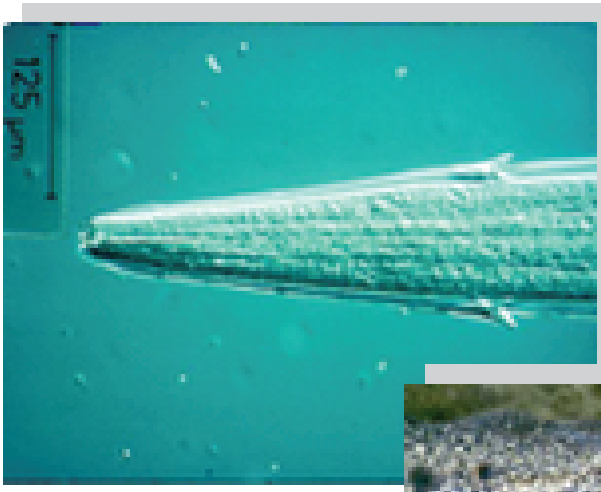
# ***Guidelines*** *resistance management and integrated parasite control in ruminants*




FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

ROME, 2004

# Guidelines resistance management and integrated parasite control in ruminants

Animal Production and Health Division
Agriculture Department
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2004

## FOREWORD

The demand for meat has grown rapidly in developing countries over the past 20 years and it is likely that this trend in consumption of livestock products will continue during the next three decades. It is estimated that some 360 million cattle, 560 million sheep and goats and 190 million more pigs would be necessary to satisfy the world's nutritional demand. This growth in livestock products is an opportunity for the 675 million rural poor who depend on livestock to improve their living conditions. Substantial evidence exists that livestock production is a useful tool for income generation and subsequent poverty reduction for the landless poor, women and small and medium scale farmers. This does, however require an enabling environment including access to credit, development of infrastructure and the supply of animal production and health services. Farmers also need access to information on disease control and livestock management in order to make informed decisions about where to invest their resources to increase production and productivity.

The predicted increase in the animal population is not without risks. There are environmental effects caused by overstocking during dry or cold periods or where grazing is conducted on land cleared from rainforest, and the transmission of animal diseases could also affect food security and safety.

Globally, parasitic and other endemic diseases continue to be a major constraint on profitable livestock production and productivity, particularly in developing countries. They are rarely associated with high mortality and usually the only clinical signs and effects are lower outputs of animal products, by-products, manure and traction, all contributing to production and productivity losses. Throughout the last century the pharmaceutical industry has continuously developed effective new drugs and parasiticides for the treatment and control of a large number of economically important diseases and as a result have reduced associated production and productivity losses. Ready access to chemicals (insecticides, acaricides and anthelmintics) and the ease with which they can be applied, combined with the immense progress made in the knowledge of the epidemiology of parasites of ruminants, has led to a period of relative success in the control of endo and ectoparasites. This was particularly true in more industrialized livestock production systems.

However, the false assumption that parasite control is easily accomplished by the use of chemical means alone has lead to the development of parasite resistance, creating ecological imbalances and leaving drug residues in meat, milk and wool, resulting in the erosion of farmer confidence in the efficiency of current and future parasite control programmes.

In view of this, FAO has promoted partnerships with research institutions that have systematically investigated and tested alternatives for diagnosis and control of parasites. It is clear that parasite control needs to move away from the reliance on parasiticide treatment only, to a more integrated and sustainable form of parasite control.

These guidelines, which are the result of a three-year collaborative effort between the Animal Health Service of FAO and the FAO-Working Group of Parasite Resistance will assist veterinarians and farmers to make qualified decisions regarding the most appropriate diagnosis and control strategy for their production system.

I would like to extend my thanks to all the contributors to these guidelines but particularly to the FAO-Industry Contact Group of the Veterinary Pharmaceutical Industry for their active collaboration and positive criticism of the guidelines.

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# EXECUTIVE SUMMARY

## Introduction

Population growth, rapidly increasing urbanization and growth in income in developing countries are creating a tremendous increase in the demand for food of animal origin. This "livestock revolution" is demand-driven, illustrated by the fact that meat consumption in developing countries grew approximately three times more than it did in the developed world during the period from the early 1970s to the mid 1990s. During the same period the production of animal food products also grew most dramatically in the countries with the increased demand. In fact the meat production in developing countries, with the exception of sub-Saharan Africa, grew at more than five times the rate in the developed countries.

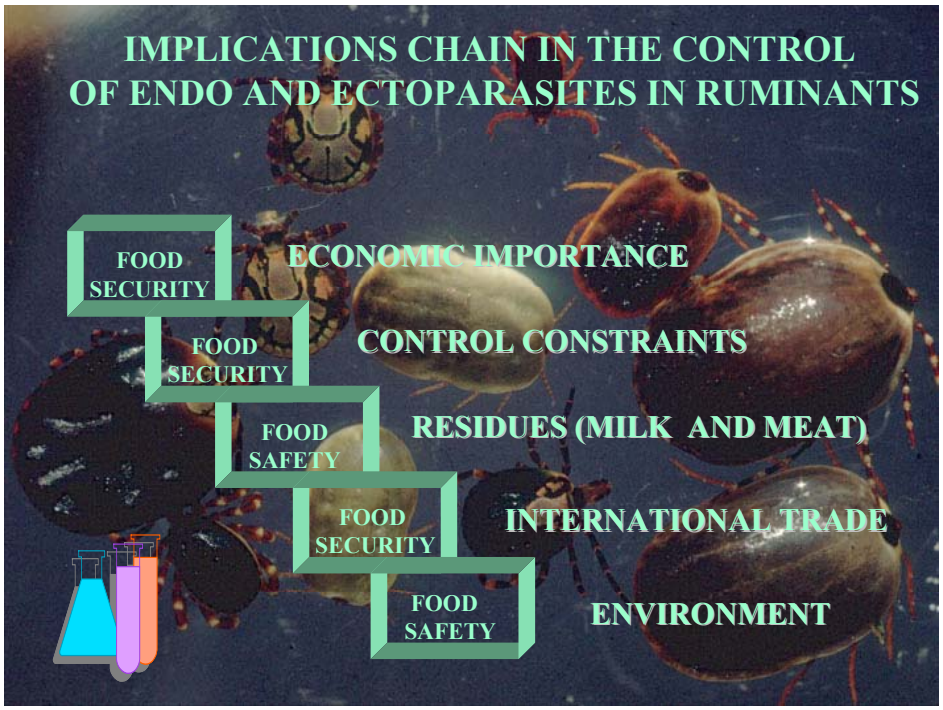
The projections of the International Food Policy Research Institute (IFPRI) using IMPACT (International Model for Policy Analysis of Agricultural Consumption) are that the consumption of meat and milk in developing countries will grow by about 3 percent per year between now (2003) and 2020 (Delgado *et al.*, 1999).

It is likely that this will improve the livelihood of small and medium scale market oriented farmers but only if an enabling environment is created including access to credit, development of infrastructure and animal production and health services. Farmers need access to information regarding disease control and livestock management supporting their ability to decide where to invest their resources to increase production and productivity.

## Parasitic diseases. A global problem

Globally, parasitic and other endemic diseases continue to be a major constraint on profitable livestock production. They are rarely associated with high mortality and easily identifiable clinical signs and their effects are usually characterized by lower outputs of animal products, by-products, manure and traction, all contributing to production and productivity losses. However, parasitic diseases are repeatedly identified by livestock owners, particularly small ruminant producers, as constraints to animals reaching their full production potential. It is generally accepted that the cost of control of most parasitic and endemic diseases is the responsibility of the animal owner.

Although specific parasites may be distributed throughout the world, they have different impact according to production system, management and geo-climatic conditions. In industrialized production systems the greatest impact is not through loss of productivity but more likely through the cost of control, with considerable resources spent on anti-parasiticides. There is however, a possible shift in this as the importance of parasite resistance continues to grow, resulting in decreased efficiency and a likely increase in production losses in industrialized production systems. For example there is a real risk that the dependency of Australian wool growers on drenching to control worms will become untenable within 10 years, due to anthelmintic resistance. Consequently the cost of gastro-intestinal nematodes to the Australian wool industry, currently estimated at AU$ 220 million, could soar to AU$ 700 million within the same time frame.




In mixed farming and extensive grazing systems in the tropics and subtropics the environment is usually ideal for parasite development and the variety and prevalence of parasitic diseases, which are much greater than in temperate climates, add substantially to the disease problems. The result is often reduced productivity expressed in the form of reduced weight gain, delayed and weak oestrus and lower calving rates, but perhaps an even greater impact is the lost production potential.

The importance of ticks and tick-borne diseases (TBDs), tsetse flies and trypanosomosis has been recognized for many years, and the structure and functions of many government veterinary services in the tropics and sub-tropics reflect the fact that the public good required them to assume responsibility for the control of these diseases. There is however, also a growing awareness of the importance of less dramatic (overtly damaging) parasitic infestations (helminths, flies, mites, lice) that are part of the multitude of factors that constrain the productivity of livestock in developing countries.

An Office Internationale des Épizooties (OIE) commissioned, FAO implemented survey of OIE member countries tried to assess the perception of the national veterinary services concerning the importance and ranking of parasitic diseases, dividing them into five groups; helminths, ticks, mange mites, flies and lice (Nari and Hansen, 1999). Unfortunately, more than 65 percent of the countries surveyed have not carried out any detailed studies, and the result is based on estimates. In order to establish a balanced and accurate profile of the member countries' perceptions, the rankings of the parasite groups were weighted according to the number of 1$^{st}$, 2$^{nd}$, 3$^{rd}$ and 4$^{th}$ order rankings received and allocated points accordingly. This led to helminths being assigned 38.9 percent of the total points for all categories, ticks, 23.7 percent, mange mites 15.4 percent, Diptera of veterinary importance, 15.2 percent and lice 6.8 percent, reflecting the opinion of the OIE member countries replying to the questionnaire.

Many of the countries (85.7 percent) considered that they have a significant market for antiparasitic agents. Because chemical compounds have, with very few exceptions, been the sole means used by agricultural and livestock producers for controlling parasitic diseases, comparing

the market for antiparasitic agents with that for other consumables (antibiotics, vaccines, sera) is a good indicator of the relative importance attributed to parasite control by the livestock owners of different countries. In 2000, the global market for animal health products was in the region of US$11 030 million (IFAH, 2002). During the same period, the demand for antiparasiticides represented 28.1 percent of the total sales, even though it differed from one geo-economic area to another depending on production systems and the composition of parasite populations. Cattle and sheep, with one third of all sales, still represent an important portion of the market of animal health products.

## Economic impact

Parasites affect host behaviour and health in a variety of ways that directly or indirectly reduce the productivity of the animal. The major effects are related to reduced food intake and to altered protein metabolism. Protein losses caused by parasites stimulate the synthesis of replacement proteins at the cost of energy and protein, which would normally be channelled into the production of wool, meat and milk.

The losses can be categorized into those affecting the productivity of individual animals and those influencing herd productivity. Among the first are mortality, lower market value (slaughter house condemnations), reduction in body weight gain, reduced wool and milk yield, reduced draught power, reduced dung output (fuel, fertilizer) and reduced efficiency in feed conversion. The second category includes the reduced productive life span of animals, the disturbance of the genetic selection effort and the possibility of immunosuppression and increased susceptibility to other diseases. In addition there may be associated costs of parasitic diseases of livestock on human welfare because of a reduced supply of protein, as well as adverse effects on community development related to reduced traction and dung output. The picture would not be complete without a calculation of the effect that handling and treating of livestock to control parasites has on their productivity, as well as the capital cost of equipment and parasiticides.

Thus there is general agreement that parasitic diseases are important and cause considerable losses in the developing world, but the extent of the losses needs to be accurately calculated. It has however, proved difficult to evaluate the economics of diseases and animal health interventions due to the complex relationships between animal health and their impact on production, access to markets and the non-production benefits of livestock. In addition the knowledge base regarding economics of treatment is inadequate.

Estimated or potential losses caused by endo- and ectoparasites have been compiled from published material. The methods used to assess the losses are often subjective but they do provide an estimate of the impact caused by these parasites. In Nigeria one study estimated that 11 percent of the value of sheep and goat production was lost per year and another estimated the annual losses caused by GI nematodes in northern Nigeria to be approximately US$ 40 million. In Kenya, losses due to *Haemonchus* infection have been estimated as US$ 26 million annually. The losses experienced in an industrialized production system can also be substantial. During the late 1980s, the United States Department of Agriculture estimated losses due to biting flies at approximately US$ 500 million per year, mites US$ 259 million, ticks US$ 104 million, lice US$ 38 million and other insects US$ 296. The annual losses in ruminants caused by GI nematodes were estimated at US$ 300 to 400 million and the expenses incurred to control worms were about US$ 140 million. Similarly the livestock industry in Australia has incurred substantial losses. Here, the annual cost of major parasites was calculated in 1994 using a costing model that separated production losses from the control costs. According to this report the losses caused by ticks to cattle production was AU$ 132 million and the losses for sheep, including worms, lice and blowflies were AU$ 552 million.

