabendazole	12	No	No	No	Yes	No
Imidazoles /tetrahydroxy-pyrimidines	Broad	Cholinergic agonists	Levamisole	7.5	Yes	No	Yes	No	No
			Morantel	7.5	yes	No	yes	No	No
Macrocyclic lactones	Broad	Glutamate-gated chloride channel potentiation	Ivermectins	0.2	Yes	No	Yes	No	Yes
			Moxidectin	0.2	yes	No	yes	No	Yes
Subsituted Salicylanides	Narrow	Uncouple oxidative phosphory-lation	Oxyclozanide	15	No	No	No	Yes	No
			Rafoxanide	7.5	No	No	No	Yes	Yes*
			Nitroxynil	10	No	No	No	Yes	No
			Closantel	10	Yes	N	No	Yes	Yes*

Notes: *=variable action, ** = dose double than nematode

1.3.1 BROAD SPECTRUM ANTHELMINTICS

These are generally divided into three action families namely the benzimidazoles, imidazoles/tetrahydroxypyrimidines and the macrocyclic lactones.

1.3.1.1. Benzimidazoles

Benzimidazoles are a group of chemicals that are more frequently used in the treatment of gastrointestinal parasite of livestock. All benzimidazoles have the same central structure, which is 1, 2 diaminobenzene. Thiabendazole was the first drug of this group that was commercially introduced in 1960 (Prichard *et al.*, 1981); after that several drugs of this group were introduced from late 1960s to late 1980s. McKellar and Scott (1990) have reviewed the available benzimidazoles. Various benzimidazole drugs that are in use and their spectrum of activity is given in Table 1.2.

Most benzimidazoles are given orally. In the rumen, they bind to rumen particulate matter and get released as this passes down into the abomasum. The drug is absorbed in the blood stream, resecreted and much is recycled (Marriner and Borgan, 1981). Benzimidazoles need the low pH of the abomasum or gastric stomach to render them soluble and certain disease conditions including GI parasitism can increase gastric pH, this reduces solubility and absorption of the drug with faster excretion rate (Prichard, 1985). The more potent anthelmintics of the benzimidazole group of compounds have slower absorption and elimination rates (Prichard *et al.*, 1978a).

not in Bibliography

1.3.1.2. Mode of action and mechanism of resistance of benzimidazoles

The mechanism of resistance for benzimidazoles is the best understood of all anthelmintics at the present time (Conder and Campbell, 1995).

Microtubules are the main structural components of eukaryotic cells and are composed of one α-tubulin and one β-tubulin polypeptide (Lacey and Prichard, 1986). Microtubules are involved in various functions such as mitotic spindle formation during cell division, maintenance of cell shape, cell motility, cellular secretion, nutrient absorption and intracellular transport (Lacey, 1988).

Benzimidazoles bind to β-tubulin of the nematode parasite and this alters the tubulin-microtubule equilibrium and causes depolymerisation of microtubules of parasites (Lacey, 1988). The binding of benzimidazoles to tubulin may affect the metabolism and functioning of many intracellular pathways. For example there is inhibition of reductase reaction in nematodes by thiabendazole (Prichard, 1970).

Various workers have conducted molecular and genetic studies on anthelmintic resistance in nematodes. Lacey and Snowdon (1988) reported a binding assay using radiolabelled mebendazole and found partially purified tubulin from mebendazole sensitive strains of *H. contortus* and *T. colubriformis* were bound to radiolabelled mebendazole at significantly higher levels than tubulin from resistant strains of the parasite. The study showed a decrease in β- tubulin restriction fragments and a decrease in high affinity binding sites in a resistant population of the parasite (Lubega and Prichard, 1990).

There was no difference in the α-tubulin from benzimidazole-resistant and susceptible populations of *H. contortus* (Roos *et al.*, 1990). However, Roos *et al.*, (1990) and Geary *et al.*, (1992a) both found only one Isotype 1β-tubulin fragment from resistant strains of *H. contortus*. Furthermore Roos *et al.* (1990) described a 9kb fragment of β-tubulin probe reactive DNA fragment that was always present in resistant parasite populations and in some susceptible populations. Studies support the hypothesis that these loci named tcb-1 and gru-1 loci are major determinants of BZ susceptibility and hence the major target of BZ-resistance selection (Grant and Masvord, 1995).

Le Jambre (1990), studied susceptible and resistant strains of *T. colubriformis* and also found this Isotype 1 in resistant strains. Kwa *et al.* (1994) identified a Phenylalanine (Phe) to Tyrosine (Tyr) mutation at amino acid 200 in Isotype 1 β-tubulin of benzimidazole-resistant isolates of *H.contortus* and *T. colubriformis* by using an allele-specific PCR technique. In the future, DNA technology could be in use for both species identification and the diagnosis of drug resistance (Humbert *et al.*, 2001).

Geary *et al.* (1992a) has found a second β- tubulin isotype named as Isotype 2 and genes but did not find any difference between resistant and susceptible strains of the parasite.

Conder and Campbell (1995) concluded that benzimidazole use selects for changes in β- tubulin isotype patterns through at least two mechanism i.e firstly, by selection of a pre-existing 9kb Isotype 1 β-tubulin gene and secondly, by elimination at higher resistance levels of individuals with Isotype 2 genes.

In conclusion, the mechanism of resistance in nematodes appears to be the loss of high affinity tubulin binding for benzimidazoles (Sangster *et al.*, 2002). However, the possibility that multiple mechanisms are contributing to benzimidazole resistance in parasitic helminth needs to be explored further (Kerboeuf *et al.*, 2003)

1.3.1.3 Imidazothiazoles/ tetrahydropyrimidines

This anthelmintic action family includes the imidathiazole, levamisole and the collection of terahydropyrimidine anthelmintics including salts of pyrantel, morantel and oxantel. All share similar mode of action. Levamisole is the most commonly used anthelmintic in this family. It is chemically different than other drugs like pyrantel, morantel and oxantel group. Levamisole is also available in combination with benzimidazoles for control and treatment of sheep nematodes.

Morantel has been used in a sustained-release bolus in cattle to control nematode infections.

1.3.1.4. Mode of action and mechanism of resistance of levamisole

Levamisole acts as a cholinergic agonist causing reversible paralysis due to sustained muscle contraction (Prichard *et al.*, 1980; Booth and McDonald, 1988).

Various workers have conducted research works on levamisole resistance mechanism at the phenotypic and molecular level. It is concluded that multigenic inheritance of levamisole occurs in a strain of *H. contortus* (Sangster and Bjorn 1995). In contrast, Martin and McKenzie (1990) indicated that levamisole resistance in *T. colubriformis* is inherited as a sex-linked recessive trait. Dobson *et al.* (1996) and Sangster (1996) examined the inheritance of levamisole resistance in *H. contortus* by using mating and in vitro assays and they concluded that it is inherited as an autosomal recessive trait and more than one gene is involved.

The levamisole resistance in *C. elegans* appears to involve a loss of cholinergic receptors (Sangster *et al.* 1996). Lewis *et al.* (1980) found that several genes in the free living nematode, *Caenorhabditis elegans* can confer levamisole resistance with mutation at any of seven loci. All the resistant mutants lack acetylcholine receptors.

Despite the research reviewed above it is clear that the molecular mechanism of resistance in this drug is not fully understood (Conder and Campbell, 1995). Further studies/research works would make easier to understand the mechanism of drug resistance to levamisole in nematodes.

1.3.1.5 Macrocyclic lactones

This group of anthelmintic comprise the avermectins and the milbemycins.

1.3.1.5.1 Ivermectin

The avermectins were discovered in 1975 and after 6 years of this discovery the semi-synthetic ivermectin was first used commercially for animal use. Due to the unique combination of killing endo and ectoparasites by the avermectins the term "endectocide" was used to describe them. They have revolutionized antiparasitic control since that time (Shoop *et al.*, 1995). Since 1981, ivermectin has been released in over 60 countries for use in cattle, sheep, goats, horses, swine, dogs, camels, reindeer, bison and man (Shoop *et al* 1995).

Several other avermectin molecules have also been released commercially. The related milbemycin are also commercially available in the form of moxidectin. Milbemycin was discovered by Sakyo scientists in 1973 (Shoop *et al.*, 1995). Moxidectin is the third avermectin or milbemycin that was discovered in late 80s.

Chemically, the avermectins and milbemycins are closely related by sharing a central, 16-membered macrocyclic lactone structure. Both the avermectins and milbemycin are produced by a fermentation process from actinomycetes of the genus *Streptomyces* and have similar biological activities (Shoop *et al.*, 1995).

The avermectins are produced from fermentation of *Streptomyces avermitilis* and these natural components are denoted as A1a, A1b, A2a, A2b, B1a, B1b and B2b (Shoop *et al.*, 1995). Ivermectin is the 22, 23-dihydro-avermectin B1 and it has been commercially available for animal treatment from 1981 (Shoop *et al.*, 1995). Ivermectin is a large molecule with two sugar rings and two hydroxyl groups composed of >80% dihydroavermectin B1a and <20% dihydroavermectin B1b (Campbell, 1989).

Ivermectin is a white powder which is soluble in organic solvents but largely insoluble in water. There are different formulations that are commercially available such as oral, injection, rumino-reticular, slow release devices and cutaneous (pour-on) delivery systems; ivermectin is rapidly absorbed via all these routes and attains

high concentrations in most of the tisues because of its lipophilicity, especially in the liver and fat tissues. It is concluded that the long acting injectable formulation of ivermectin has potential therapeutic advantages over over currently available ivermectin formulations (Errecalde, *et al.*, 1998).

It can be concluded that due to its wide spectrum of activity, wide safety margin, different routes of delivery, ivermectin is a popular choice for treatment of nematode and arthropod parasitism in animals. The macrocyclic lactone (MLs) class of drugs was readily accepted as a significant proportion of the world population of livestock and companion animals (Conder and Campbell, 1995). Macrocyclic lactones are only remaining drug class with minimal resistance for use in sheep (Barber *et al.*, 2003). The other avermectins now marketed for use in food producing animals include abamectin (the parent compound of ivermectin), eprinomectin and doramectin.

1.3.1.5.2. Doramectin

Doramectin, 25-Cyclohexyl-avermectin B1, is the fourth ivermectin or milbemycin endectocide released for production animals (Shoop *et al.*, 1995) and it was discovered from a mutant strain of *Streptomyces avermitilis* with a C-25 substitution different than ivermectin (Conder and Campbell, 1995). Doramectin like other avermectins has therapeutic as well as prophylactic activity against ecto and endo parasites.

1.3.1.5.3. Moxidectin

Moxidectin is the the only milbemycin released for farm animals. Chemically it is 23-methoxymin LL-F28249α, produced from *Streptomyces cyaneogriseus* by fermentation process. In sheep it is given at a dose rate of 200µg per kg of body weight as an oral or injectable form and is also available as a pour-on solution and sustained release ruminal bolus. Moxidectin is also used in cattle and horses. Study showed that moxidectin injected at a dose rate of 0.2mg/kg live weight is a

highly effective anthelmintic in cattle and free from obvious undesirable side-effects (Samson *et al.*, 1992).

Results indicate that moxidectin and doramectin may be ineffective against ivermectin-resistant *Cooperia* spp. infecting cattle (Vermunt *et al.*, 1996). However, moxidectin has long persistant activity in comapared to other ivermectins. Another study has established that moxidectin has significantly higher therapeutic efficacy against resistant strain of *O. circumcincta* than chemically related ivermectin (Sutherland *et al.*, 1999).

1.3.1.6 Mode of action and mechanism of resistance of macrocyclic lactones

The mechanism of action of macrocyclic lactone is not fully understood, it appears these compounds exerts their effects on muscle membranes by irreversible opening of chloride channels (Martin and Pennington, 1989). Originally it was concluded that these chloride channels were associated with Gamma aminobutyric acid (GABA) receptors (Turner and Schaeffer, 1989) but some authors has indicated no GABA association. Experiment had demonstrated that the coexpressed HG1 gene and GAB-1 receptors are GABA-responsive and provide evidence for the possible involvement of GABA receptors in the mechanism of ivermectin resistance (Feng *et al.*, 2002).

It has subsequently been shown that the anthelmintic activity of the macrocyclic lactones is mediated by an interaction with a glutamate-gated chloride channel (Arena *et al.*, 1992). Glutamate-gated chloride channels (GluCls) are targets for the avermectin anthelmintics and experiments on model organisms have provided strong evidence that avermectins exert their anthelmintic and insecticidal activities by irreversible binding to and opening, glutamate-gated chloride channels (Cheeseman *et al.*, 2001). Most of the research work has been with ivermectin and it is likely that all macrocyclic lactones anthelmintics share the same mechanism of action (Shoop *et al.*, 1995).

It is suggested that the high fat solubility of avermectins and a large fat reservoir in sheep may be contribute to the long persistence of avermectins in plasma at low concentrations because of less less blood supply to the fatty tissue (Atta and Abo-Shihada, 2000).

The concentration vs. time curves for doramectin (DRM) and ivermectin (IVM) were similar for 2-3 days, parent molecule of the MLs were detected in each group for 15 days post-treatment for IVM, 30 days for DRM and for all time point (40days) for moxidectin (Barber *et al.*, 2003).

Therapeutic efficacy of anthelmintics is an important factor in influencing how quickly drug resistance develops (Sutherland et al., 2003). The low plasma concentration of ivermectins in sheep might be related to the previously reported emergence of resistant nematodes in sheep (McKeller and Bechaoui, 1996). The greater reductions in trichostrongylid worm counts in goats suggest a difference in the efficacy of ivermectin in between lambs and goats (Gopal *et. al*, 1999).

It has been suggested that the rates of development of resistance differ between ML molecules with resistance to macrocyclic lactones in *H. contortus* occuring more slowly with moxidectin than ivermectin (Ranjan, *et al.*, 2002). Macrocyclic lactone resistance includes resistance to all the individual drugs in this class including ivermectin (Sangster *et al.*, 2002). *In-vitro* assays measuring AM effect on larval development and motility indicate that AM resistance *in vivo* can be associated with reduced sensitivity to AM inhibition on larval motility and/or development (Gill and Lacey, 1998). Macrocyclic lactone resistance in *H. contortus* field strains is a dominant, autosomal trait, largely controlled by single gene (Le Jambre *et al.*, 2001).

The mechanism of ivermectin/milbemycin (AM) resistance is complex and various studies had expanded the understanding of this mechanism but it is still far from completely understood.

1.4. DEFINITIONS OF ANTHELMINTIC RESISTANCE

Drug selection like all selection, work on natural genetic diversity occurring in a population (Shoop 1993). A variety of definitions of anthelmintic resistance have been proposed.

Taylor and Hunt (1989) described anthelmintic resistance as "a heritable change in the ability of individual parasites to survive the recommended therapeutic dose of anthelmintics."

Shoop *et al.* (1995) defined anthelmintic resistance as "a change in gene frequency of a population, produced by drug selection which renders the minimal, effective dosage previously used to kill a defined portion (e.g. 95%) of the population equally effective."

Similarly, Sangster and Gill (1999) defined it as "a decline in the efficiency of an anthelmintic against a population of parasites that is generally susceptible to that drug and is genetically determined".

Likewise Conder and Campbell (1995), defined anthelmintic resistance as "a heritable reduction in the sensitivity of a parasite population to the action of a drug, the reduction being expressed as a decrease in the frequency of individual parasites affected by exposure to the drug."

According to the World Association for the Advancement of Veterinary Parasitology (WAAVP), anthelmintic resistance is defined as failure to reduce faecal nematode egg counts (FEC) by at least 95% (Coles *et al.*, 1992) and this definition is

generally accepted diagnostically. Interestingly anthelmintic resistance has never been formally defined in terms of failure to reduce nematode burdens.

When discussing anthelmintic resistance there are several categories of anthelmintic resistance such as side-resistance, cross-resistance and multiple-resistance.

Side-resistance is defined as occurring among the chemically related drugs that share the same mode of action such as among the group of benzimidazole, macrocyclic lactones and salicylanilide drugs (Shoop, 1993).

Cross-resistance occurs when resistance to one anthelmintic results in resistance occurring among chemically unrelated compounds such as between morantel and levamisole.

Multiple-resistance involves one nematode species being resistant to at least two chemical drugs that are chemically unrelated and have a different mode of action. This is usually due to selection of resistance to each action family independently. Multiple-resistance is generally observed in mixed population of nematodes that is resistant to one or more anthelmintic drugs.

Multigeneric resistance refers to several genera in one location demonstrating anthelmintic resistance. This may or may not also be multiple-resistance.

Reversion is return towards susceptibility of a resistant nematode in absence of the selecting drug (Jackson and Coop, 2000). Reversion is a state in which there is a return to or toward anthelmintic drug susceptibility of a parasite population after it has become resistant to a particular drug (Leathwick *et al.*, 2001).

In the field, reversion is not usually separable and two processes by which reversion may occur. The first one is when fitness disadvantage is associated with

the resistant genotype and the second process is counter selection that occur as a result of different selection pressure applied through the the use of alternative anthelmintics (Leathwick *et al*., 2001).

1.5. ANTHELMINTIC RESISTANCE IN SHEEP AND GOATS IN NEW ZEALAND

Anthelmintic resistance is becoming a serious problem in the southern hemisphere especially in Australia and New Zealand as well as other large pastoral sheep/goat rearing areas of South Africa and South America. All gastrointestinal nematodes in sheep and goats are involved including *Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus colubriformis* and to a lesser extent *Nematodirus* and *Cooperia* species.

In New Zealand, anthelmintic resistance in sheep nematodes was first diagnosed in 1979 (Vlassoff and Kettle, 1980) and warnings of possible consequences soon followed (Leathwick *et al*., 2001). Although no formal survey has been conducted for 20 years, since 1979, the prevalence of anthelmintic resistance on New Zealand farms has steadily increased (Leathwick *et al*., 2001).

The high efficacy and relatively low cost of modern broad-spectrum anthelmintics allowed many farmers to rely almost exclusively on heavy drench usage to control nematode parasites in their farms, as a consequence, resistance emerged to both benzimidazole and levamisole drenches (Vlassoff and McKenna, 1994). The occurrence of a field strain of *Haemonchus contortus* resistance to thiophanate (BZ) is reported for the first time in New Zealand (Pomroy *et al*., 1985). A study showed multiple-resistance in goat-derived *Ostertagia circumcinta* was resistant to ivermectin, oxfenbendazole, fenbendazole and levamisole (Pomroy *et al*., 1992). More recently ivermectin/milbemycin resistance had also been recorded in sheep nematodes although resistance in these same species on goat farms has been seen for many years.

McKenna (1995) reported that many early cases involved benzimidazole resistance in *Nematodirus spathiger* but resistance is now found to both benzimidazole and levamisole in all common sheep trichostrongylids. He also mentioned that among parasitological submissions to animal health laboratories in 1993, levamisole resistance was evident in 29%, benzimidazole in 66% and resistance to the combination drench (benzimidazole + levamisole) in 16% of cases.

The incidence of anthelmintic resistance in sheep nematodes is steadily increasing. Leathwick *et al* (2000) reported the first cases of ivermectin resistance in *O. circumcincta* in sheep in 1999/2000. Gopal *et al*. (1999) and Vickers *et al* (2001) have confirmed ivermectin resistance in *T. colubriformis* and *H. contortus* in sheep respectively. Reduced persistant activity of moxidectin in a sheep flock has been noticed in USA.

There are several contributing factors for anthelmintic resistance, it is concluded that frequent dosing selects for resistance more strongly than less frequent dosing regimes and underdosing is another major contributor in selecting for resistance (Conder and Campbell, 1995).

Similarly, farm management factors contribute in a variety of ways to the generation of resistance in nematodes. A study on levamisole resistance in *H. contortus* showed that selection pressure is important in determining the rate of development of resistance (Sangster and Bjorn, 1995). Studies on lambs showed considerable genetic variation in faecal egg counts following natural, predominantly *O. circumcincta* infection and this genetic variation is aquired and not innate, the most likely response is the immune response (Stear *et al*., 1999).

There are several reports of benzimidazole, levamisole and macrocyclic lactones resistance in sheep/goat gastrointestinal nematodes from various countries that are shown in the Table 1.1

1.6. DIAGNOSIS OF ANTHELMINTIC RESISTANCE

A variety of techniques have been used to diagnose the prevalence of resistance in sheep/goat nematodes. These tests involve parasitological, biochemical and molecular techniques for diagnosis of anthelmintic resistance.

1.6.1 *IN VIVO* TESTS

There are two *in vivo* tests currently in use for diagnosis of anthelmintic resistance in animals. These tests are (1) Faecal egg count test (FECRT) and (2) controlled efficacy test.

1.6.1.1 Faecal egg count reduction test

The faecal egg count reduction test (FECRT) relies on comparision of faecal egg counts from animals before and after anthelmintic treatment and this provides an estimation of anthelmintic efficacy. This test has been recommended by the World Association for Advancement of Veterinary Parasitology for monitoring resistance in nematode of animals (Coles *et al.*, 1992). This test is widely used for determination of anthelmintic resistance even today. Generally, faecal samples from 10-12 animals are taken for this test. The arithmetic mean is more appropriate over geometric mean in relation with faecal egg counts interpretation (Das *et al.*, 1988).

Several modifications and protocols have been described. Vizard and Wallace (1997) have described a modification of the FECRT; in this method no pre-treatment samples are taken but both treatment and control goups are taken post-treatment and compare same population mean.

The FECRT may not provide sufficient information for correct interpretation (Taylor *et al.*, 2002). While FECRT is a very practical means of assessing drench performance, the success of the procedure largely depends on the use of adequate numbers of animals and careful administration of anthelmintic (McKenna,

1990). To determine the species of nematode involved with resistance, larval cultures can be used in conjunction with the FECRT (McKenna, 1997) but Presidente (1985) mentioned that the culture conditions may favour the development of one species over another and potentially confuse the interpretation.

The FECRT seems to be relatively insensitive and unable to detect resistance in benzimidazoles when the population of resistant genes is below 25% (Martin *et al.*, 1989). The study demonstrated that due to maturation of immature stages, the FECRT based on faecal egg counts taken at 11 or more days after treatment may give results indicative of levamisole resistance despite the absence of levamisole resistant strain of gastrointestinal nematodes (Grimshaw *et al*, 1995).

1.6.1.2 Controlled efficacy test

In this test, animals are grouped into two groups and one group is infected with infective stage (L3) nematode larvae and treated with anthelmintic after 21 days of infection and another group is kept as control. Animals in both the control and the treatment group are slaughtered 10-14 days after treatment and their worm burdens counted.

It is also possible to determine LD_{50} or ED_{50} of the drugs for the parasite, if the series of increasing dose rate are used in several treatment groups. The guideline for conducting this test has been given by the WAAVP (Wood *et al.*, 1995). This test is most reliable for determining anthelmintic efficacy but it is also the most costly (Boersema, 1987).

1.6.2 *IN VITRO* TESTS

There are several *in vitro* tests for diagnosis of anthelmintic resistance in livestock nematodes. These tests may vary in their application in field and research work. These tests include the egg hatch assay (EHA) for benzimidazoles and

levamisoles, larval development assay, larval paralysis and motility assay, adult development test, biochemical tests, molecular/genetic assays.

1.6.2.1 Egg hatch assay for benzimidazoles

The egg hatch assay can be used for routine diagnosis of benzimidazole resistance and relies on ovicidal activity of the drugs. The resistant strains of GI nematodes embryonate and hatch in higher concentrations of the drug whereas susceptible strains could not survive and do not hatch. In this test, it is recommended to incubate nematode eggs in a serial dilutions of the drugs for 24 hours at 26°C temperature, after that add lugol's iodine solution as a stain and then observe and count eggs that hatch or die at various drug concentrations (Le Jambre, 1976; Coles and Simpkin, 1977; Hall *et al.*, 1978; Johansen, 1989; Whitlock *et al.*, 1980 and Donald, 1983).

This test seems to be more realiable than the FECRT (Hall *et al.*, 1978).

1.6.2.2 Egg hatch assay for levamisole

The egg hatch assay (EHA) is used for detetection of levamisole resistance in sheep GI nematodes. Dobson *et al.* (1996) has developed this test to determine the effect of levamisole in paralyzing the first stage larvae in eggs of trichostrongylid nematodes.

In this method fresh undeveloped eggs are recovered. A known number per ml of egg solution is determined and incubated in microtitre plates at 26°C until one hour before hatching then the anthelmintic is added. Further incubation is to be done for 24 hours, the plates are snap cooled at 15°C for 5 minutes and after that chilled formaldehyde solution is added and finally the number of hatched larvae are counted. This technique requires good timing for conducting the test. The EHA is not useful for field diagnosis of anthelmintic resistance (Dobson *et al.*, 1986 and Johansen, 1989).

1.6.2.3 Larval development assay

The larval development assay (LDA) is an *in vitro* test and the general principle of this assay is to incubate strongylid nematode eggs with media in serial dilutions of various anthelmintic drugs for 6-7 days at 20-26°C; then the number of eggs and various larval stages (L1, L2 and L3) are counted after staining with lugol's iodine and their proportion is determined. This is corrected against the number in control wells and the LD_{50} and dose response curve can be determined. The practical use of this test for determining anthelmintic resistance against various drugs in nematode species is described by Ibarra and Jerkins (1984), Coles *et al.* (1988), Taylor (1990), Lacey *et al.* (1990), Hubert and Kerboeuf (1992) and Gill *et al.* (1995).

Ibara and Jenkins (1984) initially developed this test by employing it on free living stages of trichostrongylid nematodes; they used distilled water and a faecal suspension of rat faeces as the growth medium for the larvae. Hubert and Kerboeuf (1984) further improved this test by using nutritive media containing yeast extracts and Earle's balanced salt solution.

Coles *et al.*, (1988) used lyophilised *Eschericha coli* as a nutrient source when determining benzimidazole resistance in *Trichostrongylus colubriformis* and *Haemonchus contortus* using various concentration of cambendazole (benzimidazole). They also used levamisole and detected levamisole resistance in *H. contortus* and concluded the LDA is more sensitive than the egg hatch assay and this assay can be run with any anthelmintic to which resistance is suspected. Giordano *et al.* (1988) used this test for detection of ivermectin resistance in *T. colubriformis* in lambs. However, it's major drawback is that it is not useful for detecting ivermectin resistance in *Ostertagia circumcincta*.

Taylor (1990) has described a modified larval development assay for detection of resistance to benzimidazoles, levamisole and ivermectin and he used yeast extract

and Earle's balanced salt solution as a growth medium. He also concluded that this test had potential for detecting anthelmintic resistance in the field.

Hubert and Kerboeuf (1992) made a further variation in the test and used nematode eggs, Yeast extract, Earle's balanced salt and *E. coli* in the medium in 5 ml tubes and used a higher volume of anthelmintic solution in the assay. They were able to calculate LD_{50} values and demonstrated a linear dose response curve. This test appeared useful for detection of anthelmintic resistance to benzimidazoles and levamisole and an avermectin analogue (Amarante *et al.*, 1997).

Further modifications of the LDA were described by Lacey *et al.* (1990), by using a 96 well microtitre plate. In this method nematode eggs in a nutrient solution were added onto an agar matrix containing various concentrations of drugs. The amount of anthelmintic varied between wells in the microtitre plate, usually as doubling concentrations. This test is termed as the microlarval development assay and has mainly been used for investigating resistance in sheep nematodes. The use of agar was reported to eliminate solubility problems with avermectins and this led the LD_{50} values and dose response curve for ivermectins (Taylor *et al.*, 2002).

Furthermore, CSIRO, Australia has developed a commercial larval development assay plate "DrenchRite Larval Development Assay" (Horizon Technology Pty Limited, Australia, 1986) for detection of anthelmintic resistance in sheep and goat nematodes for benzimidazoles, levamisole and ivermectin. Subsequently in this thesis this commercial assay will be referred to as DrenchRite LDA.

The DrenchRite LDA, is based on the methods described by Gill *et al.* (1995) and Lacey *et al.* (1990). Various concentrations of drugs are mixed with agar in 96 well microtitre (LDA) plates and seal packed; nutrient media is also provided. The manual provided with this has detailed guidelines for nematode egg recovery, use of nutrient media and interpretation of results with relation to species of sheep

nematodes. This has been marketed by Horizon Technology Pty. Limited, Australia. In Australia, many hundred sheep farms have been sucessfully tested with this commercial LDA plate. In this DrenchRite LDA plate, the first well in each of 8 rows (A, B, C, D, E, F, G and H) is kept as a control and it does not contain any anthelmintic concentration. The other wells (2-12 well) of various rows contain increasing concentrations of various anthelmintics. The first two rows (row A and B) contain thiabendazole (TBZ), the C and D row contain levamisole (LEV), the E and F row contain combination drug (benzimidazole + levamisole) and G and H row contain analogs of ivermectin (avermectin/milbemycin).

Tandon and Kaplan (2004) have recently reported some of the concentrations used in the DrenchRite LDA plate. There are eleven concentrations of TBZ, IVM-1, IVM-2 and eight concentration of LEV. They did not formally indicate which ivermectin analogoue were used even though they reported the concentrations as IVM-1 and IVM-2. For TBZ the concentrations (µM) are: 0.010, 0.020, 0.040, 0.078, 0.156, 0.313, 0.625, 1.250, 2.500, 5.000 and 10.000, for IVM-1 (nM): 0.500, 0.970, 1.900, 3.900, 7.800, 15.600, 31.250, 62.500, 125.000, 250.000 and 500.000, for IVM-2 (nM): 0.970, 1.900, 3.900, 7.800, 15.600, 31.250, 62.500, 125.000, 250.000, 500.000 and 1000.000 and for LEV (µM): 0.195, 0.390, 0.780, 1.560, 3.125, 6.250, 12.560 and four replicate of 25.000.

The concentration of combination drugs used in this DrenchRite LDA plate (row C and D) is not known and it is not possible to calculate the LC_{50} and a dose response curve with actual drug concentrations.

A major advantage with this assay is that it has been calibrated for benzimidazole and levamisole resistance in *Haemonchus, Ostertagia* and *Trichostrongylus* spp. by controlled slaughter studies.

It can be concluded that the LDA technique is more reliable, rapid, inexpensive and suitable for use in the field for detection of anthelmintic resistance in sheep and

goat nematodes. Several workers (Taylor (1990); Amarante *et al.* (1997); Varady and Corba (1998) and Craven *et al.* (1999)) have used this technique for the detection of anthelmintic resistance in sheep/goats and horse nematodes and found it suitable for field investigation for anthelmintic resistance in these species of animals. DrenchRite LDA also been evaluated for detection of anthelmintic resistance in cyathostomin nematodes of horses (Tandon and Kaplan, 2004).

1.6.2.4 Adult development test

Taylor and Hunt (1989) described an *in-vitro* nematode culture technique that allows the development of infective third stage larvae to the adult stage of many nematode parasites. Stringfellow (1984, 1986) was able to culture *H. contortus* through to the adult egg-laying stage and described this technique. There had been little further progress in this field due to complexity of the culture technique.

1.6.2.5 Larval paralysis and motility assays

Martin and Le Jambre (1979) have developed this test for detection of levamisole and morantel resistance in nematodes. In this technique, infective third stage nematode larvae are incubated for 24 hours in serial dilutions of anthelmintics and the percentage of paralysed larvae is determined at each concentration and a dose response curve is plotted (Taylor *et al.*, 2002). This can be compared with a known reference strain of nematode parasite. A modification of this technique that is suitable for detecting thiabendazole resistance in nematodes has been described by Sutherland and Lee (1990).

Anthelmintic effect on motility of parasites led to the development of a micromotility meter as described by Bennet and Pax (1986, 1987). This equipment uses microprocessor technology to measure light reflection at miniscal interface and it is claimed that when larvae move in the solutions alter the angle of light reflection at miniscal linterface; the light deviation is measured and information passed to a computer to give a motility index (Taylor *et al.*, 2002). However, there has been no

commercial development of this approach and no recent publications are available where it has been used.

1.6.2.6 Biochemical tests

A tubulin binding test is based on the principle of mechanism of the benzimidazole resistance, i.e. the mechanism appears to be associated with a reduced affinity of tubulin binding for the anthelmintics. Lacey and Snowdon (1988) developed this test by using benzimidazole carbamates. This test is claimed to be rapid, robust, highly reproducible and sensitive to major changes in the parasite population but the requirement for large number of larvae make it unsuitable for field use (Taylor *et al.*, 2002).

Another type of biochemical test was developed by Sutherland and Lee (1989). This test compares the non specific acetylcholine esterases of benzimidazole-resistant and susceptible trichostrongylid nematode strains. In resistant trichostrongylid nematode strains there is an increased acetylcholine esterase activity. It is a simple colorimetric assay in which samples are compared either by visual examination or by the use of a densitometer. It has been reported that it is difficult to conduct in mixed worm infections and it require atleast 1,000 larvae to run the test

1.6.2.7 Molecular / genetic techniques

Molecular and genetic tests are limited and available for detecting benzimidazole resistance (Taylor *et al.*, 2002). Roos *et al.* (1990) used restriction fragment length polymorphism (RFLPs) in the genome of benzimidazole resistant and susceptible population of larvae and adult *H. contortus*. In their study they have used α and β-tubulin probes and found a maximum of six different fragments in susceptible worms compared to only one or two in resistant worms. An allele-specific polymerase chain reaction (PCR) is used to detect amino acid substitution (Phe to Try) at position in 200 in β-tubulin isotype 1 gene in H. contortus to confirm the resistance (Kwa *et al.*, 1994).

The PCR assay focus on earliest detectable genetic changes associated with benzimidazole resistance (BZ-R) selection (Roos *et al.*, 1995). Elard *et al.* (1999) have reported allele specific PCRs for genotyping benzimidazole resistant *O. circumcincta* species and it can also detect mutation in position 200 of β- tubulin gene. Studies showed no difference in ligand-binding properties of a levamisole receptor between the levamisole-resistant and susceptible *H. contortus* (Sangster *et al.* 1990).

Blackhall *et al.* (1998) analysed cloned fragments of the α- subunit from strains of H. contortus by using single strand polymorphism and reported that the resistance to ivermectin appeared to be associated with changes in the frequencies of two alleles of this subunit gene.

P-glycoproteins (pgp) act as drug transporter at the cell membrane and change in their expression appears to contribute to anthelmintic resistance. Xu *et al.* (1998) have reported research on the role of P-glycoprotein in ivermectin resistance. The current tools used to test the presence of Pgp in nematodes belong to three groups i.e. detection of corresponding genes, quantification of mRNA and analysis using monoclonal antibodies (Kerboeuf *et al.*, 2003). No validated assays using their approach to detect ivermectin resistance have yet been published.

1.7. COMPARISION OF VARIOUS TECHNIQUES FOR PERFORMING LARVAL DEVELOPMENT ASSAY

A brief summary of use of this technique, laboratory conditions for proper growth and modifications by various workers is described in the Table 1.3.

Some of the researchers also compared various tests for detection of anthelmintic resistance in sheep and other animals. Varady and Cobra (1999) concluded that the larval development assay and egg hatch assay were the most suitable *in vitro* tests for field screening of sheep nematodes for benzimidazole and levamisole

resistance by comparing six *in vitro* assays such as egg hatch assay, egg hatch paralysis assay, larval paralysis assay, larval paralysis assay with physostigmine, larval development assay and larval micromotility test.

