1. GENERAL INTRODUCTION

1.1 BLACK SCOUR WORM

Nematodes in the family Trichostrongylidae are parasites most commonly infecting the gastrointestinal tracts of ruminants, pigs, birds, horses and leporids (Georgi, 1980). Important parasites of domestic animals include the genera *Haemonchus*, *Teladorsagia*, *Ostertagia*, *Nematodirus*, *Cooperia* and *Trichostrongylus*.

Trichostrongylus colubriformis, *T. vitrinus* and, less commonly, *T. rugatus* (found in arid areas where they do not pose a serious disease threat) are collectively referred to as Black Scour Worm, after the typical symptoms of infection. They infect the small intestines of ruminants, mainly sheep and goats, but have been reported from cattle, camels, alpaca and several species of wild ruminant. While they are acknowledged as separate species, *T. colubriformis* and *T. vitrinus* are morphologically similar, share the same hosts, parasitize the same microhabitats within their hosts, have like life cycles and produce similar symptoms.

Trichostrongylus spp. have separate sexes (Figure 1.1). The males range from 4 to 5.5 mm in length and are slightly smaller than the females, ranging from 5 to 9 mm in length (Lapage, 1962). Both are reddish-brown in colour with a narrow anterior end, three-lipped mouth and rudimentary buccal cavity (Watson, 1960). The excretory pore is located close to the anterior end in a notch on the ventral side of the worm.

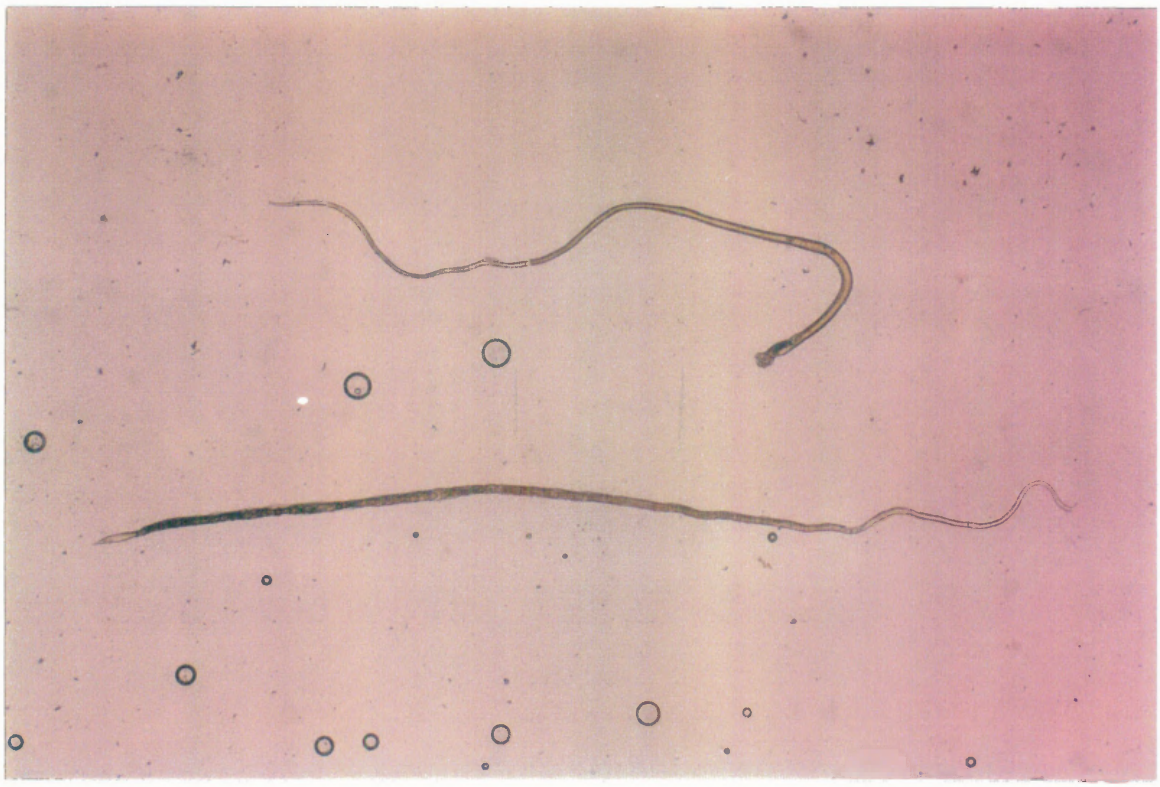

Figure 1.1: Adult *Trichostrongylus* sp. The male (above) is 5 mm long, the female is 7 mm long.

Males have a copulatory bursa with large lateral lobes and a reduced dorsal lobe. There are two, equal-sized, similarly shaped spicules that are ridged and pigmented brown. An elongate, spoon-shaped gubernaculum is present (Watson, 1960; Lapage 1962). Both the pattern of the bursal rays and the size and shape of the spicules vary between species.

The vulva of the female is located towards the posterior of the worm and the two uteri extend anteriorly and posteriorly (Watson, 1960; Georgi, 1980). Eggs are oval, slightly green in colour and thin-shelled. They are embryonated to the morula stage when they leave the host (Watson, 1960; Lapage, 1962) and are not easily identifiable from many other genera of Trichostrongylidae (Georgi, 1980). *Trichostrongylus* eggs range in length from 75 to 86 μm and in width from 34 to 45 μm (Lapage, 1962).

1.1.1 Life Cycle

Trichostrongylus spp. have life cycles very similar to that of other Trichostrongylidae (Figure 1.2). It is a direct life cycle including both parasitic and free-living stages (Lapage, 1962: Martin, 1989). Eggs leave the host in the faeces, hatching to first stage larvae (L1) which moult to give rise to the second stage larvae (L2). The first and second stage larvae are free-living, feeding on bacteria in the dung. The second moult gives rise to the third stage, the infective larva, which retains the cuticle of the previous stage, i.e. is "ensheathed". This stage may also be recognized by its large, club-shaped pharynx (Watson, 1960). The sheath serves as a protective covering for the infective larvae that migrate onto the foliage to be ingested by a host animal. Depending on temperature and humidity, infective larvae of some Trichostrongylidae genera can survive on pasture for up to eighteen months (Bairden, Armour & McWilliam, 1985).

