STUDIES ON THE OCCURRENCE OF ANTHELMINTIC RESISTANCE IN GOAT PARASITES IN NEW ZEALAND

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ABSTRACT

Two studies were conducted to investigate anthelmintic resistance in goat parasites in New Zealand. In Study 1 parasites from goats on a farm with a long history of problems with anthelmintic efficacy were used to infect sheep for a controlled slaughter study. Nineteen lambs were acquired, effectively drenched and housed. Each was infected with a mixture of larvae comprising *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *Oesophagostomum venulosum*. After 28 days lambs were restrictively randomised into 3 groups based on faecal egg counts. Group 1 was left untreated (n=6), Group 2 (n=6) was given a single dose of abamectin (0.2mg/kg) + levamisole HCL (8mg/kg) + oxfendazole (4.5mg/kg) ("Matrix Oral Drench for Sheep"®, Ancare, New Zealand) and Group 3 (n=7) was treated at twice the dose rate of Group 2. Fourteen days after treatment all animals were killed for total worm counts. The mean burdens of *T. circumcincta* in Group 1 was 337, in Group 2 was 68 (efficacy 80%) and in Group 3 was 10 (efficacy 97%). The mean burdens of *T. colubriformis* in Group 1 was 375, in Group 2 was 220 (efficacy 41%) and in Group 3 was 81 (efficacy 78%). Although the worm burdens in these lambs were low, all animals were infected with each of these two species except for *T. circumcincta* in Group 3 where only 3 lambs were infected. Efficacy against other species was 100%. These results clearly indicate that a single dose of a combination drench was ineffective against two species and even when a double dose was used the efficacy against *T. colubriformis* was only 78%. In Study 2 a survey of drench efficacy was conducted on 17 goat farms using the DrenchRite® larval development assay. Evidence of concurrent resistance to benzimidazoles, levamisole and ivermectin was detected in *T. colubriformis* and *T. circumcincta* on 11/17 and 3/14 respectively. Only 5 of 14 farms had previously undertaken some form of testing for drench resistance prior to this survey. Evidence from these two studies suggests that severe anthelmintic resistance is common on goat farms in New Zealand.

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GLOSSARY OF ABBREVIATION

AAD	Amino-acetonitrile derivative
ACH	Acetylcholine
BZ	Benzimidazole
cm	Centimetres
DR	DrenchRite
EC_{50}	Effective concentration$_{50}$
EC_{90}	Effective concentration$_{90}$
ED50	Effective dose$_{90}$
EHT	Egg hatch test
epg	Egg per gram
FEC	Faecal egg count
FECRT	Faecal egg count reduction test
g	Grams
GABA	Gamma-aminobutyric acid
ha	Hectare
HCL	Hydrochloride
hr	Hour
IVM	Ivermectin
kg	Kilograms
L	Litres
L_1	First larval stage
L_2	Second larval stage
L_3	Third larval stage
L_4	Fourth larval stage
L_5	Fifth larval stage
LDA	Larval development assay
LEV	Levamisole
LP	Larval paralysis
ML	Macrocyclic lactone
mg	Milligrams
ml	Millilitres

MUAEC	Massey University Animal Ethics Committee
nAChRs	Neuronal acetylcholine receptors
NaCl	Sodium chloride
PPP	Pre-patent periods
R^2	Coefficient of Determination
RR	Resistance ratio
SOP	Standard operational procedure
U.S.A	United States of America
WAAVP	World Association for Advanced Veterinary Parasitology
WC	Worm count
µl	Microlitres
µm	Micrometres
µM	Micromoles
nM	Nanomoles
°C	Temperature in degrees centigrade
+	Positive

CHAPTER ONE

1.1 INTRODUCTION

Parasitism is one of the most important constraints on farming systems in New Zealand and elsewhere around the world. It is well understood that gastrointestinal nematode infections can limit livestock productivity. A variety of consequences may result including reductions in liveweight gain, diarrhoea, dehydration, blood loss, poor wool growth and/or quality, reduced fertility and milk production, possible rejection of carcasses or organs for human consumption and in some cases death.

In New Zealand, the cost of internal parasitism to the country's sheep and wool industries is considerable. Anthelmintic use allows some, but by no means all, of the cost of parasitism to be recovered. However, declining efficacy of drenches due to anthelmintic resistance threatens this. The annual losses to the sheep industry attributable to resistance were estimated at NZD18 million and based on the calculations this figure will be increasing to NZD60 million within 20 years (Leathwick, 2004). These estimates are now quite dated and are likely to be considerably higher. By comparison, the estimated production losses to the Australian sheep industry due to parasitism were estimated to be about AUD 700 million/year by 2010 (Welsman, 2001).

Actions undertaken by farmers to minimise losses due to gastrointestinal nematodes generally involve regular anthelmintic treatments as the cornerstone of parasite population control. Nevertheless, regular use of anthelmintics has been shown worldwide to result in the development of potentially severe levels of resistance (Kaplan, 2004). In New Zealand, many of the major gastrointestinal nematodes which can infect sheep and goats, including *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *Haemonchus contortus* have developed resistance to existing anthelmintics (Gopal et al., 1999; Hughes et al., 2007; Waghorn et al., 2006; West et al., 2004) excluding the recently released monepantel. This has also occurred in many overseas countries (Howell et al., 2008; Love et al., 2003) and the problem continues to increase. Anthelmintic resistance now involves all three broad spectrum groups of anthelmintics that were available prior to the release of monepantel and has become a growing issue faced by many small ruminant farmers.

1.1.2 Development of resistance in goats

The phenomenon of anthelmintic resistance in sheep has been well studied, but there is less information for goats. Interestingly, the situation for goats in New Zealand hasn't been assessed for several years, but there is considerable concern that severe levels of anthelmintic resistance are now common in goat nematodes.

In goats, anthelmintic resistance might have occurred because of overuse of anthelmintics (Scherrer et al., 1990) and also due to the limited bioavailability of anthelmintics in goats (Coles et al., 1989; Hennessy et al., 1993). One difficulty in ascertaining the actual efficacy of anthelmintics in goats is lack of knowledge of the appropriate dose rates since many are uncertain for this host compared to what is known for sheep. There has been little research conducted on this subject, but one approach is to see what happens when sheep are infected with goat-derived parasites. This approach was therefore used in the studies in this thesis.

1.2 GOATS VERSUS SHEEP

Goat and sheep farming have made massive gains in productivity worldwide. Both species provide humans with meat, milk, wool and skins. Goats are well known as a hardy animal that can be productive in harsh environments that are not suited for sheep and cattle (Mason, 1984). By comparison with sheep, goats have a good ability for walking large distances, are known to select the most nutritive plants and can make use of bushes and shrubs (Morand-Fehr et al., 2004).

1.2.1 Nematode species infecting goats

Sheep and goats share the same species of gastrointestinal nematode parasites (Beveridge et al., 1987; Brunsdon, 1960; Chartier and Reche, 1992; McKenna, 2009). In New Zealand, the three species of nematode parasites that are most important to goats are *H. contortus, T. colubriformis* and *T. circumcincta* (Brunsdon, 1960; Buddle et al., 1988). However, *H. contortus* has only been reported in the more northerly parts of the country. *Nematodirus* has also been found to be a problem species in goats (Brunsdon, 1960; Pomroy et al., 1988) as has been observed in Texas as well (Craig, 1982). *N. spathiger* was found to be a problem in sheep in the North Island (Middelberg and McKenna, 1983) However, it appears that the prevalence of this species in sheep increased in the South Island as well (Vlassoff and Bisset, 1991).

Other parasites observed included *Cooperia curticei* (Andrews, 1973) which is commonly found in sheep but in goats appears to be more pathogenic (Edgar, 1936). Edgar (1936) also reported a Saanen goat that was infected with 30,000 *Cooperia curticei*, which are small intestine dwelling worms, in a three year old host. Other species observed in goats include *Trichostrongylus capricola* which have been reported in feral goats (Andrews, 1973) and *Oesophagostomum/Chabertia* may be present as well (Brunsdon, 1960). However, infections with many of these species are not likely to be as severe or important as those involving *H. contortus, T. colubriformis* and *T. circumcincta.*

Studies in feral goats in South Australia reported *Trichostrongylus rugatus* as the dominant species in the pastoral zone mirroring what occurs in sheep (Beveridge et al., 1987). In addition, *Haemonchus longistipes* which is normally found in camels has also been found to readily establish in goats (Hussein et al., 1985; Kumsa and Wossene, 2007).

Both sheep and goats can readily transmit nematode parasites to each other (Gopal et al., 1999; Watson, 1994; Watson et al., 1996). Cross-infection of resistant nematode parasites between these two hosts may thus occur and must be a significant cause for concern in countries like New Zealand where goats and sheep may share the same farming system.

1.2.2 Comparison between goats and sheep

Due to the greater ability of goats to select nutritive plants and feed on shrubs, studies in desert areas have shown that they are unlikely to get infected with nematode parasites (Jacquiet et al., 1992; Vercruysse, 1983). Goats prefer browsing, while sheep will rely almost entirely on grazing pastures. Browsing reduces L_3 intake since higher numbers of infective larvae are only found very close to the base of pastures (Familton and McAnulty, 1997). Hoste et al. (2001) noted that higher faecal egg counts were reported in the Angora goats due to the differences in feeding behaviour between this breed and Saanen goats. In this study, Angora goats were reported as more of a grazing animal whereas Saanen goats were browsers. Thus, sheep are more likely to get infections with gastrointestinal nematodes due to grazing behaviour, however, goats do not develop the same level of immune response to the gastrointestinal nematode

parasites as sheep. Evidence has been found in a number of studies when comparing the level of nematode infection in goats and sheep during common grazing when placed in various conditions. When comparisons were made between adult Romney sheep and New Zealand feral goats grazing mixed grass and clover swards with no access for browsing, the goats were found to have significantly higher faecal egg counts (Pomroy et al., 1986). Other studies have also reported that goats are typically more parasitised than sheep under grazing systems (Huntley et al., 1995; Jallow et al., 1994; Le Jambre and Royal, 1976). Therefore, goats are very challenging animals to farm at the present time.

Results from another study involving sheep and goats between 6 and 8 months of age grazing naturally infected pasture (Watson and Hosking, 1989) indicated that after 23 weeks, young goats had significantly higher faecal egg counts than those of the young sheep. In a study in Malaysia the mean faecal egg counts of sheep decreased from the age of 8 months onwards whereas this only happened in goats from 12 to18 months onwards (Dorny et al., 1995b). They concluded that as goats utilised browse behaviour they have a smaller parasite infection as compared to sheep but are also slower to develop an immune responses to gastrointestinal nematodes compared to sheep. Similar observations were also made by Le Jambre et al. (1976) whose studies indicated that FEC in sheep declines more rapidly due to the earlier development of an effective immune response.

1.3 THE LIFE CYCLE OF NEMATODE PARASITES

In New Zealand and elsewhere, infection with nematode parasites represents the most significant threat to the health of grazing livestock. About 29 species of nematode parasites have been recorded in sheep whilst 22 species were found in goats in New Zealand (McKenna, 2009). *H. contortus, T. circumcincta* and *T. colubriformis* are the three species of nematode parasites that are thought most damaging to these hosts. Pastoral ruminants are exposed to parasite infections due to their grazing habit and environmental factors for which pastoralism favours the survival and development of the free-living stages of helminth parasites (Sykes, 1997). The proportion of roundworm parasite populations that live outside the host, in faeces, on the pasture and in soil, is probably more than 90% to 99% of the total (Familton and McAnulty, 1995).

Therefore, it is important to understand the nematode parasites' life cycle both inside and outside of the host.

There are six stages in the life cycle of the nematode parasites consists of egg, four larval stages and the adults (Soulsby, 1982). The development of nematodes can be divided into two phases; the free living phase and the parasitic phase. The adult females and males mate in the gut of the host, and the uteri of the females become filled with eggs. The females lay their eggs, which are passed in the faeces of the host. Temperature, oxygen and water play important roles in egg hatching and development through the initial larval stages (Vlassoff, 1982). This requires a warm environment (the optimum temperature being 25°C to 27°C) and one that is sufficiently moist (about 80% relative humidity). When conditions are favourable, the eggs hatch and the free-living first larval stage or L_1 emerges. The presence of liquid water within the faecal mass is important both for eggs to hatch and for larval development to occur.

First stage larvae feed on bacteria and grow before undergoing the first moult to become L_2. The second stage also feeds on bacteria, grows then moults again, to produce the infective stage for many nematode species; the L_3 larva (Figure 1). This second moult is incomplete with the L_3 retaining the cuticle of the L_2 as a protective sheath. These "ensheathed" L_3 are usually less than 1mm long and migrate out of the faeces, eventually becoming distributed in soil as well as on pasture (Vlassoff, 1973). Once out of the faeces they are ready to be eaten by the grazing host. By being ingested, the L_3 manage entry into the definitive host. In the digestive tract of the host, they exsheath and enter the mucosal glands before moulting to the L_4. There is one final moult to the adult stage and sexual maturity. Some texts refer to a L_5 larva, but this is essentially the immature adult and since no further moults occur in development the term L_5 has generally fallen into disuse. The females and males mate thus completing the life cycle. The pre-patent periods (PPP) of the parasitic species vary considerably, but many are somewhere between 2 to 4 weeks and many are within 18-21 days (Soulsby, 1982). The minimum PPP is the minimum time needed to complete development from the infective stage (L_3) to sexual maturity. At the end of this period, the female parasites are starting to lay eggs.

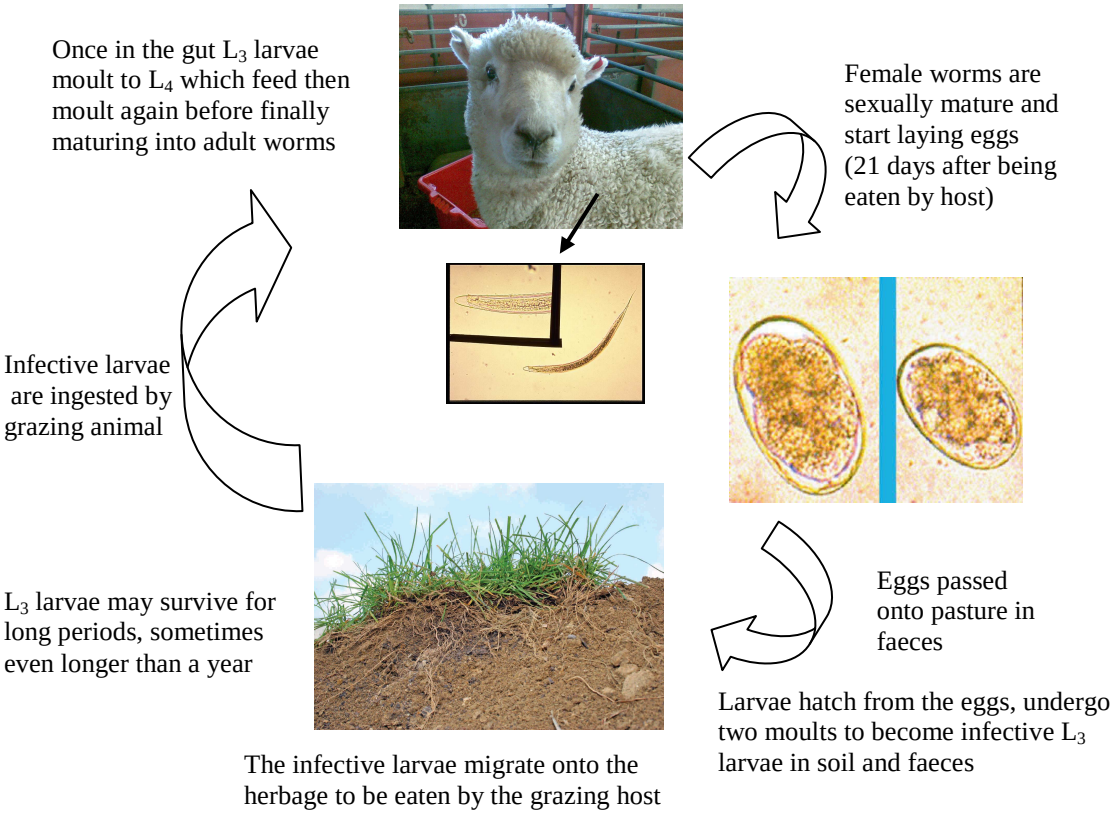


Figure 1.1: Life cycle of nematode parasite (Soulsby, 1982).

1.3.1 Geographic variations

In New Zealand, all strongylid nematodes found in sheep and goats are present on the pasture at all times throughout the year due to the moist moderate climate which is favourable for larvae development and survival (Vlassoff and Bisset, 1991). *Haemonchus* are more of a problem in the warmer areas of the north because they require a higher temperature range for development. *Nematodirus* causes more problems in the colder south as it is adapted to cool, short summers, and its larvae survive the cold winters on the pasture (Vlassoff, 1982). Most species show a tendency to inhibit through the winter (Familton and McAnulty, 1994).

1.3.2 Development and survival of eggs and larvae on pastures

The rate of deposition of eggs on pasture can be considerable, especially when the grazing area is heavily stocked with infected flocks. Thus, susceptible grazing hosts can become exposed to heavy parasite infection, due to the intake of relatively high numbers of larvae from pasture.

1.3.2.1 Hatching

Many studies on the temperature requirements, oxygen and moisture for egg hatching of various species of nematode parasites have been done. Temperature plays an important part in determining the success of egg hatching (Vlassoff, 1982). Different ranges of temperature have been cited for different species; minimum of 9°C for *H. contortus*, *T. axei* and *T. vitrinus*, and 4°C for *T. circumcincta* (Crofton, 1965). At 5°C, about 20% of *T. circumcincta* eggs hatch whereas 5% of *T. colubriformis* eggs develop to L_3 stages (Rossanigo and Gruner, 1995). It generally accepted that below the minimum temperature of 10°C, egg development is very slow, but may still occur in the presence of moisture and oxygen. Familton et al. (1994) indicated that even in the winter both *T. circumcincta* and *T. colubriformis* eggs hatched. A majority of *T. circumcincta* eggs hatched and developed into infective larvae at a constant 23°C, whereas a maximum number of *T. colubriformis* eggs developed within the range of 25°C to 28°C (Rossanigo and Gruner, 1995).

In laboratory experiments under cold exposure, studies have shown that after 3 days at 4°C, nematode egg viability was less than 60% for *H. contortus*, more than 80% for *Trichostrongylus* and over 90% for *Teladorsagia* (McKenna, 1998b). After 12 days, no *H. contortus* eggs survived whereas more than 30% of *Teladorsagia* and *Trichostrongylus* were recovered as infective larvae following subsequent culturing. During the winter months under field experiments, studies have shown that significant number of both *T. colubriformis* and *T. circumcincta* eggs had survived and hatched after 30 days (Familton and McAnulty, 1994) due to the temperature and moisture effects. Sakwa et al. (2003) indicated that in the winter, *H. contortus* eggs did not survive and only remained viable for a week within the faeces.

Less information exists on the oxygen concentrations available within faeces for egg development. The existence of oxygen within the faecal mass favours development of eggs in order for hatching to occur (Gronvold, 1989). A plentiful supply of oxygen is needed and if the faecal mass becomes anoxic, egg development becomes inhibited (Gronvold, 1989).

Observations of egg hatching also suggest that egg development also requires moist conditions (Vlassoff, 1982). It was argued that at low humidities, eggs failed to hatch. In summer, with dry conditions, the faecal mass tends to dry out. Higher temperatures can encourage rapid drying of faecal pellets and the soil surface level (Berbigier et al., 1990). However, egg hatching may occur due to the protective crust that develops on the outside of the faecal pellets which may ensure that the interior of the faecal mass is still moist (Gronvold, 1989) and with adequate rainfall, larvae will be released from the eggs (Young and Anderson, 1981). Furthermore, these effects also closely relate to other factors such as pasture and soil condition, cloud cover, wind, rainfall distribution and evaporation.

1.3.2.2 Development to L_3

It is clear that at low temperatures, development to L_3 is slow, whereas in warmer temperatures it is faster. Studies have reported that eggs and infective larvae of *T. circumcincta* and *T. colubriformis* develop at lower temperatures than *H. contortus* (Donald, 1968; Gibson, 1973). Others have confirmed and extended these studies and indicate that *T. circumcincta* populations are more successful in developing to the infective stage under colder conditions, than *T. colubriformis* (Vlassoff et al., 2001).

Temperature changes play an important part in determining the numbers of infective larval stages that develop at any given time. For *H. contortus* and *T. colubriformis*, the development of infective stages occurs optimally at 20°C and 25°C, respectively. They took a minimum of 4 days for the former and 3 days for the latter to become L_3 (Hsu and Levine, 1977). These findings were also supported by Vlassoff et al. (1991) who suggested 5 to 7 days as the optimum conditions for development to L_3. At temperatures above 30°C and 35°C *T. colubriformis* become L_3 stages after 2.5 days, while *H. contortus* takes longer at 3 days (Hsu and Levine, 1977). It appears that at 30°C, the time for development to L_3 of *H. contortus* eggs is approximately 3.5 days whilst at 37°C, they take about 2.5 days (Smith, 1990). Above 30°C, development is rapid, but there is a high death rate.

Larval development also depends on presence of moisture (Rose, 1963). L_1 and L_2 stages are very susceptible to desiccation. The absence of moisture can limit the success rate of larval development. It can be considered that in dry conditions, larvae

of some species may not be killed, but that there might be a delay of their development due to the lack of moisture. Under laboratory conditions, it has shown that the development of *H. contortus* eggs to L_3 stages occurred at 23°C by adjusting the faecal moisture content to between 15% to 85% relative humidity (Rossanigo and Gruner, 1995). For both *T. colubriformis* and *T. circumcincta*, the optimal faecal moisture was reported at 65% and 60% respectively at 23°C. The authors also suggested that the success rate of *T. colubriformis* and *T. circumcincta* were higher than that of *H. contortus.*

1.3.2.3 Movement of L_3

Moisture is a hugely important factor with regard the transition of the parasite population from faeces onto the pasture. Moisture is necessary to provide a film of water for larvae to migrate from the faecal pellet onto the grass or soil (Vlassoff and Bisset, 1991). In spring and late autumn, if humidity is low, movement of larvae onto the grass may take from 2 to 10 weeks (Vlassoff and Bisset, 1991). However, during wetter periods there are increased numbers of larvae on the pasture due to sufficient moisture on the grass. This factor also increases the development of larvae with a high survival rate of L_3.

1.4 ANTHELMINTICS

Over the past forty years, the availability of cheap and effective anthelmintic drugs has led to an almost complete reliance on these chemicals for parasite control in small ruminants. Prior to 1938, few anthelmintics were available on the worldwide market. In 1960 to 1980, rapid progress by the pharmaceutical industry resulted in three major classes of broad spectrum chemical compounds: the benzimidazoles, the imidazothiazoles, and the macrocyclic lactones (Harder et al., 2003). Initially all the products were available in the market as only single actives. More recently in New Zealand combinations of two or three of these anthelmintic classes have become available. In 2009, a new broad spectrum class; the amino-acetonitrile derivatives (AADs) was launched in New Zealand but to date has only been licensed for use in sheep. Two further novel anthelmintics of note include the cyclooctadepsipeptides and derquantel. The former has one product licensed for use in cats and the latter is not yet in the market in any form. The greatest constraint in the commercial development of new anthelmintics is the enormous costs involved.

Anthelmintics can be classified as broad- and narrow-spectrum as discussed below according to whether the drug can kill a broad or narrow range of parasite species.

1.4.1 Benzimidazoles (BZs)

Thiabendazole was introduced as the first drug with broad-spectrum activity in this class in 1961. This was followed by parbendazole in 1967, oxibendazole in 1973, fenbendazole in 1974, oxfendazole in 1975, albendazole in 1976 and ricobendazole in 1987 (McKellar and Scott, 1990). Febantel, netobimin and thiophanate which are known as probenzimidazoles are other drugs that are included in this group. These inactive, proBZs are designed to undergo the activity of enzymatic and/or non-enzymatic reactions in the treated animal to form active BZs compounds. From these listed anthelmintics, fenbendazole, oxfendazole and albendazole are generally referred to as tertiary BZs and have the highest efficacy amongst the BZs group. Fenbendazole, oxfendazole and albendazole can be used effectively at a dosage of 5 mg/kg for livestock but not cattle. These drugs have high efficacy against lungworm, tapeworm and gastrointestinal nematodes of cattle and sheep, including adults, developing and inhibited larvae.

All BZs are given by oral dosing that deposit the drug directly within the rumen of cattle, sheep and goats. The rumen thus acts as a reservoir for these relatively water-insoluble drugs, slowly releasing the chemical into the abomasum. Nevertheless, problems may occur in some animals when the dose bypasses the rumen due to esophageal groove closure leading a proportion of the dose being directed to the abomasum. As a consequence, the clearance of the drug from the animal is more rapid. Thus, this physiological phenomenon may contribute to treatment failure in animals as the resulting shortened blood phase reduces the exposure of the parasite to the drug and consequently its efficacy.

1.4.1.1 Mode of action

The activity of this group is directly linked to various interactions of BZs with tubulin which is a constituent protein present in microtubules, plasma and mitochondrial membrances (Prichard, 1986). These drugs work by affecting the formation of microtubules leading to disorders of intracellular homeostasis within the cells of parasites. Disruption of microtubules will interfere in processes such as mitotic spindle

formation during cell division, cell structure, cellular secretion and nutrient transportation (Lacey and Snowdon, 1988). BZs inhibit the polymerization of microtubules. The beta-tubulin subunit is known as the primary binding site of the BZs thus inhibiting dimer-formation and polymerization (Prichard, 2001). Death of most parasites occurs within about three days of treatment.

1.4.2 Imidazothiazoles/tetrahydropyrimidines

The most important drugs in this group, levamisole, morantel and pyrantel have similar pharmacologic effects even though they are of different chemical types. Levamisole is a broad-spectrum anthelmintic that has proven high efficacy against gastrointestinal nematodes and lungworms, and can be administered orally, by injection or by pour-on (the latter in cattle only). It is well tolerated at a dose rate of 7.5 mg/kg of body weight; but has no effect on either tapeworms or liver fluke. Levamisole together with the other anthelmintics in this group cause rapid paralysis in the parasites through mimicking the action of acetylcholine (Sangster et al., 2005). The function of acetylcholine action may be inhibited due to higher concentration of these drugs.

1.4.2.1 Levamisole

Levamisole (LEV) is a synthetic imidazothiazole derivative and has a chiral center. This drug was developed as a single enantiomer in which the anthelmintic activity was only found for the *L*-isomer. It is commercially available in two salts, a phosphate and a hydrochloride (HCl). LEV is not only effective against intestinal nematodes but lungworms as well. Severe side effects have been observed following as little as a double dose treatment.

In sheep and goats, there is an exception with regards the dose rate for LEV, which is that 1.5x sheep dose rate should be given to goats. LEV has a shorter half-life in goats than with sheep at 8mg/kg dose rate as a result of reduced efficacy of LEV in goats at the sheep dose rate. Coles et al. (1989) suggested that 12mg/kg is a recommended as effective dose rate in goats with no potential of toxicosis. Experimental studies of LEV in goats given the sheep dose rate in New Zealand have demonstrated a failure of the host to remove *Teladorsagia* although they effectively remove other common gastrointestinal parasites (Elliott, 1987; Pomroy et al., 1992).

It has also been suggested that this drug enhances the function of macrophages and T-lymphocytes as well and may also play a role in reducing activity of suppressive lymphocytes. Sajid et al. (2006) suggested that LEV has the ability to modulate the immune reactions to coccidiosis in broilers, and increase antibody production against canine parvovirus and the blastogenic activity of bovine lymphocytes.

1.4.2.1.1 Mode of action

LEV acts as a cholinergic agonist upon ligand-gated ion channels of the nematode parasites and mediates excitatory neurotransmission at the neuromuscular junctions of nematodes (Rew and Fetterer, 1986). Initially, LEV may act as a ganglion-stimulating compound and later on it may induce a neuromuscular inhibition of the depolarizing type causing spastic contraction and paralysis of nematode muscle.

1.4.3 Macrocyclic lactones

Several macrocyclic lactones (ML), such as ivermectin, moxidectin, abamectin and doramectin, are commercially available for the treatment of nematode parasites of livestock. Beside gastrointestinal nematodes, this group also has activity against lungworms (Egerton et al., 1979) and some ectoparasites (Campbell et al., 1983). Nevertheless, it has no measurable effect against liverflukes or tapeworms. In 1981, ivermectin was the first antiparasitic drug of this group to be released onto the market and became a very widely used anti-parasite medication for cattle, sheep and goats. Its use in these animals has been limited in recent years by the increased levels of anthelmintic resistance (see later). Within the ML group are two main classes of compound, the avermectins and the milbemycins. The MLs are the most potent anthelmintics on the market, their effective doses being measured in micrograms per kilogram rather than in milligrams.

1.4.3.1 Abamectin

The first ML molecule discovered, abamectin, was isolated from fermentation extracts of *Streptomyces avermitilis.* This drug has since been widely used to combat nematode parasitism especially in cattle (Heinze-Mutz et al., 1993; Kaplan et al., 1994), ivermectin-resistant strains of *T. circumcincta* in sheep (Leathwick et al., 2000) and ivermectin-resistant strains of *T. colubriformis* in sheep (Alka et al., 2004).

1.4.3.2 Ivermectin

Ivermectin (IVM) is a synthetic derivative of abamectin, is prepared using cultures of *Streptomyces avermitilis*. IVM work wells not only against nematodes but also has a wide spectrum of activity against ectoparasites. Wolstenholme et al. (2005) indicates that IVM exerted its activities by stimulating the glutamate-gated channels in the membrane of nerve cells in the invertebrates at low concentrations. Abamectin and IVM have the same mode of action, IVM is less potent against some nematode, including *Teladorsagia* and *Haemonchus* in particular but arguably has a better safety profile than abamectin. Both abamectin and IVM are examples of avermectins.

1.4.3.3 Moxidectin

Moxidectin is a milbemycin, the second group of ML anthelmintics, and was produced through fermentation of *Streptomyces cyanogriseus*. This drug is known as a particularly long-acting anthelmintic due to its greater persistence in the tissues of treated animals and it appears to be the most potent ML available (Shoop et al., 1993), but not perhaps in all instances. Moxidectin can be given orally to sheep at the same dose rate of 0.2mg/kg (Larsen et al., 2009) as ivermectin and abamectin. This drug is likely to be effective at this dose rate in removing *T. circumcincta* and *T. trifurcata* in goats (Pomroy et al., 1992).

1.4.3.4 Mode of Action

The MLs affect the nervous systems of parasites by binding to glutamate-gated chloride ion channels, which are involved in nematode feeding, reproduction and locomotion (Yates et al., 2003). Turner et al. (1989) noted that the irreversible mechanism of the drugs had opened chloride channel muscles of the pharynx, and through glutamate-gated ion channels associated with *gamma* aminobutyric acid or GABA receptors. Due to this interaction, nematode parasites become paralyzed and starve to death. It appears that different organs have different sensitivities to this group of anthelmintics. Studies with IVM have shown that the pharyngeal muscles of nematodes are significantly more susceptible than somatic muscle (Sangster et al., 2005). In studies with *H. contortus*, IVM works effectively on pharyngeal pumping processes and leads to paralysis (Geary et al., 1993).

1.4.4 Narrow-spectrum anthelmintic

A number of narrow-spectrum anthelmintics, such as closantel or rafoxanides are mainly anticestodal or antitrematodal compounds and have been used to control some highly pathogenic gastrointestinal nematode parasites, which have developed resistance to the broad-spectrum anthelmintics, in particular the BZ- and LEV-resistant *H. contortus* (Waruiru, 1997, 2002).

1.4.4.1 Closantel

Closantel has marked activity against liver flukes and can be used against multiple resistant strains of *H. contortus* in sheep and cattle as mentioned above. This drug is well tolerated at a dose rate of 10mg/kg. The mode of action appears to be due to the uncoupling of oxidative phosphorylation (Bossche et al., 1979), and it binds strongly to plasma proteins hence its activity against haematophagous parasites such as *H. contortus* and *F. hepatica* (Borgsteede et al., 2008). The mechanisms of action are resembles that of the BZs and results in reduced energy storage leading to starvation of the parasites.

1.4.5 Amino-acetonitrile derivatives (AADs), Monepantel

In 2009, a new chemical drug (monepantel) reached the market belonging to the amino-acetonitrile derivative (AAD) group (Ducray et al., 2008; Kaminsky et al., 2008a; Kaminsky et al., 2008b). AADs are a class of low molecular mass compounds and has more than 600 compounds which were synthesized and evaluated for anthelmintic activity (Kaminsky et al., 2008a). AADs have a broad spectrum of activity against gastrointestinal nematodes (Hosking et al., 2009) and have been shown to be effective against worms resistant to currently available broad-spectrum anthelmintic classes.

At a dose rate of 2.5 mg/kg body weight in sheep, monepantel is effective against a broad spectrum of the gastrointestinal nematode genera, including *Haemonchus*, *Teladorsagia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Chabertia* and *Oesophagostomum* (efficacy of monepantel was >95% on farms tested in New Zealand) (Mason et al., 2009). The AADs have been shown to have almost 100% efficacy against L_4 stages of five major nematode species and 90% to 100% efficacy against *Nematodirus spathiger, H. contortus, T. circumcincta* and *T. colubriformis* (Kaminsky et al., 2008a).

1.4.5.1 Mode of Action

The mechanism of action of the AADs is to cause hypercontraction of the body wall muscles of the parasites leading to paralysis, spasmodic contractions of the anterior portion of the pharynx and ultimately death. This is based on studies done with *C. elegans* (Kaminsky et al., 2008a). These compounds were also confirmed to have similar effects in adult *H. contortus.* It appears that in *C. elegans,* the AAD cause moulting defects and vacuolation in cells plus retardation of growth (Kaminsky et al., 2008a).

1.4.6 Paraherquamide

Paraherquamide (Yamazaki and Okuyama, 1981) and marcfortine A (Zinser et al., 2002) are both derived from the fermentation products of an oxindole alkaloid of fungal origin (*Penicillium paraherquei*). The anthelmintic activity of paraherquamide was identified using jirds infected with immature *T. colubriformis* (Ostlind et al., 1990). It has been documented that paraherquamide is more potent than the BZs, imidazothiazoles and tetrahydropyrimidines, but less potent than the MLs (Shoop et al., 1990). It is also known to work effectively against parasites resistant to the other broad-spectrum anthelmintics *(Shoop et al., 1990). As a potent anthelmintic, paraherquamide works effectively in sheep against adult H. contortus, T. colubriformis, T. axei, T. circumcincta* and *C. curticei,* including an IVM-resistant *H. contortus* and BZ/IVM-resistant *T. colubriformis* (Sargison et al., 2001; Shoop et al., 1990). However, low anthelmintic activity was shown against *Oe. columbianum.*

In calves, paraherquamide works effectively against *H. placei, O. ostertagi, T. axei, T. colubriformis, C. oncophora, N. helvetianus, Oe. radiatum* and *Dictyocaulus viviparus* at a dose rate of 1 to 4mg/kg (Shoop et al., 1992). On the other hand, this compound was reported to be ineffective in dogs against most nematode parasites from 0.5 to 2 mg/kg (Shoop et al., 1991).

Derquantel, or 2-desoxoparaherquamide, is a derivative of paraherquamide with an improved safety profile and is likely to be the first compound of this class to reach the market (Sutherland and Scott, 2010).

1.4.6.1 Mode of Action

Paraherquamide caused the paralysis of L_3 larvae of *H. contortus*, *T. circumcincta* and *T. colubriformis* in sheep (Gill and Lacey, 1993). Thompson et al. (1996) noted that paraherquamide may cause paralysis of parasitic nematodes in culture without an effect on ATP, and paraherquamide does not act as a metabolic poison. By using *in vitro* studies on *Ascaris suum*, three (*N*-, *L*- and *B-)* subtypes of cholinergic receptor were present and have effects on the body wall muscle in parasitic nematodes (Robertson et al., 2002). These studies proved that multiple receptor subtypes of the acetylcholine receptor (= ACh = primary excitatory transmitter in nematodes) and nAChRs (neuronal acetylcholine receptors) can be activated by different cholinergic anthelmintics including paraherquamide.

1.5. PHARMACOKINETICS OF ANTHELMINTICS IN GOATS

The actual efficacy of anthelmintics in goats has always been somewhat questionable as they are known to metabolise anthelmintics more rapidly than sheep as discussed in Section 1.1.2 (Swan and Gross, 1985). Consequently, dose rates of anthelmintics are uncertain for goats (Hall et al., 1981). Due to these reasons, it is believed that goats are effectively under-dosed when treated at sheep dose rates. As discussed above, goats are less likely to develop an effective immunity to gastrointestinal nematodes infections and thus more frequent treatments are required. This may be another reason that resistance appears in goats before sheep.

The relationships between the pharmacokinetic behaviour and the anthelmintic efficacy of oxfendazole against host species have been studied (Bogan et al., 1987; Sangster et al., 1991b). Gillham et al. (1985) reported that the plasma level of oxfendazole decreases rapidly in goats when compared to sheep. Controlled trials in goats using 5, 10 and 20mg/kg of oxfendazole have been conducted to improve the efficacy of this drug in goats as 5mg/kg given is the actual sheep dose rate (Sangster et al., 1991b). The authors reported that dosing goats with twice the sheep dose rate resulted in a similar peak plasma profile for, although the repetition of two or three doses at intervals of 12 to 24 hr appeared to be more effective in achieving a similar efficacy. Oxfendazole activity depends less on the peak concentration and more on the duration (Barragry, 1984).

For LEV, like oxfendazole, plasma levels were found to be lower and disappeared more rapidly in goats than in sheep (Gillham and Obendorf, 1985; Kettle et al., 1983). In comparison to the BZs, the activity of LEV is more likely to depend on absorption and resecretion (Arundel, 1983). The peak plasma levels and elimination half-life of LEV in goats are only 60% of those in sheep after oral administration of these two hosts with the same dose (Galtier et al., 1981). The authors also indicated that plasma clearance occurred 2 to 4 times faster in goats than sheep.

Morantel at a dose of 10mg/kg in goats and sheep was more effective against *Trichostrongylus* and *Teladorsagia* in the sheep than in the goats (Elliott, 1987) but this drug was still effective against *Haemonchus, Bunostomum* and *Oesophagostomum* in goats (Chandrasekharan et al., 1973). Therefore, these results have shown that the dose rate in goats is still uncertain. However, as this drug has a high safety index in sheep, the best way to achieve good efficacy may be by increasing the dose rate in goats.

For IVM, the bioavailability of this drug in goats is less than in sheep and cattle (Alvinerie et al., 1993; Gonzalez et al., 2006). From anecdotal reports, doubling the dose rate is recommended for goats as this drug has a wide margin safety. Baynes et al. (2000) suggested extending the withholding time for milk from lactating goats to nine days whereas for meat goats to 14 days.

1.6. DEFINITIONS OF RESISTANCE

Anthelmintic resistance is the ability of the parasites to survive dosages of drug that would normally kill the same species of parasites, and at the different stages of larval development. It is also defined as: “Greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and is heritable” (Prichard et al., 1980). Resistance to each group of anthelmintics is controlled by different genes, meaning that resistance develops to each class of drugs individually. It will arise when there are genetic variances in a population and a selection of resistant genotypes is produced. There are several different terms of anthelmintic resistance which are stated below (Waller, 1985):

Side resistance: occurs when nematodes are resistant to one class of anthelmintic, or drugs with the same mechanism of action. This is observed in the BZ group of compounds; although the level of resistance to the different BZs might be different.

Cross resistance: occurs when nematodes are resistant to anthelmintics that is chemically unrelated. For example; LEV-resistant nematodes can be cross-resistant to morantel due to the similarities of their mechanisms of action.

Multiple resistance: occurs when nematodes are resistant to at least two major classes of anthelmintics with different mechanisms of action family, or when more than one species of nematode is resistant to the same anthelmintic.

i) Multidrug resistance is when one nematodes becomes resistant to more than one class of anthelmintics resulting either from either selection occuring in parallel or by cross resistance.

ii) Multigeneric resistance is when different genera of nematodes become resistant to one or more classes of anthelmintics. In extreme cases, multigeneric and multidrug resistance can occur at the same time.

1.7. THE INCIDENCE OF ANTHELMINTIC RESISTANCE BETWEEN SHEEP AND GOATS IN NEW ZEALAND

Anthelmintic resistance in gastrointestinal nematodes now poses potentially significant problems to the livestock industry in New Zealand. Anthelmintic resistance has emerged faster in sheep and goats, as opposed to cattle. Intensive grazing of sheep and goats, often with too few cattle, has favoured the development and spread of resistant populations within these two hosts as well as the country. To date, the situation has worsened as multiple anthelmintic resistance has been reported more frequently both here and overseas.

Table 1.1: Milestones of sheep and goat resistance cases in New Zealand.