Table 1.3: Summary of different techniques for conducting a larval development assay.

Method developed by	Egg recovery and No. of nematode eggs used	Media and its volume	Tubes or microtitre plate	Anthelmintic used	Incubation temperature	Nematode spp.
Ibrra and Jenkin (1984)	Faecal suspension of rat (one part)	Distilled water (one part)	-	BZ	-	-
Hubert and Kerboeuf (1984)	Through sieving/centrifu-gation/magnesium sulphate soln. cleaning	Yeast and Earlie's saline solution	Petridish + agar	-	22 to 23°c for 8 days	*T.colubriformis* and *O. circumcincta*
Coles *et al.* (1988)	Sieving and saturated sucrose Floatation. (1000 eggs/ml)	Water +E.coli (lyophilized)	Sterilin multiwell plates	BZ, LV	Incub-ation for 6 to 7 days at 22°C	*N. dubius, H.contortus*, and *T.colubriformis*
Taylor (1990)	Seiving, cent-rifugation and saturated salt solution 200-300 egg/ml	(Yeast extract + Earle's balanced salt soln.) Same as Hubert and Kurboef (1984)	Petridish + agar	BZ, LV	22 to 23°C	*H.contortus* and *O.circumcincta*
Lacey *et al.* (1990)			96 well microtite plate + agar	ML	22 to 23°C	GI nematodes
Hubert and Kurboeuf (1992)	Seiving and centrigation in magnesium sulphate solution (1200-1300	As decribed by Hubert and Kerboeuf (1984)	5 ml tubes	BZ, LV, Pyrantel and IVM	22 to 23°C	*H.contortus, O. cicumcincta,* and *T. colubriformis*

	eggs/ml) + amphotericinB					
Gill *et al.* (1995)	From faecal slurries by centrifugati-on sugar soln.	As described by Hubert and Kerboeuf (1984)	96 well microtitre plate + 200µl agar	Avermectin B1, avermectin B2, avermectin mono-saccharide and avermectin aglycone	25°C	*H. contortus, T. colubriformis* and *O. circumcincta*
DrenchRi-te LDA method	Seiving, use of various sugar solutions, centrifugati-on.(4,000eggs /ml) + amphotericin B	Based on above methods (used own medium; composition kept secrete)	96 well microtitre plate + coloured agar	BZ, LV, Combination of BZ+LV and Ivermectin analogues	25°C	Recom-mended for *H. contortus ,T. colubriformis* and *O. circumcincta*
Inhouse LDA method (Used by Sharma, 2003)	Seiving use of two sugar soln, centrifugation and sedimentation (3000-4,000 eggs /ml.+ amphotericinB	Earle's balanced salt soln. + yeast extract	96 microtitre well	BZ, LV, Ivermectin (Bomectin, Erase MPC), ivermectin aglycone	24 to 25°C	Used mainly in *Trichostrongylus spp.* and also in mixed nematode infections

1.8. EPIDEMIOLOGY OF NEMATODES IN NEW ZEALAND

New Zealand has a temperate and moist climate. There is year round rainfall which is beneficial for pasture growth. The current prevalence and distribution of parasitic nematodes are largely governed by a combination of their ecological requirements for development and survival outside the host, prevailing local climate and farm management practices (Vlassoff et al., 2001). Seasonal conditions are favourable for the development of most of the parasite species for at least part of each year in all districts (Vlassoff, 1982). The seasonal pattern of GI nematodes infections in sheep tends to follow a relatively similar pattern from to year to year (Brunsdon, 1970).

In New Zealand, *Trichostrongylus* and *Ostertagia* predominate in all areas but *Haemonchus* and *Cooperia* occur with greater frequency in the North Island (McKenna *et al.*, 1974). It seems that *Nematodirus filicollis* occur mostly in the South Island areas of New Zealand because of cool climatic conditions. A chart

related to epidemiology of GI nematode in sheep and goat is given below in Figure 1.1

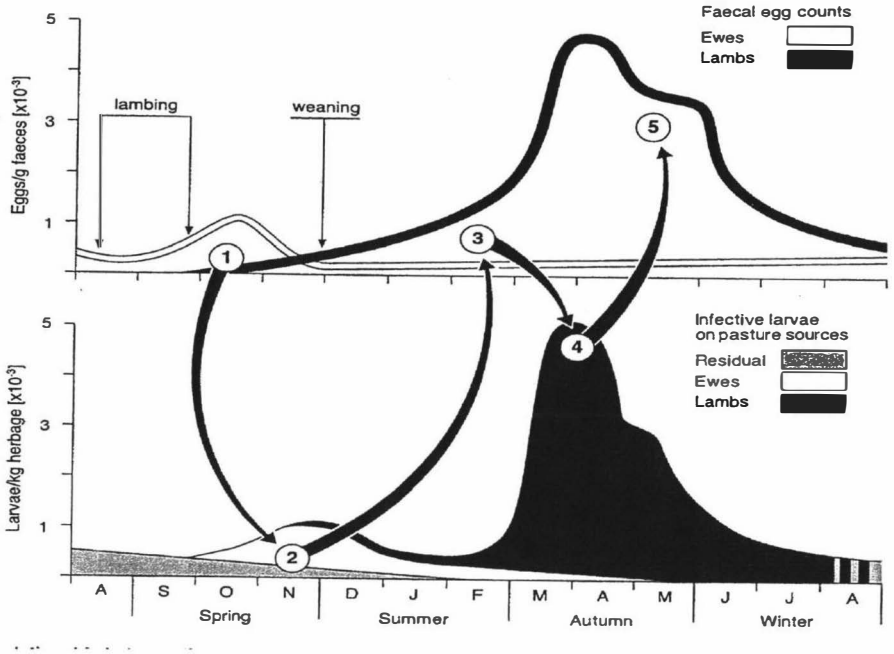


Figure 1.1. The inter-relationship between the pasture contamination by untreated lambs and ewes and infective larvae on pasture. (Adapted from Vlassoff *et al.*, 2001).

1. The post-parturient rise in FEC of breeding ewes.
2. Over-wintered L3 and those from spring peak contribute first generation of worms.
3. Eggs deposited by lambs in summer/autumn as a source of larvae on pasture.
4. L3 from autumn peak that contribute 2nd generation of worms in lambs.
5. Decrease in egg development in late autumn/winter due to low environmental temperarture.

Generally sheep harbour mixed parasitic nematode burdens in their gastrointestinal tract. The high pasture contaminations mainly contribute to nematode infections and is usually associated with grazing of untreated ewes and lambs in the paddocks. The spring peak of L3 on pasture is mainly due to a post-

parturient rise in faecal egg counts (FEC) of breeding ewes that contribute to pasture contamination.

The over-wintered L3 and those from the spring peak give rise to the first generation of worms which accumulate in lambs over summer months. The eggs deposited by lambs in summer/early autumn are the main source of the autumn/winter population of larvae on pasture. The L3 from the autumn peak produce the second generation of worms in lambs. Once development to the L3 stage is complete, larvae are considered most resistant to adverse conditions and they can survive 3-12 months.

There is a decline in nematode egg development in autumn/winter due to low environmental temperature. Early infections in lambs are usually dominated by *Strongyloides* spp.. *Nematodirus* spp. tend to predominate in late spring followed by *Ostertagia* spp and *H. contortus* and *Trichostrongylus* spp. in late summer/autumn (Vlassoff *et al.*, 2001).

A mathematical model of worm fecundity showed that the maximum egg production in *Trichostronglus colubriformis* was estimated to be 700 eggs per day (Barnes and Dobson, 1989). Lambs have less ability to resist nematode infection and develop immunity about 10-12 months of their age. Trials conducted in Australia showed that at 36 weeks of age, pasture-reared sheep had aquired a high level of resistance to infection with *T. colubriformis* (Dobson *et al.*, 1990). Adult sheep develop immunity to parasitic infections due to frequent exposure to the infection but the ability to maintain high level immunity fails at times of nutritional stress, periparturient period or illness.

The increasing incidence of anthelmintic resistance on gastrointestinal nematode poses challenge to the sheep industry (Leathwick *et al.*, 2001). It is necessary to adopt parasite management programmes for sustainable livestock farming into the future. Anthelmintics, together with an understanding of the epidemiology of

parasitism including the immune response and the nutritional requirements of sheep, currently enable satisfactory management of the problem (Vlassoff *et al.*, 2001).

The life cycle of gastrointestinal nemtodes is well documented and the trichostrongylids have a direct lifecycle with six stages (Soulsby, 1982).. Most nematodes infecting sheep share the same basic lifecycle (Vlassoff *et al.*, 2001) Adult nematodes mate in the gastrointestinal tract of the host and female worm lay eggs containing embryos, which pass out in faeces of sheep. The egg in the faeces undergo further development to L1 and L2 stages then to L3 which is the infective stage that migrates onto the herbage. Following ingestion by a suitable host the L3 ex-sheath in response to changes in carbon dioxide concentration, temperature and pH before reaching the site of infection. They undergo further moults to L4 and immature adults and develop as sexually mature adults in about 15-21 days.

1.9. NEMATODE CONTROL MEASURES

In New Zealand, parasite prevention and management programmes for lambs/hoggets and adult sheep is achieved by using anthelmintics at regular intervals or during times of risk. Most of the farmers adopt a 5-6 drench programme for their young lambs commencing at about weaning and then drenching every 4 weeks. They also drench at the time of high risk of the infection. Rams and adult ewes are also drenched once or twice in a year.

The role of ewes as a source of pasture contamination should be considered when developing parasite control regimes on farms (Stafford *et al.*, 1994). Monitoring faecal nematode egg counts from ewe flocks to limit drenching and sheep/cattle interchange grazing is also recommended to reduce the level of pasture contamination. Increasing the frequency of drenching may give rise to anthelmintic selection pressure to gastro-intestinal nematodes and this may increase the incidence of anthelmintic resistance.

Anthelmintics will play a central role in nematode control program of livestock. However, to prevent emergence of resistance, anthelmintics need to be either so effective that there would not be survivors or treatment should be so infrequent that those survive make little or no contribution to subsequent generation of the parasite population (Waller, 1993).

Mixed grazing of cattle and sheep is much more commonly practiced procedure and can be very effective form of parasite control for both livestock species, provided grazing alterations are linked with seasonal trends in larval availability on pasture (Waller, 1997).

Therefore, it is necessary to reduce pasture contamination in the farm, maintain the adequate level of nutrition, quarantine drench to newly introduced sheep from outside source, adoption of proper drenching methods, effective drug use/drench rotation and proper diagnosis of anthelmintic resistance problem.

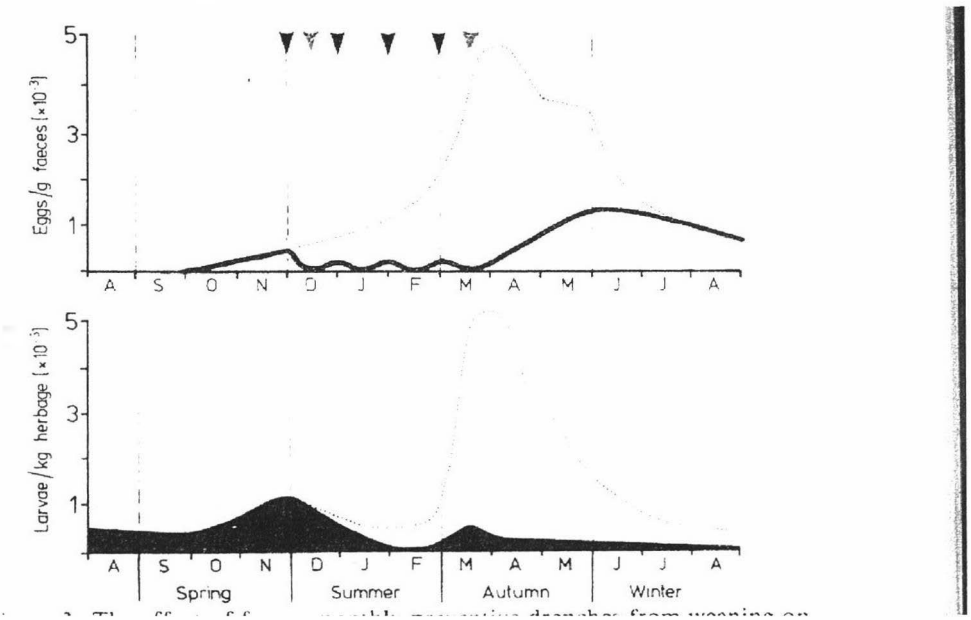


Figure 1.2. The effects of monthly preventive drenches (4-6), from weaning on the faecal egg output of lambs and pattern of larval availability on pasture. The arrow shows anthelmintic treatment. (Adapted from Brunsdon and Vlassoff, 1982).

Studies on anthelmintic resistance and parasite control measures in New Zealand have been conducted from time to time. The only large scale survey in both the North and South Island was conducted in 1981-1982 (Kettle et al., 1982). A survey on anthelmintic resistance on goat farms in Manawatu region in the North Island was conducted in 1988 (Scherrer *et al*., 1988). A smaller survey of aparasite control measures used by sheep farmers in lower North Island was conducted in 1995 (Macchi, 1997). These studies have given valuable informations regarding anthelmintic resistance and control measures adopted in various parts of New Zealand.

CHAPTER TWO

SURVEY ON ANTHELMINTIC RESISTANCE ON VARIOUS SHEEP FARMS IN NEW ZEALAND, USING LARVAL DEVELOPMENT ASSAY TEST

2.1. Introduction

Anthelmintic resistance in the sheep/goat nematodes is a growing worldwide problem. The New Zealand farming system is based on intensive grazing management system; sheep and goat farming depends moderate to heavily on anthelmintics to control gastro-intestinal nematode infections. In New Zealand, anthelmintic resistance in sheep nematodes was first detected in 1979 (Vlassoff and Kettle, 1980). A formal survey of anthelmintic resistance in sheep nematodes was conducted in the North and South Island in 1982 (Kettle *et al.*, 1981, 1983). Since then the only information on the occurrence of anthelmintic resistance is from laboratory data and occasional case reports. The prevalence has steadily increased on New Zealand sheep/goat farms. McKenna (1995) reported resistance to levamisole in 29%, benzimidazole in 66% and combination drench 16% of the parasitological submission to animal health laboratories in 1993.

The most commonly involved nematode species are *Haemonchus contortus, Ostertagia circumcinta, Trichostrongylus* spp. and to a lesser extent *Nematodirus* and *Cooperia* species.

There are several tests for detection of anthelmintic resistance in sheep/goat nematodes (see review in 1.6). The larval development assay (LDA) is an *in vitro* test and it has been considered the most reliable, inexpensive and suitable for use in the field for detection of anthelmintic resistance in sheep and goat nematodes. The LDA technique has been used for detection of anthelmintic resistance in sheep/goat nematodes by many workers (Taylor, 1995; Amarante *et. al.*, 1997,

Varady and Cobra, 1998; Craven *et al.*, 1999 and Gopal 2000). Lacey *et al.* (1990) described modifications of the LDA, by using 96 well microtitre plates. This technique is termed the microlarval development assay. In this technique nematode eggs in nutrient media are added on to an agar matrix containing various concentrations of anthelmintics. CSIRO, Australia has developed a commercial LDA assay system "DrenchRite Larval development Assay" (Horizon Technology Pty Limited) in 1996. This technique is based on methods described by Gill *et al.* (1995) and Lacey *et al.* (1990).

This chapter reports the results of a survey of anthelmintic resistance using the LDA. This survey was conducted by collecting faecal samples from randomly selected farms from various regions of New Zealand from April 2003-December 2003. The original intention was to use the commercially available larval development assay (DrenchRite LDA) but at the commencement of the survey the DrenchRite LDA plates were not available and inhouse larval development assays were used in most cases.

2.2. Materials and Methods

2.2.1. Sample collection and egg recovery

Fifty farms were randomly selected using the Agribase farm database. Altogether 25 farm samples, each farm containing 10 faecal samples were received from the field representatives of Schering Plough (New Zealand Limited) from various parts of New Zealand from the larger selection of 50 farms. Fresh faeces were collected in plastic bags from lambs and hoggets and transported by courier service and received at the Veterinary Parasitology Laboratory; Institute of Veterinary, Animal and Biomedical Sciences, Palmerston North within two days of the sample collection. These samples were processed immediately on arrival in the lab. The egg recovery procedure used was largely as described by Hubert and Kerboeuf (1992) and the DrenchRite LDA method (Horizon Technology Pty Ltd, Australia, 1996). The nematode egg count of each sample was initially estimated using a

Modified McMaster technique where each egg counted represents 50 eggs/g (as detailed in Appendix 2.1). The LDA procedure, including egg recovery is detailed in Appendix 2.1 in detail. In brief egg recovery was achieved as follows. About 30-100g of the pooled faecal sample was taken from each farm, depending on faecal egg counts (FEC) and made into slurry with water. This slurry was sieved through a 1mm aperture sieve, then through a 100µm aperture sieve with eggs subsequently collected on a 20µm aperture sieve. The eggs were further concentrated by centrifugation over a two sugar gradient solution (sucrose solutions of 10% and 25%) at 1000g for 7 minutes. The eggs were recovered from the interface of the two sugar solutions. They were then washed over a 20µm sieve which retained the eggs but removed the surplus sugar solution. The eggs were recovered into water and transferred to a 50ml centrifuge tube and allowed to stand for 30 minutes. Debris/ fine particles were removed from the supematant solution. The clean eggs were collected from the bottom of the tube. The eggs were recovered, washed with distilled water and the number of eggs/ml estimated on the basis of 5-10 counts. The egg concentration was adjusted in order to provide approximately 50, 60, 74 or 80 eggs per 20µl.

2.2.2. DrenchRite Larval Development Assay

The larval development assay and the procedure used in this trial is largely as described in the manual accompanying the DrenchRite assays as described as DrenchRite Standard Operating Procedure (Horizon Technology Pty Limited, Australia, 1996). The tests were performed in a 96 well microtitre well that contain various anthelmintic concentrations in 150µl of agar in each well. Tandon and Kaplan (2004) have reported that eleven concentrations of TBZ, IVM-1, IVM-2 and eight concentration of LEV are included in the standard DrenchRite assay. For TBZ these concentrations (µM) as as follows: 0.010, 0.020, 0.040, 0.078, 0.156, 0.313, 0.625, 1.250, 2.500, 5.000 and 10.000; for IVM-1 (nM): 0.500, 0.970, 1.900, 3.900, 7.800, 15.600, 31.250, 62.500, 125.000, 250.000 and 500.000; for IVM-2 (nM): 0.970, 1.900, 3.900, 7.800, 15.600, 31.250, 62.500, 125.000, 250.000,

500.000 and 1000.000; and for Lev (µM): 0.195, 0.390, 0.780, 1.560, 3.125, 6.250, 12.560 and four replicate of 25.000.

The sealed plates were opened immediately before commencing the assay from each farm samples. This assay was performed by adding 50-70 eggs in each well and incubated at 25 °C. On the Day 2 about 10-20µl of nutrient media was added in each well when control wells had 60-80% hatching. These plates were further incubated at 25°C until 7th day of incubation. The LDA plates were then refrigerated at 4°C until examined. On Day 7 of the incubation the liquid phase from each well was removed and the number of eggs, L1, L2 and L3 at each well of the drug concentrations were counted at 100x-200x under a compound microscope after staining with Lugol's iodine. The strongylid genera/ species were identified on the basis of their measurements and morphological features. All counts were done within two days of the end of incubation.

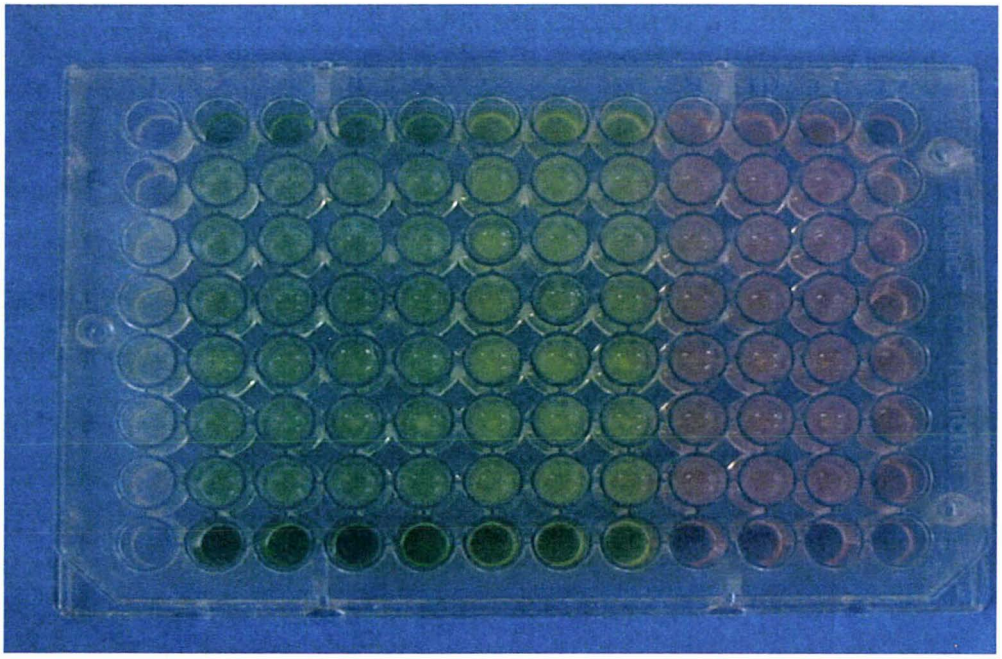


Plate 2.1. DrenchRite LDA plate

2.2.3. Larval Culture

The pooled faecal sample was mixed with vermiculite and it was cultured at 27°C for 7 days. The infective larvae (L3) were recovered by using the Baermann technique as described in Appendix 2.1. The percentage of various species of trichostrongylid genera/species were identified from the cultured samples.

2.2.4 Inhouse LDA Assays

Altogether 15 Inhouse LDA plates were prepared for the Larval Development assay of various farm samples. These LDA plates were used for study of detection of anthelmintic resistance on various sheep farms in conjunction with DrenchRite LDA

The Inhouse larval development assay (Inhouse LDA) used in this trial is largely as described by Gill et al (1995) and prepared to be similar to DrenchRite assays although with some variation of anthelmintic analogue used and some minor variation in anthelmintic concentrations.

A detailed protocol is described in Appendix 2.3. In brief, thiabendazole (99.3% powder, donated by MSD AgVet, New Zealand Ltd.), levamisole hydrochloride (Rycozole, oral drench, Youngs Animal Health, New Zealand), ivermectin aglycone (99.2% powder donated by Virbac laboratories Ltd, New Zealand) were used and two different sources of ivermectin were used: IVM-1 (Bomectin oral, Bomac laboratories Ltd; ivermectin 0.1% w/v) and IVM-2 (Erase MPC, Schering Plough Animal Health Ltd; 16g/L, ivermectin). The stock solutions of above drugs were prepared in dimethyl sulphoxide (DMSO) and serially diluted 1:2 with DMSO to give 12 concentrations. Similarly, stock solution of levamisole (618µg/ml) was prepared in distilled water and it was serially diluted 1:2 with distilled water to give 12 concentrations. The molar concentrations of these anthelmintics (2µl of the drug concentrations in 150µl of the agar) were also determined.

The tests were performed in 96 well microtitre plate (Falcon, Becton Dickinson Labware, USA) by using various anthelmintic drug concentrations. The Inhouse LDA plates were prepared by adding 2µl of the various drug concentrations in all wells of various rows except control wells. 2µl of distilled water was added for levamisole control wells and 2µl DMSO was added for thiabendazole, ivermectin analogue-1 and 2 and ivermectin aglycone control wells. Each plate contained two rows of wells for thiabendazole, levamisole and IVM-1. It also contained one row of wells for each of IVM-2 and ivermectin aglycone. Then 150µl of 2% agar (Bacto-agar, Y-1000 Sigma) was added in each of 96 wells and allowed to set.

After 1 hour of preparation of the LDA plates, 50-70 clean nematode eggs in 20µl of distilled water was added to each of the 96 wells. The number of nematode eggs per well was determined on the basis of availability of total clean eggs in the sample.

These plates were incubated at 25°C for 7 days. On Day 2 of the incubation, 10-20µl of nutrient media (one gram of yeast extract (Y- Sigma) in 90ml of 0.85% saline solution and Earle's Balanced salt solution (E7510, Sigma) was added to every 9ml of yeast solution) (Hubert and Kerboeuf, 1984 and some modification) was added in each of 96 wells when control wells had >60% egg hatching. The LDA plates were scanned every 2nd day to check for drying of the wells and whenever drying is noticed, 10µl of distilled water was added and this was recorded.

On Day 7 of the incubation the liquid phase from each well was removed and the number of eggs, L1, L2 and L3 at each well of the drug concentrations were counted at 100x under compound microscope after staining with Lugol's iodine. The strongylid genera/ species were identified on the basis of their measurements and morphological features. All counts were done with in two days of the end of incubation.

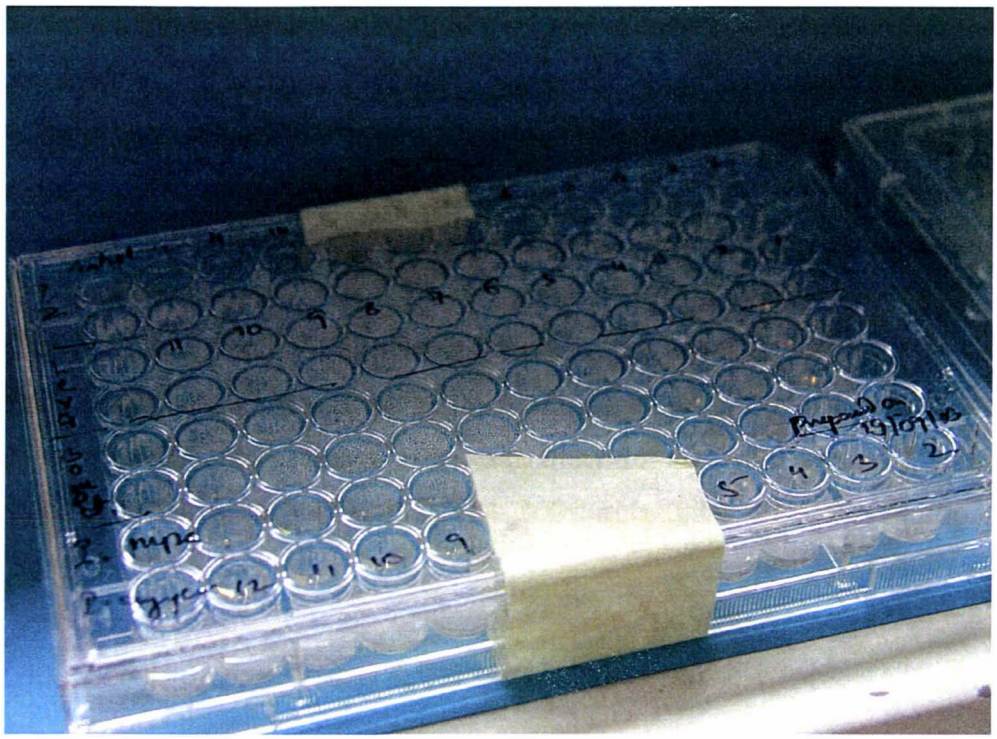


Plate 2.2 Inhouse LDA plate.

2.2.5 Data analysis

The eggs, 1st stage larvae (L1), 2nd stage larvae (L2) and third stage larvae (L3) from each well were counted and recorded. The number of third stage (L3) larvae in the test wells were compared to the number that developed in control well for each trichostrongylid genus. This adjusted proportion was fitted to a sigmoid curve (dose response curve) to calculate LC_{50} values, after log transformation, using SlideWrite version 5.01 (Advance Graphics Software Inc., U.S.A.).

The interpretation of both assays were calculated by LC_{50} well number and compared to the Drenchrite LDA manual comparison table of efficacy (see Appendix 2.x). For *Trichostrongylus* this manual states that where the LC_{50} occurs the efficacy can be interpreted as follows: For benzimidazoles Well 4.5 indicates an efficacy of 94%, Well 5 an efficacy of 87%; For levamisole Well 5.0 indicates an efficacy of 94% Well 5.5 an efficacy of 83%; For the combination of benzimidazole and levamisole (BZ+LEV) Well 6 indicates an efficacy of 93%. DrenchRite can be

used to detect the presence of resistance in ivermectin analogues but not as yet, to quantify efficacy. For ivermectin analogues the LC_{50} Well (mean) 5.5 and above, can be interpreted as indicating some level of resistance.

The molar concentration of the LC_{50} was also calculated by using the software programme SlideWrite version 5.01 (Advance Graphics Software Inc, U.S.A.) and expressed as micromole (µM) and µg/ml in the agar phase. The goodness of fit (R^2 value) of the sigmoid curve is also shown. For those farms where both DrenchRite and Inhouse LDA plates were used the results were compared by using NCSS and PASS 2000 (Hintze J., 2001, NCSS and PASS Number Cruncher Statistrical System, Kaysville)

2.3. Results of DrenchRite and Inhouse LDA tests

All together samples from 25 farms were processed that contained 10 samples from each farm (total 250 samples). Five farms had zero faecal egg counts and these samples could not be assessed for LDA.

Samples from 14 farms were assessed with DrenchRite LDA plates and results from 12 farms were recorded for data analysis. Results are shown in Table 2.2 and Fig. 2.2.

Similarly, Samples from 15 farm samples were assessed with Inhouse LDA plates for detection of the level of anthelmintic resistance. Of these, useful results for analysis were achieved for 12 farms but for 3 farms LDA plates were discarded due to poor larval growth in various control wells. Results are shown in Table 2.3 and Fig. 2.1. The details of data obtained from DrenchRite and Inhouse LDA is given in Appendix 2.6, that is included in the compact disk (CD).

The predominant genera (n=20), on LDA plates and larval cultures was *Trichostrongylus* spp. (78%), *Chabertia* and *Oesophagostomum* spp. (17%) and *Haemonchus controtus* (6%). The results are shown in the Table 2.1.

An example of a dose response curves is shown in the Figure 2.4 and various dose response curves from various farm samples are given in Appendix 2.4.

Samples from 6 farms were put onto both DrenchRite and Inhouse LDA plates and were available for comparision. Both showed a similar LC_{50} and LC_{50} well for benzimidazoles (see Table 2.4) which is consistent with the similar concentration of thiabendazole used in the two assays. The exception was for Farm TN04664 where there was a wide discrepancy but this was also a plate where there was substantial fungal contamination in the Drenchrite LDA plate.

The actual concentrations of levamisole in DrenchRite and Inhouse LDA plates were different. Drenchrite had four replicates of 25µM in wells 9, 10, 11 and 12 and DrenchRite Plates generally had 2-3 fold higher concentration than the equivalent Inhouse LDA well. The comparison of LC_{50} between the two assay systems is shown in Table 2.4. It was not sensible to compare the LC_{50} wells but even the calculated LC_{50} concentrations were different between the two assay systems. The result was difficulty in interpretating the Inhouse LDA levamisole results.

As the analogues used for assessing ivermectin resistance in the Drenchrite LDA plate were not officially known it was not possible to formally compare the two assay systems for this action family of anthelmintics.

The interpretation of DrenchRite LC_{50} results as per the Drenchrite manual is shown in detail in Appendix 2.5. Note it varies between different anthelmintics. However, in general a LC_{50} of Well 4.5-5 and above is considered to be suspected resistance (SR) and a LC_{50} of Well 8 and above is considered to be a high level resistance (R).

With the Drenchrite LDA suspected resistance for benzimidazole was found in 5 farms (n=12) and resistance (R) in 1 farm. In total 50% of the farms demonstrated some level of resistance in *Trichostrongylus* spp. with this assay. With the Inhouse LDA suspected resistance for benzimidazole was also demonstrated in 5 farms (n=12) and a high level resistance in 1 farm. Overall, as with the other assay, the Inhouse LDA also demonstrated that 50% farms (*Trichostrongylus* spp. in 5 farms and *Haemonchus contortus* in 1 farm) demonstrated a level of resistance with thiabendazole.

The actual LC_{50} concentration values of benzimidazole for suspected resistance and resistance ranged from 0.028µM to 0.29µM. The LC_{50} well wise interpretation was done on the basis of guidelines provided by DrenchRite manual and the concentrations stated in Tandon and Kaplan (2004) .

The LC_{50} Well of 4.5 and above in DrenchRite LDA for levamisole are shown in Table 2.2 and demonstrated a level of resistance in 9 of the 12 farms (75%) of which 7 farms were in *Trichostrongylus* and 2 farms in *Oesophagostomum/Chabertia* for which there is no validated comparison to determine the appropriate LC_{50} well.

For the combination of BZ and levamisole in Rows 5 and 6 of the DrenchRite plate there were 3/12 farms (25%) that demonstrated resistance and all were in *Trichostrongylus*.

The LC_{50} Well of 6 and above in DrenchRite assays for ivermectin analogues was observed in only one farm (Farm WO00501; n=12), which implies only 8.3% of sampled farms were demonstrating possible resistance to this anthelmintic. This poor efficacy was to *Trichostrongylus*. A second farm (Sample 5, Farm TN04346) had a LC50 Well of 5 indicating a suspicious level of efficacy to *Trichostrongylus*.

Another farm had a LC_{50} Well of 5 for *Oesophagostomum/Chabertia* but it is uncertain how to interpret this finding.

For levamisole, based on the molar value for LC_{50} calculated for Drenchrite, the LC_{50} Well 4.5 in DrenchRite is similar to that of Well 6 in the Inhouse assay. Using this comparison resistance was demonstrated in 25% farms.

For Inhouse LDA ivermectin aglycone there were usable data for only 6 farms. Only one farm had an LC_{50} well of Well 5 and above (8.3%) and this was to *Trichostrongylus* and it was notably higher than other cases where this genus was represented.

The results of DrenchRite LDA also showed that one farm (WO00501) out of twelve farms (8.3%) had a high level of resistance for all drug action families (benzimidazole, levamisole, combination and ivermectin). This was to *Trichostrongylus*.

Including results from both assay systems it can be concluded that anthelmintic resistance in *Trichostrongylus* (either suspected or high level) was demonstrated in 60% of the farms (9/15) to benzimidazole, resistance to levamisole in 66% of farms (10/15), combination drench (BZ+LEV) on 43% of farms (3/7) and ivermectin on 1 of 8 farms (ignoring Inhouse LDA result Farm WT05469). For those farms where *Trichostrongylus* was the predominant genus there was resistance to at least one anthelmintic on all tested farms (n=12). Of note is Farm 6 with the Drenchrite LDA (Farm WO00501) demonstrated resistance in *Trichostrongylus* to all anthelmintics.

The combination drug containing benzimidazole and levamisole was also tested in DrenchRite assay but not used in Inhouse assays. Data from DrenchRite assays showed that the combination drug is more effective as compared to BZ or LEV alone. Inhouse assays results also showed that ivermectin analogues are more effective as compared to other drugs in sheep nematodes.