Some impact assessments have also been made in Brazil, Argentina and Uruguay. It is known that the mortality in untreated sheep flocks in Rio Grande do Sul is approximately 40 percent. Those surviving will produce 300 to 500 g less wool and 2 to 5 kg less meat per animal. In addition the mating age will be delayed for 6 to 12 months. For Rio Grande alone, with more than 11 million sheep, the losses will be substantial if no alternative treatment strategies become available. The impact in Argentina has been calculated for four geographical zones with different degrees of anthelmintic resistance. The total annual loss in this country attributable to parasitism of sheep is approximately US$ 20 to 21 million. Predictions in Uruguay have shown that the estimated mortality in a scenario in which sheep are not treated to control parasites will be approximately 50 percent, in addition to a reduced body and fleece weight in survivors of 25 percent initially and 29 percent after the first year. The estimated total losses of fleece weight alone are estimated at 18 million kg, which amounts to an economic loss of about US$ 42 million.

## Chemical control

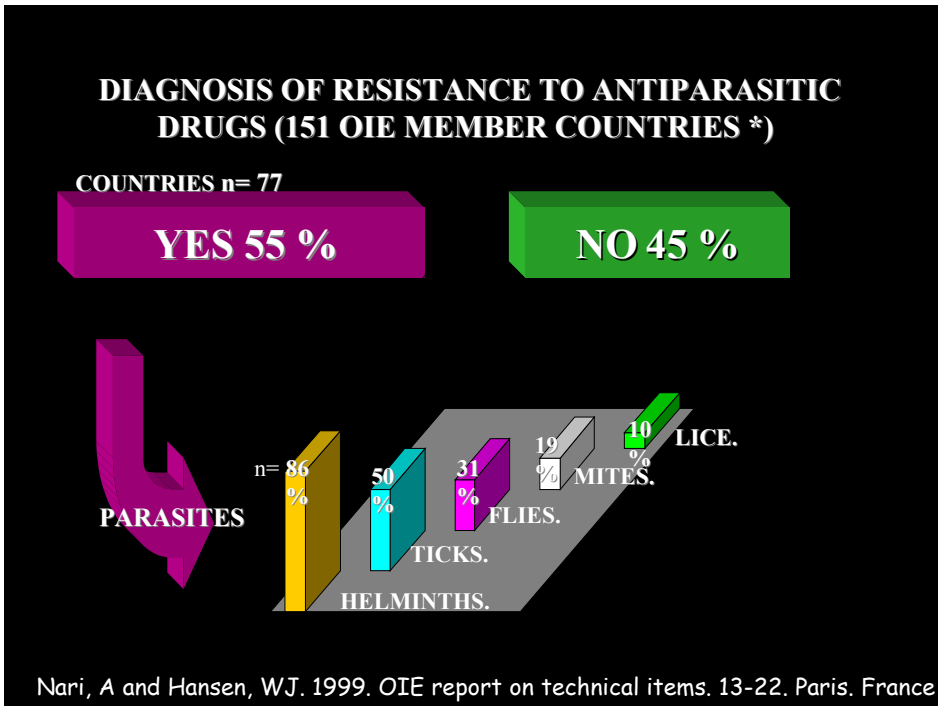
There is a tendency for host-parasite relationships to evolve into a situation of endemic stability, in which the host, parasite and vector are all present, yet clinical disease occurs rarely due to exposure of young animals with subsequent immunity. It is well known that many indigenous livestock breeds are innately relatively resistant or resilient to parasites. With the intensification of production and the introduction of new livestock breeds into areas with populations of parasites to which they were susceptible, the need for control of parasites and pests increased. Throughout the last century the pharmaceutical industry was able to continuously develop effective new compounds for the treatment and control of a large number of economically important parasites and their associated diseases.

Ready access to these efficient drugs and chemicals and the ease by which they could be applied, combined with improved diagnostic tests and the immense progress made in our knowledge of the epidemiology of parasites of ruminants, led to a period of relative success in the control of pests, particularly in industrialized livestock production systems. However, the false assumption that parasite control is easily accomplished by the use of chemical means alone, without an epidemiological database, was promoted. This has prevented or delayed the epidemiological studies that are a pre-requisite for effective control (Barger, 1998).

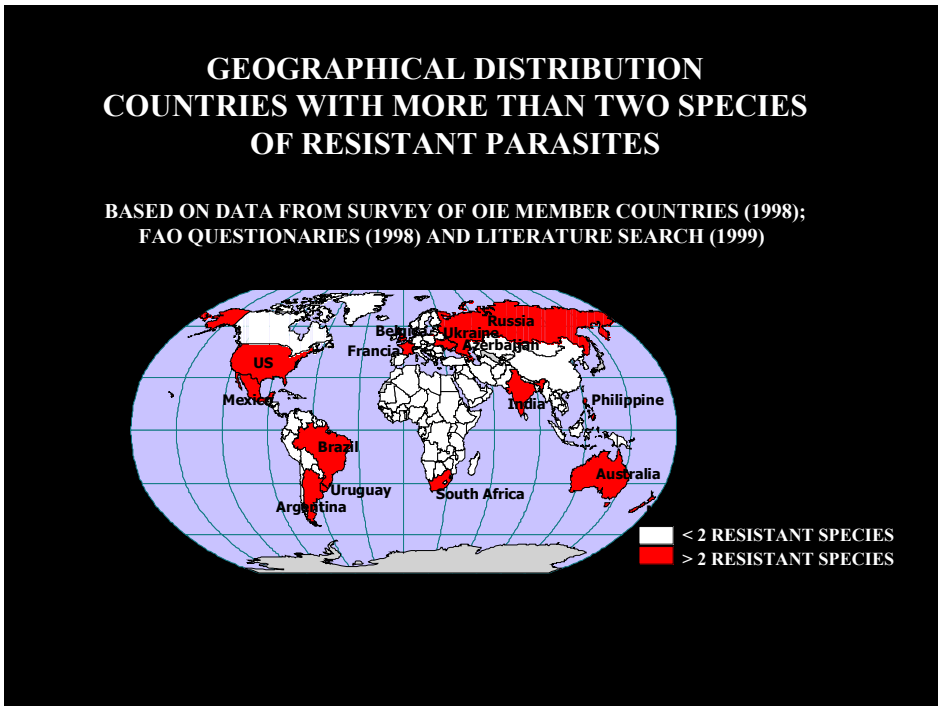
Current and future parasite control programmes are complicated by the occurrence of resistance of all the economically important parasite species of sheep and goats and some of the parasites of cattle to all four groups of anthelmintics. In the last decade, almost one century after the first reports of arthropod resistance to pesticides for agricultural use (Melander, 1914), we have seen an almost exponential increase in new cases of resistance in various parasite species affecting agriculture and public health in many geo-climatic areas of the world (Georghiou and Lagunes-Tejeda, 1991; Nari *et al*., 1996; Sanyal, 1998; Waruiru *et al*., 1998; Echevarria *et al*., 1996; Maciel, 1998).

## Parasite resistance

The definition of resistance used in this report is the detection, by means of sensitive tests, of a significant increase in the number of individuals within a single population of a species of parasite that can tolerate doses of drug(s) that have proved to be lethal for most individuals of the same species.




Resistance is probably an inevitable consequence of the use of antiparasiticides, and there is substantial evidence that when a parasite has developed resistance to one chemical or drug from a certain group, it will usually also be resistant to other products from the same chemical group. The speed with which a parasite develops resistance will depend on how severe the selection pressure is on the parasite population. It is known that this is linked to frequency of treatment. Widespread, excessive use of antiparasitic compounds, without consideration of the epidemiology or ecology of the parasites, has led to the development of resistance of parasites to all existing classes of drugs. Depending on the mode of inheritance of resistance, the common practice of under dosing can increase the selection pressure for resistance. There is also evidence that strategic treatments, particularly at times when the free-living stages of gastro-intestinal nematode populations have been small, have contributed to the development of resistance (van Wyk, 2002). Cheap, sub-standard drugs have been available on the market and this is quite likely to have contributed to the problem. However, what remains the key issue is that a moderate loss of efficacy (generally not perceived at field level) represents an enormous loss in terms of an antiparasitic agent's cost-effectiveness, and a significant step along the path of no return in terms of parasite resistance. For example, a reduction of 20 percent in the efficacy of an anthelmintic or acaricide at the field level not only means a loss of efficacy equivalent to US$ 20 for every US$ 100 invested in veterinary products, but it also represents a genetically based problem for the future sustainability of the whole chemical group (Nari and Hansen, 1999).




In theory, any population of parasites can develop resistance to any chemical compound or group of compounds. When resistance invalidates the use of an acaricide, traditional practice by farmers has been to change to an acaricide of a different chemical group. However, it is frequently the case that, due to lack of knowledge and training, this has often been only a change to a different trade name of the same group. A recent survey of resistance to parasiticides carried out by the OIE in 77 of their 151 member countries revealed that those ecto- and endoparasites with the heaviest relative impact on production are also those that have the widest distribution of resistance. Eighty six percent of these countries have diagnosed resistance to anthelmintics, 50 percent to acaricides in ticks, 31 percent to insecticides in flies of veterinary importance, 19 percent in mange mites to acaricides and 10 percent in lice to insecticides. There is also a problem with overlapping of resistance across parasite groups. Twenty four percent of the countries reported resistance in more than three groups of parasites, with 22 percent having it in helminths and ticks which are considered to be the two groups of greatest economic impact.

## Resistance diagnosis and management

Diagnosis and control are two essential elements of any health programme, but in the case of resistance to antiparasitic agents, their relationship becomes even more crucial. In this case, it is not sufficient to merely know the species of parasites, it is also necessary to determine, as early as possible, the degree of sensitivity of the parasite populations to the available chemical groups (side, cross, or multiple resistance).

In the OIE survey referred to above, 54.5 percent of the replying countries indicated that they had diagnosed resistance in at least one group of parasites. There is almost certainly under-recording of the phenomenon, since the resistance profiles of helminths, for example, are not normally reported to government veterinary services. It was also observed that 24.4 percent of the replying countries have to cope with the problem of resistance in more than three groups of parasites. The problem of resistance in several parasite species is rarely taken into account in the development of control programmes. It is a more serious problem when the same products are used to control different species of parasites and where the epidemiology of the different species

infestations also differs. Thus it is increasingly common for the producer to experience "multi-resistance," which has developed simultaneously, not only to several groups of antiparasitic agents (Nari and Hansen, 1999), but also in different species of parasites. Accordingly, any rational control programme should start by developing the capability to differentiate the effect of the antiparasitic agent on the target species for control, from those non target species affected by the antiparasitic agent.

A lack of diagnosis does not mean that the problem does not exist; on the contrary, it often indicates a series of shortcomings, ranging from a failure to perceive the problem in the field, to a lack of laboratory diagnostic capability. Another difficulty that has been of concern is a lack of infrastructure for the flow of information from the field to laboratories and back. The lack of effective, standardized techniques for diagnosing resistance is one of the main impediments to a resistance monitoring system. Many governments lack information about the real impact of these problems, hindering the planning of proper control measures (Cheneau, 1999; Fleming and Patrick, 1996).

## Potential consequences

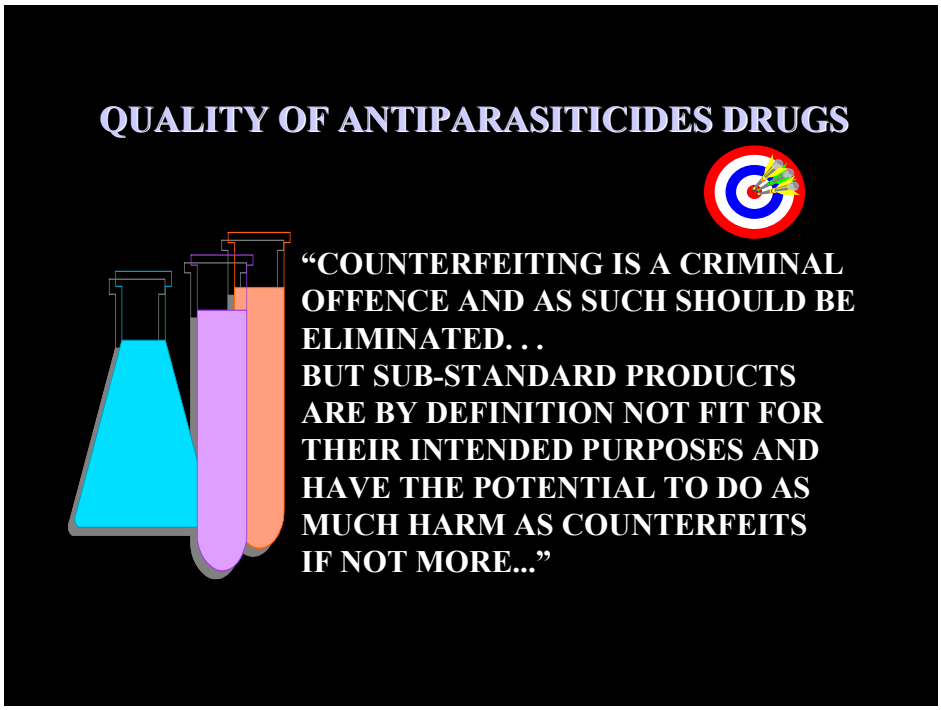
In view of the increasing problem with parasite resistance, it will be difficult to maintain an approach to control that is based primarily on the use of chemical antiparasitic agents. This is a serious problem, which is likely to be complicated by the lack of expectation of new drugs in the near future. The pharmaceutical industry has seen continuously escalating research and development costs for the registration of new drugs. The cost of developing a new product is between US$100 and US$230 million, and is for a process that can take more than 10 years from the discovery of a potential candidate, to market (De Alva, 1995; Soll, 1997). Antiparasitic compounds for livestock must compete for funding with products for the benefit of other more profitable patients, specifically humans (Soll, 1997). Thus, the antiparasitic agents for use in animal health should be viewed as a non-renewable resource and it is essential that we preserve the drugs we have available by using them wisely.