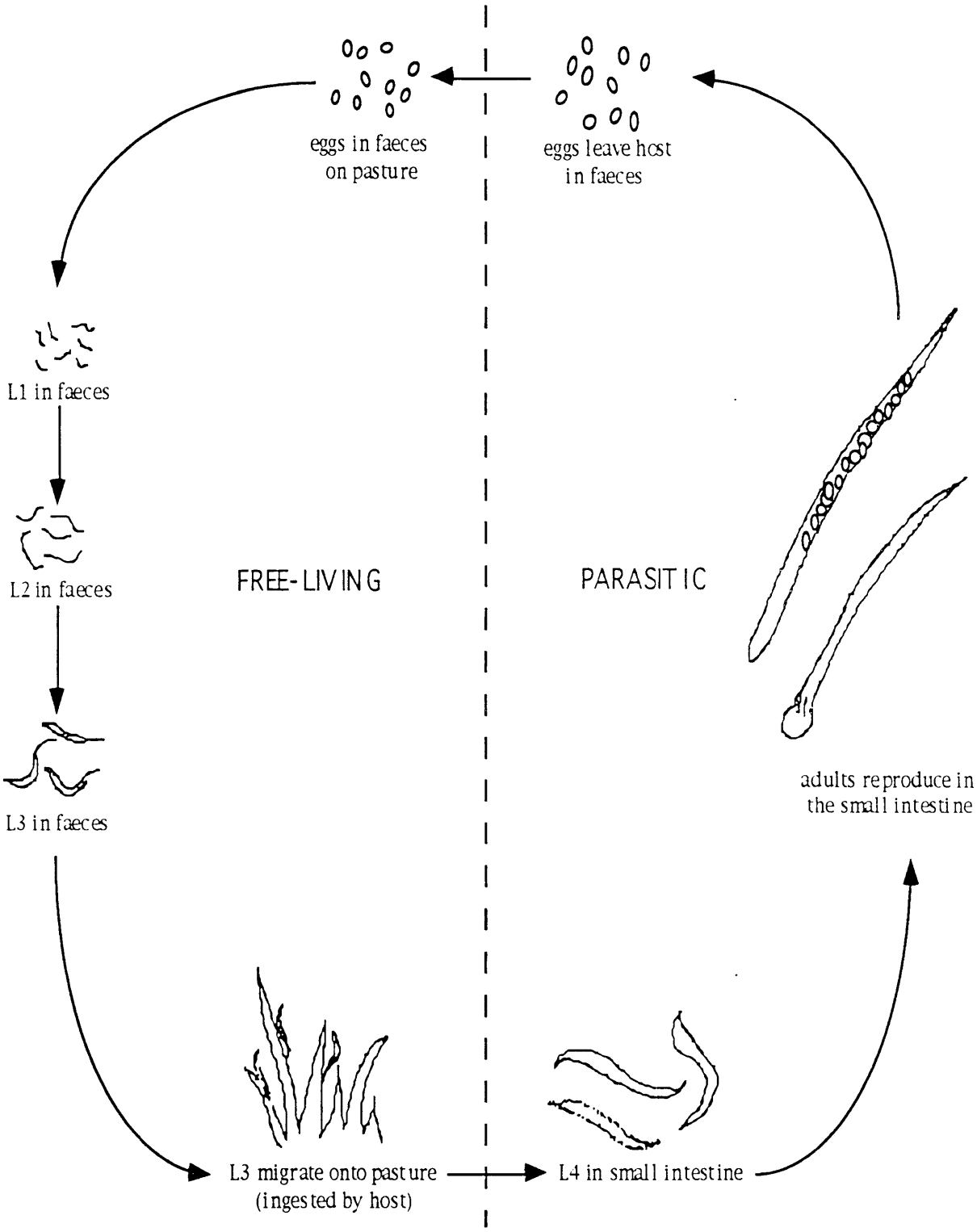

Figure 1.2: Life cycle of trichostrongylid nematode showing parasitic and free-living stages..

It has been estimated that development from egg to infective larva takes from one to two and a half weeks for *T. vitrinus* in southern Australia (Callinan, 1979), although this is dependent on rainfall and temperature, from two to eight weeks in *T. colubriformis* in northern New South Wales (Southcott, Major & Barger, 1976) and from one to eight weeks for *T. colubriformis* in the UK (Gibson & Everett, 1967).

Once ingested, the infective larva buries into the mucosa of the small intestine. It infects the first 10 metres of the intestine, but prefers the first three (Beveridge, Pullman, Phillips, Martin, Barelds & Grimson 1989). The third stage larva moults again, exsheathing, to give rise to the parasitic fourth stage larva (Watson, 1960; Lapage, 1962; Martin, 1989). After the final, fourth moult the adult emerges from the mucosa to mate and produce eggs. The average time taken from infection to the release of eggs in the faeces is 14 to 21 days (Watson, 1960; Lapage, 1962; Reid & Murray, 1973) and egg production is at a maximum after 28 days.

1.1.2 Climate

T. colubriformis and *T. vitrinus* are both found throughout the world and often in the same regions (Beveridge *et al.*, 1989). Where they do occur together it is not uncommon for one species to dominate depending on climatic conditions. This is thought to be due to the temperature and humidity preferences of the eggs and free-living larvae (Beveridge & Ford, 1982). *T. vitrinus* tends to dominate in cooler, winter rainfall areas (Beveridge & Ford, 1982), whereas *T. colubriformis* apparently prefers warmer, summer rainfall regions (Southcott *et al.*, 1976; Beveridge & Ford, 1982; Martin, 1989).

Conditions favouring development of eggs and larvae have been calculated for both *T. colubriformis* and *T. vitrinus* in Australia. Callinan (1979) estimated that a mean monthly maximum of 22.5°C, a mean monthly minimum of 12.2°C and a monthly precipitation of 97.6 mm were ideal for *T. colubriformis*. Anderson (1973) and Callinan (1979) estimated that a mean monthly maximum air temperature below 15.5°C (Callinan, 1979) and a mean relative humidity at 3 o'clock pm greater than 60% were optimal for *T. vitrinus*.

T. colubriformis eggs develop best when deposited in summer and early autumn (Callinan, 1979). The eggs are resistant to desiccation and in a semi-desiccated state are more likely to survive extreme temperatures. Development of *T. colubriformis* eggs is inhibited by cold. *T. vitrinus* eggs survive best if deposited during autumn and winter (Callinan, 1979) with hot, dry conditions proving fatal to both eggs and larvae (Rose & Small, 1984). Any larvae surviving these conditions usually do not develop further (Callinan, 1979).

1.1.3 Black Scour Disease

Black Scour Disease, or trichostrongylosis, is a result of chronic infection with any of the intestinal *Trichostrongylus* spp. but is usually associated with *T. colubriformis* or *T. vitrinus*. Symptoms include a dark-coloured diarrhoea, the so-called "black scour" and reduced appetite leading to rapid weight loss and loss of meat and wool production (Lapage, 1962; Reid & Murray, 1973; Beveridge *et al.*, 1989). Sheep will often scour after ingesting infective larvae, even if the infection does not persist. The reason for this is thought to be the altering of protein metabolism by the incoming

larvae, causing a loss of protein into the gut and hence scouring (Pullman, Beveridge & Martin, 1991). In his work on wool growth in sheep infected with *T. colubriformis*, Barger (1972) has shown a reduction in wool production of 17% to 42% in naive sheep showing patent infections and in those with induced resistance to infection that have been challenged with *T. colubriformis* L3.

Subtotal or total atrophy of the small intestinal villi (Coop, Angus & Sykes, 1979; Beveridge *et al.*, 1989) and the development of a thick mucous covering (Lapage, 1962; Reid & Murray, 1973) effectively reduce the available surface area and, therefore, the absorption of water, electrolytes and nutrients. This malabsorption causes scouring and weight loss (Sykes, Coop & Angus, 1979) and a reduction in wool quality in sheep (Reid & Murray, 1973). More recent evidence suggests that the scouring is a result of a hypersensitivity reaction in the gut to the incoming larvae (Larsen, Anderson, Vizard, Anderson & Hoste, 1994; Larsen, Vizard & Anderson, 1995; Larsen, Vizard, Webb Ware & Anderson, 1995).

There is evidence that symptoms are more severe when an infection is dominated by *T. vitrinus* (Beveridge *et al.*, 1989) with more severe epithelial erosion leading to gut leakage. Histology of the small intestine following infection with *T. vitrinus* shows many intra-epithelial tunnels not seen after an infection with *T. colubriformis*, suggesting that *T. vitrinus* has a greater tendency to migrate. The greater damage to the gut caused by *T. vitrinus* results in a greater decrease in host food intake which leads to a greater production loss.

1.1.4 Cost to Industry

In 1972, Barger described the manner in which parasites could reduce the profitability of sheep production. These included mortality, reductions in quality and quantity of wool production and of live weight gain, costs of anthelmintics and labour to administer them, and loss of opportunity due to spelling pastures that may otherwise have been grazed. Martin (1989) agreed that while loss of meat and wool production were the major costs, labour, capital and anthelmintics necessary to treat sheep added to the expense.

