A SURVEY OF ANTHELMINTIC RESISTANCE IN RELATION TO MANAGEMENT OF SMALL RUMINANTS IN PENINSULAR MALAYSIA

KHADIJAH BINTI SAAD

UNIVERSITI SAINS MALAYSIA

2007

A SURVEY OF ANTHELMINTIC RESISTANCE IN RELATION TO MANAGEMENT OF SMALL RUMINANTS IN PENINSULAR MALAYSIA

by

KHADIJAH BINTI SAAD

Thesis submitted in fulfilment of the requirements for the degree of Master of Science

APRIL 2007

ACKNOWLEDGEMENTS

I wish to extend my sincere thanks and appreciation to my main supervisor, Professor Abdul Wahab Abdul Rahman, Parasitology lecturer of Universiti Sains Malaysia for his encouragement and extensive assistance throughout this research.

I would also like to thank my field supervisor, Dr. Chandrawathani Panchadcharam, Head of Parasitology Department, Veterinary Research Institute, Ipoh who warmly accepted me to be a part of her team in this nationwide research. Being in her team had not only improved my knowledge in laboratory techniques but also enabled me to learn to be a successful researcher in the field of veterinary parasitology. The never-ending motivation and encouragement from a great leader and a wonderful woman like her had greatly improved my self-confidence throughout this research.

My deepest appreciation also goes to these individuals who have made this research possible;

- The Director General, Department of Veterinary Services, Ministry of Agriculture for the permission to conduct this study and to publish a scientific paper on this topic.
- The Director of Veterinary Research Institute, Ipoh; Dr. Sharifah Syed Hassan for the permission to use the institute's equipment, materials and transportation during the research

- The Directors, Department of Veterinary Services in all states of Malaysia who generously gave us the permission to conduct this research in their areas.
- Staff of Parasitology Department, Veterinary Research Institute, my ever dear friend and team mate; Ms. Nurulaini Raimy, my mentors; Madam Jamnah Omar, Mr. Adnan Musbah and Mr. Zaini Che Mamat and not forgetting Mr. Ramachandran. Their guidance and assistance especially in laboratory work in this research is highly appreciated. Being a part of this team has improved my laboratory skills as well as social skills, and also taught me the meaning of teamwork and friendship.
- Dr. Peter J. Waller, a world-renowned Parasitology lecturer of Swedish Agriculture University for his ideas, stimulating suggestions and motivation. His inspiring encouragement has made the publication of this research possible.
- Dr. Mohd Naheed Hussein, Madam Vasuge Marimuthu and Mr. Hasan Gulap Din who unselfishly taught the skills of proper handling and management of animals on the field.
- Prof Chan Lai Keng, lecturer of Universiti Sains Malaysia for the guide and advice in statistical analysis.
- Officers from Department of Veterinary Services in all States of Peninsular Malaysia and officers from the District Veterinary Department for the assistance in the field throughout this research.
- The staff of Veterinary Research Institute; especially Dr. Maria Jamli (head of Bacteriology Department), Mr. Ibrahim, Mr. Shuhaili, Mr.

Roszeman, Mr. Adnan, Mr. Aziz for the guidance and help during my stay in the institute.

- The farmers involved in this research for their cooperation during the study in their farms.

Most of all, to my dear parents, brothers and my best friend Norasmah Basari for their never-ending love, moral support and help throughout this research. Thank you very much!

いつもありがとうございます！

TABLE OF CONTENTS

4.0 RESULTS

5.0 DISCUSSION

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF PUBLICATIONS & SEMINARS

TINJAUAN KERINTANGAN UBAT CACING BERKAITAN DENGAN PENGURUSAN RUMINAN KECIL DI SEMENANJUNG MALAYSIA

ABSTRAK

Ujian Pengurangan Kiraan Telur Cacing (FECRT) telah dijalankan terhadap 5 ladang ruminan kecil milik kerajaan dan 21 ladang ruminan kecil milik persendirian di Semenanjung Malaysia untuk menilai status kerintangan cacing terhadap empat jenis ubat cacing iaitu oksfendazol, moksidektin, levamisol dan klosantel. Didapati bahawa kesemua 5 ladang milik kerajaan mempunyai populasi cacing yang rintang terhadap semua ubat cacing yang diuji. Larva infektif yang telah diperolehi daripada kultur tinja di ladang milik kerajaan menunjukkan bahawa *Haemonchus contortus* merupakan cacing utama dalam bebiri dan kambing dengan 78%, diikuti *Trichostrongylus* spp. (22%). Bagi ladang milik persendirian, didapati bahawa populasi cacing di kesemua ladang (100%) rintang terhadap klosantel, 14 ladang (70%) rintang terhadap oksfendazol, 11 ladang (52%) rintang terhadap levamisol dan 6 ladang (29%) rintang terhadap moksidektin. Larva infektif yang telah diperolehi daripada kultur tinja menunjukkan bahawa *Haemonchus contortus* merupakan cacing gastrointestinal utama yang menjangkiti haiwan di ladang-ladang tersebut dengan 62%, diikuti *Trichostrongylus* spp. (24%), *Bunostomum* spp. (8%), *Oesophagostomum* spp. (3%) dan *Cooperia* spp. (3%). Berdasarkan keputusan ini, jelaslah masalah kerintangan terhadap ubat cacing semakin meruncing dan kaedah lain untuk pengawalan cacing adalah diperlukan untuk mengurangkan masalah ini.