Genetically based resistance in parasite populations has developed within the context of a world undergoing far-reaching political, social and economic changes which must be taken into consideration when attempting to implement sustainable prevention and control measures. The scenario for the twenty-first century will be characterized by meat, wool and milk markets that are ever more globalized, competitive and demanding, especially with regard to freedom from residues and prevention of environmental contamination. Governments and industry will not have the same operational freedom as in the past and it is most unlikely that there will be drugs to which parasites cannot develop resistance. Therefore even more attention needs to be directed towards preventing ecological problems (Floate, 1998; Kunz and Kemp, 1994; Sprat, 1997) and residues in meat, milk and wool. The failure to do this could result in the proliferation of non-tariff barriers to trade between countries. Another impediment to trade, both within and between countries, is the risk of introducing resistant parasites through the transfer (Nari and Hansen, 1999) or importation of live animals. This is a widely recognized fact in the case of arthropods and is becoming more evident in helminths (Dorny *et al*., 1994; Himonas and Papadopoulos, 1994; Requejo-Fernandez *et al*., 1997; Sangster, 1998; Yarandy *et al*., 1993).

## Registration and quality control of antiparasiticides

In many cases, it is difficult for governments to maintain specialized staff and adequate facilities to support the registration and continuous quality control of antiparasiticides. Of the countries included in the above-mentioned study, 49.3 percent reported that they were having difficulties in ensuring proper registration of antiparasitic agents. This basic government function is fulfilled only with great difficulty in developing countries, which are much more susceptible to problems of adulteration and the introduction of low-quality drugs. The principal difficulties mentioned by

the Member Countries include: lack of proper legislation; lack of a specific registration unit; registration by other government units not specialized in animal health; total or partial lack of infrastructure to carry out the necessary analytical testing for each type of compound; the impossibility of ensuring continuing control over the quality of antiparasitic agents; the failure to link registration with the occurrence of resistance in the field.




During the present decade, generic antiparasitic agents have come to stay. It is not difficult these days to find countries where the same active ingredient is marketed under more than 20 different trade names. Competition between different formulations is healthy, provided of course that quality is maintained (which does not mean only the correct concentration of the active ingredient). This situation and the lack of training among users increase the consumption of cheap and often low-quality drugs. Without doubt this is the greatest challenge facing countries that do not yet have the capability to control the toxicity, residues and efficacy of antiparasitic agents. Parasite resistance and the use of substandard drugs continue to be of vital importance to animal health. More professionals in the field of animal production and health, and in the pharmaceutical companies, are now inclined to combine their efforts to promote prudent use of drugs, recognizing that parasite resistance is not only a problem caused by farmers' misuse of drugs.

## Future needs

It is important that all the parties involved – governments, the pharmaceutical industry, private and international organizations and most importantly veterinarians, extension personnel and livestock producers – realize that the time of easy parasite control, based on the use of antiparasiticides only, is over. It is essential that perceptions and attitudes change with an understanding that for parasite control in the future, we will have to rely on combinations of strategies which will most likely require more work and more monitoring.

The development and management of future sustainable integrated parasite control strategies require capabilities in the disciplines of epidemiology, diagnosis of parasites and their resistance, integrated pest management and the registration and quality control of antiparasiticides.

**Epidemiology**

The most important single requirement for the successful implementation of rational and sustainable parasite control programmes in grazing animals is a sound knowledge of the epidemiology of the parasites as they interact with their hosts in specific climatic, management and production environments (Barger, 1998). Epidemiology is the basis upon which the rational control of resistance must be built, and although the importance of epidemiological knowledge in the prevention and control of resistance to antiparasitic agents is widely accepted, the epidemiological database of many developing countries is still incomplete. The availability of molecular techniques and appropriate mathematical models will provide tools for a better understanding of parasite population dynamics and help to minimize the selection for resistance.

**Diagnosis**

It is necessary to establish the capability, or strengthen the existing capability, for diagnosing resistance using rigorously standardized and harmonized techniques that make it possible to implement control measures without the inappropriate use of antiparasitic agents.

**Integrated parasite control (IPC)**

Total dependence on a single method of control has proved to be non-sustainable and cost-ineffective in the long term (De Castro, 1997; Holmes, 1997; Tatchell, 1992; Vergara Ruiz, 1996; Waller, 1999; FAO, 2001). The term "integrated pest management" refers to a system where multiple approaches for control (chemical, non-chemical) are utilized, following the consideration of economic factors, epidemiology, resistance status, and the production system and management structure in place. Thus it is impossible to prepare standardized control strategies, that are applicable in all situations, and the preferred approach is to thoroughly analyse the situation and apply the 'best bet' options effectively.

It is not anticipated that the use of chemicals can be completely replaced with other interventions. However, the selection pressure for resistance can be reduced by lowering the number of treatments, by only treating the animals which need treatment and by timing the treatments according to epidemiology, making sure that susceptible refugia of parasites remain in the environment. The potential problem of exposing the total population of parasites to the selection pressure can best be illustrated by the example of mites. When treating for mange mites, an obligate parasite with a proportionately small refugia population, development of resistance will be enhanced by simultaneously exposing all parasitic stages to the treatments.

The principle of selective treatment has been practised in the past, using a specified concentration of worm eggs per g of faeces as a threshold for treatment. More recently the principle has been applied based on monitoring of the level of anaemia caused by *Haemonchus* using the FAMACHA® technique, a system originally developed in South Africa (van Wyk, 2002). The process of field validation, training of personnel and distribution of the system is ongoing with FAO collaboration in South Africa, Paraguay and Uruguay.

The implementation of an IPC programme involves elements that are sometimes difficult to achieve in developing countries. These include the availability of results of applied research in epidemiology and control, a change of policy to further the application of methods that are less dependent on antiparasitic agents and the participation of the producer and veterinary advisor in training programmes. Some IPC systems are complicated to implement, but the routine use of computerized models makes it possible to rationalize control measures in a more global and economical manner (Barger *et al*., 1990; CRCTPM, 1997; Kariuki *et al*., 1997).

### Registration and quality control of antiparasitic agents

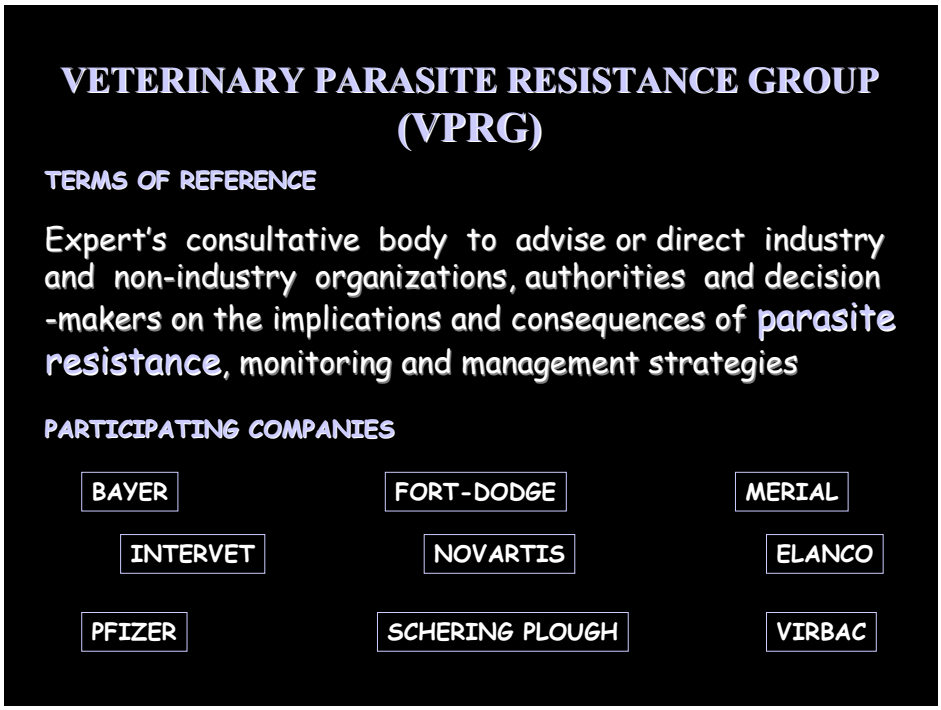
It is during the registration process that the government authority approves the sale and use of an antiparasitic agent, after having evaluated the efficacy of the product and its safety to animals, public health and the environment. This process is often difficult for many developing countries because it requires sophisticated infrastructure and specialized personnel to carry out the tests. Notwithstanding this, some countries have made important advances both nationally and regionally. Another obligation which has proved to be complicated to implement and for which the poorer countries are on their own, is the continuous monitoring of the quality of antiparasitic agents to prevent abuses, including adulteration, the sale of substandard preparations and drug combinations of dubious stability.

The privatization of veterinary services in some countries has resulted in a dramatic reduction in their capabilities for disease diagnosis and monitoring. This is jeopardizing resistance management and it is desirable that developing countries and those in transition maintain, or establish a critical mass of specialized professionals who can manage the four areas discussed above.

## FAO perspective

The parasite control programme of the Food and Agriculture Organization of the United Nations is continuously involved in the collection, analysis, collation and distribution of information related to the epidemiology, diagnosis and control of parasites and their associated diseases. This is done through regular contact with world leading scientists attending FAO or FAO/WHO Expert Consultations, interaction with FAO Collaborating Centres and the constant monitoring of the scientific literature.

Requests for assistance from member countries related to their fight against ecto- and endoparasites and the subsequent technical input have often been aimed at improving diagnostic capability and establishing the epidemiological knowledge base to create the basis for the introduction of cost-effective and sustainable control systems. The integrated programme to control ticks and tick transmitted diseases in Africa and the eradication of *Amblyomma variegatum* in the Caribbean are examples of these programmes (Caribbean *Amblyomma* Programme, 2002).




With the growing problem of resistance to antiparasitic agents, the FAO's Animal Health Service, together with its Regional Offices, have made resistance management an important component of its programme. A permanent FAO working group, "The Working Group on Parasite Resistance (WGPR) was established in 1997. This is a panel of experts, which advises the FAO on resistance management and integrated parasite control (IPC). The WGPR gathers, organizes and analyses information on the epidemiology, diagnosis and control of parasites and management of resistance to parasites, assisting the FAO in preparing guidelines for diagnosis and control.

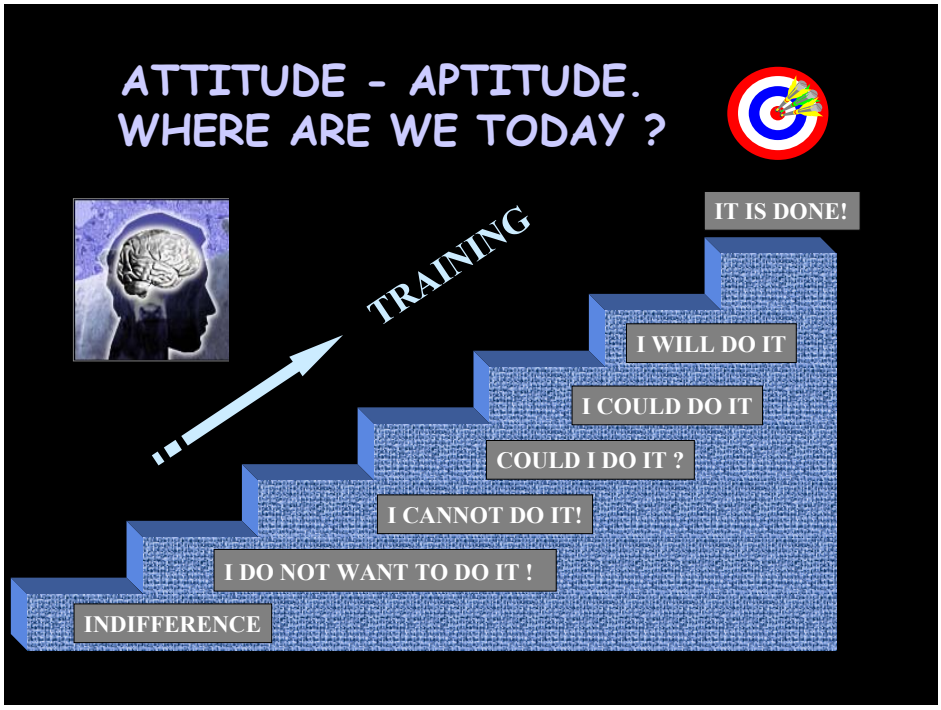
The FAO and the FAO/WGPR collaborate with the pharmaceutical industry through the FAO Industry Contact Group on Parasite Resistance. Industry is represented by the Veterinary Parasite Resistance Group (VPRG), a specialized consultative group whose mandate is to advise industry and non-industrial organizations on the implications and consequences of resistance to parasites, and on monitoring and control strategies.