Table 2.1. Average FEC and percentage of different genera of trichostrongylids on larval culture from various sheep farms

				Percentage of Trichostrongylid species				
S.No.	Farm ID	Sample date	Av.EPG	*Trichostrongylus spp.*	*Ostertagia spp.*	*Cooperia spp.*	*Haemonchus -contortus*	*Oesopagostomum/Chabertia*
1	A	10/06/2003	185	82	0	10	0	8
2	J	10/06/2003	165	3	0	3	0	94
3	I	10/06/2003	90	96	0	4	0	0
4	TN04664	10/07/2003	260	79	0	20	0	1
5	NP00844	15/07/2003	355	61	5	29	0	5
6	TN04346	15/07/2003	795	80	0	16	0	4
7	WO00501	17/07/2003	530	84	10	0	0	6
8	WO00544	22/07/2003	2,215	78	0	11	0	11
9	TI00352	26/08/2003	230	71	0	0	0	29
10	AS00539	28/08/2003	165	18	0	2	0	80
11	HS00039	04/09/2003	0	0	0	0	0	0
12	SE04795	04/09/2003	270	40	4	52	0	4
13	WK01091	09/09/2003	305	76	7	7	0	10
14	HB00312	10/09/2003	0	0	0	0	0	0
15	SE00470	10/09/2003	165	75	10	4	0	11
16	OT00695	11/09/2003	25	0	0	0	0	0
17	RU05209	17/09/2003	90	100	0	0	0	0
18	HS00039*	17/09/2003	115	54	2	2	42	0
19	TP00301	19/09/2003	450	86	0	8	0	6
20	GI00163	08/10/2003	175	79	0	6	6	9
21	WM00679	14/10/2003	5	0	0	0	0	0
22	WK04066	15/10/2003	305	36	0	4	52	8
23	OT00695*	22/10/2003	0	0	0	0	0	0
24	TI00133	30/10/2003	0	0	0	0	0	0
25	WT05469	20/11/2003	400	87	5	0	0	7

Note:- Most of the faecal samples were collected from lambs/hoggets.

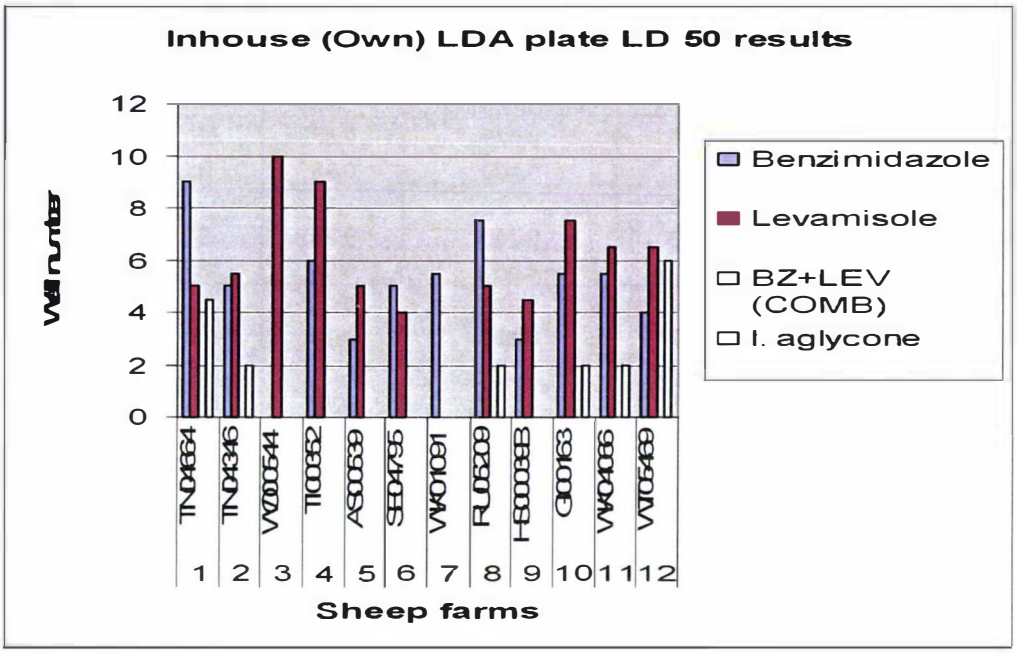


Figure 2.1. Bar chart showing Inhouse LDA plate LC_{50} wells for benzimidazole, levamisole and ivermectin aglycone for 12 sheep farms.

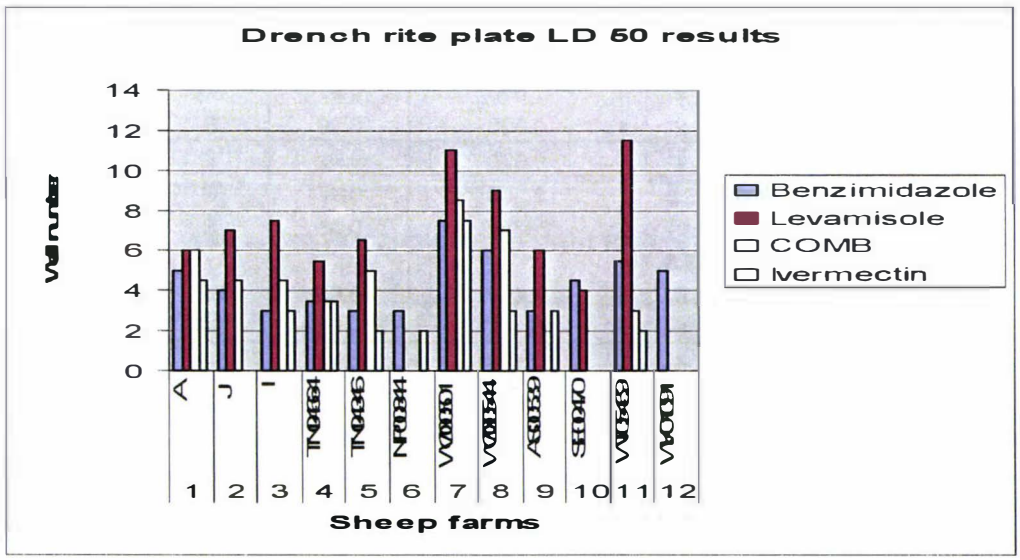


Figure 2.2. Bar chart showing DrenchRite LDA LC_{50} well for benzimidazole, levamisole, Comb (BZ+LEV) and ivermectin aglycone in various sheep farms.

Table 2.2. Summary of DrenchRite LDA results with the LC_{50} well, LC_{50} concentration, R^2 value of the dose response curve, status and predominant genera for which the assay could interpret resistance status.

S.No.	Farm ID	Drug[1]	LC_{50} well	LC_{50} (µM/nM)[2]	R^2 value	Status[3]	Predominant spp.
1	A	TBZ	5	0.029	0.97	SR	*Trichostrongylus spp.*
		LEV	6	3.100	0.98	R	" "
		COMB	6				
		IVM-1	3	0.750	0.98	S	" "
		IVM-2	4.5	4.350	0.94	S	" "
2	J	TBZ	4	0.030	0.99	S	*Oesophago/Chabertia*
		LEV	7	6.100	0.79	SR	" "
		COMB	4.5				
		IVM-1	5	1.210	0.79	SR	" "
3	I	TBZ	3	0.010	0.99	S	*Trichostrongylus spp.*
		LEV	7.5	7.800	0.93	R	" "
		COMB	4.5				
		IVM-1	5.5	3.000	0.97	S	" "
		IVM-2	4.5	3.100	0.93	S	" "
4	TN04664	TBZ	3.5	0.029	0.96	S	*Trichostrongylus spp.*
		LEV	5.5	1.930	0.95	SR	" "
		COMB	3.5				
		IVM-1	3	1.180	0.97	S	" "
		IVM-2	3.5	2.700	0.99	S	" "
5	TN04346	TBZ	3	0.018	0.97	S	*Trichostrongylus spp.*
		LEV	6.5	3.000	0.98	SR	" "
		COMB	5				
		IVM-1	5	1.600	0.97	SR	" "
		IVM-2	2	0.900	0.99	S	" "
6	NP00844	IVM-2	2	0.620	0.92	S	*Trichostrongylus spp.*
7	WO00501	TBZ	7.5	0.280	0.97	R	*Trichostrongylus spp.*
		LEV	11	26.000	0.98	R	" "
		COMB	8.5				
		IVM-1	6	6.400	0.98	SR	" "
		IVM-2	8	37.000	0.93	SR	" "
8	WO00544	TBZ	6	0.165	0.98	SR	" "
		LEV	9	11.000	0.89	R	" "
		COMB	7				
		IVM-1	5	2.950	0.95	SR	" "
		IVM-2	2	2.100	0.98	S	" "
9	AS00539	TBZ	3	0.015	0.99	S	*Oesophago/Chabertia*
		LEV	6	4.800	0.98	SR	" "
		IVM-2	3	1.700	0.98	S	" "
10	WK01091	TBZ	5	0.090	0.98	SR	*Trichostrongylus spp.*
11	SE00470	TBZ	4.5	0.080	0.94	SR	*Trichostrongylus spp.*
		LEV	4	0.990	0.97	S	" "
12	WT05469	BZ	4	0.090	0.77	S	*Trichostrongylus spp.*
		LEV	11.5	60.000	0.69	R	" "
		COMB	3				
		IVM-1	2	0.022	0.99	S	" "

		IVM-2	2	0.750	0.95	S	"	"

1. TBZ =:thiabendazole, LEV = levamisole, IVM-1 = ivermectin analogue 1, IVM-2 = ivermectin analogue 2
2. Concentration of active ingredient in agar phase as micromoles (µM) for all active ingredients or nanomoles (nM) for ivermectin.
3. Anthelmintic resistance status in the assay based on DrenchRite manual, resistant(R) , susceptible (S) or suspected resistance (SR)

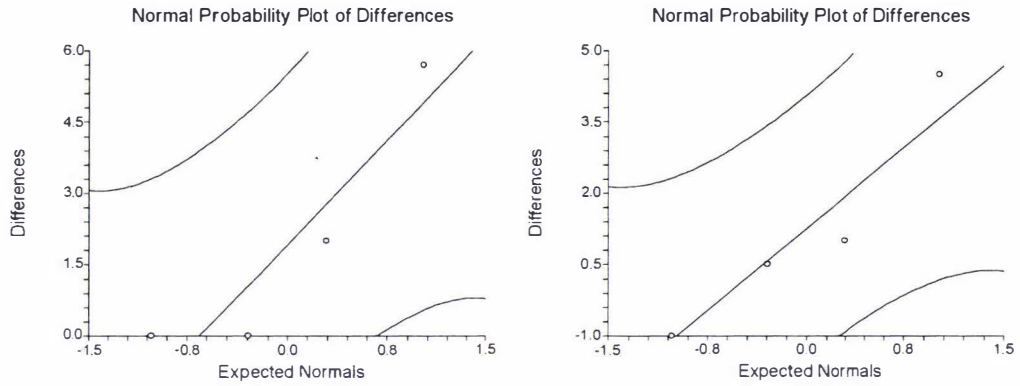


Figure 2.3 Showing normal probability Plots for comparision of DrenchRite and Inhouse LC_{50} wells with benzimidazole (left) and levamisole (right)

Table 2.3 Summary of Inhouse LDA results with the LC_{50} well, LC_{50} concentration, R^2 value of the dose response curve, status and predominant genera for which the assay could interpret resistance status.

S.NO.	Farm ID	Drug[1]	LC_{50} well	LC50(µM/nM)[2]	R^2 value	Status[3]	Genus tested
1	TN04664	TBZ	9	1.250	0.94	R	*Trichostrongylus spp.*
		LEV	5	0.820	0.94	S	" "
		IVM-B[2]	2.5	0.210	0.97	S	" "
		IVM-agly[3]	2	0.001	0.93	S	" "
2	TN04346	TBZ	5	0.090	0.98	S	*Trichostrongylus spp.*
		LEV	5.5	0.400	0.98	S	" "
3	WO00544	LEV	10	1.700	0.9	R	*Trichostrongylus spp.*
4	TI00352	TBZ	6	0.250	0.87	SR	*Trichostrongylus spp.*
		LEV	9	1.860	0.98	SR	" "
5	AS00539	TBZ	3	0.010	0.96	S	*Oesophago/Chabertia*
		LEV	5	0.110	0.96	S	" "
6	SE04795	TBZ	5	0.350	0.99	S	*Trichostrongylus spp.*
		LEV	4	0.110	0.96	S	" "
		TBZ	4.5	0.065	0.99	S	*Cooperia spp.*
		LEV	4	0.180	0.92	S	" "
7	WK01091	TBZ	5.5	0.090	0.97	SR	*Trichostrongylus spp.*
8	RU05209	TBZ	7.5	0.270	0.98	R	*Trichostrongylus spp.*
		LEV	5	0.180	0.99	S	" "
		IVM-B	3	0.400	0.98	S	" "
		IVM-agly	2	0.480	0.92	S	" "
9	HS00039B*	TBZ	3	0.009	0.99	S	*Oesophago/Chabertia*
		LEV	4.5	0.060	0.98	S	" "
10	GI00163	TBZ	5.5	0.090	0.99	SR	*Trichostrongylus spp.*
		LEV	7.5	0.990	0.93	SR	" "
		IVM-agly	2	0.400	0.98	S	" "
11	WT05469	TBZ	4	0.020	0.99	S	*Trichostrongylus spp.*
		LEV	6.5	0.180	0.96	S	" "
		IVM-B	2	0.020	0.97	S	" "
		IVM-E					
		IVM-agly	6	9.900	0.9	SR	" "
12	WK04066	TBZ	5.5	0.950	0.98	SR	*Haemonchus contortus*
		LEV	6.5	0.500	0.99	SR	" "
		IVM-B	2	0.250	0.99	S	" "
		IVM-E					" "
		IVM-agly	2	0.009	0.97	S	" "
	WK04066	TBZ	5	0.040	0.98	SR	*Trichostrongylus spp.*
		LEV	6	0.450	0.98	S	" "
		IVM-B	2	0.250	0.98	S	" "
		IVM-agly	2	9.900	0.97	S	" "

1. TBZ =:thiabendazole, LEV = levamisole, IVM-B = ivermectin B2, IVM-agly = ivermectin aglycone
2. Concentration of active ingredient in agar phase as micromoles (µM) except nanomoles (nM) for ivermectin.
3. Anthelmintic resistance status in the assay based on DrenchRite manual, resistant(R) , susceptible (S) or suspected resistance (SR)

Table 2.4 Comparison of DrenchRite and Inhouse assay results:-

S.NO.	Farm ID	Assay	LC_{50} well	Drug	LC50(µM)	R^2 value	Status	Genus tested
1	TN04664	DrenchRite	3.5	TBZ	0.029	0.96	S	*Trichostrongylus spp.*
		Inhouse	9	TBZ	1.250	0.94	SR	" "
		DrenchRite	5.5	LEV	1.930	0.95	SR	*Trichostrongylus spp.*
		Inhouse	5	LEV	0.820	0.94	SR	" "
2	TN04346	DrenchRite	3	TBZ	0.020	0.97	S	*Trichostrongylus spp.*
		Inhouse	5	TBZ	0.090	0.98	S	" "
		DrenchRite	6.5	LEV	3.000	0.98	SR	*Trichostrongylus spp.*
		Inhouse	5.5	LEV	0.420	0.98	SR	" "
3	WO00544	DrenchRite	9	TBZ	0.165	0.98	SR	*Trichostrongylus spp.*
		Inhouse	10	TBZ	1.700	0.90	R	" "
4	AS00539	DrenchRite	3	TBZ	0.015	0.99	S	*Oesophag./Chabertia*
		Inhouse	3	TBZ	0.010	0.96	S	" "
		DrenchRite	6	LEV	4.800	0.98	SR	*Oesophag./Chabertia*
		Inhouse	5	LEV	0.450	0.96	SR	" "
5	WK01091	DrenchRite	5	TBZ	0.090	0.98	SR	*Trichostrongylus spp.*
		Inhouse	5.5	TBZ	0.090	0.97	SR	" "
6	WT05469	DrenchRite	4	TBZ	0.090	0.77	S	*Trichostrongylus spp.*
		Inhouse	4	TBZ	0.020	0.99	S	" "
		DrenchRite	11.5	LEV	60.000	0.69	R	*Trichostrongylus spp.*
		Inhouse	6.5	LEV	0.180	0.960	SR	" "

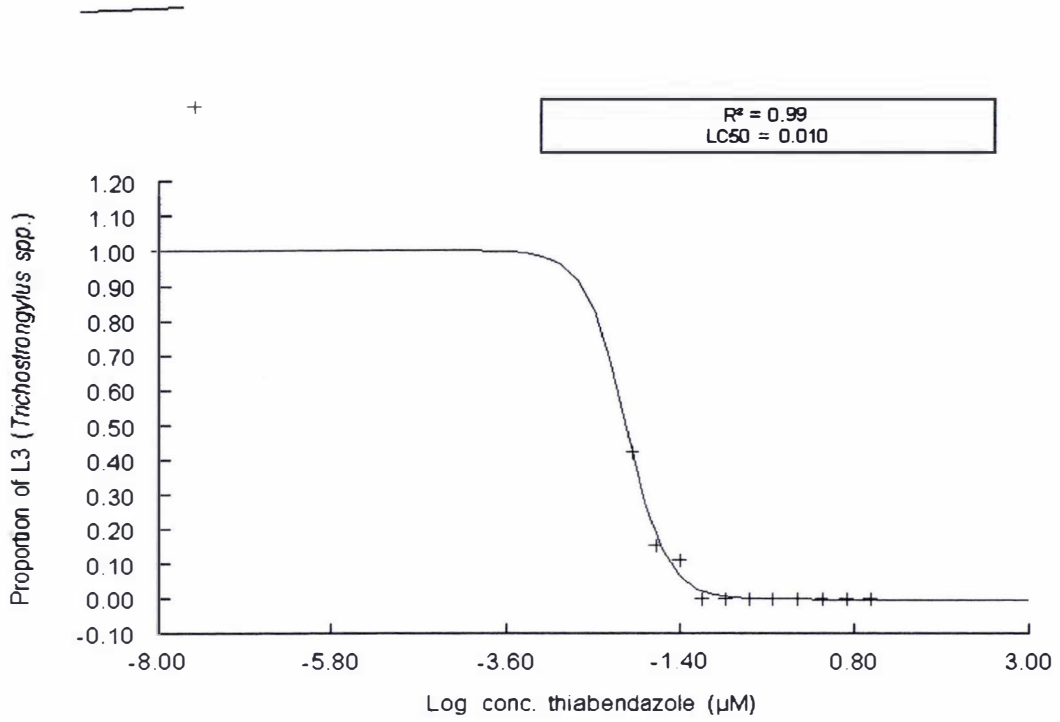


Figure 2.4. Showing a typical dose response curve relating to the proportion of L3's of *Trichostrongylus* spp. to log concentration (µM) of thiabendazole.

2.4. DISCUSSION

The original intention of the study was to conduct DrenchRite larval development assays for detection of anthelmintic resistance in sheep nematodes. However, due to unavailability of DrenchRite LDA plates during the study it was necessary to make similar Inhouse LDA plates to conduct tests in additional farm samples.

When developing the Inhouse LDA a few problems were encountered, especially with the nutrient media that initially contained yeast extract with Earle's balanced salt solution and lyophilised *E.coli*. Initially, 20µl of this nutrient media was supplemented in each well on the Day 2 of the incubation which is a similar volume to that used in DrenchRite assays. The accumulation of dark brown dirt-like

material and death of most of the larvae was observed on the 3rd and 4th day of incubation. These problems continued despite using new supplies of various ingredients to make new nutrient media.

Then, the media supplied by DrenchRite was used in freshly prepared Inhouse LDA plates, this gave better results and this did not have problems but this media was not in enough quantity i.e. it was just enough for the DrenchRite plates with which it was supplied.

The problem with the Inhouse media was eventually resolved by using Nutrient media prepared with Yeast extract solution prepared in 0.85% saline solution, Earle's Balanced salt solution (without *E.coli*) and using it in lower volume/well (7-10µl) i.e. half of the earlier quantity of the nutrient media. These problems limited the number of comparisons that were undertaken between Drenchrite and Inhouse LDA.

For comparision between DrenchRite LDA and Inhouse LDA, only 6 samples were available to compare development in increasing concentrations of benzimidazole of which four were *Trichostrongylus* spp. and another two were *Oesophagostomum/Chabertia*. The four samples that had predominantly *Trichostrongylus* showed a similar LC_{50} well and similar concentration of benzimidazole in comparision wells for benzimidazole but one sample (TN04664) had quite a large difference of a few wells. This may be due contamination of the DrenchRite plate, in which fungal growth was noticed. In case of *Oesophagostomum/Chabertia* both had a similar LC_{50} well. Overall it is concluded that Drenchrite and Inhouse LDA can be considered comparable with BZs.

For comparision between DrenchRite and Inhouse LDA with levamisole, only 4 samples were available. It is known that the actual concentration of levamisole in various wells was different between DrenchRite and Inhouse LDA plates. The actual concentration of levamisole in DrenchRite had four replicates of 25µM in

wells 9,10,11 and 12 and DrenchRite Plates had about 2-3 wells at a higher concentration than Inhouse LDA Plates. This difference had made it difficult to compare the cutoff wells and also to compare LC_{50} values for levamisole between the two systems. It is more feasible to just compare the LC_{50} concentration perhaps than the actual well number. There was a notable difference between the LC_{50} concentration between the two assay systems for which there is no obvious explanation. It was difficult to determine a dose-response curve with the concentrations of levamisole used in the Drenchrite LDA.

Inhouse LDA plates had a similar volume of agar (150µM in each well), a similar number of eggs per well (50-60 eggs/20µl of distilled water) and a similar volume of nutritive media (20µl per well) was used initially. However, 20µl nutritive media did not give satisfactory results and later this was reduced to 7-10µl of nutritive media and it gave good results compared to 20µl per well. Therefore, there was difference of 10µl nutritive media (less in Inhouse LDA plates). At least for the benzimidazole comparison this did not appear to influence the comparison between the two assay systems.

Drying of wells was observed in DrenchRite LDA plates more than in Inhouse LDA plates which probably reflect the fact that Inhouse had been recently made, whereas Drenchrite had been made for some time and even though they were stored in a sealed foil container there was opportunity for the agar to dry out a little. About 10µl of distilled water was added at the time of the start of assays and generally within two days of assays in DrenchRite plates, so DrenchRite Plates had 20µl more volume of distilled water. In some DrenchRite Plates, fungal growth was also observed in some wells which compromised some results. A lower volume of agar was observed in some of the DrenchRite LDA plates reflecting poor quality control. This may have resulted in slight variation in LC_{50} values. Since there was no opportunity to replace these plates they were used regardless.

In all farms there was only one predominant genus in each assay for which an interpretation could be made with the exception of *Oesophagostomum* and *Chabertia* which are combined together because their L3 larvae are so similar morphologically. Therefore, no conclusions can be made about other genera on these farms. In most of the farm samples (n=18) the predominant trichostrongylid genus was *Trichostrongylus*, (14 farms out of 18 farms, i.e. 78% of farms) followed by *Oesophagostomum/ Chabertia* (3 farms out of 18 farms, i.e. 17% of farms) and *Haemonchus* (1 out of 18 farms). Most of the interpretations of the results were based on *Trichostrongylus* spp.

The DrenchRite manual indicates that no comparative slaughter study was conducted to allow efficacy comparison between a LC_{50} value and reduction in worm burden for *Oesophagostomum* or *Chabertia* so the results reported above can only be regarded as suspicious for resistance. Interestingly, resistance is suspected on several farms to these genera, especially to levamisole for *Oesphagostomum/Chabertia*.

The actual drug concentrations of combination drench (benzimidazole + levamisole) in various wells were not known and so it was not possible to calculate actual LC_{50} concentration of the drugs. In Inhouse assays the combination drugs were not used. In samples assessed with Drenchrite LDA, a LC_{50} well of 6 and more was recorded in 3 farms (n=12) this accounts 25% that had some level of resistance to combination drug (BZ+LEV).

In DrenchRite assays a suspicious level of resistance in ivermectin was recorded in 3 farms in *Trichostrongylus* (2 farms) and *Oesophagostomum* spp. (1 farm) that had LC_{50} well 5 and above for ivermectin analog-1 or for ivermectin analog-2. Overall average this accounts suspected level of resistance for ivermectin analogs in 16.6% farms assessed with the Drenchrite LDA. In Inhouse assays a further farm was recorded with a suspicious level of resistance to ivermectin aglycone also in *Trichostrongylus* spp., Despite the low number of farms surveyed in this study

these results suggests there is emergence of resistance to ivermectins in a several farms which could be more serious in future.

Of significant concem was the assessment of one farm (Farm WO00501) where it was determined that resistance was present to all anthelmintics. To date there have been no confirmed reports from sheep farms in New Zealand of this occurring. This farm will be discussed together with others in Chapter 4. Most cases of multiple-resistance involving avermectins have occurred where goats are farmed. In this case there is no mention of goats in the questionnaire response but this farm had brought in large number of lambs (2,500 lambs, age of 12 weeks) on between 1 July 2002 and 30 June 2003.

A measure of the dose response curve is the R^2 value which reflects the goodness of fit of the data to the curve. Data were only analysed if this was high, preferably >0.9. As can be seen in Tables 2.2 and 2.3 this was achieved on most occasions for benzimidazole, levamisole and ivermectin analogs.

Although comparing these present results with earlier published figures is difficult it is apparent that the level of levels of benzimidazole and levamisole resistance in sheep farms in New Zealand are slightly increased since 1994 where the prevalence of resistance was 74% resistance for benzimidazoles, 23% in levamisole and 30% for combination drench in 1995 (McKenna, 1994). Most of the drench resistance tests in New Zealand are conducted using Faecal Egg Count Reduction Tests and the LC_{50} values of various drugs of this result could not be directly compared with other surveys of anthelmintic resistance so the level of resistance found here is not comparable. It may be that the prevalence is similar but where it occurs the level of efficacy is lower? The use of different test methods at the same time of study would help to make comparisions and agreement between the tests.

Overall the two assays were useful in assessing the resistance status of the farms surveyed. Despite initial problems the Inhouse LDA performed well once developed. Interpreting results from the Inhouse LDA are now easier since the publication of the concentrations of thiabendazole and levamisole in the Drenchrite Assay (Tandon and Kaplan, 2004) which occurred after the laboratory work for this present survey was completed. In this present study consistent results were achieved for BZ but not for levamisole which suggests further work may be required for assessing efficacy to this anthelmintic in an Inhouse LDA. The absence of information about the avermectin analogues included in Drenchrite LDA limit the use of an Inhouse assay for this action family. It can be concluded that there is widespread resistance in *Trichostrongylus* spp. in sheep with benzimidazole, levamisole and the combination of both drugs and this problem is steadily growing.

CHAPTER THREE

Survey on anthelmintic resistance and parasite management practices on sheep farms in New Zealand: a questionnaire survey

3.1 INTRODUCTION

There has been great concem about anthelmintic resistance in nematodes of goat, sheep, cattle and horses and now this is a worldwide and growing problem due to the frequent use of various anthelmintics and other parasite management practices on livestock farms. There are several reports of anthelmintic resistance in sheep nematodes from various countries of the world.

In New Zealand, anthelmintic resistance was first detected in 1979 (Vlassoff and Kettle 1980). This is now a serious problem in Australia and New Zealand where 40-60% sheep farms are affected; which is reported by various authors.

A survey on parasite management practices on sheep farms helps to provide updated information on the various aspects that would identify the risk factors involved in the development of anthelmintic resistance and thus help to improve management practices to promote sustainable parasite control. Parasite management practices such as stocking density of animals, grazing practices, mixed farming, pasture management, drenching procedures and use of various anthelmintic drugs on the farm influence the emergence of anthelmintic resistance in nematodes of sheep and other animals. Pasture grazing and favourable climatic conditions for nematode development in New Zealand would have influenced the development of anthelmintic resistance. The first large scale survey on parasite management of sheep was conducted in 1981 (Kettle *et al.*, 1981), in goats 1989 (Scherrer *et al.*, 1989) and a smaller survey in sheep was conducted in 1995 (Macchi, 1995).

This present limited survey was conducted to determine the present status of parasite management practices on sheep farms in New Zealand.

3.2 MATERIAL AND METHODS

3.2.1. Selection of sheep farms and questionnaire design

The parasite management survey was conducted from April to November 2003 in conjunction with a survey for anthelmintic resistance reported in Chapter Two. The survey sought to obtain information about the use of anthelmintics and parasite control for the farms in the year July 2002/ June 2003. This survey was supported by Massey University and Cooper's (a Division of Schering-Plough Animal Health Limited, New Zealand).

Altogether 100 sheep farms were randomly selected from the Agribase database using the criteria that of farms with greater than 1000 breeding ewes (Sanson pers. communication). Survey forms were completed in a face to face interview by Schering-Plough sales represesntatives when they visited the farms. The 6-page questionnaire included four sections which include details of farm and livestock numbers, management of lambs, drenching policies and procedures and farmers own opinion on issuses on anthelmintic resistance. The questionnaire is shown in the Appendix 3.1.

The survey was conducted in both the South Island and the North Island. The farms surveyed were from the following areas: Amberley, Asburton, Aria, Beautiful valley, Benneydale, Cheviot, Denneverke, Darfield, Dunluce, Eltham, Gore, Hexton, Inglewood, Mangamingi, Napier, Oamaru, Ohenewai, Onewhero, Pleasant Point, Rere, Southbum, Tapanui, Taupo, Taumarunui, Te Mata, Templeton, Timaru, Waerenga-O-Kuri, Waimate, Waipawa, Waipukuru.

The livestock numbers present in these farms were used to determine stocking rates by converting the number of each class of stock units according to

procedures described by Scherrer *et al.*, (1989), The stocking rates were calculated by converting the number of various animals to stock units (SU) using the following criteria: lambs/hoggets = 0.5SU, sheep older than 1 year = 1SU, goats < 1year = 0.5 SU, goat, 1 year =1 SU, cattle up to 1 year = 3 SU, cattle older than 1 year = 6 SU, deer > 1 year = 1.2 SU, deer > 1 year = 1.9SU.

3.3. Statistical analysis

The data from the returned survey questionnaire forms were analysed using with the help of Chart Wizard and Excel Formula and Function (Microsoft Office XP Professional 2002, Microsoft Inc., U.S.A).

3.4 RESULTS:-

The overall response and return rate to the questionnaire from various sheep farms including both North and South Island was 38%. These 38 returned questionnaires from various sheep farms were available for data analysis. A summary of the individual farms response is given in Appendix 3. 2 which is in the compact disk (CD) attached to this thesis.

3.4.1. Details of farm and livestock numbers

According to 38 respondents, on an average there were 708 lambs/hoggets on a farm ranging from 0 to 3000; a few farms (n=4) had no lambs during the survey period because those farms have sold out the lambs/hoggets.

According to 38 respondents, on an average there were 2,389 ewes and 38 rams in a farm. Similarly, the average number of dairy and beef cattle in a farm was 28 and 161 respectively. About 84% farmers rear beef cattle with sheep. Only 5.2% farmers of the total (n=38) rear goats with sheep.

According to 38 respondents, the average effective grazing area in a farm is 508 hectares. The total stocking units of sheep (average) in a farm was 2,782 and on an average the total stocking units in a farm was 4,272. For these farms the mean

stocking density was 8.4 SU/ha (n=38). The details of farms and livestock numbers are shown in Table 3.1.

Table 3.1 Details of the farm and livestock numbers

Variables/covariables	**N**	**Mean**	**Median**	**S. D.**	**Min.**	**Max.**
Av. number of lambs/hoggets in a farm	38	708	520	631.8	0	3000
Av. number of deer in a farm	38	8	0	34.3	0	154
% of farms that rear sheep with deer	38	5.26	0	-	-	-
Av. number of dairy cattle (< 1year of age)	38	28.42	0	34.4	0	600
% of farms that rear sheep with dairy cattle	38	13.16	-	-	-	-
Av. number of beef cattle (< 1year)	38	97.50	62.5	126.8	0	660
% of farms that rear beef cattle(<1 year)	38	76.32	-	-	-	-
% of farms that rear sheep with goats	38	5.6	-	-	-	-
Av. number of ewes in a farm	38	2389	1970	1757.5	162	9,000
Av. number of rams in a farm	38	38.05	30	28.13	0	150
Av. number of beef cattle (>1year) in a farm	38	161	124.5	192.9	0	1060
% of farms that rear beef cattle (>1 year)	38	84.21	-	-	-	-
Av. effective grazing area (ha) in a farm	38	508.7	392	479.8	120	2995
% of farms that had quarantine drench in 2002/2003	38	55.25	-	-	-	-
Stocking unit (SU), sheep	38	2,782	-	-	-	-
Total stock Units	38	4,272	-	-	-	-
Stocking density (SU/ha)	38	8.4	-	-	-	-

Table 3.2 Details of management of lambs/drenching policy

Variables/covariables	N	Mean	Median	S.D.	Min.	Max.
Average number of lambs at docking	38	2,903	2,575	2,015	300	10,800
Average number of drench to lambs/hoggets	38	6.07	6.25	1.96	2	13

According to 37 respondents, 58% farmers performed quarantine drenching of brought-in sheep while 37% farmers did not perfom quarantine drenching in their farms and 4% farmers did not respond.

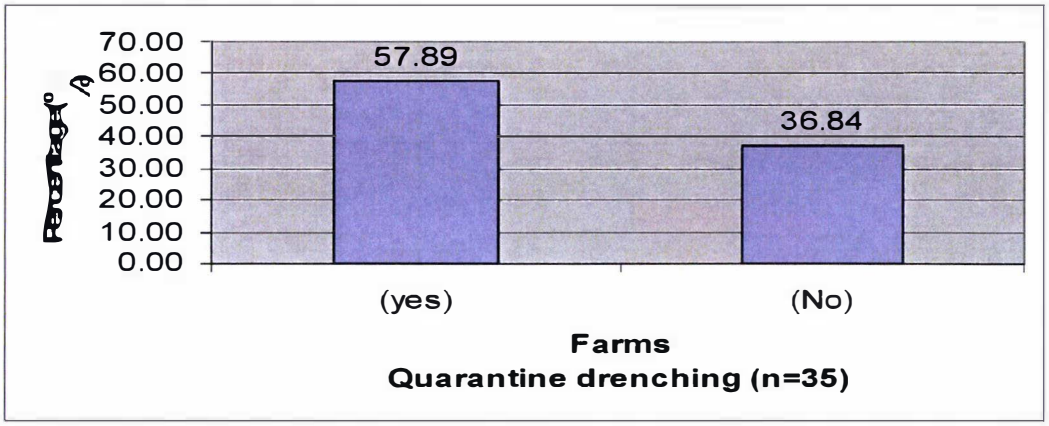


Figure 3.1 Quarantine drench policy in sheep farms.