In the past, loss of wool and meat production due to trichostrongylid infection has been estimated at 14.6% (Anderson, 1972, 1973), with untreated sheep weighing, on average, 0.52 kg less than treated sheep. In 1985, Beck, Moir & Meppem estimated the loss per sheep per year (in a year of "normal" weather conditions) due to nematode infection to be AUD$2 to AUD$4. The yearly figure totalled for the portion of the Australian sheep industry located in regions where nematodes were prevalent was divided into AUD$309m for production losses and AUD$53m for anthelmintics and their administration.

More recently, CSIRO (1990, quoted by Emery, McClure & Wagland, 1993) estimated the cost of control of internal parasites and production loss at around AUD$500 million. Overend, Phillips, Poulton & Foster (1994) suggested that the cost could actually be higher than estimated due to the unknown effect of anthelmintic resistance.

1.2 ANTHELMINTICS

Treatment with anthelmintic drugs is, at present, the only certain way to deal with the parasite problem. There are a number of families of anthelmintics available on the market, the three major ones are benzimidazoles, levamisole and the macrocyclic lactones (including avermectins and milbemycins).

1.2.1 Benzimidazoles

Benzimidazoles (BZ) are a group of compounds used both as anthelmintics and fungicides (Sangster, Prichard & Lacey, 1985; Russell & Lacey, 1991) with a broad spectrum of activity (Martin, 1989; Prichard, 1990a). All BZ have the same double ring structure with the length of the side chain affecting the efficacy of individual compounds. Compounds with longer side chains are more effective at lower doses (Martin, 1989) due to slower metabolism by the host and, therefore, increased availability to the parasite.

The mode of action of BZ was previously thought to be the inhibition of the fumarate reductase enzyme system in the fermentation pathways (Prichard, Hall, Kelly, Martin & Donald, 1980), thus reducing the parasite's energy production capacity (Martin, 1989). BZs are indeed inhibitors of the fumarate reductase system, but the major mode of action is now thought to involve tubulin binding (Sangster *et al.*, 1985; Russell & Lacey, 1991; Russell & Lacey, 1992). Tubulin is the cytoskeletal protein and a major component of microtubules. When BZs bind to tubulin the tubulin-microtubule equilibrium is altered, causing depolymerization of the microtubules (Lacey, 1988). Kohler (1990) and Prichard (1990b) agree with

Lacey (1988) showing that the microtubules from intestinal cells of susceptible nematodes disappear after treatment with BZ.

There are at least two components in the interaction between BZ and tubulin in nematodes, irreversible (or pseudo-irreversible) binding and reversible binding. Binding is temperature dependent (Russell & Lacey, 1991) with BZ-tubulin binding maximized at 37°C in fully BZ-susceptible *T. colubriformis*. Nematodes resistant to BZ show a reduction in BZ-tubulin binding (Russell & Lacey, 1991), suggesting a mutation or deletion of a tubulin isotype involved in BZ binding. Binding may still occur in BZ-resistant individuals, however this is maximized at 10°C rather than 37°C, a temperature not encountered in the host.

The free-living nematode, *Caenorhabditis elegans*, and the sheep parasite, *Haemonchus contortus*, are known to share a similar mechanism for resistance to BZ. In *C. elegans* it is the deletion of one β-tubulin gene, known as ben-1. Russell & Lacey (1992) have suggested that a specific tubulin isotype, extensively involved in BZ binding at 37°C is "lost" in *T. colubriformis* individuals showing the BZ-resistance phenotype. A further suggestion from the same workers is that BZ-susceptibility is determined by the proportion of tubulin isotypes involved in BZ binding. When the subpopulation of tubulin dimers responsible for irreversible (pseudo-irreversible) binding is deleted, there is a decrease in the amount of irreversible binding and an increase in the amount of reversible binding, resulting in BZ-resistance. Le Jambre (1990) adds to this theory with the suggestion that an alteration in the β-tubulin gene in question prevents irreversible binding but maintains gene function. Prichard (1990a) adds that it is an alteration in, not a deletion of, a β-tubulin gene that is responsible for BZ-resistance in *T. colubriformis*. Evidence supporting these theories is the

rapid development of resistance to BZ in selected laboratory strains. Many more mutable sites would be needed to eliminate a gene than to merely prevent irreversible binding at 37°C, and this would greatly increase the time it would take for resistance to develop (Le Jambre, 1990).

1.2.2 Levamisole

Levamisole (LEV), morantel (MOR) and pyrantel (PYR) are anthelmintics thought to share a mode of action (Harrow & Gration, 1985) and, therefore, mutual or side resistance would be expected. In practice nematodes that are resistant to LEV show some or no resistance to MOR, but MOR-resistant nematodes do show a high level of resistance to LEV. Since LEV is the anthelmintic of choice from this group in Australia, they will be considered as one anthelmintic, called LEV.

In the nematode *Ascaris suum* LEV has been shown to act as a cholinergic agonist (Harrow & Gration, 1985). Presence of LEV causes depolarization of the muscle bag membranes and muscle contraction resulting from an outpouring of sodium ions (Aubry, Cowell, Davey & Shevde, 1970; Coles, East & Jenkins, 1975).

Resistance to LEV has been studied using the free-living nematode *Caenorhabditis elegans* as a model for parasitic nematodes. In LEV-resistant strains of *C. elegans* there is no response to any cholinergic agonists due to the lack of acetylcholine receptors found in LEV-susceptible worms (Lewis, Wu, Levine & Berg, 1980). Lewis, Fleming, McLafferty, Murphy & Wu (1987) observed differences in the binding of LEV to LEV-resistant and LEV-susceptible worms *in vitro*. Summing up, Prichard (1990a; 1994) states that resistance to LEV is due to a reduction in the number of cholinergic receptors or a reduction in the affinity of these receptors for LEV.

1.2.3 Macrocyclic Lactones

This class of anthelmintic is the newest on the market and includes the avermectins (avermectin, ivermectin, doramectin) and milbemycins (moxidectin). Avermectins and milbemycins are considered to be separate chemical groups. However, they are both 16-membered macrolides and have similar ring structures (Shoop, Haines, Michael & Eary, 1993). The avermectins were first derived from the mycelia of the microfungus *Streptomyces avermitilis* (Miller, Chaiet, Cole, Cole, Flor, Goegelman, Gullo, Joshua, Kempf, Krellwitz, Managhan, Ormand, Wilson, Albers-Schönberg & Putter, 1979) and were first available in Australia in 1985 as avermectin B_1 injectable formulation for cattle, or Avomec® (Merck, Sharp & Dohme). These compounds are effective against a wide range of nematode and arthropod parasites including parasites of sheep, cattle, horses and poultry (Campbell, 1989).

There has been some debate in the literature about whether the avermectins and milbemycins share the same mode of action against parasites and, therefore, whether there could be any mutual or side resistance to the compounds. In a letter to a journal (Shoop, 1992, quoted by Mudd & Baldwin, 1992) it was suggested that there was evidence showing mutual resistance to ivermectin (IVM) and moxidectin (MOX). This claim was refuted by Mudd & Baldwin (1992) because the recommended dose rates (RDR) of the two compounds were not directly compared.

The data Shoop referred to were subsequently published (Shoop *et al.*, 1993), showing that the dose rates compared were those necessary to kill 95% of the nematode population. IVM and MOX were tested on IVM-

susceptible and IVM-resistant *Tel. circumcincta* and *T. colubriformis* that had not previously been exposed to MOX. The dose rates required to kill 95% of the resistant nematodes were 23 and six times larger than those required for the same mortality of susceptible nematodes for IVM and 31 and nine times larger for MOX. Similar results have been reported by Pomroy & Whelan (1993) and Leathwick (1995).