Year	Host	Anthelmintic	Parasites	Results	Reference
1979-80	Sheep	BZ	*H. contortus*	i) First case of BZ resistance - highly resistant to thiabendazole and albendazole	(Vlassoff and Kettle, 1980)
1980-81	Sheep (54 farms)	BZ	*Haemonchus, Trichostrongylus*	BZ resistance on four farms (87-93% reduction in a FECRT) and resistance to LEV on three farms (72-89% reduction in a FECRT)	(Kettle et al., 1981)
1980-81	Sheep (43 farms)	BZ, LEV	*H. contortus, T. circumcincta, T. colubriformis*	100% BZ/LEV on 32 farms, <100% BZ on one farm and <100% LEV on seven farms	(Kettle et al., 1982)
1983	Goats (47 farms)	BZ, LEV, Morantel	*Haemonchus, Trichostrongylus Teladorsagia*	BZ resistance on 17 farms, LEV or morantel resistance on two farms and LEV+morantel resistance on 18 farms	(Kettle et al., 1983)
1988	Goats	BZ, IVM, Morantel	*T. circumcincta, T. trifurcata, Trichostrongylus*	i) Multiple anthelmintic resistance ii) First cases of IVM resistance in *T. circumcincta*	(Watson and Hosking, 1990)
1986-88	Sheep	BZ	*Haemonchus, Teladorsagia, Nematodirus, Trichostrongylus, Strongyloides, Oe. venulosum*	First cases of BZ resistance in several nematode parasites on one single sheep farm	(McKenna, 1989)
1990	Goats	IVM	*T. circumcincta*	IVM efficacy was 87% against *T. circumcincta*	(Badger and McKenna, 1990)
1986-92	Sheep	BZ, LEV, BZ+LEV	strongyle genera, *Nematodirus*	MAF laboratory data showed that 63% of requests for FECRT in sheep	(McKenna, 1994)

				showed that resistance was present and 74% resistance to BZ, 23% to LEV and 30% to BZ+LEV	
1993	Ovine	BZ, LEV, BZ+LEV	*Nematodirus*	MAF laboratory data showed that cases requested for FECRT of North and South Island indicated that BZ resistance on 61% and 72%, LEV resistance in 29% and 29% and BZ+LEV resistance in 11% and 22%	(McKenna et al., 1995)
1993	Goats	Moxidectin, IVM	*Teladorsagia*	IVM and moxidectin failed to reduce FECs - probably due to the high levels of resistance of *Teladorsagia*	(Leathwick, 1995)
1992-94	Ovine	BZ, BZ+LEV, LEV/morantel	Most involved: *Trichostrongylus, Teladorsagia, Nematodirus* Less involved: *Oesophagostomum, Chabertia, Cooperia, Haemonchus*	i) Resistance in a single nematode genus occurred in 45% of cases in *Trichostrongylus* (52%), *Teladorsagia* (17%) and *Nematodirus* (11%) ii) Anthelmintic resistance involving only *Haemonchus* was found in just 3 cases	(McKenna et al., 1995)
1996-97	Sheep	BZ, LEV, BZ+LEV	n.a	MAF laboratory data showed that 68% of requests for FECRT showed BZ resistance, 42% LEV resistance and 39% BZ+LEV	(McKenna, 1998a)
1999	Goats	IVM	*T. colubriformis*	First case of ivermectin resistance in *T. colubriformis* which was also resistant to BZs and possibly to LEV	(Gopal et al., 1999)

1999-00	Sheep	IVM	*T. circumcincta*	Ivermectin resistance in sheep confirmed	(Leathwick et al., 2000)
2001	Sheep	Moxidectin, IVM	*H. contortus*, *T. circumcincta*, *T. axei*	i) First case of ML resistance in *H. contortus* in sheep ii) First case of resistance in more than one parasite species at a time	(Vickers et al., 2001)
2006	Sheep	BZ, LEV, BZ+LEV, IVM	*Trichostrongylus*, *Teladorsagia*, *Nematodirus*	Reported high prevalence of multiple resistance on sheep farms in New Zealand	(Waghorn et al., 2006)
2006	sheep	i) IVM+LEV+ albendazole ii) abamectin+ LEV+ oxfendazole	*H. contortus*, *T. circumcincta*, *T. axei*	IVM+LEV+albendazole was reported highly effective against these three parasites species whereas not effective for abamectin+LEV+oxfendazole	(Wrigley et al., 2006)
2008	sheep	i) IVM ii) BZ+LEV	*H. contortus*, *T. circumcincta*, *T. colubriformis*	i) Multiple, multi-generic anthelmintic resistance was confirmed on a sheep farm ii) First case of IVM resistance in *T. colubriformis*	(Sutherland et al., 2008)

n.a: not applicable

1.8. GENETICS OF RESISTANCE IN NEMATODES TO DIFFERENT ANTHELMINTICS

Anthelmintic resistance arises when there is a change in the susceptibility of the nematode population with continued use of an anthelmintic. When a drench is used for the first time, the resistance gene or genes may already exist within the populations of individual nematode species. Genetic inheritance influences the rate of development of resistance, with resistance coded by dominant genes developing faster than that coded by recessive genes (Barger, 1997). The resistance genes can be present in the population already at a low frequency (pre-adaptive) even before the drug is used for the first time, or arise later by mutation and can also enter a population by migration or gene flow (Silvestre and Humbert, 2002).

As anthelmintic resistance develops further in the parasite population over subsequent generations, the predominant genotype changes from the naïve, susceptible population with rare heterozygotes, through the intermediate phase of mainly heterozygotes, to the final phase where resistance has become fixed in the population. If resistance genes are dominant, heterozygotes will survive anthelmintic treatment as well as homozygotes and resistance can arise very rapidly.

1.8.1.1 Benzimidazoles

Research into BZ resistance in *H. contortus* identified genetic changes of β-tubulin encoding genes (Prichard, 1970). Further work on fungi identified a mutation in β-tubulin that correlated with the degree of resistance to this drug class (Davidse and Flach, 1977). Differences in the binding characteristic for BZ of purified β-tubulin from resistant and susceptible parasites were detected *in vitro* (Lubega and Prichard, 1991). It was suggested that by changing the beta-tubulin amino acid sequence at only one position, codon position 200, BZ resistance could be conferred (Kwa et al., 1995; Kwa et al., 1994). This may not however be the only mechanism operating in cases of BZ resistance and the mechanisms may differ between nematode species and between nematodes of the same species with different levels of resistance (Prichard, 2001).

Studies in *H. contortus* and *T. colubriformis* have demonstrated that BZ resistance is an incomplete recessive trait, which appears to involve the selection of two or more

independent genes (Dobson et al., 1996; Roos, 1997). The mechanisms which are currently thought to be involved in resistance to BZs include changes in both isotype 1 and isotype 2 β-tubulins that result in reduced affinities for the BZs (single nucleotide changes at codon 200 and potentially codons 167 and 198 as well) and isotype 2 genes which are eliminated from highly resistant worms (Conder and Campbell, 1995; Drogemuller et al., 2004; Ghisi et al., 2007).

1.8.1.2 Levamisole

The rate of development of resistance to LEV commonly appears to be slower in *H. contortus,* in which this drug remains effective even against BZ- and avermectin-resistant isolates. Early work in the 1980s suggested that LEV resistance in *T. colubriformis* was likely to be controlled by a single dominant gene (Waller et al., 1985). Further work indicated that LEV resistance in *T. colubriformis* was inherited by a single and sex-linked recessive gene (Martin and McKenzie, 1990). In contrast, other studies have shown that LEV resistance in *H. contortus* is due to a recessive, autosomal trait that is not sex-linked (Dobson et al., 1996). Sangster et al. (1998) suggested that resistance for LEV in *H. contortus* is not a completely recessive characteristic and is likely to involve more than one gene. Earlier studies on the development of LEV resistance in *H. contortus* also suggested a polygenic inheritance (Sangster et al., 1991a). LEV binds to nicotinic acetylcholine receptors, but thus far the physiological/pharmacological basis of resistance remains obscure.

1.8.1.3 Macrocyclic Lactone

A few studies have documented that IVM resistance in *H. contortus* is inherited as a dominant autosomal trait. However differences were seen between larvae and adults, since in adult worms resistance was influenced by sex (Dobson et al., 1996; Le Jambre et al., 2000). Other genetic evidence suggests however that ML resistance is polygenic. Field studies on *T. colubriformis* derived from goats reported that inheritance of IVM resistance was as a partially dominant trait and was probably not under the control of single gene (Gill and Lacey, 1998; Gopal et al., 1999). There are suggestions that selection in the field differs from experimental selection using lower doses of MLs. Thus ML resistance in the field is less likely to be polygenic

Studies in molecular genetics of ML-resistant *H. contortus* have shown differences in the transmembrane transporter P-glycoprotein (Njue et al., 2004). The action of P-glycoprotein is to reduce the concentration of toxic molecules such as MLs in the tissues of nematodes. Drogemuller et al. (2004) reported that repeated treatment with a ML (IVM) resulted in the selection of specific P-glycoprotein alleles in *H. contortus* and *Onchocerca volvulus.*

Interestingly, there appears to be an association between BZ resistance and ML resistance. Studies with *H. contortus* and *Onchocerca volvulus* indicated that there is a possible link between the beta-tubulin-codon-200 SNP (conferring BZ resistance) and ML resistance (Eng et al., 2006). Results showed that the proportion of worms with the codon 200 changes was significantly increased within IVM-selected nematode populations.

1.8.2 Reversion

The term 'reversion' refers to a return towards susceptibility of a resistant nematode when the selecting drug is withdrawn. Reversion can occur only if the resistant organisms are in some way less fit to survive than their more susceptible contemporaries. The occurrence of reversion to the susceptible state may differ between species resistant to the same anthelmintics, and between populations within species resistant to different anthelmintics, as genetic changes associated with resistance can differ between nematode species. Thus far the reversion of resistant worms to a state of susceptibility has not been reported.

1.9. DIAGNOSIS OF RESISTANCE

Numerous different assays and techniques have been used to detect anthelmintic resistance in populations of nematodes (Johansen and Waller, 1989). Several *in vivo* and *in vitro* tests can be used to detect anthelmintic resistance and work is ongoing to standardize and validate these tests.

1.9.1 *In Vivo* Tests

1.9.1.1 Faecal egg count reduction test

The faecal egg count reduction test (FECRT) has become the principle means of diagnosing anthelmintic resistance under field conditions. It does not require the

sacrifice of experimental animals or the use of elaborate equipment to conduct the test. This procedure compares the pre-treatment parasite levels (as indicated by the number of eggs shed in faeces) with the levels remaining after treatment (Presidente, 1985). Use of an untreated control group is recommended to monitor any changes in nematode egg counts that might occur during the test period that might otherwise lead to an under- or over-estimation of efficacy. The guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) have recommended the use of 10 animals per treatment group, but at least six animals are adequate for evaluating anthelmintic efficacy (Wood et al., 1995). Resistance is generally defined as being present when the reduction in faecal egg count (FEC) is < 95% (Coles et al., 2006). This assumes that efficacy was originally well in excess of this figure.

Since counts of strongylid eggs do not distinguish which species of parasite are present, larval cultures of the control and treatment groups are essential to identify the nematode that is resistant to the anthelmintic tested to at least the genus level.

The timing of when the post-treatment faecal sample is collected is important. If samples are collected late, then worms ingested after the treatment was given may have had time to mature and may now themselves be producing eggs. This is particularly true for parasites with short pre-patent periods e.g. *Cooperia* and thus samples should be collected no later than 2 weeks post-treatment. Conversely, if samples are collected too quickly after treatment then the phenomenon of suppression of egg output may lead to an over-estimation of efficacy. This occurs when the anthelmintic suppresses the egg output of the females but did not remove them. This suppression of egg output is usually temporary.

LEV suppresses egg output and post-treatment samples should be taken at least seven days after treatment, particularly in *T. circumcincta* and *H. contortus* (Grimshaw et al., 1996). In contrast, other studies have documented that in IVM-resistant *T. circumcincta* (Jackson and Coop, 1995) and *H. contortus* (Le Jambre, 1993) the suppression of egg output following IVM treatment may be longer and the post-treatment sample collected at least 14 days after treatment

1.9.1.2 Controlled slaughter test

To treat animals and then slaughter them to directly count the number of worms surviving treatment is considered the "gold standard" test for anthelmintic resistance but is severely limited by the expense of conducting it, not least because animals have to be sacrificed so that worm counts can be performed. The controlled slaughter test is conducted with adequately infected groups of animals that are then treated with anthelmintic. After 10 to 14 days post-treatment all animals are killed, including untreated controls, and all abomasa, small and large intestines are collected. The parasites in a 5% or 10% aliquot from the abomasal, small and large intestinal contents and washings, and from digests of the abomasal mucosa are identified and counted. The International Harmonisation of Anthelmintic Efficacy guidelines have indicated that aliquot size should be at least 2% (Vercruysse et al., 2001). Wood et al. (1995) noted that efficacy is calculated as the difference between the geometric mean worm counts in the untreated control group and the treatment group, expressed as a percentage of the geometric mean worm counts in the control group. The controlled slaughter test is the most expensive and is also time consuming and thus it is not recommended for the routine diagnosis of anthelmintic resistance.

Initial detection of anthelmintic resistance status in a field situation may include both the FECRT and a controlled slaughter test in the animals (Gopal et al., 1999; Pomroy and Whelan, 1993; Vickers et al., 2001). Both tests are conducted to better confirm the presence and extent of the resistance, since the FECRT may not be sufficient on its own. Kahn et al. (2001) described the limitations of the FECRT: "There are a number of disadvantages associated with FEC and larval differentiation, including considerable variation in FEC between faecal samples taken from the same sheep, and variation between nematode species in their development during culture. Furthermore, FEC are unable to indicate a worm burden until egg laying commences at three to four weeks after infection, by which time the worms are well established and the host may already be suffering adverse effects.

1.9.2 *In Vitro* Tests

Several attempts have been made to establish different *in vitro* tests for the detection of anthelmintic resistance as described in the following sections.

1.9.2.1 Egg Hatch Test

The egg hatch test (EHT) was principally developed for use with the BZs. In the EHT undeveloped eggs are recovered from pooled fresh faecal samples and are incubated in serially increasing concentrations of anthelmintic. All eggs and larvae are counted as dead, embryonated or hatched L_1 at each concentration and the drug concentration required to inhibit hatching of 50% of nematode eggs (ED_{50}) is ascertained (Coles et al., 2006; Le Jambre, 1976). The percentage of eggs counted is corrected for the natural mortality of those hatched in control conditions (i.e. no anthelmintic present), and the percentage of unhatched eggs or those which die, is plotted against each different drug concentration (Boersema, 1983). The estimation of the ED_{50} values on a logarithmic scale can be calculated using a variety of software applications.

The EHT has also been used to detect resistance to LEV in sheep and goat nematodes (Coles et al., 2006; Dobson et al., 1986). LEV has to be added for a short time, one hour prior to commencement of hatching. This requires a subjective appraisal of the eggs to determine whether hatching is imminent (the eggs become transparent and larvae can be seen actively moving within the egg envelope), but it can be hard to predict hatching accurately.

The advantage of the EHT is that it can still be applied even if the FEC is low as long as the eggs are undeveloped. However, several difficulties with the procedures have been identified, for example, the method of drug preparation, the sequence of sample preparation and setup and the storage of eggs post collection (Hunt and Taylor, 1989).

1.9.2.2 Larval paralysis and motility assay

The larval paralysis assay was originally developed to detect resistance to LEV and morantel (Martin and Le Jambre, 1979). It is based on the determination of the percentage of L_3 that are paralyzed after about 24 hr in different concentrations of LEV and morantel. The larvae are considered paralyzed when no movements are observed for 5 seconds. A few studies have been conducted and an evaluation was made with different parameters; using susceptible and resistance *O. ostertagi*, incubation period (24, 48 and 72 hr), incubation temperature (20 or 25°C) and observation period of the larvae (5 or 15 seconds). Boersema et al. (1983) reported that at high concentration of LEV, reversibility of the paralysis of the larvae may occur. This result is also

supported by Barton et al. (1983) who suggested that when lower concentrations of LEV were used, it is more effective in paralysing the L_3 stages than when higher concentrations are used. Issues also include the length of the period for observing paralysed larvae (Boersema, 1983). On the other hand, Geerts et al. (1989) reported no difficulties in conducting larval motility test and indicates that the parameters as stated above had no statistical influence on the test results.

Studies in *H. contortus* have documented the use of a larval motility assay to detect IVM-resistance (Gill et al., 1991). After a 24 hr incubation of L_3 stages on an IVM-containing agar matrix the larval motility was determined by counting sinusoidal movement of larvae. A 50% inhibition of motility (LP_{50}) was used in detecting the efficacy of IVM to the species tested. Paraherquamide is also an inhibitor of larval motility in *H. contortus, T. colubriformis* and *T. circumcincta* (Gill and Lacey, 1993). The authors indicate that LP_{50} values for *H. contortus, T. colubriformis* and *T. circumcincta* were 2.7, 0.058 and 0.033µg/ml respectively. It appears that IVM-resistant isolates of *H. contortus* were significantly more sensitive to paraherquamide in inducing paralysis than IVM-susceptible isolates of *H. contortus* (Gill and Lacey, 1993).

1.9.2.2 Adult development assay

An adult development assay for use in detecting BZ resistance in nematode parasites has been reported. In one study, *H. contortus* was cultured all the way through to the adult egg-laying stages *in vitro* (Stringfellow, 1984, 1988). Nevertheless, this test was mainly developed for research purposes, requires expertise in culture techniques and has not generally been replicated successfully.

1.9.2.3 Tubulin binding assay

This assay was developed to detect resistance to the BZs (Lacey and Snowdon, 1988). BZ resistance appears to be associated with a reduced affinity of nematode tubulin for the anthelmintics. The test involves the incubation of a crude tubulin extract from infective larvae with a tritiated benzimidazole until equilibrium is reached. After the incubation, the unbound drug is removed by using charcoal. The tritium-benzimidazole-tubulin complex is counted by liquid scintillation spectrophotometry. The tubulin binding assay was used to identify susceptible and resistant isolates in a

mixed population of *H. contortus, T. colubriformis* and *T. circumcincta* by showing that tubulin extracts from susceptible parasites bind BZ substantially more strongly than resistant parasites.

The test is claimed to be accurate, robust and sensitive to minor changes of the level of resistance in the parasite populations. The disadvantages of this test are that it requires large numbers of larvae, expensive laboratory equipment and handling of radioactive reagents.

1.9.2.4 Larval Development Assay

The larval development assay (LDA) is commonly used for the detection of resistance to the BZ, LEV and IVM in sheep and goat gastrointestinal nematode parasites (Coles et al., 2006) namely for *H. contortus, T. colubriformis* and *T. circumcincta* (Lacey et al., 1990). It was originally described in the early 1980s (Coles, 1988; Ibarra and Jenkins, 1984) and subsequently improved by others (Coles et al., 2006; Gill et al., 1995; Hubert and Kerboeuf, 1992).

To date, one LDA, DrenchRite® has been successfully commercialised and was introduced as a tool for detecting anthelmintic resistance in the field. It was developed at CSIRO's McMaster Laboratory (NSW, Australia) and has been made available commercially by Horizon Technology Pty Limited (DrenchRite® User Manual). The DrenchRite® assay has been used most commonly in Australia since it was released in 1995 (Lloyd, 1998; Palmer et al., 1998). As well as its use in sheep, this assay has also been successful for detecting anthelmintic resistance in goat parasites (Howell et al., 2008; Kaplan et al., 2007) and horse parasites in U.S.A (Tandon and Kaplan, 2004; Young et al., 1999).

The principle of this assay is to isolate nematode eggs from a pooled faecal sample and dispense the eggs into wells containing agar with serially increasing drug concentrations. A nutrient solution is then added 24 hours later after approximately 80% of eggs have hatched. The eggs are cultured to third stage infective larvae in an incubator. All eggs and larvae ($L_1/L_2/L_3$) are then counted. An EC_{50} value (anthelmintic concentration where L_3 development in 50% of the larvae is blocked) is calculated against the number of larvae in the control wells and a dose response curve

can be plotted. By comparing the EC_{50} values for the tested isolates (resistant versus susceptible isolates), a resistance ratio can be calculated.

In early studies, good results were reported for BZs and LEV, however, in subsequent work they were unable to demonstrate a complete IVM dose-response (Coles, 1988). Previous studies using the DrenchRite® assay had shown that when this assay was used to detect resistance to BZ, LEV and a combination of BZ and LEV especially high prevalence of resistance to these drugs was observed on Australian farms (Overend et al., 1994; Palmer et al., 1998). However, for IVM resistance, this assay was found to be insensitive in detecting resistance on sheep farms especially with *T. circumcincta* (Palmer et al., 1998).

The limitations of the DrenchRite® assay are that it is a relatively expensive tool, needs expertise to conduct the assay and is also less sensitive for detecting resistance for *Oesophagostomum columbianum* and *Chabertia ovina* (Dobson et al., 1998). Various authors suggest more tests should be done to test the DrenchRite® sensitivity on these parasites as compared to *H. contortus*, *T. colubriformis* and *T. circumcincta*. The volume of the nutritive medium with the right incubation time also influences the development of the eggs. The issue of oxygen levels in the wells can also limit development of the larvae. One advantage of this assay is that it can provide quick results with minimal effort by the farmers.

Table 1.2: Milestones of the DrenchRite® LDA studies in sheep and goats.

Country	Results	Reference
Australia	Sensitivity in detecting resistance to BZ, LEV, BZ+LEV in *Teladorsagia*, *Trichostrongylus* and *H. contortus.* Insensitivity in detecting resistance to IVM in *Teladorsagia.* Highest critical well reported (Row G/H): 9.0 for *Teladorsagia;* 8.0 for *Trichostrongylus*	(Palmer et al., 1998)
Australia	Sensitivity in detecting resistance to BZ, LEV, BZ+LEV in *Teladorsagia*, *Trichostrongylus* and *H. contortus.* Insensitivity in detecting resistance to IVM in *Teladorsagia.* Highest critical well reported (Row G/H): 10.5 for *Teladorsagia;* 10.5 for *Trichostrongylus;* 6.5 for *H. contortus*	(Lloyd, 1998)
Australia	Sensitivity in detecting resistance to BZ, LEV, BZ+LEV in *Teladorsagia*, *Trichostrongylus* and *H. contortus.* No samples were tested in IVM. Highest critical well reported (Row G/H):9.5 for *Trichostrongylus.* Insensitivity in detecting resistance to *Oe. columbianum* and *C. ovina* in anthelmintic tested	(Dobson et al., 1998)
U.S.A	Sensitivity in detecting resistance to BZ, LEV, BZ+LEV, Moxidectin in gastrointestinal nematode parasites	(Terrill et al., 2001)
U.S.A	Sensitivity in detecting resistance to IVM and Moxidectin in *T. colubriformis* and *H. contortus.* Row G=IVM monosaccharide (IVM-1) Row H= IVM aglycone (IVM-2) Widespread of Moxidectin resistance in this country	(Kaplan et al., 2007)
U.S.A	Sensitivity in detecting resistance to BZ, LEV, IVM, Moxidectin in *T. colubriformis* and *H. contortus.* Highest critical well reported (IVM): 8.5 for sheep*;* 12.0 for goats Highest critical well reported (Moxidectin): 12.0 for sheep*;* 12.0 for goats	(Howell et al., 2008)

CHAPTER TWO

MULTIPLE RESISTANCE STATUS OF A FIELD STRAIN DERIVED FROM GOAT OF *Teladorsagia, Trichostrongylus* AND *Haemonchus* IN SHEEPS TO SINGLE AND DOUBLE DOSE OF COMBINATION OF OXFENDAZOLE, LEVAMISOLE AND ABAMECTIN

2.1 INTRODUCTION

Anthelmintic resistance in gastrointestinal nematodes now poses problems to small ruminant farmers in New Zealand. Documented reports of resistance to anthelmintics indicate a definite upward trend in prevalence and severity in recent years both in New Zealand (Leathwick et al., 2001) and worldwide (Kaplan, 2004). Following the first report in New Zealand of BZ resistance in *H. contortus* in 1980 (Vlassoff and Kettle, 1980) and later on in other reports (Kettle et al., 1982; Kettle et al., 1981), surveys were conducted in both goats (Kettle et al., 1983) and sheep (Kettle et al., 1982) which indicated resistance was already widespread, particularly to BZs but also to LEV and involved all common genera including *Haemonchus*, *Trichostrongylus* and *Teladorsagia*. ML-resistance was first reported in *T. circumcincta* in goats in 1988 (Watson and Hosking, 1990) and in sheep in 1999 (Mason et al., 1999). The first report of ML-resistance in *T. colubriformis* in New Zealand was again from goats (Gopal et al., 1999) which also showed evidence of BZ resistance. The first report has been in sheep of IVM-resistance in *T. colubriformis* was about 10 years later (Sutherland et al., 2008). ML-resistance has also been reported in *H. contortus* (Vickers et al., 2001).

A limited number of reports of anthelmintic resistance in goats from overseas were documented during the 1980s including from Australia (Barton et al., 1985), the U.S.A (Uhlinger et al., 1988), France (Kerboeuf and Hubert, 1985) and the United Kingdom (Scott et al., 1989). Regrettably, anthelmintic resistance studies involving sheep have received more attention and there is little information about the current situation for goats. More recently, a series of reports have indicated that severe levels of anthelmintic resistance now occur in goat parasites across the South-eastern United States (Howell et al., 2008; Kaplan et al., 2007; Mortensen et al., 2003; Zajac and Gipson, 2000) especially in *H. contortus* and *T. colubriformis* and to all currently

available anthelmintics. In New Zealand, the situation in goats hasn't been assessed for several years but there is considerable concern that anthelmintic resistance is now common in nematodes of goats.

There is a broader concern as goats and sheep share the same helminth parasite fauna and isolation of resistant parasites from goats augurs poorly for the sustainability of anthelmintic use within the sheep industry. Moreover, the actual efficacy of anthelmintics in goats has always been somewhat questionable as they are known to metabolise anthelmintics more rapidly than sheep and hence the appropriate dose rates are uncertain. Studies have shown that LEV has inadequate efficacy in goats at the effective sheep dose rate (Gillham and Obendorf, 1985). Other studies also indicated that the recommended dose rate of oxfendazole in goats should be double that recommended for sheep (Sangster et al., 1991b). The aim of this present study was to confirm the anthelmintic resistance status of suspected highly-resistant parasites from goats by infecting and then treating young sheep. The present paper also describes the use of a larval development assay (LDA, DrenchRite®) to compare results achieved with this assay to the actual worm count reduction. The sheep were infected with field-derived larvae from goats suspected of being infected with multiple resistant *T. circumcincta*, *T. colubriformis* and *H. contortus* and subsequently treated with the recommended dose or double dose of a triple combination anthelmintic comprising a BZ, LEV and abamectin.

2.2 MATERIALS AND METHODS

2.2.1 Herd History

A dairy goat farm grazing about 20 to 30 goats on 4ha had a history of anthelmintic failure which had developed progressively over many years. Within the last 1 to 2 years goats have been treated with a combination of moxidectin, levamisole and fenbendazole given at the same time. However, goats generally still had strongylid eggs in their faeces after treatment. Even treating with moxidectin at 0.4mg/kg per os and treating with fenbendazole at 5mg/kg for 3 to 5 days only marginally improved the apparent efficacy. Coproculture indicated that *Teladorsagia* and *Trichostrongylus* were the genera of nematodes surviving these treatments. Sporadic faecal samples obtained from this herd commonly had egg counts >1000 eggs/g.

2.2.2 Experimental Design and animals

Source larvae: T. circumcinta, T. colubriformis and *H. contortus* and *Oesophagostomum /Chabertia* infective larvae which were obtained from infected animals at different times on this farm were pooled as the source of larvae to infect the experimental sheep. These larvae were stored at 10°C. The susceptible isolate strains of *T. circumcincta* and *H. contortus* were laboratory isolates courtesy of H. Simpson (Massey University), and the *T. colubriformis* was a laboratory isolate courtesy of AgResearch, Grasslands Research Centre, Palmerston North, New Zealand.

Lambs: 19 lambs were purchased, effectively treated with an anthelmintic and housed indoors in pens. They were fed with ad libitum access to lucerne chaff and water throughout the study. The study commenced 12 days after housing.

Experimental Design: Lambs were all infected on Day 0 by stomach tube with a mixed culture estimated to be 3780 *H. contortus*, 1260 *Teladorsagia* and 3192 *Trichostrongylus* and 168 *Oesophagostomum/Chabertia*. Animals were ranked by egg counts estimated on Day 22, divided into groups of 3 and within these groups randomly allocated to one of 3 treatment groups. The spare animal with the lowest egg count was allocated to Group 3. Group 1 was the untreated control group. On Day 22, Group 2 animals were treated with a recommended sheep dose of a combination formulation of abamectin (0.2mg/kg) + oxfendazole (4.5mg/kg) + LEV (8mg/kg) ("Matrix Oral Drench for Sheep"®, Ancare, New Zealand) given per os and Group 3 animals were treated with a 2X sheep dose of the same combination comprising abamectin (0.4mg/kg) + oxfendazole (9mg/kg) + LEV (16mg/kg). Each animal in Group 2 and Group 3 were weighed (Micropower® 2000) and treated to their particular weight. On Day 36 (14 days after treatment) all sheep were killed for total worm counts. Faecal egg counts were estimated on Day 22, 24, 27, 29, 31 and 34.

2.2.3 Parasitology Techniques

Faecal egg counts

Faecal egg counts (FECs) were estimated with a modified McMaster technique where 2g faeces were mixed with 28mls saturated NaCl (specific gravity 1.2), then passed through a coarse sieve and the retentate discarded. After effective mixing of the filtrate, the two chambers of a McMaster slide were filled where the volume under each

grid was 0.15ml. Each egg counted within the grids of both chambers represents 50eggs/g. A full SOP for this procedure is given in Appendix 2.1. A simple flotation method was also conducted on animals with zero egg counts with the McMaster technique. A full SOP for this procedure is given in Appendix 2.2.

Faecal larval cultures

Faecal larval cultures were undertaken after every sampling for FEC. Faecal samples from each group on each sampling occasions were pooled, mixed with vermiculite and cultured at 20°C for at least 14 days. Infective L_3 larvae were extracted by baermannisation, and the first 100 L_3 identified to their genera and counted. A full SOP for this procedure is given in Appendix 2.3. For each group on each sampling occasion, the proportion of different genera identified was then applied to the mean FEC to estimate the number of eggs for each genus.

Larval development assay (LDA)

DrenchRite® 96 well plates (Microbial Screening Technologies, New South Wales, Australia), a commercially available test developed to estimate anthelmintic efficacy, were used to perform the LDA. These have duplicate rows testing: BZ, LEV, combination of BZ+LEV and a single row of each of 2 different ivermectin (IVM) analogues (IVM-1 and IVM-2). The range of drug concentrations for some of the rows were detailed by (Tandon and Kaplan, 2004) and for BZ (μM) are as follows: 0.010, 0.020, 0.040, 0.078, 0.156, 0.131, 0.625, 1.250, 2.500, 5.000 and 10.000; for LEV (μM): 0.195, 0.390, 0.780, 1.560, 3.125, 6.250, 12.560 then four replicates of 25.000; 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, 7.800, 15.600, 31.250, 62.500, 125.000, 250.000, 500.000 and 1000.000. Column 1 in each row serves as a control well without anthelmintic. In DrenchRite® LDA IVM-1 is known to be ivermectin monosaccharide and IVM-2 is known to be ivermectin aglycone (Kaplan et al., 2007).

The LDA was conducted on Day 23 for Group 1 (control) and Day 35 for Group 2 (treated) (Kaplan et al., 2007). The nematode eggs were isolated from pooled faeces of each group by washing faeces through a 60μm sieve and retaining eggs on a 20μm sieve before centrifugation in sugar gradients. 60 eggs were dispensed per well followed by an additional 40μl of nutrient solution 24 hr later containing yeast,

Escherichia coli and amphotericin B. The plate was incubated at 25°C for 13 to 14 days to grow eggs through to infective larvae (Gill et al., 1995). Larvae from each well were then put onto a slide followed by one drop of Lugol's iodine to kill them and each larva was subsequently counted and identified at 100X magnification. The proportion of L_3 developing corrected for the number in the mean of the control wells was plotted against the Log_{10} of the anthelmintic concentrations for BZ, LEV, IVM-1 and IVM-2. The software package Prism (version 5.00 GraphPad Software, Inc., U.S.A) was used to fit a curve to estimate the EC_{50} and EC_{90} values. EC_{50} and EC_{90} values are defined as the concentration of anthelmintic at which 50%/90% of the eggs did not develop to L_3. A resistance ratio (RR) could then be calculated by dividing the EC_{50} and EC_{90} values by the respective value for the susceptible isolate. The Coefficient of Determination (R^2) value could be estimated for each fitted curve. The critical well was determined as the well where only 50% of the L_3 developed from that genus. If this occurred between wells then the critical well was defined as the ½ well between these two. Efficacy percentage for BZ, LEV, BZ+LEV combination drugs were estimated using the guidelines supplied in the DrenchRite® User Manual (see Appendix 2.6). For IVM-1 and IVM-2, the DrenchRite® User Manual supplied a table showing the range of critical wells for a susceptible species of *H. contortus, T. circumcincta* and *T. colubriformis* but not a table of estimated efficacy values. To further interpret data for all anthelmintics the mean percentage of L_3 developing in Wells 9 to 12 compared to the control wells were also calculated. These 4 wells contain the highest concentration of anthelmintic.

Total worm counts

Organs were removed immediately after slaughter and stored at -20°C until processed. Each organ was thawed, separated, opened and washed repeatedly for worm recovery. A 10% aliquot was taken of the abomasal contents, small intestinal contents and a 50% aliquot of the contents was removed from the large intestine. The abomasums were digested in 600mls water, 2.5 g pepsin A powder (BDH®) and 10mls concentrated HCL. Prior to counting, all the abomasal, small intestinal and pepsin digest contents were passed through a 38μm sieve while the large intestinal contents were passed through a 106μm sieve. All adult worms present were identified to genus. Up to 50

male worms (if available) were identified to species. The proportion of species identified was used to allocate all worms in a genus to a species.

All animal manipulations were approved by the Massey University Animal Ethics Committee (MUAEC 106/08).

Statistical analysis

The reduction in faecal egg counts and worm counts (WC) were estimated for each genus using the following equations (Presidente, 1985);

$$\text{Reduction (\%)} = \frac{\text{FEC}_{\text{(control)}} - \text{FEC}_{\text{(treatment group)}}}{\text{FEC}_{\text{(control)}}} \quad \text{or} \quad \frac{\text{WC}_{\text{(control)}} - \text{WC}_{\text{(treatment group)}}}{\text{WC}_{\text{(control)}}}$$

Statistical analysis was performed using the computer program STATISTIX® 8.0 (Analytical Software, Tallahassee, U.S.A). Faecal egg counts and worm burdens were compared using Kruskal-Wallis non-parametric analysis of variance with results applied at the 5% level of significance.

2.3 RESULTS

Results of the arithmetic mean FECs on Day 34 (12 days post treatment) and the mean numbers of identified worms found in the three treatment groups as well as the significance of the Kruskal-Wallis statistic test values are presented in Table 2.1. Results of FEC on other occasions are shown in Appendix 2.8.

2.3.1 Total Worm Counts

In this study worm counts post-treatment showed that all animals were infected with *T. circumcincta* and *T. colubriformis* except for Group 3 where only 3 lambs were infected with *T. circumcincta*. These data show that an abamectin+oxfendazole+LEV combination drench at the rate recommended for sheep (Group 2) was ineffective against *T. circumcincta* and *T. colubriformis* and when a double dose (Group 3) was used the efficacy for *T. circumcincta* had improved to 97% but against *T. colubriformis* was only 78%. No statistically significant differences ($p<0.05$) were found for *T. circumcincta* or *T. colubriformis* between Group 1 and 2. However, there was a significant difference ($p<0.05$) for both species between Group 3 and the other two

groups, indicating the number of worms had been significantly reduced although not totally eliminated.

2.3.2 FEC

The percentage reduction in arithmetic mean faecal egg counts (FECs) were calculated on Day 34, 12 days after treatment. When egg counts were allocated to genera the reduction for *T. colubriformis* for Group 2 (single dose) and Group 3 (double dose) was estimated at 98% and 96.7%, respectively whereas for *T. circumcincta* the reduction was estimated at 99.6% for both treatment groups. For *H. contortus*, the reduction was estimated at 100% for Group 2 and 99.9% for Group 3.

2.3.3 LDA Results

For different species of both test isolate and respective susceptible isolate the results can be interpreted by examining: the critical well; estimated efficacy (%); the EC_{50} values and resulting RR values; the EC_{90} values and resulting RR values; and all the mean number of larvae recovered in the Wells 9 to 12 (with the highest concentration of anthelmintic) (Table 2.2, 2.3 and Figure 2.1, 2.2).

For *T. colubriformis* with BZ; the critical wells were 5 to 5.5 wells higher for Group 1 and Group 2 than for the susceptible isolate with an estimated efficacy of only 2% and 0%, respectively. For Group 1, a similar mean number of larvae to the susceptible isolate developed in Wells 9 to 12. However, the estimated RR values for the EC_{50} values was low (1.5) for Group 1 but slightly higher for Group 2 (5.0). For the EC_{90} values the RR values were substantially higher (42) for Group 1 and Group 2 (1579).

For *T. colubriformis* with LEV; the critical well was 2.5 (Group 1) to 3 (Group 2) wells higher than for the susceptible isolate and the estimated efficacy was 19% (Group 1) and 1% (Group 2). The mean number of larvae that developed in Wells 9 to 12 for Group 1 was found to be 9.8% with approximately double this (20%) for Group 2 whereas almost none were found for the susceptible isolate. When comparing the EC_{50} values the RR was only 4.3 for Group 1 but lower for Group 2 at only 1.1. When comparing the EC_{90} values for *T. colubriformis,* the RR values for Group 1 was 2.9 but much higher for Group 2 at 2691.

For *T. colubriformis* with the BZ+LEV combinations; the critical well for Group 1 was 3 wells higher than for the susceptible isolate with a very poor estimated efficacy of 48%. There were 17.4% of L_3 in Wells 9 to 12 compared to 0% for the susceptible isolate. Insufficient larvae developed for interpretation to be made for Group 2.

For IVM with *T. colubriformis;* for Group 1 the critical well was 1 to 2 wells higher than for the susceptible isolate for IVM-1 and IVM-2 but for Group 2 IVM-1 was 3 wells higher whilst IVM-2 was 4.5 wells higher than for the susceptible isolate. When comparing the mean number of larvae developing in Wells 9 to 12, 4.3% of larvae were found for IVM-1 and somewhat higher for IVM-2 at 13% for Group 1. However this is similar to be values for the susceptible isolate of 1% and 13%, respectively. For Group 2 a very high percentage of mean larvae developed for both IVM-1 and IVM-2 being 40.6% and 51.5%, respectively. The EC_{50} values were lower for Group 1 than for the susceptible isolate whilst for Group 2 they had a RR at 5.9 (IVM-1) and 33.4 (IVM-2). Interestingly, the EC_{90} values for Group 1 were only slightly higher than for the susceptible isolate whereas for Group 2 they were much higher with RR values of 21,939 and 1729 for IVM-1 and IVM-2, respectively.

The R^2 values for fitted curves for *T. colubriformis* were relatively constant at about 0.80 for Group 1 for BZ, LEV and IVM-2 but only 0.34 for IVM-1. For Group 2 the R^2 were very low for all the anthelmintics tested but for the susceptible isolate they were all $\geq$0.95 except only 0.82 for IVM-1.

For *T. circumcincta* with BZ; the critical well was 5 to 5.5 wells higher than the susceptible isolate for both Group 1 and 2. The estimated efficacy was 25% for both groups. The mean number of larvae that grew in Wells 9 to 12 was only 8.8% (Group 1) and 4.6% (Group 2) but none grew in these wells for the susceptible isolate. The RR with the EC_{50} values was 3.3 for Group 1 but only 1.0 for Group 2. However, the RR with the EC_{90} values was significantly higher for Group 1 (68) and especially higher for Group 2 (316).

For *T. circumcincta* with LEV; the critical well for Group 1 and Group 2 was 3.0 to 2.5 wells higher than for the susceptible isolate with an estimated efficacy of 70% and 79% respectively. No larvae grew in Wells 9 to 12. Meanwhile, the EC_{50} values for Group

1 was higher compared to the susceptible isolate with a RR for Group 1 of 6.2 but only 1.6 for Group 2. The RR with EC_{90} values was not substantially different with the value for Group 1 being only 2.0 and Group 2 being 3.6.

For *T. circumcincta* with BZ+LEV data is only available for Group 1. The critical well was 3 wells higher than the susceptible isolate with an estimated efficacy of 81% with this combination of anthelmintics. The mean number of larvae growing in Wells 9 to 12 was 11% compared to 0% for the susceptible isolate.

For IVM with *T. circumcincta;* for Group 1 the critical well was only 2.0 or 0.5 wells higher than for the susceptible isolate for IVM-1 and IVM-2 respectively. For Group 2 the critical well was only 1 or 0.5 wells higher for IVM-1 and IVM-2 respectively. However, in general a high percentage of larvae grew in Wells 9 to 12 compared to the susceptible isolate except only 4.4% of mean larvae grew for IVM-1 for Group 1. By comparison, for the susceptible isolate no larvae grew in Wells 9 to 12 for IVM-1 and only 2.9% for IVM-2. The RR from the EC_{50} values were low at only 0.1 for IVM-1 and 0.8 for IVM-2 for Group 1 but were higher for Group 2 being 11 and 19 for IVM-1 and IVM-2 respectively. The EC_{90} values for Group 1 were 8244 for IVM-1 but a lower value of only 4.7 for IVM-2 whilst for Group 2 the RR was 4 for IVM-1 and 65 for IVM-2.

The R^2 values from the fitted curves for *T. circumcincta* for Group 1 was 0.73 for BZ and 0.85 for LEV but lower for IVM-1 and 2 being ≤ 0.41. For Group 2, the R^2 value for LEV was higher compared to other anthelmintics. The R^2 values for the susceptible isolate were all ≥ 0.90 for BZ, LEV and IVM-2 but only 0.84 for IVM-1.

For *H. contortus*; Group 2 was not assessed due to insufficient larvae developing. For Group 1 with BZ, the critical well was 2 wells higher than for the susceptible isolate. The estimated efficacy was predicted at 89% with approximately 4.3% mean larvae growing in Wells 9 to 12. No larvae grew in Wells 9 to 12 for the susceptible isolate. The RR from the EC_{50} values was 2 but higher at 33 from the EC_{90} values.

For *H. contortus* with LEV; the critical well was 2 wells higher than for the susceptible isolate but with a very good estimated efficacy (99%) and no larvae grew in Wells 9 to 12. Meanwhile, the RR for EC_{50} and EC_{90} values were 0.3 and 0.5 respectively.

For *H. contortus* with IVM; the critical well was 2 to 3 wells higher than for the susceptible isolate for IVM-1 and IVM-2 with zero larvae growing in Wells 9 to 12. The EC_{50} values for the two IVM analogues were higher than for the susceptible isolates with an RR of 2.5 (IVM-1) and 1.4 (IVM-2). The RR from the EC_{90} values was higher for IVM-2 than IVM-1 at 9.6 and 4 respectively.

The R^2 values from the fitted curves for *H. contortus* in Group 1 were $\geq$0.69 for BZ, IVM-1 and IVM-2 but only 0.43 for LEV. For the susceptible isolate the R^2 values were $\geq$0.90.

Table 2.1: Arithmetic mean faecal egg counts (FECs), arithmetic mean worm counts and reductions (%) in worm burdens in animals treated with either a single dose of a triple combination (Group 2) of abamectin (0.2mg/kg) + oxfendazole (4.5mg/kg) + levamisole (8mg/kg) per os or a double dose (Group 3) of the same triple combination compared to the control untreated animals (Group 1).

