Problems in the Control of Nematode Parasites of Small Ruminants in Malaysia: Resistance to Anthelmintics and the Biological Control Alternative

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Abstract

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Nematode parasitic disease, attributed mainly *Haemonchus contortus,* is the greatest disease problem of small ruminants in Malaysia*.* This thesis comprises 6 studies on the emergence of anthelmintic resistance in these parasites, and how control may be managed by exploiting the nematophagous fungus *Duddingtonia flagrans.*

A survey of the prevalence of anthelmintic resistance (AR) on Peninsular Malaysia revealed 50% of the sheep (n=39) and 75 % of the goat farms (n=9) were infected with nematode parasites that were resistant to the benzimidazoles. Resistance to levamisole, closantel and ivermectin was also detected. Another study on a government sheep breeding farm, showed multiple AR involving the benzimidazoles, levamisole and ivermectin (plus suspect moxidectin resistance). Continuing these investigations to eastern Malaysia, showed anthelmintic failure to all the broad-spectrum groups on all the government small ruminant breeding farms. New approaches to worm control were urgently required.

Studies on the potential of biological control by the use of nematophagous fungi against the free-living stages on pasture, were carried out to assess its suitability and efficacy for use in the tropical environment that is typical for Malaysia. In a survey for the presence of nematode trapping fungi from faecal samples of livestock, several nematophagous species including *D. flagrans* and *Arthrobotrys oligospora* were identified. Mass production of *D. flagrans* spores on various local media was attempted, and the product was also tested in pen trials where sheep were fed the spores at different dose rates, either as a supplement, or in feed blocks. Daily fungal feeding by both means resulted in an 80-95% reduction in infective larvae in faecal cultures. Similar results were found in small scale grazing experiments, where sheep were fed daily with fungal spores for 3 consecutive months. Pen trials comparing goats and sheep, showed no difference between the two livestock species. Larger scale field trials where *D. flagrans* was combined with rapid rotational grazing, showed excellent parasite control. This indicates that the integration of biological control with measures such as rotational grazing provides viable options for sustainable production of small ruminants in the tropics, where AR is becoming a major threat to livestock production.

Keywords: nematode parasites, sheep, goats, anthelmintic resistance, biological control, *Duddingtonia flagrans*

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Contents

Appendix

Papers I – VI

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Chandrawathani, P., Adnan, M. & Waller, P.J. 1999. Anthelmintic resistance in sheep and goat farms on peninsula Malaysia. *Veterinary Parasitology 82*, 305-310.

II. Chandrawathani, P., Waller, P.J., Adnan, M. & Höglund, J. 2003. Evolution of high level, multiple anthelmintic resistance on a sheep farm in Malaysia. *Tropical Animal Health and Production 35*, 17-25.

III. Chandrawathani, P., Yussof, N. & Waller, P.J. 2004. Total anthelmintic failure to control nematode parasites of small ruminants on government breeding farms in Sabah, east Malaysia. *Veterinary Research Communications,* (in press).

IV. Chandrawathani, P., Jamnah, O., Waller, P.J., Höglund, J., Larsen, M. & Zahari, W.M. 2002. Nematophagous fungi as a biological control agent for nematode parasites in Malaysia: a special emphasis on *Duddingtonia flagrans*. *Veterinary Research 33*, 685-696.

V. Chandrawathani, P., Jamnah, O., Waller, P.J., Larsen, M., Gillespie, A. & Zahari, W.M. 2003. Biological control of nematode parasites of small ruminants in Malaysia using the nematophagous fungus *Duddingtonia flagrans. Veterinary Parasitology 117*, 173-183.

VI. Chandrawathani, P., Jamnah, O., Adnan, M., Waller, P.J., Larsen, M. & Gillespie, A.T. 2004. Field studies on the biological control of nematode parasites of sheep in the tropics, using the microfungus *Duddingtonia flagrans*. *Veterinary Parasitology 120*, 177-187.

Approval has been obtained from each of the respective scientific journals for the inclusion of these manuscripts in this thesis.

Abbreviations

ACIAR	Australian Centre for International Agricultural Research
BC	Biological control
B. decumbens	*Brachiaria decumbens*
B. humidicola	*Brachiaria humidicola*
D. flagrans	*Duddingtonia flagrans*
DVS	Department of Veterinary Services in Malaysia
EL4	Early fourth stage larvae
EPG	Trichostrongyle nematode egg per gram in faeces
ES	Excretory / Secretory Proteins in helminths
FAMACHA	Fafa Malan Chart for anaemia determination
FAO	Food and Agriculture Organisation of the United Nations
FECRT	Faecal egg count reduction test
GDP	Gross Domestic product
ha	hectares
H. contortus	*Haemonchus contortus*
IgE	Immunoglobulin E
KDa	Kilo Dalton
L3	Third stage infective larvae
LDA	Larval development assay
MT	Metric Tonne
NAP	National Agriculture Policy
PCV	Packed Cell Volume
PFF	Palm Fibre Fines
PKC	Palm Kernal Cake
POME	Palm Oil Mill Effluent
RTM	Radio Television Malaysia
SSL	Self Sufficiency in Livestock
S. papillosus	*Strongyloides papillosus*
T. colubriformis	*Trichostrongylus colubriformis*
VEP	Veterinary Extension Programme
VRI	Veterinary Research Institute, Ipoh

Introduction

Malaysia consists of two parts; namely West Malaysia, or Peninsular Malaysia (131,313 sq km), and East Malaysia (201,320 sq km), which are separated by 640km by the South China Sea. West Malaysia is bordered on the north by Thailand, on the east by the South China Sea, on the south by Singapore and on the west by the Straits of Malacca and the Andaman Sea. East Malaysia is bordered on the north by the South China Sea and the Sulu Sea, on the east by the Celebes Sea and on the south and west by Indonesian Borneo (Kalimantan). Both East and West Malaysia have mountainous interiors and coastal plains, with over two thirds of the land area being forested (see Figure 1).

Figure 1: Map of Malaysia

Lying close to the equator (between 1° - 6°N and 100° - 104°E), the country has a tropical rainy climate. The climate is governed by the north-east and south-west monsoon winds which blow alternately during the course of the year. This made the country a natural meeting and exchange point for traders from the east and west in the days of the sailing ships. The north-east monsoon blows from mid November until March and the south-west monsoon between May and September, the periods of change between the two monsoons being marked by heavy rainfall. The period of the south-west monsoon is drier for the whole country as it is sheltered by Sumatra.

The average temperature throughout the year is constantly high (26°C) and the diurnal temperature range is about 7°C, with the humidity also being high (average 80%). This in turn, produces high rates of evaporation and as a consequence frequent heavy thunderstorms, with rainfall exceeding 2500mm per year. Sea breezes modify the temperature in the coastal regions, resulting in a more equable climate.

The natural vegetation of Malaysia is essentially natural tropical forest, consisting of mangrove forests in the muddy estuaries, which yield to the lowland dry forests of predominantly the timber species (Dipterocarpaceae), which are comparatively gloomy due to the thick overhead canopy. The hilly mountain vegetation consists primarily of Malaysian oaks (*e.g. Enterolobium saman, Pterocarpus indicus, Delonix regia* and *Peltophorum ferrugineum*), ferns and epiphytes. Despite the opening up of land for oil palm and rubber plantation schemes, over 50% of the country is still under forest.

The population of Malaysia is approximately 18 million, of which 80% live on Peninsular Malaysia. Of this, 60% are Malays, 25% Chinese and 10% Indians. The other 5% are the indigenous peoples such as Ibans, Dayaks and Kadazans. Historically, Malaysia has had Portuguese (15th Century), Dutch (17th Century) and English (19th Century) influences in shaping its economy, agriculture and modernisation. Malaysia achieved independence from the English in 1957, by which time the rubber and oil palm industries, as well as tin mining activities, were already well established. Over the past 50 years, Malaysia has become an important producer of rubber, tin, palm oil, crude petroleum and petroleum products, electronics, logging and textiles.

Subsistence agriculture remains the basis of livelihood for about 20% of Malaysians and agriculture provides about 15% of the gross domestic product (GDP). Rice is the staple food while fish supplies most of the protein in the human diet. Malaysia has one of the highest standards of living in south-east Asia, largely because of its expanding industrial sector which propelled the country to an 8 - 9% yearly growth rate from 1987-1997. The major cities of Peninsular Malaysia are connected by rail and an extensive network of roads, which have further contributed to is development.

Agriculture plays an important part in the overall economic development of the country and is geared towards the production of export commodities, such as natural rubber and palm oil as well as sawn timber, cocoa, pepper, pineapple and tobacco. As Malaysia is the world's primary producer of natural rubber and oil palm, the total area under rubber cultivation is 1.8 million ha and oil palm cultivation accounts for another 2.6 million ha, as of the year 2000.

The large acreage under oil palm and rubber plantation mooted the idea for the expansion of livestock industries such as cattle, sheep and goats in the 1970's. As a result, large consignments of livestock were imported from neighbouring Asian countries as well as from Australia, New Zealand, Europe and South America for breeding and increasing the ruminant livestock population. This exercise proved to be very useful in that the Department of Veterinary Services (DVS) was involved with the health management of these animals thereby highlighting several key factors in the management of large herds of ruminants in the tropics. One such factor which determined the success of the various imported breeds to adapt to local climate and management conditions was the ability to withstand helminth infections, which together with pasteurellosis, were the biggest cause of mortality in sheep. In the case of cattle, Zebu cross bred animals were prone to tick borne parasitic diseases such as babesiosis, anaplasmosis and theileriosis as well as to cutaneous myiasis caused by the screw worm fly, *Chrysomia bezziana*.

In 1996, the government launched The Seventh Malaysia Plan (1996-2000), to further accelerate the growth in the economy with special emphasis in agriculture and technology. The main objectives were to put into place a productivity-driven growth system, sustainable development in technology in various sectors, poverty alleviation and macroeconomic stability, all of which involved the upgrading of major agricultural productivity including livestock development.

History of small ruminant production in Malaysia

The small ruminant sub-sector in Malaysia is very small compared to the poultry and beef cattle sub-sectors, and contributed only 0.4 percent to the total annual ex-farm value of livestock products in 2003 (Anonymous, 2003). Traditionally, sheep farming was characterized by its small flock size (three to ten animals per farmer) and with a generally disorganized system of production, whereby animals survived by foraging for vegetation in and around villages, wastelands, roadside fringes and the like. Under this system, the level of productivity was low, thus resulting in unsatisfactory growth in the industry. The farmers regarded the animals as a form of saving, or cash reserve, rather than as production units.