A SURVEY OF ANTHELMINTIC RESISTANCE IN RELATION TO MANAGEMENT OF SMALL RUMINANTS IN PENINSULAR MALAYSIA

ABSTRACT

Nematode faecal egg count reduction tests (FECRT) was conducted on 5 government owned and 21 privately-owned small ruminant farms located in Peninsular Malaysia to evaluate the status of resistance to types of anthelmintics i.e. oxfendazole, moxidectin, levamisole and closantel. It was found that nematode populations on all the government farms (100%) showed resistance to all anthelmintics tested. Third stage infective larvae collected from faecal cultures of the farms showed *H. contortus* as the major gastrointestinal nematode in the animals with 78%, followed by *Trichostrongylus* spp. (22%). As for the private farms, it was found that nematode population on all 21 farms (100%) showed resistance to closantel, 14 farms (70%) showed resistance to oxfendazole, 11 farms (52%) showed resistance to levamisole and 6 farms (29%) showed resistance to moxidectin. Third stage infective larvae collected from faecal cultures from the farms showed *Haemonchus contortus* as the major gastrointestinal nematodes in the animals with 62%, followed by *Trichostrongylus* spp. (24%), *Bunostomum* spp. (8%), *Oesophagostomum* spp. (3%) and *Cooperia* spp. (3%). From all these findings, it is clear that anthelmintic resistance in Malaysia is escalating and alternative methods of worm control are needed to alleviate this problem.

1.0 INTRODUCTION

The 2004 figures from the Department of Veterinary Services, Malaysia recorded the total number of sheep and goat populations as 109,511 and 225,520 respectively (Department of Veterinary Services, http://agrolink.moa.my/jph).

These numbers, which constitute the small ruminant industry of Malaysia, is an important component to the overall livestock sector for the country. Furthermore, the objectives of the government in the National Agriculture Policy (NAP3) are: to increase small ruminant production through the importation of exotic breeds of sheep and goats, increasing the number of female animals and conducting intensive research in small ruminants and their diseases (Department of Veterinary Services, http://agrolink.moa.my/jph).

Infestation by parasitic worms (helminthiasis) in small ruminants has been identified as one of the major limiting factors of animal productivity in farming enterprises which rely on grazing animals on pasture (Waller, 1985). Trichostrongyle worms, from the superfamily of Trichostrongyloidea are responsible for considerable mortality and widespread morbidity. The most important genera in Trichostrongyles are *Ostertagia, Haemonchus, Trichostrongylus, Cooperia, Nematodirus, Hyostrongylus, Marshallagia* and *Mecistocirrus* (Urquhart *et al.*, 1996).

Humid climate throughout the year in Malaysia is very favourable for the development of free living stages of trichostrongyle nematodes and it is likely that all times of the year, infective larvae are available on the pasture for grazing animals (Ikeme *et al.*, 1987).

With this in mind, farmers depend heavily on anthelmintic treatments to control helminthiasis problem. Benzimidazoles, Imidothiazoles, Tetrahydropyrimidines, Avermectins and Salicylanilides (Bogan and Armour, 1987) are examples of anthelmintic groups that are available in the market – to be chosen by farmers or veterinarians to treat animals with helminthiasis.

Unfortunately, irresponsible usage of the anthelmintics (not used in a recommended manner, being often overused, misused or applied incorrectly) has led to the development of resistance nematode against anthelmintics in small ruminants (Waller, 2006).

Anthelmintic resistance is a major problem faced by livestock farmers throughout the world. Countries such as South Africa, Australia and Uruguay reported million dollars of annual losses due to helminthiasis (Waller, 2006). As anthelmintic resistance will result in failure of anthelmintics to control helminth, the losses are expected to increase.

Based on this, a lot of interests are shown by researchers, mainly veterinarians and parasitologists, to study this problem and explore the possible alternative measures to control the problem.