Group	*T. circumcincta*		*T. colubriformis*		*H. contortus*		*Oe. venulosum*	
	Day 34 mean FEC (range; efficacy)	Mean worm burden (range; efficacy)	Day 34 mean FEC (range; efficacy)	Mean worm burden (range; efficacy)	Day 34 mean FEC (range; efficacy)	Mean worm burden (range; efficacy)	Day 34 mean FEC (range; efficacy)	Mean worm burden (range; efficacy)
1	498^{a} (48-1248)	337^{a} (40 – 460)	374^{a} (36-936)	375^{a} (320 – 410)	2181^{a} (210-5460)	668^{a} (420– 1080)	31^{a} (3-78)	9^{a} (0 – 25)
2	2^{b} (0-11; 99.6%)	68^{ab} (10 – 190; 80%)	6.5^{b} (0—39; 98%)	220^{a} (160– 450; 41%)	0^{b} (0; 100%)	1.7^{b} (0-10; 99%)	0^{b} (0; 100%)	0^{b} (0; 100%)
3	2^{b} (0-6; 99.6%)	10^{b} (0 – 30; 97%)	12^{b} (0-43.5; 96.7%)	81^{b} (10 – 150; 78%)	0.1^{b} (0-0.5; 99.9%)	0^{b} (0; 100%)	0^{b} (0; 100%)	0^{b} (0; 100%)

Within a column mean values with different superscripts are significantly different ($p < 0.05$)

Table 2.2: Critical well, the estimated efficacy based on the values determined in the DrenchRite® User Manual (DrenchRite®, Mircobial Screening Technologies, New South Wales, Australia) and mean (%) of larvae/well in Wells 9-12 compared to control wells for each genus.

Group	Drug	*T. circumcincta*			*T. colubriformis*			*H. contortus*		
		Critical well	Estimated efficacy (%)	Mean (%) of larvae/well for wells 9 to12[a]	Critical well	Estimated efficacy (%)	Mean (%) of larvae/well for wells 9 to12[a]	Critical well	Estimated efficacy (%)	Mean (%) of larvae/well for wells 9 to12[a]
Group 1	BZ	8.5	25	8.8	9.5	2	15.3	5.5	89	4.3
	LEV	7.5	70	0.0	7.5	19	9.8	4.5	99	0.0
	BZ+LEV	6.5	81	11.0	8.5	48	17.4	3.5	100	0.0
	IVM-1	9.5	n.a	22.1	6.5	n.a	4.3	6.5	n.a	0.0
	IVM-2	8	n.a	4.4	6.5	n.a	13.0	5.5	n.a	0.0
Group 2	BZ	8.5	25	4.6	10	0.0	21.6	n.s	n.s	n.s
	LEV	7	79	0.0	8.5	1	20.2	n.s	n.s	n.s
	BZ+LEV	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
	IVM-1	8.5	n.a	32.8	8.5	n.a	40.6	n.s	n.s	n.s
	IVM-2	8	n.a	56.3	9	n.a	51.5	n.s	n.s	n.s
Susceptible	BZ	3.5	100	0.0	4.5	94	0.0	3.5	100	0.0
	LEV	4.5	100	0.0	5.5	83	0.6	3.5	100	0.0
	BZ+LEV	3.5	100	0.0	5.5	100	0.0	3	100	0.0
	IVM-1	7.5	n.a	0.0	5.5	n.a	1.1	4.5	n.a	0.0
	IVM-2	7.5	n.a	2.9	4.5	n.a	12.9	2.5	n.a	0.0

critical well: the well with only 50% of L_3 compared to control wells

[a]mean (%) of larvae/well in Wells 9-12 compared to control wells

n.s (no sample): insufficient larvae to estimate EC_{50}/ EC_{90}

n.a (not applicable): Estimated efficacy for BZ+LEV, IVM-1 and IVM-2 is not indicated for this genus in the DrenchRite® User Manual

Table 2.3: EC_{50} /EC_{90} values with the R^2 and resistance ratio (RR) for BZ (μM), LEV (μM), IVM-1 (nM) and IVM-2 (nM) on Day 23 (Group 1) and on Day 35 (Group 2). Values are also shown for susceptible isolates.

Group	Drug	*T. circumcincta*			*T. colubriformis*			*H. contortus*		
		EC_{50} (RR)	EC_{90} (RR)	R^2	EC_{50} (RR)	EC_{90} (RR)	R^2	EC_{50} (RR)	EC_{90} (RR)	R^2
Group 1	BZ	0.10 (3.3)	2.70 (67.5)	0.73	0.60 (1.5)	6.22 (41.5)	0.77	0.07 (2.3)	1.33 (33.3)	0.73
	LEV	7.60 (6.2)	11.84 (2.0)	0.85	8.47 (4.3)	13.52 (2.9)	0.80	0.16 (0.3)	1.31 (0.5)	0.43
	IVM-1	0.70 (0.1)	1.563e+006 (8243.7)	0.08	0.43 (0.1)	457.70 (2.6)	0.34	4.04 (2.5)	40.14 (4.0)	0.77
	IVM-2	17.17 (0.8)	1625 (4.7)	0.41	4.85 (0.7)	582.80 (1.5)	0.83	1.89 (1.4)	48.79 (9.6)	0.69
Group 2	BZ	0.03 (1.0)	12.64 (316.0)	0.33	0.20 (5.0)	236.80 (1578.7)	0.19	n.s	n.a	n.s
	LEV	1.98 (1.6)	20.55 (3.6)	0.77	2.20 (1.1)	12592 (2690.6)	0.09	n.s	n.s	n.s
	IVM-1	61.20 (10.5)	12,236 (64.5)	0.44	33.84 (5.9)	8.405e+006 (21,939.0)	0.23	n.s	n.s	n.s
	IVM-2	391.80 (18.5)	45890 (132.4)	0.09	241.10 (33.4)	661913 (1727.8)	0.25	n.s	n.s	n.s
Susceptible	BZ	0.03	0.04	0.95	0.04	0.15	0.97	0.03	0.04	1.00
	LEV	1.22	5.63	0.92	1.96	4.68	0.95	0.56	2.57	0.90
	IVM-1	5.83	189.60	0.84	5.78	177.80	0.82	1.60	11.84	0.95
	IVM-2	20.63	346.60	0.90	7.23	383.10	0.95	1.34	5.09	0.99

EC_{50} / EC_{90}: anthelmintic concentration where L_3 development in 50%/90% of the larvae (BZ: benzimidazole, LEV: levamisole, IVM: ivermectin); n.s (no sample): insufficient larvae to estimate EC_{50}/ EC_{90}; RR (Resistance Ratio): EC_{50}/ EC_{90} values test isolate/ EC_{50}/ EC_{90} values susceptible isolate;
R^2: Coefficient of Determination of the fitted curve

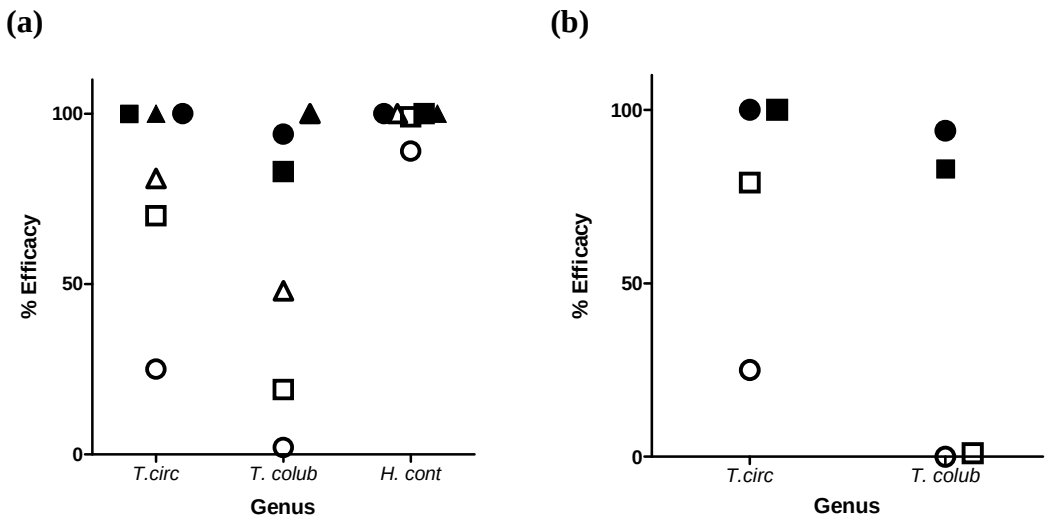


Figure 2.1: Efficacy (%) of BZ (O), LEV (□) and BZ+LEV Combination (Δ) for different genera at Day 23 for Group 1 (a) and Day 35 for Group 2 (b) by comparison with the chart supplied with the DrenchRite® User Manual. Values are also shown for susceptible isolates of BZ (●), LEV (■) and BZ+LEV (▲).

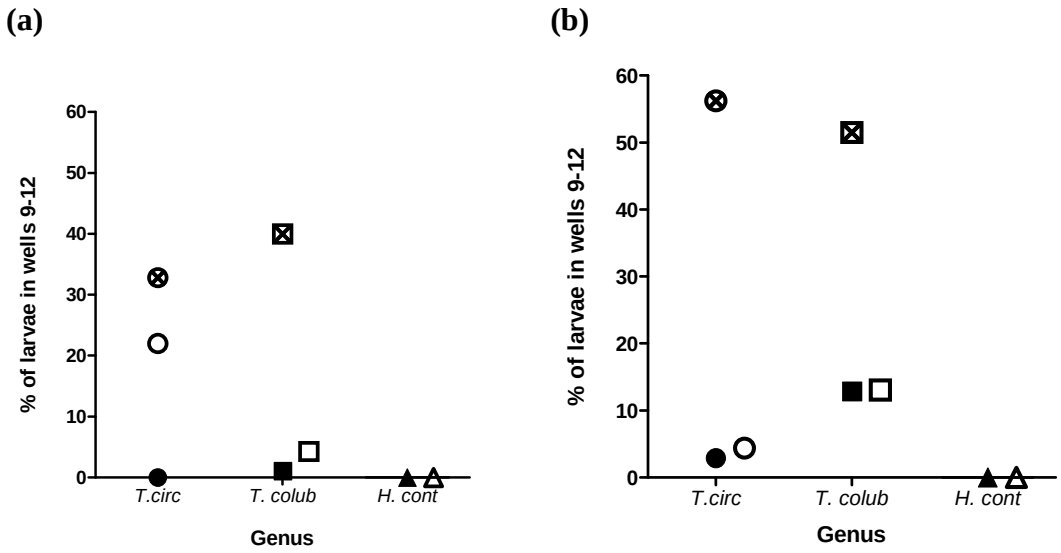


Figure 2.2: Mean number (%) of L_3/well in Wells 9 to 12 compared to control wells for *T. circumcincta* (O), *T. colubriformis* (□) and *H. contortus* (Δ) at Day 23 for Group 1 (pre treatment) and for *T. circumcincta* (⊗) and *T. colubriformis* (☒) at Day 35 for Group 2 (post treatment). Values for susceptible isolates of *T. circumcincta* (●), *T. colubriformis* (■) and *H. contortus* (▲) are also shown. Graph (a) is for IVM-1 and Graph (b) for IVM-2.

2.4 DISCUSSION

This study has confirmed that *T. circumcincta* and *T. colubriformis* from this goat farm were highly resistant to a triple combination of oxfendazole, LEV and abamectin. It was notable that the efficacy of double the recommended sheep dose rate of the three anthelmintics only achieved an efficacy of 78% against *T. colubriformis* indicating this was a highly resistant isolate. Double the recommended dose was somewhat more effective against *T. circumcincta* but the single dose was ineffective. The *H. contortus* isolate demonstrated a slightly reduced efficacy for BZ but there was no convincing evidence of reduced efficacy with LEV or IVM.

Anthelmintic resistance has been recognised as an issue for goats in New Zealand since 1983 when Kettle et al. (1983) found evidence of either BZ or LEV resistance on 79% of dairy goat farms surveyed with *Haemonchus*, *Trichostrongylus* and *Teladorsagia* all involved. Since IVM was introduced into the New Zealand market in the early 1980s goat farmers have, of necessity, used various members of the ML family of anthelmintics to control parasites. The first report of IVM resistance in *T. circumcincta* was made in 1988 (Watson and Hosking, 1990) and was an isolate from goats. It was several years later when the first report of ML resistance in *T. circumcincta* isolated directly from sheep was made (Mason et al., 1999). The first report of resistance to BZ, LEV and an ML given synchronously was based on results of a FECRT in goats where even double the sheep dose rate of all three actives failed to effectively reduce the FEC (West et al., 2004). In sheep the first confirmed case was slightly later in 2006 (Wrigley et al., 2006) and was based on worm counts. By 2004/2005 a national survey of sheep farms in New Zealand revealed that ML resistance in *Teladorsagia* was evident on 48% of sheep farms (Waghorn et al., 2006). BZ and LEV resistance were also common in this same species but no groups were tested to determine if efficacy of a combination of all three anthelmintics was effective on these farms. Consequently, confirming triple resistance in *T. circumcincta* on the study farm is somewhat disturbing but was not particularly unexpected.

The first report of ML resistance in *T. colubriformis* was in 1987 and also from goats (Gopal et al., 1999). Multiple resistance in *Trichostrongylus* to BZ, ML and LEV given synchronously was first reported, based on a FECRT in goats, in 2004 (West et al.,

2004) and also involved a similarly resistant *Teladorsagia*. The first case in nematodes directly isolated from sheep was in 2008 where the nematodes were also resistant to a combination of BZ+LEV although the triple combination was not tested (Sutherland et al., 2008). This present study represents the first case of triple resistance in *T. colubriformis* in New Zealand confirmed by observing the actual reduction in worm counts. Not surprisingly the nematodes were isolated from goats as they seem to invariably have more severe problems with anthelmintic resistance than sheep.

Some previous studies where goats have been used to assess the resistance status will have been compromised by the issue of choice of dose rate. It is well established that goats generally metabolise all anthelmintics more rapidly than sheep (Hennessy, 1994; Reinemeyer and Pringle, 1993; Sangster et al., 1991b) and hence use of the sheep dose rate is not necessarily appropriate (Gillham and Obendorf, 1985; McKenna, 1984). This is reflected in the small number of anthelmintic formulations that actually have a label claim for use in goats in New Zealand. In the present study this issue was avoided by taking the isolates from goats and assessing them in young sheep where more confidence exists about the appropriate dose rates. That a double dose of the triple combination was not effective against *T. colubriformis* reflects the experience of the authors on the source farm where an increased dose of moxidectin together with a sheep dose of LEV plus 3 consecutive days of a standard sheep dose rate of fenbendazole was not effective in reducing the faecal egg count to a low level. The consequence of this to the goat farmer was that control of nematodes was almost impossible before the release of monepantel in New Zealand in 2009. With this new anthelmintic, it appears that control of goat nematodes on this farm is now achievable even though it is not yet registered for use in this species.

The results from the FECRT conducted along with the controlled slaughter study are quite revealing about the sensitivity of this approach. As the initial infection included a majority of *H. contortus* and this was shown to be sensitive to the triple combination it could be expected that the fecundity of this species would make interpreting the results of the FECRT difficult. However, when FEC were allocated to genera based on larval culture results there were in excess of 350 eggs allocated to both *Teladorsagia* and *Trichostrongylus* in the control group. It is somewhat surprising that on Day 34 (12 days after treatment) the FECs were zero for all genera except for one animal with a

FEC of 50 eggs/g in Group 2 and 2 animals with 50 eggs/g in Group 3. This is suggestive of some form of egg count suppression. Similar results have been reported for IVM-resistant isolates of *H. contortus* (Le Jambre, 1993), *T. colubriformis* and *T. circumcincta* (Jackson and Coop, 1995) where egg counts were zero 14 days after treatment but IVM resistance was subsequently confirmed. The actual worm burdens of animals in Groups 2 and 3 were low and this might have affected the ability to detect eggs but for *T. colubriformis* in Group 2 the mean count was still 220 worms and FEC were estimated several times post-treatment (see Appendix 2.3). It does highlight the deficiencies of our reliance on using FECRT, especially with ML- resistant isolates.

The opportunity was taken to compare the results of the controlled slaughter study with those from an LDA, in this case the commercially available DrenchRite® assay. The value of this commercial assay is that it has been validated to estimate the efficacy of BZ, LEV and BZ+LEV for *H. contortus*, *Trichostrongylus* and *Teladorsagia* (see DrenchRite® User Manual, Appendix 2.6). Hence a comparison could be made with these highly resistant isolates. In this present study, a comparison was also made with the susceptible isolates. For the *T. circumcincta* susceptible isolate the efficacy was estimated as 100% for BZ, LEV and their combination. However, the estimates of efficacy for the *T. colubriformis* isolate suggested slightly less than 100% efficacy for both BZ and LEV although the combination of BZ+LEV was 100%. The estimate of efficacy for BZ and LEV for this species was 94% and 83% respectively. This may be an aberration of the assay or more likely suggests some contamination of this laboratory isolate with resistant isolates at some stage.

The usefulness of this LDA for determining ML resistance is less well established. LDA results are available for Group 1 and 2 but not for Group 3 as there were insufficient eggs to run the assay. A problem with the LDA is that fecund species such as *H. contortus* can dominate the number of larvae per well and the genera of interest may be only present in small numbers. This was the case for Group 1 so the estimate of efficacy using EC_{50} or EC_{90} values is based on only small numbers of larvae, especially for *T. circumcincta* (see Appendix 2.12 for raw data). Not surprisingly the Coefficient of Determination was not that high for either *T. circumcincta* or *T. colubriformis* and hence interpretation of the RR value is difficult. For Group 2 the number of eggs initially available was low and hence the same situation applies even

though no *H. contortus* were present (see Appendix 2.13 for raw data). For the susceptible isolates only one species was present at each time and the proportion of eggs that developed into larvae was quite high (see Appendix 2.14 for raw data). Nevertheless it was still surprising that the RR values calculated from the EC_{50} values for Group 1 did not clearly indicate a severe level of resistance for any anthelmintic or the combination of BZ+LEV. Kotze et al. (2002) compared the RR value for ML resistance from the EC_{50} with that for the EC_{99} and found that the former was a poor estimate of the level of resistance in an isolate of IVM-resistant *H. contortus* whereas the RR value from the EC_{99} was a much better indicator of the presence of resistance. In the present study the RR values from the EC_{90} values were more indicative of resistance, particularly for BZ but even so the values for the other anthelmintics were not very high. RR values predicted for Group 2 for BZ, LEV and their combination were higher than for Group 1 as expected, but again the value for *T. circumcincta* with LEV was still only 3.6 which is not very convincing of the presence of resistance. The RR values from the EC_{50} were low for both *T. colubriformis* and *T. circumcincta* with neither suggesting the presence of ML-resistance. Even the RR value from the EC_{90} values was low except for IVM-1 with *T. circumcincta*. Thus, in this experiment the LDA did not give a clear indication as to the resistance status of these nematodes.

Using the concept of establishing the critical well and the estimated efficacy from that figure provides a clearer picture of the resistance status of the nematodes from this goat farm. The efficacy of BZ for both *T. circumcincta* and *T. colubriformis* are both very low from Group 1 data whilst the efficacy of LEV in this group was also poor for *T. colubriformis*, although it was estimated at a somewhat higher value of 70% for *T. circumcincta*. Interestingly for both these two species the estimate of efficacy of the combination of BZ+LEV was higher than for either active alone. This is not surprising and is consistent with the theory behind the use of combinations where not all nematodes will have resistant genes to both present at the same time (Leathwick et al., 2001). The estimate of efficacy for *H. contortus* on this goat farm indicates a low level of BZ resistance but essentially no evidence of LEV resistance and hence the combination of BZ+LEV was also fully effective. This is somewhat surprising given the poor efficacy seen in the other two species. Why this species is not resistant whilst the other two have multiple resistance is difficult to understand. It may reflect that this *H. contortus* isolate has been recently imported onto this property. Small numbers of

animals have been introduced, generally as individual male animals for breeding and this is a possible source.

The estimate of efficacy for IVM using the critical well approach and the number of larvae growing in Wells 9 to 12 which have the highest concentration of anthelmintic is somewhat more successful in establishing their ML resistant status, especially for *T. circumcincta*. The critical wells for *T. circumcincta* were higher than those of the susceptible isolate as well as the range of critical wells indicated in the DrenchRite® User Manual (which is also based on susceptible isolates). This is interesting as it has been acknowledged that a failing of the LDA is its inability to detect ML resistance in *T. circumcincta* (Palmer et al., 1998). In addition there were substantially more larvae growing in Wells 9 to 12, particularly for IVM-1 than for the susceptible isolate (Table 2.2) which further supports the conclusion that these *T. circumcincta* were ML resistant and hence the results are consistent with the worm count data. For *T. colubriformis* the results are somewhat less convincing. The critical wells from Group 1 were only just higher than the range for susceptibles for IVM-1 and within the range for IVM-2. Kaplan et al. (2007) noted that IVM-2 yields higher resistance ratios than IVM-1 for *T. colubriformis* and is those authors' choice for detecting ML-resistance. Based on this data, ML resistance in *T. colubriformis* would not have been detected. For Group 2 after treatment with a single dose of the triple combination of anthelmintics the critical wells were higher and the proportion of larvae developing in Wells 9 to 12 was higher than for the susceptible isolate with both IVM-1 and IVM-2 such that the RR value would have been indicative of ML-resistance being present. However, in the field the situation represented by Group 1, where the animals have not been recently treated, is the proposed approach to use the LDA and in this case would not have been very effective. The number of larvae of *T. colubriformis* was small and the resulting R^2 values were low which in part explains the limitations. It does, however, highlight potential difficulties using the LDA under field conditions.

The critical wells and estimated efficacies of the susceptible isolates generally confirmed their susceptible status for *T. circumcincta* and *H. contortus*. However, for *T. colubriformis* there is a suggestion that BZ and LEV were less than fully effective. The combination of these two was fully effective. The explanation for this is unclear but may reflect some contamination of this isolate with a resistant strain or may just

highlight deficiencies in the estimate of efficacy using the DrenchRite® LDA plates. As no other means of determining the efficacy of the various anthelmintics in these susceptible isolates was possible in this study, hence a full explanation is not possible. It does mean that the estimate of RR values for *T. colubriformis* using the EC_{50} or EC_{90} values may, in reality, be conservative.

These results clearly indicate that these isolates of *T. circumcincta* and *T. colubriformis* were resistant to synchronous treatment with BZ, LEV and abamectin. For this particular goat farmer the options for future parasite control are generally limited to the use of monepantel. It is necessary to also institute other control options to limit the development of resistance to this new anthelmintic with its unique mode of action (Leathwick et al., 2009). The DrenchRite® LDA results were not particularly successful in determining the full resistant status of these nematodes and nor was evaluating the reduction in faecal egg counts. This highlights the risk with these indirect diagnostic approaches to evaluate the survival of nematodes to therapeutic doses of anthelmintic. Whilst sheep farmers have been continuously warned of the dangers of anthelmintic resistance these results indicate that considerable emphasis should be made with those farming goats as well. The existence of multiple resistant strains such as those documented here also highlight the need for effective quarantine drenching of incoming stock by both sheep and goat farmers to avoid the dispersal of these genes for resistance onto their farms.

CHAPTER THREE

A NON-RANDOM SURVEY OF ANTHELMINTIC ESTIMATE ON 17 GOAT FARMS IN NEW ZEALAND USING THE DRENCHRITE® COMMERCIAL LARVAL DEVELOPMENT ASSAYS

3.1 INTRODUCTION

The progressive development of resistance to BZ, LEV, Moxidectin and IVM in goat parasites has been recorded in New Zealand (Gopal et al., 1999; McKenna, 1990; Scherrer et al., 1989) and worldwide, including Australia (Barton et al., 1985; Love, 1999), Europe (Jackson et al., 1992; Maingi et al., 1997), U.S.A (Howell et al., 2008; Kaplan et al., 2007), Africa (Mwamachi et al., 1995; Waruiru, 2002), and Southeast Asia (Chandrawathani et al., 1999; Dorny et al., 1995a). However, the extent of the problem has not been well documented. There are concerns that the level of anthelmintic resistance is increasing and consequently goat farmers have fewer anthelmintic products available to use. Invariably the level of resistance will be different on each farm.

A variety of tests have been used to assess anthelmintic efficacy (see Section 1.9). In the present study, the larval development assay, (LDA; DrenchRite®) was used to estimate the efficacy of BZ, LEV, the combination of BZ+LEV and IVM in gastrointestinal nematode parasites in goats. The LDA has never been used commercially in New Zealand but was used for several years in Australia. Consequently, most of the reported studies were conducted in Australia during the 1990s (Lloyd, 1998; Palmer et al., 1998). DrenchRite® assay has also been used to detect anthelmintic resistance in goats in the U.S.A (Howell et al., 2008; Kaplan et al., 2007). The DrenchRite® LDA has a limitation in its ability to assess IVM efficacy compared to a reasonable degree of confidence for assessing efficacy with the BZs and LEV. The assay was developed prior to the widespread occurrence of ML resistance and hence validation studies were not possible. Two different analogues of IVM (IVM-1 and IVM-2) are included on the plate as it was considered these would give the highest resistance ratio with ML resistant isolate.

The aims of the experiments in this chapter were to investigate the prevalence of anthelmintic resistance in *Teladorsagia* and *Trichostrongylus* on goat properties in New Zealand at the farm level by using the DrenchRite® LDA.

3.2 MATERIALS AND METHODS

3.2.1 Farm

Veterinary contacts and a request through an email list to meat and fibre goat farmers were undertaken to recruit goat farmers. Each respondent was sent a reply pack containing 10 empty pottles, gloves, a simple questionnaire and a return courier pack. Respondents were requested not to send samples from goats treated with an anthelmintic in the last 4 weeks. It was also requested that all samples were sent no later than Thursday to ensure they arrived prior to a weekend when no courier deliveries were made. As a non-random survey selection bias cannot be avoided. However, given the voluntary nature of farm selection it is likely that this survey included those goat farmers who are more enthusiastic.

3.2.2 Questionnaire

Each respondent was asked to complete a short questionnaire. The questionnaire was divided into three sections and consisted of 16 questions over two pages (See Appendix 2.7). The first part of the questionnaire requested the respondents' names and addresses but both were voluntary. For the second part, the respondents were requested to provide the background details of their respective farms. In the last part of the questionnaire, questions enquired about their farms management, in particular drenching practices that might influence the occurrence of anthelmintic resistance of all gastrointestinal nematode parasites. The majority of the 16 questions in the questionnaire were closed. The questionnaire was designed to take 10 minutes to complete so as not discourage farmer participation.

3.2.3 Parasitology techniques

Faecal egg counts (FEC)

FEC were estimated using the modified McMaster technique where each egg counted represents 50 eggs/g (as described in Section 2.2.3 and in Appendix 2.1).

Larval development assay (LDA)

The commercially available DrenchRite® LDA was used to estimate anthelmintic efficacies in this study. The 96 well microtitre plate was removed from the foil pouch and warmed to room temperature during the process of egg isolation. All wells were examined for moisture content. In this study no sign of cracked agar was detected which would indicate rehydration of these wells was required. This assay was conducted as described previously in Chapter 2, Section 2.2.3 and in detail in Appendix 2.5. For each anthelmintic or combination the critical well was estimated as the average of the two rows where 50% of the larvae of genus failed to develop by comparison with control wells. For BZ, LEV and a combination of both drugs (BZ+LEV), the approximate efficacy of the anthelmintic against different types of worm was estimated using the table and guideline supplied in the DrenchRite® User Manual (see Appendix 2.6). The efficacy estimated with IVM-1 and IVM-2 was also performed as described in Chapter 2. IVM-1 is known to be IVM monosaccharide and IVM-2 as ivermectin aglycone (Kaplan et al., 2007).

Egg recovery

The procedure from recovering eggs is as described in Section 2.2.3 and in detail in Appendix 2.5. The DrenchRite® protocol can be performed on faecal samples with an egg count more than 100 egg/g. In brief, nematode eggs were isolated from a 100g to 200g pooled faecal sample taken from each farm. The faeces were mixed with water to make a faecal slurry. The faecal slurry was then washed through a 60µm sieve and the residue on top of the mesh was discarded. The filtrate was then passed through a 20µm sieve. Eggs in retained particulate matter were further cleaned by centrifugation in sugar gradients. 60 eggs were dispensed per well in the 96 well DrenchRite® microtitre plate followed by an additional 40µl of nutrient solution containing yeast, *Escherichia coli* and amphotericin B 24 hr later. The plate was incubated at 25°C for 13 to 14 days to grow eggs through to infective larvae. The infective larvae (L_3) were differentiated by species on their overall length and if necessary on morphological features of tubercles after being exsheathed (McMurtry et al., 2000).

Statistical analysis

Mean faecal egg counts (FECs) were calculated for each farm.

The EC_{50} values for BZ, LEV, IVM-1 and IVM-2 were calculated by non-linear fitting to sigmoid dose-response curves of variable slope using the software package GraphPad Prism 5.00 (GraphPad Software, Inc., U.S.A) using the information on drug concentrations as supplied by (Tandon and Kaplan, 2004). The estimated efficacies were plotted for each farm in this study. The EC_{50} values for each genus and anthelmintic involved were also plotted as well as the Coefficient of Determination values (R^2) for the fitted curve being recorded as an indication of the goodness of fit of the data. The resistance ratio (RR) was calculated by comparison with the values achieved for susceptible isolates as determined in Section 2.2.3.

To estimate the resistance status for each IVM analogue, two criteria were used. If the critical well was above that nominated in the DrenchRite® User Manual it was considered R and if the number of larvae in Wells 9 to 12 was >10% for IVM-1 with both species or >10% for IVM-2 with *Trichostrongylus* or >20% with *Teladorsagia* then it was considered positive (+) for resistance. Overall if a farm was positive for resistance with at least 2 of these 4 categories it was considered to have IVM resistance in that genus.

(a) **(b)**

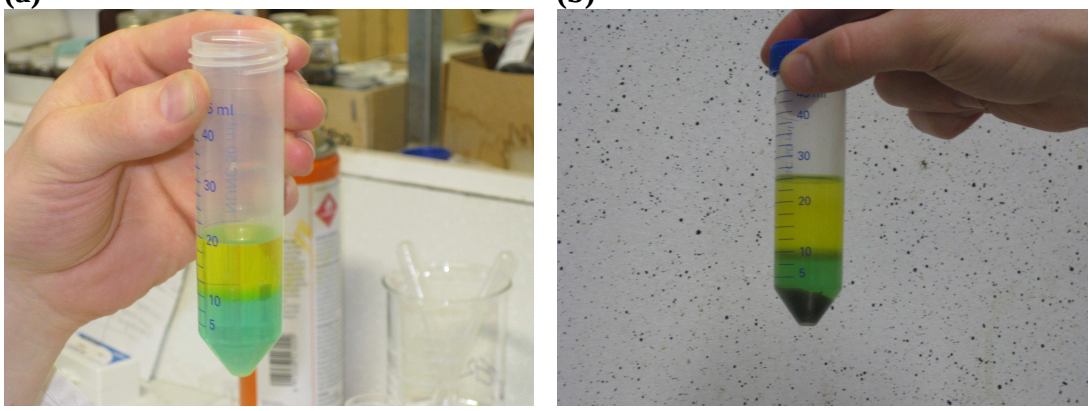


Plate 3.1: Photo of 50ml Falcon tube of sugar gradients with 10% (yellow) and 25% (blue) of sucrose solution (a) and eggs were recovered from the interface of both sugar solutions and debris/rubbish settling the bottom of the tube (b).

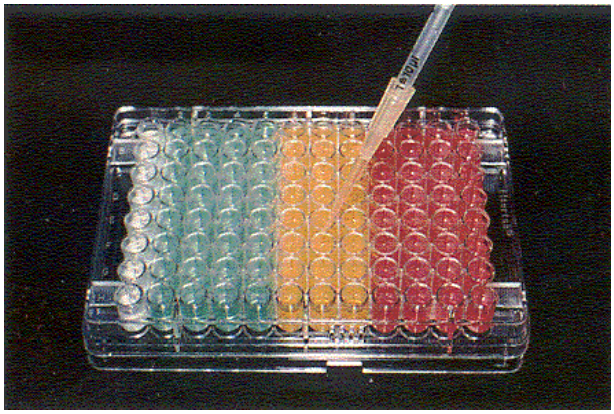


Plate 3.2: Photo of the DrenchRite® plate. The different colours indicate the status if the critical well occurs in that colour band. Interpretation of critical well status: Lane 1- control; Lane 2 to 5 (green) -susceptible; Lane 6 to 8 (yellow) -weak or intermediate resistance; Lane 9 to 12 (red) -high resistance.

3.3 RESULTS

From the initial 20 farms, 17 farmers were contacted and they provided their addresses for mailing purposes. 13 goat farms in the North Island and 4 goat farms in South Island were included in this study of which 16 were meat/fibre producers and 1 was a dairy producer. All goats used in this study were not treated with an anthelmintic for at least 4 weeks before faecal collection for the assay. This was done to ensure that goats were infected with sufficient nematode parasites representative of the farm in question for the purpose of this study.

3.3.1 Questionnaires Results

Responses to the questionnaires are presented in Table 3.1 in this study. Results of questionnaires are summarized in Appendix 3.1. A response of 82.4% (14 from 17) was achieved. Missing responses were considered as a zero. Faecal samples that were submitted from the respondents (17 from 17 farms) were test for FECs and the estimated faecal egg counts were communicated back to the respondents as soon as they were obtained. High FECs were seen on a few farms (Figure 3.1). All estimated results for the actual drench resistance study (LDA) took at least a month to be completed and were also sent to the respondents as soon as they were obtained.

Table 3.1: Summary statistics of enterprise descriptors of respondent farmers in questionnaires.

Variable	Level of variable	Count/farm	Total
Responses	Yes =1 No = 2	1 = 14/17 2 = 3/17	82.4% 17.6%
Enterprise	Meat = 1 Dairy = 2	1 = 13/14 2 = 1/14	92.9% 7.1%
Farm size (ha)	0-50 = 1 50-100 = 2 100-200 = 3 >200 = 4	1 = 12/14 2 = 0/14 3 = 1/14 4 = 1/14	86% 0% 7% 7%
Goat numbers (range)	0-50 = 1 50-200= 2 200-500 = 3 500-1000 = 4 >1000 = 5	1 = 5/14 2 = 5/14 3 = 2/14 4 = 0/14 5 = 2/14	36% 36% 14% 0% 14%
Other animals	None = 1 Sheep = 2 Cattle = 3 sheep+cattle = 4 Horse = 5 Deer = 6	1 = 5/14 2 = 1/14 3 = 5/14 4 = 1/14 5 = 1/14 6 = 1/14	36% 7% 36% 7% 7% 7%
Drenching dose rate	Missing responses = 0 Sheep dose rate =1 > sheep dose rate = 2 1+2 = 3	0 = 1/14 1 = 3/14 2 = 9/14 3 = 1/14	7% 22% 64% 7%
Time of faecal samples collected after last drench	4-8 weeks = 1 >8 weeks = 2	1 = 4/ 14 2 = 10/14	29% 71%
Previous investigation of drench resistance study	Missing responses = 0 Yes =1 No = 2	0 = 1/14 1 = 5/14 2 = 8/14	7% 36% 57%
Results of drench resistance study	Response given =1 No response = 2	1 = 2/5 2 = 3/5	40% 60%
Mean FECs (±range)	Each 17 farms	17/17	1 = 5010 (300-12500) 2 = 3450 (0-13500) 3 = 285 (100-800) 4 = 4079 (300-17400) 5 = 315 (50-800) 6 = 970 (150-2450) 7 = 980 (200-2800) 8 = 710 (200-1700) 9 = 800 (100-2300) 10 =1930 (100-3500) 11 = 733 (0-1600) 12= 2840 (200-7900) 13 = 2060 (300-7400) 14 = 910 (200-2300) 15 = 530 (100-1500) 16= 1710 (900-3200) 17 = 3090 (700-6600)

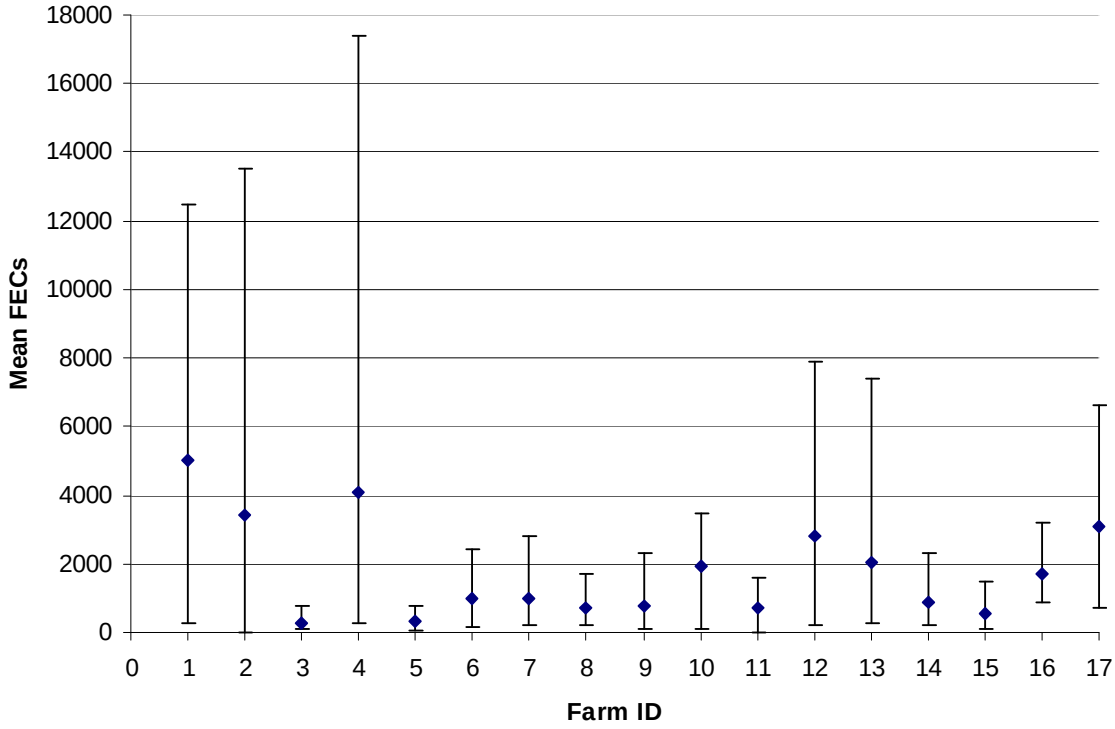


Figure 3.1: Arithmetic mean faecal egg counts (±range) from 17 farms.

Farm 1, 10, 14 and 15 (4-8 weeks since last treated with anthelmintic)
Farm 2, 3, 5, 6, 7, 8, 9, 12, 16 and 17 (> 8 weeks since last treated with anthelmintic)
Farm 4, 11 and 13 (missing responses)

3.3.2 LDA Results

LDAs to test for resistance to BZ, LEV, combination of BZ+LEV and IVM-1 and IVM-2 were performed on 17 farms but not all genera were present on all farms. Results were examined based on the critical well; estimated efficacy (%), the EC_{50} values and resulting resistance ratio (RR), R^2 values and the mean number of larvae recovered in the Wells 9 to 12 (which have the highest concentration of anthelmintic) compared to the control wells. Results for *T. colubriformis* and *T. circumcincta* are tabulated in Tables 3.2 to 3.6. Efficacy estimates for *T. colubriformis* and *T. circumcincta* are shown in Figures 3.2 to 3.4. The percentage of larvae growing in Wells 9 to 12 for IVM-1 and IVM-2 are shown in Figure 3.5. A summary of anthelmintic resistance status for *T. colubriformis* and *T. circumcincta* from the 17 goat farms are presented in Table 3.7.

For BZ with *Trichostrongylus*; the critical well from the DrenchRite® User Manual was 4.0 and for the susceptible isolate was at 4.5. Of the 17 farms, 13 farms were found to

have their critical well above this value (range, 5.5 to 9.5), indicating that suspected resistant *Trichostrongylus*, was present. Poor efficacy for BZ was predicted for 14 farms (range; 2% to 78%) whereas on 3 other farms it is clear that BZ is very effective against this genus (100% estimated efficacy). Surprisingly, for almost all the respondent farms (15 of 17 farms) there were larvae that grew in Wells 9 to 12 whereas none grew in these wells for the susceptible isolate. The EC_{50} values varied from 0.02μM to 1.72μM compared to susceptible isolates which was recorded as 0.04μM. This is also reflected in the RR for each farm (range, 0.8 to 43.0). The R^2 values for *Trichostrongylus* with BZ varied from as low as 0.48 to 0.92.

For BZ with *Teladorsagia*; the critical well from the DrenchRite® User Manual was 4.0 and for the susceptible isolate was 3.5. The critical wells for 11 out of 14 farms (range; 6.5 to 9.5) were above this value, indicating suspected resistance in this genus was present. There were 3 farms which were not assessed due to insufficient *Teladorsagia* larvae. There is also an indication of very poor estimated efficacy for BZ predicted for 11 of 14 farms (range; 9% to 67%) whilst for 3 other farms the efficacy was predicted to be 99% or 100%. Of these 11 farms with resistant *Teladorsagia* there were 9 for which larvae grew in Wells 9 to 12. The EC_{50} values were ranged from 0.03μM to 1.51μM compared to the susceptible isolate at 0.03μM. The RR values for the 11 farms with predicted resistance varied from 3.3 to 50.7. However, the R^2 values for this genus with BZ were generally poor varying from 0.29 to 0.78.

For LEV with *Trichostrongylus*; the critical well from the DrenchRite® User Manual was 4.5 and for the susceptible isolate was 5.5. The critical wells ranged from wells 3.5 to 5.5 for 6 farms, indicating that these isolates susceptible to LEV whereas for 10 other farms the critical well was higher, indicating they were considered to be resistant. The results also indicated that poor efficacy of LEV on 15 farms were predicted, with a range of 0% to 83% efficacy whilst good efficacy was predicted for 2 farms at 99% and 100%, respectively. The discrepancy between the critical well comparison and the estimated efficacy relates to the higher critical well for the susceptible isolate. By comparison 100% of the farms had larvae which grew in Wells 9 to 12 whereas almost none grew for the susceptible isolate. A wide variation of EC_{50} values were estimated ranging 0.87μM to 732.5μM compared to 1.96 μM for the susceptible isolate, resulting

in estimates of RR varying from 0.4 to 373.7. R^2 values were above 0.90 for 4 farms whilst for the other 13 farms the value was below this with the lowest at 0.08.