The anthelmintic used for quarantine drenching on these farms (n=25) were as follows: 4% farmers used benzimidazoles only, 8% farmers used levamisole only, 44% farmers used macrocyclic lactones only, 8% farmers used a double combination drench (benzimidazole + levamisole) and 8% farmers used a triple combination drench (benzimidazole + levamisole + macrocyclic lactone) but about 28% farmers did not mention the drugs used for quarantine drenching in the year 2002/2003. This data also shows that 52% farmers used macrocyclic lactones alone or macrocyclic lactones in combination with other anthelmintics for quarantine drenching in their farms in 2002/2003.

When asked if this was the same drench as was used on the lambs for the remainder of the season about 24% farmers of total (n=13) respondents reported they used same drench whereas 11% used a different product. The remainder did not respond.

Figure 3.2 Same drench used for quarantine drenching as in lambs throughout the year.

3.4.2 Management of lambs

The questions on management of lambs are further divided into the following categories.

3.4.2.1 Number of docking done in the farms

On an average, 2903 lambs were docked per farm in the year between 1 July 2002 and 30 June 2003.

3.4.2.2 Grazing management of lambs after weaning

According to 38 respondents the majority (90%) of lambs were born in the month of August-September. The rest (10%) lambs were born in the month of June/July or October.

According to 38 respondents, 41% of the farmers used 2-10 day shifts, 44% used greater than 10 day shifts and 15% farmers used set-stocking of lambs for the first two months after weaning (Figure 3.4).

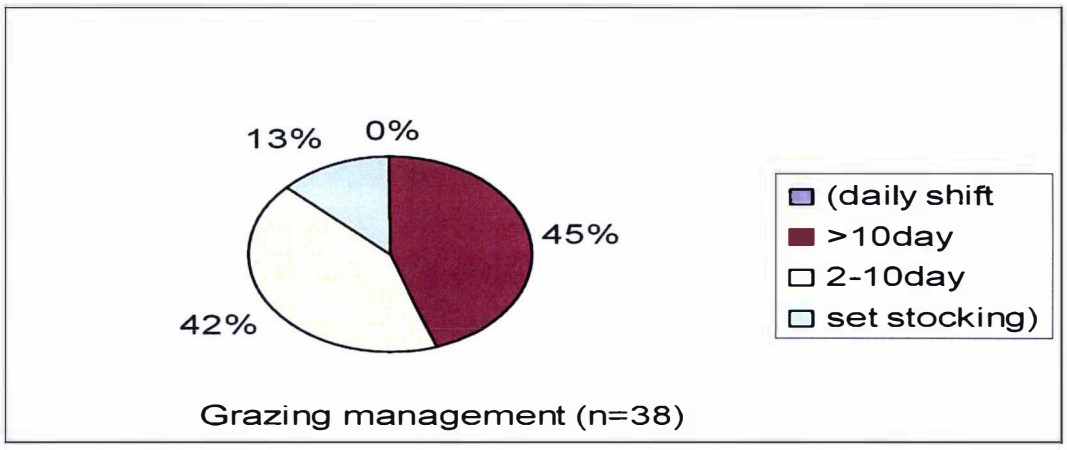


Figure 3.3 Grazing management of lambs

3.4.2.3 Proportion lambs weaned onto paddocks not grazed by lambing ewes since June 2002

According to 37 respondents, about 13% farmers weaned all of their lambs, 16% farmers weaned more than half, 26% farmers weaned less than half and 42% of the farmers did not wean any of their lambs onto paddocks not grazed by lambing ewes since 1 June 2002.

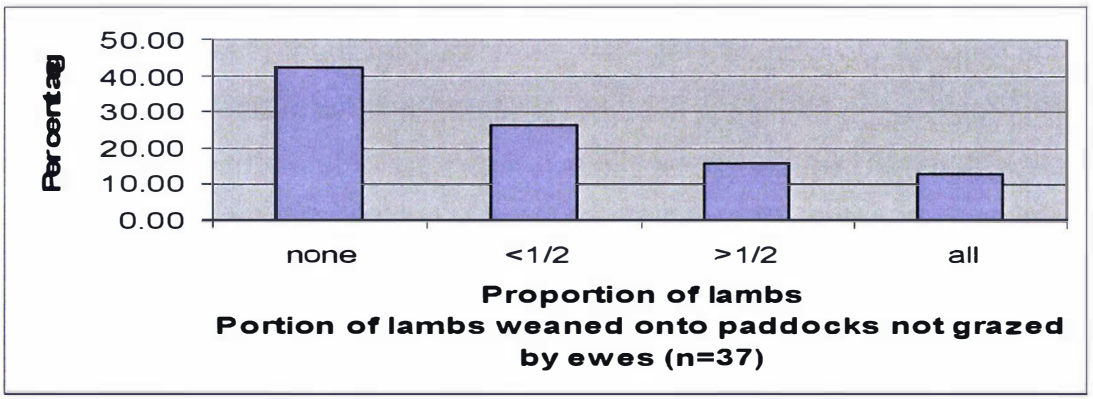


Figure 3.4 Proportion of lambs weaned onto paddocks not grazed by lambing ewes (n=37).

3.4.2.4 Average time lambs to be grazed on pasture previously grazed by ewes and lambs

For those farmers (n=23) who did wean at least some of their lambs onto pasture not grazed by ewes, 23% took 0-1 month, 23% farmers took 1-2 months and 13%

farmers took more than 2 months before these lambs grazed pastures used by lambing ewes. The remainder did not mention the time before the lambs were put on a pasture previously grazed by ewes and lambs.

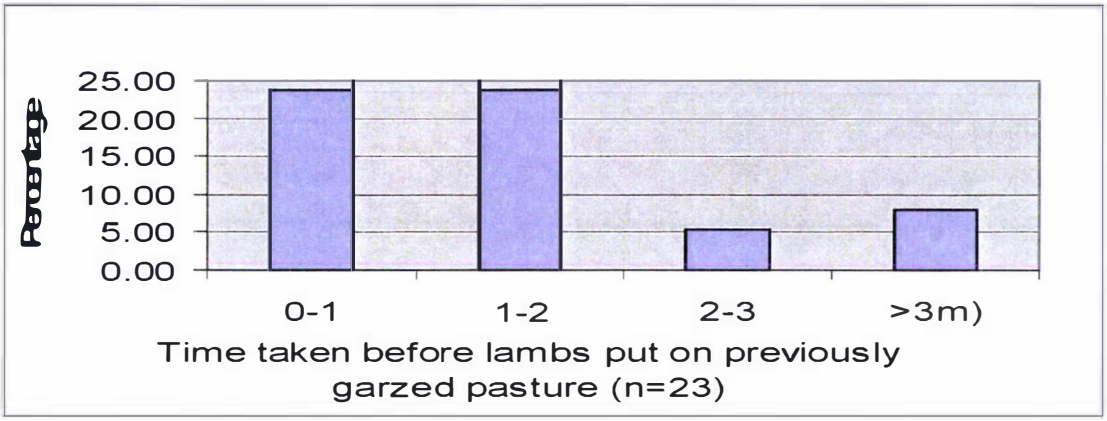


Figure 3.5 Average time taken before lambs moved to paddocks that were grazed by ewes and lambs.

3.4.2.5 Cattle/deer grazing on pasture between period of lamb grazing

According to 37 respondents 19% farmers never grazed, 40% farmers occasionally grazed, 30% farmers often grazed while 11% farmers always grazed cattle/deer on pasture between period of lamb grazing.

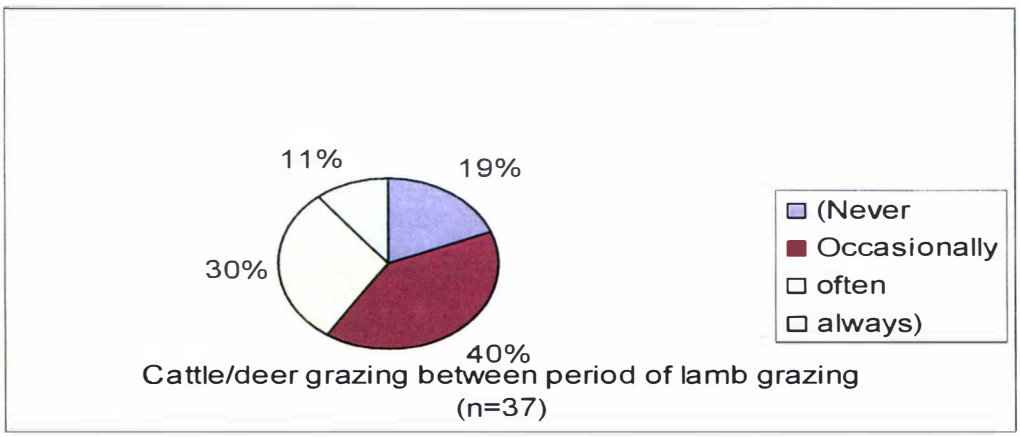


Figure 3.6 Proportion of farms where cattle or deer were grazed on pasture between periods of lambs grazing

3.4.2.6 Proportion of farm grazed by lambs between 1 July 2002 and 30 June 2003

According to 38 respondents, less than ¼ area of the farm was grazed by lambs on 11% farms, ¼ and ½ area of the farm on 34% farms, between ½ and ¾ area was grazed on 29% farms and greater than ¾ area by 26% farms between 1 July 2002 and 30 June 2003.

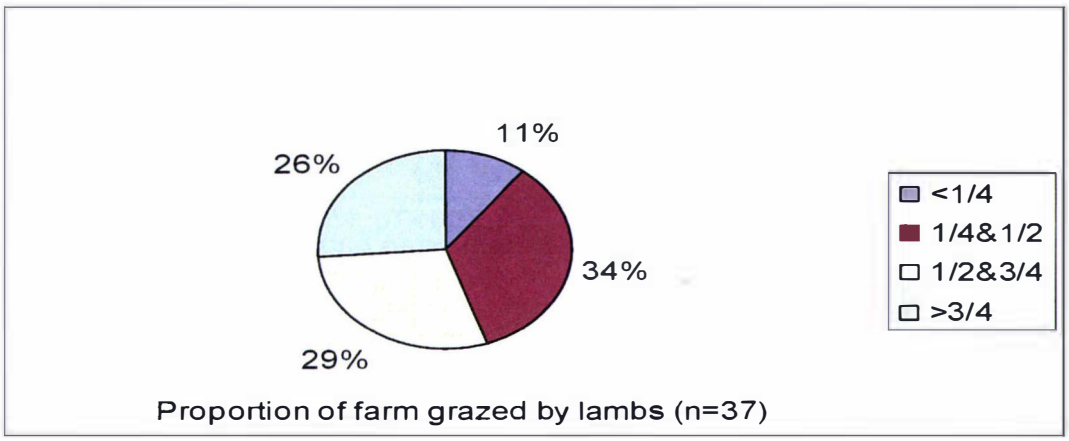


Figure 3.7 Proportion of farm grazed by lambs

3.4.3 Drenching policy and procedures

Drenching policy and procedures has following sub-questionnaires.

3.4.3.1 Anthelmintic use in lambs

In a question where farmers could choose more than one answer option about 78% (n=37) of farmers indicated they followed a 5-6 drench programme by drenching every 3-4 weeks from weaning and 21% of farmers indicated they drenched their lambs regularly (every 4 weeks) throughout their whole first year.

Regarding other reasons for drenching such as drenching because of a high FEC, signs of scouring or only when you perceive they are at risk; most of the farmers ticked more than one. Therefore, the exact percentage of these other drenching condition could not be obtained from the data.

3.4.3.2 Number of drenches to lambs/hoggets (born in 2002) between 1 July 2002 and 30 June 2003

Farmers (n=38) gave on an average 6.1 drenches to their lambs/hoggets in the year (between 1 July 2002 and 30 June 2003) for lambs that remained on the farm for the whole year.

Analysis of the data from 38 respondents showed that 50% farmers used more than 6 to 7 drenches and about 16% farmers used more than 7 drenches to lambs/hoggets in a year. Only about 8% of farmers are using 2-3 drenches per year while 50% farmers are using 2-6 drenches per year for lambs/hoggets in a year.

3.4.3.3 Names of drenches used for lambs/hoggets

Analysis of the data of anthelmintic usage in lambs on farms showed that about 2.7% farmers of the total (n=38) used only benzimidazole, 7.9% farmers used only levamisole, 10.5% farmers used only ivermectin, 7.9% farmers used only moxidectin, 2.7% used only abamectin and 10.5% farmers used a benzimidazole + levamisole combination throughout the season. Other farmers (57.8%) used various anthelmintics throughout the season.

Data showed that 71% farmers used macrocyclic lactone for lambs/hoggets on at least one occasion either alone or in combination with other anthelmintics in the year 2002/2003. The details of drugs used in various farms in the year 2002/2003 in lambs/hoggets is given in the Appendix 3.4.

3.4.3.4 Change of anthelmintics in between 2001/2002 and 2002/2003

For those farmers who changed drenches between 2001/2002 and 2002/2003, about 61% farmers (n=31) stated it was part of a planned annual drench family rotation, 13% farmers changed due to a veterinarians's advice while 8% farmers changed as a result of a person's advice other than a veterinarian. None of the

farmers made changes in the type of drench due to a cheaper price or because of drench failure or other reasons. These answers implied that 31 of 38 farms changed the action family of drench used over these two years.

3.4.3.5 Change of anthelmintic in next season

According to 38 respondents 50% farmers intend to use the same drenches next year, 39% farmers do not intend to use the same drench while 11% farmers do not know whether to use the same drench or to make change in drenches in 2003/2004 as last year.

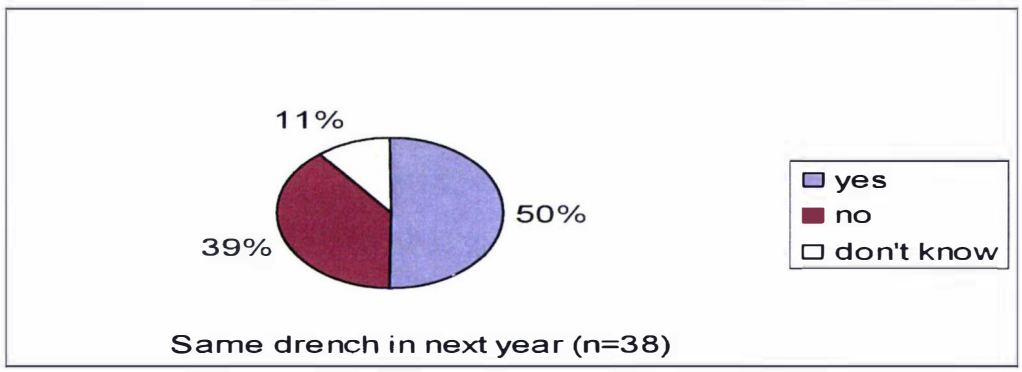


Figure 3.8 Farmer's intention of using of same drench in the following year.

3.4.3.6 Anthelmintic use in 1-2 year old sheep

On an average farmers (n=37) used 1.4 drenches in two tooths between 1 year of age and mating in 2002/2003. This reflected 10.5% farmers who did not drench, 40% who drenched 1 time and 47.5% farmers who drenched 2 times. The reasons (n=36) for two-tooth drenching were stated as: 61% farmers followed a predetermined drenching programmme, 10.5% because sheep were daggy or in poor condition, 8% due to high faecal egg counts while 16% of farmers mentioned other reasons such as haemonchosis etc.

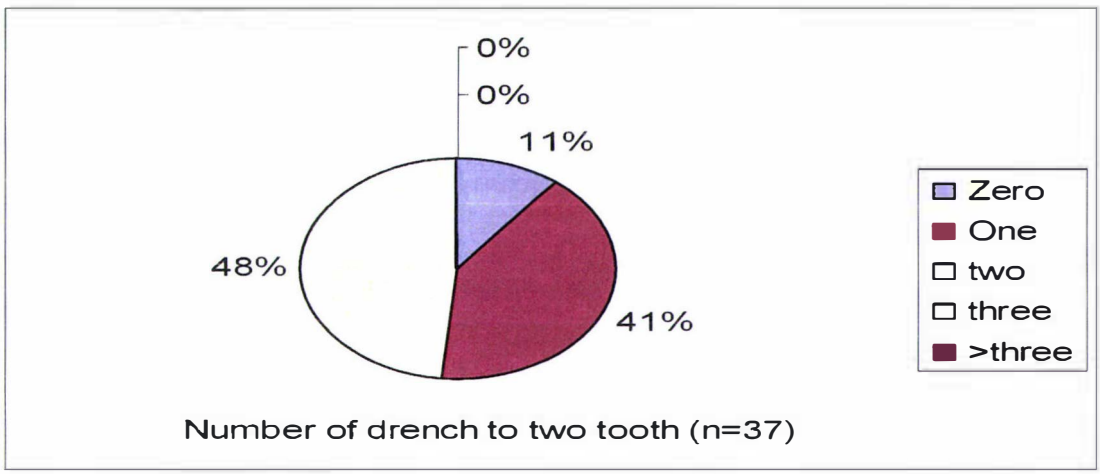


Figure 3.9 Number of anthelmintic treatments given to two tooths in the year 2002/2003.

3.4.3.7 Anthelmintic use in ewes

On an average, ewes received 1.8 anthelmintic treatments in the year 2002/2003 (n=38). According to respondents, 50% farmers used 2-3 treatments, 39.5% farmers used 1 treatment, 2.3% farmers used 4-5 treatments while about 8% farmers did not use any treatment to their ewes in July 2002/June 2003.

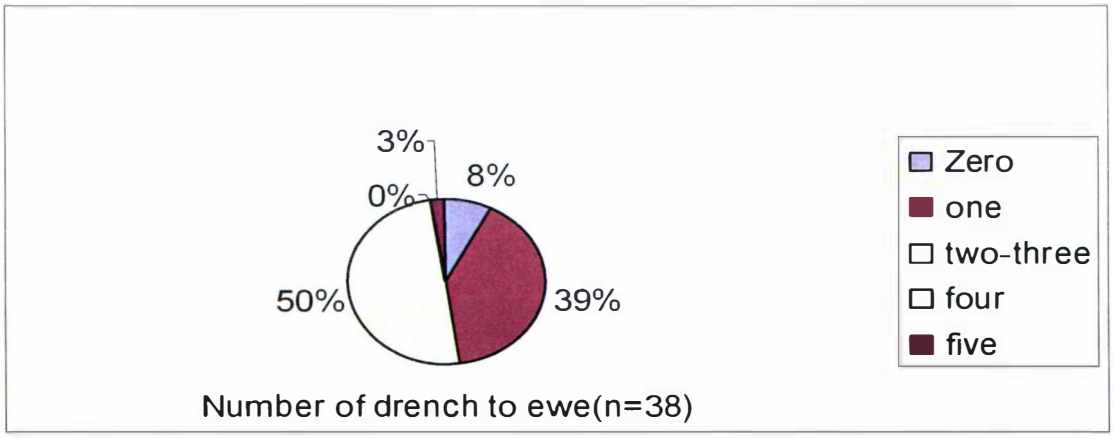


Figure 3.10 Number of anthelmintic treatments given to ewes in the year 2002/2003.

About 60% farmers (n=38) always drenched their ewes before lambing, 39.5% farmers always drenched their ewes before mating while 13% farmers also mentioned that they always drenched at docking in the year 2002/2003. About 19%

farmers also drenched their ewes at other times of the year when they showed apparent signs of parasitism.

Details of use of various drugs in ewes is given in Table 3.3 below and in Appendix 3.5.

Table 3.3 Anthelmintics used in ewes before lambing in 2002.

S. No.	Drugs	Action family[1]	Total number of farms (N)	Number of farms	% of farms	Remarks
1	Albendazole	BZ	38	1	2.6	
2	Extender 100 (albendazole)	BZ	38	4	10.5	Sustained release
3	Nilverm/leviben	LEV	38	3	7.8	
4	Arrest	BZ+LEV	38	1	2.6	
5	Ivomec	ML	38	2	5.2	
6	Maximiser CR capsules	ML	38	6	15.8	Sustained released
7	Eweguard, Cydectin, Vetdectin ±LEV[2]	ML	38	11	29.0	*Persistant activity
8	Closal		38	1	2.6	
9	Abamectin (oral or injectable)	ML	38	4	10.5	
10	Drugs not mentioned	.....	38	5	13.15	

1. BZ = benzimidazole; LEV = levamisole; ML = macrocyclic lactone

2. One response indicated some lambs were treated with a ML and some with LEV.

Of note is the use of some form of persistant action anthelmintics by 21 out of 38 farmers (55%) as a prelambing treatment. This includes the use of "Extender 100" controlled release capsules, "Ivomec Maximiser CR Capsule for Adult Sheep" and moxidectin (given by either parenteral or the oral route). It exclude the 4 farms who indicated they used abamectin as it is not clear if this was by the oral or parenteral route.

Other choices of prelambing treatment included 10.5% of farmers who used benzimidazoles, 8% who used levamisole, 5% farmers used

levamisole/macrocyclic lactones and 3% of the farmers used a combination drug containing benzimidazole + levamisole About 13% farmers did not mention the drugs used.

3.4.3.8 General monitoring and use of anthelmintics

About 31.5% farmers (n=20) that used FECs at some stage indicated that they were performed by veterinarians, another 13% indicated FECs were performed by others who were not vets and 8% farmers performed FECs themselves. This response implies that 20 out of 38 farmers used FECs at some stage.

About 42% farmers (n=38) farmers always check the volume of the drench released by the drenching gun before starting drenching whereas 21% of the farmers rely on the gun being accurate. About 31% of farmers occasionally check the volume of the drench released by the gun during use but 8% farmers check both at start and at the finish of drenching. According to 38 respondents, 76% check the seal/return-valve integrity of the drenching gun while 24% farmers do not check it.

When asked how they estimated the bodyweight of sheep when they last drenched them, 66% farmers (n=38) estimated the weight on the basis of eye appraisal whereas 34% of farmers used scales for estimation of body weight.

A majority (60.5%) of farmers (n=38) based drenching on the heaviest individual in the mob whereas 34% farmers based it on the average weight of the heaviest group of animals in the mob. None responded that they used the average weight of the whole mob or individual weight of an animal.

About 76.5% (29/38) farmers (n=38) had never carried out a test for drench resistance but 18.4% (7/38) farmers had undertaken some form of drench resistance test. There were 5% farmers who responded that they were uncertain

whether any test for drench resistance had been conducted . In relation to all surveyed farms only 11% of farms (4 farms out of 38 farms) had confirmed drench resistance to at least one type of anthelmintic but 57% (4/7) of those farmers who had tested did identify resistance on their farms. This was to the benzimidazole or combination (benzimidazole + levamisole) drench. None of the farmers had confirmed resistance to macrocyclic lactone anthelmintics.

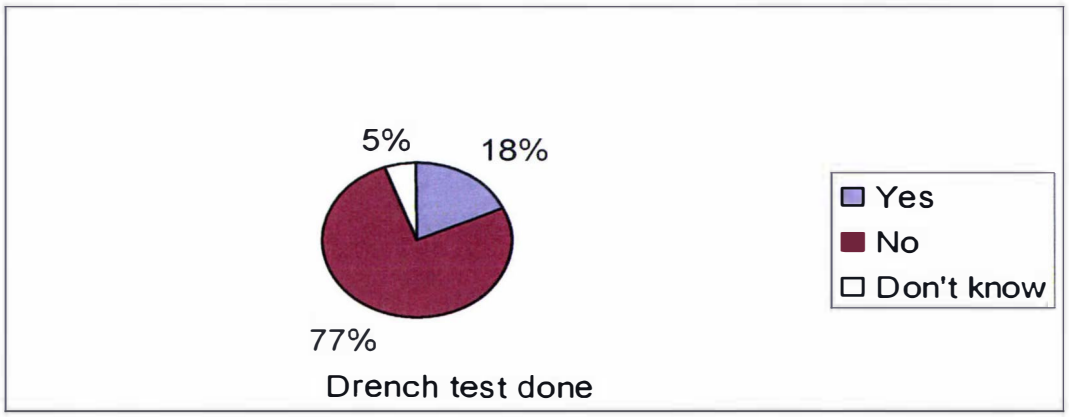


Figure 3.11 Proportion of farmers who had conducted some form of test for the presence of drench resistance at some stage.

3.4.4 Farmer's opinion

A series of questions were designed to enquire about farmers understanding of some issues associated with parasite control. These were as follows.

3.4.4.1 Period considered that grass pasture must be spelled in summer/ autumn before it becomes safe

About 60% farmers (n=38) considered it required a 1 week to 12 weeks when asked how long a grass pasture needed to be spelled over summer/autumn to be safe while 16% farmers considered it would never be safe.

3.4.4.2 Important way to delay the emergence of drench resistance

Most of the farmers (90%) considered proper dosing, drench rotation, clean pasture as an important way of delaying emergence of drench resistance on their farms.

3.4.4.3 Effectiveness of overall drenching programme

Only 8% farmers (n=38) considered their drenching programme to be very effective, 82% farmers considered it effective, 3% farmers considered it not effective and 5% farmers indicated "not known" as to whether their drench programme was effective.

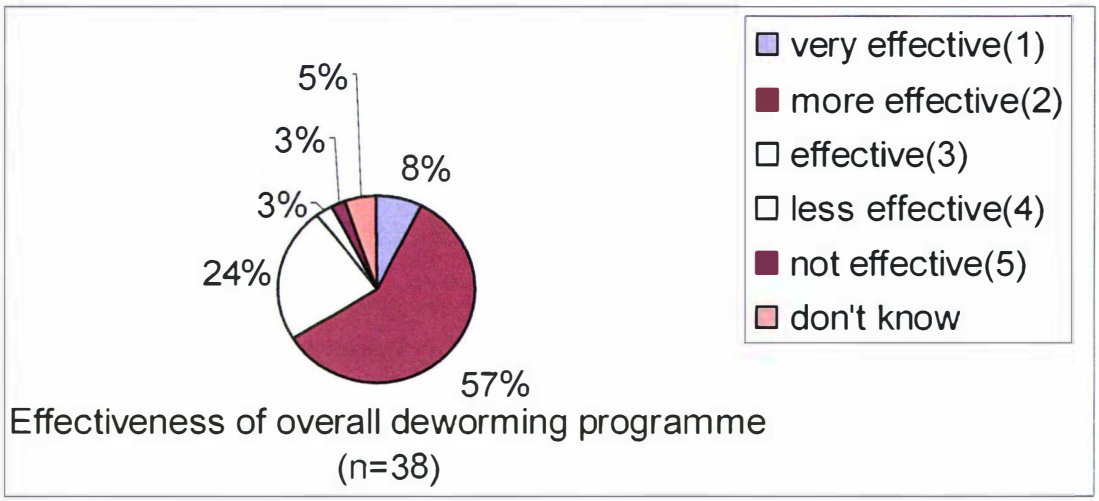


Figure 3.12 Opinion of farmers as whether their worm control was effective.

3.4.4.4 Cause of important economic losses and drench resistance problem for the industry

Worms were considered "very important" by 55% farmers (n=38) for the cause of economic losses in their farm, whereas 42% farmer considered it "important". Only 5% farmers considered worms "unimportant" as a cause of economic loss on their farms. Besides worms most farmers considered fly strike to be an important cause for economic losses in their farms. Pneumonia and facial eczema were generally ranked as less important than "worms" or "flystrike" but some farmers were from areas that did not have problems with facial eczema.

Drench resistance was considered as a "serious problem" today for the industry by 47% farmers (n=38), whereas, 34% farmers believe it as a problem but "not

serious one today" for the industry. Similarly 8% farmers believe that it is "not yet serious but will be in next 5 years" and 10.5% farmers believe it will be "serious in the next 20 years".

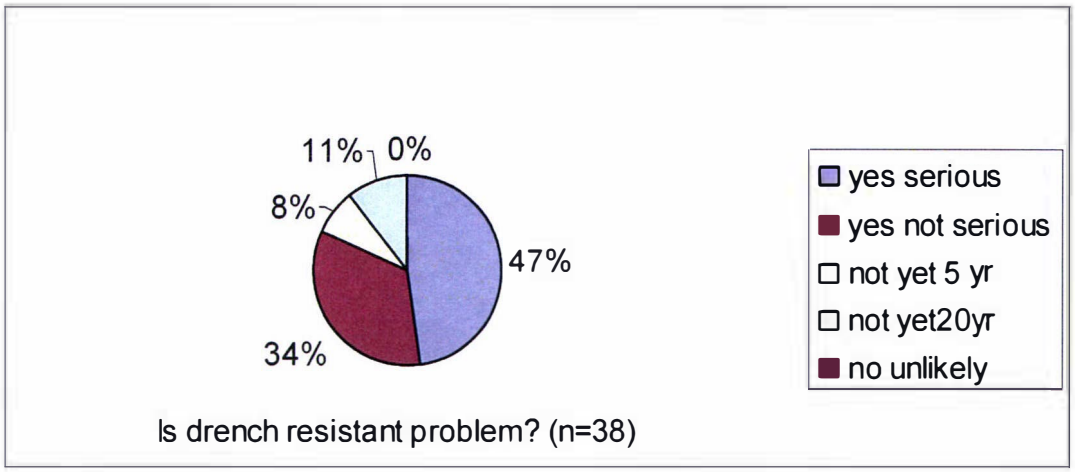


Figure 3.13 Farmers views as to whether drench resistance is a problem.

About 93% farmers considered that drench resistance definitely requires action from farmers, 97% farmers considered it definitely requires action from drench companies, 34% farmers considered it definitely requires action from MAF and 65% farmers considered it definitively requires action from vets.

3.4.4.5 Farmers suggestions or comments

Only 31.5% farmers have given their suggestion or comments regarding anthelmintic resistance. Some of the farmers suggested for independent voices and awareness and some of the farmers commented that worm control is based on computer models but not in reality and some considered pasture management is important. The comments given by farmers is given in the Appendix 3.2 in compact disk (CD).

3.5. DISCUSSION

Livestock farming (sheep, goat, cattle, deer) in New Zealand is mainly based on pastoral grazing systems and the use of various anthelmintics for control of gastrointestinal parasites has been practiced since the advent of modem

anthelmintics in the 1960s. Only three broad spectrum anthelmintic action families are available for control of gastrointestinal nematodes which are the benzimidazoles, levamisole and the macrocyclic lactones. These are also available as combination drenches such as the combination of benzimidazole + levamisole or the combination of benzimidazole + levamisole + macrocyclic lactones. The use of anthelmintics is critical to parasite control and that is reflected in the responses received in this survey.

The original intention of this survey was to obtain data from 100 farms. Unfortunately only 38 replies were received which is adequate to give an overview of practices but too few to enable any detailed analysis of the interaction of various responses.

In this survey, average effective farm size is 508 hectares. The mean number of breeding ewes (average 2400) in a farm which is similar to 1982 survey findings. This survey showed average 9.4, total stocking rate per hectare which is moderate by overall New Zealand standard.

Sheep grazing together with goats is associated with a risk of tranferring resistant nematodes. Studies have shown that anthelmintic resistance is more serious in goat farms due to high frequency of anthelmintic treatments i.e. more than 10 treatments per year. In a survey in 1989 it was found that 9 out of 10 goat farms in the Manawatu had benzimidazole resistance (Scherrer *et al.*, 1990). An earlier study also showed highed level of resistance with 97% of 47 goat farms demonstrating benzimidazole resistance in New Zealand (Kettle *et al.*, 1983). There are published experiments that demonstrated that sheep are at risk from grazing pasture contaminated by goat-derived ivermectin-resistant *Ostertagia* spp. (Gopal, 2000). Interestingly, this present survey showed only 5.6% farmers (2/38) rear sheep and goats together in their farms. There was nothing obvious in these 2 responses that suggested any more problems with parasites than others although detailed analysis was not possible. This suggests that overall there is

limited risk for cross-transfer of resistant parasites from goat to sheep or for goats to compromise the sheep parasite control programme in these surveyed farms, mainly because of limited numbers of farmers who keep both species.

Only a small number (13%) of farmers weaned all of their lambs onto paddocks not grazed by lambing ewes since 1 June 2002 but other farmers weaned less than 50% to all of their lambs onto paddocks grazed by ewes in this period. This indicated that preparation of safe pasture by not grazing with other sheep is only practiced by a small percentage of farms. The previous study conducted in Manawatu District in 1997 also showed that a majority of the farmers (54%) returned their lambs to the same paddocks that was grazed by ewes (Macchi, 1997). Failure to have pasture with low levels of infective larvae for weaned lambs implies that reliance on anthelmintics is high.

Past survey results (Macchi, 1997) and this survey result showed that sheep farmers are sensitive to increasing anthelmintic resistance problem for the industry. About 48% farmers (18/38) believe that drench resistance is a serious problem today and 34% farmers believe it is a problem but not a serious one. None of the farmers considered it unlikely that it will ever be a problem. When asked if any action should be taken to at least slow the development of resistance it was clear that this problem was for all sectors of the industry. Their concern about anthelmintic resistance is also supported by the question response indicating that farmers are clear that action needs to be taken at all levels of the industry. This survey showed that 93% farmers considered that the drench resistance requires action definitely from farmers but 97% farmers also considered that it requires action definitely from drug companies as well and a large percentage also believe central government (i.e. MAF in this case) as well as veterinarians should be taking steps to research this problem.

This survey result indicated that only a small percentage (8%) of farmers are using benzimidazoles alone or levamisole but most of the farmers are using more

expensive combination drenches and macrocyclic lactones for parasite control in their farms. So although very few have formally investigated anthelmintic resistance on their farms it would appear that most have moved away from using benzimidazoles and levamisole, presumably because they are no longer satisfied that they are effective. Instead they are using the more expensive anthelmintics which are also less likely to demonstrate resistance as shown in Chapter 2.

There is a general trend of macrocyclic lactones use in lambs/hoggets and ewes that accounts for up to 63% (24/38) of ewe treatments. In ewes 41.6% (10/24) farms have used moxidectin in 2002. Moxidectin has a long persistant activity and farmers might have used it when other anthelmintics did not give satisfactory results or might have used it to protect from strongylid infections in ewes for longer period of time. The emergence of moxidectin resistance in sheep nematodes may lead to serious economic losses in the farms and there would not be any anthelmintics left over for effective parasite control.

Underdosing and lack of proper weight estimation methods during drenching is considered to be a predisposing factor which may lead to the emergence of anthelmintic resistance. In this survey, nearly 2/3rd (25/38), farmers estimated body weight of their mob of sheep on the basis of eye appraisals and only 34% farmers used scales for estimation of body weight. About 34% farmers (13/38) based their drenching on the average weight of the heaviest group of animals in mob. These results indicate that a large proportion of farmers may be underdosing some of their sheep and this may contribute to the emergence of anthelmintic resistance on sheep farms.

This survey showed that a large proportion of farms, 76.5% (29/38), has never carried out a drench resistance test and only 18% (7/38) of farms has conducted a test, out of which 57% farms (4/7) diagnosed drench resistance to benzimidazoles and combination drenches of benzimidazole + levamisole. In relation to all the surveyed farms, including those that had not conducted a drench test, this

accounts for only 11% (4/38). It is interesting that farmers have serious concern about anthelmintic resistance with 93% indicating farmers should take some actions yet so few have actually investigated the resistance status of nematodes on their farms. There was no opportunity in this survey to determine if the tests that were conducted on these 4 farms were part of a routine procedure or in response to a clinical problem.