In the mid-1980s, the DVS and the Ministry of Agriculture, initiated an ambitious program of developing the small ruminant sub-sector, particularly the sheep industry, as a means of meeting the increasing demand for mutton and for increasing farmer's income. Due to this effort, the more enterprising farmers have taken sheep production seriously. These farmers rear larger numbers of sheep per flock and provide better management, with the objective of gaining a considerable amount of income from this enterprise. The economic downturn in the early 1980's also prompted many primary crop plantation owners to become involved in small ruminant husbandry, particularly sheep raising, using them as a means of biological weed control in plantations, as well as a means of diversifying their economic activities.

Thus more smallholder farmers, as well as plantation owners, are now involved in commercially managed sheep farming businesses. Subsequently, the sheep population has increased from approximately 59,000 head in 1980 to 119,000 in 2003 and the mutton production from sheep has increased from negligible amounts to 976 MT in 2001 (Anonymous, 2003). The small ruminant industry has also undergone considerable structural changes; features such as bigger farm size, integration with plantation crops, commercially managed enterprises, more important source of rural income and involvement of large business enterprises have become more dominant. The success of the industry is the result of an integrated effort of many agencies and of various sectors.

Despite the emphasis and priority received from the government, the ruminant sub-sector still faces considerable hurdles. Cattle, buffalo, goat and sheep constitute the ruminant sub-sector and are mostly reared by smallholders, although the numbers raised by individual farmers is increasing. Keen interest in integrating ruminants (cattle and sheep) in the plantations is on the increase and is probably the most feasible system for beef and mutton production (Ibrahim, 1996). The small ruminant industry is relatively small and contributed only 0.36% to the total ex-farm value of the livestock sector in 1993. Goats are raised for both milk and meat production, however sheep provide the greatest proportion of locally produced mutton in Malaysia. The number of goats has been on a downward trend for several years and is now fairly stable at about 280,000 head. The unpopularity of goat raising is generally attributed to the animals behaviour of browsing and feeding on vegetables and ornamental plants.

Current policy towards the small ruminant industry

Due to low small ruminant population, low per capita mutton consumption and the comparative advantage of horticulture, oil and rubber plantations in the utilization of agricultural land, it is not prudent for Malaysia to strive towards self-sufficiency in mutton supply. However, in the total context of environmental awareness and the agricultural industry, sheep production in integration with plantation crops can contribute significantly to a better environment and supplement the income of estate workers and plantation owners, and also for smallholders.

The New Agriculture Policy (NAP) did not specify any target of self-sufficiency of livestock (SSL) for mutton production (Anonymous, 1993). However, government policy makers envisaged that with an estimated area of 0.5 million hectares of rubber and oil palm plantation, the projected resultant increase would amount to 3,400 MT in the year 2000 which would be maintained at that level until the year 2010. The projected increase, together with the present level of production, would substantially raise the SSL to 43.3% in the year 2000 and by another 20.5% in the year 2010 (Anonymous, 1993).

To achieve this objective, the means of promotion to be undertaken by the government include the importation of exotic breeds, increase in the female base population, expansion in research and development. Emphasis will be placed on the importance of environmental adaptation, nutrition, disease control and prevention. Special emphasis will also be given in overcoming the problems with regards to productivity (weight gains) and the need to increase lambing percentage (fertility).

Extension Activities

In terms of development of the small ruminant industry, the DVS has played a major role, and is expected to do so for years to come. Some of the activities carried out by the DVS include recommendations for the importation of exotic breeds suitable to the local climate, establishing breeding programmes on DVS farms for distribution of progeny to farmers, disseminating knowledge in animal husbandry through keeping close contact with smallholders, and providing veterinary services.

The DVS has allocated a substantial amount of funds for development of the small ruminant industry in the past decades. For instance, in 1988 and 1989, the amount was approximately 17% of its total development budget (Anonymous, 1990). The DVS officers gave approximately 325 interviews and site discussions through the national radio channel (RTM) from 1988 to 1990, printed 29,500 copies of books and booklets (four titles) and about 200 farmers were trained in small ruminant husbandry during the same period.

In 1990, DVS initiated the Veterinary Extension Programme (VEP) as a vehicle to accelerate the growth of the Malaysian livestock industries, particularly the ruminant sub-sector, to be more modern, commercial and sustainable. VEP integrates the philosophy of prevention and information/technology transfer incorporating it with extension and economic principles. In this programme, livestock farms are categorized into three groups, viz:

1. corporate and private
2. commercial and potentially commercial
3. small holder

The second group, were identified as potential clients of the VEP. Besides extension activities and providing health and veterinary services and advice, the DVS also distributes sheep parent stock to farmers. This stock is distributed by the so-called `Pawah' scheme, whereby the DVS provides initial stock ranging from ten to fifty head per farmer, which should be reimbursed by the project participants as per agreement between DVS/participant after project participants acquire a population target. DVS then redistributes the reimbursed stock to other qualified farmers. For example, in 1988/89, about 8,900 rams and ewes were distributed to 220 farmers (Anonymous, 1990). It is hoped that such a system will assist in increasing the sheep population of the country and promote better participation by other interested farmers

Sheep breeds

During the early 1980's, the main exotic sheep breeds imported into Malaysia were Dorset Horn, Polled Dorset, Suffolk, Corriedale and the Merino. Since many of these breeds suffer from acclimatization problems, a large number of Thai Long Tail sheep were imported in later years. From 1986 - 1990, 24,300 head of sheep were imported from Australia and 27,300 head from Thailand. From 1991 - 1994, the number of imported sheep was increased, with approximately 43,000 arriving from Australia and 28,000 from Thailand. These figures include both breeding and slaughter animals (Anonymous, 1995). In a recent survey of smallholder sheep farms in Peninsular Malaysia, Badariah *et al.*, (1996) found that 85% of the respondents had Malin and Thailand Long Tail breeds on their farms. The wool breeds were mostly found on the large plantations and government farms.

Crossbreeding programs on government farms have been undertaken for the long-term strategy of up-grading the local breeds raised by the small holders. The offspring of Dorset Horn and Polled Dorset with the local Malin breed were reported to perform well under sheep-tree crop integration systems (Johan & Jamaluddin, 1996). A multinational estate plantation, Kumpulan Guthrie, has recently successfully developed two new crossbreds, namely the Guthrie Dorsimal (Dorset X Malin) and the Guthrie Suffrimal (Suffolk X Malin) (Johan & Jamaluddin, 1996). Besides the wool sheep breeds, crossbreeding with exotic tropical hair sheep has also been implemented. A hair sheep from Africa, called the Bali-Bali, has been crossed with Malin to produce Balin (Bali-Bali X Malin) and with Dorset-Malin to produce Badorlin. From early 1998 The DVS embarked on a scheduled importation programme of hair breeds of sheep such as the Barbados Black Belly, St.Croix and Santa Ines, to upgrade and improve the existing sheep population. The hair breeds are now found on the 2 large government farms (Chalok and Gajah Mati), where they are bred for distribution to small holders. Additionally, a significant number are found on some of the large estates.

Small ruminant feeding and management systems

Feed resources and feeding systems are important elements in the development of the small ruminant industry in Malaysia. Until recently, the feeds and feeding systems for small ruminants were not given much emphasis by smallholders since green natural forage on road sides, rice bunds, waste vegetation and areas previously used for mining were available. However, due to the economic development throughout the nation, these areas are becoming scarce and together with a shift in attitude, the smallholders are now looking for cheap feed resources that will enable them to enhance their farm performance.

The major sources of nutrition are improved grasses for intensive systems of management, native undergrowth under tree-crop integration with crop residues and agro-industrial by-products as supplementation. Improved pasture species like *Digitaria setivalva* and *Panicum maximum* are common, but the easily cultivated, high yielding grass *Bracharia decumbens* is toxic to goats and sheep (Salam Abdullah *et al.*, 1987), although cattle can graze this plant with impunity. *B. decumbens* is a copper accumulating plant and it has been reported that sheep show signs of liver damage and photosensitization which may result in death (Abas *et al.*, 1983). However closely related species, such as *B. humidicola*, do not have this undesirable characteristic (Schweick, 1982), and thus are popular pasture species on sheep and goat farms in Malaysia.

However, in view of the high cost of pasture establishment and maintenance, and competitive uses of available land, the more preferable source of forage is the undergrowth in plantations. The botanical composition, yield and quality of the ground vegetation will vary with factors including the agro-management practices, types and ages of the primary crops, soil types, rainfall, terrain which will produce differences in animal performance under integrated systems. Besides the native and improved pastures, agro-based by products from the oil palm, cocoa and coconut industry, such as palm kernel cake (PKC), cocoa meal and coconut meal respectively, as well as coffee pulp-hull mixture, copra cake, tapioca chips and soya bean waste are being utilized to a limited extent (Tajuddin & Zahari, 1992). Other agro-based by products, such as palm oil mill effluent (POME) and palm fibre fines (PFF) cannot be fed singly to livestock due to their low overall nutritive value, quality variability and inconsistency in supply. However these latter products have to be formulated into balanced diets.

Although the integration of sheep under plantation crops has been regarded as a novel sheep production system in Malaysia, the viability of these intensive production systems still needs to be established. From a survey of 130 sheep small holders farms in Peninsular Malaysia, Badariah *et al.*, (1996) found that semi-intensive management systems are the most prevalent (98%). Under this type of system, the animals are let out to graze for 4 - 6 hours each day and then housed each night. The main grazing areas are the oil palm estates (57%), rubber estates (25%) and orchards (8%). Other grazing areas include unused land, roadsides and paddy fields. It was also established that these farmers incurred very little expense on feed supplementation. It was also found that approximately 40% of the farmers do not provide any additional feed to their animals. Other farmers supplemented their animals with cut grass (26.3%), small ruminant pellet formulations (18.6%), sago palm and sago waste (11.8%), oil palm fronds (1.8%) and molasses (0.8%).

Small ruminant health and diseases

The two main diseases that cause high morbidity and mortality in both goats and sheep in Malaysia are pneumonic pasteurellosis and endoparasitism (Sani & Chandrawathani, 1996). Much research has been directed towards assessing the occurrence, incidence and understanding of the pathogenesis of pasteurellosis in order to develop effective vaccines, which are presently being produced and assessed by the Veterinary Research Institute (VRI). Mortality rates between 12 and 15% due to pneumonic pasteurellosis, have been reported to occur in sheep and goats in Malaysia following transportation and other stresses (Zamri-Saad *et al.*, 1992). Several vaccines have been used to control the disease with different success rates (Chandrasekaran *et al.*, 1991; Wan Mohamed *et al.*, 1988; Zamri-Saad *et al.*, 1992).