For LEV with *Teladorsagia*; the critical well from the DrenchRite® User Manual was 5.5 and for the susceptible isolate was 4.5. The critical well for 10 farms ranged from 2 to 5.5, indicating susceptibility. The critical wells for the other 4 farms were slightly higher ranging from 6.5 to 7.5. Consequently the estimated efficacy was 98% to 100% for 10 of the 14 farms. On the other 4 farms, the efficacy was estimated to be less than the desired level of 95% being 70% to 87%. Interestingly, some larvae grew in Wells 9 to 12 for 7 farms including Farm 12 and 16 both of which had estimates of efficacy >95%. The EC_{50} values were found to be varying from 0.01μM to 5.50μM as compared to 1.22μM in the case of susceptible isolate, giving an RR value of 0 to 4.5. The R^2 values were generally low ranging from 0.33 to 0.98.

For the two rows with the combination of BZ+LEV drugs with *Trichostrongylus*; the critical well from the DrenchRite® User Manual was 5.5 and for the susceptible isolate was 3.5. The critical well was higher than 5.5 for 11 farms indicating resistance was present on these farms with the estimated efficacy varying from 28% to 87% whilst on the other 6 farms the efficacy were predicted to be high (range; 98% to 100%). However, results also indicated that larvae grew in Wells 9 to 12 for 16 of the 17 farms examined whereas no larvae grew in these wells for the susceptible isolate.

For the two rows with the BZ+LEV combination of drugs with *Teladorsagia*; the critical well from the DrenchRite® User Manual was 5 and for susceptible isolate was 3.5. The critical well was higher than Well 5 for 8 farms. However, the estimated efficacy was less than 95% for only 4 farms. Larvae grew in Wells 9 to 12 for only 2 of these 4 farms. Overall, almost all farms (10 out of 14 farms) were predicted to have 100% efficacy whilst the other 4 farms had shown poorer estimated efficacy (range; 81% to 94%). The other 3 farms were not assessed due to insufficient *Teladorsagia* larvae.

For IVM-1 with *Trichostrongylus*; the critical well from the DrenchRite® User Manual was 4.5 (range, 4 to 5.5) and for the susceptible isolate was 5.5. The critical wells varied from 6.5 to 9.5 for 16 farms, indicating resistance was possibly present. Only

one farm had a critical well within the normal range nominated in the DrenchRite® User Manual. Results also showed that 100% of the farms had larvae which grew in Wells 9 to 12 (range, 0.5% to 40.1%) whereas only 1.1% grew in these wells for the susceptible isolate. A wide range of EC_{50} values were estimated, giving RR values from 0.6 to 40.0. The R^2 values for 5 farms were $\geq$ 0.90 whilst less (range, 0.40 to 0.89) for the other farms.

For IVM-1 with for *Teladorsagia*; the critical well from the DrenchRite® User Manual was 5.5 (range, 5.0 to 6.5) and for susceptible isolate was 7.5. No larvae grew in Wells 9 to 12 for the susceptible isolate for 12 of the 14 farms tested. The critical wells for 7 farms were higher than 7.5 (range, 8.5 to 10.5), indicating the isolate was likely to be resistant. The EC_{50} values were lower than the susceptible isolate (5.83nM) for 6 farms and slightly increased for 8 farms. The RR with EC_{50} values were from 0 to 42.2 for all farms. The R^2 values were generally low being $\leq$0.89 for these farms.

For IVM-2 with *Trichostrongylus*; the critical well from the DrenchRite® User Manual was 5.5 (range, 5.0 to 7.0) and for susceptible isolate was 4.5. The critical wells were 6.5 to 10.5 for all 17 farms reported. Thus only two of these were within the range given by the DrenchRite® User Manual and all were higher than the well value for the susceptible isolate suggesting resistance was present on at least 15 of 17 farms. Although 12.9% of larvae grew in Wells 9 to 12 for the susceptible isolate, it was greater than this for 15 farms. The EC_{50} values for 17 farms were higher (range 0.2 to 108.1) than for the susceptible isolate (7.23nM) which giving the RR values varying from 1.4 to 108.1. The lowest R^2 values were 0.34 but most were higher ranging to 0.98.

For IVM-2 with for *Teladorsagia*; the critical well from the DrenchRite® User Manual was 6.0 (range, 5.5 to 7.5) and for the susceptible isolate was 7.5. The critical wells ranged from 5.0 to 7.5 for 9 farms, indicating no evidence of resistance was present. For the other 5 farms, the critical wells ranged from 8.0 to 10.5, indicating resistance was possibly present. However, larvae grew in Wells 9 to 12 for 10 farms reported. The EC_{50} value was higher than the susceptible isolate for 7 farms with the RR values from 1.3 to 5.8. R^2 values ranged from 0.26 to 0.86.

H. contortus was only detected on 10 of 17 farms. However, only 6 farms (Farm 1, 2, 4, 7, 13 and 17) had *H. contortus* present with sufficient numbers of larvae to draw useful conclusion.

For BZ, the critical well from the DrenchRite® User Manual was 4.0 and for the susceptible isolate was 3.5. The critical wells for all 6 of these farms were above this value (range, 5.5 to 9.5), indicating suspected resistant *H. contortus* were present. Farm 1, 2, 4, 7, 13 and 17 had estimated efficacies of 6%, 23%, 46%, 89% and 89% respectively.

For LEV, the critical well from the DrenchRite® User Manual was 5.5 and for the susceptible isolate were 4.5. The critical wells ranged from 3.5 to 5.5 from all 6 farms, indicating they were considered to be susceptible. The estimated efficacies ranged from 94% to 100%.

For BZ+LEV, the critical well from the DrenchRite® User Manual was 5 and for susceptible isolate was 3.5. All farms had the critical wells below this value and consequently the estimated efficacy were >95%.

For IVM-1, the critical well from the DrenchRite® User Manual was 5.5 (range, 5.0 to 6.5) and for the susceptible isolate was 7.5. The critical wells for all 6 farms were lower than this value and zero larvae were grew in Wells 9 to 12, indicating *H. contortus* was likely to be susceptible.

For IVM-2, the critical well from the DrenchRite® User Manual was 5.5 (range, 5.0 to 7.0) and for susceptible isolate was 4.5. The critical wells for all 6 farms ranged from 2.5 to 4.5 and no larvae grew in Wells 9 to 12, indicating *H. contortus* was likely to be susceptible.

Table 3.2: Critical well and estimated efficacy (%) for BZ by comparison with the chart supplied with the DrenchRite® User Manual with EC_{50} values, RR and R^2 for different genera for 17 farms. Mean number (%) of L_3/well in Wells 9 to 12 compared to control wells also shown for each genus.

Farm ID	Benzimidazole (BZ)											
	Teladorsagia						*Trichostrongylus*					
	Critical well	Estimated efficacy (%)	Mean (%) of larvae in Wells 9 to 12[a]	EC_{50} (µM)	RR	R^2	Critical well	Estimated efficacy (%)	Mean (%) of larvae in Wells 9 to 12[a]	EC_{50} (µM)	RR	R^2
1.❶	n.s	n.s	n.s	n.s	n.s	n.a	9.5	2	14.4	1.26	31.5	0.48
2.❷	7.5	46	0.0	0.47	15.7	0.58	6.5	56	1.7	0.12	3.0	0.76
3.❸	7.5	46	12.7	0.16	5.3	0.40	7.5	32	4.1	0.57	14.3	0.85
4.❹	6.5	67	0.0	0.38	12.7	0.25	5.5	78	2.3	0.12	3.0	0.86
5.❺	6.5	67	2.6	0.48	16.0	0.29	3.5	100	0.0	0.07	1.8	0.82
6.❻	3.5	100	0.0	0.05	1.7	0.68	3.5	100	0.0	0.03	0.8	0.88
7.❼	4	99	0.0	0.03	1.0	0.51	6.5	56	2.0	0.07	1.8	0.90
8.❽	2.5	100	0.0	0.04	1.3	0.42	3.5	100	3.2	0.03	0.8	0.73
9.❾	n.s	n.s	n.s	n.s	n.s	n.a	7.5	32	7.2	0.50	12.5	0.92
10.❿	n.s	n.s	n.s	n.s	n.s	n.a	7.5	32	5.3	0.20	5.0	0.86
11.▼	8.5	25	10.0	0.84	28.0	0.64	9.0	6	13.2	0.42	10.5	0.79
12.★	8.5	25	8.8	0.24	8.0	0.36	8.5	13	10.1	0.33	8.3	0.90
13.◈	7.5	46	14.8	0.10	3.3	0.14	7.5	32	8.4	0.69	17.3	0.78
14.⊙	9.5	9	20.3	1.52	50.7	0.57	9.5	2	25.0	1.72	43.0	0.71
15.◆	8.5	25	6.3	0.81	27.0	0.56	9.5	2	14.8	1.23	30.8	0.68
16.×	8.5	25	6.8	0.58	19.3	0.57	7.5	32	9.4	0.39	9.8	0.90
17.□	8.0	35	5.0	0.60	20.0	0.45	8.5	13	3.3	0.49	12.3	0.88
□[b]	3.5	100	0.0	0.03	n.a	0.97	4.5	94	0.0	0.04	n.a	0.98
DR[c]	4.0	99					4.0	99				
R		79							82			

[a]mean (%) of larvae/well in Wells 9 to12 compared to control wells; [b]susceptibles isolates; [c]critical wells and estimated efficacy based on values in the table in the DrenchRite® User Manual; n.s (no sample): insufficient larvae to evaluate LDA; n.a (not applicable); R: percentage of resistance farms (efficacy <95%)

Table 3. 3: Critical well and estimated efficacy (%) for LEV by comparison with the chart supplied with the DrenchRite® User Manual with EC_{50} values, RR and R^2 for different genera for 17 farms. Mean number (%) of L_3/well in Wells 9 to 12 compared to control wells also shown for each genus.

Farm ID	Levamisole (LEV)											
	Teladorsagia						*Trichostrongylus*					
	Critical well	Estimated efficacy (%)	Mean (%) of larvae in Wells 9 to 12[a]	EC_{50} (µM)	RR	R^2	Critical well	Estimated efficacy (%)	Mean (%) of larvae in Wells 9 to 12[a]	EC_{50} (µM)	RR	R^2
1.❶	n.s	n.s	n.s	n.s	n.s	n.s	10.0	0	67.0	732.5	373.7	0.65
2.❷	4.5	100%	0.0	0.01	0.0	0.65	5.5	83	3.6	0.87	0.4	0.74
3.❸	2.0	100%	0.0	0.08	0.1	0.76	5.5	83	5.0	2.69	1.4	0.87
4.❹	4.5	100%	0.0	1.92	1.6	0.50	8.5	1	8.9	4.66	2.4	0.80
5.❺	4.5	100%	0.0	0.29	0.2	0.84	5.5	83	1.0	1.57	0.8	0.99
6.❻	2.0	100%	0.0	0.28	0.2	0.38	5.5	83	2.0	1.35	0.7	0.90
7.❼	2.5	100%	0.0	0.09	0.0	0.33	4.5	99	5.0	1.51	0.8	0.92
8.❽	4.5	100%	0.0	0.09	0.1	0.37	3.5	100	9.0	1.02	0.5	0.85
9.❾	n.s	n.s	n.s	n.s	n.s	n.s	8.5	1	51.8	18.25	9.3	0.53
10.❿	n.s	n.s	n.s	n.s	n.s	n.s	6.0	68	23.3	2.60	1.3	0.70
11.▼	2.5	100%	0.9	0.31	0.3	0.98	8.5	1	20.0	7.46	3.8	0.84
12.★	5.5	98%	2.9	0.11	0.1	0.72	8.5	1	42.2	16.85	8.6	0.92
13.◈	7.5	70%	7.4	0.01	0.0	0.67	10.5	0	52.7	25.39	13.0	0.77
14.⊙	6.5	87%	5.2	4.65	3.8	0.74	11.5	0	55.9	28.64	14.6	0.82
15.◆	6.5	87%	8.9	5.55	4.5	0.39	11.5	0	76.4	38.45	19.6	0.08
16.×	5.5	98%	2.7	2.19	1.8	0.83	10.5	0	51.1	26.48	13.5	0.75
17.□	6.5	87%	5.0	4.55	3.7	0.65	9.0	0	46.1	16.70	8.5	0.79
□[b]	4.5	100	0.0	1.22	n.a	0.95	5.5	83	0.6	1.96	n.a	0.97
DR[c]	5.5	98					4.5	99				
R		29%							88%			

[a]mean (%) of larvae/well in Wells 9 to12 compared to control wells; [b]susceptibles isolates; [c]critical wells and estimated efficacy based on values in the table in the DrenchRite® User Manual

n.s (no sample): insufficient larvae to evaluate LDA; n.a (not applicable); R: percentage of resistance farms (efficacy <95%)

Table 3. 4: Critical well and estimated efficacy (%) for BZ+LEV combination drugs by comparison with the chart supplied with the DrenchRite® User Manual for different genera for 17 farms. Mean number (%) of L_3/well in Wells 9 to 12 compared to control wells also shown for each genus.

Farm ID	Benzimidazole + Levamisole (BZ+LEV)					
	Teladorsagia			*Trichostrongylus*		
	Critical well	Estimated efficacy (%)	Mean (%) of larvae in Wells 9 to 12[a]	Critical well	Estimated efficacy (%)	Mean (%) of larvae in Wells 9 to 12[a]
1.❶	n.a	n.a	n.a	8.5	48	32.7
2.❷	5.5	100	0.0	6.5	87	1.8
3.❸	2.5	100	0.0	5.5	98	3.6
4.❹	4.5	100	0.0	6.5	87	1.9
5.❺	5.5	100	0.0	3.5	100	0.5
6.❻	4.5	100	0.0	4.5	100	0.0
7.❼	4.5	100	0.0	4.5	100	2.6
8.❽	2.5	100	0.0	4.5	100	3.9
9.❾	n.a	n.a	n.a	7.5	48	30.8
10.❿	n.a	n.a	n.a	5.5	98	18.3
11.▼	2.5	100	0.0	8.5	48	8.8
12.★	5.5	100	0.0	6.5	87	24.6
13.◈	6.5	81	3.7	8.5	48	31.3
14.⊙	9.5	81	5.2	9.5	28	41.7
15.◆	6.5	81	0.0	7.5	69	38.0
16.✕	5.5	100	0.0	8.5	48	37.3
17.□	6.0	94	10.0	8.5	48	37.0
□[b]	3.5	100	0.0	5.5	100	0.0
DR[c]	5.0	100		5.5	98	
R		29%			65%	

[a]mean (%) of larvae/well in Wells 9 to12 compared to control wells; [b]susceptibles isolates; [c]critical wells and estimated efficacy based on values in the table in the DrenchRite® User Manual; n.s (no sample): insufficient larvae to evaluate LDA; R: percentage of resistance farms (efficacy <95%)

Table 3. 5: Critical well, EC_{50} values, RR and R^2 for IVM-1 for different genera for 17 farms. Mean number (%) of L_3/well in Wells 9 to 12 compared to control wells also shown for each genus.

Farm ID	Ivermectin -1 (IVM-1)									
	Teladorsagia					*Trichostrongylus*				
	Critical well	Mean (%) of larvae in Wells 9 to 12[a]	EC_{50} (nM)	RR	R^2	Critical well	Mean (%) of larvae in Wells 9 to 12[a]	EC_{50} (nM)	RR	R^2
1.❶	n.s	n.s	n.s	n.s	n.s	8.5	7.8	25.04	4.3	0.40
2.❷	7.0	44.0	66.33	11.4	0.34	7.5	25.0	17.26	3.0	0.52
3.❸	8.5	21.8	2.09	0.4	0.23	7.5	4.5	16.11	2.8	0.80
4.❹	7.5	11.7	4.41	0.8	0.32	6.5	3.1	15.41	2.7	0.92
5.❺	6.0	35.8	60.13	10.3	0.69	5.5	7.2	6.36	1.1	0.97
6.❻	6.5	0.0	4.33	0.7	0.81	7.5	0.5	4.46	0.8	0.83
7.❼	3.5	0.0	0.25	0.0	0.79	7.5	2.0	4.24	0.7	0.81
8.❽	7.5	18.1	1.64	0.3	0.40	8.5	4.7	3.74	0.6	0.65
9.❾	n.s	n.s	n.s	n.s	n.s	8.5	21.4	37.11	6.4	0.89
10.❿	n.s	n.s	n.s	n.s	n.s	8.5	8.9	12.41	2.1	0.80
11.▼	8.5	22.6	9.26	1.6	0.46	8.5	32.3	76.71	13.3	0.75
12.★	8.5	52.5	246.0	42.2	0.47	9.5	26.0	44.37	7.7	0.89
13.◈	6.0	12.9	7.37	1.3	0.47	7.5	10.6	29.21	5.1	0.90
14.⊙	10.5	52.0	7.78	1.3	0.89	9.5	40.1	231.60	40.0	0.56
15.◆	10.0	33.0	99.17	17.0	0.28	9.5	22.7	63.84	11.0	0.92
16.✕	8.5	16.4	0.47	0.1	0.34	8.5	10.2	22.57	3.9	0.82
17.□	10.5	50.0	80.50	13.8	0.55	8.5	8.0	34.93	6.0	0.93
□[b]	7.5	0.0	5.83	n.a	0.94	5.5	1.1	5.78	n.a	0.93
DR[c]	5.5 (5.0-6.5)					4.5 (4-5.5.0)				
R	71					94				

[a]mean (%) of larvae/well in Wells 9 to12 compared to control wells; [b]susceptibles isolates; [c]critical wells based on values in the DrenchRite® User Manual; n.s (no sample): insufficient larvae to evaluate LDA; n.a (not applicable); R: percentage of resistance farms (upper limit from the susceptible range wells supplied by the DrenchRite® User Manual)

Table 3. 6: Critical well, EC_{50} values, RR and R^2 forIVM-2 for different genera for 17 farms. Mean number (%) of L_3/well in Wells 9 to 12 compared to control wells also shown for each genus.

Farm ID	Ivermectin -2 (IVM-2)									
	Teladorsagia					*Trichostrongylus*				
	Critical well	Mean (%) of larvae in Wells 9 to 12[a]	EC_{50} (nM)	RR	R^2	Critical well	Mean (%) of larvae in Wells 9 to 12[a]	EC_{50} (nM)	RR	R^2
1.❶	n.s	n.s	n.s	n.s	n.s	9.0	43.0	293.8	40.6	0.75
2.❷	6.5	7.4	26.50	1.3	0.67	9.0	28.0	106.1	14.7	0.89
3.❸	8.0	10.9	1.99	0.1	0.63	8.5	51.9	50.44	7.0	0.75
4.❹	7.5	0.0	1.69	0.1	0.64	7.5	15.5	96.74	13.4	0.69
5.❺	6.5	25.6	76.99	3.7	0.81	6.5	24.5	10.46	1.4	0.98
6.❻	7.0	0.0	0.37	0.0	0.44	7.5	7.2	11.00	1.5	0.71
7.❼	5.0	0.0	13.30	0.6	0.86	7.5	14.2	22.54	3.1	0.85
8.❽	7.0	6.0	13.36	0.6	0.66	7.5	11.5	17.92	25.0	0.96
9.❾	n.s	n.s	n.s	n.s	n.s	9.0	45.7	267.70	37.0	0.60
10.❿	n.s	n.s	n.s	n.s	n.s	8.5	27.8	16.35	2.3	0.34
11.▼	8.5	9.4	119.10	5.8	0.50	8.5	42.6	154.20	21.3	0.42
12.★	7.5	5.8	15.82	0.8	0.71	7.5	27.4	37.54	5.2	0.92
13.◈	7.0	0.0	6.03	0.3	0.48	8.0	27.0	105.30	14.6	0.85
14.⊙	8.5	8.3	75.32	3.7	0.78	10.5	55.8	781.3	108.1	0.63
15.◆	7.5	7.6	35.75	1.7	0.63	10.5	45.4	304.80	42.2	0.70
16.×	8.5	5.5	50.57	2.5	0.68	8.5	23.4	63.27	8.8	0.75
17.□	10.5	20.0	85.28	4.1	0.26	9.5	30.0	44.81	6.2	0.61
□[b]	7.5	2.9	20.63	n.a	0.96	4.5	12.9	7.23	n.a	0.98
DR[c]	6.0 (5.5-7.5)					5.5 (5.0-7.0)				
R	36					88				

[a]mean (%) of larvae/well in Wells 9 to12 compared to control wells; [b]susceptibles isolates; [c]critical wells based on values in the DrenchRite® User Manual; n.s (no sample): insufficient larvae to evaluate LDA; n.a (not applicable); R: percentage of resistance farms (upper limit from the susceptible range wells supplied by the DrenchRite® User Manual)

Table 3. 7: Summary of anthelmintic resistance status of *Teladorsagia* and *Trichostrongylus* from 17 goat farms.

Farm ID	*Teladorsagia*					*Trichostrongylus*				
	BZ	LEV	BZ+LEV	IVM-1	IVM-2	BZ	LEV	BZ+LEV	IVM-1	IVM-2
1.	n.a	n.a	n.a	n.a	n.a	R	R	R	R^s	R^+
2.	R	S	S	S^+	S^s	R	R	R	R^+	R^+
3.	R	S	S	R^+	R^+	R	R	S	R^s	R^+
4.	R	S	S	S^+	S^s	R	R	R	R^s	R^s
5.	R	S	S	S^+	S^+	S	R	S	S^s	S^+
6.	S	S	S	S^s	S^s	S	R	S	R^s	R^s
7.	S	S	S	S^s	S^s	R	S	S	R^s	R^s
8.	S	S	S	S^+	S^s	S	S	S	R^s	R^s
9.	n.a	n.a	n.a	n.a	n.a	R	R	R	R^+	R
10.	n.a	n.a	n.a	n.a	n.a	R	R	S	R^s	R^+
11.	R	S	S	R^+	Rs	R	R	R	R^+	R^+
12.	R	S	S	R^+	S^s	R	R	R	R^+	R^+
13.	R	R	R	S^+	S^s	R	R	R	R^+	R^+
14.	R	R	R	R^+	R^s	R	R	R	R^+	R^+
15.	R	R	R	R^+	S^s	R	R	R	R^+	R^+
16.	R	S	S	R^+	R^s	R	R	R	R^s	R^+
17.	R	R	R	R^+	R^+	R	R	R	R^s	R^+

n.a : not applicable

R: Farm with resistance status for that anthelmintic

S: Farm with susceptible status for that anthelmintic

R^+: Farm with resistance status and the number of larvae in Wells 9 to 12 was >10% for IVM-1 with both species or >10% for IVM-2 with *Trichostrongylus* or >20% with *Teladorsagia*

S^+: Farm with susceptible status and the number of larvae in Wells 9 to 12 was >10% for IVM-1 with both species or >10% for IVM-2 with *Trichostrongylus* or >20% with *Teladorsagia*

R^s: Farm with resistance status and the number of larvae in Wells 9 to 12 was <10% for IVM-1 with both species or <10% for IVM-2 with *Trichostrongylus* or <20% with *Teladorsagia*

S^s: Farm with susceptible status and the number of larvae in Wells 9 to 12 was <10% for IVM-1 with both species or <10% for IVM-2 with *Trichostrongylus* or <20% with *Teladorsagia*

: Isolate indicating resistance to BZ, LEV and IVM

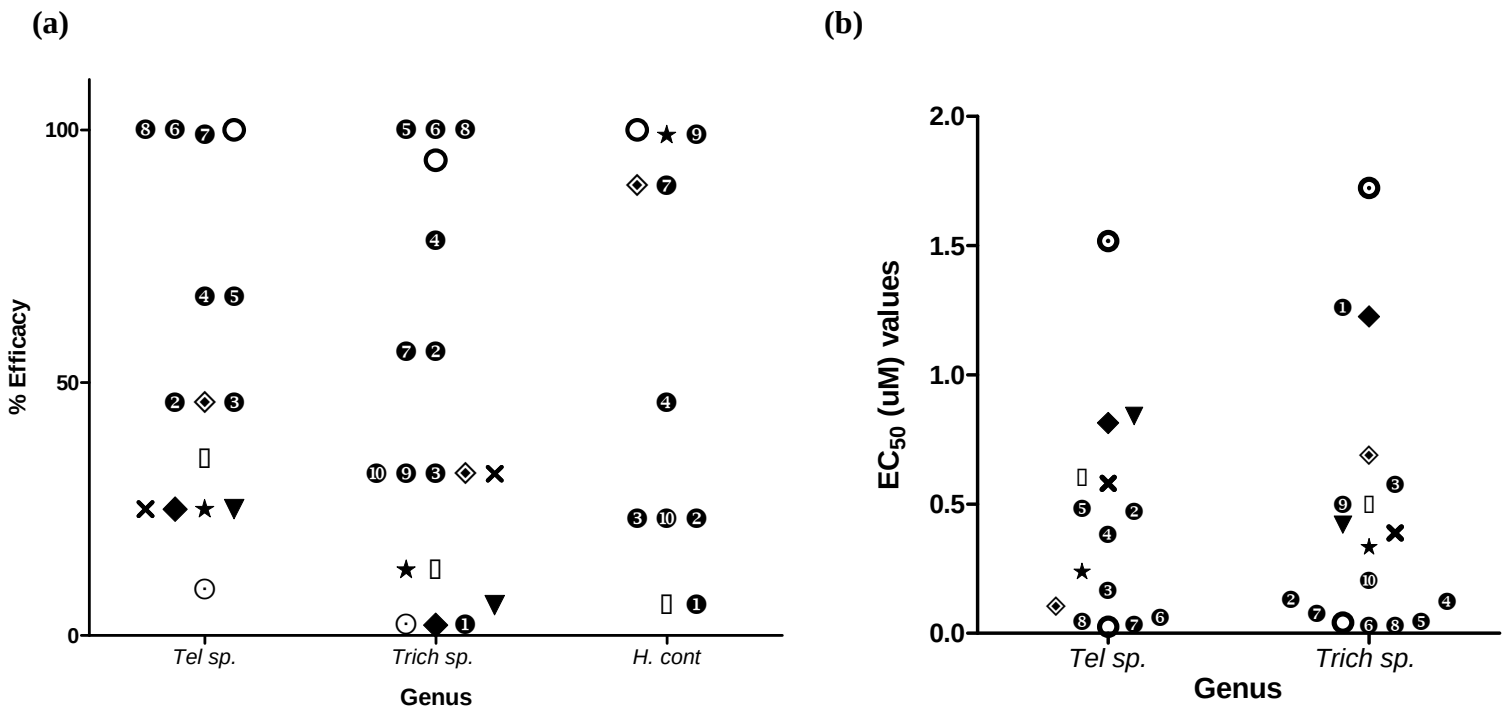
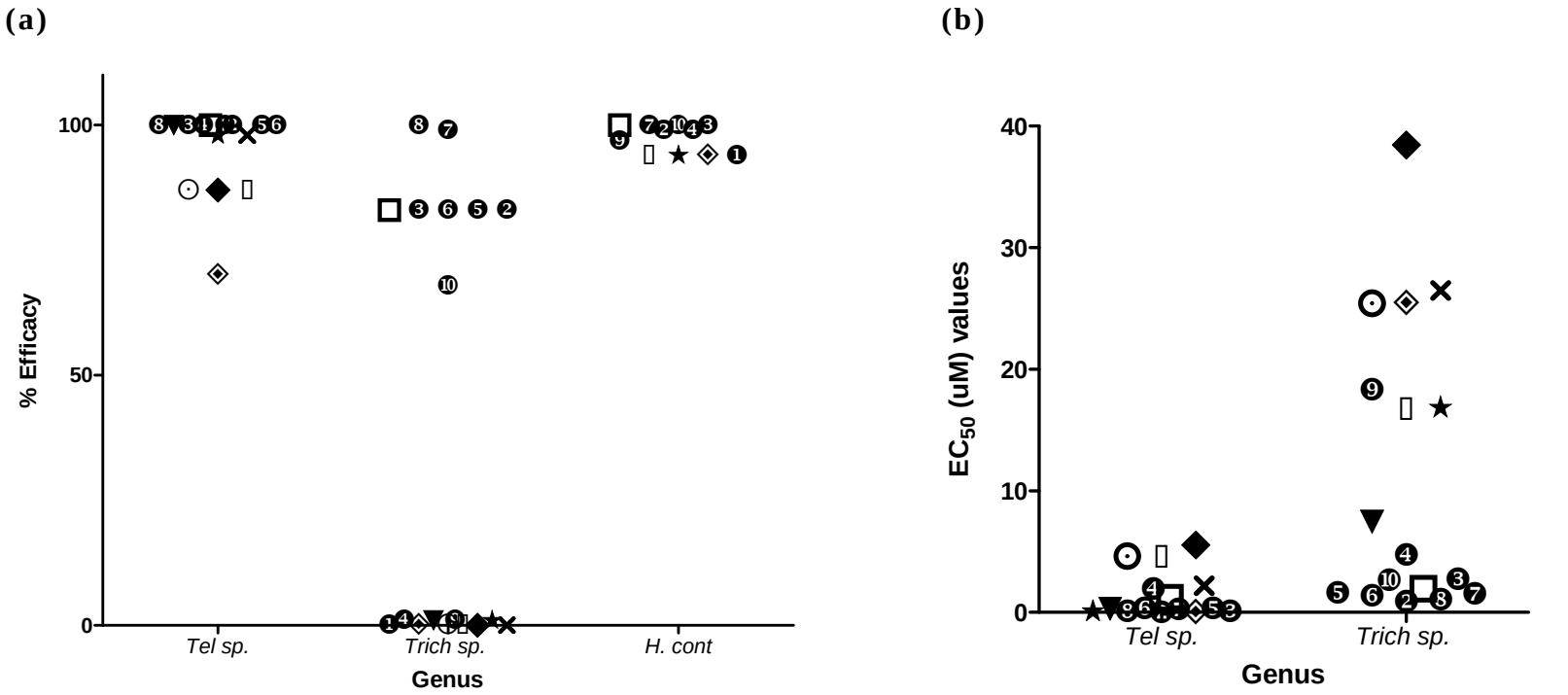


Figure 3.2: Efficacy (%) of BZ (a) by comparison with the chart supplied with the DrenchRite® User Manual and EC_{50} values (b) for different genera on 17 farms. Values are also shown for susceptible isolates of BZ (○).



Outlier for farm❶ (*Trichostrongylus*) = 732.5µM

Figure 3.3: Efficacy (%) of LEV (a) by comparison with the chart supplied with the DrenchRite® User Manual and EC_{50} values (b) for different genera on 17 farms. Values are also shown for susceptible isolates of LEV (□).

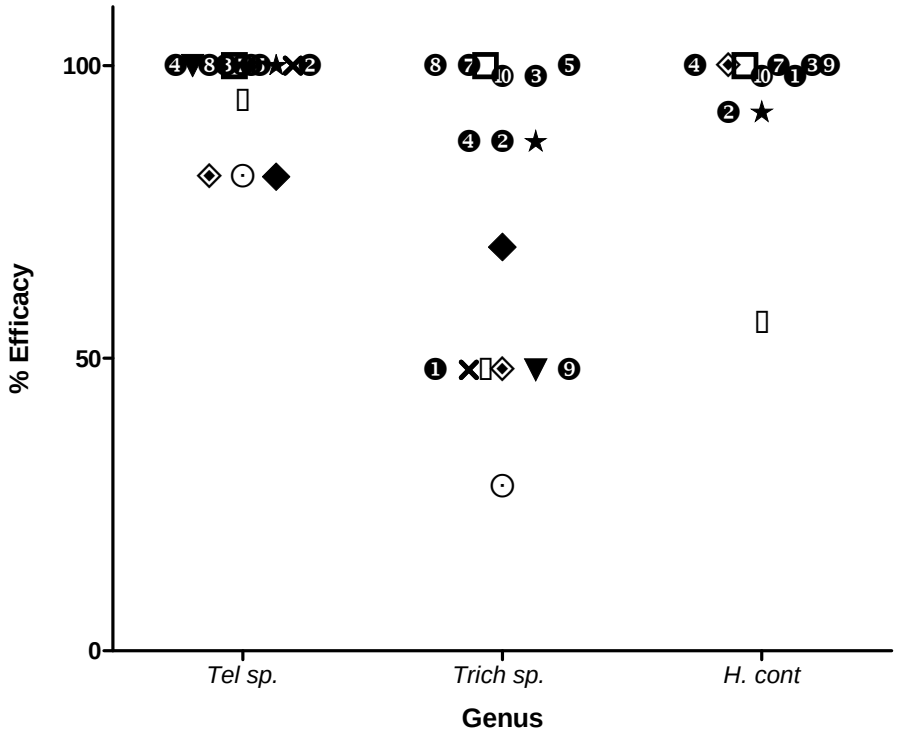


Figure 3.4: Efficacy (%) of BZ+LEV combination by comparison with the chart supplied with the DrenchRite® User Manual for different genera on 17 farms. Values are also shown for susceptible isolates of BZ+LEV (○).

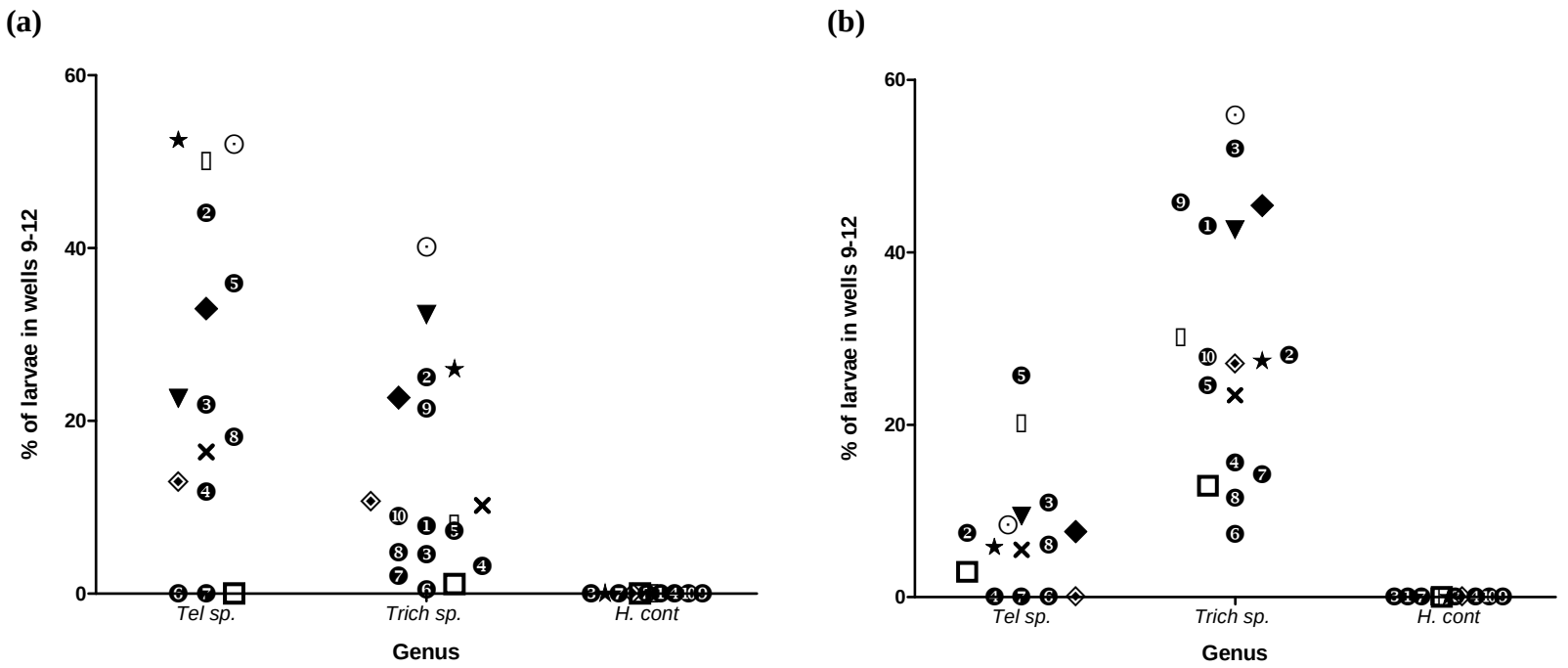


Figure 3.5: Mean number (%) of L_3/well in Wells 9 to 12 compared to control wells for different genera on 17 farms for IVM-1 (a) and IVM-2 (b). Values are also shown for susceptible isolates of IVM (□).

3.4 DISCUSSION

In this study the anthelmintic resistance status of 17 farms was assessed using a larval development assay. By the criteria used severe levels of anthelmintic resistance were found on a large number of these farms (Table 3.7). In particular multiple resistance to all three existing anthelmintics was considered to be present in *Trichostrongylus* on 12 of the 17 farms which is of serious concern. For one of these the combination of BZ and LEV was apparently still effective but not for the other 11. For *Teladorsagia* multiple resistance to all three existing anthelmintic groups was considered to be present on 5 of the 14 farms assessed. As the parasite burdens in the goats at the time of the year this survey was conducted were dominated by *Trichostrongylus* and *Teladorsagia*, only limited data was available for *H. contortus* and not for other gastrointestinal nematode parasites.

The commercially available DrenchRite® LDA was used in this survey and it includes duplicate wells containing BZ, LEV and a combination of a BZ+LEV. When this assay was developed a considerable amount of work was undertaken to compare the results from this LDA with the results for a number of experimentally isolated resistant strains where the resistant status was known based on worm count reduction (Dobson et al., 1998; Lacey et al., 1993). Hence the assessment of efficacy for these two actives and their combination is reasonably robust. The assessment of efficacy for LEV is less straight forward than for the BZ as it is necessary to consider the component which is highly resistant and grew in Wells 9 to 12 together with the remainder of the results from the assay (see Appendix 2.6). Nevertheless, the assessment has been shown to be reflective of the actual drench efficacy for both LEV and BZ+LEV. This is also demonstrated in the present study for the susceptible isolates. However, as discussed in Section 2.4, the estimates of efficacy for the *T. colubriformis* isolate suggested 94% and 83% efficacy for BZ and LEV respectively although the combination of BZ+LEV was 100% indicating this isolate may be demonstrating a low level of resistance to these two anthelmintics. Consequently a comparison of test farms with this *T. colubriformis* isolate is likely to be conservative in terms of determining their resistant status and particularly when considering the RR value calculated from values obtained from this isolate.

The assessment of ML resistance is less straight forward. The DrenchRite® plate also includes one row containing doubling concentrations of IVM monosaccharide (IVM-1) and the second row IVM aglycone (IVM-2). These two analogues of IVM were chosen as they appeared to maximise the RR for known resistant isolates of *H. contortus* (Gill et al., 1998; Le Jambre et al., 1995). At the time this assay was developed ML resistance was rare even for *H. contortus* and effectively unknown for *T. circumcincta* and *T. colubriformis* and hence it was not possible to undertake a comparison with known resistant isolates for these other two species. Instead the critical wells for known susceptible strains were determined and a range also given (Table 3.5 and 3.6). For this survey these have been used as one criteria to determine resistance. In addition, an arbitrary decision was made to consider the percentage of larvae for each species which developed in Wells 9 to 12 for both IVM-1 and IVM-2 and compare this for each farm with the results for the susceptible isolates (see Table 3.5 and 3.6). Consequently for each farm there are 2 IVM analogues and two criteria applied to each of these analogues. For each farm resistance to a species was considered to be present if at least two of these four criteria indicated resistance. During the 1980s and 1990s it became apparent that the DrenchRite® assay had difficulty determining the resistance status of *T. circumcincta* (Palmer et al., 1998). In that report they had evidence of insensitivity to detect a low-level of resistance of this species and also a difficulty when dealing with a minority genus in an assay. Consequently the results from this present survey will invariably underestimate the prevalence of ML resistant *T. circumcincta* with the criteria used. In contrast, for *T. colubriformis* the ability of DrenchRite® to detect ML resistance is more established, especially using IVM aglycone (IVM-2). Kaplan et al. (2007) reported that they found IVM-2 much more sensitive in detecting ML resistance and the RR values invariably much higher to the extent that they ignored the results from IVM-1 in a recent survey of resistance in goat parasites in the U.S.A. In the present survey, RR values were estimated but as the Coefficient of Determination (R^2) value was often less than 0.90 it was generally considered to be of less value than the more straight forward determination of the critical well. This is particularly the case for *T. circumcincta* which was generally the less numerous genus present and a small variation in count of L_3 would more severely influence the R^2 value. Interestingly, the R^2 values for the two susceptible isolates were high being ≥ 0.93 when considering all fitted curves.

Anthelmintic resistance has only been formally surveyed in goats once in New Zealand in 1983 (Kettle et al., 1983). In that survey only dairy goats were involved and resistance was detected using a faecal egg count reduction test with 79% of 47 farms having egg count reductions of <80%. Resistance to BZs occurred on 74% of farms with *Trichostrongylus* being predominant in post-treatment larval cultures on 17 farms, *Teladorsagia* on 17 farms and *Haemonchus* on 4 farms. In contrast the most recent data on resistance in sheep nematodes in New Zealand was from a national survey in 2004/5 (Waghorn et al., 2006) and found 41% of 80 farms had evidence of BZ resistance in *Teladorsagia* and 21% of 80 farms had evidence of BZ resistance in *Trichostrongylus*. Whilst a smaller number of farms were surveyed in the current survey the level of BZ resistance was somewhat more severe than both of these earlier surveys with 14 farms of the 17 having resistance in *Trichostrongylus* and 11 of 14 farms having resistance to *Teladorsagia*. For both of these genera the level of resistance was generally severe with efficacy being <50% for the majority of those showing resistance. This indicates that BZs are now of very limited use for these controlling nematodes in goats in New Zealand.

For LEV, the earlier survey of dairy goats (Kettle et al., 1983) found resistance to LEV on 42% of the 47 goat farms with *Trichostrongylus* being predominant in post-treatment larval cultures on 17 and *Teladorsagia* on 23 of these farms. Again in contrast the recent survey of sheep nematodes (Waghorn et al., 2006) found 24% of 80 farms with evidence of resistance in *Teladorsagia* and 14% of 80 farms with evidence of resistance in *Trichostrongylus*. Interestingly only 4 of 14 farms in the current survey had evidence of LEV resistant *Teladorsagia* which is similar to the recent sheep nematode survey and less than the earlier survey in goats. However, by contrast the situation with *Trichostrongylus* in the current survey indicated the level of LEV resistance was more severe with 15 of the 17 farms having resistance and for most the estimate of efficacy was <50%. This is higher than for either earlier survey and generally more severe than either as well. Thus for *Teladorsagia* LEV is still showing reasonable efficacy and consequently useful whilst for *Trichostrongylus* LEV would appear to be of limited use.