Thus, the objective of this study is to report on;

1. The prevalence of anthelmintic resistance in government and also private small ruminant farms in Peninsular Malaysia.

2. The management of animals in government and private small ruminant farms in Peninsular Malaysia.

3. The prevalent species of nematode existing in government and private small ruminant farms in Peninsular Malaysia.

2.0 LITERATURE REVIEW

2.1 Helminth infection in small ruminants

Helminth infection causes problems and financial losses to the livestock industry (Waller, 2006) as it is one of the most prominent causes of mortality and morbidity in Malaysia (Fatimah *et al*., 1985, Sani and Rajamanickam, 1990, Sani *et al.,* 2004). This is due to the grazing activities of the livestock in pastures contaminated with third stage infective larvae of parasitic nematodes. Fatimah *et al.* (1985) reported that parasitic gastroenteritis was the main cause of mortality in Dorset Horn Sheep hogs while for the adults, parasitic gastroenteritis was one of the causes of mortality besides pneumonia, bacterial enteritis, trauma and septicaemia. Sani *et al.* (1985) reported that from 72 goats necropsied in Universiti Putra Malaysia, *Haemonchus contortus* was found to be the major helminth found (67%), followed by *Moniezia expansa* (51%), *Oesophagostomum columbianum* (42%) and *Trichostrongylus colubriformis* (38%). Work done by Dorny *et al.* (1995) on sheep and goats from three farms in Peninsular Malaysia showed that *H. contortus* and *Trichostrongylus* spp. were the most important strongyles in sheep and goats.

Helminth infection (helminthiasis or helminthiasis) is mainly caused by trichostrongyle nematodes such as *H. contortus*, *Oesophagostomum* spp., *Cooperia* spp. and *Trichostrongylus* spp. The parasite that causes the most problems to small ruminants is the bloodsucker nematode; *H. contortus*, better known as the "barber’s pole" worm, which is generally considered as the most pathogenic parasite of small ruminants (Soulsby, 1982). This parasite is a problem to small ruminants, not only in countries with warm and wet climates such as Malaysia but also in temperate countries such as Sweden (Waller and Chandrawathani, 2005).

2.1.1 *Haemonchus contortus*

This blood-sucking abomasal nematode is responsible for the extensive productivity losses in sheep and cattle industry, especially in tropical areas. The nematode can be found worldwide, but it is most important in tropical and subtropical areas (Urquhart *et al.*, 1996). The infective larvae of *H. contortus* can survive dessication as the second stage cuticle becomes less permeable as it dries (Ellenby, 1968).

The eggs of *Haemonchus* spp. hatch to first stage larvae (L_1) on the pasture and may develop to third stage infective larvae (L_3) in a period of five days but development may be delayed for weeks or months under cool conditions (Urquhart *et al.*, 1996). Four days after infection, all the worms are at the fourth stage larvae (L_4) and the third stage larvae (L_3) are totally absent. The worms spent between 7 to 11 days after infection in the mucosa before emerging, as late L_4 larvae and establishing themselves in the lumen (Rahman and Collins, 1990a).

The late L_4 larvae developed piercing lancet before the final moulting which enables them to obtain blood from the mucosal vessels. As adults, they move freely on the surface of the mucosa. The prepatent period is 2-3 weeks in sheep and four weeks in cattle (Urquhart *et al.*, 1996).

The main feature of *Haemonchus* spp. infection is anaemia as both the adult and the fourth larval stage (L_4) sucks blood, moves and leaves wounds which haemorrhage into the abomasum. Infection by an extremely large number of *Haemonchus* spp. results in a rapidly developing severe anaemia, dark-coloured faeces and sudden death due to acute blood loss. There is also a severe haemorrhagic gastritis and death may occur in the prepatent period of such heavy infections (Soulsby, 1982).

When young susceptible animals become heavily infected, the anaemia may develop rapidly, accompanied by hypoproteinaemia and oedema (i.e. bottle jaw) and death will occur. The most common clinical signs in infected animals are weakness, unthriftiness and emaciation (Soulsby, 1982).

Rahman and Collins (1991) reported that infections caused by goat-derived strain of *H. contortus* in goats caused greater weight loss compared to infections by sheep–derived strain. The study also reported that the reduction in packed cell volume (PCV) values and haemoglobin concentration was significant at day 14-28 after infection. This coincides with worm maturation and active feeding of the worms at that particular period (Rahman and Collins, 1990a).

It was reported that each worm removes about 0.05 ml of blood per day by ingestion and leakage from the lesions (Urquhart *et al.*, 1996). Thus, an amount of 5000 larvae of *H. contortus* can cause death in goats (Rahman and Collins, 1991) with an estimation of 250 ml blood loss daily (Urquhart *et al.*, 1996).

2.1.2 *Oesophagostomum* spp.

Oesophagostomum spp. are parasites in the small and large intestines of cattle, sheep, pigs and primates. These nematodes are often referred to as nodular worms, owing to the fact that several species cause nodule formation on the wall of the intestine (Soulsby, 1982). The prepatent period is about 45 days (Urquhart *et al.*, 1996).

Oesophagostomum columbianum is a serious pathogen of sheep where extensive nodular formation seriously interferes with absorption, bowel movement and digestion. The nodules may rupture on the peritoneal surface, causing peritonitis and multiple adhesions. Though the adult worms do not suck blood as in *H. contortus*, they can cause marked thickenings of the bowel wall, cause massive congestion and produce large amount of mucous. In infected lamb, the first sign of infection is marked by persistent diarrhoea, which results in exhaustion and death. This diarrhoea begins on the sixth day after a severe infection and coincides with the time when the larvae leave the nodules (Soulsby, 1982).