The issue of acaricide resistance in ticks has attracted the attention of governments, international institutions, the pharmaceutical industry and academic organizations (Coles *et al*., 1992; Kemp *et al*., 1998). This led to the establishment of the FAO's World Acaricide Resistance Reference Centre (WARRC) in Berlin, Germany (Thullner, 1997). Unfortunately this Centre has been defunct since 1996 due to funding problems, and the functions that were formerly carried out by WARRC are now being transferred to the FAO Regional Reference Laboratories for Diagnosis and Monitoring of Acaricide Resistance and other parasites of veterinary importance. Two Centres have been established, one in Mexico (CENAPA[1]), and one in Uruguay (DILAVE "Miguel C. Rubino"[2]) During the first phase, the Centres have the task of developing the region's

[1] CENAPA: Centro Nacional de Investigaciones Disciplinarias en Microbiología

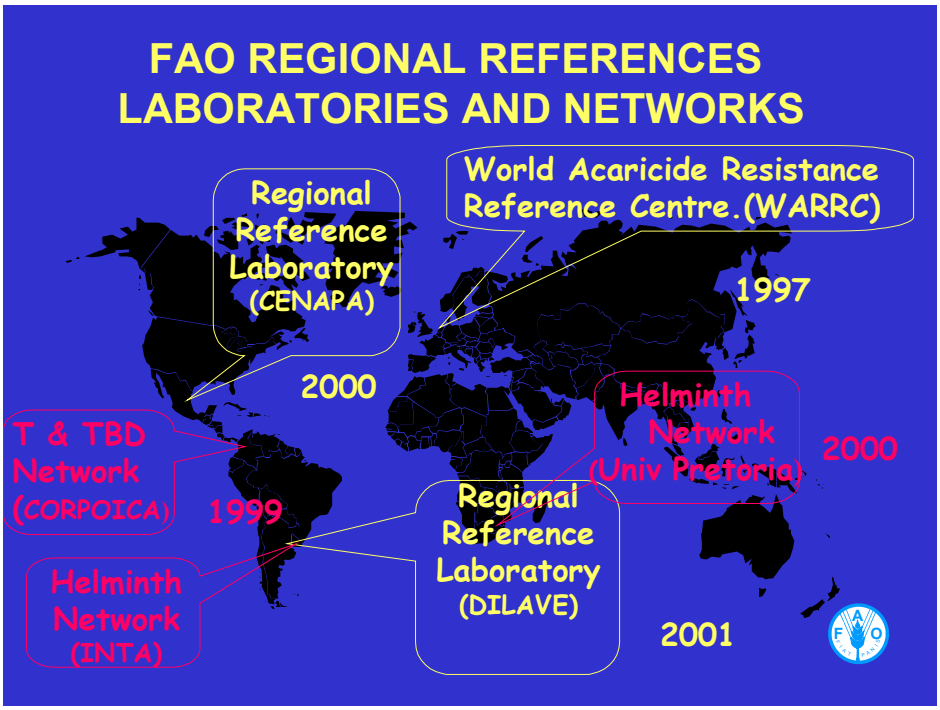
[2] DILAVE: Dirección de Laboratorios Veterinarios "Miguel C. Rubino"

technical capability for diagnosing and monitoring tick resistance to acaricides. The results of activities and information related to the work of the Reference Laboratories will be disseminated through the FAO network on Ticks and Tick-Borne Diseases, coordinated by CORPOICA[3] in Bogota, Colombia. This Network also provides timely information on training courses, seminars and working meetings and the availability of duly harmonized and standardized diagnostic test kits.




In addition, the FAO has promoted and provided financial support to the creation of two electronic networks on helminths – one in Latin America (coordinated by INTA[4], Castelar, Argentina) and the other in Africa (coordinated by the Veterinary Faculty, Pretoria University, South Africa). In addition to the specific functions performed by these networks, the FAO will encourage interconnection and interaction between these and other existing networks.

3 CORPOICA: Corporación Colombiana de Investigación Agropecuaria
4 INTA: Instituto Nacional de Tecnología Agropecuaria




In short, the collaboration between the FAO, the WGPR, the INDUSTRY Contact Group, the FAO Regional Reference Laboratories and other international institutions and organizations, aims to provide the required information and training for development of sustainable integrated pest management (IPM) and the appropriate management of resistance to parasites.

## The purpose of the FAO guidelines

The guidelines contain five modules, one for each of the five economically important parasite groups: ticks, helminths, flies, mites and lice. Each module is presented in a standardized format and is intended to be used by laboratory technicians, scientists and advisers in the field. It is the intention that each of the modules will serve as a guide to the diagnosis of resistance and act as a decision support system for selecting the 'best bet' options for integrated control and resistance management.

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# MODULE 1. TICKS: ACARICIDE RESISTANCE: DIAGNOSIS, MANAGEMENT AND PREVENTION

## 1 INTRODUCTION

Ticks and the diseases they transmit are widely distributed throughout the world, particularly in tropical and subtropical regions. It has been estimated that 80 percent of the world's cattle population is exposed to tick infestation (FAO, 1984). Although species of ticks and tick borne diseases (TBDs) differ among ecological regions, their impact on animal production is important wherever they occur.

Losses attributable to ticks are caused either directly, through tick worry, blood loss, damage to hides and udders and the injection of toxins, or indirectly through mortality or debility caused by the diseases transmitted by or associated with the ticks. The most quantitative assessments of the economic impact of ticks and TBDs are from studies of *Boophilus microplus*. These losses have been expressed either in terms of grams of live weight gain or milk production lost per tick engorging (for example 0.7g/tick) or in terms of total average financial loss (production losses plus cost of control) per animal per year (e.g. US$7.3/head/year). Losses due to ticks and TBDs tend to be lowest in areas where indigenous animals, the tick vector and the TBDs have co existed, resulting in endemic stability. Endemic stability arises when there is sufficient early exposure of young animals to ticks and TBD organisms to ensure that there is a high level of immunity in the host population, reducing the incidence of clinical disease. Losses due to ticks and TBDs tend to be highest when exotic animals susceptible to ticks and TBDs, are introduced into tick infested areas or when ticks and their TBDs are introduced to areas where cattle have not previously been exposed. Considerable livestock mortality occurred during the late nineteenth and early twentieth century, resulting from extensive human and animal migration (FAO, 1984).

Structural changes in the provision of veterinary services, associated with reduced budget allocations, economic and social changes in livestock production systems, increased costs of acaricides and labour, combined with the increasing incidence of acaricide resistance in ticks have led to a demand for more cost effective and sustainable approaches to the control of ticks and tick borne diseases.

## 2 RESISTANCE DEVELOPMENT: TICKS

The resistance of ticks to acaricides is an inherited phenomenon. It results from exposure of populations of ticks to chemical parasiticides (acaricides) and survival and reproduction of ticks that are less affected by the acaricide. The higher reproductive rate of ticks that have heritable resistance factors and the resulting increase in the proportion of the population of ticks that carry genes for these factors is known as selection.

Resistance to a given acaricide or insecticide can be described as a reduction in susceptibility of a parasite to the acaricide or insecticide when it is used at the recommended concentration and according to all of the recommendations for its use.

In most cases, it is likely that genes that confer resistance are already present at very low levels in the tick population before the introduction of a new acaricide. The rate at which a resistant allele becomes established in the population and the time it takes for the control of ticks to break down is dependent upon many factors. These include the frequency of the original mutation in the population before treatment, the mode of inheritance of the resistant

allele (dominant, co-dominant or recessive), the frequency of acaricide treatment, the concentration gradient of the acaricide and the proportion of the total tick population that is not exposed to the acaricide (refugia).

Although the frequency of resistant genes initially only increases slowly, by the time declining efficiency of dipping or treatment is noticed, the rate of increasing frequency of resistance genes is usually high (Nolan and Schnitzerling, 1986). In the initial phase, the frequency of heterozygous resistant individuals (single allele mutation) within the population is low and the rate of increase in the frequency of the resistant allele is low. In the next, emerging phase, given continued exposure to a drug, the frequency of heterozygous resistant individuals within the population increases. Finally, the sustained selection pressure results in increasing numbers of homozygous resistant individuals, which ultimately predominate in the population.

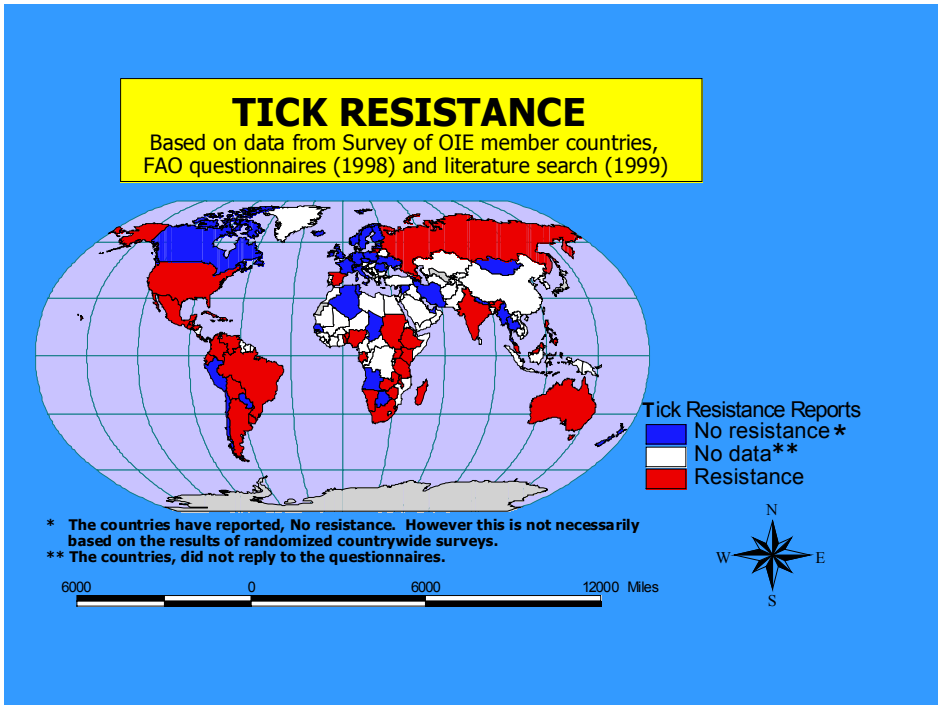
In Latin America the increasing spread of horn fly (*Haematobia irritans*) during the last decade has resulted in frequent spraying of cattle (mainly with synthetic pyrethroid based compounds). Usually, lower concentrations are used than are necessary to kill any ticks also present on the host. It is likely that this also contributes to an increase in the rate of the evolution of resistance to acaricides in the ticks. A similar situation has been developing in Australia with *Haematobia irritans exigua*.

It is increasingly common for livestock producers to experience multispecies resistance in parasite populations exposed to acaricides or insecticides (Morales *et al*., 1999). This sometimes involves multiple tick species (e.g. *Amblyomma variegatum* and *B. microplus*) or multiple taxa (e.g. *B. microplus* and *H. irritans*). The increasingly frequent use of endectocides in livestock production systems may accelerate this trend, not only inducing resistance in ticks and insects but also in helminths. Training of producers must be a priority for the implementation of successful control programmes for ticks.

## 3 CURRENT STATUS

A lack of standardized techniques for diagnosing acaricide resistance appears to be the main difficulty in creating and maintaining a tick resistance monitoring system. The issue of diagnosis of resistance has attracted the attention of governments, international institutions and academic organizations. It was one of the principal objectives of the FAO's World Acaricide Resistance Reference Centre (WARRC) based in Berlin, Germany. The WAARC had to be discontinued in its original physical form in 1997, due to funding problems. Its vital support functions, however, will need to be included in any future plans for global acaricide resistance monitoring (Nari and Hansen, 1999).

Ticks resistant to organophosphate (OP) and synthetic pyrethroid (SP) acaricides are widespread and amitraz resistance has been reported but is less common (Tables 1a and 1b). Resistance to macrocyclic lactones by *Boophilus microplus* has been reported recently (Martins and Furlong, 2001). Notification of failures in the effect of fluazuron and fipronil have not been detected or reported in the literature to date.




## 4 DIAGNOSIS OF RESISTANCE IN TICKS

In selecting a suitable laboratory test for acaricide resistance, the following requirements must be satisfied. The test should be sensitive enough to identify resistance early in its emergence. It should also cover the full range of chemical groups that are in use, including the most recently developed active ingredients. The diagnostic test should be simple and inexpensive. It should provide a rapid and reliable result, and be suitable for standardization among laboratories in many countries. The most widely used *in vitro* tests are bioassays applied to larvae and engorged female ticks. None of them meet all of the above requirements, and improvement of protocols for diagnosis of acaricide resistance should be a continuing goal.

Resistant strains of ticks can be diagnosed without having internationally recognized standardized test protocols and reporting methods. However, to facilitate global monitoring and provide a basis for comparison of test results, standardized diagnostic methods should be adopted. In view of this and following the advice of experts since 1975, FAO has promoted the use of the Larval Packet Test (LPT) for field investigations of acaricide resistance. The WARRC supported FAO member countries in determining patterns of resistance, especially to OP and SP acaricides (Kemp *et al*., 1999). Nevertheless, other methods continue to be used and improved (**Table 2**) (Baxter *et al*., 1999).