Substantial evidence for a common mode of action has also been supplied by Conder, Thompson & Johnson (1993). They measured changes in membrane conductance induced by IVM or MOX in leg muscles of crabs as well as looking at clearance of IVM-resistant and IVM-susceptible *H. contortus* from jirds treated with either IVM or MOX. Their results showed MOX to cause a rapid loss of membrane resistance in the muscle which was approximately 50% reversible with a chloride channel-blocker, the same result seen when IVM was used. In the jirds, MOX only killed up to 47.2% of IVM-resistant worms with a dose that killed over 98% of IVM-susceptible worms. While there is no question that the RDR of MOX kills IVM-resistant nematodes (Pankavich, Berger & Simpkins, 1992), these results, combined with those of Pomroy & Whelan (1993), Shoop *et al.* (1993) and Leathwick (1995), appear to indicate side resistance and a similar mode of action for these two classes of anthelmintic

In 1989 Turner & Schaeffer (quoted by Prichard, 1990a) reviewed the mode of action of IVM, writing that IVM binds to a specific, high affinity receptor in target organisms (nematodes and arthropods). The same authors have recorded the purification of the receptor from the free-living nematode *Caenorhabditis elegans.* When IVM binds with the receptor the permeability of the membrane to chloride ions increases. Prichard (1994) adds that the receptors are glutamate receptors in a membrane chloride channel.

The standard route for anthelmintic treatment is oral , however, there are injectable and topical ("pour-on") formulations of anthelmintics in this class. Borgsteede (1993) has shown that while both the oral and injectable IVM formulations available on the market control IVM-susceptible strains of *H. contortus*, *C. curticei* and *T. vitrinus*, the injectable formulation did not reduce egg output by 100% where the oral formulation did. He goes on to confirm results published by other workers (Armour, Bairden, Batty, Davison & Ross, 1985; Egerton, Eary & Suhayda, 1981; Campbell & Benz, 1984) that oral IVM has greater efficacy against intestinal worms than injectable IVM, however, there is no apparent persistent effect. Injectable IVM was found to have a persistent effect on all three species investigated for at least 10 days. Zajac, Thatcher, Brock, Umberger & Notter (1992) recorded a significant effect of administration route of IVM on parasite control and weight gain in lambs. They recommend that oral IVM should be used more frequently for parasite control to be as effective as injectable IVM because the oral formulation has no residual effect in sheep.

The residual effect of MOX has been examined by Peter, Boelema, Grove & Rall (1994). The injectable formulation was found to be greater than 80% effective in more than 80% of treated sheep against challenge with incoming *H. contortus*, *Tel. circumcincta*, *Gaigeria pachyscelis* and *Oesophagostomum columbianum* at 28 and 35 days after treatment. They found the oral formulation to have the same efficacy against *G. pachyscelis* at 28 days post treatment but to be only about 60% effective in about 60% of treated sheep against the other three species at 28 days post infection. The results show injectable MOX to have a greater residual effect than the oral formulation but both MOX formulations have a much greater residual effect than IVM.

1.3 THE PROBLEM OF ANTHELMINTIC RESISTANCE

While anthelmintics are the most commonly used method of parasite control at present, this may not be the case for much longer. Resistance has been recorded to all classes of anthelmintics available on the market (Prichard, 1994) with resistance problems identified in many countries.

The existence of anthelmintic resistant parasite populations has been well documented in Australia with resistance to BZ and LEV a serious problem in most sheep-producing areas (Anon, 1989). Anthelmintic resistance to the modern anthelmintics was first recorded to a BZ, thiabendazole (TBZ), in *H. contortus* on the northern tablelands of New South Wales (Smeal, Gough, Jackson & Hotson, 1968), BZ resistance in *T. colubriformis* (Hotson, Campbell & Smeal, 1970) and *Ostertagia (Teladorsagia) circumcincta* (Hall, Campbell & Carroll, 1979) were reported in following years. Reports of LEV resistance soon followed in *T. colubriformis* and *Tel. circumcincta* (Sangster, Whitlock, Russ, Gunawan, Griffin & Kelly, 1979). Multiple resistance, where a nematode population shows resistance to more than one family of anthelmintics, has also become a problem (Dash, 1986b).

Since these early reports various groups have undertaken anthelmintic resistance surveys covering most Australian states. Beveridge, Ellis, Riley & Brown (1990) surveyed 68 farms in South Australia estimating the prevalence of resistance to BZ at 70% and LEV at 20% for the genera *Haemonchus, Ostertagia* and *Trichostrongylus* combined. Overend, Phillips, Poulton & Foster (1994) surveyed large commercial sheep flocks in all

Australian states except Queensland. Overall they found 85% of farms had nematodes resistant to BZ, 65% with resistance to LEV and 34% with resistance to a combination of BZ and LEV, no resistance to IVM was detected. Only 9% of farms surveyed showed little or no resistance to all classes of anthelmintics tested. The predominant nematode species were *Tel. circumcincta*, *Trichostrongylus* spp., *Chabertia ovina* and *H. contortus*.

There have been two reported incidences of ivermectin resistance in field populations in Australia, one in *Ostertagia* spp. in Western Australia (Swan, Gardner, Besier & Wroth, 1994) and one in *H. contortus* in New South Wales (LeJambre, 1993). With high levels of resistance to the other classes of anthelmintics, producers are tending to rely more on ivermectin or moxidectin. The increased usage of these drugs is likely to accelerate the development of resistance to them in the nematode populations.

The problem of anthelmintic resistance is not restricted to Australia. Multiple resistance, to BZ, LEV and IVM, was detected in goats exported from New Zealand to Czechoslovakia in 1991 (Varady, Praslicka, Corba & Vesely, 1993; Praslicka, Várady & Corba, 1994). The species involved were from the genera *Ostertagia* and *Trichostrongylus*.

Anthelmintic resistance has also been recorded in sheep and goat nematodes in Malaysia. BZ resistance has been identified in *H. contortus* infecting goats in peninsular and west Malaysia (Dorney, Claerebout, Vercruysse, Jalila & Sani, 1993; Rahman, 1994a; 1994b). Sivaraj, Dorny, Vercruysse & Pandey (1994) reported multiple resistance of *H. contortus* in sheep to BZ and IVM and of *T. colubriformis* in sheep to BZ and LEV.

Resistance to BZ has been reported in *H. contortus* and *Cooperia curticei* in the United States of America (Uhlinger, Fleming & Moncol, 1992; Lyons, Drudge, Tolliver & Stamper, 1992). In the West Indies, on Martinique, BZ resistance has been reported in *H. contortus* from sheep (Gruner, Kerboeuf, Beaumont & Hubert, 1986).

In southern Latin America levels of anthelmintic resistance were surveyed on 65 farms in Argentina (Eddi, Caracostantogolo, Peña, Schapiro, Marangunich, Waller & Hansen, 1996), 182 farms in Brazil (Echevarria, Borba, Pinheiro, Waller & Hansen, 1996), 37 farms in Paraguay (Maciel, Giménez, Gaona, Waller & Hansen, 1996) and 252 farms in Uraguay (Nari, Salles, Gil, Waller & Hansen, 1996). The percentage of farms with sheep/goat nematodes (*H. contortus*, *Ostertagia* spp. and *Trichostrongylus* spp.) showing resistance to BZ was 40% in Argentina, 90% in Brazil, 73% in Paraguay and 80% in Uraguay. For LEV the levels of resistance in the four countries were 22%, 84%, 68% and 71%, respectively. The levels of resistance to the avermectins were 6% in Argentina, 13% in Brazil, 73% (oral formulation) and 47% (injectable formulation) in Paraguay and 1.2% in Uraguay. A combination of BZ and LEV was tested in Argentina and Brazil with levels of resistance being recorded at 11% and 73%, respectively. The efficacy of closantel, a narrow spectrum anthelmintic used for the control of *H. contortus*, was tested in Brazil, with nematodes on 20% of farms showing resistance. Resistance to more than one class of anthelmintic (multiple resistance) was identified on many farms.