2.1.3 *Bunostomum* spp.

Bunostomum trigonocephalum is a hookworm which occurs in the small intestine (ileum and jejunum) of sheep and goats in many parts of the world. The parasite is more important in warmer climates like Malaysia than colder climates. Infection usually occurs along with other gastrointestinal strongyles and the hookworms contribute to the general effects of parasitism (Soulsby, 1982). The adult worms attach themselves to the intestinal mucosa and suck blood. The main clinical signs when substantial number of hookworms occurs are progressive anaemia, hydraemia and oedema especially in the intermandibular region (i.e. bottle jaw). Diarrhoea is infrequent and the faeces may be dark in colour due to the presence of altered blood pigments (Soulsby, 1982).

2.1.4 *Cooperia* spp.

In temperate areas, members of the genus *Cooperia* usually play a secondary role in the pathogenesis of parasitic gastroenteritis of ruminants, although they may be the most numerous trichostrongyle present. However, in some tropical and subtropical areas, *Cooperia* spp. are responsible for severe enteritis in calves (Urquhart *et al.*, 1996).

The adult worms penetrate the mucosa of the small intestine. There is no consequence from a light infection of the worms, but young cattle and sheep may be affected by heavy infections, which are usually acquired from moist pastures (Soulsby, 1982).

Loss of appetite, poor weight gains, diarrhoea, severe weight loss and submandibular oedema are the common clinical signs for animals infected with *Cooperia* (Urquhart *et al.*, 1996).

2.1.5 *Trichostrongylus* spp.

Trichostrongylus spp. is rarely a primary pathogen in temperate areas, but is usually a component of parasitic gastroenteritis in ruminants. By contrast, in the sub-tropics, it is one of the most important causes of parasitic gastroenteritis (Urquhart *et al.*, 1996).

It was reported that all free-living stages of *T. colubriformis*, with the exception of the L_1 larvae survived exposure to freezing temperatures in contact with water. The L_3 larvae was found to be the most resistant stage. (Wharton and Allan, 1989).

The exsheathment of the L_3 larvae occurs in the abomasum. Under optimal conditions, development from egg to the infective stage (L_3) occurs on 1-2 weeks. In ruminants, the interval between infection of the larvae and the first ability to detect the diagnostic stage from that host (prepatent period) is within 2-3 weeks (Urquhart *et al.*, 1996). *Trichostrongylus colubriformis* was found to stay in the mucosa of goats until about 11 days after infection, and the immature adults emerge and mature in the lumen (Rahman and Collins, 1990b). Twenty one days after infection, it was found that about 57% of the female worms had eggs in their uteri and around this time the eggs were first detected in the faeces (Rahman and Collins, 1990b).

In sheep and goats, young animals are susceptible to the infection of this worm. When a severe infection is acquired within a short time, the disease may be acute and may rapidly lead to death. Animals usually do not show emaciation or anaemia, but they become weak in the legs and are unable to stand shortly before they die. Mild anaemia, emaciation, alternating constipation and diarrhoea are visible in chronic cases (Soulsby, 1982).

2.2 Anthelmintics for helminth control

As helminth infections cause serious problems to small ruminants and sometimes death, farmers depend on anthelmintics to control the infections. A wide variety of anthelmintics from various chemical groups, are used for the treatment of helminth parasites. These chemical groups include: the benzimidazoles, imidothiazoles (levamisole), salicylanilides (closantel) and macrocyclic lactones (ivermectin) (Chandrawathani *et al*., 1994).

According to Behm and Bryant (1985) anthelmintics can be divided into four major categories according to their mode of action:

1. drugs that interfere with neurophysiology or neuromuscular coordination and exploit the differences in neurophysiology between host and parasite (e.g. ivermectin and levamisole),

2. drugs that interfere with essential energy metabolism (e.g. salicylanilides)

3. drugs that interfere with essential biosynthetic pathways and

4. drugs that interfere with essential cellular processes (e.g. substitutes of benzimidazole).

2.2.1 Benzimidazole anthelmintics

All benzimidazole anthelmintics remove most of the adult parasites which include *Bunostomum* spp., *Chabertia* spp., *Cooperia* spp., *Haemonchus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Ostertagia* spp., *Strongyloides* spp. and *Trichostrongylus* spp. (Brander *et al.,* 1982).

The benzimidazole anthelmintics bind to the nematode tubulin, thus inhibiting the formation of microtubules. Subsequently, the nematode is unable to transport secretory granules or secrete enzymes within the cell cytoplasm.

This eventually results in cell lysis (Lacey, 1988). Prichard *et al.* (1978) suggested that the benzimidazole anthelmintics; oxfendazole, cambendazole, fenbendazole and thiabendazole affect parasitic helminths in a similar manner, i.e. inhibit the fumarate stimulated oxidation of NADH in parasite's mitochondria. Figure 2.1 shows the chemical structure of oxfendazole.