**Table 1a. Bibliographic references for worldwide acaricide resistance in different tick genera and species of *Boophilus* reported in the literature, principally since 1993**

| Chemical | *B. microplus* | *B. decoloratus* | *B. annulatus* | *Hyalomma* spp. | *Amblyomma* spp. | *Rhipicephalus* spp. |
|---|---|---|---|---|---|---|
| **Synthetic pyrethroids (SPs):** | | | | | | |
| permethrin | Davey and George; 1998, Beugnet, 1996; | | | Smirnova, 1994. | | |
| cypermethrin | Romero *et al*., 1997; Arantes *et al*., 1996; Benavides, 1995; Schnitzerling *et al*., 1989; FAO, 1987; Martins *et al*., 1995c; Ortiz *et al*., 1995. | Bruce and Mazhowu, 1992. | | | | |
| alpha cypermethrin | Arantes et al., 1996; Romero *et al*., 1997; Rosario *et al*., 1997. | | | Bagherwal *et al*., 1995. | | |
| deltamethrin | Arantes *et al*., 1996; Benavides, 1995; Romero *et al*., 1997; Martins *et al*., 1995c; Ortiz *et al*., 1995; Beugnet *et al*., 1994; Beugnet and Chardonnet, 1995; Brun, 1992; Hagen, 1997. | | | | | |
| cyhalothrin | | | | | | |
| flumethrin | Beugnet, 1996; Benavides, 1995; Martins *et al*., 1995c; Ortiz *et al*., 1995; Beugnet et al., 1994; Beugnet and Chardonnet, 1995; Hagen, 1997. | Bruce and Mazhowu, 1992. | | | | |
| fenvalerate | Beugnet *et al*., 1994; Beugnet and Chardonnet, 1995. | | | | | |
| cyfluthrin | Arantes *et al*., 1996; Hagen, 1997. | | | | | |
| **BHC/Cyclodines**: | | | | | | |
| dieldrin | Arantes *et al*., 1996; Ortiz *et al*., 1995; Romero *et al*., 1997; Kagaruki, 1991. | Regassa and de Castro, 1993; Kagaruki, 1991. | | | Kagaruki, 1991. | Kagaruki, 1991. |
| toxaphene | Benavides, 1995. | Regassa and de Castro, 1993. | | | | |
| lindane | Ortiz *et al*., 1995; Kagaruki, 1991. | Kagaruki, 1991. | | | Kagaruki, 1991. | Kagaruki, 1991. |
| **Carbamate:** | | | | | | |
| carbaryl | Basu and Haldar, 1997. | | | | | |

**Table 1b. Bibliographic references for worldwide acaricide resistance in different tick genera and species of *Boophilus*; reported in the literature, principally since 1993**

| Chemical | *B. microplus* | *B. decoloratus* | *B. annulatus* | *Hyalomma* spp. | *Amblyomma* spp. | *Rhipicephalus* spp. |
|---|---|---|---|---|---|---|
| **Organophosphates (OPs):** | | | | | | |
| diazinon | Benavides, 1995; Ortiz *et al*., 1995; Romero *et al*., 1997. | | | | | |
| coumaphos | Benavides, 1995; Coronado, 1995; Ortiz *et al*., 1995; Romero *et al*., 1997; Rosario *et al*., 1997. | | | | | |
| ethaphos | | | Smirnova, 1994. | Smirnova, 1994. | | |
| cyclophos | Benavides, 1995. | | | Smirnova, 1994. | | |
| trichlorfon | Arantes *et al*., 1996; Benavides, 1995. | | | Smirnova, 1994. | | |
| dioxathion | Ortiz *et al*., 1995. | Bruce and Mazhowu, 1992; Luguru *et al*., 1987. | Smirnova, 1994. | | Luguru *et al*., 1987. | |
| chlorpyriphos | Ortiz *et al*., 1995. | | | | | |
| ethion | Benavides, 1995; Ortiz *et al*., 1995. | | | | | |
| chlorfenvinphos | Coronado, 1995; Romero *et al*., 1997. | | | | | |
| dimethoate | Ortiz *et al*., 1995. | Luguru *et al*., 1987. | | | | Luguru *et al*., 1987. |
| Amitraz | Benavides, 1995; Martins *et al*., 1995c. | | | | | |


**Table 2. A summary of methods used for the diagnosis of acaricide resistance in the tick, *Boophilus microplus*, in some Latin American countries (Nolan, 1994)**

| Country | Test method | | | | Age of larvae when used (days) | Types of resistance tested |
|---|---|---|---|---|---|---|
| | AIT | LPT | SLIT | Other | | |
| Argentina | X | X | | Stall | | OP, SP |
| Brazil | X | X | X | | 14 to 21 | OP, SP, AM |
| Colombia | X | X | X | | 14 to 20 | OP, SP, AM |
| Mexico | X | X | | | 7 to 14 | OP, SP |
| Uruguay | X | X | | Stall | 7 to 14 | OP, SP |
| Venezuela | X | | | | | |

AIT, Adult Immersion Test; LPT, Larval Packet Test; SLIT, Shaw Larval Immersion Test; OP, organophosphorus; SP, synthetic pyrethroid; AM, amidine

A recent questionnaire that was circulated by the FAO Working Group on Parasite Resistance (WGPR), revealed that the method most widely used in laboratories involved with the diagnosis of resistance was the Adult Immersion Test (AIT). This has been taken into consideration when deciding which tests should be included among the FAO recommended standardized tests and protocols for the future. Protocols for the LPT and AIT are described below. The adoption and recommendation by FAO and the FAO WGPR of standardized LPT and AIT methods does not imply that other methods for diagnosis of tick resistance are not important and useful.

In order to facilitate regional acaricide resistance monitoring and management, an FAO Regional Reference Laboratory (RRL) for the diagnosis of tick resistance was inaugurated in Mexico in 2000. Further RRLs are expected to be established in Colombia and Uruguay. These RRLs will have the role of:

- producing and distributing test papers impregnated with acaricides for LPT;
- maintaining colonies of tick reference strains;
- collection, analysis and transmission of data;
- assisting in technology transfer of the standardized LPT and AIT tests through training exercises.

## 5 DIAGNOSTIC METHODS: *IN VITRO*

The fact that there are several tests in use for the diagnosis of acaricide resistance in ticks should serve to indicate that none of the tests is perfect in all circumstances. The larval packet test is considered to be the most repeatable, although it is limited by the length of time that it takes. Hence it remains the test of choice for surveys and for definitive confirmation of a diagnosis of resistance. The adult immersion test with a discriminating dose has recently been recommended as a preliminary screening test for resistance because it is relatively rapid, but further work is required to determine just how sensitive and specific the test is for acaricide resistance. In the meantime it is probably most appropriately used to provide rapid supporting evidence when control breaks down in the field.

***The larval packet test (lpt)***

Results for this larval bioassay for the diagnosis of resistance in *B. microplus* takes about 6 weeks and is based on protocols used for many years by the Commonwealth Scientific and Industrial Research Organization (CSIRO) and the Queensland Department of Primary Industries (DPI) in Australia. However, it can be used for other species of Ixodid ticks and has also been widely employed in Latin America and Africa. Following the adoption of this test by the FAO as the preferred means of diagnosis of resistance in ticks, it was promoted in the form of the FAO Acaricide Resistance Testing Kit. It is anticipated that it will continue to be prepared and distributed by the RRLs in Latin America and possibly elsewhere. In this test, tick larvae are exposed to chemically impregnated filter papers and their subsequent mortality is quantified after 24 hours. The kit contains standardized materials and procedures enabling data obtained from different parts of the world to be directly compared and discussed. Adequate training is essential in order to achieve a high degree of confidence in this technique.

More recently, protocols have been added for macrocyclic lactones (MLs). There are some problems with the application of the standard LPT and AIT protocols for susceptibility to amitraz, requiring specific modifications for this acaricide.

The LPT cannot be used for acarine growth regulators such as fluazuron.

***The Larval Immersion Test (LIT)***

This larval bioassay (Shaw, 1996) is not so widely used for the diagnosis of resistance and has not been promoted by FAO. The method provides a result in six weeks, the same time as the LPT. Comparative studies have indicated LIT results can be compared with LPT results as there is good agreement between results of the test methods. The inability of the LPT to diagnose potential resistance to fluazuron also applies to LIT.

It may be worth considering the LIT test when developing new tests for the detection of resistance to MLs in tick larvae. The LPT can be used for MLs but preliminary results at CSIRO, Australia, have shown that the LIT is much more sensitive.

***The Adult Immersion Test (AIT)***

The AIT is a bioassay applied to engorged, female ticks. The AIT was described by Drummond *et al.* (1973) and was used to determine the relative effectiveness of new acaricides against a number of tick species. It was adapted for resistance testing in several laboratories but standard protocols have not previously been developed. A protocol provided and modified by the Agricultural Research Service (ARS), United States Department of Agriculture (USDA), the organization that initially developed the test, is described in this module. Protocols for technical and injectable formulations of ML's and fluazuron are also included.

In the near future, the RRLs of the FAO will use the classical AIT (with different concentrations of product) and a standardized AIT that uses discriminating doses (DD).

***Detection of acaricide resistance: protocols for the FAO recommended methodologies***

Most of the following discussion on the diagnosis of acaricide resistance in ticks is based on techniques developed for the one-host tick *Boophilus microplus*. The procedures have, however, been adapted and used successfully for other species of tick, including 3-host ticks.

***Collection and submission of ticks for resistance testing***

A pre requisite for effectively performing the tests is to obtain high quality samples of ticks from the field for testing. Veterinarians, extension staff and farmers need guidance in the collection, handling and submission of samples to ensure that suitable test material reaches the diagnostic laboratories.

### 5.1.1 Introduction

Resistance is usually first recognized as a failure of treatment to eliminate tick burdens from cattle. Although failure of treatment is often the result of incorrect preparation or application of acaricide, the persistence of ticks after frequent, correctly applied treatments indicates that acaricide resistance is likely.

Because of the technically exacting requirements of the bioassays for resistance, very specific requirements exist with regard to the number, stage and age of the ticks and will vary according to the purpose of the test. For example, the requirements are different if the testing involves the diagnosis and characterization of a new form of resistance, if it is part of a resistance survey or if it is a follow up to treatment failure.

### 5.1.2 What to collect

Regardless of the type of test to be used, engorged female ticks must be collected.

### 5.1.3 How many ticks to collect

This will depend on the type of test applied, the number of acaricides that are to be tested and how many concentrations of each acaricide will be used.

For the LPT, a sample of 10 to more than 50 fully engorged female ticks is desired. However, it should be kept in mind that the LPT can be conducted with fewer ticks because sufficient larvae will hatch from the eggs of a few engorged female ticks. Where ticks are collected from animals recently treated with acaricides, the results might suggest a higher frequency of resistance than in the population of all ticks from the farm.

For the AIT, the general practice has been to use a minimum of 10 engorged female ticks for each acaricide and the same for controls, but 20 ticks for each would be preferred.

The rule of thumb is: **"collect as many engorged females as possible".** Collecting enough, undamaged, fully engorged female ticks can sometimes be difficult, particularly when carrying out surveys, or outside the principal tick season.

### 5.1.4 When to collect

***Time of the day:*** Because most engorged ticks drop off the host in the early morning, holding tick infested animals overnight in pens or by tethering them is recommended. Engorged female ticks can then be collected from the animals and/or from the ground around them, early the following morning.

***Time in relation to treatment:*** The optimum time will depend on the acaricide used in the context of whether it has residual effect, strong 'knock down' effect or if the maximum effect is delayed. As species of *Boophilus* are all one host ticks, moulting from larvae to nymph and from nymph to adult takes place on the host. During the moulting periods, the ticks are less susceptible to dipping and spraying due to the fact that the acaricide has to penetrate the double cuticle surrounding these stages; i.e. they have a greater 'physical' resistance. Engorged female ticks dropping 9 to 14 days after treatment are likely to have been engorging nymphs and nymphs in the moult at the time of treatment, and are therefore less likely to have a true, genetically based resistance. Engorged female ticks, collected 3 to 8 or 14 to 17 days after treatment, are therefore more suitable for resistance testing. Where macrocyclic lactones are used, it is essential to allow 3 days or so after treatment, before collecting ticks; for fluazuron (Acatak) at least 15 days should be allowed.

***Time in relation to purpose of test:*** For surveys, sampling before treatment is essential in order to avoid a bias in the population sample. For suspected resistance, inevitably, samples will have to be taken after treatment.

### 5.1.5 Where and how to collect ticks

Usually ticks are easily available from farms with problems of resistance. The sample should be collected from as many of the cattle as possible and if several dips and/or yards for holding cattle during treatment are present on the property, ticks should be collected separately from the various groups. Each sample must be clearly identified, including time and date, place, group number, treatment and owner.

### 5.1.6 Storage of ticks

In cases where sufficient numbers of ticks are not available, it is possible to collect them over several days, thus creating a pool of refrigerated (4ºC) engorged female ticks. Storage conditions are more critical for AIT than for LPT, because the test is conducted on the collected ticks for AIT whereas for LPT it is conducted on their progeny. Factors influencing the viability of the engorged female will have a direct effect on AIT results but not on LPT results. Additionally, egg laying before application of the AIT invalidates the results. It appears that engorged female ticks can be refrigerated for up to 5 days without adverse effect on their response to AIT, provided they are stored **immediately** after detachment. For AIT they can be refrigerated for up to 2 days if they were kept at moderate field temperature for 1 day prior to refrigeration (Spickett *et al*., 1983).

### 5.1.7 Transport of ticks

For transport, ticks should be put into small boxes, preferably made of cardboard, with a few small holes allowing air to circulate. If the ticks are to be transported over long distances, they

should be placed between layers of slightly moistened paper towels in order to keep the environment humid and protect the ticks from damage. Alternatively, freshly cut, green grass or herbage (of a type that has no direct adverse effects on ticks) can be used to provide moisture. More problems occur with samples that are too wet than too dry, provided that transport times and temperatures are not excessive.

Where large numbers of ticks are submitted for AIT, the cardboard containers should be placed in a cool box with a cooler brick (wrapped in paper towels) and transported to the laboratory by the fastest route.