The results of the combination of BZ+LEV are as predicted by the individual BZ or LEV results with no apparent additive effect. Thus if either BZ or LEV were still

effective then the combination was effective but if individually both had evidence of resistance the efficacy of the combination was also not fully effective. This is in contrast to the situation seen in the field with sheep (Waghorn et al., 2006) where the use of both together often resulted in good efficacy even though neither component was fully effective on their own. In this current survey this failure to get any useful additive efficacy probably reflects the very severe nature of the resistance seen with either BZ or LEV.

IVM was not yet released for sheep when the earlier survey in goats occurred. In the recent survey of sheep nematodes IVM resistance was found in *Teladorsagia* on 27% of 52 farms which is a relatively similar percentage to the results seen in the current survey. However, as mentioned above, the ability of the LDA to detect ML resistance in *Teladorsagia* is limited so this estimate may be an under-representation suggesting the general prevalence of ML resistance could be much higher in *Teladorsagia* on goat farms than these figures suggest. In the national sheep nematode survey only 3% of farms were determined to have ML-resistant *Trichostrongylus* which is in stark contrast to the current survey where 16 of the 17 farms were considered to have IVM resistant *Trichostrongylus.* This represents a serious issue for the New Zealand goat industry but also potentially a serious issue for the sheep industry as well. ML-resistant *T. colubriformis* were first reported in New Zealand in 1999 (Gopal et al., 1999) and erratic reports have been made of their occurrence since that time including (West et al., 2004) who detected a multiple-resistant *Trichostrongylus* and *Teladorsagia* in goats using a FECRT where even a double dose of all three actives did not effectively reduce the egg count for either species. The most disturbing finding is the occurrence of so many farms that had evidence of multiple resistance to all three anthelmintic types in *Trichostrongylus.* The study reported in Chapter 2 is the first to experimentally infect sheep and conclusively demonstrate multiple resistance in *T. colubriformis*. Others (Watson et al., 1996) have also taken goat isolates and assessed the efficacy of anthelmintics after infecting sheep with these isolates which removes the question about efficacy of different dose rates for various anthelmintics in goats. The results from this present survey suggest that such isolates are common in goats in New Zealand. This is a potentially very serious situation as it suggests that only the recently released new anthelmintic monepantel (Hosking et al., 2008; Sager et al., 2009) is

likely to be of use on most of these goat farms from now on, even though it is currently not licenced for use in this species.

H. contortus was only present in sufficient number on 6 farms and the only resistance detected was to BZ on all of them. This is a higher prevalence than reported for sheep of 8% as recorded by Waghorn et al. (2006), suggesting again more severe level of resistance in parasites from goats. Absence of resistance to LEV is consistent with these earlier findings in *H. contortus* in sheep as is absence of IVM resistance.

That anthelmintic resistance is more serious in goats than sheep is not surprising as there a number of factors that are likely to have contributed to this occurring. These include the poor immune response that is developed by goats. Although adults goats can develop some immunity to *T. colubriformis* (Pomroy et al., 1992) in general the level of immunity is sufficiently poor that adults goats frequently require anthelmintic treatment (Scherrer et al., 1989) whereas adult sheep in New Zealand rarely need anthelmintic treatment and can effectively control nematodes without anthelmintic assistance. In 1983 kids were reported to be treated with anthelmintic 12.5 times per year whereas for does (older than one year) 13.4 times per year (Kettle et al., 1983). Studies in 1988 reported similar results as adult goats were reported to be treated with anthelmintic 10 or more times per year (Scherrer et al., 1990). Another factor that is likely to have contributed is the rapid metabolism of anthelmintics by goats which means that use of sheep dose rates effectively means that goats are underdosed. Underdosing is a critical issue that needs to be addressed in goats. Goats are known to metabolize anthelmintics more rapidly than sheep and generally require a higher dose rate to maximize the efficacy of anthelmintics (Hennessy, 1994; Reinemeyer and Pringle, 1993). Studies suggested that higher doses of LEV (McKenna and Watson, 1987), albendazole (Hennessy et al., 1993) and oxfendazole (Bogan et al., 1987; Sangster et al., 1991b) were necessary in goats than in sheep. Use of lower dose rates is recognised as a precipitating factor for anthelmintic resistance (Leathwick et al., 2009; Leathwick et al., 2001).

Results from the survey show that the respondents generally have a very small farm size with a small number of goats. Five respondents (36%) indicate that goat numbers ranged from 0-50 and another 5 respondents had 50-200 goats whereas 2 respondents

(14%) had 200-500 goats. Another 2 respondents (14%) have shown that the number of goats they had were >1000. Whether the size of the farm is relevant to the development of resistance is unknown but these observations are consistent with the current farming practices of goats in New Zealand where they are believed to be largely on small farms.

Goat farmers always have the tendency to think that the dose rates for sheep are the same for goats. Results from the questionnaires have shown that 22% of the goat farmers still treated goats at the sheep dose rate. As there are differences in metabolism and pharmacokinetics between sheep and goats, goats generally require a higher dose compared to sheep (Elliott, 1987; McKenna and Watson, 1987; Pomroy et al., 1992). On the positive side it is apparent that approximately 64% of the goat farmers followed this recommendation by giving more than the sheep dose rate. One of the respondent (7%) indicated he/she varied between using the sheep dose rate and greater than the sheep dose rate. As this question did not relate to specific anthelmintics this likely reflects different dose rates with different anthelmintics as some such as IVM actually have a valid claim for efficacy using the same dose rate as for sheep.

Results from the survey indicate that 14% (2 from 17 farms) of the respondents kept sheep together with goats. This is a reduced proportion compared with the situation in New Zealand in the 1980s where up to 87% of goat farmers were reported to graze sheep on goat farms and 41% grazed them together (Pearson and MacKenzie, 1986). Sharing grazing with sheep is potentially hazardous as cross infection is likely to happen as it has been shown that sheep can be readily infected with parasites derived from goats (Gopal et al., 1999; Watson, 1994; Watson et al., 1996) (results from Chapter 2). Given the high proportion of severely resistant parasites found in the current study, cross-grazing will promote the movement of these parasites into the general sheep population.

This survey showed that 7% of farms cross-grazed with cattle, deer or horses. Such a practice would facilitate better parasite control as these hosts do not effectively share parasites with goats. It is somewhat disturbing that so few goat farmers were adopting this policy to endeavour to improve their parasite control and reduce reliance on anthelmintics.

From the questionnaire only 36% (5 from 14 farms) of the respondents had conducted a drench resistance study on their farm. Of these, only 40% (2 from 5 farms) had mentioned the results for their drench resistance study. One farm (Farm 3) indicated evidence of IVM resistance in *Teladorsagia* whilst the second (Farm 9) reported that BZ, LEV and IVOMEC only achieved 67%, 68% and 47% efficacy respectively. Given the results from this survey the fact that so few goat farmers were aware of their resistance status is disturbing and would suggest that probably many have not been achieving good parasite control.

The faecal egg counts in submitted samples were high on many farms with 8 having a mean count >1000 eggs/g. No specific request was made to allow animals to become parasitized prior to sample submission except to not submit within 4 weeks of treatment. Whilst interpretation of egg counts in goats is poorly understood such high egg counts are surely much higher than desirable and suggest parasitism is an issue on these farms. Interestingly, for the 4 farms who submitted samples within 4 to 8 weeks of treatment the FEC were all generally high, being >500epg and 3 of the 4 had triple resistance in *Trichostrongylus* and 2 of these also had triple resistance *Teladorsagia*.

Time after last drench is very important. In this study, 29% (4 of 17 farms) had sent their faecal samples from goats that were 4 to 8 weeks after the last drenched whereas 71% (10 of 17 farms) were more than 8 weeks. Three more of the goat farms were indicated as missing responses. All farmers complied with the request to not submit faeces within 4 weeks of treatment. The significance behind this time length is associated with the pre-patent period (PPP) for trichostrongylids. The minimum PPP is 2 to 4 weeks which means 3 weeks after treatment with anthelmintic means that parasites picked up in the first 1 to 2 days after treatment will be starting to lay eggs. These parasites need to be 4 to 5 weeks of age to be at full egg laying capacity (Soulsby, 1982). Therefore, at least 4 weeks after treatment is the time to get a more meaningful interpretation of resistance status of parasite on the farm. If samples had been submitted earlier after drenching there would have been a bias as it would have been only parasites surviving the drench that had contributed the eggs.

Last but not least is regarding the surveys in this study. It is believed that giving back feedback and useful information to the participants will encourage them to participate further in future surveys and other research related activities.

In conclusion, the present study has provided evidence of anthelmintic resistance in gastrointestinal nematodes in goats in New Zealand. The DrenchRite® LDA has proven to be useful in detecting resistance in three predominant species of worms to BZ, LEV, BZ+LEV combination drugs and IVM in New Zealand. However, as only this single assay was being used for diagnostic purposes in this study, further studies are needed to improve our knowledge to properly interpret the efficacy of the anthelmintic and at the same time to improve the quality of the results. High levels of multiple resistance are much more severe than is commonly recognized is a disturbing finding. In future, goat farmers need to gain more knowledge on various aspects of goat parasitology and parasite management. A better understanding of goat-parasite interactions in the host and more sustainable use of anthelmintics, especially for new anthelmintics are required. For many farmers in this survey the only effective anthelmintic will be monepantel and they need to take action to conserve its effectiveness for as long as possible.

CHAPTER FOUR

GENERAL DISCUSION

The results presented in Chapter 2 and Chapter 3 illustrates the presence of severely resistant *T. circumcincta* and *T. colubriformis* in goats in New Zealand. In Chapter 2 the presence of a triple resistant *T. circumcincta* was a new confirmation using worm count data of this occurring but in many respects expected given previous reports. However, the presence of an isolate of *T. colubriformis* that was able to survive a double dose of this combination of anthelmintics is a somewhat more sinister discovery although West et al. (2004) had indicated a similar situation based on FECRT data. In general *T. colubriformis* is a more pathogenic parasite than *T. circumcincta* (Dobson et al., 1992; Sykes et al., 1988) so the presence of these parasites will result in clinical disease and possible death on farms where such parasites occur unless use of monepantel is adopted.

The comparisons of reduction in worm counts with results for the FECRT illustrate the potential limitations of this latter approach. Despite the poor efficacy demonstrated by the worm counts the reduction in egg counts did not indicate severe resistance. Other reports have made similar observations and as most surveys rely on use of egg count reduction some reservations should be made about their results. It would appear this is particularly a problem with the ML group of anthelmintics (Jackson and Coop, 1995).

Use of the DrenchRite® LDA with the confirmed resistant isolates in Chapter 2 was somewhat frustrating as again the results were not consistent with the results of the worm count reduction. In many respects this reflects the small number of larvae of *Teladorsagia* and *Trichostrongylus* which grew, especially for Group 1 where *Haemonchus* dominated the egg count. The Coefficient of Determination (R^2) values were consistently low which is again consistent with small numbers of larvae present. The use of critical wells was a more convincing approach and the estimate efficacy indicated that both *Teladorsagia* and *Trichostrongylus* were resistant to BZ, LEV and the combination of BZ+LEV. However, interpretation of the ML efficacy is less consistent although observing the number of larvae growing in Wells 9 to 12 did indicate many more larvae of *Teladorsagia* grew for the resistant isolate than for the

susceptible isolate. Again this was not consistent for *Trichostrongylus* where there was no real difference between the numbers in Wells 9 to 12 for the susceptible and the resistant isolate. These results could suggest the interpretation of LDA results in Chapter 3, especially for IVM, is conservative.

The survey in Chapter 3 reveals that severe levels of anthelmintic resistance to BZ, LEV and ML are common on goat farms for *Trichostrongylus* and *Teladorsagia*. Although not randomly selected, these results must still be generally indicative of the situation throughout New Zealand. Use of the LDA made the survey logistically possible but does present problems with interpretation of ML resistance, at least in *Teladorsagia*. The absence of validated efficacy comparison for IVM also limits interpretation. However, the combined uses of different criteria as detailed in Chapter 3 provide a somewhat more robust level of interpretation. Nevertheless, this approach is likely to be conservative and only detect severe levels of ML resistance. As the survey was generally conducted over the autumn/winter period the dominance of *Trichostrongylus* is not surprising as that has generally been reported from sheep (Familton and McAnulty, 1994). However, there were still enough *Teladorsagia* to make interpretation possible for most farms. Not surprisingly *H. contortus* was present in sufficient numbers on only 6 out of 17 farms.

These results indicate that goat farmers in New Zealand are now likely to be highly dependent on the use of monepantel. It is imperative that approaches are adopted to conserve the efficacy of this novel anthelmintic and any others that may be released in the future. These approaches have been discussed in several recent reviews (Leathwick et al., 2009; Leathwick et al., 2001). Many possible measures could be put in place on such farms and this is likely to vary from farm to farm. In general goat farmers need to understand the concept of maintaining a refugia of unselected nematodes and take measures to achieve effective refugia population on their farm. There have been very few recent surveys of anthelmintic resistance in goat parasites anywhere in the world except for recent studies in the Southeast United State. Although dominated by some different genera, the levels of anthelmintic resistance in the present study are similar to those reported by Howell et al. (2008) in these southeast states of the U.S.A. This supports the widely held view that anthelmintic resistance in goat parasites is a global problem.

It is also clear that good quarantine procedures need to be put in place to avoid importing such resistant parasites with any animal purchases. The occurrence of these multiresistant parasites in goats poses significant issues for the sheep industry. Whilst only 2 of the 14 respondents in the survey also farmed sheep this still highlights the possibility of these parasites being transferred to the general sheep population. It is generally accepted that these goat-derived parasites are equally infective for sheep as shown in Chapter 2.

The biggest challenge in culturing *Teladorsagia* and *Trichostrongylus* eggs in the LDA plate was to get enough number of mature L_3 in each well so that response curves for each species can be generated with a reasonable R^2 value. In the present study, 60 eggs were dispensed in each well, thus suggesting the probability of getting enough L_3 was a challenging task and this highlighted the limitation of LDA in detecting resistance. However, based on the DrenchRite® User Manual the optimum egg per well is stated as 50 to 70 eggs. If the number of dispensed eggs is >70, it will make the counting relatively slow and difficult. Therefore, it is imperative that cleaning and preparation of nematode eggs to minimize plate contamination is undertaken to ensure the development of L_3.

Another issue with the LDA is a high estimate of efficacy but still observing larvae in Wells 9 to 12. For example, when the reading of estimated efficacy was >95%, larvae still grew in the high concentrations in Wells 9 to 12, thus suggesting that some resistant larvae was present on these goat farms. Another issue is regarding the pattern of distribution of larvae per well. In the present study, it was observed that the number of larvae detected fluctuated between each of the wells which are will also affect the EC_{50} and R^2 values as well particularly when only low numbers were present. It is possible that a few goat farms in the present study may have been incorrectly classified because of the wrong interpretation of resistance ratio (RR) resulting in farms being classified as having no resistance whereas resistance was actually present. Due to the method of interpretation it is much less likely the reverse interpretation of declaring resistance when they were truly susceptible was made. For *Teladorsagia*, the DrenchRite® study in Australia in the 1990s reported that the critical well was 10.5 for IVM-2, thus suggesting larvae were expected to grow in Wells 9 to 12 (Lloyd, 1998). Interestingly, results from this study indicate that almost all goat farms had larvae

which grew in Wells 9 to 12. Thus increasing the anthelmintic concentration of this assay is somewhat necessary in order to provide more data points for the higher concentrations of this anthelmintic.

The survey results also indicate concern about the ability of farmers to know or estimate the efficacy of different anthelmintics available to treat goats on their farms. It appears that few were aware of the poor efficacy shown in the survey for their farm. Their future success is not only about controlling parasites but also to know the resistance status of nematodes on the farms; for example by performing an *in vitro* LDA or FECRT on a regular basic. Any farmer should use an anthelmintic with high efficacy if they are seeking to achieve good parasite control.

An isolate of *T. colubriformis* and *T. circumcincta* was shown to demonstrate severe levels of resistance to a combined dose of BZ, LEV and abamectin given together. The results from the survey of 17 farms using the LDA indicate that such severe levels of resistance are not uncommon on goat farms in New Zealand.

APPENDICES: STANDARD OPERATING PROCEDURE (SOP) FOR CHAPTER 2 AND 3

Appendix 2.1: Modified McMaster technique for counting of eggs SOP

Equipment/chemical

Bowl (100ml capacity)

Coarse sieve (0.85mm)

Counter (Clay Adams)

Electronic balance (accuracy ±0.1g)

McMaster egg counting slide

Microscope

Pasteur pipette and rubber bulb

Saturated sodium chloride solution; NaCl (specific gravity 1.2)

Teaspoon

Universal bottle (28ml capacity)

Procedure

1. Bowl, sieve and teaspoon were place on the balance and press tare to adjust the weight to zero.
2. Two grams of faeces were weighed.
3. The faeces were mixed manually with 28 ml of saturated salt solution in a bowl thoroughly a sieve and the residues in the sieve were discarded.
4. The contents were mixed well in bowl thoroughly with the teaspoon using a to and fro action followed by two samples were removed with Pasteur pipette and both chambers of McMaster counting slide were filled.
5. The slide was placed on microscope and all eggs were counted when seen within the ruled areas of both the chambers.
6. The number of eggs was multiplied with 50 to get eggs per gram of faeces.

Appendix 2.2: Simple faecal flotation SOP

This method was used when the egg count was zero. The remaining contents in the bowl were placed in a universal bottle and filled up with saturated NaCl to form a meniscus. A coverslip was placed on top of it and left for 15 minutes. The coverslip was then removed in a positive upward motion, placed on a microscope slide and eggs present were counted at 100x magnification.

Appendix 2.3:Larval culture SOP

Equipment/chemical

Bowl

Conical flask (100ml)

Counter (Clay Adams)

Deionised water

Fine paper tissue

Glass jar with lids

Incubator (27°C)

Nuplex fine grade vermiculite

Scoop

Sieve (22cm diameter)

Spoon

Stand for funnel with rubber tubing and clamps

Procedure

1. Faeces were broken up in the bowl by spoon.
2. The vermiculite was added plus water if the faeces are too dry. The correct consistency of the faeces should be moist and crumbly but not wet.
3. The mixture was put in the culture jar to about ¼ to ½ marks and was capped with leaving some air space at the top.
4. The jar was then was placed in an incubator for at least 10 days.
5. The little water should be added if the cultures start to dry out.
6. After 10 days, larvae were recovered by the Baermann technique as follows:
    i. The clip of the rubber tubing attached to the stem of the funnel was closed.
    ii. The funnel was filled to 2 cm of the top with deionised water.
    iii. The faeces from the culture jar were placed on the wire mesh sieve with the layer of tissues underneath the faeces.
    iv. More water was added in the funnel so that the faeces were submerged.
    v. The suspension was left to stand for 24 hours.

vi. The clip was release and 50 ml of the sediment containing the larvae was tapped off from the rubber tubing into a 50 ml plastic centrifuge tube.

vii. The above was then left to another 24 hours to allow the larvae to settle at the bottom of the tube.

viii. The supernatant was poured off up to the 100 ml mark.

7. A few drops from the contents were taken using Eppendorp pipette and was put on a slide with a drop of Lugol's iodine.
8. It was then covered with cover slip.
9. The slide was put under the low power of the microscope and examines systematically.
10. A total 100 larvae were counted and identified at 100x magnification.

Appendix 2.4: Total worm counting SOP

Equipment/chemical

Agee jar (1L)

Aluminum foil

Beakers (50ml, 250ml)

Bucket (10L)

Concentrated Hydrochloride acid (HCL)

Counter (Clay Adams)

Gloves

Gut scissors

Pepsin BDH

Petri dish (grid marked on it)

Sieve (38μm, 106μm)

Scoop (50ml)

Stereo microscope

Stirring rod

Trays

Water bath

Procedure

(a) Contents

1. The abomasums, small and large intestine were tied off and then separated using fingers. These were then frozen at -20°C whilst waiting for further processing.
2. Abomasums and small intestine was placed in a bucket for thawed, and then were cut along its length with scissors.
3. The mucosal surface was washed under a light stream of water with constant manual manipulation to remove any adhered worms and/or mucus.
4. The volume of the contents were made up to 2L or 4L and mixed thoroughly with a to and fro motion with the stirring rod. At the same time, 10% by volume was removed and placed in a Agee jar. For a spare sample, another 10% by volume was removed and placed in a Agee jar plus with a formalin for preservation.

5. The contents from Agee jar was placed in a 38μm sieve and washed gently under running water until clear. As for large intestine, wash the contents thorough a 106μm sieve. The materials on top of the mesh were transferred into beaker and by using a wash bottle rinsed the sieve. Beaker was placed in a tray when filling to make sure no loss due to spillage.
6. The contents from the organs were transferred in a small portions to the counting dish and examined under a stereo microscope under 15-20X magnification. Another option for large intestine contents were transferred in small portions to a black tray and the contents were examined under a magnifying lamp. The worms were pulled out with a probe and examined under a stereo microscope.
7. The genus and stage of larvae were counted and identified.
8. During counting 50 male nematodes of each genus (if available) were recovered into formalin. These were individually examined and identified to genus.

(b) Pepsin digest

1. The section of small intestine or abomasums were cut into small pieces and placed into a beaker containing a pepsin solution (600mls water+2.5g pepsin+10ml concentrated HCL).
2. The beaker was then covered with aluminum foil and incubated for 2 hours at 37°C in a water bath.
3. The digested abomasums or small intestine was placed in the bucket and was washed under a light stream of running water.
4. The volume of the contents were made up to 2L or 4L and mixed thoroughly with a to and fro motion with the stirring rod. At the same time, 10% by volume was removed and placed in a Agee jar. For a spare sample, another 10% by volume was removed and placed in a Agee jar plus with a formalin for preservation.
5. The contents from Agee jar was placed in a 38μm sieve and washed gently under running water until clear. The materials on top of the mesh were transferred into beaker and by using a wash bottle rinsed the sieve. Beaker was placed in a tray when filling to make sure no loss due to spillage.

6. The contents from the organs were transferred in a small portions to the counting dish and examined under a stereo microscope under 15-20X magnification.
7. The genus and stage of larvae were counted and identified.

Appendix 2.5: Larval development assay (DrenchRite®) SOP

Equipment/chemical

96 well DrenchRite® plate

Beakers (50ml)

Bucket

Centrifuge

Cover slips

Deionised water

Distilled water

Electronic balance

Eppendorp pipette

Incubator @25°C

Counter (Clay Adams)

Gloves

Sieve (20μm, 60μm)

Wash bottle

Procedure

(a) Egg recovery

1. FEC were carried out to estimate the total number of eggs required for the assay before recovering the nematode eggs.
2. The pooled feacal samples were mixed with water by hand to make faecal slurry.
3. The faecal slurry was worked through a 60μm sieve and then the washings in a bucket underneath were collected. The residual in the sieve was discarded.
4. The filtrate was then washed through a 20μm sieve by running a hand underneath the sieve until the water runs clean into the sink. The particulate matter (eggs/debris) was retained on top of the sieve.
5. The eggs were collected in a 50ml Falcon tube by using a wash bottle to rinse the sieve and filled to the 50ml mark. These tubes were centrifuged at 3000g for 7 minutes to concentrate the eggs at the bottom.
6. The supernatant were discarded and the egg residue was thoroughly mixed. The egg residue was then transferred into a 50ml Falcon tube with two layers of

sugar gradient (10ml of each; 10% sucrose solution at the top and 25% sucrose solution at the bottom of the tube) in it with the tube at an angle of approximately 45 degrees. The residue containing the eggs were added on top the sugar gradients using a disposable pipette.

7. The tubes were then placed in the centrifuge buckets ensuring they were accurately balanced. Water was added if need to weight the accuracy and then centrifuged at 3000g for another 7 minutes.
8. The eggs plus residues were collected from the interface of both layers sugar solutions (approximately 9-12ml per tube) into clean 50ml Falcon tube and filled to the 50ml mark with deionised water. Water was used to remove all sucrose. Centrifuged again at 3000g for another 7 minutes.
9. The water and sugar supernatant were discarded leaving approximately 10ml containing the clean eggs at the bottom of the tube.
10. The final volume of eggs suspension was adjusted to approximately 60 eggs per 60µl.

(b) Sugar solution

1. Two sugar solutions were made from saturated solution of 60g sugar+40ml deionised water.
2. 10% sugar solution was dissolved in 10ml saturated solution+90ml deionised water.
3. 25% sugar solution was dissolved in 25ml saturated solution+75ml deionised water.

(c) Nutritive media

15ml of 1% of yeast solution was added with 15ml of 0.015% *of E. coli* suspension plus

45µl of *Amphotericin* B solution (approximately 30ml of medium for 6 plates) and were

stored @10°C until used.

(i) *Escherichia coli* (powder in freezer)

0.008g of lyophilised cells of *E. coli* (strain W (ATCC) 9637, Sigma) was added to 50ml of deionised water. The suspension was dispensed into 5ml bottles, sterile by

autoclaving and stored @10°C until used.

(ii) Yeast

0.50g yeast extract (Y-1000 Sigma) was added to 45ml phosphate buffered saline plus

5ml sterilised (by filtration) Earle's balanced salt solution (E7510, Sigma). The suspension was dispensed into 5ml bottles, sterile by autoclaving and stored @10°C

until used.

(iii) *Amphotericin* B solution

50mg of *Amphotericin* B solubilised (Sigma A-9525) was added to 10ml of deionised

water. The suspension was stored @10°C until used.

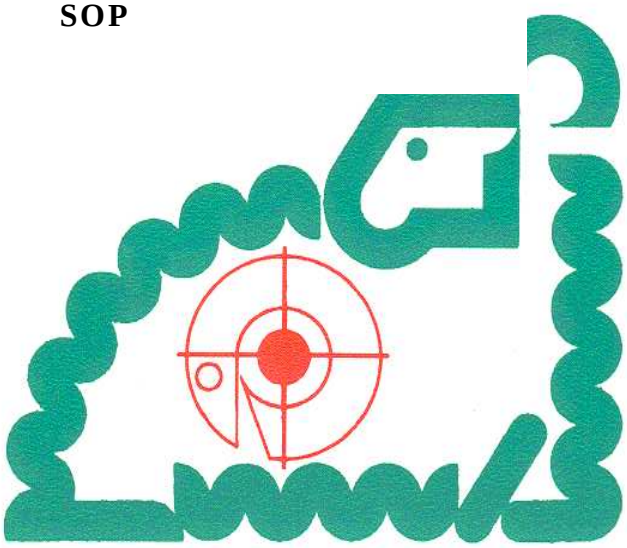
(iv) Phosphate buffered saline

$^1/_4$ tablet, (0.5g) (sigma P4417) was dissolve in 125ml of deionised water. The suspension was placed in a bottle at room temperature until used.

(d) LDA plates preparation

1. The plates were placed in a plastic container with moistened paper towels in the bottom and incubated at 25°C for 13 to14 days.
2. The control wells were examined to ensure that more than 80% of eggs were hatched after 24 hours. 40µl of nutritive media was added to each well.
3. The plates were checked daily to ensure that oxygen and moisture covered the agar of each well and 10µl of deionised water was added to any wells that looked dry.
4. The plates were removed from the incubator and stored at 10°C after the larvae have reached L_3 stage.

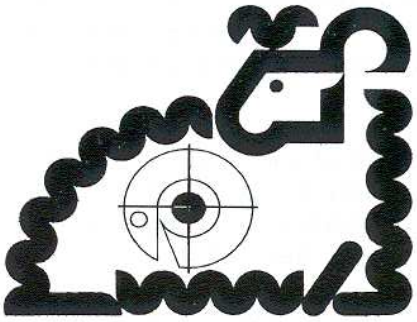
Appendix 2 6: Drenchrite® User Manual SOP



DrenchRite*

LARVAL DEVELOPMENT ASSAY

A PRODUCT OF CSIRO RESEARCH



TARGETTING RESISTANT WORMS

DrenchRite*

A LARVAL DEVELOPMENT ASSAY FOR THE DETECTION OF ANTHELMINTIC RESISTANCE

STANDARD OPERATING PROCEDURES

A Product of CSIRO Research

Horizon Technology Pty Limited
PO Box 598, Roseville NSW 2069. Australia.
Telephone: 02 805 1941
Facsimile: 02 887 4428
*Trademark

DrenchRite User Manual

Warranty

The DrenchRite Larval Development Assay and this Standard Operating Procedure are based on considerable scientific study and experiment carried out at the CSIRO, Division of Animal Production, McMaster Laboratory, Blacktown NSW Australia. Relevant research publication can be found in the following:

Avermectin Inhibition of Larval Development in *Haemonchus contortus* - Effects of Ivermectin Resistance.
Gill J.H., Redwin J.M., van Wyk J.A. and Lacey E. 1995. *International Journal for Parasitology* 25: 463-470.

The Larval Development Assay as an Alternative to Faecal Egg Reduction Tests for Field Diagnosis of Broad Spectrum Anthelmintic Resistance.
Lacey E., Craven J.H., Gill J.H. and Baker P.J. 1993. The Australian Society for Parasitology, Programme and Abstracts of Papers presented at the Annual Scientific Meeting of the Society.

A Larval Development Assay for the Simultaneous Detection of Broad Spectrum Anthelmintic Resistance.
Lacey E., Redwin J.M., Gill J.H., Demargheriti V.M. and Waller P.J. 1990. Resistance of Parasites to Antiparasitic Drugs (Edited by Boray J.C., Martin P.J. and Roush R.T.), pp. 177-184. MSD AGVET, Rahway, NJ.

Use of the DrenchRite Assay is conditional on you, the customer, agreeing to the terms set out on the DrenchRite product insert included in the box containing the microtitre plates. Do not open the sealed foil pouch containing the assay plate until you have read and accepted all the terms of the warranty. Acceptance shall bind you and all your employees to the terms of the warranty. Opening the foil pouch will be deemed to be your acceptance of the terms. If you do not accept the terms return the kit unopened to Horizon Technology and any purchase fee you have paid will be refunded.

Introduction

DrenchRite is an in vitro assay for the detection of resistance to benzimidazole, levamisole, benzimidazole/levamisole combination and avermectin/milbemycin drenches in the major gastrointestinal nematode parasites infecting sheep, *Haemonchus contortus, Trichostrongylus colubriformis and Ostertagia circumcincta.*

In this assay, nematode eggs are placed into the wells of a microtitre plate and hatched larvae develop to the L3 stage in the presence of anthelmintic. The concentration of anthelmintic required to block development is related to an anticipated in vivo efficacy.

The DrenchRite assay plate contains a single lane (Lane 1) for Control development (no dye) and eleven lanes (Lanes 2 to 12) which contain increasing concentrations of a drug from each drench class. The DrenchRite plate is colour coded; green for susceptible, yellow for weak to intermediate resistance and red for highly resistant.

Each drench class is tested in duplicate. The benzimidazoles are tested in rows A and B, levamisole in rows C and D, benzimidazole/levamisole combinations in rows E and F and the avermectin/milbemycins in rows G and H (Figure 1).

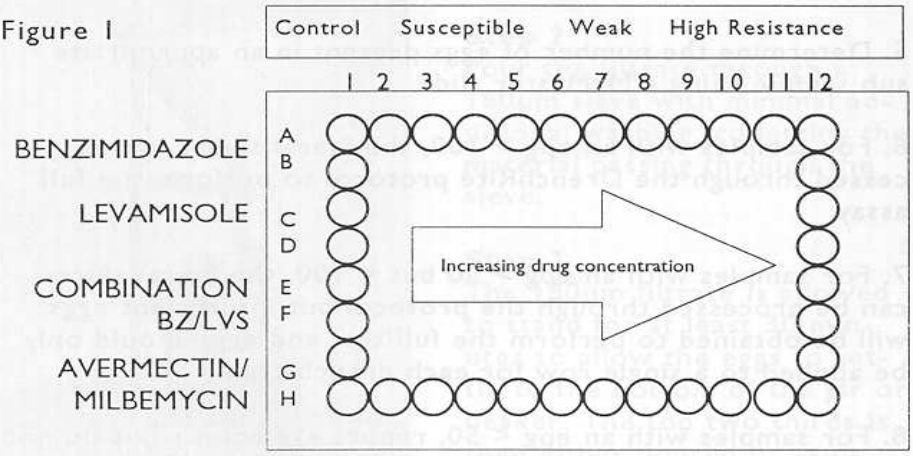
Parasite eggs isolated from faecal samples submitted by producers are applied to wells of the assay plate. After hatching, the first stage larvae are fed to sustain development through to the infective L3 stage over the next five days. In the presence of the drenches, development is blocked. By scanning the plate under the microscope the number of the well in which a shift, from Control well approximate L3 development to substantially no L3, is observed for each drench. This important shift may occur between adjacent wells (e.g. say between well 5 & 6) making the critical well the average of the two wells (5.5 in the above example). When a more gradual reduction, in successful development to L3 occurs over a range of wells, then the critical well is that in which the L3 counted is approximately half the Control well L3 counts.

If the larvae are resistant to a drench, the critical well in which the shift from Control L3 development to where half Control, or less, L3 development first occurs, will be in the yellow or red region of the plate. That is, depending on just how resistant the larvae are - the critical well will move from the green to the yellow and subsequently to the red region of the plate for that drench.

The well numbers so determined for each drench class are then used to estimate drench efficacy from a supplied table. For the avermectins/milbemycins, where resistance in the field is rare at present, the well number is used in a slightly different way. DrenchRite is used to identify any emerging resistance.

The following Standard Operating Procedure details methodology for performing the DrenchRite test, how to get service for statistical analysis of your results, and includes the equipment and chemicals required to carry out the assay. Appendix 1 sets out the abstract of a paper presented on the LDA.

Figure 1



PREPARATION OF FAECAL SLURRY

1. Weigh the bag.

2. Transfer approximately 200g of faeces to a plastic beaker, add 1mL of tap water per gram of faeces. Soak for at least 30 minutes to soften the pellets.

3. Break up the faeces by gloved hand or mortar and pestle and add a further 2mL of water per gram of faeces to give a slurry. (**Do not use a blender as blending may damage nematode eggs.)**

4. Sample slurry for egg count - eggs per gram (epg), and faecal culture:

a. To get a representative sample of faeces, for determination of species present, set aside 20 to 30g of faeces slurry for a standard faecal culture. Add vermiculite to absorb some of the water from the slurry sample. Incubate the sample in a jar with a loose lid at 25°C for 6.5 days. (See Day 7 Page 14).

b. To determine the epg weigh out an 8g sample of the slurry into a 50mL jar on a top loading balance (equivalent to 2g of faeces). Dilute to 50mL with saturated saline, mix well. **Note: A random selection of faecal pellets for egg count and culture is not recommended.**

5. Determine the number of eggs present in an appropriate sub sample using a McMaster slide.

6. For samples with an epg > 100, the faecal slurry is processed through the DrenchRite protocol to perform the full assay.

7. For samples with an epg > 50 but < 100, the faecal slurry can be processed through the protocol but insufficient eggs will be obtained to perform the full test and eggs should only be applied to a single row for each drench type.

8. For samples with an epg < 50, report egg counts but do not further process using this protocol.

The single most critical aspect of the *DrenchRite* test is the isolation of eggs relatively free of faecal debris. Failure to achieve a clean suspension will result in erratic performance of the assay and possibly the complete failure of the larvae to develop through to the infective L3 stage, even in Control wells. Avoid contaminating the plate with airborne spores - the plates are provided sealed in a sterilised pouch.

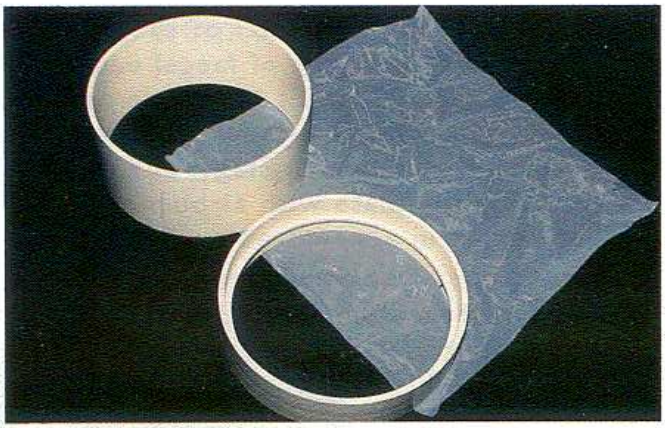
Step 1
Wash the slurry through a 250µm sieve with tap water, Collect washings (approximately 4L) in a large jar or beaker.
Note: Losses of eggs at this stage can be high with the bulk of the faecal debris being retained on the sieve. Wash the faeces through this sieve in two batches, move the material on the sieve with a gloved hand while washing.

Step 2
Pour the filtrate through a 180µm sieve with minimal additional washing, collecting the material passing through the sieve.

Step 3
The 180µm filtrate is allowed to stand for at least 30 minutes to allow the eggs to settle to the bottom of the jar or beaker. The top two thirds is then either poured or aspirated off.

PREPARATION OF SIEVES

75 & 25µm sieves 15 & 10cm in diameter respectively can be constructed using nylon mesh (Swiss Screens) and plastic drainage pipe.
Full equipment list Page 24-25.



Attach the mesh using joining sleeves for each pipe size.
Cut the sleeve in half to make two sieves from each sleeve. Drainage pipe obtainable from any hardware outlet. N.B. Do not glue the mesh to the pipe.
Dismantle the sieves after each use and thoroughly wash the nylon mesh.

Step 4

Wash the concentrated filtrate through a 75µm sieve with a fine stream of water (attach a pasteur pipette to a tap by an appropriately sized flexible tubing). Collect the filtrate in approximately 2 litres of water.
Optional: Allow the filtrate to settle for at least 20 minutes. Pour or aspirate off, the top two-thirds.

Step 5
Pour the concentrated filtrate from the 75µm sieve on to a 25µm sieve under vacuum to collect nematode eggs.

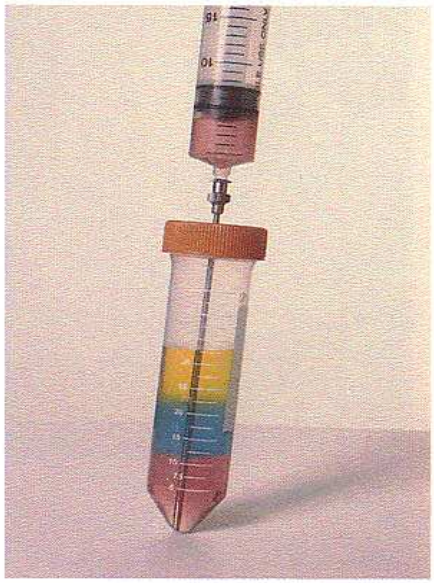
Step 6
Transfer material on 25µm sieve to small beaker with minimal amount of water and allow to settle.

Step 7
Prepare gradients in 50mL centrifuge tubes adding 10mL each of the yellow and blue gradient solutions and 15mL of the red gradient solution. The kit supplied, contains one vial of 50mL each yellow and blue gradients and two vials each red gradient.

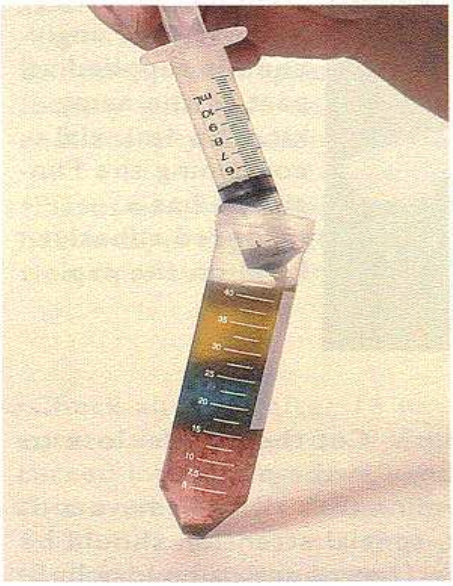
Order of Gradient Addition:
Withdraw 10mL of yellow gradient solution from the vial capped with the gold aluminium cap. Add to the centrifuge tube.

Withdraw 10mL blue gradient solution from the vial capped with the blue aluminium cap. Add to the centrifuge tube with the cannula placed at the base of the centrifuge tube.

Withdraw 15mL red gradient solution from one of the vials capped with the red aluminium cap. Add to the centrifuge tube again with the cannula placed at the base of the centrifuge tube. Aspirate excess water from solution containing eggs (Step 6).



Step 8
Using a 10mL syringe, carefully dispense no more than 15mL egg slurry on top of yellow gradient by holding outlet against side of centrifuge tube. Avoid mixing slurry with gradient.



Step 9
Spin gradients at 3500 rpm in a bench top centrifuge for 7 minutes. Accelerate the tubes gradually up to 3500 rpm. Make sure the tubes are balanced and not overfull.

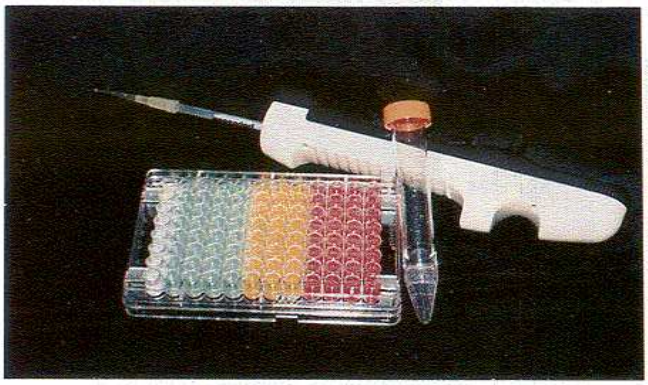
Step 10
Use a pasteur pipette to remove eggs from the interface of the yellow and blue phases of the sedimentation solution. Collect onto a small 25µm sieve.

Rinse eggs thoroughly with water to remove sugar. Transfer to a 15mL tube using distilled water.
Allow eggs to settle. Aspirate off excess water containing fine suspended debris. Repeat this step for dirty preparations. Excess heavy debri settling with the eggs can be removed by repeating Step 7.

Loading the microtitre plate

Step 11

•Count the eggs and dilute 40 eggs with 10µl of distilled water.
Note: 50-70 eggs per well is optimum, more than 70 eggs per well makes counting slow and difficult.