In Africa BZ resistance in sheep nematodes has been recorded in Cameroon (Ndamukong & Sewell, 1992), Tanzania (Ngomo, Kassuku & Ruheta, 1990), Kenya (Maingi, 1991; Mwamachi, Audho, Thorpe & Baker, 1995) and South Africa (VanWyk & Malan, 1988). LEV resistance has been

described in Kenya (Maingi, 1991) and multiple resistances have been recorded in Kenya (Mwamachi *et al.*, 1995) and South Africa (VanWyk & Malan, 1988). One experiment by Mwamachi *et al.* (1995) using six month old lambs from Kenya showed nematode reduction percentages of only 42%, 92%, 77% and 13% following treatment with BZ, LEV, IVM (injectable formulation) and IVM (oral formulation), respectively. These results indicate a high level of resistance to BZ and oral IVM, moderate resistance to injectable IVM and low level resistance to LEV, the species involved were *H. contortus*, *Trichostrongylus* spp. and *Oesophagostomum*spp. The first incidences of IVM resistance ever recorded were in *H. contortus* in South Africa (Carmichael, Visser, Schneider & Stoll, 1987; VanWyk, Malan, Gerber & Alves, 1987; VanWyk & Malan, 1988).

Gill (1993) surveyed five sheep farms in India and found resistance to both BZ and LEV on all farms but no IVM resistance. Multiple resistance to BZ and LEV was identified in *H. contortus* on another Indian farm by Yadav, Kumar, Uppal & Verma (1995), again there was no resistance to IVM, or to closantel.

In Europe and the United Kingdom anthelmintic resistance is also widespread. IN the UK, BZ resistance has been recorded in *C. curticei* from sheep in England (Hunt, Hong, Coles, Simpson & Neal, 1992) and multiple resistance (to BZ and IVM) in *Tel. circumcincta* in goats in Scotland (Jackson, Jackson & Coop, 1992). Examples of resistance recorded from Europe include, BZ resistance in sheep nematodes in Belgium (Vercruysse, Dorny & Meurrens, 1989) and The Netherlands (Boersema, Borgsteede, Eysker, Hendrikx, Jansen & Smith-Buys, 1987; Borgsteede, Schavemaker, Van der Burg, Gaasenbeek & Pekelder, 1991).

1.4 ALTERNATIVE METHODS OF PARASITE CONTROL

Widespread anthelmintic resistance in sheep nematodes in Australia, as well as a desire for fewer chemical residues in animal products and the environment, have led researchers to work on alternatives to traditional chemotherapy as a means of parasite control. Some of these alternative methods include flock management, manipulation of nutrition, vaccination, breeding for resistance and biological control.

1.4.1 Flock Management

Many workers have endeavoured to establish flock/herd management routines to significantly reduce worm burden in stock in an effort to control parasitism without relying totally on repeated anthelmintic treatments. Ways by which this has been attempted include mixed, alternate or rotational grazing of two or more classes of host, grazing crop stubble or rotating pasture with crops and making anthelmintic treatments with existing drugs more effective.

Mixed, alternate or rotational grazing uses two or more different classes of host, such as cattle and sheep or cows and calves. The success of such systems depends on host specificity of the parasites involved and longevity of the free-living stages of the parasites. Host specificity varies among the gastrointestinal nematodes affecting stock, with little or no cross-infectivity seen in *Ostertagia/Teladorsagia* while some species of *Trichostrongylus* can sucessfully reproduce in either host.

Longevity of free-living stages can be affected by climate, with free-living stages surviving longer in cooler climates. In temperate regions of Australia L3 of gastrointestinal nematodes have been recorded surviving on pasture for longer than six months (Southcott, Major & Barger, 1976; Donald, Morley, Waller, Axelson & Donnelly, 1978), while in other parts of the world they have been recorded surviving for at least 18 months (Bairden, Armour & McWilliam, 1985). For the temperate regions of Australia, Donald *et al.* (1978) have shown that resting a paddock for three months will significantly reduce numbers of *Trichostrongylus* available to infect sheep, but not *Ostertagia*.

There are many examples of farmers already using mixed, alternate or rotational grazing as a means of parasite control (Gettinby, Armour, Bairden & Plenderleith, 1987) and work carried out in Australia (Barger & Southcott, 1975) has successfully shown a significant reduction in *Ostertagia* and *Cooperia* L3 available to calves grazing cattle paddocks that had been stocked with sheep for 6, 12 or 24 weeks. However, an experiment conducted by Bairden, Armour & Duncan (1995) showed that annual alternate grazing of sheep and cattle did not prevent parasitic disease in calves. They did, however, suggest that a longer rotation including a crop, which would effectively lengthen the pasture's "resting" period, would give a greater degree of control.

The effects of rotational grazing have also been examined in wet, tropical environments where temperature and rainfall allow development of nematode eggs to L3 on pasture throughout the year. Barger, Siale, Banks & LeJambre (1994) have shown that rotational grazing can significantly reduce the number of anthelmintic treatments required by goats. They suggested that rotational grazing could easily be implemented in such environments by

means of fencing, tethering or herding stock. The work of Banks, Singh, Barger, Pratap & LeJambre (1990) on development times and survival of nematode larvae on pasture in Fiji suggests that a rotational grazing system could aid in nematode parasite control in that Pacific region as well.

The "drench and move" technique involves animals being treated with an effective anthelmintic before moving onto "safe" pastures. "Safe" pastures can be those that have been spelled for an extended period, grazed by alternate hosts, crop stubble, or pasture reseeded after cropping, so that few or no L3 remain to infect stock. However, the method of drenching animals then moving onto clean pasture may actually increase selection pressure for anthelmintic resistance. If any worms survive the anthelmintic treatment they are highly likely to be resistant to that anthelmintic and, therefore, so would offspring contaminating the clean pasture. LeJambre (1978) has suggested that this type of treat and move regime may cause anthelmintic resistance to develop more quickly than more traditional treatment regimes in one paddock. Donald (1983, quoted by Echevarria, Armour, Duncan & Pinheiro, 1993) suggested that this may not be the case if animals grazing these pastures are slaughtered, if pastures are grazed next by cattle, or if pastures are cropped the following year. This theory was the basis for work carried out in Brazil (Echevarria, Armour, Duncan & Pinheiro, 1993) using pastures reseeded after a soyabean crop. Results of this work showed that little immediate risk of infection was present for sheep and cattle grazing such pastures, and that this method could be used for parasite control in southern Brazil.

Another way to control nematode parasites using management strategies is to increase the efficacy of available anthelmintic treatments or to increase their persistence. Prichard, Hennessy & Steel (1978) showed

persistence to be important in the level of efficacy and the spectrum of BZ anthelmintics. They postulated that persistence would be just as important in other anthelmintic classes.

After controlling three species of BZ-resistant nematodes with a BZ administered in a sustained-release device, LeJambre, Prichard, Hennessy & Laby (1981) concluded that the efficacy of an anthelmintic could be increased by increasing its persistence

Ali & Hennessy (1993, 1995) have shown that anthelmintic efficacy of BZ can be increased so that significantly higher percentages of BZ-resistant nematodes are removed by the RDR. This is done simply by withholding food from sheep for some time prior to anthelmintic treatment. The temporary reduction in food intake slows gastric flow, keeping the drug in the animal's system for longer. Ali & Hennessy (1993) have suggested using this technique not only to extend the useful lives of anthelmintics that have lost efficacy, but with drugs that have no resistance problems as yet. The theory is that combining this technique with strategic drenching programmes minimising anthelmintic use may delay the onset of resistance to these drugs.