Do not submit ticks in airtight containers, plastic bags or glass tubes.

Do not place ticks in cotton wool.

Do not expose ticks or containers to excessive heat/sunlight.

It is vital to complete the standardized FAO Tick Resistance Information Sheet, supplied with the kit, providing as much of the required information as possible.

### 5.1.8 Laboratory handling of ticks

***Engorged female ticks:*** Immediately upon arrival at the laboratory, engorged female ticks should be washed, preferably in distilled water, to remove any eggs laid during transport. Those that have started laying eggs should not be used for the AIT.

***Conditions for all tick stages before testing*:** For all Ixodid ticks, incubation conditions for engorged female ticks, eggs and larvae before testing should be 27 to 28°C and 85 to 95 percent relative humidity (RH).

***Age of larvae for testing:*** For all Ixodid tick larvae, the recommended age is 14 to 21days. It may be found more convenient to use different ages for multi host tick species. However, it must be borne in mind that whatever the tick species or developmental stage, its age greatly affects its susceptibility to acaricides; standard ages for the testing of each species and stage should therefore be established.

***Conditions during testing:*** For all Ixodid tick larvae, the conditions for holding treated ticks should be 27 to 28°C and 85 to 95 percent RH and without illumination. Incubator hygrometers should be calibrated twice a year. The incubator should allow air exchange but does not require fan circulation. It is recommended that a different incubator be used for SP testing. If this is not possible, then SP testing could be conducted in the same incubator used for other acaricide groups, but at a different time. Following use with SP acaricides, the incubator should be cleaned with acetone and permitted to dry with adequate ventilation.

### 5.1.9 Quality control of ticks before testing

For the AIT, it is important that the engorged female ticks are healthy and are tested as soon as possible after collection. The size and weight of an engorged female tick will influence its egg laying capacity. If female ticks are below a certain weight the eggs produced are no longer proportional to the weight and ticks below this weight (150 to 350 mg is suggested) should be rejected. Because weighing individual ticks is time consuming, it is recommended that all the engorged female ticks are put on a sieve, with a mesh size that permits those below this weight range to pass through and be rejected, before commencing the AIT.

For the AIT, the criterion for resistance to an acaricide is the production of eggs following treatment. Hence, ticks from the field that have already started to lay eggs (4 to 5 days after collection, depending on the temperature) are not suitable for testing. Damaged and discoloured ticks should also be rejected**.** Healthy ticks should also be seen to move around. A useful

approach is to put the ticks under a source of light (e.g. a desk lamp), which will cause healthy ticks to move away.

### 5.1.10 Rearing methods for engorged female ticks, eggs and larvae

Up to five clean, engorged female ticks are placed in a 150 mm glass rearing tube, which is then closed firmly with a ventilated stopper and placed in an incubator maintained at 27 to 28°C and 85 to 95 percent RH.

All eggs should be collected 7 days from commencement of incubation. Each tube containing the first week's egg production should be labelled with the date, to enable the selection of more uniform larvae for each LPT.

Under optimal rearing conditions, the engorged female ticks of most species will begin to lay eggs within 2 to 7 days. *Boophilus* spp. begin to lay eggs after 2 to 3 days and will continue for 12 to 15 days. After 21 to 28 days, *Boophilus* spp. larvae begin to hatch.

## A. *IN VITRO* TEST PROTOCOLS

## The FAO Larval Packet Test – LPT (as further refined at the WARRC)

**Acaricide impregnated papers**
Dieldrin is included as an indicator of toxaphene and lindane resistance. Flumethrin and cypermethrin are indicators of resistance against synthetic pyrethroids. Diazinon is a general indicator of organophosphorus (OP) resistance.

If OP resistance is identified with the diazinon test, the only way of specifically determining which OPs are affected is to test them individually. In such a case, additional, specific acaricides must be used and have to be sent individually from the FAO RRL.

**a. Storage and handling of acaricide–impregnated papers**

The papers should be kept in their original, individual isolating aluminium foil envelopes in a refrigerator and protected from high humidity, high temperature and light (especially direct sunlight) and opened only immediately before use. Individual papers should be handled with forceps and used only once for a test. After removing a paper, its foil envelope should be immediately resealed with adhesive tape. Self-protective, disposable gloves and facemasks must be worn.

**b. Guidance notes before commencing a test**

1. Two control packets and two for each of the concentrations per acaricide active ingredient (AI) are used for each tick sample suspected of having developed resistance.

2. The control papers are always prepared first, followed by the acaricide impregnated papers per active principal, each of these series being handled in ascending order of concentration

3. The use of a white tray enables any accidentally fallen larvae to be seen and subsequently trapped on adhesive tape

4. Instructions for the introduction of tick larvae into the acaricide impregnated paper packets should be read carefully before commencing, including a practice run first without any tick larvae being involved

**c. Composition of the FAO test kit**

a) The kit contains sealed, aluminium foil envelopes containing equal numbers of impregnated papers for each of the following acaricide active ingredients (AIs), and standardized FAO data report forms.

| Chemical | Percentage active ingredient in formulation on paper |
|---|---|
| BHC | |
| dieldrin | 0.20 |
| OPs | |
| chlorfenvinphos | 0.20 |
| coumaphos | 0.20 |
| diazinon | 0.10, 0.20 |
| SPs | |
| cypermethrin | 0.20 |
| cyfluthrin | 0.03 |
| deltamethrin | 0.06 |
| flumethrin | 0.0036 |
| cyhalothrin | 0.05, 0.1 |
| Amidine | |
| amitraz | 0.4, 0.1 and 0.025 |
| MLs | |
| cydectin | 1.0 |
| Control | |
| solvent only | |

b) Additional equipment required, but NOT supplied with the FAO kit, consists of:

| | |
|---|---|
| Tubular plastic clips | Log/probit graph paper (one per assay) |
| Plastic stands | 40 mm rubber bung |
| Fine paint brushes | One glass Petri dish, 90 mm diameter |
| Pointed glass rods | One glass Petri dish, 150 mm diameter |
| Glass conical flask | Two glass beakers |
| Polystyrene blocks | White enamel tray |
| Needles | Two forceps |
| Cotton wool | Scissors |
| Adhesive tape | Disposable gloves and masks |
| Double sided adhesive tape | Tally counter |
| Small aerated cardboard boxes for tick collection, 150mm deep glass tubes with ventilated but larva-proof stoppers for tick rearing | Magnifying glass (×2) |

**Preparation of the packets for introduction of the tick larvae**

An aluminium envelope containing the control papers (impregnated with solvent only) is opened and a single paper removed with forceps.

The envelope is resealed.

The paper is folded in half horizontally, with its identification mark (AI and concentration) on the inside.

A single tubular plastic clip is slid up each short side of the paper, starting from the folded end. Alternatively, bulldog clips can be used.

The packet formed, with its unfastened end upwards, is then put on a stand by pushing the side clips down over the stand's two nails.

Pushing the side clips gently towards the middle of the packet forces it to open slightly.

The process is repeated to make a second control paper packet that is also set up on a stand.

**Introduction of reared tick larvae into the packets**

**a) The equipment:**

1. 150 mm glass rearing tubes, 10 to 15 mm diameter, with 14 to 21 day old tick larvae, closed
2. 40 mm diameter rubber bung, with a 10 mm hole drilled into its underside, to a depth of 15 mm
3. 1 small (e.g. 90 mm diameter) and one larger (e.g. 150 mm diameter) glass Petri dish
4. One small beaker of water with detergent added and a second beaker with water only
5. Small conical flask holding glass rods and fine paintbrushes
6. White enamel tray on which to set up the above equipment
7. Water with a wetting agent (detergent)
8. Double sided adhesive tape
9. Several supports (one for each strain) for the test packets during introduction of tick larvae.

**b) Setting up the equipment:**

A closed glass rearing tube of tick larvae, obtained directly from the incubator, is supported upright in the hole in the inverted rubber bung.

The tube of larvae, supported in the rubber bung, is stood upright inside the small Petri dish within the larger one to form a moat.

The whole unit is placed on the white enamel tray.

Water containing the wetting agent (detergent) is added to the moat, to trap any tick larvae that escape from the rearing tube.

The conical flask holding the brushes and rods for transferring larvae, two supports for acaricide test packets, the two beakers of water, one of which also contains detergent, and some cotton wool wetted with water, are also placed on the tray.

The edge of the tray has a continuous band of double-sided adhesive tape to trap any escaping tick larvae.

**c) Introduction of the tick larvae into the packets:**

**NOTE**: Control packets (with the solvent only) are prepared with tick larvae first, followed by those with acaricide active ingredients and for each acaricide in order of increasing concentration.

The glass rearing tube is freshly removed from the incubator, opened and tick larvae permitted to aggregate freely at its top rim.

A small cluster that will contain approximately 100 larvae is picked up from the rim of the open tube using the fine brush and, with the aid of a glass rod, eased into the control packet.

Care should be taken to ensure brush and rod do not come into contact with the packets, which should only be handled by the clips.

Brush and rod can be cleared of excess or tenacious tick larvae by rubbing on the wetted cotton wool which was placed in the tray.

The control packet with its tick larvae is removed from its stand and closed by sliding a tubular plastic clip along its open, top paper sides. The top clip must touch both of the side clips to form a completely continuous seal to prevent subsequent escape of any tick larvae during the incubation period.

The closed packet is laid on a tray ready for subsequent placement in the incubator.

This entire procedure is repeated for the second control packet.

The packets are arranged on trays or in racks, without contact with each other.

The procedure is repeated with two packets for each concentration of each acaricide, always working in ascending order of their concentration per acaricide.

Extra care should be taken to avoid contact between brushes, glass rods and the paper packets, particularly in the case of those impregnated with acaricides, to avoid their contamination. As an additional precautionary measure, between the introduction of tick larvae into the control packets and those of each concentration of each type of acaricide, the brush and rod can be cleaned in the detergent solution and rinsed in water from the beakers, and then allowed to dry thoroughly at ambient temperature. Acetone can also be used for this purpose. A substitute brush and rod are used while waiting for the others to dry.

The packets are placed in the incubator at a temperature of 27 to 28°C and 85 to 95 percent RH for 24 h. Variations in temperature during the incubation period can have a considerable effect on the toxicity of the chemical. They should therefore be avoided or at least carefully noted with the test results if they do inadvertently occur.

**NOTE**: Packets impregnated with amitraz and cydectin should be left with their larvae in the incubator for 48 hours.

For the LPT incubation procedure specifically for amitraz and cydectin, see later.

**Tick larval mortality counts**

**a) The equipment:**

1. Polystyrene block or thick sheet (approximately 300 × 100 × 10 mm)
2. Two entomological needles
3. Wetted cotton wool
4. Fine paintbrush
5. Beaker containing water
6. Forceps
7. White enamel tray
8. Standard FAO Acaricide Resistance Test Kit report forms
9. Disposable gloves and face mask
10. Tally counter
11. Magnifying glass (×2)

**b) Counting the larvae:**

The packets are examined in the same order as they were prepared and filled with tick larvae. This is an attempt to minimise variation in duration of exposure to test acaricide.

The recommended mortality criterion is the inability of tick larvae to walk. Only those larvae capable of walking are considered to be alive. For assessment of walking ability, a magnifying glass and lamp should be used. Ticks can be stimulated by gently breathing directly onto them. All other larvae, including those that move their appendages but do not walk, are counted as if dead.

A control packet is opened by holding it by one side clip and lying it on the polystyrene block with the top opening to one side of the block, The top clip is removed and the bottom side of the paper packet secured to the block with a pin. The remaining clips are removed and the packet secured to the block in the open position with the other pin.

The live larvae are removed with the paintbrush and immobilized on cotton wool moistened with a wetting agent (detergent) in water. The dead larvae remaining are then counted and recorded, followed by the living larvae that have been trapped in the cotton wool. Extensive experience with *B. microplus* has shown that mortality of larvae in the control, untreated papers is usually zero. Counting of larvae is not necessary if it is evident that they are all or very predominantly alive (i.e. considered to be zero percent mortality).

The second control packet is opened and its tick larvae examined and counted.

If control mortality is greater than 10 percent, then the test conditions may be faulty. The test method should be checked carefully and the entire test repeated correctly.

If control mortality is less than 10 percent, the experimental tick packets are opened one by one**,** in the ascending order of acaricide concentration in which they were prepared with larvae for incubation**.** In each lot, live larvae are first removed and trapped in moistened cotton wool for counting as before. The dead larvae are counted *in situ* on the paper of the opened packet. Detailed counting is not necessary if the larvae in a packet are clearly all dead; such lots are automatically considered to be of 100 percent mortality.

**Treatment of results**

If counting reveals larval mortality to be "very low" (<5%), then the direct mortality figures can be utilized.

If they are found to be "low" (5 to 10%) in the control, then the percentage mortality in all of the experimental batches of larvae will have to be corrected by applying Abbott's formula:

$$\text{Corrected percent mortality} = \frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} x100$$

*Tabulation of results*: the mean value for each of the two results for control and each concentration of each acaricide are recorded on the standard results forms provided in the FAO kit.

If a full dose-mortality test has been undertaken, results should be plotted: percent concentration (x-axis) by Probit mortality (y-axis) for each acaricide in the kit using log/probit graph paper. Alternatively, the data can be submitted to *Polo-PC* for analysis.