With regards to parasitic gastro enteritis, the nematode parasite *Haemonchus contortus* is by far the most important problem (Zamri-Saad *et al.*, 1992; Sani *et al.*, 1994; Chandrawathani *et al.*, 1996). Although other species such as *Trichostrongylus* spp. and *Cooperia* spp. also contribute to the overall problems due to helminths, they can be regarded to be of minor importance compared with *H. contortus*, which normally constitutes approximately 90% of the nematode parasite population in small ruminants.

Epidemiology of Gastro-intestinal Nematodes of Small Ruminants in the Tropics / Sub Tropics

In the tropics and sub/tropics, the nematode species of major economic importance are *Haemonchus contortus* (Allonby & Urquhart, 1975; Schillhorn van Veen, 1978), *Trichostrongylus colubriformis* (Eysker & Ogunsusi, 1980), *Oesophagostomum columbianum* and *Gaigeria pachycelis* (Le Jambre, 1996). In addition, sheep and goats are often infected with other species, such as *Bunostomum* and *Cooperia* spp. In the cool tropical highlands, *Nematodirus* spp., *Pseudomarshallagia (Longistrongylus) elongata* and the lungworm *Dictyocaulus filaria* have also been frequently reported (Charles, 1989).

The development, survival and transmission of the free-living stages of nematode parasites of small ruminants, are influenced by climatic and environmental factors such as temperature, rainfall and humidity. The combined effects of these factors are responsible for the seasonal fluctuations in the availability of infective larvae, or third stage larvae (L3), on pasture and subsequently in the prevalence of infections and worm burdens of the hosts. In tropical environments, this seasonal variation in parasite population dynamics has been demonstrated in a number of studies of the epidemiology of nematode parasites of sheep and goats (Allonby & Urquhart, 1975; Cheijina *et al.*, 1989; Banks *et al.*, 1990; Cheah & Rajamanickam, 1997). In general, rapid translation of eggs through to L3 occurs throughout most of the rainy season, and grazing animals harbour a variable but generally significant number of worms. In areas with distinct rainy and dry seasons, the majority of L3 acquired by the grazing animals undergo arrested development at the end of the rainy season, and faecal egg counts decline and remain consistently low during the dry season (Chiejina *et al.*, 1989; Eysker & Ogunsusi, 1980). With the onset of the rainy season, when pasture larval challenge and intake of L3 are high, there is a sharp increase in the egg output. While the pattern described is the most common, there may be limited areas surrounding drinking places and permanent ponds, which may be sufficiently humid to maintain optimum larval development and transmission all year around (Allonby & Urquhart, 1975; Cheijina *et al.*, 1989).

In the wet tropics of Africa, Latin America and Southeast Asia the climatic conditions permit development of eggs and larval stages more-or-less continuously throughout the year (Banks *et al.*, 1990; Cheah & Rajamanickam, 1997). Although seasonal variations in the availability of L3 can also be found in these areas, the oscillations tend not to be as great, but the overall availability tends to be much higher, than in those areas with distinct dry seasons. Another important feature is that although the translation of eggs through to L3 is rapid (usually less than 5 days), their survival on pasture is very short (typically 4 – 6 weeks), due to the fact that the L3 are constantly active and rapidly exhaust their food reserves (Okon & Enyenihi, 1977; Charles, 1989; Aumount & Gruner, 1989; Banks *et al.*, 1990; Barger *et al.*, 1994). Studies on the bionomics of trichostrongyle infection of small ruminants in the humid tropics showed that sheep can safely graze in an area for 3 – 4 days, but they must be moved at this time to avoid auto-infestation. However animals could be safely returned to the original pasture after 5-6 weeks (Barger *et al.*, 1994; Sani *et al.*, 1995).

With regard to transmission, it is anticipated that a higher stocking rate may cause higher infection rates in the animals. In extensive grazing areas with high temperatures and low humidity, there may be little, or variable, association between animal density and worm burdens (Macpherson, 1994; Eckert & Hertzberg, 1994).

As indicated above, the larvae of some genera of nematodes are able to delay the maturation to adult stages (a phenomenon known as hypobiosis). Resumption of their development usually coincides with the onset of the rainy season, the most favourable period for larval development and transmission on pasture (Agyei *et al.*, 1991). However, this phenomenon may also occur as a manifestation of acquired immunity (Urquhart *et al.*, 1962), as well as the result of prior exposure to adverse climatic conditions during the stages as free-living larvae. While the onset of arrested development has been linked to falling temperatures in some temperate zones (Allonby & Urquhart, 1975), the stimulus in tropical areas is clearly different. The arrested larvae accumulate in the abomasal and intestinal mucosa at the end of the rainy season, persist in this form over the dry season and complete the development at the start of the next rainy season (Vercruysse, 1985; Chiejina *et al.*, 1989).

The phenomenon of peri-parturient rise in faecal egg count of ewes is of great importance in the epidemiology of gastrointestinal nematodes of sheep and has been extensively studied (Connan, 1976; Barger, 1993) and also been reported under tropical conditions (Agyei *et al.*, 1991; van Geldrop & Schillhorn, 1976). A peri-parturient rise in nematode egg counts was observed in some instances as early as two weeks before lambing and persisted up to eight weeks post-partum when lambing took place at the end of the wet season (van Geldorp & Schillhorn, 1976; Agyei *et al.*, 1991; Zajac *et al.*, 1988). Thus, pregnant/lactating ewes become the major source of infection for the new-born lambs.

Although the same helminth parasites species were found to infect both sheep and goats, the former usually suffer heavier worm burdens because of the difference in their grazing habits. While sheep will rely almost entirely on grazing, goats prefer to browse trees and shrubs if given the choice, often reducing their intake of L3 considerably. An additional factor which may increase the worm burden of sheep is their ability to graze very close to the roots of the grasses during times of feed shortage, inevitably ingesting higher numbers of infective larvae. However, if goats are forced to graze, rather than allowed to browse, then it has been found that they are particularly susceptible to nematode parasite infection, not only of ovine but also bovine parasites (Sangster, 1990).

Epidemiological Studies in Malaysia

Within the three farming systems for small ruminants in Malaysia, namely the institutional farms, the plantation and the smallholder farming operations, animals are exposed to the same disease challenges. Gastrointestinal nematode infections, primarily due to *H. contortus*, are the major causes of death in all age classes of sheep and goats, especially in poorly managed farms. Zamri Saad *et al.*, (1994), established that even in mild haemonchosis (2500 + 151 adult worms) in goats appeared to cause sufficient stress to induce immunosuppression, which in turn allowed the development of the experimentally induced pneumonic pasteurellosis. Daud-Ahmad (1991) recorded a mortality of 80% among goats under one year of age in a herd, and post-mortem examination revealed that one-third of the deaths were primarily due to *H. contortus* infections. Evidence of acquired immunity to nematode infection in sheep was observed at around 8 months of age. A marked rise in egg shedding was observed in ewes 6 weeks before and after parturition. Following from these studies, a standard recommendation was made that de-worming programs should direct attention towards sheep under 8 months of age and peri-parturient ewes (van Geldrop & Schillhorn, 1976).

Figure 2: “Biological weed control” in a Malaysian oil palm plantation

Epidemiological studies conducted on nematode parasite infections in sheep have shown that in smallholder systems, animals commence shedding parasite eggs at the age of 1-2 months; in an open pasture management at 4 months of age; and in an integrated production system with rubber at 5 months of age (Dorny *et al.*, 1995). These differences in the onset of egg shedding were dependent on the age at which the lambs are allowed to graze. The smallholder systems, lambs were allowed to graze with the rest of the flock at about 2 weeks of age while in the open pasture and integrated production system managements, lambs only begin to graze at 3 months of age. Lambs appeared more susceptible to ill thrift when they began to graze under 1 month of age.

Figure 3: A typical small holder sheep and goat farm (housing shed and pastures) in Malaysia

The highest egg shedding was shown in the 4-8 months age group with geometric mean of approximately 3000 eggs per gram (epg) (small holder), in the 5-6 months age group with mean of 2200 epg (open pasture), in the 6-7 months are group with mean of 1750 epg (integrated production system). The mean epg values partly reflect the varying degrees of pasture contamination, the highest being in the small-holder system and the lowest in the integrated production system (Dorny *et al.*, 1995). The improved plane of nutrition found in the open pasture and integrated production systems was also an important factor to consider when comparing levels of parasitism between the different systems of management.

Evidence of acquisition of immunity against nematode infection in sheep was seen over 8 months of age (small holder) and at 7.5 months of age (open pasture and integrated pasture system) while in goats this occurred from 12-18 months onwards (Dorny *et al.*, 1995). However, Daud-Ahmad (1991) did not find any evidence of the development of immunity against nematode infections in the Kacang breed of goats.

A marked peri-parturient rise in egg shedding was observed in ewes starting at 2 weeks before parturition for about 3 months (smallholder) (Dorny *et al.*, 1995) and 6 weeks before and after parturition (open pasture) (Sani *et al.*, 1994).

These observations on the epidemiology of gastrointestinal nematode parasites of sheep are useful in designing control measures. It is therefore recommended in de-worming programs to direct attention towards sheep less than 8 months of age, and ewes around lambing.

In common with studies elsewhere in the humid tropics (Banks *et al.*, 1990; Barger *et al.*, 1994), studies on the bionomics of nematode parasites of small ruminants in the field during wet and dry periods in Malaysia, revealed that development of eggs to infective larvae occurred within 3 – 4 days and that most larvae died by the fifth to sixth week (Sani *et al.*, 1994; Sam Mohan *et al.*, 1995). This means that sheep can safely graze in a fenced area for 3-4 days before moving, and that a heavily contaminated area should be "rested" for 5-6 weeks.

Control of Gastro-intestinal Nematodes of Small Ruminants in the Tropics / Sub Tropics

Anthelmintics

Parasitic infections continue to play a significant role in limiting livestock productivity in the tropics, and chemotherapy continues to serve as the cornerstone of parasite control. However, effective anti-parasitic compounds constitute a limited resource and their future availability depends on a variety of factors such as escalating research costs, increased regulatory requirements and the development of resistance (Waller, 1997).