It is generally considered that the frequency of anthelmintic treatments of sheep is a factor in the emergence of anthelmintic resistance in gastro-intestinal nematodes of animals. This survey of farms in both the North and South Island showed that lambs/hoggets are drenched on an average 6.1 times (range 2-13), two tooth sheep 1.4 (range 0-3), ewes 1.8 (range 0-5) times in a year respectively. This drenching frequency is remarkably similar to that reported from a survey from South and North Island sheep farms which showed that on an average lambs and hoggets were drenched 5.6 and 7 times respectively (average = 6.3), sheep aged between 1-2 year 1.7 times and older ewes 1.2 times in a year respectively (Kettle *et al*. 1981 and 1982). The smaller survey of the lower North Island sheep farmers in 1995 showed that the drenching frequency of lambs at 6.2 times per year was also similar to the present findings (Macchi, 1997). Collectively it appears that farmers have not changed their drenching policies for young sheep over the past 2 decades despite anthelmintic resistance awareness campaigns. These drench frequency figures are lower than those reported for goats which are drenched more frequently (more than 10 times in a year) then sheep (Scherrer *et al*., 1990).

Of note is the increase in the frequency of drenching in ewes which has increased by 0.6 treatments/year as compared to 1982 survey (Kettle *et al*.,1982). The 1982 survey did not indicate time of treatment but by comparision by with the 1995 survey (Macchi, 1997), it means that ewe treatments pre-lambing have increased and docking treatment has decreased. However, this does not show the true exposure of ewes to anthelmintics as at least 55% of farmers used some form of persistant action anthelmintic in the pre-lambing period. This indicates that

considerably more selection pressure is occurring with ewe drenches than reported in the earlier surveys. It is also argued that treatment of ewes over lambing will mean that only survivors of this treatment will contaminate the pasture during the period immediately before lambing and early lactation which coincides with the relaxation in immunity shown by ewes at this time. Hence more nematodes can establish at this time and by treating ewes this will mean that only resistant survivors will be present to contaminate pastures in early spring. At this time temperatures are warming up and more development of larvae on pasture is occurring. The risk is significant because these survivors may then be taken up by young lambs and effectively multiplied up in these younger animals thus changing the gene frequency for anthelmintic resistance very quickly. Overall, anthelmintic use in sheep has effectively increased over a 20 year of period, despite farmer awareness campaigns indicating drench frequency is a risk factor for anthelmintic resistance.

Most farmers (62%) followed a predetermined drenching programme for their lambs. The majority of farmers (29/37) are following a 5-6 drench programme to their lambs/hoggets by drenching every 3-4 weeks from weaning, and a large percentage continue to drench regularly throughout their first year. The response that 78% of farmers followed a predetermined 5-6 drench programmme suggests that they find this approach is necessary for good parasite control which would imply they have found it is difficult to reduce drench frequency to any marked extent.

This survey showed that about 57% farmers (21/37) performed quarantine drenching whereas a large number of farms (38%) did not perform quarantine drenching. If resistant strains of strongylids are introduced in to the farm, this could lead to serious problem in these farms. There were 67% (13/19) of farmers used macrocyclic lactones alone or in combination drenches as a quarantine drench in the year 2002/2003. Given the acknowledged low level of macrocyclic lactone resistance, this is likely to be effective on those farms. However, this means that a

large percentage of farmers are either not using a quarantine drench or are using one that is less than optimal.

Underdosing and lack of proper weight estimation methods during drenching may lead to emergence of anthelmintic resistance. In this survey, nearly 2/3rd (25/38), farmers estimated body weight of their mob of sheep on the basis of eye appraisals and only 34% farmers used scales for estimation of body weight. About 34% farmers (13/38) based their drenching on the average weight of the heaviest group of animals in mob. This shows a large number of farmers are potentially underdosing their sheep and this is another factor considered to be important for selection of resistant parasites.

Majority (61%) of farmers follow planned drench family rotation for drug changes between last season (2001-2002) but more than 1/3rd did not follow this. This policy was promoted in the 1980s to slow down selection for drench resistant although recent reviews (Leathwick et al., 2001) argue it probably achieves very little.

Some farmers were concerned about the confusion in advice about drenching policy from drench companies, vets and other advisers, especially regarding anthelmintic resistance. Most of farmers considered proper dosing, drench action family rotation, safe pasture and cattle sheep grazing would help in delaying emergence of anthelmintic resistance on their farms. There would appear to be a need to continue to educate farmers about this issue and to attempt to achieve a consensus view on approaches that are recommended to farmers

Although, this is small scale survey conducted on sheep farms in New Zealand in the year 2003 and survey findings highlighted the present status of anthelmintic resistance on sheep farms and parasite control measures adopted by farmers at present time.

CHAPTER FOUR

GENERAL DISCUSSION

The studies here report on a limited survey of anthelmintic resistance employing larval development assays. Altogether, 25 farm samples, each farm contributing 10 faecal samples were processed using the DrenchRite LDA and/or Inhouse LDA. It was possible to compare the results of DrenchRite and Inhouse LDA plates using samples from 6. Although a very limited number, the results indicate that the benzimidazole results were comparable between the two assays but the levamisole results were not. The reasons for the latter are not obvious but may be the consequence of using very different concentrations of anthelmintic in the LDA. As the analogues of avermectin in DrenchRite are not known it is not possible to compare these with any certainty.

Anthelmintic resistance in sheep nematodes has been steadily growing in New Zealand and this is now posing a threat to the industry. Despite several campaign efforts to control this problem, these efforts seem to be not very effective. The results from the DrenchRite LDA demonstrated that 50% of the farms have a level of resistance to benzimidazole in *Trichostrongylus* spp. The Inhouse LDA also showed a level of resistance in 50% farms to benzimidazole in *Trichostrongylus* spp. and one farm with benzimidazole resistance in *Haemonchus contortus.* The predominant genera (n=20), on LDA plates and larval cultures was *Trichostrongylus* spp. (78%), *Chabertia/Oesophagostomum* spp. (17%) and *Haemonchus contortus* (6%). This will reflect the time of year that the samples were collected. As most farms could only be tested for efficacy against *Trichostrongylus* it is clearly an underestimate of the level of resistance overall on the farm as it is highly likely there will also be resistance to benzimidazoles in other genera.

Results for efficacy using levamisole could only be assessed in the farms where samples were assayed using DrenchRite LDA. Even this small number showed a

disturbingly high prevalence with resistance in 75% farms (n=12) and in 25% (3/12) farms for the combination of benzimidazole and levamisole anthelmintics. Again, this would be an underestimate of the number of farms with resistance to levamisole as other genera, especially *Ostertagia,* were not tested on these farms.

Suspected resistance to ivermectin analog-2 in *Trichostrongylus* spp. was observed in one farm (n=15) which represents 8.3% of farms. In addition, there was one farm with the Inhouse LDA that showed a high LC_{50} to an avermectin analogue. Resistance to avermectins is still considered to be rare in New Zealand and the chance of finding two such farms in such a small survey would be considered unusual unless the prevalence of such resistance is higher than expected. The observation of one farm with resistance to all action families in *Trichostrongylus* is particularly concerning.

Since, most of the studies on anthelmintic resistance in New Zealand have been conducted using a FECRT it was not possible to compare the absolute level of resistance in this survey with that reported earlier. Both the prevalence and level of resistance are of interest.

Farm management practices play an important role in the emergence of anthelmintic resistance. This present study has explored parasite management practices on various sheep farms in New Zealand. The survey only resulted in 38 replies out of the possible 100 that were originally planned. This small number limited the ability to investigate relationships between various responses. Nevertheless several interesting findings were made from this survey. These include the frequency of drenching of various age classes which appear to have changed little since the first survey in 1982/3, especially for lambs and two-tooths. Lambs which remain on the farm for the whole first year are dosed on average 6.1 times (range 2-13), two tooth sheep 1.4 (range 0-3), ewes 1.8 (range 0-5) times in a year respectively. This indicates that farmers are still reliant on anthelmintics and have apparently not changed their drenching policies for young sheep over this

time. Consistent with these drenching frequency figures is the observation that farmers mostly graze lambs on pasture previously grazed by ewes and lambs, implying they will be grazing contaminated pasture and be reliant on use of anthelmintics for parasite control. It also appears that farmers tend to have an area in which weaned lambs are grazed rather than over the whole farm and that little attempt is made to interchange grazing with other ruminant species between periods of lamb grazing.

The observations that farmers appear to remain very dependent on regular use of anthelmintics were made despite the expressed opinion of these farmers that they are concerned about anthelmintic resistance.

Of particular note is the increase in ewe drenching that would appear to have occurred. The figure in this survey represents an increase of about 0.6 anthelmintic treatments per year over the rate reported in 1982 (Kettle et al., 1982). The 1982 survey did not indicate time of treatment but by comparison with a 1995 survey (Macchi, 1997), the results in the present survey means that ewe treatments pre-lambing have increased and docking treatment have decreased. However, this does not show the true exposure of ewes to anthelmintic as at least 55% of farmers used some form of persistent-action anthelmintics in this pre-lambing period. This indicates that considerably more selection pressure is occurring with ewe drenches that indicated by the simple increase in number of treatments.

Other factors that are considered to increase selection for anthelmintic resistance were examined in this survey. The policy of quarantine drenching was adopted by about 57% farmers (21/37). Most of the farmers, (67%; 13/19) used macrocyclic lactones alone or in combination drenches containing MLs for quarantine drenches in the year 2002/2003. Given the level of macrocyclic lactone resistance in New Zealand is considered to be low this is likely to be effective on those farms. However, the other farmers who have not adopted a policy of quarantine drenching must be considered at risk of introducing resistant parasites with bought-in

livestock. In addition, a large proportion of farmers who did quarantine drench were not using very effective anthelmintics for this purpose which would limit its effectiveness.

Another factor which is considered important for selection of resistant parasites is underdosing. In this survey, nearly 2/3rd (25/38) farmers estimated body weight of their mob of sheep on the basis of eye appraisals whilst only 34% farmers used scales for estimation of body weight. About 34% farmers (13/38) based their drenching on the average weight of the heaviest group of animals in mob. This shows a large number of farmers are potentially underdosing some of their sheep.

Majority (61%) of farmers follow a planned drench family rotation for drug changes between seasons but more than 1/3rd did not follow this. This policy was promoted in the 1980s to slow down selection for drench resistance although recent reviews (Leathwick et al., 2001) indicate it probably achieves very little.

This small scale survey conducted on sheep farms in New Zealand in the year 2003 and the survey findings highlight the present status of anthelmintic resistance on sheep farms and parasite control measures adopted by farmers at the present time. A further large scale study would help to confirm these findings and provide a solid background to explore options for sustainable parasite control in the industry. Anthelmintic resistance does appear to be an issue given the results from the LDA assays, but farmers appear to be following similar drenching policies to those of 10 and 20 years ago which have lead to these levels of resistance in the first place. Indeed the change in ewe drenching policies is likely to have increased selection pressure for developing resistance. Unless new anthelmintics are developed and/or changes in parasite control policies, there must be concern for achieving sustainable parasite control in New Zealand.

APPENDICES

APPENDIX 2.1.

Modified McMaster technique for counting of eggs

Equipment and chemicals

Microscope (Olympus)

Electronic balance (Delta Range® U.S.A.) 0.1g

MacMaster counting slide (Olympic Equine Products; grid size 1cm², volume under each grid 0.15ml)

Laboratory counter (Clay Adams)

Pasture pipette

Domestic strainer 1mm aperture

Domestic plastic teaspoon

Bowl (steel)

Universal bottle, volume 28ml.

Saturated sodium chloride solution (specific gravity 1.2)

Procedure:-

1- Bowel, sieve and spoon were place on the electronic balance and their weight adjusted to zero.
2- 2 g of faeces were weighed.
3- 28 ml of saturated solution of NaCl was added into the sieve and faeces worked thoroughly sieve and the faecal residue was discarded.
4- Both chambers (0.15ml. each side) of a MacMaster counting slide were filled by a pasture pipette while stirring the bowel solution to and fro.
5- The slide was left for 2 minutes before counting to allow eggs to float to the surface.
6- The slide was placed on microscope and examined at 100x and counted the eggs present on the both chamber. The number of eggs were multiplied by 50 to get eggs per gram of the faeces.

APPENDIX 2.2.

Procedure for Larval culture

Equipment/Chemicals

Glass jars or Agee with lids

Stand for funnel

Conical flask volume 100ml

Pasture pipettes

Glass or platic funnels with rubber tubing attached and closed by a clip

Fine gauze or wire mesh

Fine paper tissue

Grooved glass slide and coverslips

Graduated cylinder, volume 500ml.

Laboratory counter (Clay Adams)

Vermiculite to retain moisture and air in the culture jars

Lugol's iodine solution (5% iodine and 10% potassium iodide in water)

Faeces 10g or more and water.

Procedure:-

1. Faeces were broken and mixed with vermiculite (not less than 20% of the volume of the faeces) and water just enough to stick together.
2. The mixture of the faeces and vermiculite was packed loosely in to glass jars, leaving large air space at the top.
3. The bottles were screwed on the lids leaving a slight air-leak
4. The bottles were incubated at 27°C for 7-10 days.
5. Water was added to make it moist and not to dry out.
6. The larvae were recovered from the sample by the Baermann's technique as follows:-

(i) The rubber tubing was closed by clip.

(ii) The funnel was filled to within 2cm of the top with deionised water.

(iii) The faeces were placed on wire mesh sieve with a layer of tissue paper underneath the faeces.

(iv) The wire mesh sieve was placed in the funnel and more water was added to submerge the faeces.

(v) The funnel containing the mixture was left to stand for 24 hours.

(vi) The clip was released carefully and 50-60 ml of water containing larvae was tapped off from the rubber tubing in to a 100 ml plastic conical flask.

(vii) It was allowed to stand for 1-2 hour so that larvae concentrate on the bottom.

(viii) The supernatant was sucked off.

(ix) Using pasture pipette few drops were place on grooved slide and a drop of lugol's iodine was put on the slide and covered with coverslip.

(x) The slide was placed under microscope and the larvae were counted at 100x magnification.

(xi) Five counts were done. The larval identification was done on the basis of their length and morphological features.

Appendix 2.3.

Procedures for Larval Development Assay

(A) Inhouse (Own prepared) LDA plate Method

Equipments

Microscope (Olympus)

Microwave (Panasonic)

Centrifuge (ILEC centra-8 International equipment company)

Incubator 26°C (Sony)

Dessicator

Electronic balance (Delta Range® U.S.A.) 0.1g

Eppendorf pipette (2µl, 20µl, 200µl, 1000µl)

Heating plate (Heidolph, Germany)

Laboratory counter (Clay Adams)

Measuring cylinders

Beakers (30ml to 500 ml)

Plastic centrifuge tubes (Falcon), volume 50ml

Plastic spoons

Sieves (1mm, 20µm, 60µm, 100µm aperture)

Grooved microscope slides (glass)

Coverslips

96-well (Falcon, Becton Dickinson, USA) round bottomed microtitre plates (volume of each well 300µl)

Deionised water, Distilled water

Chemicals

Anthelmintics

(a) Thiabendazole (99.3% powder, a gieft of MSD Agvet, New Zealand Ltd.)

Molecular weight of thiabendazole = 201.25

12.9mg powder was dissolved in 50ml of Dimethyl sulphoxide (DMSO). This gave a stock solution of 258 µg/ml of thiabendazole.

The following serial dilutions were used for thiabendazole in 150µl of agar. The final concentration is shown when 150µl of agar was added to 2µl of each thiabendazole dilution.

1. 2µl of stock solution (258µg/ml) in 150 µl of agar = 3.44µl in the agar phase (17.093µmol)
2. 1 ml of 258µg/ml +1ml of DMSO =129µg/ml of agar = 1.72µl in the agar phase (8.546µmol)
3. 1 ml of 129µg/ml +1 ml of DMSO = 64.5µg /ml of agar = 0.86µl in the agar phase (4.280µmol)
4. 1ml of 64.5 µg /ml + 1ml of DMSO =32.25 µg/ml of agar = 0.43µg/ml in the agar phase (2.136µmol)
5. 1 ml of 32.25µg/ml +1ml of DMSO =12.125µg/ml of agar = 0.22µg/ml in the agar phase (1.068µmol)
6. 1ml of 16.12µg/ml + 1ml of DMSO = 8.06µg/ml of agar = 0.11µg/ml in the agar phase (0.534µmol)
7. 1 ml of 8.06µg/ml + 1ml of DMSO = 4.03µg/ml of agar = 0.055µg/ml in agar phase (0.267µmol)
8. 1ml of 4.03µg/ml +1 ml of DMSO =2.02µg/ml of agar = 0.027µg/ml in the agar phase (0.133µmol)
9. 1ml of 2.02µg/ml + 1ml of DMSO = 1.01µg/ml of agar = 0.0135µg/ml in the agar phase (0.0667µmol)
10. 1ml of 1.01µg/ml = 1ml of DMSO = 0.503µg/ml of agar = 0.0067µg/ml in the agar phase (0.0334µmol).
11. 1ml of 0.503µg/ml + 1 ml of DMSO = 0.252µg/ml of agar = 0.0033µg/ml in the agar phase (0.01669µmol)
12. 1ml of 0.252µg/ml + 1ml of DMSO =0.126µg/ml of agar = 0.00168µg/ml in the agar phase (0.00834µmol).

(b) Levamisole hydrochloride (Rycozole®, oral drench, Young's Animal health, New Zealand)

Molecular weight of levamisole = 240.75

The concentration of levamisole in the drench =40g/litre i.e. 40µg/ml

Therefore 1ml drench contain 4,000µg of levamisole.

1,000µl (1ml)of levamisole drench + 77.5 ml of distilled water = 516µg/ml of levamisole in stock solution

The following dilutions were used for levamisole. The final concentration is shown when 150µl of agar was added to 2µl of each dilution.

1. 2µl of the stock solution (516µg/ml)in 150µl of agar = 6.88µg/ml in agar phase (28.58µmol)
2. 1ml of 516µg/ml + 1ml of dist. water = 256µg/ml = 3.44µg/ml in agar phase (14.29µmol)
3. 1ml of 256µg/ml +1ml dist. water = 128µg/ml =1.72µg/ml in agar phase (7.146µmol)
4. 1ml of 128µg/ml + 1 ml dist. water = 64µg/ml = 0.86µg/ml in agar phase (3.573µmol)
5. 1ml of 64µg/ml + 1ml dist. water = 32µg/ml = 0.43µg/ml in agar phase (1.786µmol)
6. 1ml of 32µg/ml + 1ml dist. water = 16µg/ml = 0.215µg/ml in agar phase (0.893µmol)
7. 1ml of 16µg/ml + 1ml dist. water = 8µg/ml = 0.1075µg/ml in agar phase (0.4466µmol)
8. 1ml of 8µg/ml + 1ml dist. water = 4µg/ml = 0.0537µg/ml in agar phase (0.2233 µmol)
9. 1ml of 4µg/ml + 1ml dist. water = 2µg/ml = 0.2685µg/ml in agar phase (0.1116 µmol)
10. 1ml of 2µg/ml + 1ml dist. water = 1µg/ml = 0.1342 µg/ml in agar phase (0.0558 µmol)

11. 1ml of 1µg/ml + 1ml dist. water = 0.5µg/ml = 0.6712 µg/ml in agar phase (0.0279 µmol)

(c) Ivermectin (IVM-1) (Bomectin oral, Bomac Laboratories Ltd; ivermectin 0.1% w/v)

Ivermectin (0.1%) (Bomectin oral drench for sheep and goats, Bomac Laboratories, New Zealand Ltd.)

0.1% of Ivermectin (Bomectin) drench = 1.0g/litre, i.e. 1mg/ml, i.e. 1000µg/ml

Therefore, 1ml of 1000µg/ml of Ivermectin (Bomectin) =33.3 ml of DMSO provided 24µg/ml of the stock solution.

The final concentration is shown when 150µl of agar was added to 2µl of each ivermectin dilution.

1. 2µl of stock solution (24µg/ml) in 150µl of agar = 0.32µg/ml in agar phase (0.3679µmol)
2. 1ml of 24µg/ml + 1ml of DMSO = 12µg/ml = 0.16µg/ml in agar phase (0.1839µmol)
3. 1ml of 12µg/ml + 1ml of DMSO = 6µg/ml = 0.08µg/ml in agar phase (0.0919µmol)
4. 1ml of 6µg/ml + 1ml of DMSO = 3µg/ml = 0.04µg/ml in agar phase (0.0459µmol)
5. 1ml of 3µg/ml + 1ml of DMSO = 1.5µg/ml = 0.02µg/ml in agar phase (0.02229µmol)
6. 1ml of 1.5µg/ml + 1ml of DMSO = 0.75µg/ml = 0.01µg/ml in agar phase (0.0115µmol)
7. 1ml of 0.75µg/ml + 1ml of DMSO = 0.375µg/ml = 0.005µg/ml in agar phase (0.0574µmol)
8. 1ml of 0.375 µg/ml + 1ml of DMSO = 0.1875µg/ml = 0.0025µg/ml in agar phase (0.0057µmol)
9. 1ml of 0.1875 µg/ml + 1ml of DMSO = 0.09375µg/ml = 0.00125µg/ml in agar phase (0.00143µmol)

10. 1ml of 0.0937 µg/ml + 1ml of DMSO = 0.0468µg/ml = 0.0063µg/ml in agar phase (0.00071µmol)

11. 1ml of 0.0468 µg/ml + 1ml of DMSO = 0.0234µg/ml = 0.00315µg/ml in agar phase (0.000359µmol)

12. 1ml of 0.0234 µg/ml + 1ml of DMSO = 0.01172µg/ml = 0.0016µg/ml in agar phase (0.000179µmol).

(d) Ivermectin (Erase MPC, Schering-Plough Animal Health Limited; 16g/litre, ivermectin)

16g/L of ivermectin (Erase MPC) = 1.6g/100ml

or 16mg/ml, i.e. 16000µg/ml. Therefore, 1ml of 16000µg/ml of Ivermectin (Erase MPC) in 33.3 ml of DMSO provided 384µg/ml of the stock solution. Further 12 concentrations were prepared by serial dilution.

The final concentration in the agar phase is shown when 150µl of agar was added to 2µl of each ivermectin (IVM-2) dilution.

(e) Ivermectin aglycone (99.2% powder; a gift of Virbac NZ Ltd.)

Molecular weight I. aglycone = 566.73

1molar = 566.7g in 1,000ml (i.e. 566.73µg x 1,000 x 1,000 per litre)

1µmol = 567µg/ml (i.e. 250µmol =0.567x 250= 141.75µg/ml)

Therefore, 1ml contains = 141.75µg/ml of ivermectin aglycone

Therefore 50ml contain = 7,087.5µg = 7.087mg

So, 7.0875 mg of Ivermectin aglycone powder was diluted in 50ml of DMSO = 141.75µg/ml.

The following dilutions were used for this drug and final concentration in the agar phase is shown when 150ml of agar was added to 2µl of each ivermectin aglycone dilution.

1. 2µl of stock solution of I. aglycone (141.75µg/ml) in 150 µg of agar = 1.89µg/ml in the agar phase (3.335µmol)
2. 1ml of stock solution of I. aglycone (141.75µg/ml) + 1ml of DMSO = 70.875µg/ml = 0.945µg/ml in the agar phase (1.667µmol)
3. 1ml of of I. aglycone (35.437µg/ml) + 1ml of DMSO = 35.437µg/ml = 0.4725µg/ml in the agar phase (0.833µmol)
4. 1ml of I. aglycone (17.719µg/ml) + 1ml of DMSO = 17.719µg/ml = 0.236 µg/ml in the agar phase (0.416µmol)
5. 1ml of I. aglycone (17.719µg/ml) + 1ml of DMSO = 8.86µg/ml = 0.118 µg/ml in the agar phase (0.208 µmol)
6. 1ml of I. aglycone (8.86µg/ml) + 1ml of DMSO = 4.43µg/ml = 0.118 µg/ml in the agar phase (0.104µmol)
7. 1ml of I. aglycone (4.43µg/ml) + 1ml of DMSO = 2.21µg/ml = 0.0295µg/ml in the agar phase (0.0552 µmol)
8. 1ml of I. aglycone (2.21µg/ml) + 1ml of DMSO = 1.11µg/ml = 0.1477 µg/ml in the agar phase (0.026 µmol)
9. 1ml of I. aglycone (1.11µg/ml) + 1ml of DMSO = 0.553µg/ml = 0.0073 µg/ml in the agar phase (0.013 µmol)
10. 1ml of I. aglycone (0.553µg/ml) + 1ml of DMSO = 0.227 µg/ml = 0.00369µg/ml in the agar phase (0.0065 µmol)
11. 1ml of I. aglycone (0.277 µg/ml) + 1ml of DMSO = 0.138 µg/ml = 0.0018µg/ml in the agar phase (0.00325 µmol)
12. 1ml of I. aglycone (0.137 µg/ml) + 1ml of DMSO = 0.069 µg/ml = 0.0009µg/ml in the agar phase (0.0016 µmol)

Nutritive media

1g of yeast extract (Y-1000, Sigma) was added in 90ml of 0.85% saline solution. Then, the yeast solution was autoclaved and stored at -20°C until used. At the time of the assay 1ml of Earle's balanced salt solution (E7510, Sigma) was added to every 9ml of the yeast solution.

Amphotericin B solution

25 mg of amphotericin B (A-9525, Sigma) was dissolved in 100ml of distilled water. Then, the suspension was dispensed into 0.5ml aliquots and sored in freezer.

Dimethyl sulphoxide (DMSO) (Sigma, New Zealand)

Sugar solutions

10% sugar solution was prepared by dissolving 50gm of sucrose into 450ml of distilled water. Then, few drops of food colour (yellow) were added to this solution and dispensed into 28ml universal bottles.

25% sugar solution was prepared by dissolving 100g of sucrose into 400ml of distilled water. Then, few drops of food colour (blue) were added to this solution and dispensed into 28ml universal bottles.

Both, 10% (yellow) and 25% (blue) sugar solutions were sterilized by autoclaving and stored at room temperature.

2% Agar matrix

2g of agar (Bacto-agar, Y-1000, Sigma) was dissolved in 100ml of distilled water by heating in a microwave untilled dissolved (3 minutes). The agar was kept warm (approximately 85°C) on a heating plate until added to the plates.

Lugol's iodine solution

4g of iodine and 10g of potassium iodide were dissolved in 100ml of tap water and mixed thoroughly.

PROCEDURES:-

Nematode egg recovery

Nematode eggs were recovered by method described by Hubert and Kerboeuf (1992) and DrenchRite method (Horizon Technology Pty Limited, Australia). The steps are as follows:

1. Nematode egg counts (see appendix 2.1) were carried out and eggs per gram of faeces from the faecal samples were estimated. Then, 30-200g of faeces depending upon the total number of eggs required for an assay was softened by putting it water in a beaker for about 20 minutes.
2. The faeces were mixed until the faecal material was in suspension. The suspension was poured through a 1mm aperture sieve into a 100µm sieve into a 2 litre plastic jag and the faecal residue in the sieve was discarded.
3. The suspension was then washed through a 20µm aperture sieve and eggs were retained by the sieve. The eggs were washed off the screen with a jet of water from a squeeze bottle and then collected in a clean beaker.
4. Two sugar gradients were prepared in 50ml centrifuge tubes by adding 10ml of yellow (10% sugar solution) and 15ml blue (25% sugar solution) by using a cannula or a pipette.
5. By using a 20ml syringe, carefully dispensed 10-15ml of faecal slurry on top of yellow gradient by holding outlet against side of centrifuge tube, avoiding mixing slurry with gradient
6. The tubes were centrifuged at 2,000-3,500rpm (1,500g) for 7 minutes.
7. The eggs were removed from the interface of yellow and blue phases of the sedimentation solution. These eggs were collected onto a 20µm sieve and ringed thoroughly with de-ionised water. Then transferred to a 50ml centrifuge tube by using distilled water and allowed to settle eggs in the

tube. Aspirated off excess water containing fine suspended debris. This step was repeated for dirty preparations.

8. Then eggs were counted in a grooved slide under 100x magnification of microscope and five counts were made in each sample.
9. The final volume of egg suspension was adjusted to approximately 4,000eggs/ml (80eggs/20µl).
10. 90ml fungizone or amphotericin B was added to per ml of eggs suspension (74 eggs/20µl).

Preparation of larval development assay plates (Own plates)

1. The test was carried out in 96 well microtitre plates described by Gill et al. (1995) and Drench Rite plate method. This was tried to make similar to Drench Rite method.
2. Two replicate per anthelmintic dilution were prepared for benzimidazole (thiabendazole) (row A and B), levamisole (row C and D) and ivermectin (Bomectin) (row E and F) and a single row made for ivermectin (Erase MPC)Multipurpose concentration (MPC) (row G) and ivermectin aglycone (row H) were made.
3. 2µl of DMSO was added in each control well of row A, B, E, F,G and H. 2µl of distilled water added in each control well of levamisole (row C and D).
4. 2µl of anthelmintic dilution were added to each to each well and mixed with 150µl of 2% agar. Twelve different concentrations of anthelmintics to be tested were prepared as described previously and added in order of increasing concentration across the plate from well 2 to 12.
5. The plates were allowed to stand for one hour at room temperature.

Preparation for cultures

1. 20µl of egg suspension (distilled water + amphotericin B + eggs) containing about 50-74 eggs was laid on top of agar matrix in each well with Eppendorf pipette.

2. The plates were placed in desiccator with water at the bottom to ensure high humidity and incubated at 25°C for 7 days.
3. All wells were checked after 16 hours of incubation to determine presence of free water on the agar surface. If required rehydrated by adding 10µl in the well.
4. 9ml yeast extract + 1ml Earle's balanced salt solution mixed together to make nutrient media.
5. 20µl of nutrient media was added in each well when eggs in the control wells have hatched (day 2 of the incubation).
6. Plates were scanned at 2-3 days interval to check free surface water and 10µl distilled water was added to the wells that did not have free surface water. The wells rehydrated were recorded.
7. On 7^{th} day of the culture, the liquid phase from each well was removed and transferred by to grooved slides by means of pasture pipette.
8. The number of eggs, first, second and third stage larvae were counted in each well after staining with Lugol's iodine solution.
9. The data were corrected for the mean number of larvae not developed to L3 in control wells.
10. The proportion of L3's was fitted into a sigmoid curve after log10 transformation of values for the different anthelmintic concentrations. The curve was fitted by using the software package Slide Write (Advance Graphic Software Inc., U.S.A.).
11. The LD50 (LC50) values were calculated using the above software programme.

(B) DrenchRite LDA Methods

1. The equipments and chemicals used were similar to own LDA plate method. Nematode egg recovery and the culture were done according to the Drench Rite procedure and it was similar to own LDA plate method.

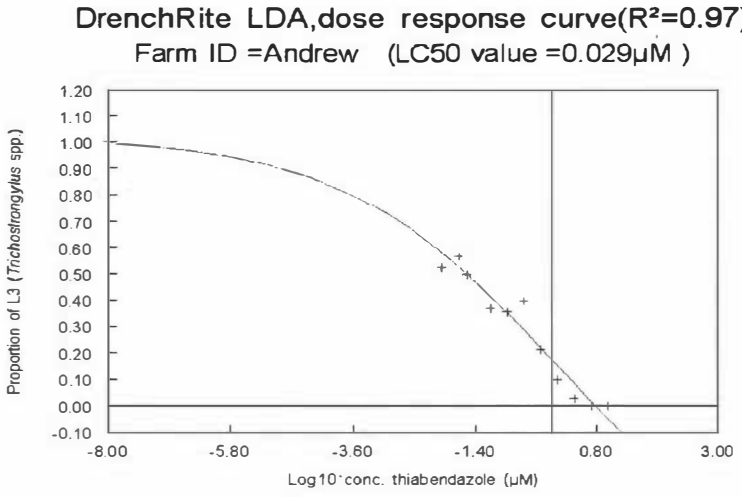
2. The DrenchRite plate was readymade for use and needed to put required concentration of egg suspension (50-80 eggs/well) on Day one.
3. The nutritive medium (growth medium) was provided with the DrenchRite LDA plates and 20µl was put in each well on Day two.
4. 10µl of distilled water was added whenever the wells get dry and it was recorded.
5. The other procedures were similar to the Inhouse LDA Plate method and the actual $\log_{10}$ concentration of the drugs and LC_{50} values could only be calculated where the concentrations of anthelmintics used in DrenchRite LDA plates were known.
6. The interpretations of the results were made according to DrenchRite guidelines. LC_{50} values were made according to average well number of the drug rows.
7. The proportion of L3s was fitted into a sigmoid curve after $\log_{10}$ transformation for values for different wells.