Within anthelmintic groups there are specific compounds having a greater persistence in the host animal. These compounds, specifically moxidectin from the macrocylic lactone group, are said to have a "tail" of efficacy. That is, a declining level of drug available to the parasite over time. There has been suggestion that this "tail" effect will increase selection for resistance with resistant larvae surviving while susceptible larvae are still being removed from the host for a period during the "tail". Dobson, LeJambre & Gill (1996) have modelled the effects of "tail" selection and shown that a drug's "tail" has a very small role in selection for anthelmintic

resistance. In fact, their model showed that a highly efficacious anthelmintic killing all larvae, resistant and susceptible, strongly selects for resistant adults surviving the treatment. Overall the model showed that rapid anthelmintic selection was mainly caused by treatment frequency.

1.4.2 Nutrition

The survival of parasitic nematodes can be greatly affected by the nutritional status of the host (Geiman, 1958), with malnourished animals being far more susceptible than well fed animals (Kates & Wilson, 1955; Bawden, 1969). Workers in the mid 1950s (Baird, Vegors, Sell & Stewart, 1954) demonstrated that the amount of protein in the diet was more important to parasitism than the total amount of food ingested.

Dobson & Bawden (1974) have shown that sheep fed higher protein diets sustained smaller populations of *Oesophagostomum columbianum*, being more immunologically competent than sheep fed low protein diets. Sheep on the high protein diet eliminated more worms, worms produced fewer eggs, worms produced eggs later in the infection and more larvae were found to show arrested development.

A number of researchers have since examined the influence of dietary protein on nematode parasite infection. Unlike Dobson & Bawden (1974), Abbott, Parkins & Holmes (1985a, 1985b, 1988) were unable to show any effect of protein on establishment of *O. columbianum*. They also were unable to show any effect on establishment of *H. contortus* in Scottish Blackface lambs. However, a high protein diet enabled Finn Dorset and Dorset Horn lambs to develop resistance to *H. contortus* when those on a low protein diet showed much more severe infection symptoms. Other work has

shown similar results, i.e. that an increased or earlier developing immune response against the nematode parasite is evoked by high dietary protein levels. This has been seen with *H. contortus* infection in goats (Blackburn, Rocha, Figueiredo, Berne, Vieira, Calvalcante & Rosa, 1991), *Teladorsagia circumcincta* and *Nematodirus battus* infections in Greyface/Suffolk lambs (Coop, Huntley & Smith, 1995; Isaf, Coop, Stevenson, Jones, Jackson, Jackson, MacKellar & Huntley, 1996) and *T. colubriformis* infections in Dorset/Coopworth lambs (Bown, Poppi & Sykes, 1991).

Kambara, McFarlane, Abell, McAnulty & Sykes (1993) showed that young Dorset Down/Coopworth lambs (8-26 weeks old) on a low protein diet showed significantly lower resistance to *T. colubriformis* than the same aged lambs on a higher protein diet, with level of protein affecting immune response as measured by *in vitro* T lymphocyte response to parasite antigen. The same effect was not seen in older lambs (33-51 weeks old). Abbot & Holmes (1990) and Shaw, Nolan, Lynch, Coverdale & Gill (1995) achieved similar results with Scottish Blackface and Merino lambs, respectively. Wedrychowicz, Abbott & Holmes (1984) showed depression of local antibody responses in yearling Blackface sheep, vaccinated or not against *H. contortus* and fed a low protein diet. However, the low protein diet had no effect on the efficacy of the vaccination.

Young animals have a higher protein requirement, and protein loss due to parasitism increases this requirement further. Protein supplementation is thought to help the lambs overcome protein deficiency caused by nematode infection (Shaw *et al.*, 1995). Endogenous protein losses into the gut, altered digestion and altered protein absorption as well as reduced food intake are the suggested mechanisms by which parasitism causes protein deficiency. The level of protein in the diet affects the development of immunity to

parasite infection (Dobson & Bawden, 1974; Abbott *et al.*, 1985a, 1988; Bown *et al.*, 1991), so young animals with a reduced protein intake suffer a delayed development of immunty.

There has been much research examining the effects of protein supplementation on production. Wallace, Bairden, Duncan, Fishwick, Gill, Holmes, McKellar, Murray, Parkins & Stear (1995) examined the influence of dietary protein on resistance to *H. contortus* in Hampshire down lambs. Their findings indicated that although mean worm burdens were not significantly different, supplemented lambs had lower faecal egg count (FEC) and an improved carcase composition. Research on penned young Merino wethers (van Houtert, Barger, Steel, Windon & Emery, 1995) has shown a significant reduction in liveweight gain in *T. colubriformis* infected lambs not fed a protein supplement, compared to supplemented sheep. Supplemented animals also showed an increase in greasy wool production and fibre diameter and a decrease in FEC in protein supplemented animals. They concluded that protein supplementation substantially reduced production losses due to *T. colubriformis* infection. Another experiment, this time in grazing Merino wether lambs infected with *T. colubriformis*, *T. vitrinus*, *T. axei*, *Nematodirus* spp. and *Teladorsagia circumcincta*, has shown similar improvements in production parameters seen in lambs fed the protein supplement (van Houtert, Barger & Steel, 1995).

1.4.3 Vaccination Against Parasitic Nematodes

Currently there are three international collaborations involving Australian scientists that are working on the development of vaccines against species of *Haemonchus*, *Ostertagia/Teladorsagia* and *Trichostrongylus*. These collaborations are using DNA technology for vaccine development

(Emery *et al.*, 1993) which should overcome the problems of technical limitations and the limited amount of antigen available. Despite these apparent problems, there were vaccines against nematode parasites developed before the advent of DNA technology. One such example is a vaccine against against hookworm in dogs (Miller 1964, 1965). This vaccine is not currently on the market due to lack of consumer demand.

Two major types of protective antigens can be used for the development of vaccines, "conventional" or "novel". Conventional antigens stimulate naturally acquired immunity while novel antigens are protective after immunity is induced by vaccination. Vaccines against blood-sucking parasites, like *Haemonchus*, can utilize novel antigens as worms ingest the serum antibody as they feed (Munn, 1993). It is thought that conventional antigens will be necessary for vaccines against browsing parasites, like *Ostertagia/Teladorsagia* and *Trichostrongylus*, by way of activation of a hypersensitivity response to remove the worms before they establish (Emery *et al.*, 1993).

At present it is possible to protect sheep against *H. contortus* using novel antigens. Greater than 90% protection has been achieved in Dorset and Clun Forest sheep using the novel antigen H11 (Tavernor, Smith, Langford, Munn & Graham, 1992; Tavernor, Smith, Langford, Graham & Munn, 1992), while around 50% protection has been achieved in Merinos using tropomyosin (Cobon, Kennedy, Wagland, Adams & O'Donnell, 1989; patent application quoted by Emery *et al.*, 1993). Despite these successes the production of a commercial vaccine is still expected to be years away (LeJambre, Knox & Gray, 1996). To be viable the vaccine must be effective against a number of nematode species and be simple and cheap to administer.

1.4.4 Breeding for Parasite Resistance

With the ever-increasing threat of anthelmintic resistance and commercial worm vaccines not expected in the near future, many workers around the world have investigated genetic resistance to parasite infection. Most of this work has fallen into one of three categories: between-breed variation, within-breed variation and identification of resistance genes (Stear & Murray, 1994).

1.4.4.1 *Between-breed Variation*

Certain breeds have been shown to resist parasite infection better than other breeds. Examples of these include the Red Masai (Preston & Allonby, 1978) and the Scottish Blackface (Abbott, Parkins & Holmes, 1985a,b). In certain instances breed substitution may be a possible means of avoiding parasite problems. A successful example of this is the substitution of *Bos indicus* breeds of cattle and their crosses for *Bos taurus* breeds to eleviate tick problems in north-east Australia.

Where highly specialized breeds fill an industry need, breed substitution is not always possible. It is in cases like these, a prime example of which is the Merino for fine wool production, that within-breed variation must be exploited.