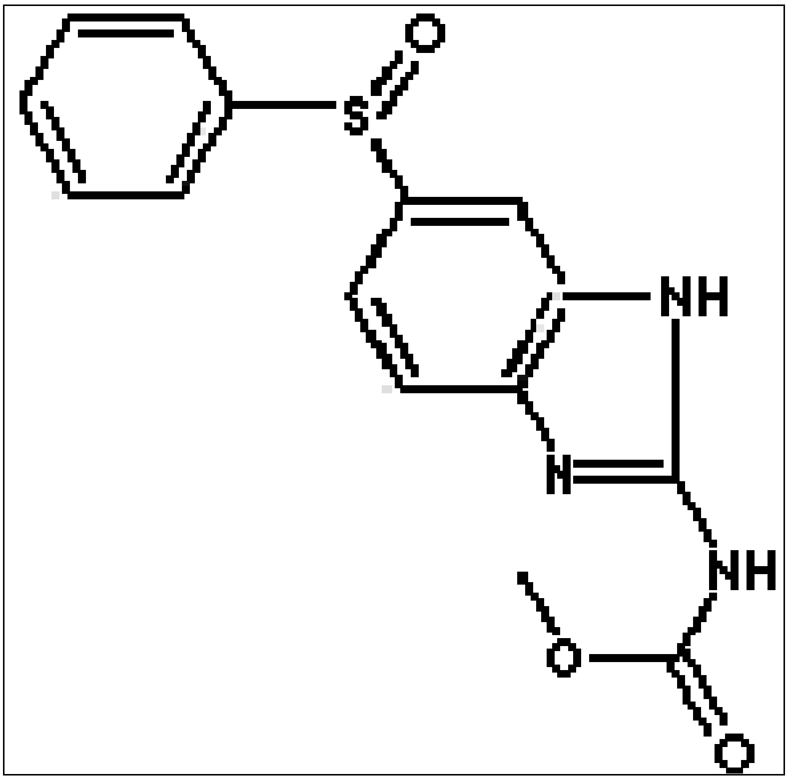

Figure 2.1: Chemical structure of oxfendazole

2.2.2 Imidazothiazole anthelmintics

Levamisole, the major drug used in this group is a broad spectrum anthelmintic and is effective against the adult and larval stages of *Haemonchus* spp., *Ostertagia* spp., *Trichostrongylus* spp., *Cooperia* spp., *Nematodirus* spp., *Bunostomum* spp., *Oesophagostomum* spp., *Metastrongylus* spp., *Ascaris* spp., and *Trichuris* spp., and the lungworm *Dictyocaulus* spp. This anthelmintic has been used against both artificial and natural infections, and has the advantage of being active at a low dosage. Because of its activity against the larval stage, it has widened the whole field of the strategic dosing of cattle and sheep to prevent larval infestation. It is also active against benzimidazole-resistant *H. contortus* and *T. colubriformis* (Brander *et al.,* 1982).

Low levels of the levamisole anthelmintic act as ganglion stimulants and give rise to muscular paralysis in parasites, whereas higher levels interfere with carbohydrate metabolism. The blockage occurs at the site of fumarate reduction and succinate oxidation (Brander *et al.,* 1982). Figure 2.2 shows the chemical structure of levamisole.