### Interpretation of results

Results similar to (a), (b) or (c) (**Figure 1**) can be found in a complete dosage mortality test. If the population is homogeneously susceptible, a straight line will be obtained as in (a). If, on the other hand, a line similar to (b) is obtained, it indicates that the population is a mixture of susceptible and resistant individuals. The horizontal portion of this line (b) will vary in position depending on the proportion of resistant ticks in the sample. If the resistance factor is very low, the flat portion may be difficult to distinguish as the displacement of the susceptible line to the right will be small.

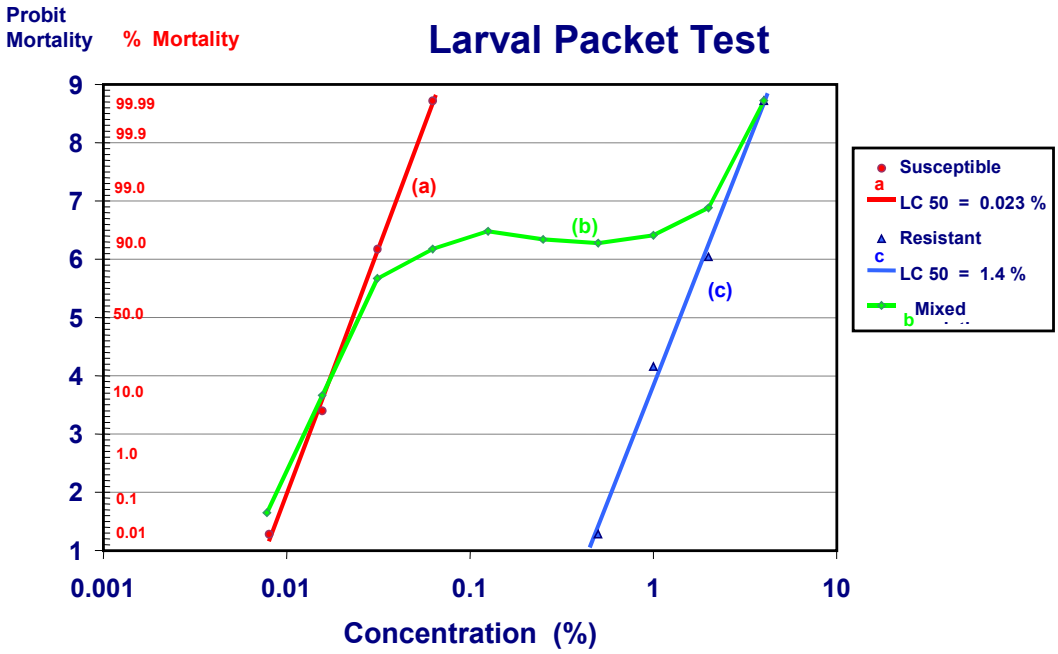



**Figure 1.** Examples of probit mortalities for samples of three populations (a, b and c) of the cattle tick, *Boophilus microplus*, subjected to a complete dosage mortality test for an acaricide. (LC = lowest concentration needed to provide a particular mortality rate)

### Discriminating dose (DD)

The use of a DD for each acaricide is a means of substantially reducing the amount of work needed to determine whether resistance is present. It is only really necessary to do a complete dosage mortality test at the first discovery of resistance to an acaricide in the field. Such dosage mortality tests, based on a series of concentrations of the acaricide, provide the means for determining the susceptibility of a tick population to a particular chemical and the homogeneity of this response. A dose mortality test and a probit analysis of the resulting data provide the basis for establishing a discriminating dose (DD). This can be used as a diagnostic tool for determining if failure of tick control by a compound is due to resistance, for estimating the proportion of a tick population that is resistant and for surveys to estimate the extent of the distribution of resistance to an acaricide.

The principle is that when a dosage mortality line can be established with confidence for a susceptible population, the concentration required to ensure 100 percent mortality of the larval population under test can be determined, as described below. If a sample of larvae is treated with this discriminating concentration and a significant number of ticks survive, it indicates that resistant ticks are present. The percentage of ticks surviving treatment at the DD can be taken as the percentage resistance to the acaricide. Care should be taken when determining what will be selected as the critical DD. Because variations in response will occur in separate tests, even using a homogeneous susceptible tick population, it is important that the DD should be determined from the mean of several tests.

**Discriminating doses (DD) for the technical acaricides in the Larval Packet Test (LPT)**

a) After the CSIRO, for *Boophilus microplus* in Australia (not necessarily 2 × LC99.9)

| **Chemical** | **Percentage concentration (v/v)** |
|---|---|
| DDT | 2.0 (w/v) |
| Dieldrin | 0.3 |
| Dioxathion | 0.28 |
| Coumaphos | 0.10 |
| Chlorpyrifos | 0.10 and 1.0 |
| Flumethrin | 0.01 |
| Cypermethrin (cis/trans 1:1) | 0.50 |
| Amitraz | 0.4, 0.1 and 0.025 |

(DD for other OP and SP acaricides were calculated but were not needed for resistance testing.)

b) The WARRC DDs, for the susceptible Yeerongpilly strain of *B. microplus*.

| **Chemical** | **Percentage concentration (v/v)** |
|---|---|
| Diazinon | 0.1, 0.2 |
| Coumaphos | 0.2* |
| Chlorfenvinphos | 0.2 |
| Dieldrin | 0.2 (w/v) |
| Cyfluthrin | 0.03 |
| Cyhalothrin | 0.05 and 0.1 |
| Cypermethrin | 0.2 |
| Deltamethrin | 0.06* |
| Flumethrin | 0.0036 |

*Statistically calculated.

**Interpretation of results**

One method of determining the DD to be used is to double the mean $LC_{99.9}$ derived from a series of tests conducted with the susceptible strain. This is, in effect, a crude substitute for an accurate estimate of an $LC_{100}$. From an operational standpoint, the procedure is probably a pragmatic solution. However, too high a DD can kill the heterozygote resistant individuals and result in a missed diagnosis when the frequency of resistant genes in a population is low. Practical experience with the LPT will allow a higher DD to be set where required in order to be certain that all surviving ticks are resistant. In particular, reference laboratories might need to set DDs for their own regions up to four or five times the $LC_{99.9}$ to account for factors such as the relative resistance of susceptible ticks. In most cases of genetically based resistance, resistance factors exceed 10 fold.

The DD would be represented by a dotted vertical line at $2 \times LC_{99.9}$ in **Figure 1**. It can be appreciated that not only does this indicate the presence of any resistance in the population, but also, from the point at which the vertical line intercepts the dosage mortality plot, an estimate of the proportion of resistant ticks in the sample can be obtained; in this example for population (b) it is approximately 8 percent. Finally, if this mixed population of susceptible and resistant individuals were to be selected, by exposing each subsequent generation of larvae to increasing concentrations of the chemical used in the test, one should eventually, after several generations, arrive at a line as represented by (c); where all the ticks are resistant.

It is only when this line (c) is finally reached that one can refer with confidence to resistance factors, although the potential of the resistance can be estimated from the chemical concentration required to produce mortality in the most resistant component of the population. This estimate is frequently calculated through a comparison of, say, the concentrations ($LC_{99}$) required to produce 99 percent mortality in the homogeneously susceptible compared with resistant populations. It is more common to quote the $LC_{50}$, or concentration required to kill 50 percent of the larvae, for comparisons between susceptible and resistant populations. Hence, in **Figure 1**, the $LC_{50}$ for the susceptible population (a) would be 0.02 percent and for the resistant strain (c) 1.45 percent, and the resistance factor would be 72 fold.

It should be re emphasized at this point that great caution should be exercised in interpreting resistance factors in terms of required field concentrations needed to combat resistance problems. Particularly, one should not use resistance factors obtained for different chemical classes to make judgements as to the seriousness of the comparative resistance problems affecting each. The laboratory test is well suited and extensively proven as useful for identifying that a resistance problem does exist for a chemical. It helps one to choose other chemicals which are not affected and which may be suitable alternatives to test in the field. Interpretation of laboratory tests should not be taken further than to establish these conclusions.

**Modification of the LPT for amitraz resistance testing**

Some modification to the LPT is required for diagnosis of amitraz resistance because resistant strains do not show a linear relationship between probit mortality and log concentration of the acaricide. (The reasons for this are unknown.) A discriminating dose ($2 \times LC_{99.9}$) can therefore not be determined and instead three concentrations of amitraz are chosen. These are 0.2, 0.05 and 0.0125 percent w/v.

The test follows exactly the LPT protocol but the packets are enclosed in plastic Petri dishes (with each replicate of packets for one concentration in a separate dish) and the exposure time is extended to 48 hours. In the past, the paper packets were enclosed in polythene bags but some polythene has been found to be toxic to larvae. Enclosing the packets in Petri dishes

allows the test to be read after 48 hours. Unenclosed packets do not give sufficient mortality until 6 days have passed.

Results are shown in **Table 3** and their presentation for analysis in **Figure 2**. Field strains, with mortality close to those of the laboratory maintained, homogeneous, amitraz resistant strains (Ulam), are probably resistant to amitraz. This is the best that can be achieved with the LPT or any other test used to date at the CSIRO or the Queensland Department of Primary Industries, Brisbane, Australia.

**Table 3. Example of results from a modified FAO Larval Packet Test procedure for suspected resistance of ticks to the acaricide Amitraz**
(S. Hughes, CSIRO, Australia, personal communication)

| **CHEMICAL :** | **Amitraz** | **LABORATORY NO. :** | **W981843C** | **DATE TESTED :** | **09 Jul 98** |
|---|---|---|---|---|---|

| **% CONCENTRATION** | **YEERONGPILLY** | | | **ULAM SELECTED** | | | **W981843C** | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. dead | Total | % mortality | No. dead | Total | % mortality | No. dead | Total | % mortality |
| 0.4 | 200 | 200 | 100.0 | 82 | 270 | 30.4 | 137 | 231 | 59.3 |
| 0.2 | Not tested | | | | | | | | |
| 0.1 | 220 | 220 | 100.0 | 58 | 218 | 26.6 | 105 | 256 | 41.0 |
| 0.05 | Not tested | | | | | | | | |
| 0.025 | 220 | 220 | 100.0 | 17 | 212 | 8.0 | 32 | 237 | 13.5 |
| CONTROL | 0 | 190 | 0.0 | 0 | 210 | 0.0 | 0 | 200 | 0.0 |

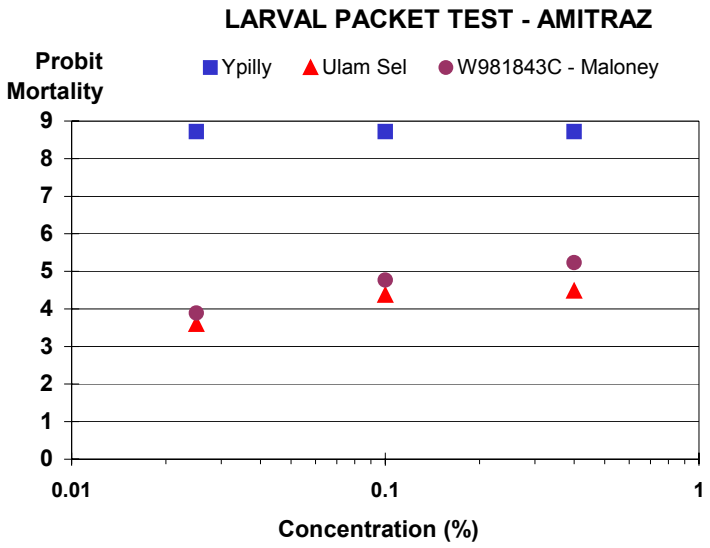



**Figure 2.** Example of results from a modified FAO Larval Packet Test procedure for suspected resistance of ticks, using technical quality amitraz. (S. Hughes, CSIRO, Australia, personal communication)

Where there is some doubt about the level of resistance of the field strain, the following additional tests might be undertaken:

After selection of larvae with 0.2 percent technical amitraz in packets, followed by infestation on donor cattle housed in stalls, the next generation of larvae should be re-tested with the larval packet test.

*Use of a positive control*. If the larvae have similar dosage mortality response characteristics of an amitraz resistant strain (e.g. Ulam) tested at the same time, this confirms amitraz resistance. However, an amitraz spray trial (in the field or in stalls) may need to be used until full confidence can be placed in the test and its results.

**USDA modification of LPT for amitraz**

The standard technique is modified in two ways.

Nylon fabric (Type 2320, Cerex Advanced Fabrics, Pensacola, FL) is used instead of Whatman filter paper as the substrate.

Formulated amitraz (Taktic 12.5% EC) is diluted in trichloroethylene and olive oil instead of technical amitraz. All other conditions are identical to the standard technique.

These modifications produce data that are suitable for the determination of resistance ratios and discriminating doses. The RR at the LC50 estimate was determined to be 26.3 (25.7–26.9) for the Santa Luiza versus Gonzalez (susceptible) *B. microplus* strains and 7.3 (5.5–9.9) at the LC50 estimate for the Panama versus Kerrville (susceptible) *Rhipicephalus sanguineus* strains. A DD of 0.03 percent was determined from the Gonzalez strain. A full description of this modification can be found in the *Journal of Medical Entomology* 39: 645–651 (2002).

**The Larval Packet Test (LPT) for macrocyclic lactones (MLs) using cydectin technical powder**

The LPT is carried out with cydectin technical powder using the standard protocol. As with the LPT for amitraz it may be necessary to incubate the larvae for 48 h to improve sensitivity of the tests. The data presented in a form for analysis (**Figure 3**) are from four replicates. The recommended DD (2 × LC99.9) would be 1 percent.