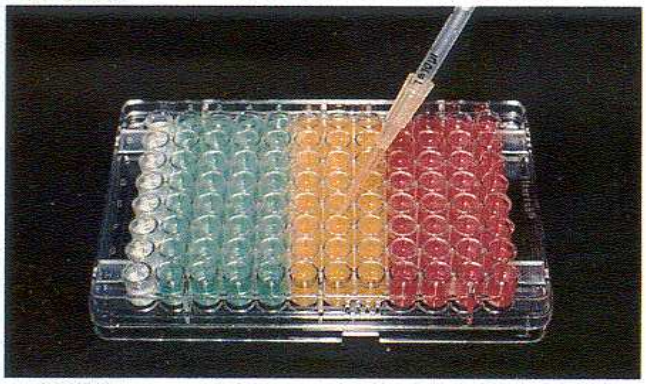
•Add 90µl of fungizone per mL of egg suspension. Fungizone is supplied as part of the assay kit. The 1mL vial containing the Fungizone has a coloured adhesive label on the cap.

Step 12

•Remove the plate from the foil pouch. Cut the pouch close to the seal to allow re-use. Retain sponge in the pouch.
•Inspect wells of the plate to determine that all wells have a film of surface water over the agar, special attention should be given to the outside rows and lanes. Detect moisture loss by holding the plate horizontally at eye level. Observe if some wells have less agar than others. Rehydrate only those wells without a film of surface moisture using 10µl of distilled water.

Step 13

Dispense 20µl egg suspension to each well of the microtitre plate.



Step 14
INCUBATION
•An incubator capable of maintaining a constant temperature of 25°C is required. Place an open tray containing water in the incubator, to avoid a dry environment.

•Carry out egg recovery procedures from Step 10 and 11 and dispense the eggs onto the plates. Return the plates to the foil pouch with sponge (under base). Seal the open end loosely with tape and place in incubator. Placing in the pouch reduces evaporation of water from the wells.

•Plates are incubated for 156 hours (6.5 days). Providing no bacterial overgrowth occurs and wells do not dry out, incubation time can be extended up to 180 hours.

Egg Hatch - Day 2

•Check all wells after 16 hours incubation to determine presence of free water on the agar surface. If absorption has occurred into the agar add 10µL of distilled water **only to those wells that do not have a film of surface water.**

•Record wells rehydrated, in case eggs were damaged by dehydration.
Note: It is essential a film of water be maintained on the agar surface throughout the assay. Eggs may be prevented from hatching/developing if wells dry. If drying occurs later during incubation larvae may migrate from the well or into the agar.

•Supplement the wells with growth medium (20µl per well) when eggs in the control wells have hatched,

•Return to incubator.
Note: After 24-30 hours there should be a high proportion of hatched eggs in the control wells. Eggs can be damaged in transit (eg. exposure to high temperature). Abandon the assay if 60% or more of eggs fail to hatch after 48 hours incubation.

Egg Hatch - Day 3-6

•Scan the plate at 2 to 3 day intervals to check there is free surface water (as noted day 2).

·Add 10µl distilled water to any well that does not have free surface water.

·Record wells rehydrated. On weekends carry out on Friday and check again Monday.

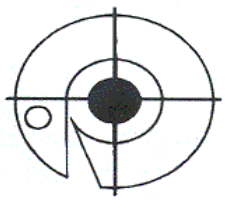
Egg Hatch - Day 7

·At 25°C insure incubation of 156 hours (6.5 days), longer may be undertaken (to 180 hours) provided **wells do not dry** and bacterial overgrowth does not occur.
Note: If wells dry at this point larvae can migrate from the wells.

·Harvest faecal culture set up from the slurry on Day 1, (Page 6).

·Differentiate larvae to determine the species composition of the sample and identify the predominant species (see Speciation Page 15). The larvae from a few (up to 4) of the Control wells (Lane 1) can also be used to determine species.

·Kill the larvae in all lanes (Lane 1 to 12) by adding a dilute solution of Lugol's iodine (10µl per well) and follow the Interpretation protocol Page 16.



Speciation of larvae can be undertaken according to the guidelines given in SCA Technical Series Report - No 28.
Speciation based on **length**, as the sole criterion, is not recommended. It is important that the speciation technique used, adequately differentiates *T. colubriformis* from *O. circumcincta* as this has important implications for the diagnosis of avermectin/milbemycin resistance status (see later). Methods based on the work of Lancaster and Hong (*Vet. Rec.* 120: 503, 1987) are preferred.

DOMINANT SPECIES: If a resistant proportion of L3s is observed to develop in the yellow and/or red lanes, for a particular drug, they can be removed for comparison with the control culture. In such lanes a large change in species composition from the controls would indicated resistance is not present in the dominant species. For example, it may be that *T. colubriformis* is the dominant species in the control culture but only *O. circumcincta* is found in the red lanes of a row. From such a result you would conclude that *O. circumcincta* is resistant while the dominant species (*T. colubriformis*) is susceptible to the drug identified with that row.

SPECIATION BETWEEN: *T. colubriformis and O. circumcincta:*
The method used in the CSIRO McMaster Laboratory, is given below. This method will not distinguish *T. axei* from *O. circumcincta*.
1. Take fresh larvae from the standard culture (or remove larvae from surface of agar in 4 Control wells - addition of a small volume of water (50µl) to wells will aid removal - and place into a clean 15mL centrifuge tube.
2. Add 5 drops per mL, sodium hypochlorite solution (12%), to the live larval suspension to cause the larvae to exsheath. To Lugol's iodine treated larvae add 7 drops per mL.
3. Monitor the exsheathment process under the microscope, live larvae will exsheathe in 15 minutes.
4. Add Lugol's iodine when the larvae have exsheathed, (1 drop per 5mL - lightly stain) then draw larval suspension into a pasteur pipette. If only small numbers of larvae are available concentrate sample by allowing pipette to stand upright for a few minutes so that the larvae settle to the tip of the pipette.
5. Examine larvae under a light microscope (x200) to differentiate species present. Speciation between *T. colubriformis* and *O. circumcincta* is based on the presence or absence of tubercules. For accurate results 100 larvae should be inspected.

Interpretation

The DrenchRite plate is inspected under a binocular microscope (x 25 magnification).

1. Wells in the Control lane (no colour) should contain >90% L3 larvae, that is, 0% to 10% L1/L2 and ignoring unhatched eggs. After a little experience with the LDA L1/L2 mortalities in Control wells are usually between 5% and 15%. See note below for L1/L2 mortalities above 15%.

2. For Rows A to H, a transition from L3 to L1/L2 larvae should be apparent as each Row is scanned from well 2 to well 12. Estimate to the nearest half-well the number of the well corresponding to the position, where, L3 development in 50% of the larvae is blocked, (by comparison with Control) for each drench class ie. half the number of L3s that can be found in the Control wells.

3. Because of ovicidal effects at high concentrations of benzimidazole, additional egg mortalities should be included in the L1/L2 tally from Lanes 5-12 in rows A and B (resistant strains only).

4. For levamisole only (Rows C and D), Wells 9 to 12 must be inspected for the presence of L3 larvae. If L3 larvae are present, the proportion of L3 larvae to the total larvae present in these wells should be determined by counting the L1/L2 and L3 larvae present in these wells.

Note: Natural mortality of L1/L2 can vary from 2% to 40% for different egg isolations and strains, but is generally consistent on a particular plate.

If Control wells contain more than 10% to 15% L1/L2 then the critical well number where 50% L3 development is blocked must be determined as follows:

Allowances for natural mortality (detected in Control wells) can be taken into account by either
(a) subtracting the average number of L1/L2 in Control wells from the L1/L2 count in each drug concentration well
or
(b) count the L3s in each well till they are reduced to 50% of the average number of L3s in the Control wells.
Both these methods can be used to quickly and fairly accurately access the critical well where half the L3s are blocked in developing (without counting all wells and stages). For example, scanning along a row there may be 20 to 30 L3s in each well, at some point say between well 5 and 6 this may decline to 10 L3s or less, then the critical well would be 5.5. In such an example a background count of 20 or so L1/L2 in wells 1-5 would be ignored (being attributed to natural mortality).

5. **Alternatively**, a statistical analysis of the data can be carried out when all L1/L2 and L3s in each well are counted. It takes about 1 to 1.25 hours to count the entire plate if approximately 60 eggs per well were added to the plate. (See Statistical Analysis page 21).

For BENZIMIDAZOLES (BZ) and BENZIMIDAZOLE/LEVAMISOLE (BZ/LVS) combinations:

The number of the well in which development is blocked in 50% of the larvae present is used to determine the estimated efficacy of the drench from Table 1. Use the section of the table which corresponds to the predominant species present.

For LEVAMISOLE (LVS):

Levamisole resistance in the field shows two distinct forms: "**High level resistance**", where the larvae are insensitive to the high LVS concentrations and develop to L3 larvae in wells 9 to 12, and "**Moderate level resistance**", where the well number in which development is blocked by 50%, shifts into the yellow and red regions with increasing resistance. Both types of resistances occur in the field, often together, and both must be estimated.

Interpretation

For LEVAMISOLE Contd....

1. Determine the average proportion of L3 larvae present in wells 9 to 12. That is, the mean number of L3s in wells 9 to 12 divided by the mean number of L3s in the Control wells. This is the proportion of "**High level resistance**" in the population. The remainder of the population is **no** and/or "**Moderate level resistance**" determined as (one minus proportion "**High level resistance**").

2. Estimate efficacy from Table 1 based on the number of the well in which development in the remainder of the L3 population is blocked by 50%.

3. If L3 larvae are present in wells 9 to 12 (red region), determine the population efficacy by multiplying the efficacy from Table 1 (determined in Point 2 above) by one minus the proportion of L3 larvae present in these wells (i.e. the proportion of worms with **no** or "**Moderate level resistance**", determined at the end of Point 1 above).

FOR EXAMPLE:

A. If a mean of 50 L3s develop in Control wells and a mean of 10 L3s are found in wells 9 to 12 then 20% of the population is classed as "**High level resistant**".
B. Subtract 10 L3s from each levamisole well and the Control mean i.e. Controls now have 40 L3s and the critical well is the one where, 20 or less L3s (after subtracting 10), is first found.
C. If the critical well was say well 3, then from Table 1 it can be assumed there is 100% efficacy for 80% of the population and zero efficacy for the resistant fraction. Therefore efficacy is read as 80%. If the critical well was 6 (say for *H. contortus*) Table 1 indicates 90% efficacy (for 80% of the population). This yields a population efficacy of about 72% - (i.e. 90%*0.8 = 72%)

Table 1: Estimated Efficacy

Estimated efficacy for benzimidazoles (BZ), levamisole (LVS) and levamisole/benzimidazole combinations (COMB) based on the predominant species present. *** Well in which development is blocked by 50% for the McMaster susceptible isolate of each species.**

Well No.	H. contortus % Efficacy			T. colubriformis % Efficacy			O. circumcincta % Efficacy		
	BZ	LVS	COMB	BZ	LVS	COMB	BZ	LVS	COMB
2.0	100	100	100	100	100	100	100	100	100
2.5	100	100	100	100	100	100	100	100	100
3.0	100	100	100	100	100	100	100	100	100
3.5	100	100	100	100	100	100	100	100	100
4.0	**100***	100	100	**98***	100	100	**99***	100	100
4.5	99	99	**100***	94	**99***	100	97	100	100
5.0	95	97	98	87	94	100	92	100	**100***
5.5	89	**94***	92	78	83	**98***	85	**98***	100
6.0	80	90	83	67	68	93	77	93	94
6.5	69	85	70	56	51	87	67	87	81
7.0	58	79	56	44	34	79	57	79	64
7.5	46	73	41	32	19	69	46	70	44
8.0	34	66	27	22	7	59	35	60	26
8.5	23	59	15	13	1	48	25	49	11
9.0	14	52	6	6	0	38	17	39	2
9.5	6	52	1	2	0	28	9	39	0
10.0	2	52	0	0	0	19	4	39	0
10.5	0	52	0	0	0	11	1	39	0
11.0	0	52	0	0	0	5	0	39	0
11.5	0	52	0	0	0	1	0	39	0
12.0	0	52	0	0	0	1	0	39	0

Interpretation

For AVERMECTIN/MILBEMYCINS:

Resistance to the avermectin/milbemycins is rare in the field by comparison with the first broad spectrum anthelmintics introduced. *DrenchRite* can be used to detect the presence of resistance but not as yet, to quantify efficacy.

Table 2 lists the well numbers for rows G and H where it is expected that development will be blocked in 50% of the larvae present for susceptible populations of *H. contortus*, *T. colubriformis* and *O. circumcincta*.
Note that they vary according to the species present.
For susceptible *H. contortus* populations these wells are in the green region of the plate, while for susceptible *O. circumcincta* populations, the indicated well falls just within the yellow region. *T. colubriformis* falls between the other two species.

Where the well, containing a 50% reduction from L3s in Control wells, falls more than 2 wells away from the indicated well for the predominant species, resistance is suspected. Further testing may be required.

The most common error in assigning resistance to this drench class in *DrenchRite* is the incorrect speciation of *O. circumcincta* larvae as *T. colubriformis*. See Speciation Page 15.

Table 2: Average Well Numbers

The susceptible range for avermectin/milbemycins in Rows G and H. Note: Rows G and H contain different drug derivatives.

Row	Predominant Species Present	Well No. Mean	Well No. Range
G	*H. contortus*	4.5	4.0-5.0
	T. colubriformis	4.5	4.0-5.5
	O. circumcincta	5.5	5.0-6.5
H	*H. contortus*	3.5	3.0-4.0
	T. colubriformis	5.5	5.0-7.0
	O. circumcincta	6.0	5.5-7.5

A service is available to testing laboratories providing an estimation of;

- drug efficacy,
- worm LD50,
- corrected (for Control well mortality) and uncorrected data plots for single or mixed populations,
- and proportions of each population if two are present.

A log-dose-logistic model for one or two (if present) populations is fitted to the raw data. The two populations are separated by their level of drug resistance. They can be either a single species with a distinct resistant and less-resistant fraction, or two species with different dose responses to the same drug. See Interpretation section Point 5 Page 17.

A fee per drug will be charged for this service.

Call Horizon Technology for pricing and to determine the appropriate method for your results to be communicated for analysis. Results can be emailed, faxed, or posted on a DOS/ Microsoft Windows formatted 3.5" disk.

PRESENTATION OF RESULTS.
Results must be in the following format (as an ASCII file if on disk):

1 Row letter A to G for the data set.
2 Total larvae and total affected in all Control wells counted.
3 Well # 1 to 12 Total $L_1+L_2+L_3$ Affected L_1+L_2 (one line for each well counted).
4 Provide raw data for all individual Control wells counted on the plate.

An example follows Page 22.

For example, a levamisole row (C or D) the data is set out as follows:

C		
1	160	28*
2	39	8
3	35	6
4	44	11
5	31	10
6	36	13
7	46	21
8	35	21
9	33	19
10	26	18
11	35	23
12	27	15

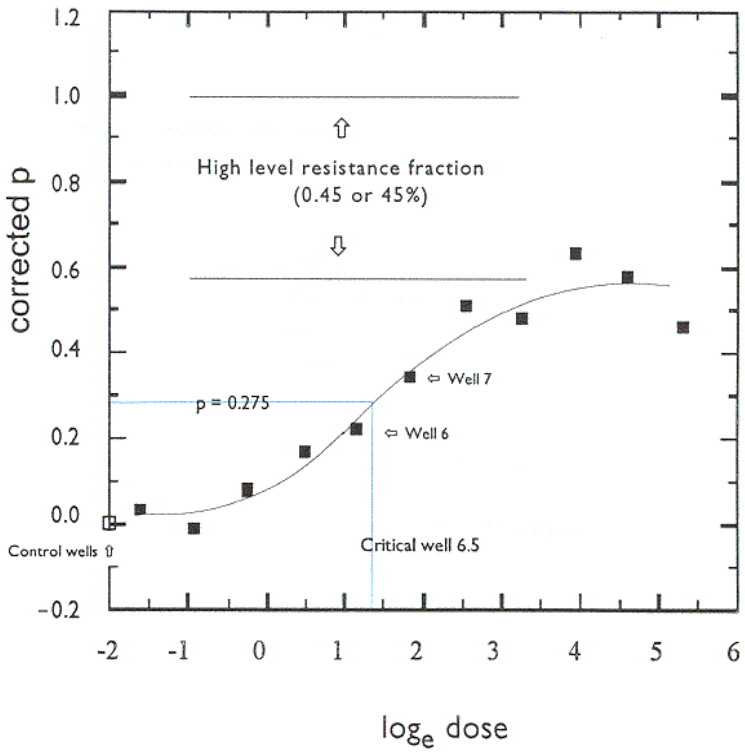
*(This is the sum from 4 Control well results & represents 17.5% affected in the Control wells.)

Note: It is not necessary to count all wells once an end point is reached (i.e. no L3 development). Be sure to include both rows of drug(s) for which analysis is required.

PRESENTATION OF RESULTS:
A plot of the above data after analysis and corrected for Control development is set out on Page 23. From the line fitted to the data it can be seen that the proportion affected by the drug ("corrected p") plateaus at about 0.55 (55%). This means about 45% of this population has "**High level resistance**". See Interpretation section for levamisole pages 17 & 18.

The 55% with "**Moderate level resistance**" has a critical well number 6.5 (i.e, where half of 55% are affected - p = 0.275). Well numbers can be counted from the data point starting on the left vertical axis where the Control well (#1) is shown as "O". If this data was *H. contortus* the efficacy of this proportion of the population (0.55) would be 73% (from Table 1). This gives an efficacy of 40% for the whole population (i.e. 0.55 * 73% and 0% efficacy for the 0.45 "**High level resistant**" fraction).

Row C - example of levamisole analysis



Equipment and Chemicals

1. A top loading balance capable of weighing up to 400g.

2. Large beakers/jars of 2L capacity (3 or 4 per sample) and small beakers of 250mL capacity (1 per sample). Preferably plastic.

3. 250µm and 180µm sieves - diameter, 20cm.

4. 75 and 25µm sieves of about 15 and 10cm diameter, respectively - can be constructed using nylon mesh (Swiss screens) and plastic drainage pipe.

5. Plastic drainage pipe used in plumbing and pipe joining sleeves used for attaching the mesh to a section of pipe for each sieve, (available from the local hardware shop). The sleeves should be cut in half so that 2 sieves can be made from each sleeve.
Note: Do not glue the mesh to the pipe, it is essential to take the sieves apart after each use to thoroughly wash the mesh.

6. Plastic funnels capable of holding sieves - polypropylene Buchner funnels are ideal and are available from Selby Scientific and other labware suppliers.

7. 3 Retort stands with ring attachments to hold funnels.

8. 2L filter flask.

9 Vacuum outlet.

10. Rubber tubing or hose attached to water outlet to supply water for washing sieves. (Attaching a plastic pipette tip or a short pasteur pipette to the hose supplying water to wash material, on the 25µm sieve, will achieve a finer jet which will be more efficient).

11. 50mL plastic centrifuge tubes with lids.

12. Gradient solutions (yellow, blue and red). Store at 2°C-8°C (Refrigerate). Supplied in the DrenchRite kit.

13. Large bore luerlock needle as long as a 50mL centrifuge tube - to position the needle for gradient preparation this should be pierced through a lid (see Step 7 Page 10).

14. Plastic syringes, one for each sedimentation solution to make the gradients and one for each sample to load the sample onto a gradient.

15. Bench top centrifuge with swing out rotor capable of holding 50mL centrifuge tubes and operating at 3500 rpm.

16. Smaller tubes - suggest 15mL plastic centrifuge tubes -to hold eggs removed from gradients once they have been washed free of the sedimentation solutions.

17. Pasteur pipettes.

18. Standard binocular dissecting microscope with 10 and 25 x objectives.

19. DrenchRite plates. Store below 8°C and do not open foil pouch until plate required for use.

20. Pipette capable of dispensing 10 and 20µL samples - multipipettes capable of dispensing up to 40 x 10µL or 20 x 20µL without refilling are available and most advantageous (e.g. Eppendorf Multipette using 0.5mL Combitips).

21. Fungizone 250µg/mL (supplied in the DrenchRite kit) Protect from light. Prolonged storage, Store below -5°C (Freeze).

22. Growth medium (supplied in the DrenchRite kit) - Store below minus 5°C (Freeze).

23. Incubator capable of maintaining temperature at about 25°C.

Suppliers such as Swiss Screens (Aust) Pty Ltd are located at Seven Hills NSW. Selby Scientific have outlets in most States.

Field Collection

1. Advise the client to herd a flock of undrenched sheep (preferably weaners) into a corner of the paddock for 10 to 15 minutes then allow the flock to disperse.

2. Collect **fresh** pellets from the ground where the sheep were standing and **tightly pack** the faeces into a good quality clear plastic bag (approximately 30 x 40 cm) to about one third full. Mould into shape suitable for posting in a 24 x 37cm post bag or postal cylinder available from Australia Post. Exclude all air from the plastic bag, fold and completely seal this bag with adhesive tape.

3. Tightly wrap the plastic bag of faeces with ample Cling/Glad wrap type plastic food wrap to ensure the sample is completely airtight. **Note. This is very important, - preventing air from reaching the faeces will preserve the worm eggs in the sample.**

4. Record name and date of collection on the sample and post **immediately** to the closest DrenchRite testing laboratory.

5. On arrival at the laboratory samples should be stored at room temperature after ensuring that the bag remained tightly sealed. For best results samples should be processed as soon as possible and within 5 days of collection. Eggs in faeces will remain viable provided all air is excluded from the container and it is not exposed to temperature extremes.

The Larval Development Assay as an Alternative to Faecal Egg Reduction Tests for Field Diagnosis of Broad Spectrum Anthelmintic Resistance

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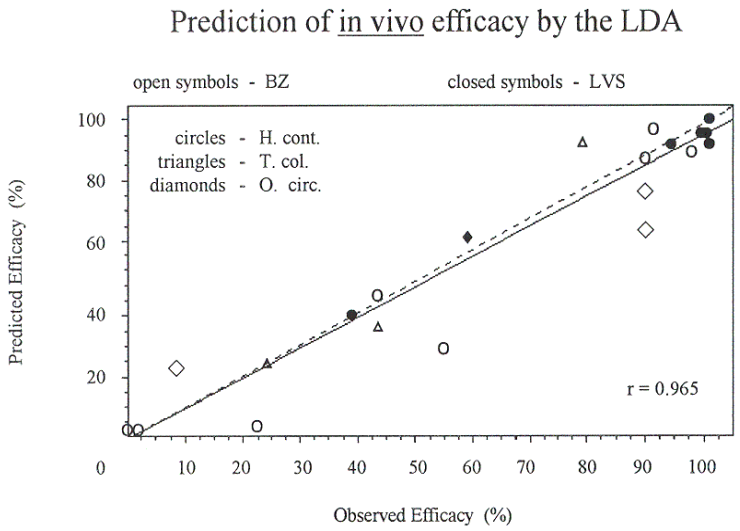
Reproduced from The Australian Society for Parasitology, Programme and Abstracts of Papers presented at the annual scientific meeting of the Society held at Heron Island September 28 - October 1, 1993

The Larval Development Assay (LDA) is an *in vitro* microtitre plate assay developed for the detection of anthelmintic resistance in the major gastrointestinal nematode parasites of sheep, *Haemonchus contortus, Trichostrongylus colubriformis* and *Ostertagia circumcincta*. The objective of the study was to correlate resistance ratios (RRs) derived in the LDA to *in vivo* anthelmintic efficacy in order to standardise the LDA for routine use in the diagnosis of anthelmintic resistance in the field.

Parasite isolates were artificially constructed to contain representatives of all three species with a defined level of resistance to both the benzimidazoles (BZs) and levamisole (LVS) ranging from susceptible to fully resistant. The resistance of each construct to BZs, LVS and BZ/LVS combination was tested in the LDA, in a faecal egg count reduction test (FECRT) and in a drench and slaughter assay.

A linear relationship was observed between the natural logarithm of the RR obtained in the LDA and % efficacy in vivo, as determined in the drench and slaughter assay, after arcsin transformation of the latter data. Highly significant correlations were obtained for BZs and LVS, individually and as a combination, against all three nematodes tested. The regression equations obtained enable RR values from the LDA to be interpreted as an expected in vivo % efficacy and will facilitate the use of the LDA in the diagnosis of resistance to BZs, LVS and BZ\LVS combinations in the field, replacing the commonly used but poorly validated FECRT.
This work was undertaken in collaboration with Horizon Agriculture Pty Limited.



Species	Isolate	Drug	Dose mg/kg	RR	Est. Eff	Confid.	Actual	Reference
H. contortus	Pretoria	BZ (FBZ)	5	13	46	31-60	44.5	Van Wyk et al. Ond J Vet Res 45:143 (1987)
		LVS	7.5	0.4	100	100	100	Van Wyk et al. Ond J Vet Res 45:143 (1987)
	W. River I	BZ (FBZ)	5	59	2	0-17	0	Van Wyk et al. Ond J Vet Res 45:143 (1987)
	VRSG	BZ (ABZ)	3.8	62	2	0-17	1.4	Hall et al. Res Vet Sci 25:364 (1978)
	Lawes	BZ (MBZ)	12.5	16	34	19-50	53.6	Green et al. Aust Vet J 57:80 (1981)
		LVS	6	>50 RF 60%	40		40	Green et al. Aust Vet J 57:80 (1981)
	Stellenbosch	BZ (OFZ)	5	3	89	74-89	97.9	Van Wyk et al. Ond J Vet Res 56:41 (1989)
		LVS	7.5	0.8	97	76-100	99.1	Van Wyk et al. Ond J Vet Res 56:41 (1989)
	W. River II	BZ (FBZ)	5	68	2	0-17	22.1	Van Wyk et al. Ond J Vet Res 56:41 (1989)
		LVS	7.5	0.8	97	76-100	100	Van Wyk et al. Ond J Vet Res 56:41 (1989)
	Badgerys Ck	BZ (TBZ)	44	2.6	95	82-100	91	Waller et al. Int J Para 19:99 (1989)
		LVS	7.5	1.6	94	72-100	100	Waller et al. Int J Para 19:99 (1989)
O. cicumcincta	Ra803 (goat)	BZ (OFZ)	5	16	35	27-44	44	Watson & Hosking, NZ Vet J 38:50 (1990)
	KR79	BZ (TBZ)	44	2	25	17-34	25	Martin et al. Aust Vet J 58:185 (1982)
	KS79	BZ (TBZ)	44	2	92	87-96	82	Martin et al. Aust Vet J 62:38 (1985)
T. colubriformis	Badgerys Ck	BZ (TBZ)	44	3.2	78	65-88	88	Waller et al. Int J Para 19:99 (1989)
		LVS	7.5	1.1	68		60	Waller et al. Int J Para 19:99 (1989)
	TAS-MRT(AR3)	BZ (TBZ)	44	RF 30% 17	22	9-29	7	Waller et al. Vet Para 21:255 (1986)
	Gunderoo	LVS	7.5	1	90		93	Waller et al. Aust Vet J 65:376 (1988)
				RF 10%				
	BCK-LVS (AR5)	BZ (TBZ)	44	2	87	75-95	88	Waller et al. Int J Para 19:99 (1989)
	AR5 - 81VM	BZ (TBZ)	44	4.1	67	54-79	88	Waller et al. Int J Para 19:99 (1989)

Appendix 2.7: Questionnaire

Massey University

"ANTHELMINTIC RESISTANCE IN GOATS IN NEW ZEALAND"

Master candidate: Juriah Kamaludeen
Supervisor: Prof W Pomroy

SECTION 1: Contact details

1.1 Name (optional)* _____

1.1 Address (optional)* _____

_____

1.3 E-mail (optional)* _____

1.4 Phone (home/work) (optional)* _____

1.5 Area/district _____

1.6 Date

QUESTIONNAIRE:

SECTION 2: Farm information

Please indicate area values and years as whole numbers. If less than one year, please write <1

2.1 What is the **effective** size (grazing area) of your farming operation? _____ ha

2.2 How many years have you been **farming this property**? _____ year(s)

2.3 How many years have you farmed **goats on this property**? _____ year(s)

2.4 Which of the following best describes your farm?
Please tick the appropriate box

Dairy goat (all ages)		Meat/fibre goat (all ages)	
0 - 50	☐	0 - 50	☐
50 - 200	☐	50 - 200	☐
200 – 500	☐	200 - 500	☐
500 – 1000	☐	500 - 1000	☐
> 1000	☐	> 1000	☐

* Please supply us with at least the district your farm is located in. If you supply contact information we will return a copy of the results to you as soon as they are completed. Faecal egg counts results will be returned within 3 to 4 days but results from the drench resistance testing may take one to two months.

Sheep (all ages)		Cattle (all ages)	
0 - 50	☐	0 - 50	☐
50 - 200	☐	50 - 200	☐
200 – 500	☐	200 - 500	☐
500 – 1000	☐	500 - 1000	☐
> 1000	☐	> 1000	☐

Other (please stipulate) _____

SECTION 3: Your animal worm control practices:

3.1 We would like to understand which drenches you are currently using. To do this we are asking you to tell us which drench was used the last time you drenched your goats. You can either tell us the brand name or the active ingredient.

_____

_____

3.2 What is the drenching dose rate you normally use for your goats?
Please tick the appropriate box

Sheep dose rate ☐ Greater than the sheep dose rate ☐

3.3 Please tell us how long ago you last drenched the goats from which you obtained the faecal samples to send to us.

< 2 weeks ☐ 2 – 4 weeks ☐

4 – 8 weeks ☐ > 8 weeks ☐

3.4 Have you ever tested for drench resistance in your goats? Yes ☐ No ☐

3.5 If yes were any resistant worms found. Please explain results.

_____

_____

Thank you for completing this survey. Your participation is much appreciated.

If you have any questions about the survey, please call either Juriah Kamaludeen on (06) 350 5600 (Ext: 4251) or email on j.kamaludeen@massey.ac.nz or William Pomroy on (06) 350 5600 (Ext: 7569) or email on W.Pomroy@massey.ac.nz

APPENDICES: RESULTS FOR CHAPTER 2

APPENDIX 2.8: FAECAL EGG COUNTS FOR CHAPTER 2.

Animal	Treatment Group	Day 22	Day 24	Day 27	Day 29	Day 31	Day 34
195	1	300	600	950	2050	1700	1950
189	1	1050	1400	2200	1900	2300	3200
177	1	1000	800	2200	1950	1500	1450
187	1	1000	1150	3250	50	3850	4000
182	1	2500	3150	5800	5150	5550	7800
193	1	1800	1450	4200	3750	3950	300
192	2	750	0	50	0 (9.5)	50 (3.0)	0 (8.0)
190	2	800	0	0	0 (6.5)	0 (1.5)	0 (2.0)
178	2	800	0	0	0 (2.5)	50 (5.0)	0 (1.0)
184	2	1700	0	0	50 (26)	0 (7.0)	50 (22.5)
181	2	1100	0	50	0 (5.5)	0 (1.5)	0 (5.5)
188	2	1350	100	0	100 (12.5)	0 (4.5)	0 (9.5)
185	3	1250	0	0	0 (1)	0 (0.0)	0 (0.0)
186	3	900	0	0	0 (2)	0 (0.0)	50 (3.0)
179	3	1000	0	0	50 (1.5)	0 (0.5)	50 (0.5)
183	3	3650	100	100	50 (1)	0 (0.0)	0 (1.0)
194	3	1600	0	0	0 (0)	0 (1.0)	0 (1.0)
191	3	2150	0	0	0 (0)	0 (0.0)	0 (0.0)
180	3	1700	0	50	0 (4.5)	0 (14.5)	0 (9.0)

( ) value for faecal float

Appendix 2.9: Larval cultures for Chapter 2.

Day 22 (Pre treatment)

Treatment	*H. contortus*	*T. circumcincta*	*T. colubriformis*	*Oe. venulosum*	Total
Group 1	70%	15%	13%	2%	100%
Group 2	85%	9%	6%	x	100%
Group 3	54%	25%	21%	x	100%

Day 24

Treatment	*H. contortus*	*T. circumcincta*	*T. colubriformis*	*Oe. venulosum*	Total
Group 1	x	x	x	x	0
Group 2	x	x	x	x	0
Group 3	x	x	x	x	0

Day 27

Treatment	*H. contortus*	*T. circumcincta*	*T. colubriformis*	*Oe. venulosum*	Total
Group 1	60%	8%	32%	0	100%
Group 2	0	0	0	0	0
Group 3	0	0	0	0	0

Day 29

Treatment	*H. contortus*	*T. circumcincta*	*T. colubriformis*	*Oe. venulosum*	Total
Group 1	64%	10%	26%	0	100%
Group 2	0	0	0	0	0
Group 3	5%	21%	74%	0	100%

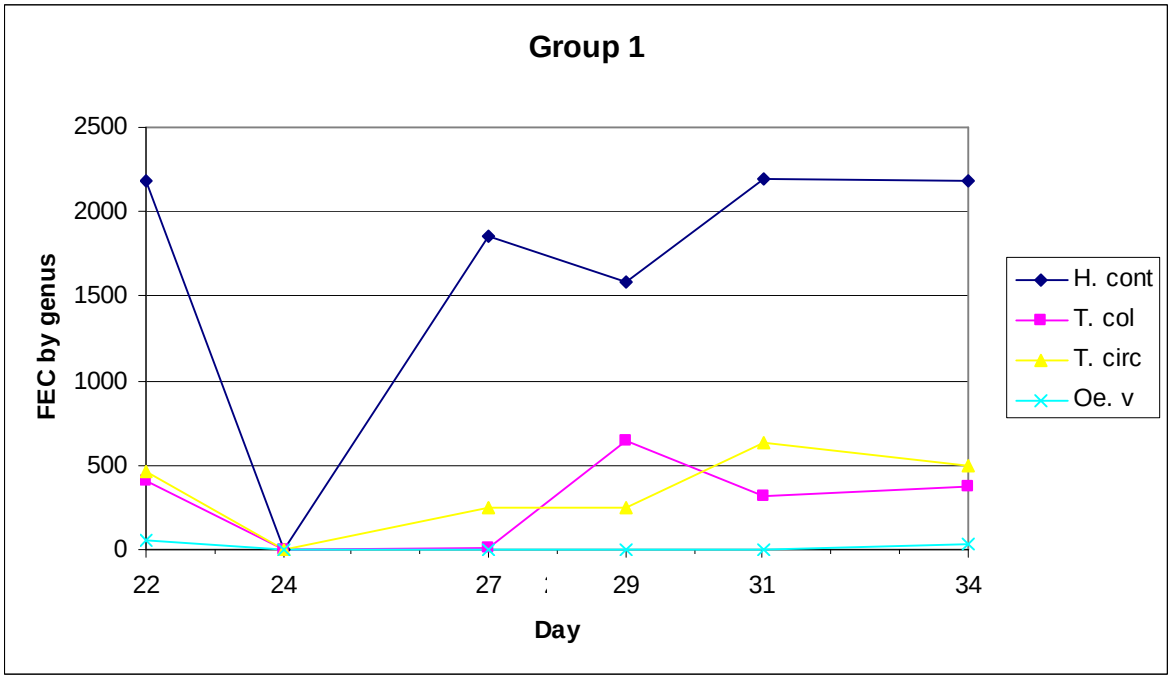
Day 31

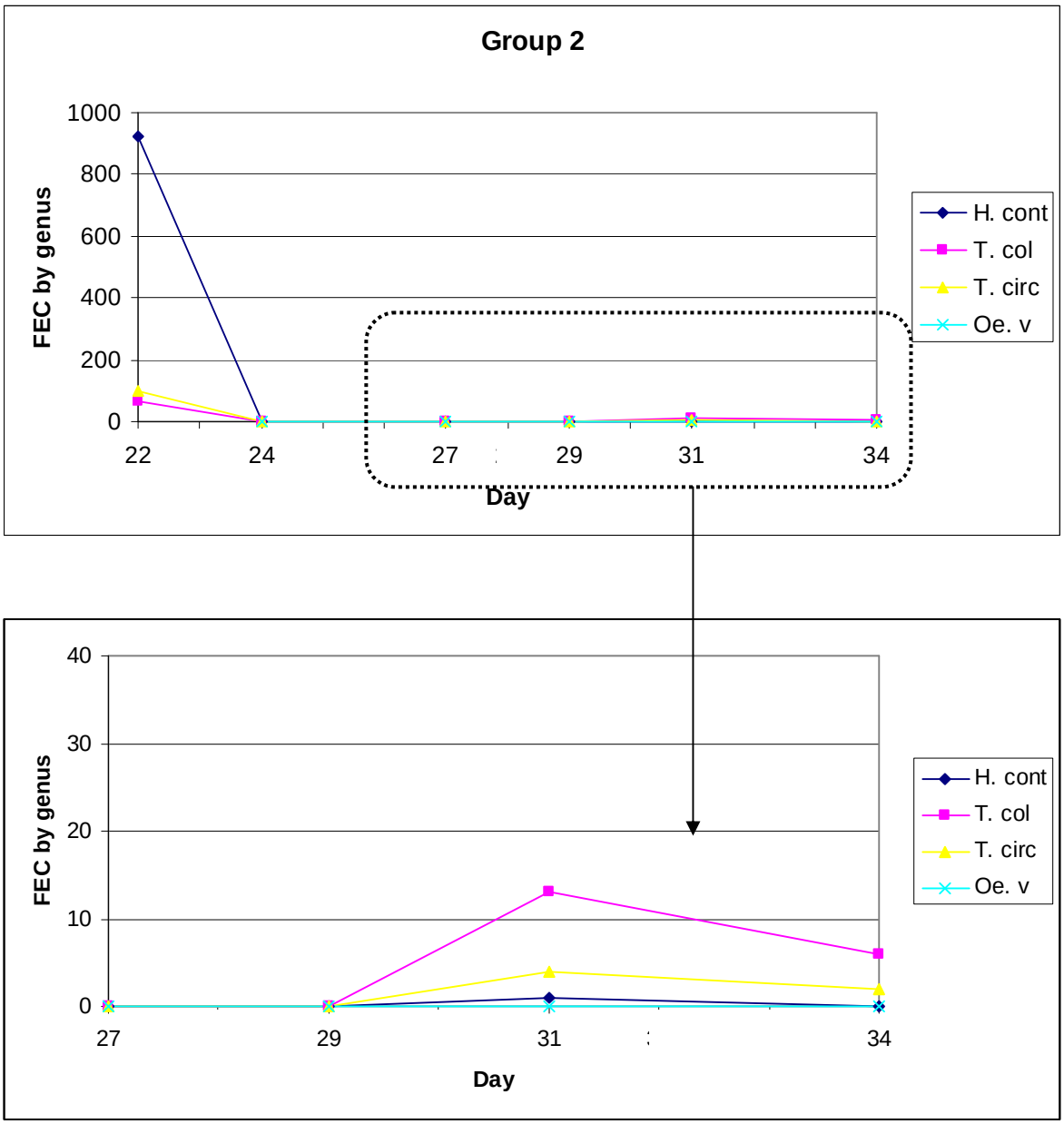
Treatment	*H. contortus*	*T. circumcincta*	*T. colubriformis*	*Oe. venulosum*	Total
Group 1	70%	20%	10%	0%	100%
Group 2	1%	23%	76%	0	100%
Group 3	0	0	0	0	0

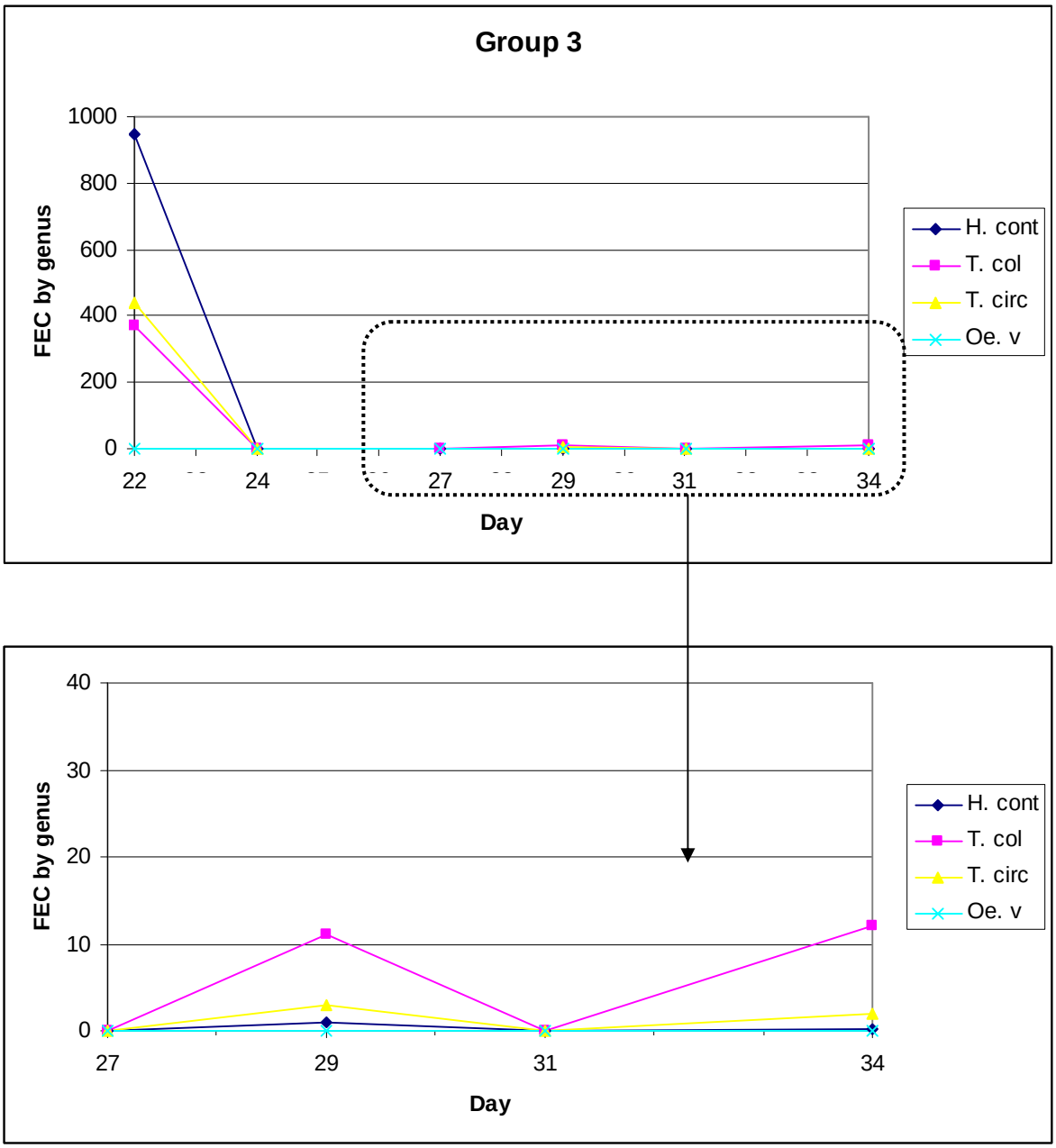
Day 34

Treatment	*H. contortus*	*T. circumcincta*	*T. colubriformis*	*Oe. venulosum*	Total
Group 1	70%	16%	12%	1%	100%
Group 2	0%	22%	78%	0	100%
Group 3	1%	12%	87%	0	100%

Appendix 2.10: Arithmetic mean FECs from Group 1 (control), Group 2 (single dose) and Group 3 (double dose). All sheep challenged with 3780 *H.contortus*, 1260 *Teladorsagia* and 3192 *Trichostrongylus* and 168 *Oesophagostomum/Chabertia* on Day 0. On Day 22, Group 2 and Group 3 were treated with anthelmintic for Chapter 2.