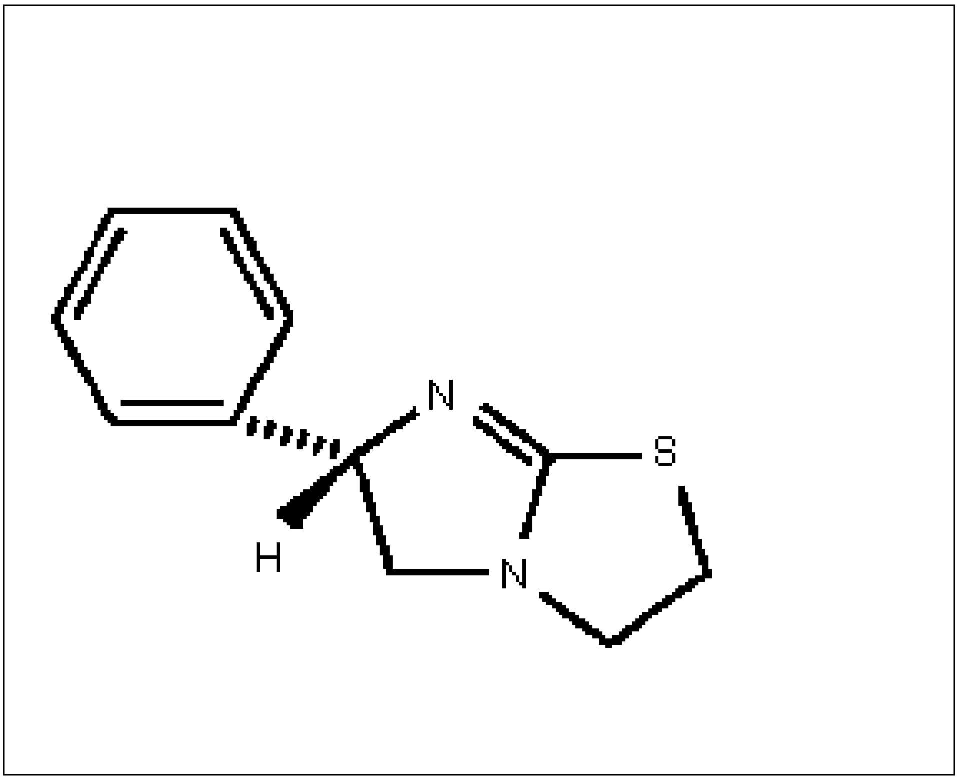

Figure 2.2: Chemical structure of levamisole

2.2.3 Macrocyclic lactone anthelmintics

Anthelmintics in this group gate the opening of glutamate gated chloride channels found only in invertebrates (Martin *et al.,* 1997) and cause inhibition of the motility and development of the free living stages of trichostrongylid nematode parasites (Le Jambre, 1996). Macrocyclic lactone anthelmintics are used for the treatment and control of gastrointestinal nematodes, lungworms, warbles, mange and sucking lice, and has been claimed to be effective against nematodes and external parasites (Brander *et al.,* 1982).

Moxidectin (a member of macrocyclic lactone anthelmintics) was found to be 100% effective in eliminating trichostrongyle egg counts by day 3 of treatment and the egg counts remained negative until 31 days post treatment (Miller *et al.*, 1994). Besides that, it was found that horn flies (*Haematobina irritants*) feeding on the blood of moxidectin-treated cattle drawn on day 21 of daily treatment, showed a decline in survival and egg production (Miller *et al.*, 1994). Moxidectin was also found to have larvacidal activity against the immature stages of the horn fly in the manure of treated cattle (Miller *et al.*, 1994).

Initial studies carried out on a government sheep farm in Malaysia showed that moxidectin was 100% efficacious against sheep infected with benzimidazole-resistant nematodes for up to 6 weeks. As an alternative to resistant anthelmintics, this drug was suggested to be used for the control of helminthiasis in sheep (Chandrawathani *et al.*, 1998). Figure 2.3 shows the chemical structure of oxfendazole.