Since the early 1960's there have been only 3 major classes of broad spectrum anthelmintics commercially released for the control of nematode parasites of ruminant livestock, namely:

1. benzimidazoles / probenzimidazoles
2. tetrahydropyrimidines / imidazothiazoles
3. macrocyclic lactones

In addition to these classes of compounds is a range of other useful drugs, but with more limited anthelmintic activity. These are generally classified as narrow spectrum anthelmintics:

- organophosphates
- substituted phenols
- salicylanilides

Broad Spectrum Anthelmintics

Benzimidazoles

The tertiary benzimidazoles, such as fenbendazole, oxfendazole and albendazole, remain in the host's body for longer periods (15 to 24 hours) after dosing as compared to thiabendazole, oxibendazole and parbendazole (4 to 6 hours) (Prichard *et al.*, 1978). Thus the tertiary benzimidazoles have in general, the highest efficacy and the broadest spectrum of activity (including lungworm and tapeworms) amongst the benzimidazole anthelmintics. The benzimidazoles act by attaching to tubulin dimers, preventing their polymerization to microtubules and thus causing the disassembly of existing cytoplasmic microtubule structures of the parasite (Prichard *et al.*, 1978). These actions reduce the absorption of nutrients in the parasite and must be maintained for some time to allow irreversible metabolic changes to occur, otherwise the parasite can survive the drug onslaught by reducing its energy demands (Le Jambre, 1996).

Tetrahydropyrimidines / imidazothiazoles

The two most important drugs in this class, levamisole and morantel, affect the neuromuscular transmission of the nematode. Following oral administration, they are absorbed by the host almost immediately and cause rapid paralysis in those parasites that are exposed (Prichard *et al.*, 1978). Once paralysed, the nematodes are swept out of the host along with the ingesta. Both compounds are active against the major intestinal parasites of sheep and cattle, with the exception of inhibited *Ostertagia ostertagi* (Le Jambre, 1996).

Macrocyclic lactones

The macrocyclic lactones, or the avermectins/ milbimycins, are a family of 16 membered macrocyclic lactones, originally isolated from an actinomycete, *Streptomyces avermitilis*. They are all potent nematocides and insecticides, thus commonly referred to as end-ectocides. Structurally, the avermectins belong to the milbemycin class of macrocyclic lactones and within the milbemycin class, 5 analogues are commercially available for the treatment of nematodes in animals, namely: ivermectin, avermectin B1, doramectin, moxidectin and milbemycin A4-5-oxime. The most popular, ivermectin, is a potent inhibitor of the motility and development of the free-living stages of trichostrongylid nematode parasites (Campbell, 1983).

Narrow Spectrum Anthelmintics

The substituted salicylanides and phenols are trematocidal and cestocidal and at normal dose rates, some are highly effective against *H. contortus* in sheep. These compounds, like closantel, are highly effective against the blood sucking stages of parasites, which acquire the drugs that become tightly bound to plasma proteins. Closantel used as a single oral dose, exerts a suppressive anthelmintic effect against *H. contortus* for 30 days after treatment, and a significant effect still remains after 60 days (Hall *et al.*, 1981). If used at the commencement of a period when there is a high probability of development of *H. contortus* eggs through to infective larvae, closantel can reduce the contamination of pastures for long periods. This attribute of the drug was exploited in the development of the "Wormkill" programme in Australia (Le Jambre, 1996).

The use of anthelmintics in small ruminants in Malaysia

In keeping with other small-holder farmers in the tropics and sub-topics, those in Malaysia often employ a variety of traditional de-worming remedies (Pond, 1996). In most traditional systems helminth control was hardly practiced, due to the lack of awareness on the part of livestock owners, the relatively high cost, or scarcity of modern anthelmintics. However, if control programs are implemented they are usually based only on anthelmintics, but often the treatments are not given correctly, or at the right time.

The lack of epidemiological data for strategic control programs, which is generally still the case for most of the regions in the tropics where livestock are raised, is still a major handicap in Malaysia. Salvage treatment is the most common practice and treated animals return to the communal, highly contaminated grazing lands. The concept of "clean" and "safe" pasture has been advocated in several studies in developing countries but is not feasible under traditional management systems.

However the results of a small postal survey of goat and sheep farms revealed that all farms practiced rotational drenching with 2 or 3 types of drugs, which they claimed was intended to delay the development of anthelmintic resistance (Chandrawathani *et al.*, 1994). Strategic treatment was advised to farmers of sheep and goats, to reduce the use of anthelmintics on their farms. To promote this concept, studies on the strategic use of closantel (Chandrawathani *et al.*, 1996a), levamisole – netobimin combination (Chandrawathani *et al.*, 1995a), moxidectin (Chandrawathani *et al.*, 1998a) and ivermectin (Rajamanickam *et al.*, 1990) use has proven to be effective in smallholder farms, whereby farmers are advised to use these drugs less than 6 times a year and if possible, rotate the use of at least 2 drug groups per year.

It was not until the development of the small ruminant industries in recent years that there has been a need, particularly by the large government and plantation farms, to resort to the frequent use of anthelmintics. Up until recently, the choice of drug was almost always dictated by price. With the arrival onto the market place of the generic drugs, prices fell and this precipitated the frequent use (at least every month and in some circumstances, every 2 – 3 weeks) of these drugs.

Anthelmintic Resistance

Broadly speaking, anthelmintic resistance is the ability of the parasites to survive dosages of drugs that would normally kill parasites of the same species and stage of development. It is inherited and selected for because the survivors of drug treatments pass genes for resistance on to their offspring (Sangster, 2001). There are several reasons identified which contribute to the development of resistance. Overuse of drenches is one of the most obvious causes, with under-dosing also being identified as a major contributor to the selection for drench resistance (Waller, 1997). Again, the need to weigh sheep, calibrate the drench gun and dose to the heaviest animal in the flock or mob needs to be adopted by the producers (Waller *et al.*, 1995). Repeated use of the same drench group increases the rate at which resistance develops. Under-dosing is one way that parasites can be exposed to sub lethal doses, thus encouraging the selection for resistance. Poor drenching technique is another way to accelerate the development of resistance. It is important that the drench gun is placed over the tongue of the animal to ensure that the drench is swallowed, this allows the majority of the drench to reach the rumen and produce maximum availability of all the active ingredient in the medication (Hennessy, 1998). The flow rate of the digesta in the gut also determines how long the worms are exposed to the drench. Slowing the movement of the digesta, increases the exposure of the worms to the anthelmintic and therefore increases its efficacy. Thus keeping the sheep off feed for 24 hours before drenching will slow the movement of digesta through the gut and improve the effect of benzimidazole and ivermectin drenches as these drenches become associated with the rumen particles and pass through the gastrointestinal tract at the same rate as the digesta (Hennessy, 1998). This effect is particularly evident when sheep are on green, highly digestible pasture.

There is an urgent need to minimise the frequency of anthelmintic treatments (Waller *et al.*, 1995). The rate of development of resistance is influenced by the proportion of the nematode population that are in refugia when anthelmintics are administered, and hence escape the selection pressure applied to adult worms (Prichard *et al.*, 1990).

Anthelmintic resistance is by far the greatest problem in the small ruminant industries throughout the world, especially so in the warmer, more humid environments such as the tropics (Waller, 2003a; 2003b). This in turn is linked to the importance of the highly pathogenic nematode parasite *H. contortus*. The use of drenches to control nematodes of small ruminants is becoming more complex and less effective with widespread development of resistance. Producers are requiring a greater understanding and sophisticated advice to develop a suitable drenching programme for their properties. Large surveys in Australia have demonstrated that 85% of sheep farms have parasite populations which are resistant to benzimidazole drenches (Waller *et al.*, 1995; Hennessy, 1998). The problem of multiple drench resistance is becoming a worldwide phenomenon as seen in South America (Echevarria *et al.*, 1996, Eddi *et al.*, 1996), South East Asia (Sivaraj *et al.*, 1994; Dorny *et al.*, 1993) and South Africa (van Wyk, 1990). With the likelihood of developing new products for worm control becoming more remote, due to high costs and risks involved, and probably poor returns compared to human pharmaceuticals (Waller, 1997) – there is an urgent need to assess and understand the situation leading to anthelmintic resistance and if possible stem the rapid rise and spread of anthelmintic resistant strains of worms.

The issue on anthelmintic resistance in small ruminant flocks in Malaysia was not seriously addressed until approximately one decade ago. At that time, a nationwide survey involving 96 randomly selected goat farms demonstrated the high incidence of resistance (34%) of *H. contortus* towards the benzimidazole group of anthelmintics (Dorny *et al.*, 1994a). Levamisole resistance was also detected on two of 10 farms investigated. Sivaraj and Pandey (1994) showed multiple resistance of *H. contortus* on an institution farm to ivermectin and benzimidazoles. Closantel, through its sustained activity was a potential drug in the strategic control of haemonchosis, and as an alternative treatment for benzimidazole and levamisole resistant *H. contortus*, was introduced into the country. It was shown that a single treatment with closantel resulted in a 72.5% to 86.8% reduction of strongyle egg deposition on pasture during a two-month period (Dorny *et al.*, 1994b). The problem of anthelmintic resistance was found to be particularly serious on institution farms. These farms allowed their animals to graze on overstocked pastures and as a consequence, de-worming at 3-4 weekly intervals was necessary to avoid high mortality. The resistant strain of nematodes that developed on these farms may have spread when these farms supplied breeding stock to smallholder farmers. These findings clearly show that there is an urgent need to monitor for anthelmintic resistance on small ruminant farms using the simple Faecal Egg Count Reduction Test (FECRT) (Anonymous, 1989; Coles *et al.*, 1992; Waller *et al.*, 1995).

Alternative Control Measures

Grazing management

In wet, tropical climates, the micro-environmental conditions in the pasture sward, favours the rapid and continuous egg hatching and larval development. However this also results in high death rates of the infective larvae on pasture (Banks *et al.*, 1990). These studies found that larvae survive for no more than 4-6 weeks on heavily contaminated tropical pastures and peak concentrations invariably occur approximately within 1 week following contamination of pastures with faeces containing nematode eggs. However, larvae were detected as early as 4 days after contamination was deposited for all major nematode species encountered eg. *H. contortus, Trichostrongylus colubriformis* and *Oesophagostomum columbianum* (Barger *et al.*, 1994). All species also had similar survival times on pasture. These workers developed a grazing system to exploit these findings. It consisted of a 10-paddock rotational grazing system, with each paddock grazed in sequence for 3.5 days, then spelled for 31.5 days. For goats managed this way, it was found that their egg counts were less than half those of similar, but set- stocked, goats. Furthermore, the set stocked goats, required approximately four times as many anthelmintic treatments over the course of a year, compared to rotationally grazed animals (Barger *et al.*, 1996a; 1996b).