Group 2
1000
800
600
400
200
0
FEC by genus
22
24
27
29
31
34
Day
H. cont
T. col
T. circ
Oe. v
40
30
20
10
0
FEC by genus
27
29
31
34
Day
H. cont
T. col
T. circ
Oe. v


Group 3
FEC by genus
1000
800
600
400
200
0
22
24
27
29
31
34
Day
H. cont
T. col
T. circ
Oe. v
FEC by genus
40
30
20
10
0
27
29
31
34
Day
H. cont
T. col
T. circ
Oe. v

Appendix 2.11: Total worm counts from abomasums, small intestines and large intestines for Chapter 2.

Animal	Treatment Group	*H. contortus*	*T. circumcincta*	*T. colubriformis*	*Oe. venulosum*
195	1	530	370	410	12
189	1	600	300	400	4
177	1	420	430	320	25
187	1	710	420	350	0
182	1	1100	460	370	8
193	1	680	40	400	6
192	2	0	10	170	0
190	2	0	60	450	0
178	2	0	50	170	0
184	2	0	190	200	0
181	2	0	30	170	0
188	2	10	90	160	0
185	3	0	0	40	0
186	3	0	0	100	0
179	3	0	0	110	0
183	3	0	20	70	0
194	3	0	20	90	0
191	3	0	0	10	0
180	3	0	30	150	0

Appendix 2.12: DrenchRite® LDA results for Group 1 (Day 23) showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 2.

Row A (BZ)				Row B (BZ)				BZ LogConc	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*		*H. cont*	*Trich*	*Tel*
Control	48	13	2	Control	25	14	7				
2	24	31	6	2	20	25	1	-2.0	47.7	114.3	105.9
3	46	21	4	3	50	24	4	-1.7	104.0	91.8	70.6
4	41	25	3	4	22	22	6	-1.4	68.2	95.9	52.9
5	21	15	1	5	18	18	1	-1.11	42.2	67.3	17.6
6	13	20	4	6	14	14	10	-0.81	29.2	69.4	70.6
7	15	29	3	7	8	8	6	-0.5	24.9	75.5	52.9
8	7	16	1	8	10	10	3	-0.2	18.4	53.1	17.6
9	6	14	1	9	10	10	3	0.1	17.3	49.0	17.6
10	0	1	0	10	0	0	0	0.4	0.0	2.0	0.0
11	0	2	0	11	0	1	0	0.7	0.0	6.1	0.0
12	0	0	0	12	0	2	0	1.0	0.0	4.1	0.0
Row C (LEV)				**Row D (LEV)**				**LEV LogConc**	**Mean proportion larval develop**		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*		*H. cont*	*Trich*	*Tel*
Control	42	17	10	Control	20	18	8				
2	3	8	8	2	30	14	4	-0.71	35.7	95.7	105.9
3	4	11	5	3	39	9	7	-0.41	46.6	87.0	105.9
4	13	19	8	4	27	8	5	-0.11	43.3	117.4	114.7
5	1	12	3	5	1	10	8	0.19	2.2	95.7	97.1
6	0	8	6	6	0	12	6	0.49	0.0	87.0	105.9
7	0	10	3	7	0	9	5	0.80	0.0	82.6	70.6
8	0	1	1	8	0	1	0	1.10	0.0	8.7	8.8
9	0	1	0	9	0	1	0	1.40	0.0	8.7	0.0
10	0	0	0	10	0	0	0	1.40	0.0	0.0	0.0
11	0	1	0	11	0	1	0	1.40	0.0	8.7	0.0
12	0	2	0	12	0	3	0	1.40	0.0	21.7	0.0

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	60	10	5	Control	44	12	6	x			
2	29	10	1	2	35	5	4	x	69.3	16.2	5.4
3	33	8	2	3	39	10	4	x	78.0	19.5	6.5
4	12	8	3	4	16	5	3	x	30.3	14.1	6.5
5	1	7	4	5	0	7	5	x	1.1	15.2	9.7
6	2	7	3	6	2	7	3	x	4.3	15.2	6.5
7	1	7	3	7	1	6	2	x	2.2	14.1	5.4
8	0	4	4	8	0	8	1	x	0.0	13.0	5.4
9	0	1	3	9	0	0	0	x	0.0	1.1	3.2
10	0	1	1	10	0	1	0	x	0.0	2.2	1.1
11	0	0	1	11	0	0	0	x	0.0	0.0	1.1
12	0	1	0	12	0	0	0	x	0.0	1.1	0.0

Row G (IVM-1)					Proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM-1 LogConc	*H. cont*	*Trich*	*Tel*
Control	43	9	3				
2	25	1	1	-0.3	54.2	8.7	17.6
3	30	7	2	-0.01	65.0	60.9	35.3
4	44	7	2	0.28	95.3	60.9	35.3
5	26	3	4	0.59	56.3	26.1	70.6
6	22	7	3	0.89	47.7	60.9	52.9
7	3	3	3	1.19	6.5	26.1	52.9
8	0	2	4	1.49	0.0	17.4	70.6
9	0	0	3	1.8	0.0	0.0	52.9
10	0	1	1	2.1	0.0	8.7	17.6
11	0	1	1	2.4	0.0	8.7	17.6
12	0	0	0	2.7	0.0	0.0	0.0

Row H (IVM-2)					Proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM-2 LogConc	*H. cont*	*Trich*	*Tel*
Control	40	11	4				
2	26	8	4	-0.01	56.3	69.6	70.6
3	19	8	4	0.28	41.2	69.6	70.6
4	17	4	1	0.59	36.8	34.8	17.6
5	31	6	5	0.89	67.1	52.2	88.2
6	1	6	3	1.19	2.2	52.2	52.9
7	4	5	6	1.49	8.7	43.5	105.9
8	0	2	2	1.8	0.0	17.4	35.3
9	0	3	0	2.1	0.0	26.1	0.0
10	0	2	1	2.4	0.0	17.4	17.6
11	0	1	0	2.7	0.0	8.7	0.0
12	0	0	0	3	0.0	0.0	0.0

Appendix 2.13: DrenchRite® LDA results for Group 2 (Day 35) showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 2.

Row A (BZ)				Row B (BZ)				BZ LogConc	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*		*H. cont*	*Trich*	*Tel*
Control	0	20	6	Control	0	12	6				
2	0	15	5	2	0	16	6	-2.0	0	66.9	50.8
3	0	14	5	3	0	29	5	-1.7	0	92.8	46.2
4	0	10	12	4	0	8	5	-1.4	0	38.8	78.5
5	0	10	2	5	0	2	1	-1.11	0	25.9	13.8
6	0	8	2	6	0	10	4	-0.81	0	38.8	27.7
7	0	18	7	7	0	7	5	-0.5	0	54.0	55.4
8	0	35	10	8	0	12	2	-0.2	0	101.4	55.4
9	0	6	1	9	0	18	2	0.1	0	51.8	13.8
10	0	11	1	10	0	5	0	0.4	0	34.5	4.6
11	0	0	0	11	0	0	0	0.7	0	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0	0.0	0.0

Row C (LEV)				Row D (LEV)				LEV LogConc	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*		*H. cont*	*Trich*	*Tel*
Control	0	24	4	Control	0	24	6				
2	0	11	4	2	0	7	6	-0.71	0	42.9	93.8
3	0	18	5	3	0	15	3	-0.41	0	78.6	75.0
4	0	12	4	4	0	7	2	-0.11	0	45.2	56.3
5	0	7	4	5	0	6	2	0.19	0	31.0	56.3
6	0	11	2	6	0	17	4	0.49	0	66.7	56.3
7	0	6	2	7	0	35	3	0.80	0	97.6	46.9
8	0	5	0	8	0	19	0	1.10	0	57.1	0.0
9	0	0	0	9	0	5	0	1.40	0	11.9	0.0
10	0	1	0	10	0	3	0	1.40	0	9.5	0.0
11	0	2	0	11	0	8	0	1.40	0	23.8	0.0
12	0	10	0	12	0	5	0	1.40	0	35.7	0.0

Row G (IVM-1)				IVM-1	Proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	25	5				
2	0	9	3	-0.3	0	39.5	56.3
3	0	18	4	-0.01	0	78.9	75.0
4	0	17	4	0.28	0	74.6	75.0
5	0	13	5	0.59	0	57.0	93.8
6	0	9	3	0.89	0	39.5	56.3
7	0	14	5	1.19	0	61.4	93.8
8	0	16	4	1.49	0	70.2	75.0
9	0	16	3	1.8	0	70.2	56.3
10	0	10	2	2.1	0	43.9	37.5
11	0	3	0	2.4	0	13.2	0.0
12	0	8	2	2.7	0	35.1	37.5

Row H (IVM-2)				IVM-2	Proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	21	5				
2	0	12	2	-0.01	0	52.6	37.5
3	0	28	5	0.28	0	122.8	93.8
4	0	20	5	0.59	0	87.7	93.8
5	0	16	7	0.89	0	70.2	131.3
6	0	10	1	1.19	0	43.9	18.8
7	0	16	3	1.49	0	70.2	56.3
8	0	9	5	1.80	0	39.5	93.8
9	0	10	4	2.10	0	43.9	75.0
10	0	21	8	2.40	0	92.1	150.0
11	0	6	0	2.70	0	26.3	0.0
12	0	10	0	3.00	0	43.9	0.0

Appendix 2.14: DrenchRite® LDA results for susceptible isolates. Separate animals used for each species. Values are also shown for numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 2 and Chapter 3.

Row A (BZ)				**Row B (BZ)**					Mean proportion larval develop		
Well	*H .cont*	*Trich*	*Tel*	Well	*H .cont*	*Trich*	*Tel*	BZ LogConc	*H .cont*	*Trich*	*Tel*
Control	63	40	45	Control	64	45	43				
2	54	33	29	2	60	35	42	-2.0	101.5	79.8	82.6
3	51	34	36	3	44	35	33	-1.7	84.6	80.9	80.2
4	5	23	4	4	5	28	0	-1.4	8.9	59.8	4.7
5	0	8	0	5	0	10	0	-1.11	0.0	21.1	0.0
6	0	3	0	6	0	2	0	-0.81	0.0	5.9	0.0
7	0	1	2	7	0	0	0	-0.5	0.0	1.2	2.3
8	0	0	1	8	0	2	0	-0.2	0.0	2.3	1.2
9	0	0	0	9	0	0	0	0.1	0.0	0.0	0.0
10	0	0	0	10	0	0	0	0.4	0.0	0.0	0.0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				**LEV LogConc**	**Mean proportion larval develop**		
Well	*H .cont*	*Trich*	*Tel*	Well	*H .cont*	*Trich*	*Tel*		*H .cont*	*Trich*	*Tel*
Control	58	42	50	Control	52	45	47				
2	47	34	39	2	48	42	35	-0.71	84.6	89.1	86.0
3	43	38	30	3	21	47	32	-0.41	57.0	99.7	72.1
4	18	33	25	4	32	31	37	-0.11	44.5	75.1	72.1
5	19	29	15	5	1	35	30	0.19	17.8	75.1	52.3
6	7	12	8	6	0	4	4	0.49	6.2	18.8	14.0
7	0	1	0	7	0	0	0	0.80	0.0	1.2	0.0
8	0	0	0	8	0	0	0	1.10	0.0	0.0	0.0
9	0	0	0	9	0	0	0	1.40	0.0	0.0	0.0
10	0	0	0	10	0	0	0	1.40	0.0	0.0	0.0
11	0	1	0	11	0	0	0	1.40	0.0	1.2	0.0
12	0	1	0	12	0	0	0	1.40	0.0	1.2	0.0

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	50	44	40	Control	50	40	41	x			
2	50	42	30	2	46	36	39	x	81.9	91.5	80.2
3	28	40	34	3	25	33	20	x	44.5	85.6	62.8
4	2	41	20	4	2	32	15	x	3.6	85.6	40.7
5	0	22	2	5	0.5	23	2	x	0.9	52.8	4.7
6	0	10	1	6	0	11	0	x	0.0	24.6	1.2
7	0	0	0	7	0	1	0	x	0.0	1.2	0.0
8	0	0	0	8	0	0	0	x	0.0	0.0	0.0
9	0	0	0	9	0	0	0	x	0.0	0.0	0.0
10	0	0	0	10	0	0	0	x	0.0	0.0	0.0
11	0	0	0	11	0	0	0	x	0.0	0.0	0.0
12	0	0	0	12	0	0	0	x	0.0	0.0	0.0

Row G (IVM-1)					Proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	46	40	40				
2	37	31	28	-0.3	65.9	72.7	65.1
3	41	21	30	-0.01	73.0	49.3	69.8
4	24	34	30	0.28	42.7	79.8	69.8
5	20	30	23	0.59	35.6	70.4	53.5
6	6	20	25	0.89	10.7	46.9	58.1
7	0	20	24	1.19	0.0	46.9	55.8
8	0	13	13	1.49	0.0	30.5	30.2
9	0	2	0	1.8	0.0	4.7	0.0
10	0	0	0	2.1	0.0	0.0	0.0
11	0	0	0	2.4	0.0	0.0	0.0
12	0	0	0	2.7	0.0	0.0	0.0

Row H (IVM-2)					Proportion larval develop		
Well	*H .cont*	*Trich*	*Tel*	IVM-2 LogConc	*H .cont*	*Trich*	*Tel*
Control	48	45	38				
2	36	31	31	-0.01	64.1	72.7	72.1
3	18	29	31	0.28	32.0	68.0	72.1
4	11	25	36	0.59	19.6	58.7	83.7
5	2	22	32	0.89	3.6	51.6	74.4
6	0	20	26	1.19	0.0	46.9	60.5
7	0	9	20	1.49	0.0	21.1	46.5
8	0	7	17	1.8	0.0	16.4	39.5
9	0	9	5	2.1	0.0	21.1	11.6
10	0	9	0	2.4	0.0	21.1	0.0
11	0	4	0	2.7	0.0	9.4	0.0
12	0	0	0	3.0	0.0	0.0	0.0

APPENDICES: RESULTS FOR CHAPTER 3

Appendix 3.1: Results from the survey from 17 goat farms for Chapter 3.

Farm ID	Responses	Enterprise	Farm Size (ha)	Goat numbers (range)	Other animals	Drenching dose rate*	Time of faecal samples collected after last drench	Results of drench resistance study
1.❶	Yes	Meat	4ha	0-50	sheep(0-50)/cattle (0-50)/duck	1	4-8 wks	no
2.❷	Yes	Meat	1.6ha	0-50	horse (2)	2	>8 wks	no
3.❸	Yes	Meat	3.2ha	50-200	cattle (0-50)	1	>8 wks	Yes (*Teladorsagia* /IVOMEC)
4.❹	no	n.a	n.a	n.a	n.a	n.a	n.a	n.a
5.❺	Yes	Meat	48.6ha	50-200	cow/calves (50-200)	1	>8 wks	no
6.❻	Yes	Meat	120ha	>1000	none	2	>8 wks	Yes (none)
7.❼	Yes	Meat	1ha	0-50	none	none	>8 wks	none
8.❽	Yes	Meat	7ha	50-200	cattle (0-50)	2	>8 wks	no
9.❾	Yes	Meat	10ha	0-50	deer (420)	2	>8 wks	Yes BZ (Reduction 67%), LEV (Reduction 68%), IVOMEC (Reduction 47%)
10.❿	Yes	Meat	14ha	50-200	none	2	4-8 wks	no
11.▼	no	n.a	n.a	n.a	n.a	n.a	n.a	n.a
12.★	Yes	Meat	1.6ha	0-50	none	2	>8 wks	no
13.◈	no	n.a	n.a	n.a	n.a	n.a	n.a	n.a
14.⊙	Yes	Meat	600ha	>1000	sheep (200-500)	2	4-8 wks	no
15.◆	Yes	Meat	20ha	50-200	cattle (0-50)	2	4-8 wks	no
16.✕	Yes	Meat	18ha	200-500	cattle (0-50)	1 and 2	>8 wks	Yes (none)
17.□	Yes	Dairy	21ha	200-500	none	2	>8 wks	Yes (none)

*Drenching dose rate: sheep dose rate (1); >sheep dose rate (2); (1)+(2)=(3); n.a: not applicable

Appendix 3.2: DrenchRite® LDA results for farm ❶ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	8	4	0	Control	18	21	0				
2	7	11	0	2	17	8	0	-2.0	57.5	49.6	0
3	10	10	0	3	11	10	0	-1.7	50.3	52.2	0
4	29	14	0	4	22	18	0	-1.4	122.3	83.6	0
5	9	8	0	5	8	12	0	-1.11	40.8	52.2	0
6	16	21	0	6	10	25	0	-0.81	62.3	120.1	0
7	14	16	0	7	10	20	0	-0.5	57.5	94.0	0
8	11	21	0	8	16	11	0	-0.2	64.7	83.6	0
9	12	9	1	9	20	12	1	0.1	76.7	54.9	0
10	3	0	0	10	5	1	0	0.4	19.2	2.6	1
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0
12	1	0	0	12	0	0	0	1.0	2.4	0.0	0
Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	16	17	0	Control	21	21	0				
2	23	18	0	2	16	14	0	-0.71	93.5	83.6	0
3	12	7	0	3	17	15	0	-0.41	69.5	57.5	0
4	19	16	0	4	15	16	0	-0.11	81.5	83.6	0
5	17	14	0	5	18	10	0	0.19	83.9	62.7	0
6	3	16	0	6	0	7	0	0.49	7.2	60.1	0
7	1	17	0	7	0	12	0	0.80	2.4	75.7	0
8	0	10	0	8	1	16	0	1.10	2.4	67.9	0
9	0	20	0	9	0	20	0	1.40	0.0	104.5	0
10	0	10	0	10	0	9	0	1.40	0.0	49.6	0
11	0	12	0	11	0	10	0	1.40	0.0	57.5	0
12	0	11	0	12	0	11	0	1.40	0.0	57.5	0

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	23	17	0	Control	25	20	0	x			
2	18	12	0	2	17	15	0	x	83.9	70.5	0
3	10	0	0	3	16	9	0	x	62.3	23.5	0
4	19	16	0	4	12	6	0	x	74.3	57.5	0
5	14	4	0	5	10	8	0	x	57.5	31.3	0
6	4	20	0	6	5	15	0	x	21.6	91.4	0
7	6	9	0	7	0	10	0	x	14.4	49.6	0
8	0	14	0	8	0	11	0	x	0.0	65.3	0
9	0	9	0	9	0	7	0	x	0.0	41.8	0
10	0	7	0	10	0	9	0	x	0.0	41.8	0
11	0	9	0	11	0	8	0	x	0.0	44.4	0
12	1	1	0	12	1	0	0	x	4.8	2.6	0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	21	19	0				
2	14	10	0	-0.3	67.1	52.2	0
3	11	9	0	-0.01	52.7	47.0	0
4	10	18	0	0.28	47.9	94.0	0
5	2	9	0	0.59	9.6	47.0	0
6	0	17	0	0.89	0.0	88.8	0
7	0	20	0	1.19	0.0	104.5	0
8	0	14	0	1.49	0.0	73.1	0
9	0	5	0	1.8	0.0	26.1	0
10	0	1	0	2.1	0.0	5.2	0
11	0	0	0	2.4	0.0	0.0	0
12	0	0	0	2.7	0.0	0.0	0

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	22	19	0				
2	24	22	0	-0.01	115.1	114.9	0
3	21	18	0	0.28	100.7	94.0	0
4	10	26	0	0.59	47.9	135.8	0
5	7	26	0	0.89	33.6	135.8	0
6	2	22	0	1.19	9.6	114.9	0
7	0	21	0	1.49	0.0	109.7	0
8	0	13	0	1.8	0.0	67.9	0
9	0	12	0	2.1	0.0	62.7	0
10	0	14	0	2.4	0.0	73.1	0
11	0	7	0	2.7	0.0	36.6	0
12	0	0	0	3	0.0	0.0	0

Appendix 3.3: DrenchRite® LDA results for farm ❷ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	40	7	4	Control	49	6	4				
2	44	6	2	2	35	5	4	-2.0	89.0	78.6	88.9
3	23	4	6	3	32	4	2	-1.7	62.0	57.1	118.5
4	39	7	2	4	32	2	0	-1.4	80.0	64.3	29.6
5	26	6	7	5	27	4	3	-1.11	59.7	71.4	148.1
6	26	4	5	6	31	3	2	-0.81	64.2	50.0	103.7
7	22	7	1	7	30	2	4	-0.5	58.6	64.3	74.1
8	24	0	2	8	23	2	0	-0.2	53.0	14.3	29.6
9	20	0	0	9	23	1	0	0.1	48.5	7.1	0.0
10	7	0	0	10	7	0	0	0.4	15.8	0.0	0.0
11	1	0	0	11	2	0	0	0.7	3.4	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0
Row C (LEV)				**Row D (LEV)**					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LEV LogConc	*Mean H .c*	*Mean Trich*	*Mean Tel*
Control	40	5	4	Control	38	8	2				
2	30	2	0	2	27	5	4	-0.71	64.2	50.0	59.3
3	33	5	0	3	25	3	0	-0.41	65.4	57.1	0.0
4	21	3	1	4	20	8	3	-0.11	46.2	78.6	59.3
5	4	1	0	5	13	5	1	0.19	19.2	42.9	14.8
6	3	2	0	6	8	3	0	0.49	12.4	35.7	0.0
7	0	1	0	7	0	3	0	0.80	0.0	28.6	0.0
8	0	0	0	8	0	0	0	1.10	0.0	0.0	0.0
9	0	0	0	9	0	2	0	1.40	0.0	14.3	0.0
10	0	0	0	10	0	0	0	1.40	0.0	0.0	0.0
11	1	0	0	11	0	0	0	1.40	1.1	0.0	0.0
12	0	0	0	12	1	0	0	1.40	1.1	0.0	0.0

Row E (BZ+LEV)				**Row F (BZ+LEV)**					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	40	7	2	Control	50	7	3	x			
2	31	3	2	2	38	6	2	x	77.7	64.3	59.3
3	26	2	0	3	29	7	3	x	62.0	64.3	44.4
4	25	6	1	4	29	8	3	x	60.8	100.0	59.3
5	28	7	2	5	25	2	2	x	59.7	64.3	59.3
6	14	10	0	6	6	2	0	x	22.5	85.7	0.0
7	11	1	0	7	5	0	0	x	18.0	7.1	0.0
8	1	0	0	8	0	2	0	x	1.1	14.3	0.0
9	0	0	0	9	0	0	0	x	0.0	0.0	0.0
10	0	0	0	10	0	1	0	x	0.0	7.1	0.0
11	0	0	0	11	0	0	0	x	0.0	0.0	0.0
12	0	0	0	12	0	0	0	x	0.0	0.0	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	50	8	1				
2	34	3	4	-0.3	76.6	42.9	118.5
3	31	7	4	-0.01	69.9	100.0	118.5
4	39	0	1	0.28	87.9	0.0	29.6
5	25	3	1	0.59	56.3	42.9	29.6
6	8	10	3	0.89	18.0	142.9	88.9
7	2	5	2	1.19	4.5	71.4	59.3
8	0	3	4	1.49	0.0	42.9	118.5
9	0	2	3	1.8	0.0	28.6	88.9
10	0	4	1	2.1	0.0	57.1	29.6
11	0	1	0	2.4	0.0	14.3	0.0
12	0	0	0	2.7	0.0	0.0	0.0

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	48	8	7				
2	38	6	3	-0.01	85.6	85.7	88.9
3	29	8	1	0.28	65.4	114.3	29.6
4	23	5	2	0.59	51.8	71.4	59.3
5	19	7	6	0.89	42.8	100.0	177.8
6	3	6	3	1.19	6.8	85.7	88.9
7	0	4	1	1.49	0.0	57.1	29.6
8	0	5	1	1.8	0.0	71.4	29.6
9	0	5	1	2.1	0.0	71.4	29.6
10	0	0	0	2.4	0.0	0.0	0.0
11	0	2	0	2.7	0.0	28.6	0.0
12	0	1	0	3.0	0.0	14.3	0.0

Appendix 3.4: DrenchRite® LDA results for farm ❸ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	5	15	3	Control	19	40	8				
2	3	20	10	2	6	30	8	-2.0	67.0	91.1	131.3
3	2	20	1	3	5	26	4	-1.7	52.1	83.9	36.5
4	2	10	0	4	7	28	5	-1.4	67.0	69.3	36.5
5	5	35	4	5	6	20	4	-1.11	81.9	100.3	58.3
6	5	25	4	6	3	21	4	-0.81	59.6	83.9	58.3
7	5	20	3	7	3	25	7	-0.5	59.6	82.0	72.9
8	3	15	0	8	5	10	6	-0.2	59.6	45.6	43.8
9	0	6	2	9	3	3	5	0.1	22.3	16.4	51.0
10	1	0	0	10	0	0	0	0.4	7.4	0.0	0.0
11	1	0	0	11	0	0	0	0.7	7.4	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	5	30	10	Control	6	34	6				
2	1	30	1	2	3	30	2	-0.71	29.8	109.4	21.9
3	1	29	0	3	2	15	6	-0.41	22.3	80.2	43.8
4	0	20	0	4	0	21	4	-0.11	0.0	74.7	29.2
5	2	17	2	5	0	20	3	0.19	14.9	67.4	36.5
6	0	6	1	6	0	15	1	0.49	0.0	38.3	14.6
7	0	7	1	7	0	15	1	0.80	0.0	40.1	14.6
8	0	5	0	8	0	7	0	1.10	0.0	21.9	0.0
9	0	0	0	9	0	0	0	1.40	0.0	0.0	0.0
10	0	0	0	10	0	6	0	1.40	0.0	10.9	0.0
11	0	2	0	11	0	3	0	1.40	0.0	9.1	0.0
12	0	0	0	12	0	0	0	1.40	0.0	0.0	0.0

Row E (BZ+LEV)				**Row F (BZ+LEV)**					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	9	29	6	Control	11	32	12	x			
2	5	25	7	2	6	20	4	x	81.9	82.0	80.2
3	6	31	6	3	1	13	1	x	52.1	80.2	51.0
4	2	18	2	4	5	19	4	x	52.1	67.4	43.8
5	1	15	3	5	1	16	1	x	14.9	56.5	29.2
6	0	12	0	6	0	13	2	x	0.0	45.6	14.6
7	0	14	1	7	0	0	0	x	0.0	25.5	7.3
8	0	3	0	8	0	5	0	x	0.0	14.6	0.0
9	0	0	0	9	0	3	0	x	0.0	5.5	0.0
10	0	0	0	10	0	3	0	x	0.0	5.5	0.0
11	0	2	0	11	0	0	0	x	0.0	3.6	0.0
12	0	0	0	12	0	0	0	x	0.0	0.0	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	7	27	7				
2	2	15	2	-0.3	29.8	54.7	29.2
3	7	24	8	-0.01	104.3	87.5	116.7
4	1	24	1	0.28	14.9	87.5	14.6
5	3	25	3	0.59	44.7	91.1	43.8
6	0	18	3	0.89	0.0	65.6	43.8
7	0	13	2	1.19	0.0	47.4	29.2
8	0	15	3	1.49	0.0	54.7	43.8
9	0	3	2	1.8	0.0	10.9	29.2
10	0	1	3	2.1	0.0	3.6	43.8
11	0	1	1	2.4	0.0	3.6	14.6
12	0	0	0	2.7	0.0	0.0	0.0

Row H (IVM-2)				IVM 2 LogConc	proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*		*H. cont*	*Trich*	*Tel*
Control	4	25	4				
2	1	26	6	-0.01	14.9	94.8	87.5
3	8	24	2	0.28	119.1	87.5	29.2
4	0	19	0	0.59	0.0	69.3	0.0
5	1	20	2	0.89	14.9	72.9	29.2
6	0	14	2	1.19	0.0	51.0	29.2
7	0	13	3	1.49	0.0	47.4	43.8
8	0	14	1	1.8	0.0	51.0	14.6
9	0	17	3	2.1	0.0	62.0	43.8
10	0	25	0	2.4	0.0	91.1	0.0
11	0	10	0	2.7	0.0	36.5	0.0
12	0	5	0	3.0	0.0	18.2	0.0

Appendix 3.5: DrenchRite® LDA results for farm ❹ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	24	30	0	Control	30	36	4				
2	14	40	2	2	21	44	2	-2.0	66.0	130.2	188.2
3	20	36	1	3	28	24	1	-1.7	90.6	93.0	94.1
4	30	30	3	4	39	20	0	-1.4	130.2	77.5	141.2
5	28	15	3	5	30	20	0	-1.11	109.4	54.3	141.2
6	28	10	3	6	21	8	0	-0.81	92.5	27.9	141.2
7	20	16	0	7	18	10	2	-0.5	71.7	40.3	94.1
8	10	8	2	8	7	3	0	-0.2	32.1	17.1	94.1
9	4	2	0	9	10	3	0	0.1	26.4	7.8	0.0
10	0	1	0	10	0	0	0	0.4	0.0	1.6	0.0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	31	28	3	Control	24	28	5				
2	29	28	2	2	30	29	0	-0.71	111.3	88.4	47.1
3	24	32	4	3	22	31	2	-0.41	86.8	97.7	141.2
4	27	28	2	4	13	14	0	-0.11	75.5	65.1	47.1
5	9	26	3	5	6	15	1	0.19	28.3	63.6	94.1
6	5	24	0	6	2	23	0	0.49	13.2	72.9	0.0
7	3	20	0	7	1	15	0	0.80	7.5	54.3	0.0
8	5	19	0	8	0	10	0	1.10	9.4	45.0	0.0
9	0	3	0	9	0	1	0	1.40	0.0	6.2	0.0
10	1	3	0	10	0	1	0	1.40	1.9	6.2	0.0
11	0	4	0	11	0	2	0	1.40	0.0	9.3	0.0
12	3	6	0	12	0	3	0	1.40	5.7	14.0	0.0

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	21	30	3	Control	27	34	1	x			
2	17	15	2	2	16	30	1	x	62.3	69.8	70.6
3	25	22	1	3	16	37	2	x	77.4	91.5	70.6
4	12	20	2	4	8	16	0	x	37.7	55.8	47.1
5	5	19	0	5	5	15	1	x	18.9	52.7	23.5
6	3	19	1	6	2	14	0	x	9.4	51.2	23.5
7	0	12	0	7	0	15	0	x	0.0	41.9	0.0
8	0	4	0	8	0	1	0	x	0.0	7.8	0.0
9	0	0	0	9	0	0	0	x	0.0	0.0	0.0
10	0	0	0	10	0	2	0	x	0.0	3.1	0.0
11	0	2	0	11	0	0	0	x	0.0	3.1	0.0
12	0	0	0	12	0	1	0	x	0.0	1.6	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	21	39	2				
2	21	35	2	-0.3	79.2	108.5	94.1
3	19	36	2	-0.01	71.7	111.6	94.1
4	16	23	0	0.28	60.4	71.3	0.0
5	11	30	1	0.59	41.5	93.0	47.1
6	7	19	0	0.89	26.4	58.9	0.0
7	0	23	2	1.19	0.0	71.3	94.1
8	0	6	1	1.49	0.0	18.6	47.1
9	0	2	1	1.8	0.0	6.2	47.1
10	0	0	0	2.1	0.0	0.0	0.0
11	0	2	0	2.4	0.0	6.2	0.0
12	0	0	0	2.7	0.0	0.0	0.0

Row H (IVM-2)				IVM 2 LogConc	proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*		*H. cont*	*Trich*	*Tel*
Control	34	33	1				
2	24	37	1	-0.01	90.6	114.7	47.1
3	20	42	1	0.28	75.5	130.2	47.1
4	10	16	1	0.59	37.7	49.6	47.1
5	4	30	1	0.89	15.1	93.0	47.1
6	1	30	0	1.19	3.8	93.0	0.0
7	1	30	1	1.49	3.8	93.0	47.1
8	0	28	0	1.8	0.0	86.8	0.0
9	0	0	0	2.1	0.0	0.0	0.0
10	0	14	0	2.4	0.0	43.4	0.0
11	0	5	0	2.7	0.0	15.5	0.0
12	0	1	0	3.0	0.0	3.1	0.0

Appendix 3.6: DrenchRite® LDA results for farm ❺ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	55	3	Control	0	52	13				
2	0	39	2	2	0	41	1	-2.0	0.0	77.1	30.8
3	0	43	1	3	0	49	11	-1.7	0.0	88.7	123.1
4	0	23	23	4	0	22	0	-1.4	0.0	43.4	235.9
5	0	11	9	5	0	18	4	-1.11	0.0	28.0	133.3
6	0	14	9	6	0	5	6	-0.81	0.0	18.3	153.8
7	0	9	5	7	0	6	0	-0.5	0.0	14.5	51.3
8	0	9	1	8	0	5	3	-0.2	0.0	13.5	41.0
9	0	0	1	9	0	1	0	0.1	0.0	1.0	10.3
10	0	0	0	10	0	0	0	0.4	0.0	0.0	0.0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	58	6	Control	0	53	1				
2	0	50	1	2	0	48	3	-0.71	0.0	94.5	41.0
3	0	47	3	3	0	46	2	-0.41	0.0	89.6	51.3
4	0	34	2	4	0	36	4	-0.11	0.0	67.5	61.5
5	0	30	0	5	0	33	0	0.19	0.0	60.7	0.0
6	0	11	0	6	0	14	0	0.49	0.0	24.1	0.0
7	0	1	0	7	0	2	0	0.80	0.0	2.9	0.0
8	0	0	0	8	0	1	0	1.10	0.0	1.0	0.0
9	0	0	0	9	0	1	0	1.40	0.0	1.0	0.0
10	0	1	0	10	0	0	0	1.40	0.0	1.0	0.0
11	0	1	0	11	0	0	0	1.40	0.0	1.0	0.0
12	0	0	0	12	0	1	0	1.40	0.0	1.0	0.0

Row E (BZ+LEV)				**Row F (BZ+LEV)**					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	50	2	Control	0	48	5	x			
2	0	44	4	2	0	41	6	x	0	81.9	102.6
3	0	29	2	3	0	34	3	x	0	60.7	51.3
4	0	16	3	4	0	25	4	x	0	39.5	71.8
5	0	18	1	5	0	25	3	x	0	41.4	41.0
6	0	14	0	6	0	13	0	x	0	26.0	0.0
7	0	4	0	7	0	7	0	x	0	10.6	0.0
8	0	1	0	8	0	2	0	x	0	2.9	0.0
9	0	0	0	9	0	0	0	x	0	0.0	0.0
10	0	0	0	10	0	0	0	x	0	0.0	0.0
11	0	0	0	11	0	1	0	x	0	1.0	0.0
12	0	1	0	12	0	0	0	x	0	1.0	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	50	6				
2	0	44	6	-0.3	0.0	84.8	123.1
3	0	30	3	-0.01	0.0	57.8	61.5
4	0	38	2	0.28	0.0	73.3	41.0
5	0	38	2	0.59	0.0	73.3	41.0
6	0	22	3	0.89	0.0	42.4	61.5
7	0	20	5	1.19	0.0	38.6	102.6
8	0	15	4	1.49	0.0	28.9	82.1
9	0	8	4	1.8	0.0	15.4	82.1
10	0	4	2	2.1	0.0	7.7	41.0
11	0	1	1	2.4	0.0	1.9	20.5
12	0	2	0	2.7	0.0	3.9	0.0

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	49	5				
2	0	33	6	-0.01	0.0	63.6	123.1
3	0	31	5	0.28	0.0	59.8	102.6
4	0	33	6	0.59	0.0	63.6	123.1
5	0	28	4	0.89	0.0	54.0	82.1
6	0	25	3	1.19	0.0	48.2	61.5
7	0	22	1	1.49	0.0	42.4	20.5
8	0	15	4	1.8	0.0	28.9	82.1
9	0	20	2	2.1	0.0	38.6	41.0
10	0	19	3	2.4	0.0	36.6	61.5
11	0	8	0	2.7	0.0	15.4	0.0
12	0	4	0	3.0	0.0	7.7	0.0

Appendix 3.7: DrenchRite® LDA results for farm ❻ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	38	4	Control	0	51	6				
2	0	42	4	2	0	50	2	-2	0.0	83.1	77.4
3	0	27	6	3	0	41	4	-1.7	0.0	61.4	129.0
4	0	9	0	4	0	17	2	-1.4	0.0	23.5	25.8
5	0	5	2	5	0	16	2	-1.11	0.0	19.0	51.6
6	0	6	1	6	0	13	0	-0.81	0.0	17.2	12.9
7	0	10	0	7	0	9	1	-0.5	0.0	17.2	12.9
8	0	6	0	8	0	4	0	-0.2	0.0	9.0	0.0
9	0	0	0	9	0	0	0	0.1	0.0	0.0	0.0
10	0	0	0	10	0	0	0	0.4	0.0	0.0	0.0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	60	5	Control	0	55	3				
2	0	35	2	2	0	42	2	-0.71	0	69.6	51.6
3	0	39	0	3	0	46	2	-0.41	0	76.8	25.8
4	0	40	5	4	0	45	1	-0.11	0	76.8	77.4
5	0	29	0	5	0	37	0	0.19	0	59.6	0.0
6	0	9	0	6	0	8	0	0.49	0	15.4	0.0
7	0	1	0	7	0	1	0	0.8	0	1.8	0.0
8	0	0	0	8	0	2	0	1.1	0	1.8	0.0
9	0	2	0	9	0	1	0	1.4	0	2.7	0.0
10	0	0	0	10	0	1	0	1.4	0	0.9	0.0
11	0	2	0	11	0	0	0	1.4	0	1.8	0.0
12	0	2	0	12	0	1	0	1.4	0	2.7	0.0

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	62	5	Control	0	54	2	x			
2	0	46	3	2	0	47	4	x	0	84.0	90.3
3	0	40	3	3	0	42	1	x	0	74.1	51.6
4	0	28	3	4	0	31	1	x	0	53.3	51.6
5	0	13	2	5	0	10	1	x	0	20.8	38.7
6	0	6	0	6	0	9	0	x	0	13.6	0.0
7	0	4	0	7	0	3	0	x	0	6.3	0.0
8	0	0	0	8	0	0	0	x	0	0.0	0.0
9	0	0	0	9	0	0	0	x	0	0.0	0.0
10	0	0	0	10	0	0	0	x	0	0.0	0.0
11	0	0	0	11	0	0	0	x	0	0.0	0.0
12	0	0	0	12	0	0	0	x	0	0.0	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	50	4				
2	0	45	3	-0.3	0.0	81.3	77.4
3	0	31	2	-0.01	0.0	56.0	51.6
4	0	38	3	0.28	0.0	68.7	77.4
5	0	26	2	0.59	0.0	47.0	51.6
6	0	32	2	0.89	0.0	57.8	51.6
7	0	30	2	1.19	0.0	54.2	51.6
8	0	2	0	1.49	0.0	3.6	0.0
9	0	1	0	1.8	0.0	1.8	0.0
10	0	0	0	2.1	0.0	0.0	0.0
11	0	0	0	2.4	0.0	0.0	0.0
12	0	0	0	2.7	0.0	0.0	0.0

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	38	2				
2	0	25	1	-0.01	0	45.2	25.8
3	0	40	1	0.28	0	72.3	25.8
4	0	37	2	0.59	0	66.9	51.6
5	0	30	1	0.89	0	54.2	25.8
6	0	35	2	1.19	0	63.3	51.6
7	0	34	1	1.49	0	61.4	25.8
8	0	14	0	1.8	0	25.3	0.0
9	0	10	0	2.1	0	18.1	0.0
10	0	6	0	2.4	0	10.8	0.0
11	0	0	0	2.7	0	0.0	0.0
12	0	0	0	3.0	0	0.0	0.0

Appendix 3.8: DrenchRite® LDA results for farm ❼ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	20	17		Control	16	21	6				
2	19	20	6	2	17	14	0	-2	105.1	69.0	74.1
3	13	11	5	3	15	17	0	-1.7	81.8	56.9	14.8
4	16	14	1	4	19	17	5	-1.4	102.2	62.9	88.9
5	15	20	1	5	5	15	2	-1.11	58.4	71.1	29.6
6	10	13	0	6	4	8	1	-0.81	40.9	42.6	29.6
7	0	6	1	7	1	3	0	-0.5	2.9	18.3	14.8
8	0	6	1	8	0	3	0	-0.2	0.0	18.3	0.0
9	0	1	0	9	0	3	0	0.1	0.0	8.1	0.0
10	0	0	0	10	0	0	0	0.4	0.0	0.0	0.0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	13	28	3	Control	16	31	4				
2	10	27	2	2	24	25	2	-0.71	99.3	105.6	59.3
3	11	25	0	3	16	19	0	-0.41	78.8	89.3	0.0
4	6	18	1	4	6	15	1	-0.11	35.0	67.0	29.6
5	4	10	0	5	1	14	0	0.19	14.6	48.7	0.0
6	3	10	1	6	0	3	1	0.49	8.8	26.4	29.6
7	0	1	2	7	0	3	0	0.8	0.0	8.1	29.6
8	0	6	0	8	0	1	0	1.1	0.0	14.2	0.0
9	0	3	0	9	0	1	0	1.4	0.0	8.1	0.0
10	0	6	0	10	0	0	0	1.4	0.0	12.2	0.0
11	0	0	0	11	0	0	0	1.4	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1.4	0.0	0.0	0.0