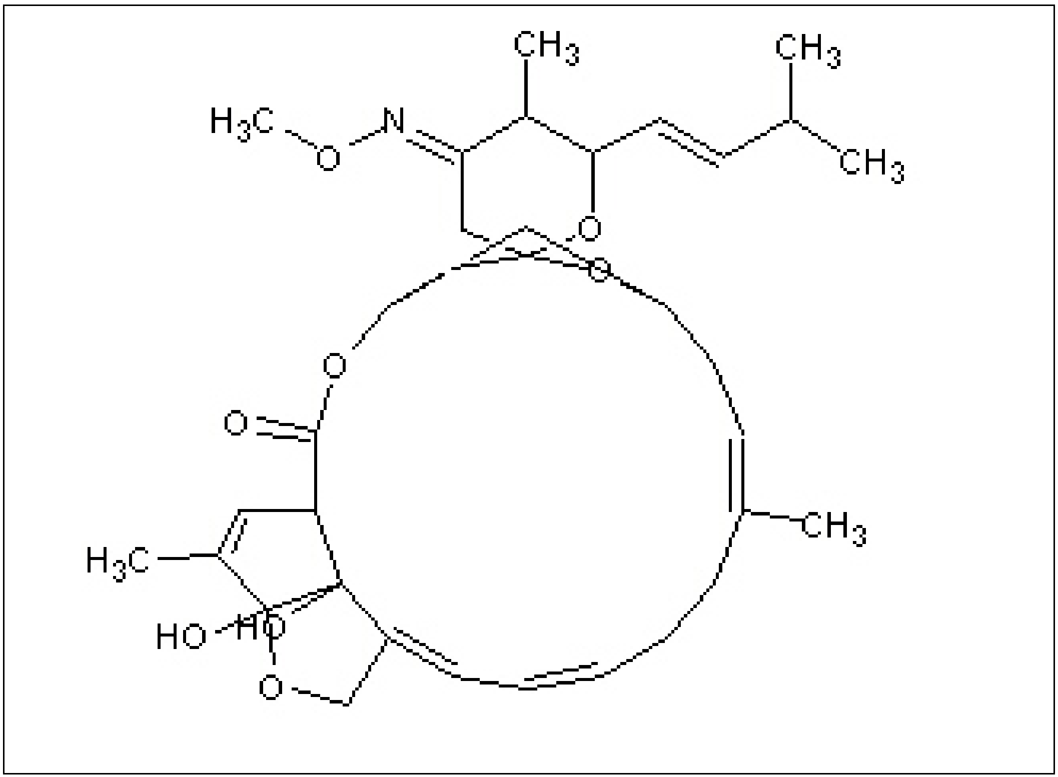

Figure 2.3: Chemical structure of moxidectin

2.2.4 Salicylanilides anthelmintics

Closantel, one of the substituted salicylanilides is a narrow spectrum, long-acting anthelmintic with activity against *H. contortus*, *Fasciola hepatica* and *Oestrus ovis*. Closantel binds to plasma protein and is toxic only to parasites that ingest and digest blood or dwell in the bile ducts (Behm and Bryant, 1985). Closantel is believed to uncouple oxidative phosphorylation and caused poisoning in the mitochondria (Martin *et al.,* 1997). However, Rothwell and Sangster (1996) suggested that closantel is a molecule with the ability to affect a number of membrane-associated processes including ion and fluid transport, and the uncoupling of oxidative phosphorylation is secondary to such a membrane effect.

This drug has been used widely for *H. contortus* control as a result of the widespread occurrence of parasite resistance to broad spectrum anthelmintics such as benzimidazoles. Figure 2.4 shows the chemical structure of closantel.

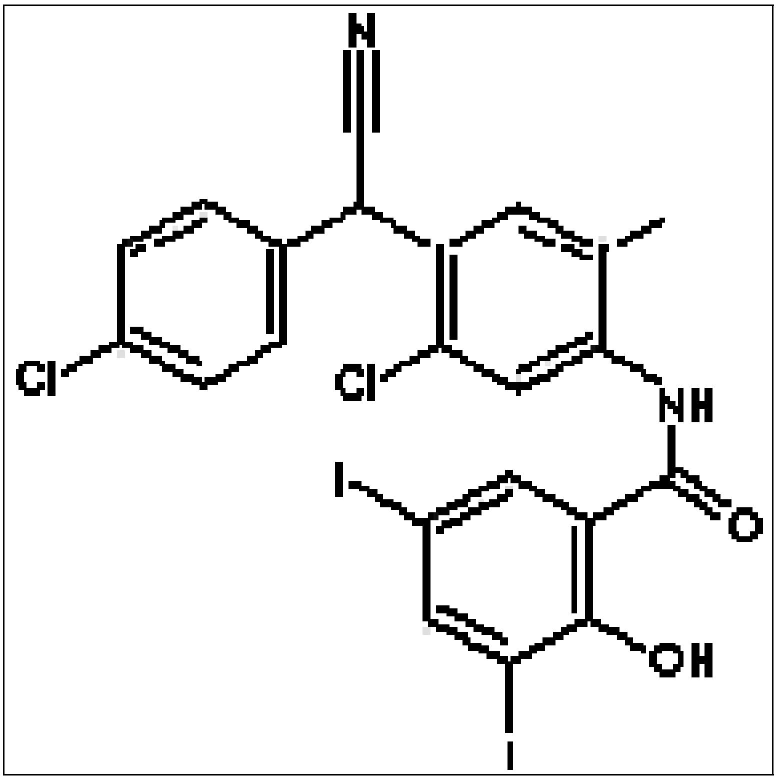

Figure 2.4: Chemical structure of closantel

2.3 Anthelmintic resistance

As defined by Prichard *et al.* (1980), anthelmintic resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species. Evolutionary theory explains the appearance of a resistant strain by proposing that the original population of worms contained a few rare individuals that possessed the ability to survive the anthelmintic. Since anthelmintics kill all susceptible worms, the next generation will consist of offsprings of the resistant minority (Le Jambre, 1985). Resistance is a heritable character (Prichard *et al.*, 1980). Therefore, many of these worms inherited their parent's ability to survive the drugs. If the character that provides resistance is controlled by a single major gene, then the resistant population will build up very rapidly (Le Jambre, 1985).

Prichard (1994) reviewed anthelmintic resistance to levamisole, benzimidazoles and ivermectin. Levamisole resistance appears to be associated with alterations in cholinergic receptors in resistant nematodes. On the other hand, benzimidazoles resistance in nematodes appear to be associated with an alteration in ß-tubulin genes which reduces or abolishes the high affinity binding of benzimidazoles for tubulin in these organisms. Alterations in the glutamate/ivermectin chloride channel receptor in nematodes were suggested as the mechanism of ivermectin resistance.

2.4 Anthelmintic resistance in small ruminants

Anthelmintic resistance is a problem for livestock industries especially in countries with hot and humid climates, an ideal condition for the development and survival of free-living stages of nematode parasites on pasture.

In South America, more than 70% of the total sheep population is found in Argentina, Brazil, Paraguay and Uruguay. Within these countries is the sub-tropical humid zone which encompasses Northern Argentina, Southern Brazil and all of Paraguay and Uruguay. The environment is ideal for parasite development and transmission and this may occur more or less continuously throughout the year. The 45 million sheep in this region are therefore exposed to frequent use of anthelmintic, resulting in anthelmintic resistance in parasite populations of the sheep (Waller *et al.*, 1996). Many reports were published on the prevalence of anthelmintic resistance in Argentina, Brazil, Paraguay and Uruguay (Eddi *et al.*, 1996, Echevarria *et al.*, 1996, Maciel *et al.*, 1996, Nari *et al.*, 1996).