1.4.4.2 *Within-breed Variation*

It has been well established that host resistance to the parasite *Haemonchus contortus* has a genetic basis (Whitlock, 1958; LeJambre, 1978; Windon, Dineen, Gregg, Griffiths & Donald, 1984) and, therefore, could be a selectable trait. The first work examining the feasibility of breeding for

parasite resistance or resilience was described by Albers, Gray, Piper, Barker, LeJambre & Barger (1987). This group worked on *H. contortus* infected Merino sheep and set out to quantify both the genetic basis for resistance and resilience (resistance is the ability to reduce numbers of parasites through preventing establishment or inhibiting development; resilience is the ability to maintain production while infected) and genetic relationships between resistance, resilience and production traits.

Their findings showed moderate heritability estimates for resistance, faecal egg count (FEC) and haematocrit ($h^2 = 0.3$), which is a similar level to other production traits that have been successfully selected for years. However, heritability for resilience traits, reduction in live weight gain and wool growth, were low (approximately 0.08) indicating a slower progression if selection were based on these traits. Genetic correlation of resistance traits with production traits were found to be extremely low, suggesting that selection for resistance would not have any undesirable effect on production (Windon & Dineen, 1984; Albers *et al.*, 1978). Once the feasibility of breeding for resistance had been established a number of projects commenced in Australia.

CSIRO *Haemonchus* Selection Lines

First described by Piper (1987), this flock was established in 1978 and consists of two divergent selection lines, resistant and susceptible, and an unselected control line. This flock has since been described in detail by Woolaston, Barger & Piper (1990). Selection for the flock is based on the maximum FEC recorded after artificial challenge with *H. contortus* infective larvae, the samples were usually taken three to six weeks post infection (pi). The heritability of resistance in this flock has been estimated at 0.23±0.03 and that of packed cell volume decline (PCVD) at 0.21±0.03.

Transformation (cube root) of the FEC data reduced selection bias due to heterogeneity of variance and increased FEC heritability to 0.29±0.03.

"Golden Ram" Flock

This flock was created in the 1980s as the result of progeny testing 60 fine and medium wool Merino sire progeny groups for parasite resistance (Albers *et al*, 1987). One extreme outlying sire group was identified, progeny of the so-called "Golden Ram". The selection criterion for this flock was FEC at 28 and 35 days pi, but as the genetic correlation of 28 and 35 day FEC was found to be 0.98, it is regarded as essentially the same trait. Heritability using both measurements was estimated at 0.24±0.04, which is slightly lower than the CSIRO flock but could be due to flock effect or different experimental protocols (Woolaston & Eady, 1995). Gray, Presson, Albers, LeJambre, Piper & Barker (1987) have demonstrated that sheep genetically resistant to *H. contortus* have FEC 50% to 95% lower than non-selected or susceptible sheep.

CSIRO *Trichostrongylus* Selection Lines

This flock was started at CSIRO in Sydney and has been described by Windon and Dineen (1984) and Windon, Dineen & Wagland (1987). It is based on medium wool Peppin-strain Merinos and differs from the previous two flocks in that animals are kept worm-free from birth in pens until they are vaccinated with irradiated *Trichostrongylus colubriformis* L3. All animals are infected as weaners and all are within a 2 week age range. FECs were measured every two weeks, from three weeks pi until 11 weeks pi, and selection was made on average FEC. In 1990 the flock was moved to Armidale and testing on pasture began. Heritability of resistance in pen-tested animals was estimated at 0.37±0.04 and in paddock-tested animals as 0.39±0.11 (Woolaston & Eady, 1995). FEC of high responder (resistant)

animals were typically 10% to 50% of FEC of non-selected or low responder animals when animals were subjected to artificial or natural challenge after vaccination (Windon & Dineen, 1984).

Hamilton Selection Lines

Described by Cummins, Thompson, Yong, Riffkin, Goddard, Callinan & Saunders (1991), this flock was established after the development of an assay to measure *in vitro* lymphocyte stimulation to trichostrongylid antigens (whole blood microtire culture assay, WBLC). There are two divergent resistance selection lines using sires that originated from Victorian fine wool Merino flocks. Heritability was estimated for log WBLC at 0.29±0.13 and log FEC (+30) at 0.42±0.14.

Rylington Flock

This flock was established in 1987 (Karlsson, MacLeod, Leelawardana, Sissoev & Simmons, 1991) with foundation rams coming from a variety of sources, including the CSIRO *Haemonchus* resistant selection line, the "Golden Ram" flock, CSIRO *Trichostrongylus* high responder line (all mentioned previously) and the CSIRO closed flock from Yalanbee field station, a flock which has not been treated with anthelmintics for more than 20 years. This flock was closed and replacement breeders were selected on production traits as well as FEC after natural challenge. Heritability estimates were found to be highly variable and selection was changed to FEC after artificial infection with *T. colubriformis* (Woolaston & Eady, 1995).

Armidale Nucleus Flock

This flock was established as a collaborative project between CSIRO Divisions of Animal Production and Animal Health and the University of

New England. The foundation animals were selected on low FEC estimated breeding values (EBVs) and originated from the CSIRO *Haemonchus* and *Trichostrongylus* selection lines and the "Golden Ram" flock. This flock has been linked to industry through sire evaluation schemes with the intent of providing resistant animals to industry.

In other parts of the world, too, selection lines (for resistance or resilience) have been established, in both Merinos and other breeds. Examples of these include Romney selection lines in New Zealand (Baker, Watson, Bisset & Vlassoff, 1990; Morris, Watson, Bisset, Vlassoff & Douch, 1995; Bisset, Vlassoff, Douch, Jonas, West & Green, 1996), and Merino selection lines in Hungary (Sréter, Kassai & Takács, 1994).

The mechanisms of genetic resistance have been the subject of many experiments (Windon, 1990, 1991; Woolaston, Barger & Piper, 1990; Gill, Gray & Watson, 1991; Pernthaner, Stankiewicz, Bisset, Jonas, Cabaj & Pulford, 1995). Gill *et al.* (1991) describe resistance to *H. contortus* as being an acquired immune response and is associated with mucosal mastocytosis, eosinophilia and anti-*Haemonchus* antibody responses. The level of resistance appears to be affected by the number of sensitizing infections, removal of adult worms by drenching and environmental factors. Supporting evidence is that treatment with the immunosupressant, dexamethasone, removed any susceptibility differences between resistant and random bred wethers. Windon (1991) reports animals from the *Trichostrongylus* selection high responder line as having increased responses across a range of immunological functions, including humoral and cellular recognition of parasite antigen, increased levels of complement component C3 and increased numbers of circulating eosinophils.

Animals selected for resistance to one nematode parasite have been shown to exhibit resistance to other nematode parasites. This has been seen in both the CSIRO *Haemonchus* and *Trichostrongylus* selection lines with animals selected for resistance to either *H. contortus* or *T. colubriformis* passing fewer *Haemonchus*, *Trichostrongylus*, *Teladorsagia* and *Oesophagostomum* eggs than unselected animals, including ewes nearing parturition or lactating (Windon, 1990; Woolaston, 1992).

The relationship between selection for resistance to nematode parasites and resistance to other diseases has also been examined (Gray, Gill & Woolaston, 1991). Results indicate that the general immune response of resistant sheep to other types of infection has not been compromised. Occasional positive correlations have been noted between nematode resistance and other diseases in Merino sheep. However, these associations are weak and Woolaston & Eady (1995) suggest that the presence of one disease may possibly have predisposed the animals to infection with the second disease.

If nematode parasites can develop resistance to anthelmintics as a survival mechanism it would appear possible that they could also adapt to a changing resistance status of host animals, particularly considering their high reproductive rate and short generation time. Windon (1991) theorizes that if selection for resistance was based on a gene of major importance, parasites could adapt more rapidly to survive these changes. Preliminary studies on the *Trichostrongylus* selection flock (Windon, 1990) have shown that *T. colubriformis* may be adapting to the high responder sheep. FEC of vaccinated sheep challenged with larvae from high responder animals were

significantly higher than FEC of those challenged with larvae from non-selected or low responder sheep.