Similar findings were also seen in grazing studies conducted in Malaysia by Chandrawathani *et al.* (1995b; 1996b). This was further investigated by ecological investigations (development and survival) of the free-living stages of infective larvae of *H. contortus* and *T. colubriformis*. Faeces containing eggs of these nematode species was spread on grass plots at 3 different times of the year (Cheah & Rajamanickam, 1997). In all three trials, development of eggs to infective larvae occurred within 1 week and their survival times were up to 7 weeks. This information confirmed the observations of Banks *et al.* (1990) and proved vital to convince managers of the government small ruminant farms for the need to establish rotational grazing systems for worm control. Also these findings provided valuable information for sheep management under plantation crops, like oil palm and rubber, which have large acreages.

The rapid rotational grazing strategy is an evasive strategy for application in the humid tropics. It relies firstly on the removal of existing nematode infections in the animals by anthelmintic treatment, secondly the movement of the treated animals to a safe pasture and thirdly (and most critical) to move animals before they re-infect (auto-infect) themselves. Thus the timing of the move needs to be less than one week. To make the procedure a simple extension message ("user friendly") it was decided that twice / week movement (the "Monday – Thursday" grazing management scheme) should be promoted (Anonymous, 2001b).

Nutritional Supplementation

The dynamics of nematode populations in ruminants is complex and influenced by host age, breed, immunological and nutritional status. In the interaction between parasitism and nutrition, two key factors play a major role; that is, firstly the influence of the parasite on the metabolism of the host and secondly the effect of host nutrition on the parasite populations and the ability of the host to withstand the patho-physiological disturbances of infection. Studies by Coop & Holmes (1996) have shown that gastrointestinal nematodes reduce voluntary fed intake and efficiency of feed utilization, by increasing endogenous loss of protein into the gastrointestinal tract. There is a re-allocation of protein from productive processes into repair of gastrointestinal tract, synthesis of plasma proteins and mucoprotein production. Thus, dietary supplementation with additional protein is advocated to counter the effects of parasitism. Although it has been found that protein supplementation does not affect initial establishment of nematode infections, the patho-physiological consequences are more transient and less severe (van Houtert & Sykes, 1996). Coop *et al.* (1995) found that the provision of a by-pass protein supplement accelerated the development of immunity to *Teladorsagia (Ostertagia) cicumcincta* in trickle-infected lambs. This was demonstrated by the supplemented lambs having a higher concentration of gastric mast cell protease, which correlated positively with the proportion of early fourth larval stages (EL4) and negatively with total worm burden. The main effect of protein supplementation is to increase immunity and resistance to re-infection, which has been associated with an enhanced cellular immune response in the gastrointestinal mucosa.

In tropical environments where the quality of feed resources is variable, nutritional deficiencies are likely to exacerbate the detrimental effects of parasitism. Knox (1996) has reported that the use of low cost supplements, such as urea/molasses feed blocks, can enhance the animal's ability to utilise the available diet as well as assist the animal to withstand the infection. However, studies in Malaysia with sheep grazing continuously on permanent pastures (Cheah & Rajamanickam, 1997), the use of nutritional blocks, coupled with regular anthelmintic treatments, failed to prevent worm establishment. Although another treatment group that was rotationally grazed, the use of feed blocks produced much lower faecal egg counts in sheep. These findings indicated that the benefits of nutritional block supplement were enhanced by rotational grazing with the recommended spelling period (Cheah & Rajamanickam, 1997). Epidemiological data collected from these Malaysian studies showed that the populations of *H. contortus* and *T. colubriformis* did not fluctuate significantly over a one-year period. Zahari *et al.* (1996) also found that the faecal egg counts of sheep fed with urea/molasses blocks, as well as those offered medicated (fenbendazole) urea/molasses blocks, had lower faecal egg counts compared to control sheep fed only on a basal diet.

Breeding for parasite resistance

European sheep breeds and particularly Merino crossbreds, are more susceptible to nematode infections than Florida Native, Barbados Blackbelly and St. Croix (Yazwinsky *et al.*, 1979). Increased parasite resistance was first reported in these indigenous breeds in the Florida Native (Zajac, 1988). Decreased faecal egg counts and increased hematocrit levels in pastured Florida Native ewes and lambs compared to Rambouillet and Hampshire sheep have been observed. Subsequently, natural trichostrongyle infections of Florida Native, Barbados Blackbelly and St. Croix sheep have been followed in several studies. Both lambs and ewes of the Florida Native and St. Croix breeds regularly show lower faecal egg counts than domestic breeds (Zajac, 1995).

It is now established that indigenous breeds in the tropics have developed a range of unique adaptive traits, which enable them to survive and be productive in a diverse and harsh environment (Baker, 1996). In the tropics, there have been some reports of sheep breeds in West Africa (Baker, 1995) and in the Caribbean (Zajac *et al.*, 1988), which are innately resistant to gastrointestinal nematodes. Also in Asia, sheep showing natural resistance to the trematode, *Fasciola gigantica*, has been reported (Wiedosari & Copeman, 1990). Although there is some evidence for parasite resistance among tropically adapted breeds of goats, this seems to be not as marked as for sheep (Baker, 1998).

The development of a program for sheep production improvement in the tropics should capitalize on the merits of the local livestock population, particularly their adaptation, year-around reproduction, tolerance to heat stress, tolerance to parasitic infection and resistance to disease, resulting in higher survival rates. If possible, these characteristics should be incorporated into a new genotype with superior performance in growth rate, milk production, prolificacy and lean meat production, provided that the feeding environment has also been improved to meet the additional demands for energy for increased productivity. Sustainability of such a breeding scheme is an issue of concern, considering that the realization of genetic responses on the population under improvement is a long-term process.

More recently, there is clear evidence that some North American sheep breeds, particularly those of Caribbean origin, are more resistant to infection. As a result of this, there is an active programme of importation of these breeds of sheep into Malaysia (Khusahry & Ariff, 1990). The government farms of the DVS have stocked St. Croix, Santa Ines and Barbados Black Belly sheep, which are now being used as foundation breeding stock for distribution to farmers in the hope of stemming the related problems of worm control and anthelmintic resistance. For the Malaysian scenario, studies by Sani *et al.* (1998; 2000) using Santa Ines sheep showed that the faecal egg counts of the pure bred animals showed that sires and ewes who are high responders (sheep that have consistently low faecal egg counts) produced offspring who had similarly low faecal egg counts. This trend suggests the possibility of segregating animals into high and low responders based on faecal egg counts, according to the methodologies used for several decades in Australia (Woolaston & Windon, 2001) for selecting within the Merino breed for parasite resistance, can also be used as a criterion for further improving resistance amongst tropically adapted and more innately resistant breeds.

Helminth Vaccines

Being metozoan organisms, helminth parasites are much more complex than viruses, or bacteria. Additionally, helminths may often change their antigenic structures between the different stages of their life cycle (Smith, 1997). The search for the few antigenic components within this complex mixture, that are involved in the natural protective immune response, requires a more sophisticated approach than the long standing use of crude preparations of parasites. Research towards a targeted approach to antigen identification has recently been developed which allows for a rational selection of candidate vaccine antigens (Smith, 1997). Further to this, studies on the immune response induced by the vaccine candidate(s) need to be analysed to ascertain whether it is protective. The mode of delivery of the vaccines as well as the way the vaccine is presented to the immune system of the host also needs to be studied. This information will have far reaching implications in the control of helminth parasites and subsequently to the small ruminant industry. However, this is still an elusive dream, which needs much more inputs in terms of new technology and funding (Meeusen, 1995).

The first worm vaccine breakthrough came in 1960's when *Dictyocaulus viviparus* larvae, attenuated by irradiation, could stimulate a high degree of protection against challenge with intact, non-irradiated infective larvae. This led to the commercialisation of the "Dictol" vaccine against the bovine lungworm, which is still sold today. Early attempts to immunise ruminants against gastrointestinal helminths, either with crude worm homogenate antigen or by ectopic infection (live worms administered intraperitoneally), met with little or no success. Attempts to extend the same principle used in "Dictol" by giving *H. contortus* irradiated larvae, unfortunately was also found to be not effective in young lambs (Smith, 1997; Bain, 1999).

Since 1980's, there has been a steady trickle of parasite vaccines onto the marketplace, which include those against *Taenia ovis* in 1989 (Johnson *et al.*, 1989), *Boophilus microplus* in 1995 (Willadsen *et al.*, 1995). In addition, the filing of numerous patents relating to specific protective antigens of parasites has taken place. In 1994, the first recombinant subunit vaccine against *B. microplus*, named Tickgard (Hoescht), was launched. Thus, there is cautious optimism for the production of efficacious vaccines also against the nematodes *H. contortus*, *Ostertagia* spp. and *Trichostrongylus* spp. will become a reality in the near future (Smith, 1997).

Most of the trials against these gastrointestinal parasites, use semi defined native antigens, larval excretory/secretory materials, and semi purified adult membrane extracts. Munn *et al.*, (1987) reported that an extract derived from the gut of *H. contortus* gave more than 90% protection against larval challenge with this parasite. On purifying this extract and vaccinating lambs, it was also found to give more than 90% protection (Travernor *et al.*, 1992). However, repeated trials by Emery (1996), have shown that protection rates varied between 40-70%. Several antigens have been cloned and expressed as proteins and these recombinant ES proteins from *H. contortus* and *T. colubriformis* (11-94 kDa antigens) have given 30-70% protection against a challenge with the homologous parasite (Emery, 1996). In line with these developments, there has been an accumulation of information on the regulation of mucosal immunity that has enabled a reappraisal of vaccination options to provide appropriate mucosal effector responses. It has been shown that the requirement for mucosal priming can be transferred by Peyer's patch T cells and involves cytokines, which in turn affects mucosal IgE responses and eosinophilia (Husband *et al.*, 1995). The success of new mucosal vaccination opportunities will depend on selection of highly immunogenic subunit antigens, coupled with strategies for cytokine manipulation and delivered with appropriate adjuvant / vehicle formulations, based on micro-encapsulation technology, to chaperone labile antigenic and genetic material to appropriate sites for mucosal stimulation after oral or systemic administration (Smith, 1997).