Appendix 2.4

Statistical analysis of dose response curve and LC50 for various farms

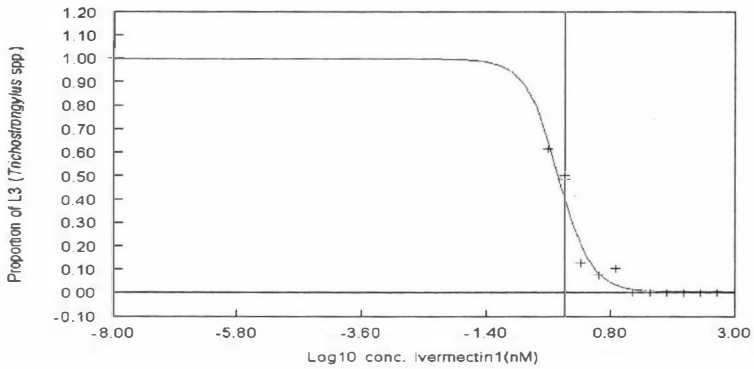
1.0 DrenchRite LDA Assays

(a) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analog -1 (IVM-1), ivermectin analog -2 (IVM-2); *Trichosrongylus* spp. of farm -Andrew



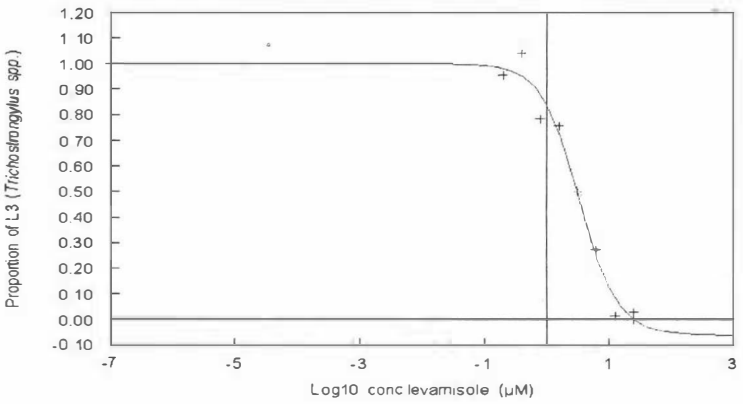
DrenchRite LDA, dose response curve(R^2=0.98)

Farm ID = Jim (LC50=0.75nM)



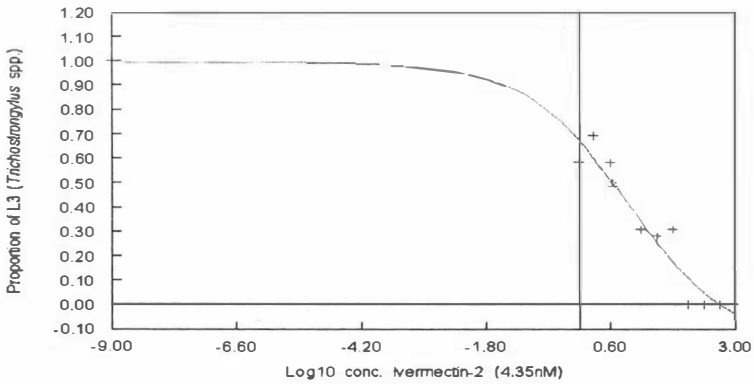
DrenchRite LDA,dose response curve(R^2=0.98)

Farm ID= Andrew (LC50 =3.1µM)

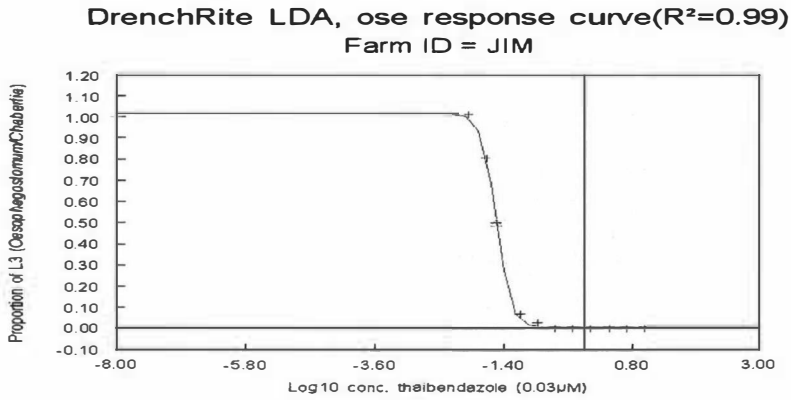


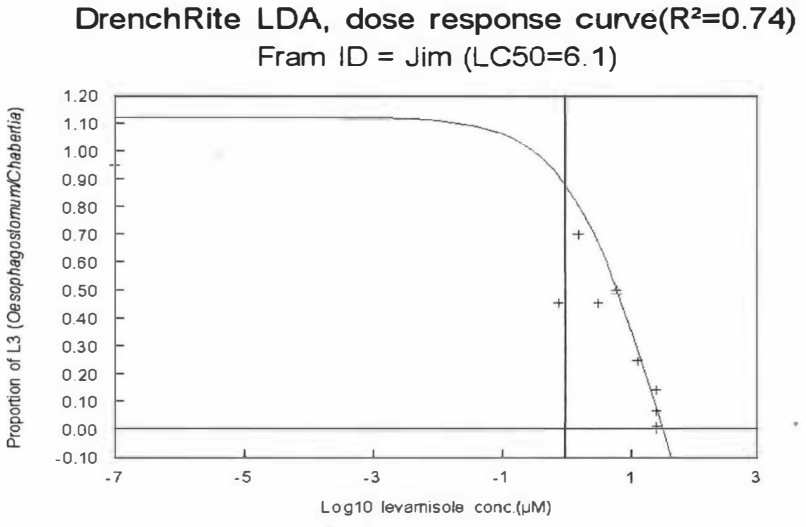
DrenchRite, dose response curve(R^2=0.94)

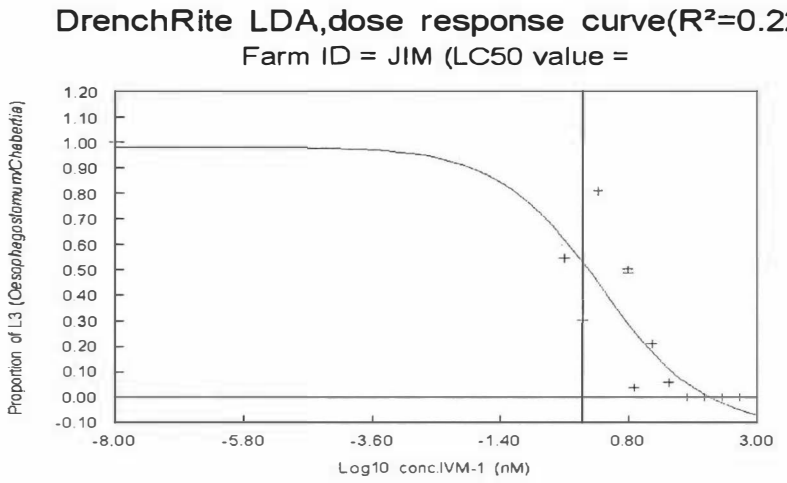
Farm ID = Andrew (LC50=4.35nM)



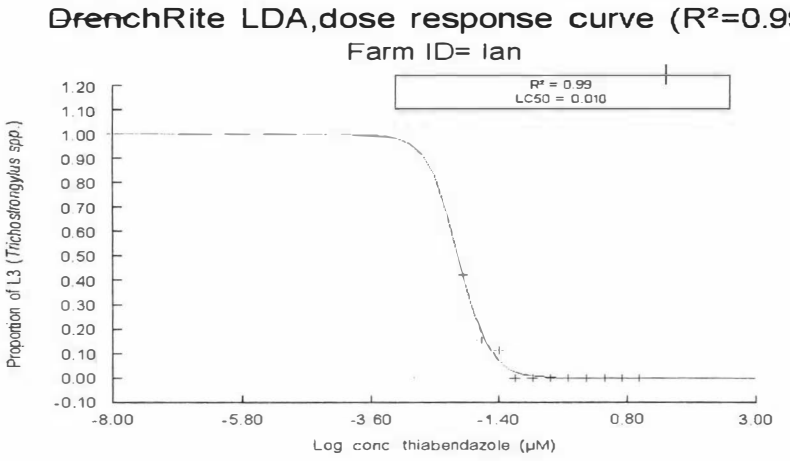
(b) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analog -1 (IVM-1); *Oesophagostomum/Chabertia* of farm – Jim.





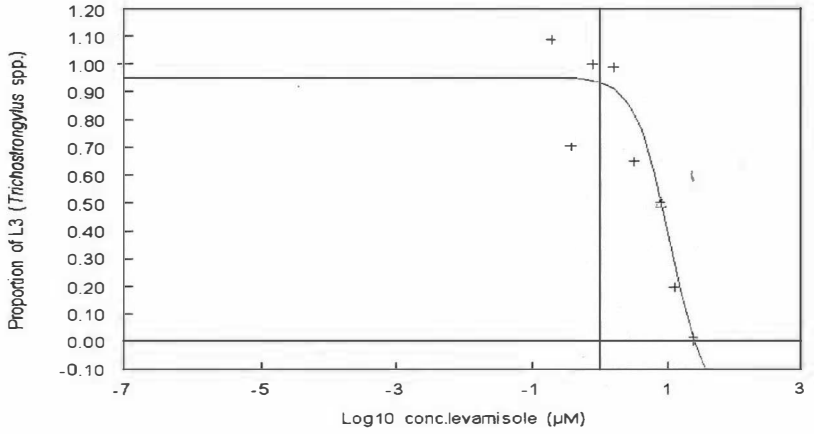


(c) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analog -1 (IVM-1), ivermectin analog -2 (IVM-2); *Trichostrongylus* spp. of farm – I



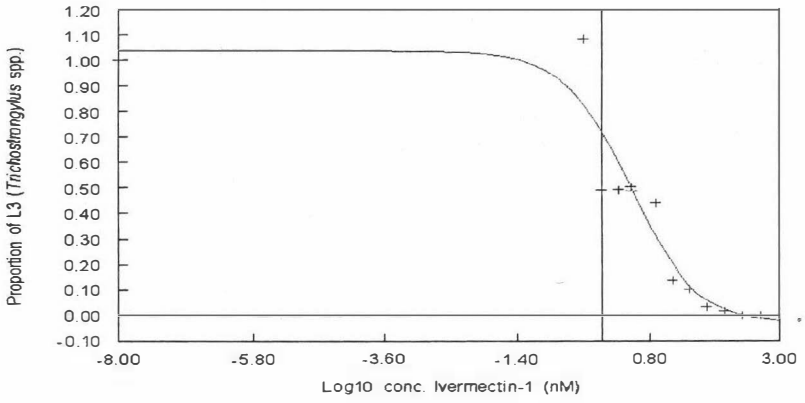
DrenchRite LDA,dose response curve(R^2=0.93)

Farm ID = Ian (LC50=7.8μM)



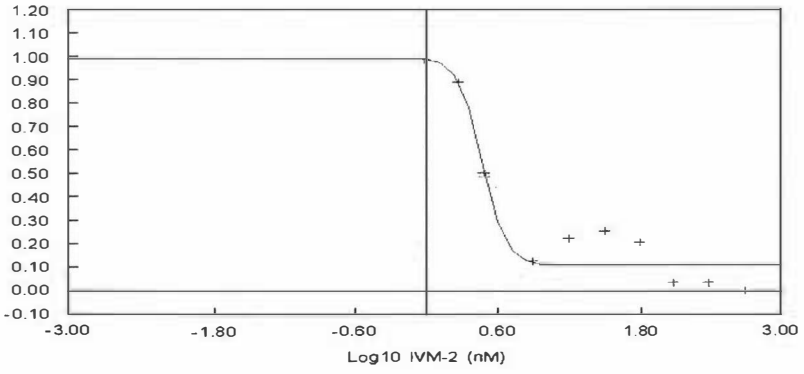
DrenchRite LDA,dose response curve(R^2=0.90)

Farm ID = Ian (LC50=3.0nM)

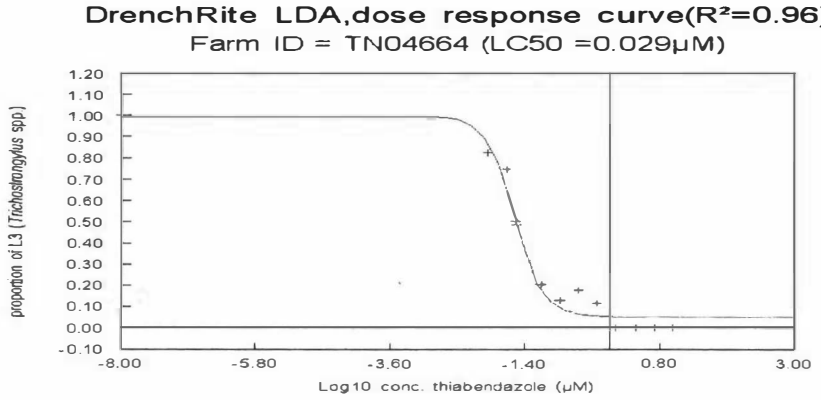


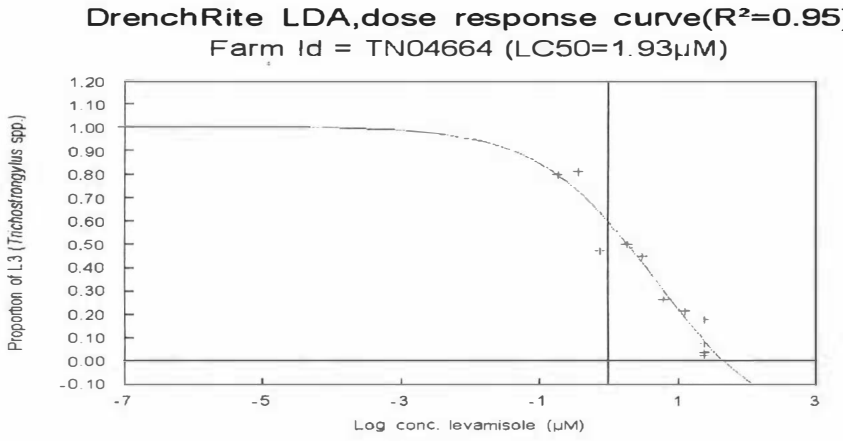
DrenchRite LDA,dose response curve(R^2=0.93)

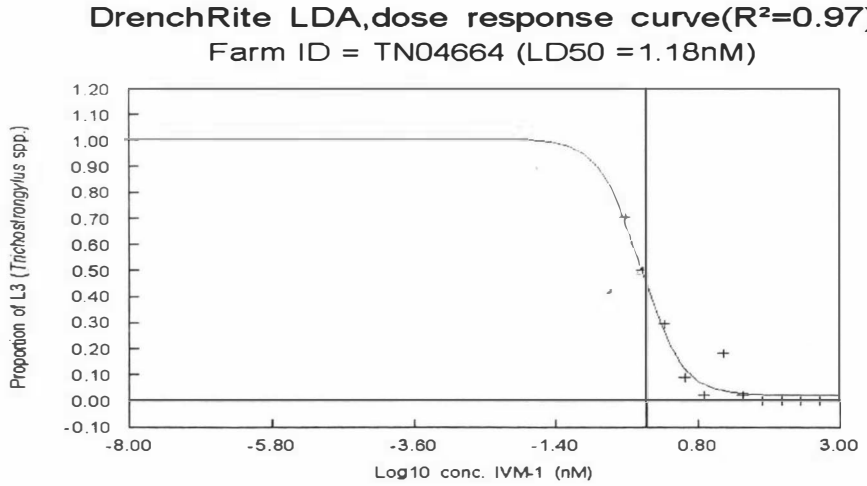
Farm ID = Ian (LC50=3.1nM)

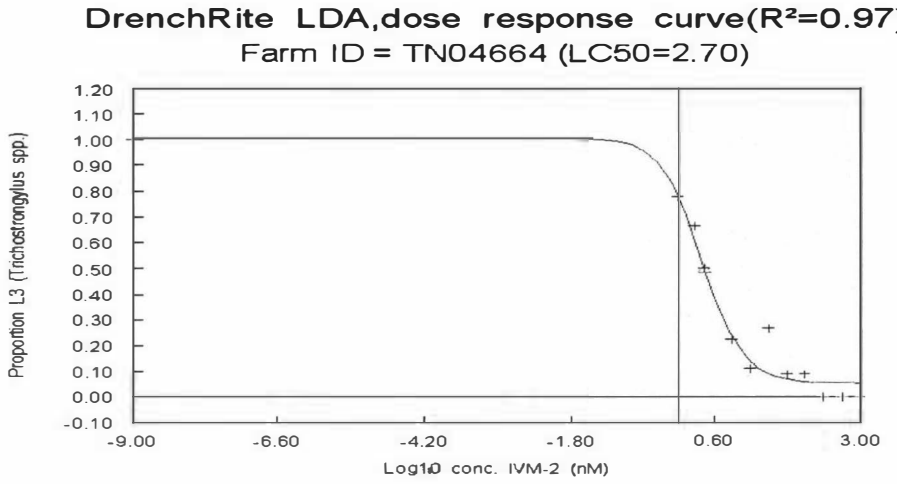


(d) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analog -1 (IVM-1), ivermectin analog -2 (IVM-2); *Trichostrongylus* spp. of farm – TN04664

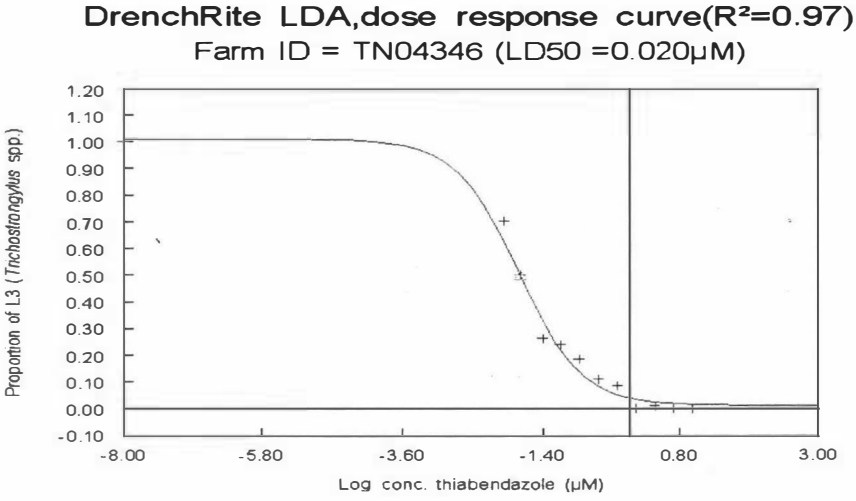




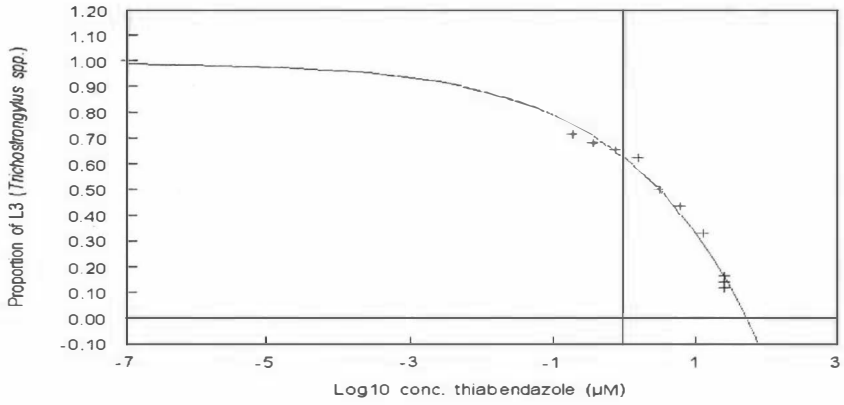




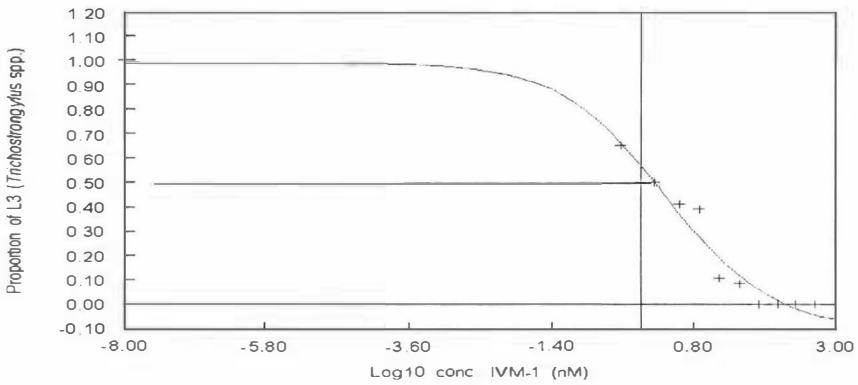
(e) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analog -1 (IVM-1), ivermectin analog -2 (IVM-2); *Trichostrongylus* spp. of farm farm – TN04346

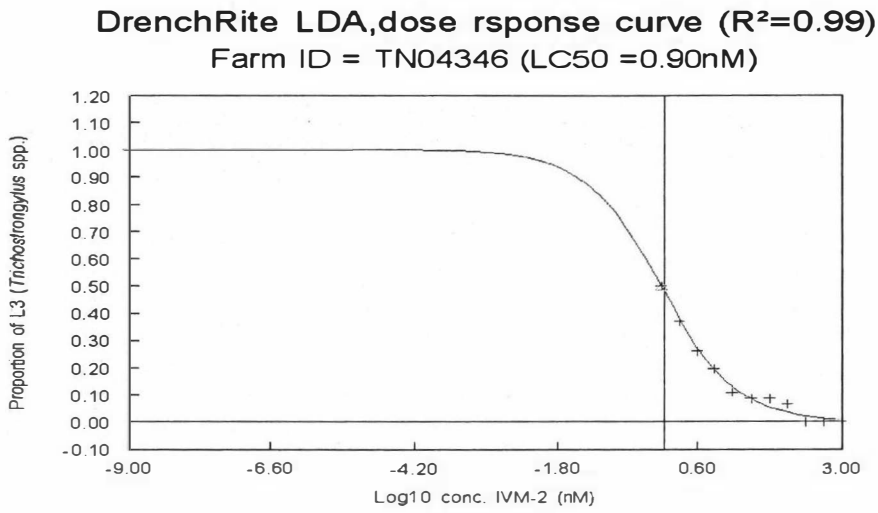


DrenchRite LDA,dose response curve(R^2=0.98)
Farm ID= TN04346 (LC50=3.1µM)

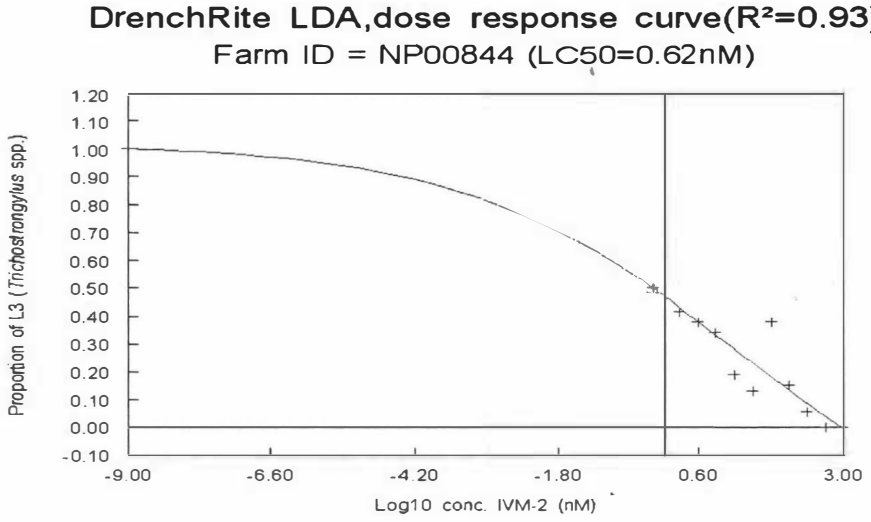


DrenchRite LDA,dose response curve(R^2=0.97)
Farm ID = TN04346 (LC50 =1.6nM)



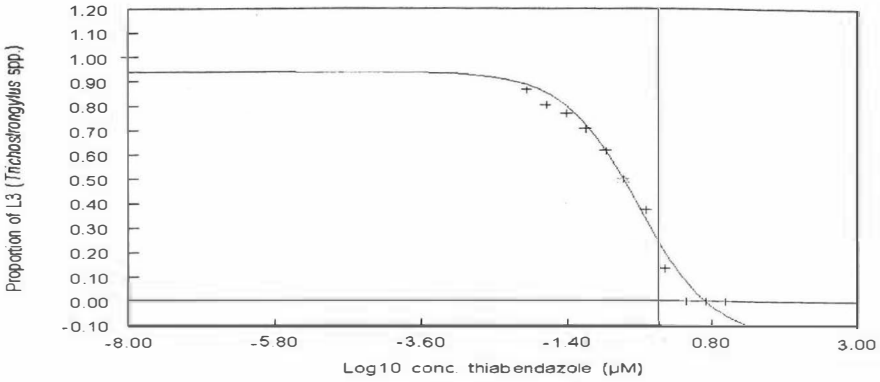


(f) Dose response curve and LC50 for ivermectin analog -2 (IVM-2); *Trichostrongylus* spp. of farm –NP00844

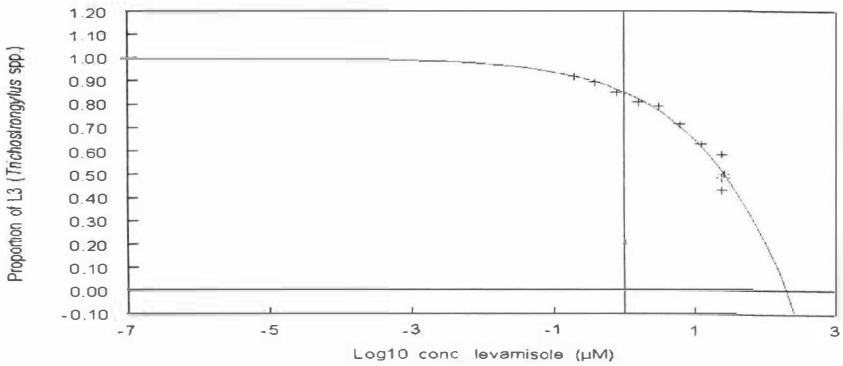


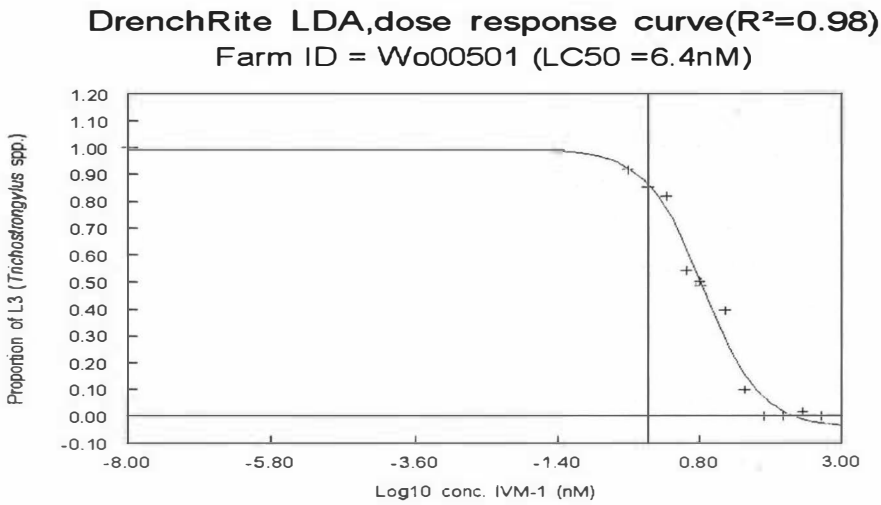
(g) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analog -1 (IVM-1), ivermectin analog -2 (IVM-2); *Trichostrongylus* spp. of farm – WO00501

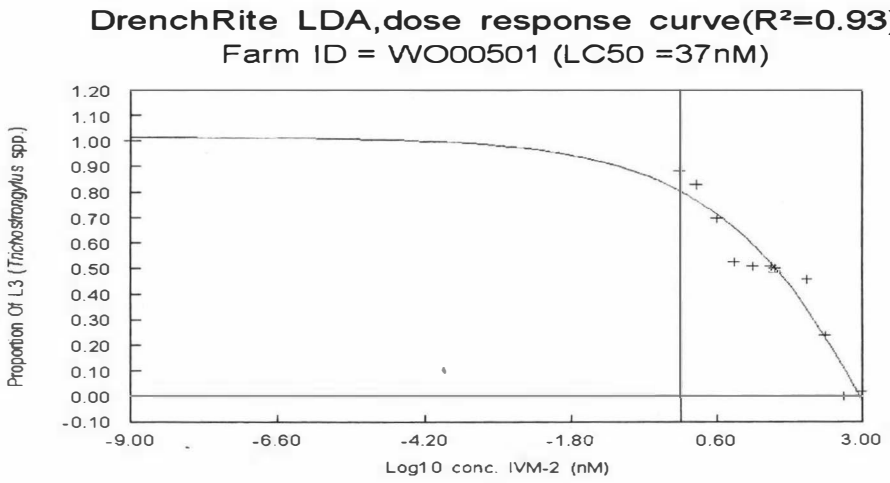
DrenchRite LDA,dose response curvr(R^2=0.97)
Farm ID = WO00501 (LC50=0.280)



DrenchRite LDA,dose response curve(R^2=0.96)
Farm ID = WO00501 (LC50=26.5µM)

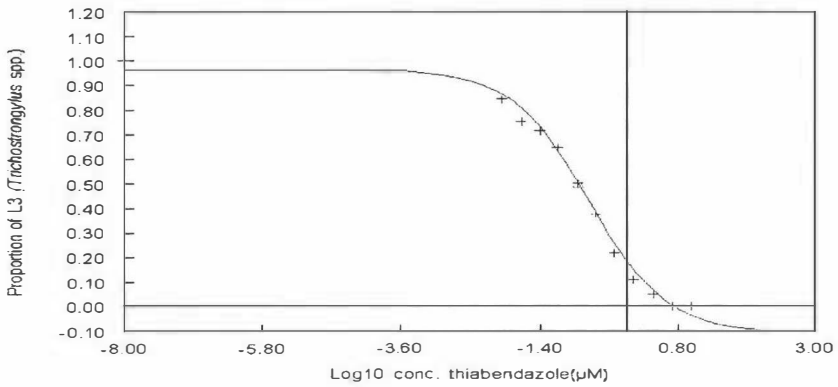




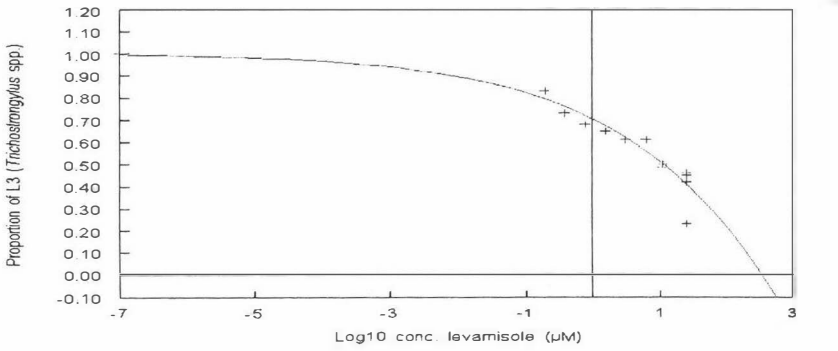


(h) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analog -1 (IVM-1), ivermectin analog -2 (IVM-2); *Trichostrongylus* spp. of farm farm – WO00544

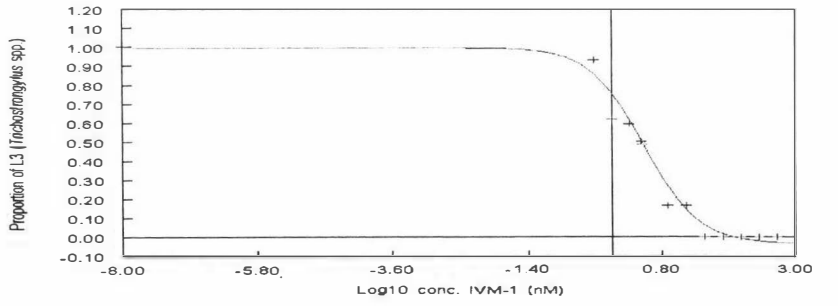
DrenchRite LDA,dose response curve(R^2=0.98)
Farm ID = WO00544 (LC50=0.165µM)

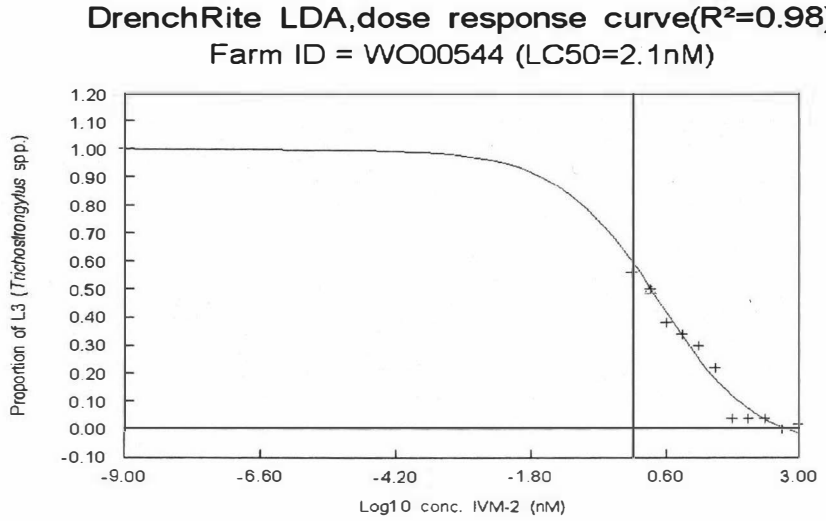


DrenchRite LDA,dose response curve(R^2=0.89)
Fram ID = WO00544 (LC50 =11µM)

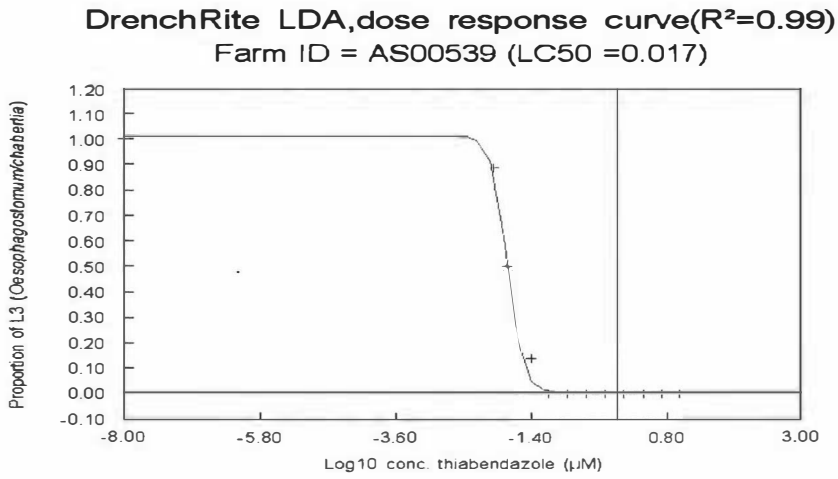


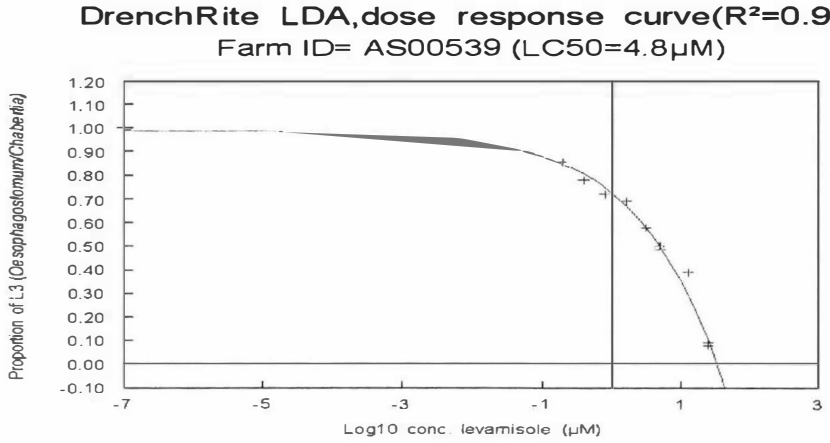
DrenchRite LDA,dose response curve(R^2=0.95)
Farm ID = WO00544 (LC50=2.95nM)