According to Eddie *et al.* (1996), from 65 sheep farms in Argentina, it was found that 40% of the farms showed resistance to benzimidazoles, 22% to levamisole, 11% to the combination anthelmintics (benzimidazole and levamisole) and 6% to ivermectin. A similar situation was also observed in Brazil where 90% of 182 sheep farms selected showed resistance to albendazole, 84% to levamisole, 73% to the combination anthelmintics (benzimidazole and levamisole) and 13% to ivermectin (Echevarria *et al.*, 1996).

According to them, the results demonstrated that the parasite control in this country is rapidly reaching a state of crisis (Echevarria *et al.*, 1996)

The level of anthelmintic resistance in sheep in Paraguay has also reached a crisis situation. A report in 1996 showed that the levels of resistance were 73% to benzimidazoles, 68% to levamisole, 73% to oral ivermectin and 47% to injectable ivermectin (Maciel *et al.*, 1996).

A study conducted on 252 sheep farms in Uruguay showed that 86% of the sheep flocks were resistant to benzimidazoles, 71% to levamisole and 1.2% to ivermectin (Nari *et al.*, 1996).

In 1993, it was reported that albendazole and oxfendazole caused a very low reduction in faecal egg counts after treatment in a goat farm in Malaysia, indicating the failure of both anthelmintics. Highly benzimidazole-resistant strain of *H. contortus* was isolated from that farm. This was the first resistance to Benzimidazole in *H. contortus* reported in Malaysia (Dorny *et al.*, 1993).

Rahman (1993) reported thiabendazole resistance cases in 19% of 48 goat farms in northern Peninsular Malaysia. Later in 1994, he also reported *H. contortus* resistance to fenbendazole in West Malaysia. In addition, he also reported that levamisole was highly effective against trichostrongyle nematodes in goats and in some farms, there was total removal of the worms (Rahman, 1994a). In another study, Rahman (1994b) reported that levamisole significantly reduced the mean worm burdens compared to reductions in animals treated with albendazole, fenbendazole, oxfendazole and mebendazole.

The first benzimidazole resistance in *H. contortus* in sheep from Southeast Asia was reported by Pandey and Sivaraj (1994) showing high nematode resistance albendazole, oxfendazole and fenbendazole in 4 sheep farms in Malaysia. In this study, no resistance to closantel and levamisole was observed (Pandey and Sivaraj, 1994).

A first case of multiple anthelmintic resistance in a sheep farm in Malaysia was reported by Sivaraj *et al.* in 1994. The study demonstrated simultaneous resistance of *H. contortus* against benzimidazoles and levamisole and of *T. colubriformis* against benzimidazoles and levamisole in the same farm. It was also reported that moxidectin was effective against the ivermectin resistant *H. contortus* (Sivaraj *et al.*, 1994).

With the expectation that the anthelmintic resistance status was developing rapidly on sheep and goat farms in Malaysia, a nationwide anthelmintic resistance survey involving 48 farms, was conducted in the 1990's. This survey reported the presence of nematode resistance to benzimidazole in 16 farms, levamisole in 7 farms, closantel in 1 farm, ivermectin in 1 farm and also resistance to the combination of levamisole and closantel in 1 farm (Chandrawathani *et al.,* 1999).

Since then, further studies of the anthelmintic resistance status on government small ruminant breeding farms have showed high levels of multiple resistance in one government sheep farm in Peninsular Malaysia (Chandrawathani *et al.*, 2003) and total anthelmintic failure of all five commercially available anthelmintic drug classes in five small ruminant government farms in Sabah, East Malaysia (Chandrawathani *et al.*, 2004).

Since government small ruminant farms in Malaysia distribute their animals to small-holder farmers throughout the country, the severity of this problem is likely to have been widely dispersed.

3.0 MATERIALS AND METHODS

3.1 Experimental animals

Faecal samples from 100 small ruminant farms in Peninsular Malaysia were collected from October 2004 to August 2006. Faecal egg counts were estimated using the modified McMaster method (Ministry of Agriculture, Food and Fisheries, 1986).

Faecal Egg Count Reduction Test (FECRT) was also carried out but they were confined to farms which meet the following criteria:

1. The farm had more than 30 animals of age, ranging from 6 months to 3 years.

2. When screening for nematode faecal egg counts, at least 10% of the animals showed mean counts exceeding 120 eggs per gram (e.p.g.) faeces.

3. The animals were generally healthy and in good condition based on gross clinical examination.

Twenty six farms were selected, of which 15 were exclusively goat farms, 9 sheep and two mixed. They were randomly distributed in the states of Perak (14 farms), Kelantan (2 farms), Terengganu (2 farms), Johor (2 farms), Penang (1 farm), Kedah (1 farm), Selangor (1 farm), Pahang (1 farm), Negeri Sembilan (1 farm) and Melaka (1 farm) (Figure 3.1).