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	20	25	1	Control	20	24	3	x			
2	14	20	2	2	15	23	2	x	84.7	87.3	59.3
3	10	12	1	3	16	17	3	x	75.9	58.9	59.3
4	6	10	1	4	5	17	2	x	32.1	54.8	44.4
5	0	8	0	5	0	6	0	x	0.0	28.4	0.0
6	0	11	0	6	0	9	1	x	0.0	40.6	14.8
7	0	6	0	7	0	3	0	x	0.0	18.3	0.0
8	0	2	0	8	0	0	0	x	0.0	4.1	0.0
9	0	0	0	9	0	2	0	x	0.0	4.1	0.0
10	0	0	0	10	0	1	0	x	0.0	2.0	0.0
11	0	1	0	11	0	0	0	x	0.0	2.0	0.0
12	0	0	0	12	0	0	0	x	0.0	0.0	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Telad*	IVM 1 LogConc	*H. cont*	*Trich*	*Telad*
Control	18	28	2				
2	13	23	1	-0.3	75.9	93.4	29.6
3	15	12	1	-0.01	87.6	48.7	29.6
4	14	18	1	0.28	81.8	73.1	29.6
5	8	9	0	0.59	46.7	36.5	0.0
6	3	10	0	0.89	17.5	40.6	0.0
7	0	12	0	1.19	0.0	48.7	0.0
8	0	9	0	1.49	0.0	36.5	0.0
9	0	2	0	1.8	0.0	8.1	0.0
10	0	0	0	2.1	0.0	0.0	0.0
11	0	0	0	2.4	0.0	0.0	0.0
12	0	0	0	2.7	0.0	0.0	0.0

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	14	24	4				
2	14	23	3	-0.01	81.8	93.4	88.9
3	5	16	1	0.28	29.2	65.0	29.6
4	5	16	3	0.59	29.2	65.0	88.9
5	0	19	2	0.89	0.0	77.2	59.3
6	0	10	2	1.19	0.0	40.6	59.3
7	0	16	2	1.49	0.0	65.0	59.3
8	0	10	1	1.8	0.0	40.6	29.6
9	0	6	0	2.1	0.0	24.4	0.0
10	0	4	0	2.4	0.0	16.2	0.0
11	0	4	0	2.7	0.0	16.2	0.0
12	0	0	0	3.0	0.0	0.0	0.0

Appendix 3.9: DrenchRite® LDA results for farm ❽ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	52	9	Control	0	60	3				
2	0	23	3	2	0	53	2	-2.0	0.0	65.0	60.3
3	0	35	2	3	0	55	3	-1.7	0.0	77.0	60.3
4	0	14	0	4	0	13	4	-1.4	0.0	23.1	48.3
5	0	13	1	5	0	15	3	-1.11	0.0	24.0	48.3
6	0	14	0	6	0	12	3	-0.81	0.0	22.2	36.2
7	0	10	0	7	0	10	2	-0.5	0.0	17.1	24.1
8	0	10	2	8	0	10	0	-0.2	0.0	17.1	24.1
9	0	10	0	9	0	3	2	0.1	0.0	11.1	24.1
10	0	0	0	10	0	1	0	0.4	0.0	0.9	0.0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	1	0	12	0	0	0	1.0	0.0	0.9	0.0

Row C (LEV)				**Row D (LEV)**				**LEV**	**Mean proportion larval develop**		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	**LogConc**	*H. cont*	*Trich*	*Tel*
Control	0	62	2	Control	0	58	3				
2	0	57	0	2	0	38	1	-0.71	0	81.3	12.1
3	0	45	2	3	0	48	3	-0.41	0	79.6	60.3
4	0	25	2	4	0	25	1	-0.11	0	42.8	36.2
5	0	15	0	5	0	17	0	0.19	0	27.4	0.0
6	0	18	0	6	0	23	1	0.49	0	35.1	12.1
7	0	14	0	7	0	16	0	0.80	0	25.7	0.0
8	0	13	0	8	0	18	0	1.10	0	26.5	0.0
9	0	5	0	9	0	6	0	1.40	0	9.4	0.0
10	0	6	0	10	0	7	0	1.40	0	11.1	0.0
11	0	2	0	11	0	10	0	1.40	0	10.3	0.0
12	0	3	0	12	0	3	0	1.40	0	5.1	0.0

Row E (BZ+LEV)				**Row F (BZ+LEV)**					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	58	4	Control	0	60	5	x			
2	0	40	3	2	0	36	4	x	0	65.0	84.5
3	0	38	1	3	0	30	3	x	0	58.2	48.3
4	0	30	0	4	0	31	0	x	0	52.2	0.0
5	0	20	1	5	0	28	2	x	0	41.1	36.2
6	0	30	2	6	0	18	0	x	0	41.1	24.1
7	0	15	0	7	0	20	0	x	0	30.0	0.0
8	0	16	0	8	0	18	0	x	0	29.1	0.0
9	0	3	0	9	0	6	0	x	0	7.7	0.0
10	0	1	0	10	0	2	0	x	0	2.6	0.0
11	0	0	0	11	0	0	0	x	0	0.0	0.0
12	0	4	0	12	0	2	0	x	0	5.1	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	59	3				
2	0	30	4	-0.3	0	51.3	96.6
3	0	34	2	-0.01	0	58.2	48.3
4	0	36	1	0.28	0	61.6	24.1
5	0	28	1	0.59	0	47.9	24.1
6	0	30	1	0.89	0	51.3	24.1
7	0	30	1	1.19	0	51.3	24.1
8	0	35	0	1.49	0	59.9	0.0
9	0	6	3	1.8	0	10.3	72.4
10	0	3	0	2.1	0	5.1	0.0
11	0	2	0	2.4	0	3.4	0.0
12	0	0	0	2.7	0	0.0	0.0

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	31	3				
2	0	50	3	-0.01	0	85.6	72.4
3	0	43	2	0.28	0	73.6	48.3
4	0	46	4	0.59	0	78.7	96.6
5	0	32	3	0.89	0	54.8	72.4
6	0	32	1	1.19	0	54.8	24.1
7	0	26	1	1.49	0	44.5	24.1
8	0	23	2	1.8	0	39.4	48.3
9	0	12	0	2.1	0	20.5	0.0
10	0	6	1	2.4	0	10.3	24.1
11	0	8	0	2.7	0	13.7	0.0
12	0	1	0	3.0	0	1.7	0.0

Appendix 3.10: DrenchRite® LDA results for farm ❾ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	6	39	0	Control	4	53	0				
2	10	43	0	2	1	59	0	-2.0	101.3	95.2	0
3	9	43	0	3	4	60	0	-1.7	119.7	96.1	0
4	4	54	0	4	5	36	0	-1.4	82.9	84.0	0
5	4	45	0	5	0	53	0	-1.11	36.8	91.5	0
6	2	45	0	6	1	44	0	-0.81	27.6	83.1	0
7	0	41	0	7	1	36	0	-0.5	9.2	71.9	0
8	1	21	0	8	0	18	0	-0.2	9.2	36.4	0
9	0	10	0	9	0	20	0	0.1	0.0	28.0	0
10	0	1	0	10	0	0	0	0.4	0.0	0.9	0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0
Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	10	48	0	Control	4	50	0				
2	7	46	0	2	9	49	0	-0.71	147.4	88.7	0
3	2	30	0	3	9	43	0	-0.41	101.3	68.1	0
4	6	44	0	4	4	37	0	-0.11	92.1	75.6	0
5	5	43	0	5	2	35	0	0.19	64.5	72.8	0
6	0	40	0	6	1	26	0	0.49	9.2	61.6	0
7	0	31	0	7	0	35	0	0.80	0.0	61.6	0
8	0	20	0	8	0	30	0	1.10	0.0	46.7	0
9	0	18	0	9	0	26	0	1.40	0.0	41.1	0
10	0	28	0	10	0	33	0	1.40	0.0	56.9	0
11	0	30	0	11	1	28	0	1.40	9.2	54.1	0
12	0	32	0	12	0	27	0	1.40	0.0	55.1	0

Row E (BZ+LEV)				**Row F (BZ+LEV)**					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	3	50	0	Control	6	56	0	x			
2	6	50	0	2	3	31	0	x	82.9	75.6	0
3	4	47	0	3	2	47	0	x	55.3	87.7	0
4	3	51	0	4	4	24	0	x	64.5	70.0	0
5	2	26	0	5	2	47	0	x	36.8	68.1	0
6	1	21	0	6	2	23	0	x	27.6	41.1	0
7	0	30	0	7	0	35	0	x	0.0	60.7	0
8	0	28	0	8	0	26	0	x	0.0	50.4	0
9	0	28	0	9	0	20	0	x	0.0	44.8	0
10	0	16	0	10	0	19	0	x	0.0	32.7	0
11	0	16	0	11	0	9	0	x	0.0	23.3	0
12	0	9	0	12	0	15	0	x	0.0	22.4	0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	6	58	0				
2	2	52	0	-0.3	36.8	97.1	0
3	7	50	0	-0.01	128.9	93.3	0
4	4	39	0	0.28	73.7	72.8	0
5	3	49	0	0.59	55.3	91.5	0
6	1	32	0	0.89	18.4	59.7	0
7	0	47	0	1.19	0.0	87.7	0
8	0	30	0	1.49	0.0	56.0	0
9	0	23	0	1.8	0.0	42.9	0
10	0	13	0	2.1	0.0	24.3	0
11	0	5	0	2.4	0.0	9.3	0
12	0	5	0	2.7	0.0	9.3	0

H (IVM-2)				IVM 2 LogConc	proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*		*H. cont*	*Trich*	*Tel*
Control	5	60	0				
2	5	49	0	-0.01	92.1	91.5	0
3	3	51	0	0.28	55.3	95.2	0
4	1	37	0	0.59	18.4	69.1	0
5	0	31	0	0.89	0.0	57.9	0
6	0	46	0	1.19	0.0	85.9	0
7	0	46	0	1.49	0.0	85.9	0
8	0	41	0	1.8	0.0	76.5	0
9	0	32	0	2.1	0.0	59.7	0
10	0	41	0	2.4	0.0	76.5	0
11	0	15	0	2.7	0.0	28.0	0
12	0.0	10.0	0	3.0	0.0	18.7	0

Appendix 3.11: DrenchRite® LDA results for farm ❿ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	4	38	0	Control	4	40	0				
2	3	30	0	2	0	50	0	-2.0	61.5	89.1	0.0
3	0	48	0	3	7	43	0	-1.7	143.6	101.4	0.0
4	4	26	0	4	3	25	0	-1.4	143.6	56.8	0.0
5	4	28	0	5	4	28	0	-1.11	164.1	62.4	0.0
6	5	20	0	6	2	30	0	-0.81	143.6	55.7	0.0
7	1	22	0	7	3	26	0	-0.5	82.1	53.5	0.0
8	1	19	0	8	4	15	0	-0.2	102.6	37.9	0.0
9	2	9	0	9	0	6	0	0.1	41.0	16.7	0.0
10	2	1	0	10	0	1	0	0.4	41.0	2.2	0.0
11	0	0	0	11	0	1	0	0.7	0.0	1.1	0.0
12	0	1	0	12	0	0	0	1.0	0.0	1.1	0.0
Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	6	48	0	Control	9	48	0				
2	2	30	0	2	2	48	0	-0.71	41.0	86.9	0.0
3	1	28	0	3	1	40	0	-0.41	20.5	75.8	0.0
4	2	27	0	4	3	31	0	-0.11	51.3	64.6	0.0
5	0	25	0	5	2	31	0	0.19	20.5	62.4	0.0
6	0	1	0	6	0	20	0	0.49	0.0	23.4	0.0
7	0	20	0	7	0	21	0	0.80	0.0	45.7	0.0
8	0	12	0	8	0	20	0	1.10	0.0	35.7	0.0
9	0	13	0	9	0	7	0	1.40	0.0	22.3	0.0
10	0	9	0	10	0	10	0	1.40	0.0	21.2	0.0
11	0.0	10.0	0	11	0	11	0	1.40	0.0	23.4	0.0
12	0.0	6	0	12	0	18	0	1.4	0.0	26.7	0.0

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	4	43	0	Control	4	47.5	0	x			
2	4	29	0	2	1	36	0	x	51.3	80.2	0.0
3	5	30	0	3	5	35.5	0	x	102.6	79.1	0.0
4	1	29	0	4	2	28.5	0	x	30.8	63.5	0.0
5	2	31	0	5	2	29.5	0	x	41.0	65.7	0.0
6	0	19	0	6	1	22	0	x	10.3	49.0	0.0
7	0	14	0	7	0	17.5	0	x	0.0	39.0	0.0
8	0	10	0	8	0	15	0	x	0.0	33.4	0.0
9	1	15	0	9	0	14.5	0	x	10.3	32.3	0.0
10	0	7	0	10	0	9	0	x	0.0	20.1	0.0
11	0	6	0	11	0	5	0	x	0.0	11.1	0.0
12	0	5	0	12	0	4.5	0	x	0.0	10.0	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	2	50	0				
2	2	30	0	-0.3	41.0	66.9	0.0
3	3	30	0	-0.01	61.5	66.9	0.0
4	2	35	0	0.28	41.0	78.0	0.0
5	3	31	0	0.59	61.5	69.1	0.0
6	1	26	0	0.89	20.5	57.9	0.0
7	2	29	0	1.19	41.0	64.6	0.0
8	0	26	0	1.49	0.0	57.9	0.0
9	0	8	0	1.8	0.0	17.8	0.0
10	0	3	0	2.1	0.0	6.7	0.0
11	0	3	0	2.4	0.0	6.7	0.0
12	0	2	0	2.7	0.0	4.5	0.0

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	6	40	0				
2	3	26	0	-0.01	61.5	57.9	0.0
3	0	18	0	0.28	0.0	40.1	0.0
4	0	28	0	0.59	0.0	62.4	0.0
5	1	20	0	0.89	20.5	44.6	0.0
6	0	32	0	1.19	0.0	71.3	0.0
7	0	27	0	1.49	0.0	60.2	0.0
8	0	22	0	1.8	0.0	49.0	0.0
9	0	15	0	2.1	0.0	33.4	0.0
10	0	12	0	2.4	0.0	26.7	0.0
11	0	12	0	2.7	0.0	26.7	0.0
12	0	11	0	3.0	0.0	24.5	0.0

Appendix 3.12: DrenchRite® LDA results for farm ▼showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	56	14	Control	0	51	11				
2	0	38	19	2	0	70	16	-2.0	0.0	97.1	132.1
3	0	48	11	3	0	60	12	-1.7	0.0	97.1	86.8
4	0	36	14	4	0	46	10	-1.4	0.0	73.7	90.6
5	0	41	19	5	0	11	0	-1.11	0.0	46.7	71.7
6	0	30	9	6	0	40	16	-0.81	0.0	62.9	94.3
7	0	38	22	7	0	35	8	-0.5	0.0	65.6	113.2
8	0	41	8	8	0	37	10	-0.2	0.0	70.1	67.9
9	0	21	3	9	0	32	3	0.1	0.0	47.6	22.6
10	0	1	0	10	0	3	1	0.4	0.0	3.6	3.8
11	0	0	0	11	0	2	0	0.7	0.0	1.8	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	60	10	Control	0	70	15				
2	0	60	10	2	0	55	10	-0.71	0.0	103.4	75.5
3	0	45	5	3	0	30	5	-0.41	0.0	67.4	37.7
4	0	40	1	4	0	44	1	-0.11	0.0	75.5	7.5
5	0	46	0	5	0	36	1	0.19	0.0	73.7	3.8
6	0	47	1	6	0	47	1	0.49	0.0	84.5	7.5
7	0	35	0	7	0	25	0	0.80	0.0	53.9	0.0
8	0	32	0	8	0	41	0	1.10	0.0	65.6	0.0
9	0	9	0	9	0	13	0	1.40	0.0	19.8	0.0
10	0	0	0	10	0	14	0	1.40	0.0	12.6	0.0
11	0	15	0	11	0	7	1	1.40	0.0	19.8	3.8
12	0	15	0	12	0	16	0	1.4	0.0	27.9	0.0

Row E (BZ+LEV)				**Row F (BZ+LEV)**					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	42	16	Control	0	61	18	x			
2	0	50	5	2	0	40	16	x	0.0	80.9	79.2
3	0	56	7	3	0	40	6	x	0.0	86.3	49.1
4	0	48	1	4	0	30	8	x	0.0	70.1	34.0
5	0	31	1	5	0	35	0	x	0.0	59.3	3.8
6	0	51	4	6	0	41	0	x	0.0	82.7	15.1
7	0	34	2	7	0	44	1	x	0.0	70.1	11.3
8	0	30	0	8	0	29	0	x	0.0	53.0	0.0
9	0	12	0	9	0	5	0	x	0.0	15.3	0.0
10	0	1	0	10	0	0	0	x	0.0	0.9	0.0
11	0	3	0	11	0	7	0	x	0.0	9.0	0.0
12	0	4	0	12	0	7	0	x	0.0	9.9	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	50	12				
2	0	49	11	-0.3	0.0	88.1	83.0
3	0	31	9	-0.01	0.0	55.7	67.9
4	0	42	18	0.28	0.0	75.5	135.8
5	0	29	4	0.59	0.0	52.1	30.2
6	0	50	10	0.89	0.0	89.9	75.5
7	0	46	10	1.19	0.0	82.7	75.5
8	0	40	10	1.49	0.0	71.9	75.5
9	0	24	5	1.8	0.0	43.1	37.7
10	0	25	5	2.1	0.0	44.9	37.7
11	0	17	1	2.4	0.0	30.6	7.5
12	0	6	1	2.7	0.0	10.8	7.5

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	55	10				
2	0	39	12	-0.01	0.0	70.1	90.6
3	0	29	11	0.28	0.0	52.1	83.0
4	0	50	15	0.59	0.0	89.9	113.2
5	0	31	10	0.89	0.0	55.7	75.5
6	0	46	14	1.19	0.0	82.7	105.7
7	0	36	10	1.49	0.0	64.7	75.5
8	0	51	3	1.8	0.0	91.7	22.6
9	0	26	2	2.1	0.0	46.7	15.1
10	0	40	2	2.4	0.0	71.9	15.1
11	0	13	1	2.7	0.0	23.4	7.5
12	0	16	0	3.0	0.0	28.8	0.0

Appendix 3.13: DrenchRite® LDA results for farm ★ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	37	6	Control	3	55	1				
2	3	41	1	2	2	49	0	-2.0	109.4	86.8	11.7
3	0	47	4	3	2	46	2	-1.7	43.8	89.7	70.0
4	1	39	5	4	3	36	5	-1.4	87.5	72.3	116.7
5	1	33	2	5	0	32	3	-1.11	21.9	62.7	58.3
6	0	36	2	6	0	30	0	-0.81	0.0	63.6	23.3
7	0	29	6	7	1	30	2	-0.5	21.9	56.9	93.3
8	1	40	6	8	0	22	1	-0.2	21.9	59.8	81.7
9	1	27	2	9	0	14	1	0.1	21.9	39.5	35.0
10	0	0	0	10	0	1	0	0.4	0.0	1.0	0.0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	5	49	2	Control	1	51	3				
2	0	48	1	2	0	46	2	-0.71	0	90.6	35.0
3	0	47	0	3	2	48	3	-0.41	43.8	91.6	35.0
4	1	48	0	4	3	49	2	-0.11	87.5	93.5	23.3
5	1	46	0	5	4	48	4	0.19	109.4	90.6	46.7
6	0	35	1	6	0	43	1	0.49	0.0	75.2	23.3
7	1	28	0	7	0	37	0	0.80	21.9	62.7	0.0
8	0	32	0	8	0	31	0	1.10	0.0	60.7	0.0
9	0	26	0	9	0	22	0	1.40	0.0	46.3	0.0
10	0	21	0	10	0	27	0	1.40	0.0	46.3	0.0
11	0	20	0	11	0	21	0	1.40	0.0	39.5	0.0
12	0	16	0	12	0	22	1	1.4	0.0	36.6	11.7

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	1	52	3	Control	2	53	5	x			
2	3	45	5	2	4	50	8	x	153.1	91.6	151.7
3	2	46	3	3	2	49	4	x	87.5	91.6	81.7
4	3	43	0	4	1	44	3	x	87.5	83.9	35.0
5	3	38	5	5	1	28	4	x	87.5	63.6	105.0
6	0	41	3	6	1	38	0	x	21.9	76.2	35.0
7	0	36	0	7	0	13	1	x	0.0	47.2	11.7
8	0	28	0	8	0	28	0	x	0.0	54.0	0.0
9	0	10	0	9	0	15	0	x	0.0	24.1	0.0
10	0	18	0	10	0	10	0	x	0.0	27.0	0.0
11	0	13	0	11	0	6	0	x	0.0	18.3	0.0
12	0	15	0	12	0	15	0	x	0.0	28.9	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	3	51	8				
2	2	41	0	-0.3	87.5	79.1	0.0
3	0	32	4	-0.01	0.0	61.7	93.3
4	4	38	6	0.28	175.0	73.3	140.0
5	0	40	5	0.59	0.0	77.1	116.7
6	0	40	7	0.89	0.0	77.1	163.3
7	0	42	1	1.19	0.0	81.0	23.3
8	0	40	6	1.49	0.0	77.1	140.0
9	0	29	3	1.8	0.0	55.9	70.0
10	0	10	2	2.1	0.0	19.3	46.7
11	0	10	4	2.4	0.0	19.3	93.3
12	0	5	0	2.7	0.0	9.6	0.0

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	1	52	8				
2	0	45	3	-0.01	0.0	86.8	70.0
3	2	49	3	0.28	87.5	94.5	70.0
4	0	40	4	0.59	0.0	77.1	93.3
5	0	38	4	0.89	0.0	73.3	93.3
6	0	25	2	1.19	0.0	48.2	46.7
7	0	23	0	1.49	0.0	44.4	0.0
8	0	25	2	1.8	0.0	48.2	46.7
9	0	5	0	2.1	0.0	9.6	0.0
10	0	20	1	2.4	0.0	38.6	23.3
11	0	24	0	2.7	0.0	46.3	0.0
12	0	8	0	3.0	0.0	15.4	0.0

Appendix 3.14: DrenchRite® LDA results for farm ◈ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	17	16	2	Control	18	16	7				
2	17	21	1	2	20	18	0	-2.0	122.3	119.1	14.8
3	16	6	0	3	18	12	3	-1.7	112.4	55.0	44.4
4	19	12	2	4	10	12	2	-1.4	95.9	73.3	59.3
5	7	21	3	5	11	20	3	-1.11	59.5	125.2	88.9
6	4	15	3	6	10	18	1	-0.81	46.3	100.8	59.3
7	6	20	2	7	4	16	3	-0.5	33.1	109.9	74.1
8	1	6	2	8	3	7	2	-0.2	13.2	39.7	59.3
9	3	6	2	9	4	4	2	0.1	23.1	30.5	59.3
10	0	0	0	10	0	1	0	0.4	0.0	3.1	0.0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	15	16	3	Control	16	14	3				
2	22	20	2	2	20	17	2	-0.71	138.8	113.0	59.3
3	15	24	0	3	17	22	0	-0.41	105.8	140.5	0.0
4	23	22	0	4	13	16	0	-0.11	119.0	116.0	0.0
5	13	13	0	5	11	12	1	0.19	79.3	76.3	14.8
6	7	16	0	6	2	9	2	0.49	29.8	76.3	29.6
7	0	12	0	7	0	11	2	0.80	0.0	70.2	29.6
8	0	10	0	8	0	10	1	1.10	0.0	61.1	14.8
9	0	10	0	9	0	8	1	1.40	0.0	55.0	14.8
10	0	8	0	10	0	11	0	1.40	0.0	58.0	0.0
11	0	4	0	11	0	7	1	1.40	0.0	33.6	14.8
12	0	10	0	12	0	11	0	1.4	0.0	64.1	0.0

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	15	18	4	Control	14	15	1	x			
2	9	12	0	2	8	14	4	x	56.2	79.4	59.3
3	10	21	3	3	12	19	2	x	72.7	122.1	74.1
4	12	13	0	4	6	13	1	x	59.5	79.4	14.8
5	6	4	2	5	5	14	3	x	36.4	55.0	74.1
6	1	16	1	6	0	16	2	x	3.3	97.7	44.4
7	0	15	0	7	0	9	0	x	0.0	73.3	0.0
8	0	11	0	8	0	12	0	x	0.0	70.2	0.0
9	0	6	0	9	0	5	0	x	0.0	33.6	0.0
10	0	8	0	10	0	6	0	x	0.0	42.7	0.0
11	0	4	0	11	0	6	0	x	0.0	30.5	0.0
12	0	3	1	12	0	3	0	x	0.0	18.3	14.8

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	15	14	6				
2	11	11	1	-0.3	72.7	67.2	25.8
3	12	16	2	-0.01	79.3	97.7	51.6
4	4	16	1	0.28	26.4	97.7	25.8
5	3	17	2	0.59	19.8	103.8	51.6
6	1	17	3	0.89	6.6	103.8	77.4
7	0	13	6	1.19	0.0	79.4	154.8
8	0	5	2	1.49	0.0	30.5	51.6
9	0	6	2	1.8	0.0	36.6	51.6
10	0	1	0	2.1	0.0	6.1	0.0
11	0	0	0	2.4	0.0	0.0	0.0
12	0	0	0	2.7	0.0	0.0	0.0

Row H (IVM-2)				IVM 2 LogConc	proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*		*H. cont*	*Trich*	*Tel*
Control	11	22	4				
2	17	17	0	-0.01	112.4	103.8	0.0
3	3	18	0	0.28	19.8	109.9	0.0
4	3	17	5	0.59	19.8	103.8	129.0
5	1	15	3	0.89	6.6	91.6	77.4
6	1	11	4	1.19	6.6	67.2	103.2
7	0	10	2	1.49	0.0	61.1	51.6
8	0	11	1	1.8	0.0	67.2	25.8
9	0	8	0	2.1	0.0	48.9	0.0
10	0	10	0	2.4	0.0	61.1	0.0
11	0	0	0	2.7	0.0	0.0	0.0
12	0	0	0	3.0	0.0	0.0	0.0

Appendix 3.15: DrenchRite® LDA results for farm ⊙ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	22	22	Control	0	23	41				
2	0	40	10	2	0	30	20	-2.0	0.0	137.3	62.5
3	0	39	11	3	0	27	21	-1.7	0.0	129.4	66.7
4	0	37	10	4	0	32	30	-1.4	0.0	135.3	83.3
5	0	37	11	5	0	27	21	-1.11	0.0	125.5	66.7
6	0	26	21	6	0	13	24	-0.81	0.0	76.5	93.8
7	0	24	28	7	0	12	23	-0.5	0.0	70.6	106.3
8	0	28	32	8	0	17	15	-0.2	0.0	88.2	97.9
9	0	22	19	9	0	18	15	0.1	0.0	78.4	70.8
10	0	6	2	10	0	4	2	0.4	0.0	19.6	8.3
11	0	0	0	11	0	1	1	0.7	0.0	2.0	2.1
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	17	39	Control	0	30	26				
2	0	26	25	2	0	31	14	-0.71	0.0	111.8	81.3
3	0	22	14	3	0	30	21	-0.41	0.0	102.0	72.9
4	0	29	20	4	0	23	13	-0.11	0.0	102.0	68.8
5	0	22	22	5	0	24	24	0.19	0.0	90.2	95.8
6	0	25	12	6	0	30	26	0.49	0.0	107.8	79.2
7	0	22	5	7	0	21	9	0.80	0.0	84.3	29.2
8	0	22	2	8	0	23	5	1.10	0.0	88.2	14.6
9	0	18	0	9	0	15	4	1.40	0.0	64.7	8.3
10	0	17	0	10	0	18	2	1.40	0.0	68.6	4.2
11	0	17	1	11	0	17	0	1.40	0.0	66.7	2.1
12	0	0	0	12	0	12	3	1.40	0.0	23.5	6.3

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	26	18	Control	0	24	27	x			
2	0	36	26	2	0	44	25	x	0	156.9	106.3
3	0	30	10	3	0	36	22	x	0	129.4	66.7
4	0	22	20	4	0	34	15	x	0	109.8	72.9
5	0	40	12	5	0	37	15	x	0	151.0	56.3
6	0	46	12	6	0	25	13	x	0	139.2	52.1
7	0	31	7	7	0	30	2	x	0	119.6	18.8
8	0	18	7	8	0	24	3	x	0	82.4	20.8
9	0	13	4	9	0	20	2	x	0	64.7	12.5
10	0	9	0	10	0	12	0	x	0	41.2	0.0
11	0	8	0	11	0	6	0	x	0	27.5	0.0
12	0	8	1	12	0	9	0	x	0	33.3	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	16	30				
2	0	35	16	-0.3	0.0	137.3	66.7
3	0	21	29	-0.01	0.0	82.4	120.8
4	0	23	28	0.28	0.0	90.2	116.7
5	0	16	22	0.59	0.0	62.7	91.7
6	0	27	22	0.89	0.0	105.9	91.7
7	0	23	31	1.19	0.0	90.2	129.2
8	0	23	30	1.49	0.0	90.2	125.0
9	0	14	22	1.8	0.0	54.9	91.7
10	0	7	10	2.1	0.0	27.5	41.7
11	0	12	14	2.4	0.0	47.1	58.3
12	0	8	4	2.7	0.0	31.4	16.7

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	28	21				
2	0	30	14	-0.01	0.0	117.6	58.3
3	0	26	21	0.28	0.0	102.0	87.5
4	0	21	21	0.59	0.0	82.4	87.5
5	0	36	17	0.89	0.0	141.2	70.8
6	0	26	21	1.19	0.0	102.0	87.5
7	0	32	20	1.49	0.0	125.5	83.3
8	0	22	16	1.8	0.0	86.3	66.7
9	0	22	7	2.1	0.0	86.3	29.2
10	0	19	1	2.4	0.0	74.5	4.2
11	0	9	0	2.7	0.0	35.3	0.0
12	0	7	0	3.0	0.0	27.5	0.0

Appendix 3.16: DrenchRite® LDA results for farm ◆ showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	16	6	Control	0	37	5				
2	0	20	7	2	0	37	18	-2.0	0.0	112.5	127.1
3	0	31	13	3	0	46	13	-1.7	0.0	152.0	132.2
4	0	16	11	4	0	33	24	-1.4	0.0	96.7	178.0
5	0	18	15	5	0	30	4	-1.11	0.0	94.7	96.6
6	0	8	11	6	0	35	5	-0.81	0.0	84.9	81.4
7	0	26	8	7	0	24	16	-0.5	0.0	98.7	122.0
8	0	24	4	8	0	15	8	-0.2	0.0	77.0	61.0
9	0	16	5	9	0	13	0	0.1	0.0	57.2	25.4
10	0	1	0	10	0	0	0	0.4	0.0	2.0	0.0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0
Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	35	14	Control	0	20	8				
2	0	42	3	2	0	38	10	-0.71	0.0	157.9	66.1
3	0	31	8	3	0	40	2	-0.41	0.0	140.1	50.8
4	0	39	18	4	0	43	3	-0.11	0.0	161.8	106.8
5	0	40	14	5	0	30	4	0.19	0.0	138.2	91.5
6	0	32	7	6	0	21	7	0.49	0.0	104.6	71.2
7	0	33	4	7	0	20	6	0.80	0.0	104.6	50.8
8	0	27	2	8	0	20	1	1.10	0.0	92.8	15.3
9	0	21	0	9	0	17	0	1.40	0.0	75.0	0.0
10	0	21	3	10	0	18	0	1.40	0.0	77.0	15.3
11	0	18	0	11	0	12	3	1.40	0.0	59.2	15.3
12	0	20	0	12	0	28	1	1.40	0.0	94.7	5.1

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0.0	53	8	Control	0.0	44	11	x			
2	0.0	44	14	2	0.0	47	9	x	0.0	179.6	116.9
3	0.0	50	2	3	0.0	33	8	x	0.0	163.8	50.8
4	0.0	42	8	4	0.0	42	13	x	0.0	165.8	106.8
5	0.0	32	4	5	0.0	31	6	x	0.0	124.3	50.8
6	0.0	31	6	6	0.0	56	4	x	0.0	171.7	50.8
7	0.0	23	2	7	0.0	33	4	x	0.0	110.5	30.5
8	0.0	18	0	8	0.0	28	1	x	0.0	90.8	5.1
9	0.0	9	0	9	0.0	13	0	x	0.0	43.4	0.0
10	0.0	12	0	10	0.0	9	0	x	0.0	41.4	0.0
11	0.0	7	0	11	0.0	6	0	x	0.0	25.7	0.0
12	0.0	10	0	12	0.0	11	0	x	0.0	41.4	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	18	8				
2	0	24	23	-0.3	0.0	94.7	233.9
3	0	21	26	-0.01	0.0	82.9	264.4
4	0	24	15	0.28	0.0	94.7	152.5
5	0	27	10	0.59	0.0	106.6	101.7
6	0	23	9	0.89	0.0	90.8	91.5
7	0	26	10	1.19	0.0	102.6	101.7
8	0	23	9	1.49	0.0	90.8	91.5
9	0	10	6	1.8	0.0	39.5	61.0
10	0	6	4	2.1	0.0	23.7	40.7
11	0	3	2	2.4	0.0	11.8	20.3
12	0	4	1	2.7	0.0	15.8	10.2

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	26	10				
2	0	36	18	-0.01	0.0	142.1	183.1
3	0	30	14	0.28	0.0	118.4	142.4
4	0	26	13	0.59	0.0	102.6	132.2
5	0	19	2	0.89	0.0	75.0	20.3
6	0	27	9	1.19	0.0	106.6	91.5
7	0	35	6	1.49	0.0	138.2	61.0
8	0	21	3	1.8	0.0	82.9	30.5
9	0	18	1	2.1	0.0	71.1	10.2
10	0	14	1	2.4	0.0	55.3	10.2
11	0	8	0	2.7	0.0	31.6	0.0
12	0	6	1	3.0	0.0	23.7	10.2

Appendix 3.17: DrenchRite® LDA results for farm × showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	53	8	Control	0	38	7				
2	0	39	6	2	0	43	0	-2.0	0.0	83.4	32.8
3	0	49	6	3	0	56	17	-1.7	0.0	106.8	125.8
4	0	32	8	4	0	30	9	-1.4	0.0	63.1	93.0
5	0	44	12	5	0	36	2	-1.11	0.0	81.4	76.6
6	0	38	11	6	0	34	10	-0.81	0.0	73.3	114.8
7	0	20	3	7	0	37	5	-0.5	0.0	58.0	43.8
8	0	28	5	8	0	17	7	-0.2	0.0	45.8	65.6
9	0	16	1	9	0	20	3	0.1	0.0	36.6	21.9
10	0	1	1	10	0	0	0	0.4	0.0	1.0	5.5
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0

Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	44	12	Control	0	50	11				
2	0	57	13	2	0	45	8	-0.71	0.0	103.8	114.8
3	0	48	8	3	0	40	4	-0.41	0.0	89.5	65.6
4	0	50	6	4	0	44	6	-0.11	0.0	95.6	65.6
5	0	51	9	5	0	44	8	0.19	0.0	96.7	93.0
6	0	53	5	6	0	49	0	0.49	0.0	103.8	27.3
7	0	36	1	7	0	36	1	0.80	0.0	73.3	10.9
8	0	34	0	8	0	42	2	1.10	0.0	77.3	10.9
9	0	31	1	9	0	28	0	1.40	0.0	60.0	5.5
10	0	33	0	10	0	30	0	1.40	0.0	64.1	0.0
11	0	15	0	11	0	28	0	1.40	0.0	43.8	0.0
12	0	16	0	12	0	20	1	1.40	0.0	36.6	5.5

Row E (BZ+LEV)				**Row F (BZ+LEV)**					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	0	48	8	Control	0	56	11	x			
2	0	46	10	2	0	44	8	x	0	91.6	98.4
3	0	49	9	3	0	41	9	x	0	91.6	98.4
4	0	44	10	4	0	37	16	x	0	82.4	142.2
5	0	32	8	5	0	31	6	x	0	64.1	76.6
6	0	40	5	6	0	41	2	x	0	82.4	38.3
7	0	29	0	7	0	31	2	x	0	61.0	10.9
8	0	22	0	8	0	31	0	x	0	53.9	0.0
9	0	21	0	9	0	22	0	x	0	43.8	0.0
10	0	20	0	10	0	18	0	x	0	38.7	0.0
11	0	20	0	11	0	16	0	x	0	36.6	0.0
12	0	15	0	12	0	15	0	x	0	30.5	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	50	16				
2	0	52	5	-0.3	0.0	105.8	54.7
3	0	30	8	-0.01	0.0	61.0	87.5
4	0	51	10	0.28	0.0	103.8	109.4
5	0	36	10	0.59	0.0	73.3	109.4
6	0	39	10	0.89	0.0	79.4	109.4
7	0	21	9	1.19	0.0	42.7	98.4
8	0	34	12	1.49	0.0	69.2	131.3
9	0	14	5	1.8	0.0	28.5	54.7
10	0	5	1	2.1	0.0	10.2	10.9
11	0	0	0	2.4	0.0	0.0	0.0
12	0	1	0	2.7	0.0	2.0	0.0

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	0	43	7				
2	0	31	5	-0.01	0.0	63.1	54.7
3	0	40	12	0.28	0.0	81.4	131.3
4	0	45	5	0.59	0.0	91.6	54.7
5	0	40	10	0.89	0.0	81.4	109.4
6	0	26	6	1.19	0.0	52.9	65.6
7	0	38	6	1.49	0.0	77.3	65.6
8	0	31	6	1.8	0.0	63.1	65.6
9	0	17	1	2.1	0.0	34.6	10.9
10	0	18	1	2.4	0.0	36.6	10.9
11	0	10	0	2.7	0.0	20.3	0.0
12	0	1	0	3.0	0.0	2.0	0.0

Appendix 3.18: DrenchRite® LDA results for farm showing numbers of L_3 for each well, Log_{10} concentration and mean proportion of larval development compared to control wells for Chapter 3.

Row A (BZ)				**Row B (BZ)**				BZ	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	15	26	5	Control	21	33	0				
2	19	29	2	2	12	30	0	-2.0	125.0	86.3	40.0
3	18	46	1	3	15	33	0	-1.7	133.1	115.6	20.0
4	3	27	3	4	17	37	2	-1.4	80.6	93.7	100.0
5	18	41	5	5	13	25	2	-1.11	125.0	96.6	140.0
6	12	25	4	6	19	26	3	-0.81	125.0	74.6	140.0
7	21	30	5	7	9	20	0	-0.5	121.0	73.2	100.0
8	17	21	1	8	6	10	1	-0.2	92.7	45.4	40.0
9	15	7	1	9	12	1	0	0.1	108.9	11.7	20.0
10	6	1	0	10	0	0	0	0.4	24.2	1.5	0.0
11	0	0	0	11	0	0	0	0.7	0.0	0.0	0.0
12	0	0	0	12	0	0	0	1.0	0.0	0.0	0.0
Row C (LEV)				**Row D (LEV)**				LEV	Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	14	23	1	Control	19	37	6				
2	18	37	3	2	16	34	5	-0.71	137.1	103.9	160.0
3	17	29	3	3	18	39	4	-0.41	141.1	99.5	140.0
4	19	34	3	4	13	31	6	-0.11	129.0	95.1	180.0
5	12	36	3	5	15	29	2	0.19	108.9	95.1	100.0
6	10	29	2	6	0	30	2	0.49	40.3	86.3	80.0
7	0	15	0	7	0	17	1	0.80	0.0	46.8	20.0
8	0	18	0	8	0	14	1	1.10	0.0	46.8	20.0
9	0	18	0	9	0	12	0	1.40	0.0	43.9	0.0
10	0	19	1	10	0	15	0	1.40	0.0	49.8	20.0
11	0	15	0	11	0	9	0	1.40	0.0	35.1	0.0
12	0	17	0	12	1	21	0	1.40	4.0	55.6	0.0

Row E (BZ+LEV)				Row F (BZ+LEV)					Mean proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	Well	*H. cont*	*Trich*	*Tel*	LogConc	*H. cont*	*Trich*	*Tel*
Control	12	26	2	Control	10	17	4	x			
2	17	20	2	2	11	24	4	x	112.9	64.4	120.0
3	14	16	2	3	19	22	2	x	133.1	55.6	80.0
4	13	16	1	4	18	23	5	x	125.0	57.1	120.0
5	11	8	3	5	20	8	1	x	125.0	23.4	80.0
6	5	14	1	6	10	22	2	x	60.5	52.7	60.0
7	3	10	0	7	8	21	2	x	44.4	45.4	40.0
8	0	6	0	8	0	17	0	x	0.0	33.7	0.0
9	0	4	0	9	0	12	0	x	0.0	23.4	0.0
10	0	13	0	10	0	14	2	x	0.0	39.5	40.0
11	0	8	0	11	0	24	0	x	0.0	46.8	0.0
12	0	13	0	12	0	13	0	x	0.0	38.0	0.0

Row G (IVM-1)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 1 LogConc	*H. cont*	*Trich*	*Tel*
Control	21	33	1				
2	13	32	2	-0.3	104.8	93.7	80.0
3	15	28	3	-0.01	121.0	82.0	120.0
4	11	31	2	0.28	88.7	90.7	80.0
5	6	32	2	0.59	48.4	93.7	80.0
6	5	26	2	0.89	40.3	76.1	80.0
7	0	32	1	1.19	0.0	93.7	40.0
8	0	19	2	1.49	0.0	55.6	80.0
9	0	8	2	1.8	0.0	23.4	80.0
10	0	1	2	2.1	0.0	2.9	80.0
11	0	2	0	2.4	0.0	5.9	0.0
12	0	0	0	2.7	0.0	0.0	0.0

Row H (IVM-2)					proportion larval develop		
Well	*H. cont*	*Trich*	*Tel*	IVM 2 LogConc	*H. cont*	*Trich*	*Tel*
Control	11	27	1				
2	7	27	2	-0.01	56.5	79.0	80.0
3	7	23	1	0.28	56.5	67.3	40.0
4	6	17	1	0.59	48.4	49.8	40.0
5	2	24	3	0.89	16.1	70.2	120.0
6	0	17	4	1.19	0.0	49.8	160.0
7	0	20	1	1.49	0.0	58.5	40.0
8	0	26	1	1.8	0.0	76.1	40.0
9	0	17	1	2.1	0.0	49.8	40.0
10	0	12	1	2.4	0.0	35.1	40.0
11	0	10	0	2.7	0.0	29.3	0.0
12	0	2	0	3.0	0.0	5.9	0.0

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