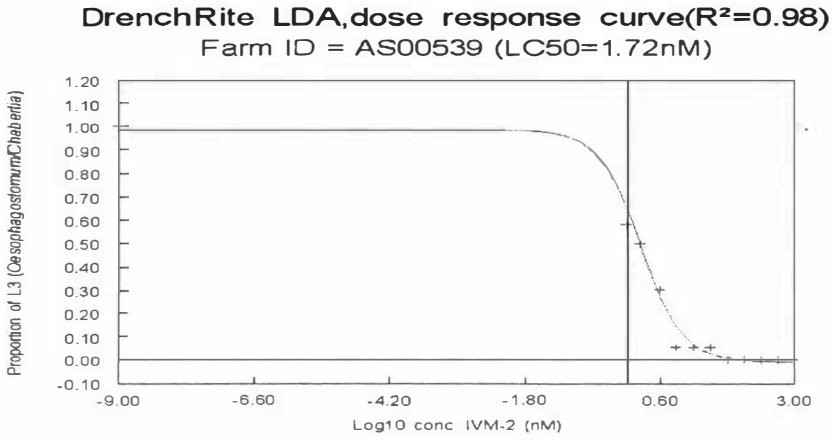




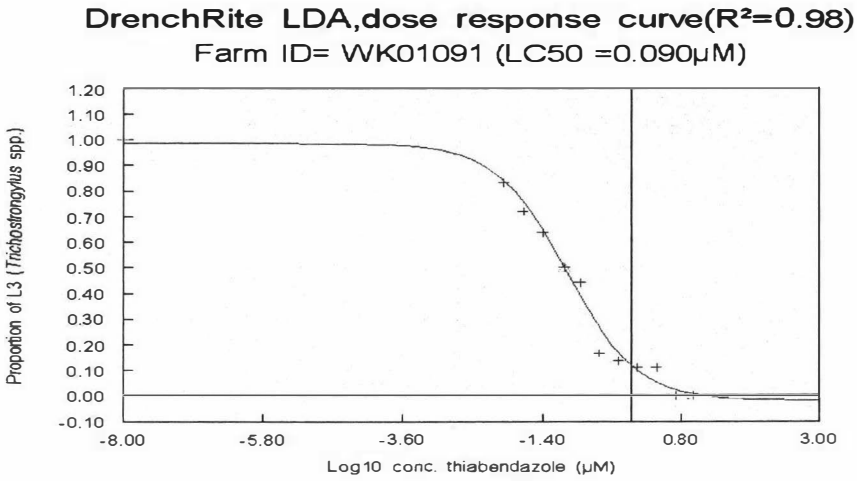
(i) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analog -2 (IVM-2); *Oesophagostomum/Chabertia* of farm - AS00539



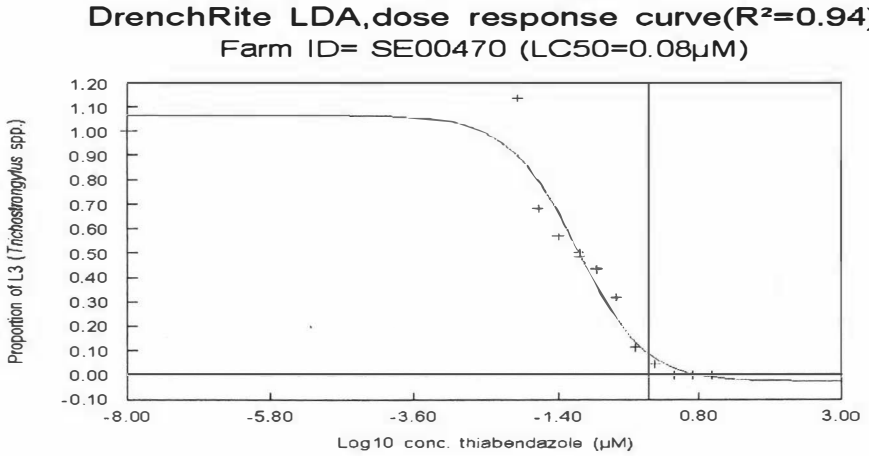


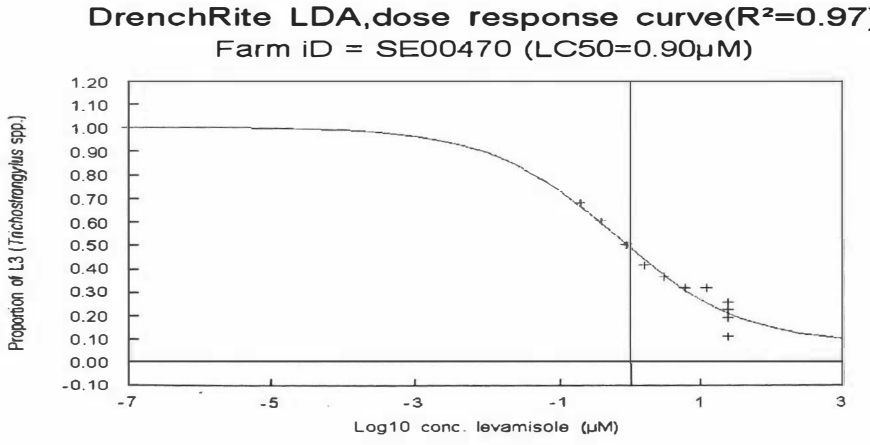


(J) Dose response curve and LC50 for benzimidazole (BZ), levamisole; *Trichostrongylus* spp. of farm – WK01091

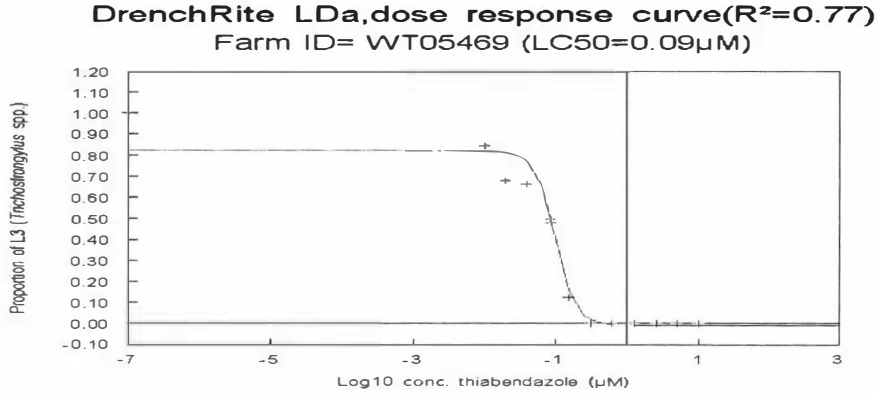


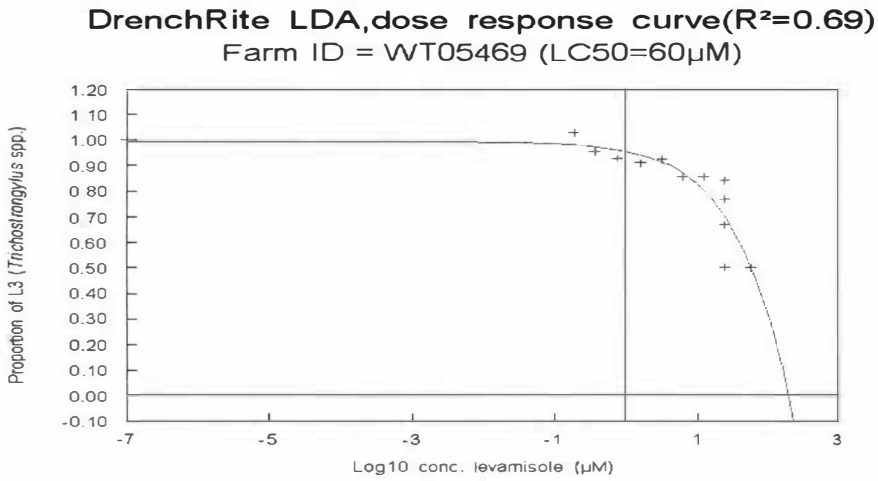
(K) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV); *Trichostrongylus* spp. of farm – SE00470

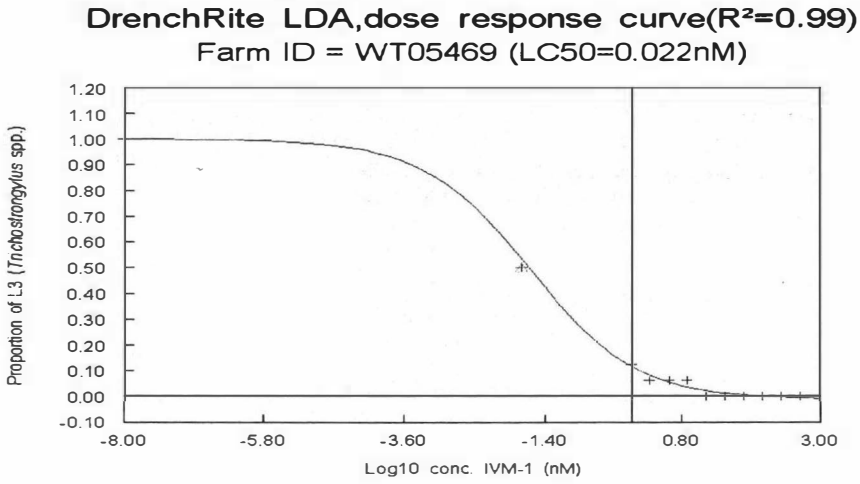


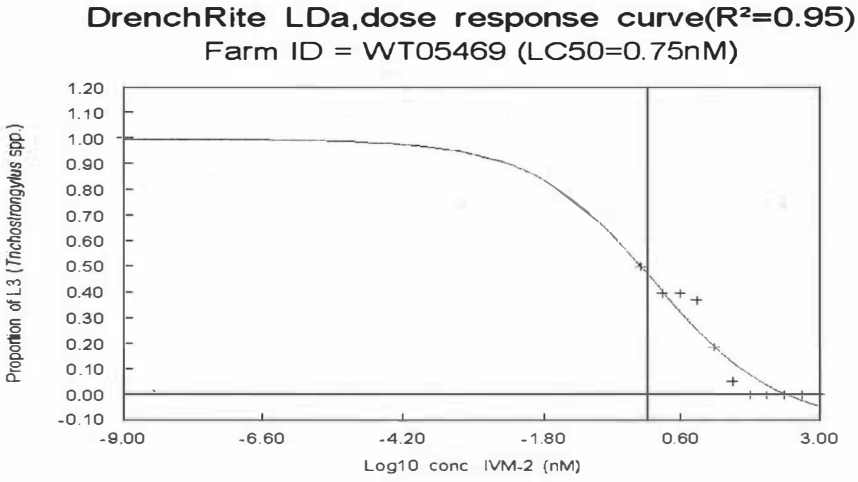


(I) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analog -1 (IVM-1), ivermectin analog -2 (IVM-2); *Trichostrongylus* spp. of farm – WT05469



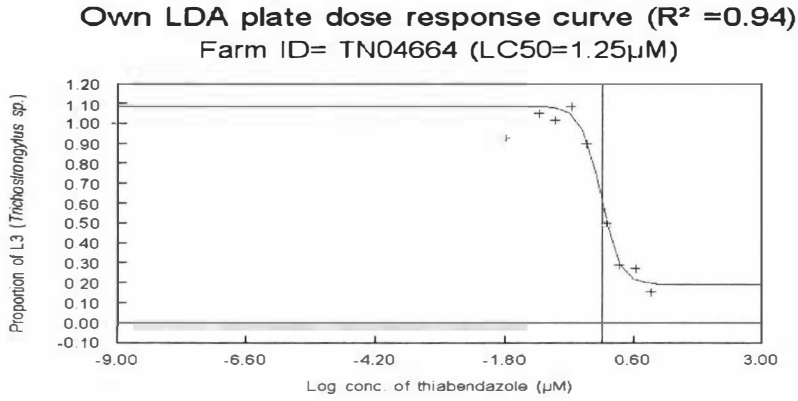


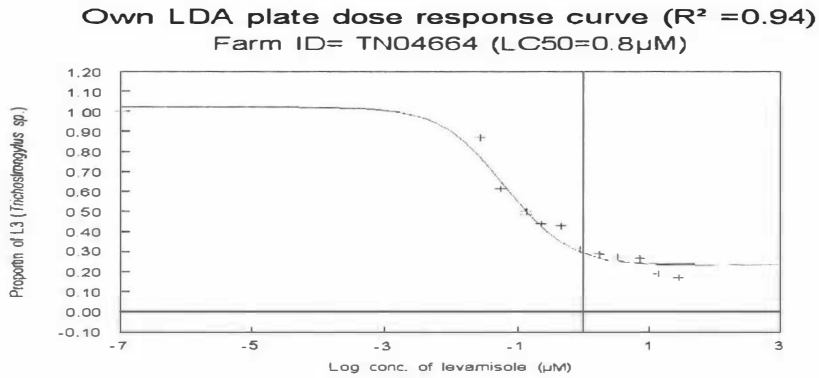


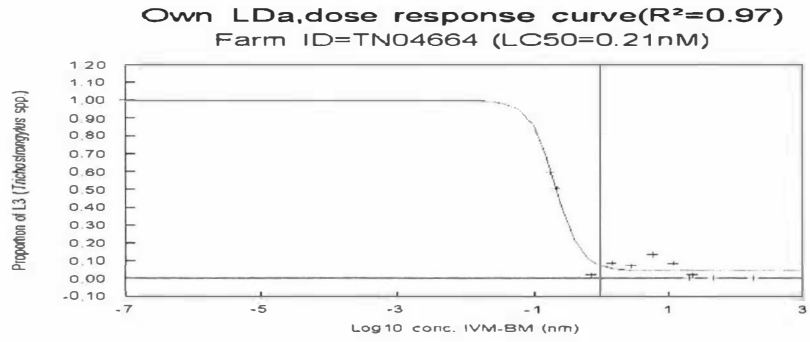


2. Inhouse LDA assays (Own prepared LDA plates)

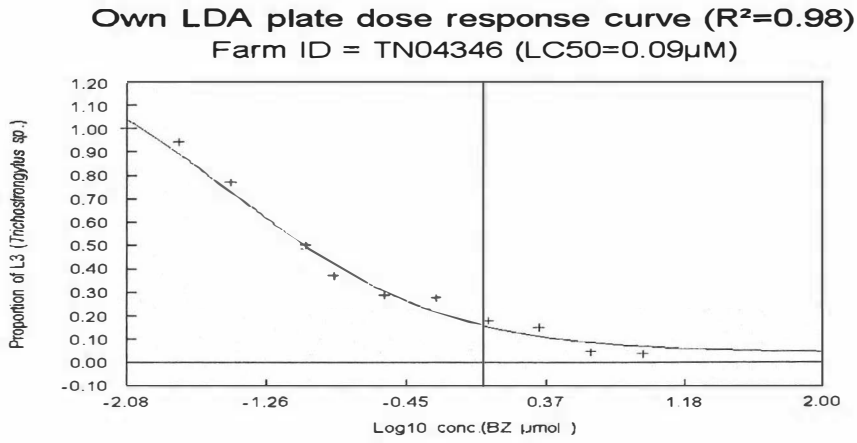
(a) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analogue-1 (bomectin); *Trichostrongylus* spp. of farm - TN04664

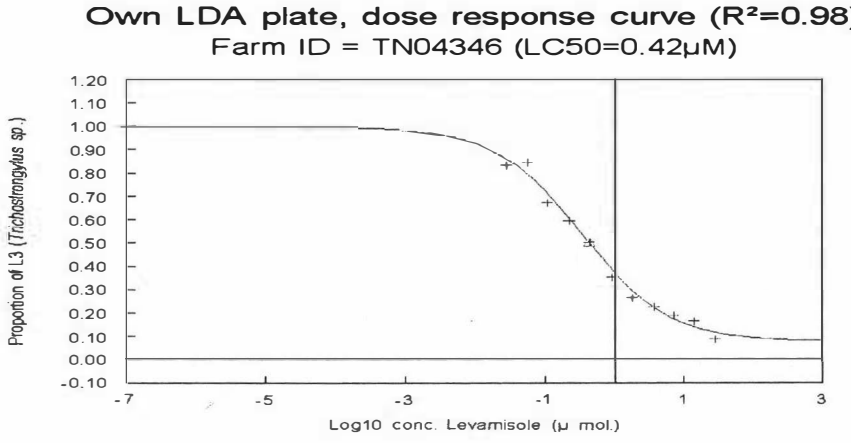




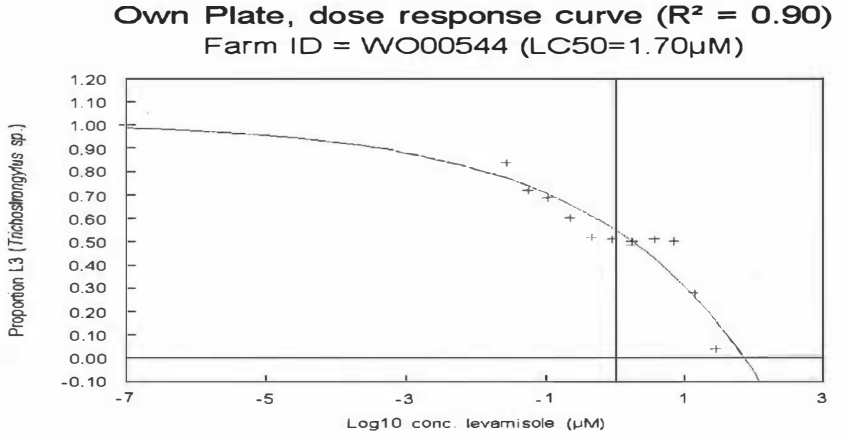


(b) **Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV); *Trichostrongylus* spp. farm - TN04346**

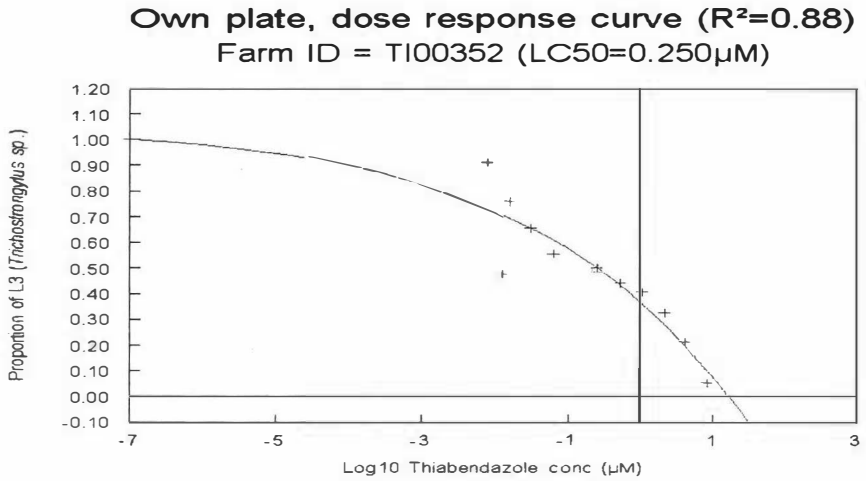


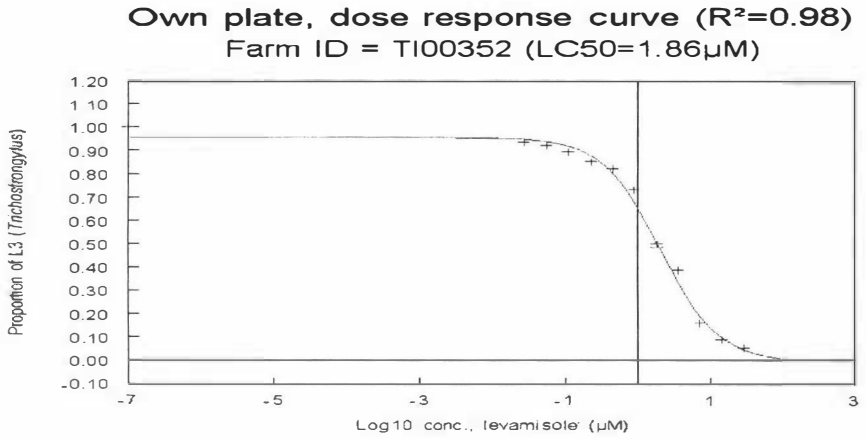


(c) **Dose response curve and LC50 for benzimidazole (BZ); *Trichostrongylus* spp. of farm – WO00544**

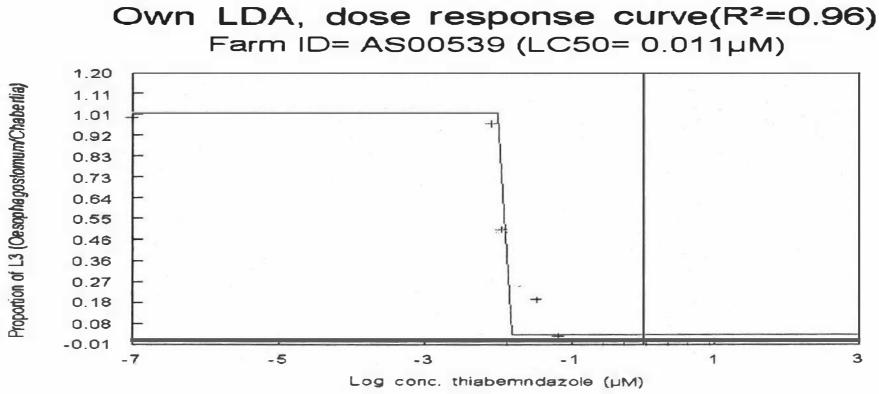


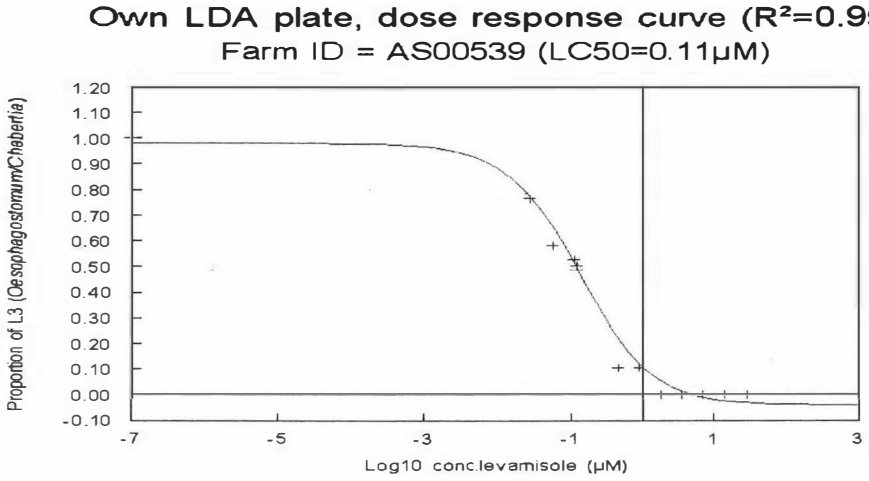
(d) Dose response curve, LC50 for benzimidazole (BZ), levamisole (LEV); *Trichostrongylus* spp. of farm – TI00352





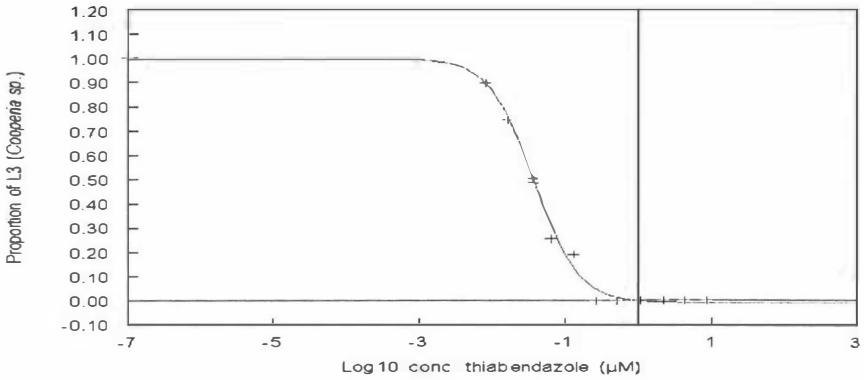
(e) Dose response curve, LC50 for benzimidazole (BZ), levamisole (LEV); *Oesophagostomum/chabertia* of farm – AS00359



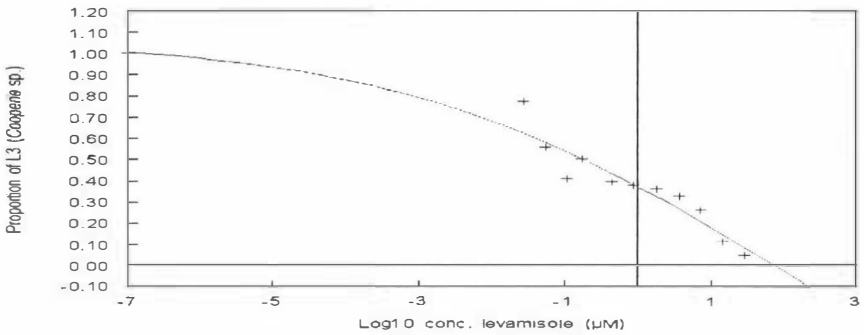


(f) Dose response curve, LC50 for benzimidazole (BZ), levamisole (LEV); *Trichostrongylus* spp. and *Cooperia* spp. of farm –SE04795

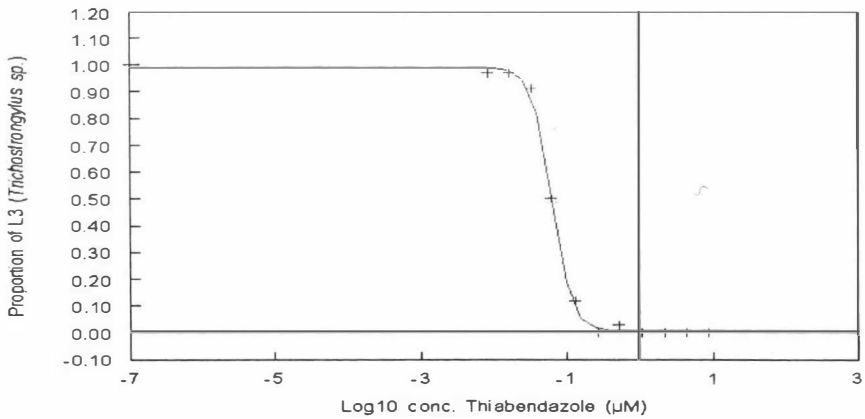
Own LDa plate, dose response curve (R^2=0.99)
Farm ID = SE04795 (LC50=0.065µM)

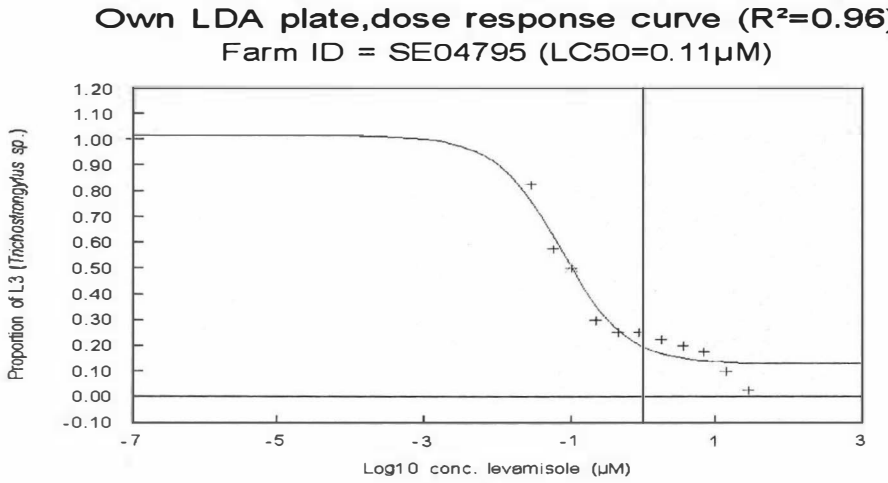


Own LDA plate dose response curve (R^2=0.90)
Farm ID = SE04795 (LC50=0.18µM)

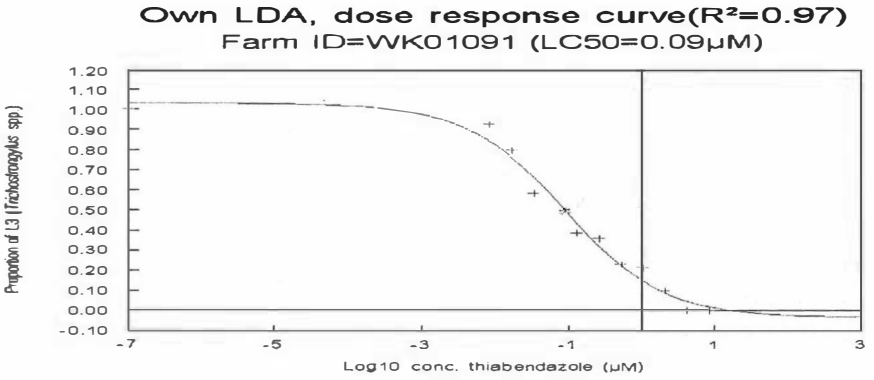


Own LDA plate, dose response curve (R^2=0.99)
Farm ID = SE04795 (LC50=0.35µM)



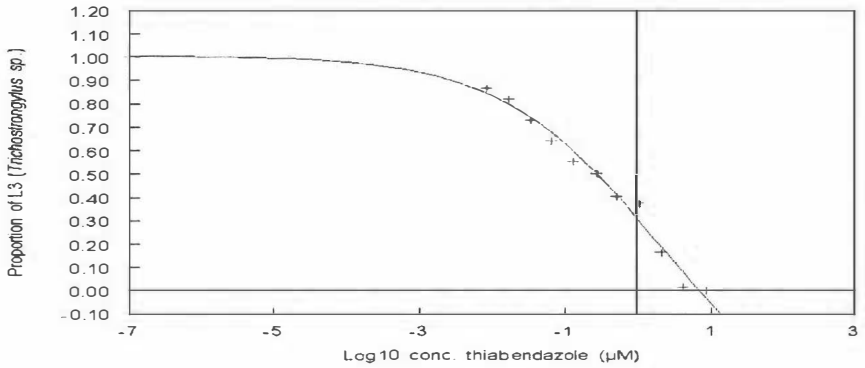


(g) Dose response curve and LC50 for benzimidazole (BZ); *Trichostrongylus* spp. of farm – WK01091

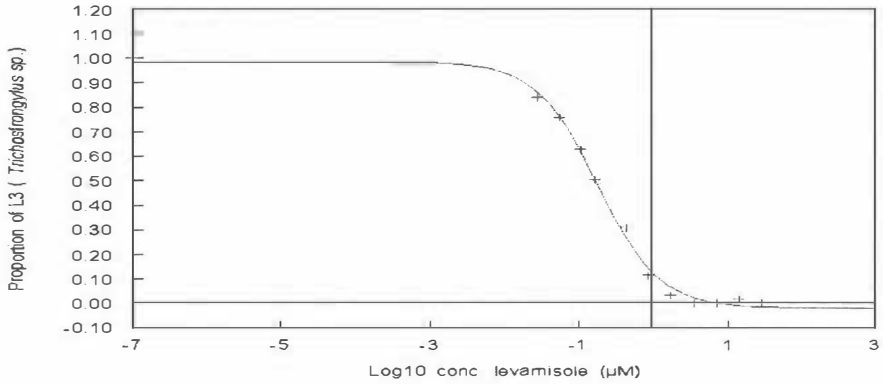


(h) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin (bomectin); *Trichostrongylus* spp. of farm - RU05209

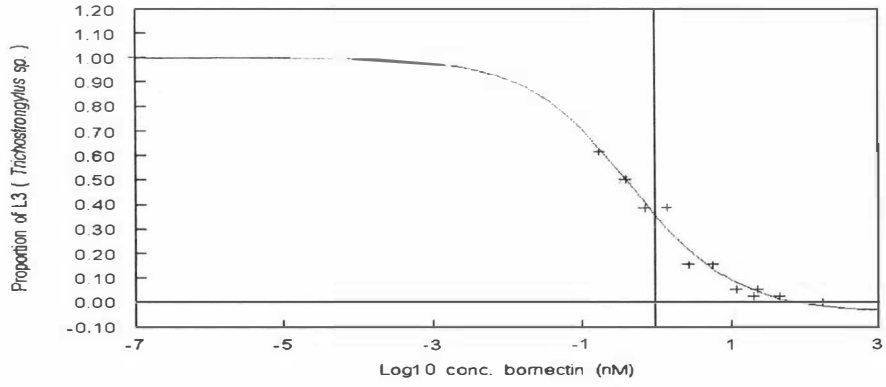
Own LDA plate, dose response curve (R^2=0.98)
Farm ID = RU05209 (LC50=0.27µM)



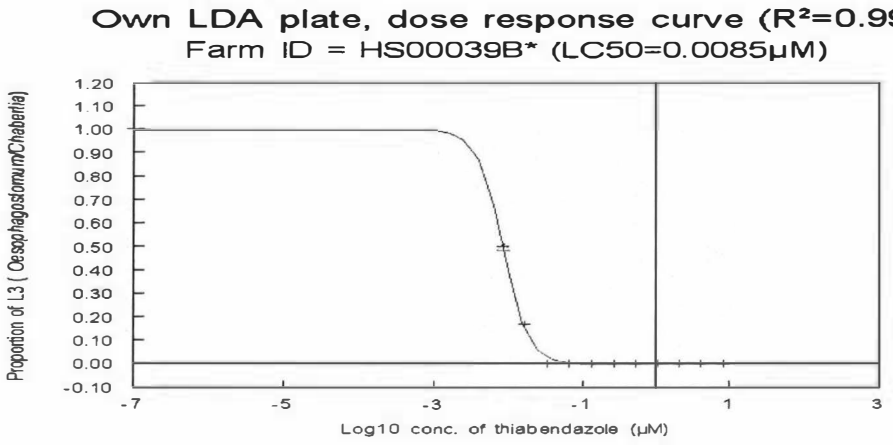
Own LDA plate, dose response curve (R^2=0.99)
Farm ID = RU05209 (LC50=0.18µM)

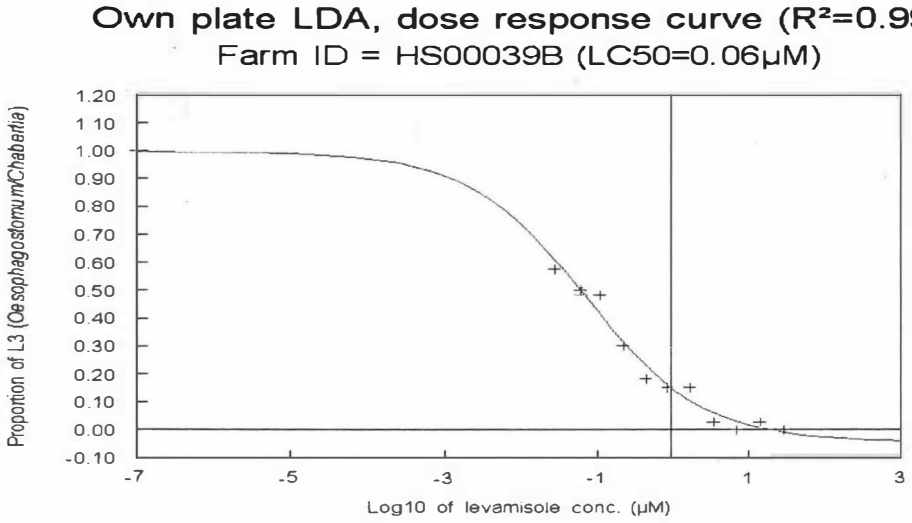


Own LDA plate, dose response curve (R^2=0.98)
Farm ID = RU05209 (LC50=0.4nM)