In contrast, work by Woolaston, Elwin & Barger (1992) has shown that populations of *H. contortus* and *T. colubriformis* maintained in the CSIRO *Haemonchus* selection lines did not show any significant divergence in their reproductive fitness (a measure of adaptation to the resistant sheep). Other workers, too, have also been unable to show any sign of parasite adaptation to a resistant host (Adams, 1988; Albers & Burgess, 1988). Barger (1989) and Barger and Sutherst (1991) have described the epidemiology of nematode parasitism and what effects husbandry practices may have had which explain above observations. Briefly, distribution of the parasites within a flock is overdispersed, meaning that a small number of animals host a large number of parasites. Even in resistant flocks there is within-flock variation in genetic resistance and, therefore, it would be expected that the majority of the parasites would be found in the less resistant animals. In such a case, the parasites would still survive in these host animals and, as it is unlikely that the following infection would be worms directly descended from the previous infection, the survival of the parasites would not be threatened. If there is no selection pressure exerted on the parasites by resistant hosts there would be no change in the parasite population to cope with such hosts.

1.4.4.3 *Identification of Resistance Genes*

Two main approaches have been followed in the third line of research, the identification of resistance genes (genes conferring resistance to parasite infection). The first approach involved the statistical examination of the distribution of parasitological variables between distinct genetic groups

(Stear & Murray, 1994). Using this approach several groups (Whitlock, 1955,1958; Whitlock & Madsen, 1958; Albers *et al.*, 1987) suggested the existence of a dominant resistance gene against *H. contortus* infection. However, later work (Woolaston, Gray, Albers, Piper & Barker, 1990) could shed no light on the question of a dominant resistance gene. If a dominant resistant gene or genes were found, they could possibly increase the rate of selection for resistance, making this method of nematode control even more commercially attractive (Albers & Gray, 1987).

The second approach is to test animals of know resistance status for specific alleles. An example of a gene suggested for testing is MHC (Major Histocompatability Complex). Associations have been described between variations of MHC and nematode resistance in sheep (Outteridge, Windon & Dineen, 1985; Stear, Baldock, Brown, Gershwin, Hetzel, Miller, Nicholas, Rudder & Tierney, 1990; Hulme, Windon, Nicholas & Beh, 1992; Blattman, Hulme, Kinghorn, Woolaston, Gray & Beh, 1993).

1.4.5 Biological Control

Roubaud & Deschiens (1941, quoted by Waller, 1993a) first suggested that control of nematode parasites could be accomplished by nematode-destroying fungi. Their small experiment showed a reduction in parasitism of lambs grazed on a plot seeded with the fungi *Dactylella bembicoides*, *Dactylaria ellipsospora* and *Arthrobotrys oligospora* when compared to a control plot. Later work (Cooke & Godfrey, 1964) listed many species of fungi with nematode-destroying capabilities, but concentrated mainly on *A. oligospora*.

Since then, many workers have studied the nematode-destroying capabilities of *Arthrobotrys* spp. (Pandey, 1973; Nansen, Gronveld, Hendrikson, & Wolstrup, 1988; Mendoza-de Gives, Zavaleta-Mejia, Quiroz-Romero, Herrera-Rodriguez & Perdomo-Roldan, 1992). Species in this genus are described as voracious predators in culture with low nutrient media, but experiments of this type do not indicate how they will act in the field. It had been shown from work on fungi affecting plant parasites that this type of culture technique can select for aggressive colonizers that are easy to culture but which are only facultative predators or have poor nematode-destroying properties (Kerry, 1984; Stirling, 1988). However, experiments were conducted (Waller & Faedo, 1993), using artificial inoculation of cattle faeces, which clearly demonstrated that *A. oligospora* is capable of reducing numbers of infective larvae in faeces under field conditions.

Waller & Faedo (1993) screened 94 species of nematophagous fungi, including predacious and endoparasitic types, to determine their ability to destroy the free-living stages of parasitic nematodes infecting sheep. The 94 species were rigourously tested on a range of laboratory media before testing directly for nematophagous properties on sheep faeces. The screening process identified eleven species as potential biological control agents, consistently reducing numbers of infective larvae by more than 80% at 100-250 conidia/gram faeces. The successful fungi were all of the predacious type and included seven species of *Arthrobotrys*, including the much-studied *A. oligospora*, two species of *Geniculifera* and two species of *Monacrosporium*.

Once nematophagous fungi were identified it was necessary to determine a method of administration that would be both practical and

economically viable. A suitable fungus would have to survive gut passage, readily colonize fresh faeces and begin trapping nematodes in the time it takes the nematode eggs to hatch. Larsen, Wolstrup, Henrikson, Dackman, Gronveld & Nansen (1991) have developed an *in vitro* assay to examine the effects of rumen and abomasal passage on the fungi. Using this assay and calf feeding trials (Larsen *et al.*, 1992), the fungus *Duddingtonia flagrans* was identified as a better biological control prospect than *Arthrobotrys* spp. Waller (1993a, 1993b) suggested that the thick-walled chlamydospores produced by some nematophagous species, including *D. flagrans*, would intuitively allow them to survive harsher conditions than species with thin-walled conidia.

Growing the fungi on grains to be used as supplements for domestic animals may well suit some husbandry practices, but where supplementary feeding is not feasible, alternative methods of fungi deployment must be developed. Sustained-release intraruminal devices have been recommended (Waller, 1993a, 1993b) as an "obvious and possibly ideal" choice. Research in this area continues.

1.5 THE CURRENT STUDY

Trichostrongylus spp. have been shown to develop resistance to all classes of anthelmintic available on the market. However, most surveys state resistance has been identified in *Trichostrongylus* spp., without identifying the species involved. When a species is identified it is usually *T. colubriformis*. Little has been published on the resistance status of the species individually, particularly *T. vitrinus*, since more often than not *Trichostrongylus* are considered as a single group due to the difficulty in

distinguishing between the eggs and larval stages of these species morphologically.

It has been well established in the literature that *T. colubriformis* and *T. vitrinus* occur together, both in the host and as free-living larvae on pasture. A mixed *Trichostrongylus* infection can be dominated by one species, dependent on climatic conditions (Southcott *et al.*, 1976; Beveridge & Ford, 1982; Martin, 1989) but anecdotal evidence suggests that, even in the same region under the same climatic conditions, species proportion in a mixed infection can vary from property to property, from paddock to paddock within a property and even from sheep to sheep within a paddock. With the possibility that in any given area *T. colubriformis* and *T. vitrinus* could show resistance to different anthelmintic groups, and hence need different treatment regimes to control infection, and with *T. vitrinus* being more pathogenic (Beveridge *et al.*, 1989), a greater understanding of factors affecting the proportions of the two species in an infection could be of considerable importance.

1.5.1 Aims

1. To identify methods for distinguishing between *T. colubriformis* and *T. vitrinus*.

2. To investigate factors which may affect the proportion of the species in *Trichostrongylus* infections.

A range of "user-friendly" techniques was examined for species identification, including morphological examination of specimens, staining techniques and molecular techniques.

There are many factors that could have an effect on species proportion in a mixed *Trichostrongylus* infection, affecting either the parasitic or free-living stages. At the parasitic stage these could include anthelmintic treatment, nutritional state of the host, host genetics, or any of the other alternative control methods described above. The free-living stages could be affected by temperature, competition with other nematode species and flock management practices involving stock rotation and pasture spelling. Factors considered for examination in this study were anthelmintic treatment, protein supplementation, host resistance status, competition in free-living stages and temperature during development of the free-living stages.