Biological control

Concerted scientific interest in the possibility of biological control of nematode parasites livestock first emerged 10 to 15 years ago. This was brought about by the serious problem of the rapid development of anthelmintic resistance in parasite populations worldwide (Waller, 1997), as well as problems in managing parasitic problems in organic livestock production systems. The aim of any biological control strategy is not to eliminate the target pest organism, but to keep these populations under a threshold level and thus to eliminate their harmful effects. With regards to nematode parasites, attention was initially directed towards the nematode destroying microfungi found in the soil-pasture-microenvironment (Grønvold *et al.*, 1988). These fungi are found in a variety of habitats especially in organically rich environments such as compost and aged faeces on pasture (Larsen *et al.*, 1994). Two main groups have been identified:

- predacious fungi and that produce nematode trapping structures such as knobs, branches and rings

- endoparasitic fungi that infect nematodes by sticky spores that are eaten or stick to the nematode and finally puncture the nematode

In using nematode trapping fungi for worm control, it has been demonstrated that very high doses of fungal spores (10^8 conidia per gram of faeces) had to be used in faecal cultures to elicit an effective response (Larsen, 1994). An important consideration in the implementation of the nematode trapping fungi is to deliver it to the animal such that the spores would not be damaged by gut passage of livestock so that they will germinate in fresh dung in close association with the free-living stages of nematode parasites. In such cases, the fungi can develop naturally and simultaneously with the nematode free-living stages thereby allowing the natural processes of larval trapping to take place. Screening studies have shown that very few species of nematode trapping fungi can withstand gut passage of livestock (Larsen, 1991; Waller & Faedo, 1993; Waller *et al.*, 1994). However one such fungus, namely *Duddingtonia flagrans*, has shown to possess an exceptional ability to withstand gut passage as well as produce prolific trapping networks and abundant thick walled resting spores, called chlamydospores (Larsen, 1991). Several studies have shown that *D. flagrans* has been able to reduce infective larvae of *H. contortus* in sheep faeces (Pena *et al.*, 2002; Anonymous, 2002). Studies in Australia using *D. flagrans* isolated from fresh sheep faeces and subjected to a number of *in vivo* investigations to determine its capacity to survive gut passage in sheep (Larsen *et al.*, 1998) has shown that between $5x10^5$ and 10^6 chlamydospores per day resulted in more than 80% reduction in the number of infective larvae. The studies on delivery methods (Waller *et al.*, 2001a) have shown that *D. flagrans* can survive the manufacturing process of compression when the fungus was incorporated into a range of block formulations, by significantly reducing larval numbers in faecal cultures from sheep fed with these blocks. This was observed in sheep, which only showed modest, or irregular, block consumption. Waller *et al.* (2001b) also showed that *D. flagrans* chlamydospores are also capable of surviving pressures of several tonnes when incorporated into matrices and pressed into tablets for the manufacture of prototype intraruminal controlled release devices. They remain viable for at least 9 months when stored at 4^oC and chlamydospores released by such a device can substantially reduce the infective larvae of *H. contortus* in faeces of sheep having this device *in situ*. This work again demonstrates that spores of *D. flagrans* can be delivered in a variety of ways depending on the suitability and management system of the farm.

Although the main emphasis of studies on biological control of nematode parasites of livestock has been with *D. flagrans*, other species of fungi, namely *Arthrobotrys* spp., were also found to be effective in controlling *Strongyloides papillosus* larvae when sprayed on cattle dung (Chandrawathani *et al.*, 1998b). Two laboratory trials conducted to determine the effect of *A. oligospora* spores on the development of *S. papillosus* in bovine faecal cultures showed that at a concentration of 2000 conidia per gram faeces, virtually all (>99%) the larvae were eliminated after 14 days incubation. This indicates the possibility of using *A. oligospora* to control *S. papillosus* infection, which is rampant in young calves in the tropics, by applying the spores of this fungus directly to the animal bedding materials.

Biological control cannot be considered as a substitute for anthelmintics. It has no chemotherapeutic effect and the worm population inside the animal will therefore not be affected. The purpose of using *D. flagrans* is to achieve prophylactic worm control, whereby future free-living parasite populations on pasture are reduced. Thus biological control should best be used in combination with other effective parasite control options, including the timely and effective use of anthelmintics. In the organic farm situation, very high standards of livestock management is required to achieve good worm control and biological control alone cannot achieve this result (Waller, 2003b).

Recent work in Denmark and Sweden (Fernandez *et al.*, 1998, Dimander *et al.*, 2003) as well as other European countries (Larsen, 1999; Anonymous 1998; Anonymous, 2001a) shows that using the fungal spores as a feed supplement in livestock has proven to be useful in reducing pasture infection. The fungus has shown beneficial effects in the control of free-living stages of nematode parasites of a range of livestock species. For example, horse (Fernandez *et al.*, 1997), cattle (Dimander *et al.*, 2003) and pig (Nansen *et al.*, 1996) parasitic nematodes.

Environmental impact studies on the effect of *D. flagrans* on soil nematode populations have been reported in field trials on sheep (Yeates *et al.*, 1997) and cattle (Yeates *et al.*, 2003; 2004). These studies showed no effect either on total numbers, or species composition of soil nematodes on pastures where animals treated with *D. flagrans* had grazed. This result, together with the findings by Faedo *et al.* (2002), which showed this fungus fails to migrate, or colonise soils surrounding dung deposits containing *D. flagrans*, clearly indicate that the deployment of *D. flagrans* as a bio-control agent against nematode parasites of livestock is environmentally benign.

Herbal De-wormers

As the livestock industry in developing countries is mainly in the hands of small-holder farmers, there has been an tendency for traditional methods of worm control to be used – the so-called ethno-veterinary medicine practices. An important component in this area is the use of herbal remedies. One such plant that has been used extensively in the Asian region, particularly amongst the Indian communities, is the neem tree (*Azadirachta indica*). The leaves of this tree have been used for generations as a multipurpose antidote for worms (fresh leaves fed orally), wounds (leaves mashed and used as a poultice) and ticks (neem leaves used as bedding in calf pens) (Anonymous, 1997). A study was carried out to assess the effect of neem fed orally to sheep to control *H. contortus* infections, showed that there was a reduction in faecal egg counts and larval recoveries after daily feeding of fresh neem leaves for 5 weeks (Chandrawathani *et al.*, 2002a). However, recent comprehensive testing of a range of plants (including neem) used by pastoralist and small-holder communities in East Africa, have failed to show any particular plant preparation had a significant anthelmintic effect against nematode parasite infections of either ruminants (Githiori *et al.*, 2002; 2003a; 2004), or monogastric animals (Githiori *et al.*, 2003a; 2003b).

Diagnosis of Parasitism

In controlling nematode parasites of livestock, one of the important aspects to be considered is the proper and timely diagnosis of helminthosis. All studies related to helminthosis and its control, have adopted standard techniques, such as the McMaster faecal egg count technique and larval differentiation by faecal culture methods (Anonymous, 2000). In Malaysia, farmers are advised to send monthly faecal samples to the nearest veterinary investigation laboratory for diagnosing helminthosis. This service is free of charge for livestock farmers, to encourage them to better manage worm problems in a proactive manner (Anonymous, 2000). Advice is also given on the type, dose and frequency of anthelmintic usage, by the field support staff. If anthelmintic resistance is suspected on a farm where a regular deworming programme has been conducted, but deaths due to helminthosis still occur, the faecal egg count reduction test (FECRT) is carried out (Anonymous, 1989; Coles *et al.*, 1992). With the FECRT, several factors play a role in the successful outcome of the results. For example, at least 10 animals should be used in each treatment group. Initially they should have a high egg count, dose of drugs to be tested have to be administered carefully according to weight, the animals should be ideally below 1 year of age and pre- and post-faecal egg counts need to be taken, entailing at least 3 trips to the farm before the test can be completed. Resistance is declared when the observed percentage reduction in faecal egg count is less than 95% and the lower 95% confidence limit of the percentage reduction is less than 90% (Anonymous, 1989).

Recently, the larval development assay (LDA) was also introduced to help in the identification of anthelmintic resistance. This assay is conducted using a 96 well microtitre plate that contains increasing (colour coded) concentrations of anthelmintic dissolved in agar. Fresh nematode eggs are deposited into each well and following feeding of the newly hatched larvae after 24 hours incubation at 25ºC, development through to the infective larval stage in wells of progressively increasing drug concentration, is assessed after 7 days of further incubation. This assay was developed for the detection of anthelmintic resistance in the major gastrointestinal nematode parasites of sheep, *H. contortus*, *T. colubriformis* and *T. cicumcincta* (Lacey *et al.*, 1990). Based on this, the "Drenchrite" assay was commercialised which enables the detection of resistance to benzimidazole, levamisole, benzimidazole/levamisole combination and avermectin/milbemycin drenches in the major gastrointestinal nematodes parasites infecting sheep.

Several other *in vitro* assays have been developed, but these are for only one anthelmintic group eg. levamisole (Dobson *et al.*, 1986); benzimidazoles (Le Jambre, 1976; Hall *et al.*, 1978) and because of this, they are generally restricted to research activities.

Another diagnostic method useful for monitoring *H. contortus* infections in small ruminants is the FAMACHA technique. This procedure was developed in South Africa and is based on the evaluation of the degree of clinical anemia caused by infection with *H. contortus* by classifying the colour of the ocular mucous membranes of sheep (Malan & van Wyk, 1992). A colour chart was developed which was based on the results of several field trials where the degree of anaemia was matched with the packed cell volume (PCV) and faecal egg counts of sheep (Anonymous, 2001a). The aim of FAMACHA is to provide confidence to stockmen and farmers so that they can detect individual animals that require drenching, rather than resorting to the treatment of the whole flock when signs of haemonchosis become apparent. The advantages of using FAMACHA, is that it substantially reduces the amount and frequency of anthelmintic treatment on farms. As a consequence of this, fewer sheep are treated and the selection pressure for anthelmintic resistance is dramatically reduced. Individual sheep that repeatedly fail to cope with worm infection, despite treatment, can be identified and eliminated. The technique is cheap, quick and can be readily integrated with other activities like vaccination, weighing, or counting. In addition, because it is based on assessing colour of ocular mucous membranes, literacy is not a requirement. However, misdiagnosis can occur when the cause of anaemia is not due to *H. contortus* (for example acute fasciolosis and trypanosomosis) and therefore it is still in the process of further evaluation.

Aims of the Study

The aims of this study were formulated as the result of several years of research and information gathered on the control of nematode parasites of small ruminants in Malaysia. There were strong reasons to believe that conventional helminth control by the use of anthelmintics was failing on the large government farms. Although the managers of these establishments were kept informed on research findings of the benefits of short-term rotational grazing, nutritional supplementation and the choice of sheep breeds with a known resistance to parasites, it became increasingly more apparent that they were relying on anthelmintic treatment as the sole means for parasite control. Although resistance to anthelmintics had been recorded in sheep and goat farms on peninsula Malaysia, this work was in the early – mid 1990s and there was no further update on the situation. Accordingly the main research objectives that embody this thesis were:

- Conduct a comprehensive investigation into the current status of anthelmintic resistance in Malaysia, particularly focussing on the large government farms that act as a source of sheep and goat stock for the small- holder farmers.
- Conduct anthelmintic resistance studies in both Peninsular and East Malaysia to ascertain whether differences occur in the two different locations.
- Conduct a survey to determine the presence of nematode trapping fungi from soil from farms and faecal samples of local livestock under the tropical environment. To identify the nematode trapping fungi and a suitable candidate for biological control programme under local tropical conditions.
- Establish if there are any differences between sheep and goats in the efficiency of nematophagous fungi to control nematode parasites.
- Determine whether there are differences between two different means of fungal spore delivery (daily supplement and incorporated into a feed block).
- Establish the use of the fungus for worm control on small-scale and large institutional farms.