(i) Dose response curve, LC50 for benzimidazole (BZ), levamisole (LEV); *Oesophagostomum/Chabertia* spp. of farm - HS0039B*

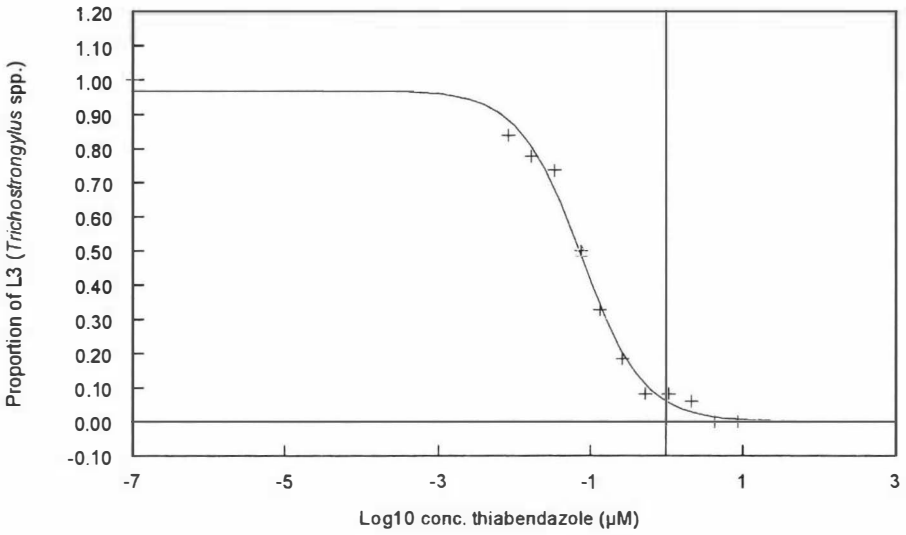




(J) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin aglycone; *Trichostrongylus* ; Trichostrongylus spp. of farm – GI00163

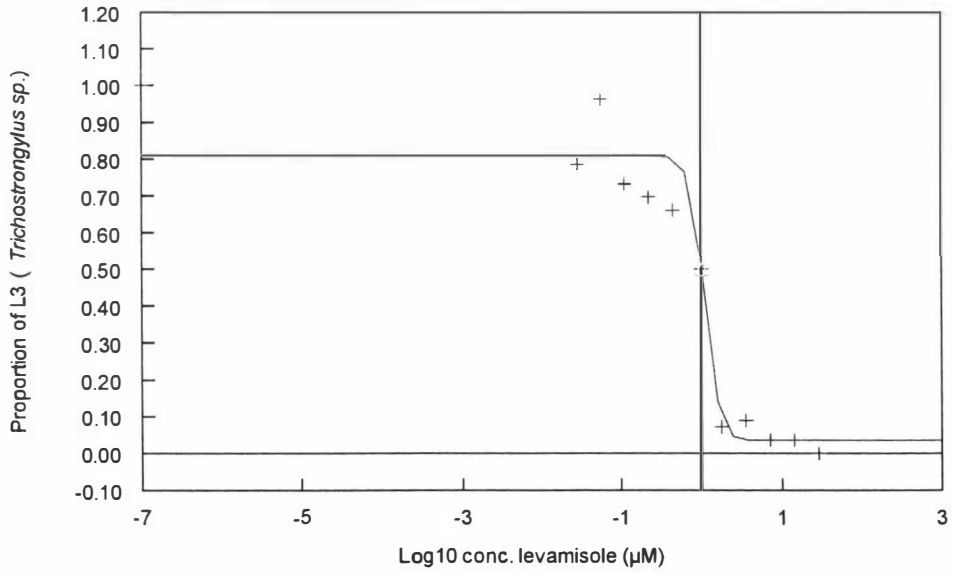
Own LDA, dose response curve(R^2=0.99)

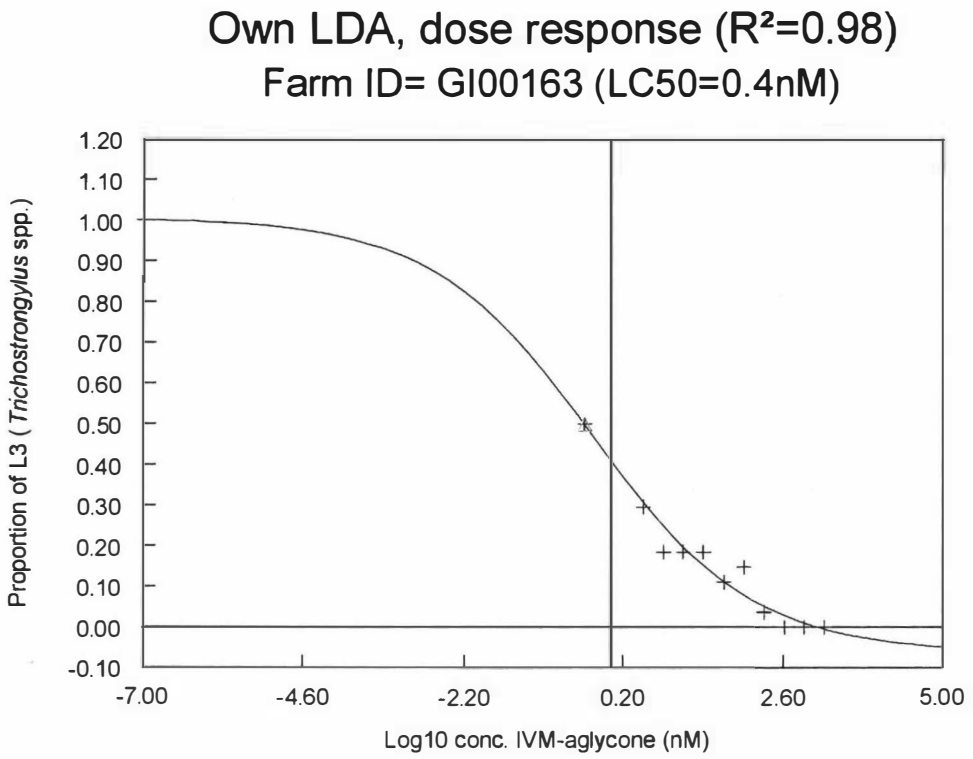
Farm ID= GI00163 (LC50=0.09µM)



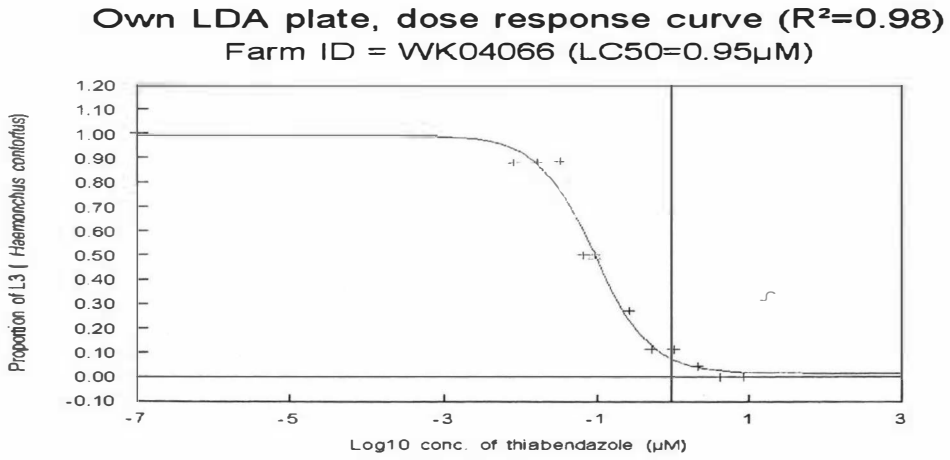
Own LDA plate, dose response curve (R^2=0.94)

Farm ID = GI00163 (LC50=1µM)

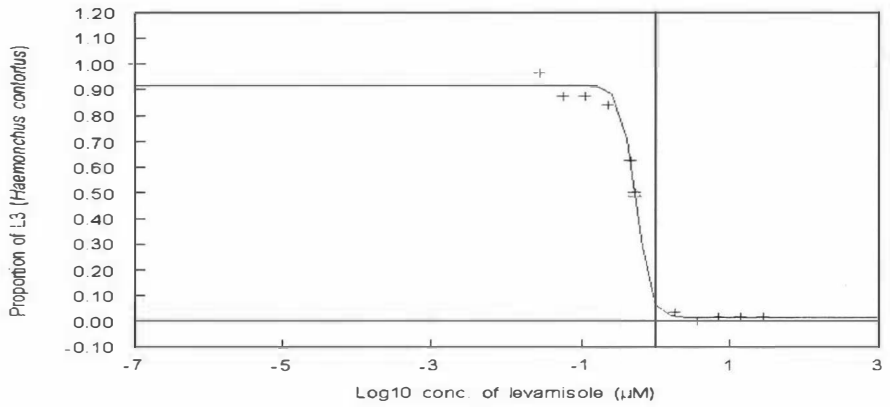




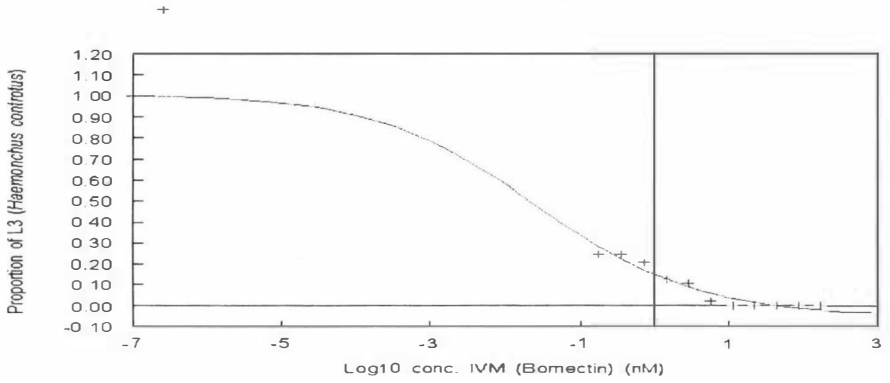
(K) Dose response curve and LC50 for benzimidazole (BZ), levamisole (LEV), ivermectin analogue-1 (bomectin); *Haemonchus contortus* and *Trichostrongylus* spp. of farm – WK 04066



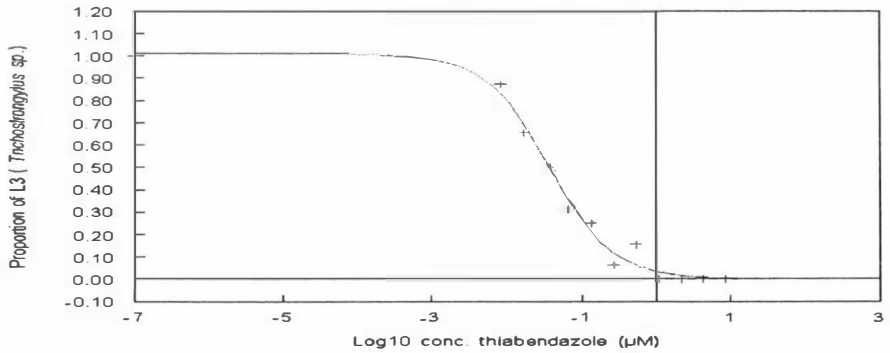
Own LDA plate, dose response curve (R^2=0.99)
Farm ID = WK04066 (LC50=0.50µM)

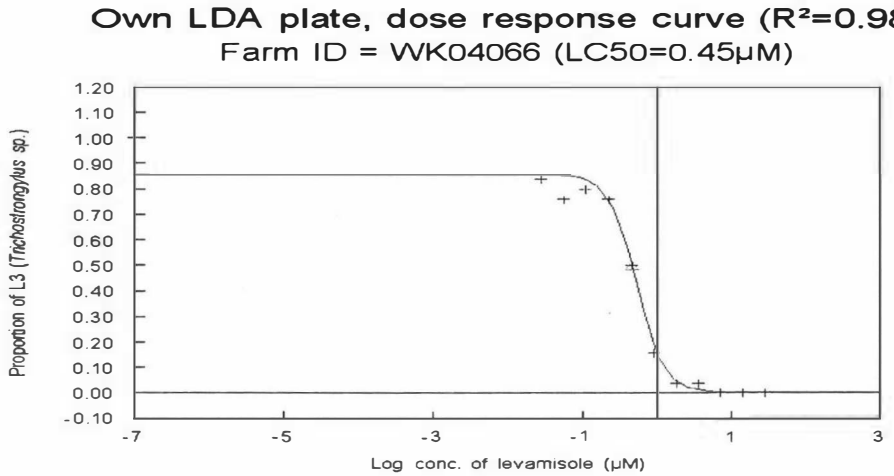


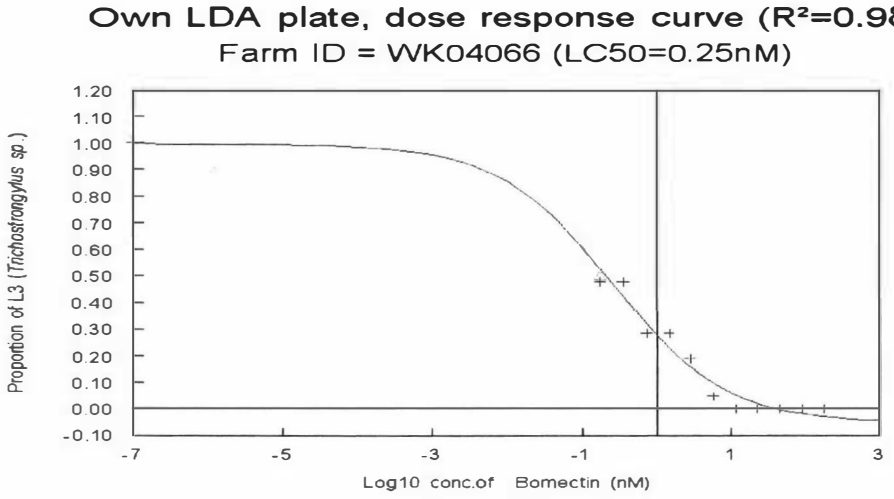
Own LDA plate, dose response curve (R^2=0.99)
Farm ID = WK04066 (LC=0.025nM)



Own LDA plate, dose response curve (R^2=0.98)
Farm ID = WK04066 (LC50=0.04µM)

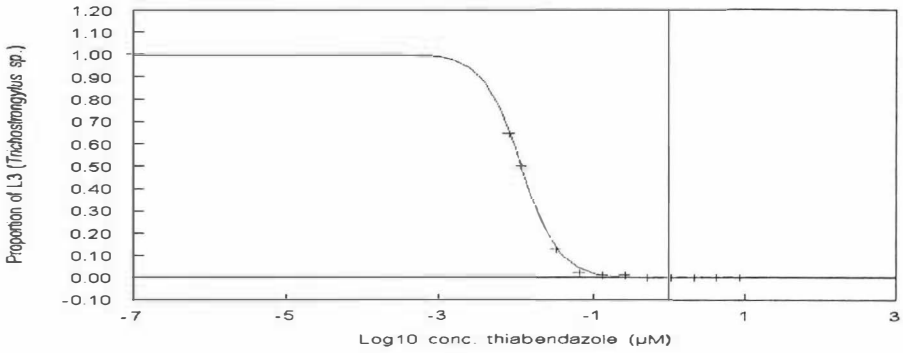




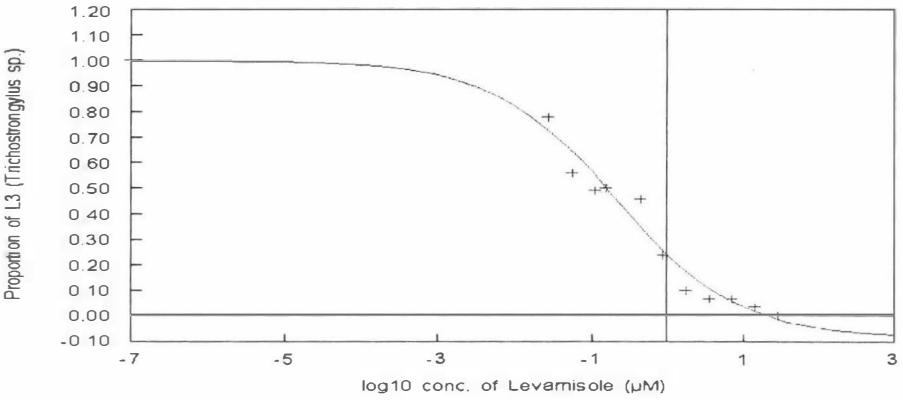


(I) Dose response curve and LC50 for benzimidazole (BZ). Levamisole (LEV), ivermectin (Bomectin) and ivermectin aglycone; *Trichostrongylus* spp. of farm – WT05469

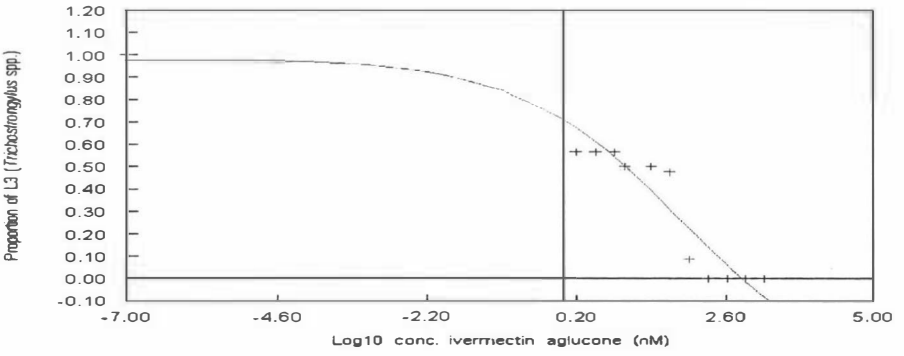
Own LDA plate, dose response curve (R^2=0.99)
Farm ID = WT05469 (LC50=0.015µM)



Own LDA plate, dose response curve (R^2=0.96)
Farm ID = WT05469 (LC=0.18µM)



Own LDA,dose response curve(R^2=0.90)
Farm ID= WT05469 (LC50=9.9nM)



Appendix 2.5

DrenchRite User Manual (Standard Operating Procedures) is included in the accompanying compact disk (CD).

Appendix 2.6

The details of data obtained from DrenchRite and Inhouse LDA is included in the accompanying compact disk (CD).

Appendix 3.1

Standard questionnaire (6 pages) used for questionnaire survey in 2003.

Massey University

PARASITE MANAGEMENT SURVEY

Farmer Name (Optional) .....

Region .....

Rep Initials .....

DATE .....

Thank-you for participating in this survey. Your farm has been chosen at random for this survey and all information will be treated in strictest confidence.

This nation-wide survey has three aims. Firstly to determine current drenching and parasite management practices on sheep farms in New Zealand. Secondly, to perform for the first time a random survey of the prevalence of resistance to all current broad spectrum drench families on New Zealand sheep farms. Thirdly to help develop an alternative to the traditional method of diagnosing drench resistance. This information will ultimately allow us to formulate improved worm control programmes.

I. Details of farm and livestock numbers

1. What livestock numbers were on your farm as at 1 July 2002.

	Younger than 1 Year
Lambs/Hoggets	
Deer	
Dairy Cattle	
Beef Cattle	
Goats	
Others	

	Older Than 1 Year
Ewes	
Rams	
Wethers	
Dairy Cattle	
Beef Cattle	
Goats	
Deer	
Others	

2. What is the effective grazing area on your farm?

[ ] Hectares or [ ] Acres

3. How many animals have you bought on or intend to introduce between 1 July 2002 and 30 June 2003. What if any quarantine drenching regime was/ will be performed?

Stock Class	Age	Drenched on Arrival		Product Used	Different to drenched used in lambs?	
		Yes	No		Yes	No

II. Management of Lambs

4. How many lambs were/ will be docked (tailed) on your farm between 1 July 2002 and 30 June 2003? [redacted]

5.

Mob ID	Date	Mob ID	Date

6. At what dates did ewes begin to lamb or are due to lamb between 1 July 2002 and 30 June 2003?

7. What system of grazing management for lambs do you use in the first two months after weaning?

☐ Daily Shifts ☐ Shifts at intervals of longer than 10 days
☐ Shifts every 2-10 days ☐ Set stocking

8. What proportion of lambs did you wean onto paddocks not grazed by lambing ewes since 1 June 2002?
☐ None ☐ Less than 1/2 (<50%) ☐ More than 1/2(>50%) ☐ All

9. Unless you ticked none, how long (on average) did it take before the lambs were put on a pasture previously grazed by ewes and lambs?

☐ 0-1 month ☐ 1-2months ☐ 2-3 months ☐ More than 3 months

10. Do you graze cattle/deer on pasture between periods of lamb grazing?

☐ Never ☐ Occasionally ☐ Often ☐ Always

11. For lambs born between 1 July 2002 and 30 June 2003 did or will you make hay, baylage, silage or another crop on land before lambs grazed. Yes / no

12. If yes what size of area?

☐ Ha ☐ Acres

13. What proportion of your farm was or will be grazed by lambs between 1 July 2002 and 30 June 2003?

☐ Less than ¼ ☐ Between ¼ & ½ ☐ Between ½ & ¾ ☐ Greater than ¾

14. When did you sell the lambs born in 2002?

Full date or month	No. of Lambs sold	Av. Weight
/ / 200		
/ / 200		
/ / 200		
/ / 200		
/ / 200		

Full date or month	No. of Lambs sold	Av. Weight
/ / 200		
/ / 200		
/ / 200		
/ / 200		
/ / 200		

III. Drenching Policy and Procedures

15. How do you assess the level of parasitism in your sheep? (May tick more than one)
- ☐ FEC
- ☐ Condition Score
- ☐ Growth Rate
- ☐ Dags
- ☐ Scouring
- ☐ Other

16. If you use FEC who does the counts?
- ☐ Vet
- ☐ Yourself
- ☐ Other

17. How many drenches did you give to your lambs/hoggets (born in 2002) between 1 July 2002 and 30 June 2003?

18. Please specify the commercial names of the drenches you used or will use on your lambs/hoggets:

July to September 2002	October to December 02	January to March 03	April to June 03

19. If you changed drenches between last season (2001-02) and this season (2002-03), please give the reason(s): (you may tick one or more answers)

- ☐ The previous drench did not appear to work
- ☐ A veterinarian recommended that you change to a different drench
- ☐ A person other than a veterinarian recommended that you change
- ☐ It was part of a planned drench family rotation
- ☐ The new drench was cheaper
- ☐ Other
  Please explain _____

20. Do you intend to use the same drenches in 2003/2004 as this season?

☐ Yes ☐No ☐Don't know

21. When do you drench your lambs/hoggets? (you may tick one or more answers)

- ☐ You follow a 5-6 drench programme by drenching every 3-4 weeks from weaning, regardless of weather or other factors
- ☐ You monitor the situation by periodically carrying out faecal egg counts and drench when they are high
- ☐ You drench the lambs when they show signs of scouring or illthrift.
- ☐ You drench the lambs regularly (~4 weekly) throughout their first year.
- ☐ You drench the lambs regularly during the period of greatest risk. Please specify months and / or reasons (eg when the season is very wet)

_____

_____

_____

- ☐ Other
  Please explain

_____

_____

_____

_____

22. Do you return the lambs after drenching to the same paddock?

☐ Never ☐ Occasionally ☐ Often ☐ Always

23. Of the lambs that do not return to the same paddock where to they go?

- ☐ Pasture re-growth from an earlier grazing with lambs?
- ☐ Pasture re-growth form another class of stock? Class/es _____
- ☐ New pasture
- ☐ Crop
- ☐ other

24. How many times (on average) did you drench your two-tooths between 1 year of age and mating in 2002/2003?

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ more than 3

25. If you did drench your two-tooths between 1 year of age and mating in 2002/03, was it because:

- ☐ Their egg counts were high
- ☐ They looked daggy or in poor condition
- ☐ You followed a predetermined drenching programme and drenched them at set time.
- ☐ Other

_____

26. How many times (on average) did you drench your ewes between 1 July 2002 and 30 June 2003? {or expect to if prior to 30th June}

☐ 0 ☐ 1 ☐ 2-3 ☐ 4-5 ☐ more than 5

27. When do you usually drench your ewes?

	Never	*Always*	*Sometimes*
Before Mating	☐	☐	☐
Before Lambing	☐	☐	☐
At tailing / docking	☐	☐	☐

28. If you drenched your ewes before lambing in 2002, what product/s did you use?
(Commercial name)

_____

_____

29. If you drench your ewes at other times of the year (other than in Q 24), when do you drench them?
(You may tick more than one answer)
☐ At regular predetermined times
☐ When they show signs of scouring/illthrift
☐ When their egg counts are high
☐ Other *(please specify when)*

_____

_____

30. When drenching your sheep, how often to you check the volume of the drench released by the gun?
☐ Occasionally before you start drenching
☐ You rely on the gun being accurate
☐ Always before you start drenching
☐ Both when you start and when you finish drenching
☐ Every _____ sheep / lambs
(Please indicate approximate number)

31. Do you check the seal integrity of your drench gun before use?

Yes No

32. The last time you drenched a mob of sheep how did you estimate their body weight?

☐ Scales
☐ Eye appraisal
☐ Other

_____

33. The last time you drenched a mob of sheep what weight did you base the drenching on?
☐ The average weight of the heaviest group of animals in the mob
☐ The average weight in the mob
☐ The heaviest individual in the mob
☐ The individual animal's weight
☐ Other

_____

34. Has a test for drench resistance **ever** carried out on this farm?
☐ Yes ☐ No ☐ Don't know

35. If yes, please fill in below the year(s) when the test was carried out, the results of the test, the products (if any) to which resistance was diagnosed and the resistant parasite species identified?

Year	*Resistance*		*Products*	*Parasite Species*
	Yes	*no*		
			to	
			to	
			to	

36. How was the last test carried out?

☐ Faecal samples were collected only after drenching the sheep (drench check)
☐ Faecal samples were collected both before and after drenching the sheep (drench test)
☐ Don't know

37. Who performed the test?

IV Your Own Opinion

38. How long do you consider a grass pasture must be spelled over the summer autumn period before it becomes "safe"?

_____

39. What is the most important way that you can delay the emergence of drench resistance on your farm?

_____
_____
_____

40. How effective do you consider your overall deworming programme? Please score it from 1 to 5.

Very effective				***Not effective***	***Don't Know***
(1)	(2)	(3)	(4)	(5)	☐

41. Do you consider the following problems to cause important economic losses on your farm?
Please score them 1 to 5.

	Very Important				***Unimportant***	***Don't Kn***
Worms	(1)	(2)	(3)	(4)	(5)	☐
Facial eczema	(1)	(2)	(3)	(4)	(5)	☐
Pneumonia	(1)	(2)	(3)	(4)	(5)	☐
Fly Strike	(1)	(2)	(3)	(4)	(5)	☐

42. Do you below drench resistance is a problem for the industry?

- ☐ Yes, it is a serious problem today
- ☐ Yes, it is a problem today, but not a serious one
- ☐ Not yet, but will be in the next 5 years
- ☐ Not yet, but will be in the next 20 years
- ☐ No, it is unlikely it will ever be a problem

43. Do you consider drench resistance requires action:

	Definitely	***Possible***	***No***	***Don't Kno***
From farmers	☐	☐	☐	☐
From drench companies	☐	☐	☐	☐
From MAF	☐	☐	☐	☐
From private vets	☐	☐	☐	☐
From other *(please specify)*	☐	☐	☐	☐

_____

44. Please feel free to add any suggestions or personal comments.

Appendix 3.2

Details of questionnaire survey 2003 from various farms is given in the compact disk (CD) accompanying this thesis.

Appendix 3.3

Drugs used in quarantine drenching in sheep farms between July 2002 and 30 June 2003.

S.NO.	Farm ID	Q drench	Products	Drug action family
1	**TN04664**	yes	Ivomec	Ivermectin
2	**TN04346**	no	....	
3	**RU05529**	no	.....	
4	**WO00271**	no	.....	
5	**WO00501**	yes	Scanda	Ivermectin
6	**WO00544**	yes	Triton	ABZ+LEV+IVER
7	**HS00039**	no	.....	
8	**TI00352**	yes	combo	Ricobendazole+LEV
9	**SE04795**	yes	Levamisole	Levamisole
10	**WM00679**	yes	White drench	BZ
11	**HB05386**	.....	....	
12	**AS00359**	No	.....	
13	**SE00470**	Yes	Scanda	IVM
14	**HB00312**	No	.....	
15	**NP00844**	No	.....	
16	**OT00695**	Yes	Genesis	Abamectin
17	**WK01091**	no	....	
18	**TP00301**	yes	Ivomec	IVM
19	**HU00174**	no	.....	
20	**KK00067**	.....	.....	
21	**TP05028**	yes	Genesis	Abamectin
22	**WT05469**	yes	Ivomec	IVM
23	**HU00323**	yes	Nilverm	Levamisole
24	**GI00459**	yes	Ivomec	IVM
25	**GI00366**	yes	Eprinex	

26	**GI00291**	yes	Triton	BZ+LEV+IVM
27	**GI00163**	yes	Scanda	IVM
28	**RU05209**	yes	Arrest	BZ+LEV
29	**FR00895**	yes	Albendazole	BZ
30	**WK04066**	.....	.....	
31	**AS00432**	yes	?	
32	**WM00107**	.....	.....	
33	**TI00048**	no	.....	
34	**TI00133**	yes	?	
35	**WM00278**	No	.....	
36	**GO00468**	no	.....	
37	**CO00481**	yes	Ivomec	IVM
38	**CL00063**	yes	?	

Appendix 3.4

The details of various drugs used in lambs/hoggets in various sheep farms in the year 2002/2003.

Uses of Various anthelmintics in lambs/hoggets drench in 2002/2003:- (Q-16)

S.No	Farm ID	July-Sept 2002	Oct-Dec 2002	Jan - Mar 03	Apr-June03
1	**TN04664**	……..	First	First	arrest
2	**TN04346**	……..	First	Genesis oral	Genesis oral
3	**RU05529**	…..	First drench/Scanda	Scanda	Scanda
4	**WO00271**	……..	First/scanda	Scanda	Scanda
5	**WO00501**	……..	First drench	Scanda/genesis	Scanda/Tandem
6	**WO00544**	……..	Nilverm/valbazen	Strategik/Valbazen/closal	Strategik/Closal/triton
7	**HS00039**	Combitape	Combitape	Cydectin	Leviben
8	**TI00352**	Leviben	Leviben	Leviben	Leviben
9	**SE04795**	……..	……..	Genesis oral	Albendazole
10	**WM00679**	Systamex	systamex	Systamex	Systamex
11	**HB05386**	Vetdectin plus	Vetdectin	Arrest	Vetdectin
12	**AS00359**	Nilverm	Ivermectin	Ivermectin	Ivermectin
13	**SE00470**	…….	Scanda	Scanda	scanda
14	**HB00312**	……..	lamb first	Arrest	Arrest
15	**NP00844**	……..	Genesis tape	Genesis	Genesis
16	**OT00695**	……..	lamb first	Genesis	Genesis
17	**WK01091**	First drench	First drench	Closal	Ivomec
18	**TP00301**	Arrest	Arrest	Arrest	Arrest
19	**HU00174**	……..	Genesis himin	Genesis	……..
20	**KK00067**	……..	Ivomec	Ivomec	ivomec
21	**TP05028**	Eweguard	First	Genesis/Moxidectin	……
22	**WT05469**	…….	Ivomec	……..	…
23	**HU00323**	……..	Nilverm/Genesis	Nilverm/Genesis	……..
24	**GI00459**	Ivomec	Rycotape	Cydectin	Ivomec
25	**GI00366**	Valbazen	Rycotape	Rycotape/Cydectin	Nilverm
26	**GI00291**	Ivomec	Triton tape	Ivomec	Ivomec

27	**GI00163**	.....	First lamb	Scanda	Scanda
28	**RU05209**	.....	Triton tape	Closal	Triton
29	**FR00895**		First drench/Ivomec Genesis	Albendazole/Ivomec	.....
30	**WK04066**	.....	tape/Arrest/valbazen	Arrest/valbazen	Arrest/genesis
31	**AS00432**	.....	Cydectin	Cydectin	Cydectin
32	**WM00107**	.....	Leviben	Leviben	Leviben
33	**TI00048**	Leviben	Leviben/Cydectin	Cydectin	Cydectin
34	**TI00133**	.....	Systamex/Valbazen	Valbazen/Levamisole/	Normadectin/Levam
35	**WM00278**	.....	Arrest	Arrest	Arrest
36	**GO00468**	Nilzan	Nilzan	Nilverm	Nilverm
37	**CO00481**	.....	.....	Levitape	Vetdectin/Ivomec
38	**CL00063**	.....	Cydectin	Cydectin	Cydectin oral

Appendix 3.5.

The details of various drugs used in ewes drenching in sheep farms in 2002.

S.NO.	Farm ID	Drench used	Generic family	
1	**TN04664**	Arrest	BZ+LEV	COMB
2	**TN04346**	Nilverm	LEV	LEV
3	**RU05529**	Closal	Closantel	CL
4	**WO00271**	Maximizer caps	IVM	ML
5	**WO00501**	Eweguard	Moxidectin	ML
6	**WO00544**	Ivimec maximiser	IVM	ML
7	**HS00039**	Eweguard	Moxidectin	ML
8	**TI00352**	Ivomec+Extender SeCo	IVM	ML
9	**SE04795**	.....		
10	**WM00679**	Extender SeCo	BZ	BZ
11	**HB05386**	Eweguard inj	Moxidectin	ML
12	**AS00359**	.....		
13	**SE00470**	Scanda/Maximizer	IVM	ML
14	**HB00312**	.....		
15	**NP00844**	Genesis	Abamectin	ML
16	**OT00695**	Eweguard	Moxidectin	ML
17	**WK01091**	Ivomec	IVM	ML
18	**TP00301**	Levicare	Levamisole	LEV
19	**HU00174**	Genesis Himin.	Abamectin	ML
20	**KK00067**	Ivomec	IVM	ML
21	**TP05028**	Eweguard	Moxidectin	ML
22	**WT05469**	Eweguard	Moxidectin	ML
23	**HU00323**	Genesis	Abamectin	ML
24	**GI00459**	Maximizer caps	IVM	ML
25	**GI00366**	Extender caps	BZ	BZ
26	**GI00291**	.....		
27	**GI00163**	Cydectin	Moxidectin	ML
28	**RU05209**	Maximizer caps	IVM	ML
29	**FR00895**	Albendazole	BZ	BZ
30	**WK04066**	Gensis	Abamectin	ML
31	**AS00432**	.....		
32	**WM00107**	Leviben	Levamisole	LEV

33	**TI00048**	Leviben/Cydectin	LEV/Moxidectin	LEV/ML
34	**TI00133**	Eweguard	Moxidectin	ML
35	**WM00278**	Ivomec maximizer	IVM	ML
36	**GO00468**	Nilverm/Extender caps	LEV/BZ	LEV/BZ
37	**CO00481**	Ivomec/Maximizer/Vetdectin	IVM/Moxidectin	ML
38	**CL00063**	Cydectin inj.	Moxidectin	ML

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