- Establish the use biological control for nematode parasites as an integrated option simultaneously with other non-chemical methods of worm control.

This information provides a better understanding of the limitations and possibilities for the control of helminth parasites in small ruminants in Malaysia. This will hopefully lead to the development of a programme which will ensure the viability of sheep and goat production systems in this country.

Results and Discussion

Field investigations conducted in the early – mid 1990's showed that anthelmintic resistance was common amongst small ruminant farms in Malaysia. Concern was also growing that despite research that demonstrated the benefits of a number of alternative parasite control methods, owners and managers of small ruminant production units were still resorting to frequent anthelmintic treatment. This concern was particularly relevant to the situation on the government owned small ruminant breeding farms, whose main purpose was to produce stock for the dissemination to the small-holder farmers throughout the country. Thus a programme of research was initiated that forms the basis of this doctoral dissertation. The work is embodies 2 complementary areas of research activity:

Anthelmintic resistance

The findings of this research are presented in detail in papers 1 – III. In summary the results showed:

Paper 1: Chandrawathani, P., Adnan, M. & Waller, P.J. 1999. Anthelmintic resistance in sheep and goat farms on peninsula Malaysia. *Veterinary Parasitology 82,* 305-310.

An anthelmintic resistance survey was conducted in Peninsula Malaysia, involving 39 sheep farms and 9 goat farms, using the conventional faecal egg count reduction test (FECRT). In carrying out this survey, it was found that *H. contortus* was the most common nematode parasite of sheep and goats on all farms (85-90%). It was also concluded that approximately 50% of the sheep farms had parasites that were resistant to the benzimidazole anthelmintics, which are some of the cheapest and commonest anthelmintics used in Malaysia. More than 50% of the farms showed levamisole and closantel resistance, or suspected resistance, and one farm showed resistance to ivermectin. Although the overall number of goat farms was much less, anthelmintic resistance to benzimidazole (75%) and levamisole (57%) was at a high level. This work demonstrated that there was evidence of an escalation in the prevalence of anthelmintic resistance in Malaysia. Clearly the situation was as of the same magnitude of importance as the more extensively researched and reported problems of resistance in the sheep industries of the temperate regions of the world. Also it demonstrated that the small ruminant industries in Malaysia were facing major problems with regards to nematode parasite control in small ruminant flocks, and thus successful further development of these livestock industries.

Paper II: Chandrawathani, P., Waller, P.J., Adnan, M. & Höglund, J. 2003. Evolution of high level, multiple anthelmintic resistance on a sheep farm in Malaysia. *Tropical Animal Health and Production 35*, 17-25.

The FECRT was carried out on a large government small ruminant farm used as a breeding and distribution centre to small-holder farmers in Peninsula Malaysia. This farm had a history of anthelmintic resistance (resistance to levamisole and suspect resistance to moxidectin and closantel) to the major drug groups and attempts were made to alleviate the problem by implementing several alternative parasite control measures. Over a period of 3 years these strategies included rotational grazing, introducing a tropical, more resistant breed of sheep (Santa Ines and Barbados Black Belly), and introducing medicated urea molasses blocks to improve the nutritional status of the animals. During the same time, the use of anthelmintics was reduced. However, faecal egg count monitoring of selected sub-flocks on this farm showed that parasite infections remained generally high (800-5000 epg), with *H. contortus* comprising 87-97% of the nematode population. In this investigation, the results of a FECRT showed resistance to benzimidazoles at both the single and double recommended dose rate, as well as when 2 single doses were given 24 hours apart. Resistance to levamisole and oxyclozanide were recorded at single and double dose rates, and resistance to ivermectin at the single dose rate. Moxidectin was found to exhibit suspect resistance status. This indicates that anthelmintic resistance can very swiftly escalate to encompass all currently available anthelmintic groups, even when specific precautions have been taken. The consequences for this situation are most serious – not only for the government breeding farm, but also for the small-holder farmers who are the recipients of these animals.

Paper III: Chandrawathani, P., Yussof, N. & Waller, P.J. 2004. Total anthelmintic failure to control nematode parasites of small ruminants on government breeding farms in Sabah, East Malaysia. *Veterinary Research Communications*, (in press).

Government small ruminant breeding farms in East Malaysia have not been previously involved in anthelmintic survey investigations. As a consequence of the above study, a comprehensive investigation was carried out to investigate the anthelmintic resistance status on the entire government small ruminant breeding farms on Sabah. These farms had reported continuing high levels of mortalities (approx. 20% per year), despite attempts at good management and health control. However from interviews it was established that intensive anthelmintic dosing programmes to control helminthiasis, (approx. every 4 weeks), with no short term rotational grazing, were common practices. As such the animals were on permanent pastures, or rotated based on availability of pasture. Again, as for the West Malaysian farms, the worm population was dominated by *H. contortus* (90%). Faecal egg count reduction tests (FECRT) conducted on the 4 government goat farms and the only government sheep farm, showed resistance to all the major anthelmintic drug groups, viz: closantel, levamisole, benzimidazole and ivermectin. This clearly indicates complete chemotherapeutic failure to control *H. contortus* on all the government small ruminant farms in Sabah. It is certain that the annual losses, which approximate 20% of these government farms, are mainly parasite induced. Thus the small ruminant breeding activities on Sabah are clearly economically unsustainable. Strong and concerted efforts are now being directed towards policy makers and managers of the small ruminant industry in Malaysia to completely overhaul the ways and means by which it is considered that these industries will be brought back to sustainability. This work has clearly indicated to the senior livestock management bureaucracy in Malaysia the need for change.

Biological Control

As a means towards developing sustainable parasite control of small ruminants in Malaysia, a series of studies were initiated on biological control. These investigations were structured to initially investigate the presence of nematophagous fungi in Malaysia. Then through a logical sequence of laboratory, pen and plot studies, to finally evaluate this non-chemical option for nematode parasite control under practical farming conditions in Malaysia. These investigations are embodied in the following 3 papers:

Paper IV: Chandrawathani, P., Jamnah, O., Waller, P.J., Höglund, J., Larsen, M. & Zahari, W.M. 2002. Nematophagous fungi as a biological control agent for nematode parasites in Malaysia: a special emphasis on *Duddingtonia flagrans*. *Veterinary Research 33*, 685-696.

As biological control is one of the options in worm control that had not been tried in Malaysia, it was with keen interest that we embarked on studies relating to this novel and exciting approach. Initial studies were based on the survey of approximately 3,000 faecal samples from various livestock and wildlife species to detect the presence of nematode trapping fungi that might be found in the tropical environment. Several species of nematode trapping fungi were isolated, such as *Arthrobotrys* spp., but just one isolate of *D. flagrans* was made. Various substrates were tested in the laboratory with the aim of producing sufficient fungal material for testing *in vivo* in penned sheep and goats. The media tested (eg. padi rice, wheat and millet) were chosen, as these are cheap and readily available in Malaysia. Further progress in scaling-up the production of *D. flagrans* spores were made. However, the amount of spores produced was very low, with this tropical *D. flagrans* isolate. Nevertheless sufficient material was obtained to conduct pen feeding trials using sheep infected with *H. contortus*, to test the efficacy of *D. flagrans* fed as a grain supplement, or incorporated into a feed block. Results showed that the fungus survived gut passage in sheep and a dose of $1x10^6$ spores / animal / day and reduced the percentage of infective larvae in faecal cultures by more than 90%. These encouraging results paved the way for further studies on the use of the fungus for local use in the small ruminant industry plagued by severe anthelmintic resistance problems.

Paper V: Chandrawathani, P., Jamnah, O., Waller, P.J., Larsen, M., Gillespie, A. & Zahari, W.M. 2003. Biological control of nematode parasites of small ruminants in Malaysia using the nematophagous fungus *Duddingtonia flagrans*. *Veterinary Parasitology 117*, 173-183.

As production of chlamydspores using the local isolate of *D. flagrans* was very poor, it was deemed more advantageous to obtain commercially produced *D. flagrans* spore material (Christian Hansen Biosystems AB) for further trials in Malaysia, as it would save time and effort. Pen studies in sheep and goats were carried out using spores as a daily feed supplement as well as incorporated into feed blocks. The spore dose rates used were 125,000 spores /kg/liveweight /day and 250,000 spores /kg/liveweight /day and both these investigations proved to be very successful in the reduction of nematode infective larvae from faecal cultures. At the lower dose rate this reduction was between 80 – 90% compared with the pre-treatment levels, and at the high dose rate there was almost complete suppression (>99% reduction) of larval recovery. A small paddock study was carried out for a period of approximately 3 months, to further assess the effects of using fungal spores to control nematode parasites in grazing animals. Three groups of sheep were fed either a daily feed supplement without fungal spores, a supplement with spores, or offered fungal blocks. Each treatment group were managed on a separate set of paddocks. The dose rate of the latter two groups was 500,000 spores / kg liveweight / day. Egg counts of the two fungal treatment groups remained relatively constant (1500 – 2200epg), but the percentage larval recovery from their faeces dropped to negligible levels. The FAMACHA scores of the 2 groups of animals showed that anthelmintic treatment was not necessary. However with the control animals, faecal egg counts steadily increased to exceed 4000epg within 1 month of commencement of the trial. There was one death in this latter group, due to haemonchosis, and salvage anthelmintic treatment was imposed. Although this immediately reduced their egg count, within 1 month these had again escalated to approximately 1000 epg and a second anthelmintic treatment was given. Tracer worm counts showed that the pastures grazed by the two fungal treatment groups had consistently negligible levels of *H. contortus* larvae, whereas the controls had levels exceeding 10 fold the number on the fungal treatments. Thus both the pen and field studies showed unequivocally that fungal spores can reduce infective larvae in faeces of animals, both goats and sheep, and over a period of three months, managed to reduce worm burdens in treated sheep by reducing pasture contamination. In contrast, sheep that were grazing and not fed the fungal spores had high faecal egg counts and had to be treated twice during the course of the trial.

Paper VI: Chandrawathani, P., Jamnah, O., Adnan, M., Waller, P.J., Larsen, M. & Gillespie, A.T. 2004. Field studies on the biological control of nematode parasites of sheep in the tropics, using the microfungus *Duddingtonia flagrans*. *Veterinary Parasitology 120*, 177-187.

The final phase of this project was to test of the use of the fungal spores under commercial farming situations in Malaysia. In this respect, two simultaneous trials were conducted in 2 government sheep farms (Infoternak and Chalok) located in different geo-climatic regions of West Malaysia and approximately 300 km separate from each other. The Infoternak trial (48 weeks) and the Chalok trial (43 weeks) compared nematode parasite control in separately managed flocks of young sheep, either short-term rotationally grazed around a suite of 10 paddocks in addition to receiving a daily supplement of *D. flagrans* spores (500,000 spores /kg liveweight/day), or similar groups of sheep being rotationally grazed alone. The group sizes were 20 and 30 lambs for the Infoternak and Chalok trials, respectively. The prevailing weather conditions at Infoternak farm were of below average rainfall conditions for the most of the trial. As a consequence, only very low worm infections (almost exclusively *H. contortus*) were acquired by the 17 sets of tracer lambs that grazed sequentially with the experimental lambs. However on all except 2 occasions in the early part of the trial, the mean tracer worm burdens were significantly lower ($P<0.05$) and the experimental lambs grew significantly better ($P = 0.054$) in the fungus treatment group. Rainfall at Chalok farm during the course of the trial was also below average. As a consequence infectivity of pastures was assumed to be relatively low based on faecal egg counts of the experimental sheep, which following an anthelmintic treatment prior to allocation, remained very low in both treatment groups. Faecal egg counts of undosed replacement lambs in the latter half of the Chalok study, showed a progressive increase in the control group to levels exceeding 3000 epg, whereas the fungus group remained static at approximately 500 epg. These findings supported the previous investigations and showed that over a period of 1 year, the worm burdens in the animals in the biological control treatment were greatly reduced. Both these investigations showed that the combination of biological control plus short-term rotational grazing points the way to an integrated, sustainable means of nematode parasite control for small ruminants in Malaysia.

Summary and Concluding Remarks

On a global basis, gastrointestinal parasitism emerges with the highest global index as an animal health constraint in resource poor communities (Perry *et al.*, 2002). Gastrointestinal parasitism encompasses haemonchosis in sheep, goats and camels and toxocariasis in cattle and buffaloes. The other nematodes implicated in causing gastrointestinal parasitism of economic importance are *Trichostrongylus*, *Teladorsagia/Ostertagia*, *Oesophagostomum* and *Strongyloides*. The high score is a reflection of the wide geographical distribution of nematode parasites in livestock, the wide host species range and the importance given to its high economic impact on the poor farmer level in all production systems particularly in small ruminants. However, the effects of parasitism is difficult and elusive to quantify and thus can be missed in many production systems and planning programmes. Gastrointestinal parasitism and specifically haemonchosis has been implicated as one of the top 10 diseases or pathogens in livestock in pastoral systems and mixed crop-livestock systems sharing the importance with other diseases like ectoparasitism, trypanosomiasis, fascioliasis, nutritional deficiencies, respiratory syndromes and foot and mouth disease. In a majority of clinical gastrointestinal parasitism cases, poor nutrition and respiratory infections are also commonly seen and sometimes these exacerbate the effects of parasitism, causing high mortalities.

Thus it is clear that parasitic diseases are a major cause in limiting productivity and this is especially so in the poor developing countries, which have inadequate infrastructure, health care and extension services for farmers. It is also timely that the importance of gastrointestinal parasitism, namely haemonchosis is recognised as one of the world's greatest killers of small ruminants and due emphasis should be given for research programmes aimed at combating this problem. In most developed countries, the problem of managing haemonchosis has relied heavily on the use of anthelmintics, as it is the easiest and generally the cheapest means available. However excessive reliance on anthelmintics alone has given rise to severe anthelmintic resistance, deeming it quite useless to go on treating. This scenario is even seen in the tropical, less developed countries of the world, including Malaysia. Baseline data that has been collated over time on anthelmintic resistance, stands us in good stead to further explore the possibilities of novel approaches to worm control. A good network for the sharing of information has led us to experiments with several new methods in worm control over the past decade, such as rapid rotational grazing, the use of medicated urea molasses blocks and breeding more worm resistant tropically adapted animals. In Malaysia, the extensive problem of multiple, high-level anthelmintic resistance created an opportunity to embark on research into the use of the biological control agent of nematode parasites, namely *D. flagrans*. The studies carried out in pen and field trials conclusively proved that it could be successfully deployed in most production systems which use grazing in Malaysia. For small-holders who graze their animals along roadsides and under plantation crops, it is an excellent means of reducing pasture contamination. If the fungal spores are incorporated into feed blocks, the effect is likely to be enhanced because of the better nutrition that will help alleviate impending helminthiasis by improving immunity in the animals. In large-scale government farms where pasture paddock grazing is practised, feeding fungal spores via a daily feed supplement would also help to reduce pasture infection. In these systems, two or more methods can be used for worm control and anthelmintics can be utilised to the minimum.

Perspectives and Future Research

Clearly the core of the problem with regards to directing the small ruminant livestock sector in Malaysia towards sustainability, is attempting to address the urgent issue of multiple, high level anthelmintic resistance that is now entrenched on the large government breeding farms (Papers II and III). Although the future appears bleak – because on economic grounds alone the government farms cannot sustain annual stock losses in the order of 20% largely due to total anthelmintic failure (Paper III) – the problem is not insurmountable.

If remedial action is to be taken, it needs to be immediate and decisive. These should include the following for each government breeding farm:

Immediate

- A substantial reduction in the stock numbers on all farms. This will provide more ease and flexibility to undertake the other measures.

- Attempt to remove resident worm infections in all stock. In the first instance this seems likely to be a difficult task, even using a combination of all available anthelmintics. However other older classes of drugs with known good efficacy against *H. contortus*, which have not been used in Malaysia should be tried, such as the organo-phosphate anthelmintics (napthalophos), or phenothiazine. These drugs, particularly the former, now play an important role in the management of multiple, high level resistance in nematode parasites of sheep in Australia (Dobson *et al.*, 2001)
- As a means of breaking the major infection cycle, prevent all pasture grazing for 2 months by implementing cut-and- carry for shed feeding of all animals during this period. The pastures selected for cutting should be from a location that has been ungrazed (thus uncontaminated) by small ruminants for the last 2 months. Ecological studies in the wet tropics have shown that survival of the free-living stages of *H. contortus* is very short, with the majority of infective larvae disappearing from pasture within 4-6 weeks (Banks *et al.*, 1990; Sani & Chandrawathani, 1996).

Short-term

- Implement and strictly adhere to the short-term rotational grazing strategy, as developed for parasite control in small ruminants in the wet tropics (Barger *et al.*, 1994; Sani & Chandrawathani 1996), and shown to work effectively in this study (Paper VI). This requires the subdivision of available pastures into small plots. Animals are then moved around these pastures in quick succession (3-4 days grazing only on each plot), returning to their original plot after approximately 30 days.
- Monitor faecal egg counts on a regular (initially every 4-6 weeks) basis in a sentinel group of young sheep. This should be coupled with the use of the FAMACHA procedure (Papers V and VI).
- Introduce molasses/mineral feed blocks to improve the general nutrition of the animals. Trial the use of medicated (fenbendazole) blocks for one grazing cycle (4 weeks) every 6 months. Previous work has shown that by using a benzimidazole anthelmintic to which resistance has developed, from non-persistent (oral drench) to persistent (daily administration in a feed block) use, can restore anthelmintic efficiency (Knox, 1996).
- Implement biological control with the micro-fungus *D. flagrans*, in addition to rotational grazing. This has been shown to be a useful adjunct to worm control of sheep and goat parasites (Papers V and VI).

Long-term

- Attempt to change the genotype of the animals, particularly sheep. The sheep genotype (Dorset Horn) of the government breeding farm in East Malaysia, is neither tropically adapted, nor naturally resistant to *H. contortus*. Changing the genotype of sheep should not prove to be particularly difficult, as there exists some excellent replacement stock (Barbados Black Belly) on government farms on Peninsula Malaysia eg. Chalok government farm (Kuala Terengganu)

(Paper VI). These animals not only are adapted to thrive in the humid tropics, they also have a high degree of innate resistance to *H. contortus*.

The priority areas of future research in attaining sustainability in the small ruminant industries in Malaysia should be directed towards the practical aspects of this endeavour. This will include monitoring egg counts and anaemia levels to ensure that adequate control against *H. contortus* is being maintained, but also monitoring the anthelmintic resistance status to determine whether reversion towards drug susceptibility occurs. Other adjuncts to control should also be trailed, if and when, they are appropriate (eg. *H. contortus* vaccine).

However, serious attention also needs to be directed towards assisting the small-holder farmers with the likely problem of anthelmintic resistance of parasites that they have unwittingly acquired, together with their animals from the government farms. First and foremost, veterinary advisers should be aware of the problem of resistance and thus should not as a matter of course simply recommend anthelmintic treatment if parasite problems are suspected. Correct diagnosis is important, based clinical signs, history and particularly on faecal nematode egg counts. In the majority of cases when animals are suffering with “ill thrift”, or diarrhoea, nutritional inadequacies rather than internal parasites is the problem. Surveys of anthelmintic resistance in the general farming community (Paper I), should continue, but also including the small-holder farmer using the modified “Drenchrite” assay that has been used with good success in obtaining estimates of anthelmintic resistance amongst small-holder sheep and goat farmers throughout Asia.

According to the critique of Asian small-ruminant research priorities, which emerged from a recent ACIAR sponsored International workshop (1996) in Indonesia, there is a need to identify indigenous resistant genotypes of sheep and goats and to exploit these through selective breeding using recent developments in quantitative and molecular genetics. It was also considered important to determine the effects of improved nutrition on the ability of small ruminants to resist parasitic infection. Research in this area should concentrate on the nutritional approaches to increase productivity, which are technologically appropriate in the various production systems in different geographical regions of Asia, concentrating on using low cost supplements and agricultural residues. In the final outcome, an assessment as to how adequate nutrition affects phenotypic expression of improved resistant geotypes needs to be made. Meanwhile, as these are long-term measures for worm control, the more practical aspects of management and grazing of small ruminants throughout Asia need to be pursued. These include knowledge on epidemiology under various climatic and topographical situations, anthelmintic usage despite the looming problems with resistance and the use of biological control needs to be integrated with suitable combinations to be successful. This needs the commitment and dedication of the farmer as well as the participatory organisations in all countries throughout Asia.

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