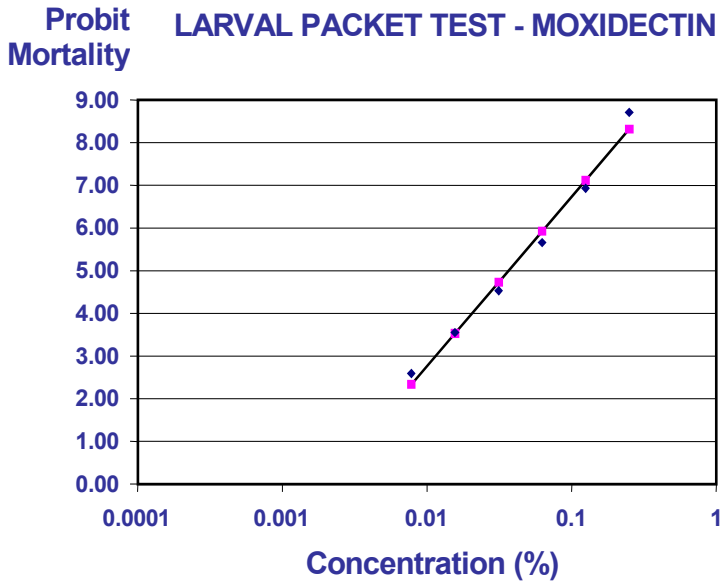



**Figure 3.** Example of interpretation of the results from a modified FAO Larval Packet Test procedure for suspected resistance of ticks to the macrocyclic lactone, moxidectin, using cydectin technical powder. LC50 = 0.037, LC99 = 0.14, incubation 24 h. (S. Hughes, CSIRO, Australia, personal communication)

## B. RESISTANCE TESTS USING ENGORGED FEMALE IXODID TICKS

The Adult Immersion Test (AIT) was first developed by Drummond *et al*, (1973). FAO is currently exploring the possibility of a rapid AIT similar to the Drummond AIT but incorporating a number of modifications including the use of a discriminating dose (DD) and the observation of egg laying but not larval hatching. Both tests are included here.

### The AIT based on a series of acaricide concentrations (for *Boophilus microplus*)

***Principle:*** Dose mortality responses of ticks to acaricides are determined by treating engorged female ticks with a range of dilutions of an acaricide and comparing treated and untreated ticks to assess the effect of a treatment on fecundity and fertility.

**1. Equipment and supplies**

1. Sample of engorged female ticks: healthy, not laying eggs
2. Ethanol, laboratory grade (formerly laboratory grade xylene)

3. Triton X-100, analytical
4. 10 ml glass measuring cylinders
5. 125 ml glass conical flasks
6. 1 ml pipettes
7. 1 ml Eppendorf tubes
8. 90 mm plastic Petri dishes
9. Ring portions of metal jar lids
10. Glass stirring rod
11. Plastic containers: (e.g. ice cream type cartons/tubs) 500 ml
12. Plastic kitchen sieves
13. Adhesive tape: double sided, 2 cm wide
14. Stoppered vials with caps modified to allow ventilation through fine mesh bolting cloth
15. Fine mesh bolting cloth (or cotton/nylon gauze)
16. Freezer, at -25°C
17. Incubator, set to 27 or 28°C and 85 to 95 percent RH

## 2. Candidate compounds

***General remarks*:** Experience with adult tests has shown that testing a maximum of two SP acaricides, amitraz, and any OP that might be causing problems in the field can provide complete information. The two SP acaricides used are flumethrin (Bayticol®, Bayer, Germany) and cypermethrin (representative of all SPs other than flumethrin) because other SPs can control some strains that are resistant to flumethrin, although if resistance to SPs other than flumethrin is shown, resistance to flumethrin can be inferred. Cypermethrin can be replaced by deltamethrin if required.

Resistance to the SPs means that amitraz or new acaricides (MLs, Fluazuron) may be used. Resistance to amitraz means that SPs (if no resistance to SPs is shown) or new acaricides (MLs, Fluazuron) can be used. Resistance to OP compounds is complex and strains resistant to one OP might be controlled by other OPs. Consult the FAO Regional Reference Laboratory (RRL) about which OPs to use or check other OPs with the LPT or AIT to find an OP that will kill the resistant ticks.

Commercial formulations such as emulsifiable concentrates or wettable powders may be used for resistance testing, but standard formulations made from technical grade active ingredient (AI) are preferred for comparisons of relative effectiveness.

Formulate active ingredient as emulsifiable concentrates containing 25 percent of AI (w/v), 65 percent xylene, and 10 percent Triton X-100.

$$\text{AI needed (g)} = \frac{\text{ml desired} \times \text{\% concentration desired}}{\text{\% purity available}}$$

Compounds not soluble in xylene are formulated in mixtures of N-methyl-2-pyrrolidone, xylene, and Triton X-100. Start with 25 percent water, 65 percent xylene, and 10 percent Triton X-100. Dilute to same initial percentage concentration as pesticide.

## Treatment procedure for the classical Drummond AIT and the FAO AITDD test

### 1. Drummond AIT

1. Perform a serial dilution from the stock formulation, using water as the diluent and beakers/plastic cups/ice-cream tubs as containers. Final volumes of diluted acaricide will be 50 ml. Use the following formula for making the initial dilution from stock material.

$$\text{amount of stock needed} = \frac{\text{final \% concentration} \times \text{final volume}}{\text{\% initial concentration}}$$

Example: need 50 ml of 1.0 percent AI from stock of 25 percent

2 ml of stock formulation diluted with 48 ml water to reach a final volume of 50 ml:

$$2 \text{ ml} = \frac{1.0\% \text{ AI} \times 50 \text{ ml}}{25\% \text{ AI}}$$

Dilution errors are a major source of experimental error. Extra care must be taken when small volumes of the stock acaricide are added to relatively large amounts of diluent. Pipettes and graduated cylinders must be clean and dry.

2. Solution for control ticks will contain the same quantities of ethanol (formerly xylene) and Triton X-100 as the initial acaricide treatment concentration. Therefore, the stock control solution will be diluted in the same way that the stock acaricide was diluted.

3. Rinse ticks in tap water to remove faeces and other debris.

4. Allow ticks to dry by placing them on paper towels. Place the ring portion of a metal jar lid around the ticks to confine them until they are dry.

5. Assign engorged female ticks at random to groups (minimum 10 ticks per group).

6. Weigh each group of engorged ticks.

7. For the control solution and each of the acaricide test solutions, and beginning with the lowest concentration, place the ticks of each group directly into a container, such as an ice cream carton/tub, with 25 ml of the treatment solution and stir vigorously with a glass stirring rod for 30 s before and after adding ticks.

8. After 30 s, the acaricide is poured off through the sieve and the ticks are retained.

9. The ticks are then placed onto a clean paper towel to dry. All ticks of each group are then deposited into a Petri dish (90 mm diameter) and sealed with tape.

10. The ticks are stored at 27 to 28°C and 80 to 95 percent RH for oviposition.

11. After 20 d, the ticks are discarded and the eggs produced by the ticks in each treatment group are weighed and then placed in a shell vial (8 dram) and sealed with a cotton plug.

12. The eggs are held in the incubator for another month and the percentage hatch of larvae is estimated visually.

13. Replicate three times. Each replicate requires the preparation of a fresh set of dilutions from the stock material. All graduated cylinders and pipettes must be carefully washed and dried before use.

**Estimation of acaricide efficacy**

The efficacy of the acaricide is determined by comparing the estimated reproduction (ER) of each group of treated ticks with that of the control ticks. Estimated reproduction is an estimate of the number of larvae produced by each female at each concentration of acaricide used in the bioassay.

First, the ER is calculated as follows:

$$\text{ER} = \frac{\text{weight of eggs laid (g)}}{\text{number of females}} \times \text{estimated hatch (\%)} \times 20\,000 \text{ (\# eggs per g)}$$

Secondly, the ER of each group of treated ticks is compared with that of its control group. The percentage control is calculated as follows:

$$\text{Control (\%)} = \frac{\text{ER control ticks - ER treated ticks}}{\text{ER control ticks}} \times 100$$

Resistance (%) = 100 - Control %

Recently, USDA scientists have begun to calculate an index of fertility (IF) rather than the ER. The equation for IF is the same as for the ER, except that multiplication by the number of eggs per g (20 000) is omitted. Therefore, IF is an estimate of the weight of larvae produced per female at each concentration of acaricide used in the bioassay.

**Mortality response.**

The software *Polo-PC* is used to produce statistics of the dose mortality response. When creating the data set for *Polo-PC*, control value percentages are rounded to the nearest integer. Use the percent control values as if they represented a number of ticks; for example: 92 percent control would represent 92 dead and 8 survivors.

**2. AIT modified with a discriminating dose (AIT-DD)**

The adult immersion test with a discriminating dose (AIT-DD) has been developed to provide a quicker and simpler test than the classical Drummond AIT. Using the AIT-DD, it is not necessary to weigh the eggs or to estimate the percentage hatch, allowing the test to be completed within 7 days rather than 4 to 5 weeks.

1. Dilute acaricides to the recommended discriminating doses.

2. Add 20 ml of the diluted acaricide to 100 ml plastic containers with screw-cap lids. Add 20 ml of water to another container as a control. Label the containers.

3. Add to each container a minimum of 10 healthy, clean, engorged female ticks taken from cattle within 48 h of the test.

4. Immerse ticks in acaricide solution for 30 minutes at about 25°C and shake containers gently.

5. After 30 minutes, pour off the acaricide solution into a safe storage container and dry the ticks gently on paper towelling.

6. Stick the ticks from one container, with ventral side up, onto double-sided sticky tape in a Petri dish.

7. Incubate the dishes in a larger polystyrene container at about 25 to 30°C for 7 days. Keep the container moist with damp paper or towelling. Do not shake the container as the egg batches from each tick have to be observed.

8. After 7 days count the number of ticks that have laid eggs.

**Treatment of results**

Ticks immersed in water only should have laid many eggs after 7 days. Ticks that have been treated with acaricide but still lay eggs are resistant. Ticks that have been treated with acaricide and do not lay eggs are susceptible. The percentage resistance is calculated as:

$$\text{Resistance (\%)} = (N_t/N_w) \times 100$$

Where, $N_t$ = number of treated ticks laying eggs

$N_w$ = number of untreated ticks laying eggs

**Suggested discriminating doses for AIT-DD**

DDs should be set at 2 × the $LC_{99.9}$ for the susceptible Porto Alegre (POA) strain to establish a sensitive test for resistance. The currently recommended DDs (from the Report of FAO Industry Sub-group 2 on AIT for acaricide resistance in *Boophilus* ticks, Guernavaca, Mexico, September 2000) are listed below, some of which are derived from POA and others from the Yeerongpilly strain. It is likely that these DDs will require modification in the future

| Acaricide | Suggested discriminating dose (g/litre) |
|---|---|
| Diazinon | 5 |
| Chlorfenvinphos, coumaphos, ethion | Same as diazinon |
| Cypermethrin | 0.05 |
| Flumethrin | 0.075 |
| Deltamethrin | Same as flumethrin |
| Amitraz | 2.5 |
| Moxidectin, ivermectin | 1 |

## The AIT for technical MLs

ML technical material is dissolved in laboratory grade ethanol and 2 percent Triton X-100 detergent (technical material does not dissolve in water and the detergent helps to wet the ticks in the ML solution). Serial dilutions are prepared in ethanol and Triton X-100, then water is added so that the final ethanol and Triton X-100 concentration is 1 percent ethanol and 0.02 percent Triton X-100. Adjustment is made if the technical material is not 100 percent pure (i.e. 100 percent AI).

An example is as follows:

1. Mix 9.8 ml ethanol with 0.2 ml Triton X-100 (2 percent)

2. Dissolve 0.1 g technical ML in 10 ml of the ethanol Triton X-100 mixture (1 percent)

3. Prepare a serial dilution of the 1 percent ML in ethanol Triton X-100 mixture. (i.e. 0.5 ml of 1 percent ML into 0.5 ml of ethanol Triton X-100 mixture)

4. These serial dilutions may be stored in a freezer (at approximately -25°C) until needed. They will not freeze at this temperature and can be used for several weeks. Maximum storage time is unknown.

5. When needed, 0.2 ml of each of the serial ML dilutions in ethanol and Triton X-100 are diluted in 19.8 ml of water and 10 female ticks are added. For controls, 0.2 ml of ethanol and 2 percent Triton X-100 is diluted in 19.8 ml of water. (This is 1/100 dilution so the maximum ML concentration is 0.01 percent, the ethanol is 1 percent and the Triton X-100 is 0.02 percent. It has been found that engorged female ticks can be immersed in 1 percent ethanol and 0.02 percent Triton X-100 for 1 hour without affecting subsequent egg laying)

6. A minimum of 10 weighed, engorged female ticks are immersed for 30 min in each dilution at approximately 25°C. The glass conical flasks (125 ml) used are held on a rocker (slow speed) or gently agitated during this time.

7. The ML is decanted through a sieve and the ticks dried on paper towelling.

8. Ticks are incubated, dorsal surface down on double sided adhesive tape (e.g. 3M Co.) in Petri dishes (90 mm diameter approx) at 27 to 28°C, 85 to 95 percent RH.

9. After 7 days, eggs are collected and weighed.

10. Mortality (percentage inhibition of fecundity) is calculated as follows:

$$\text{Index of fecundity (IF)} = \text{weight of eggs laid (g)/weight of females (g)}$$

$$\text{Percentage mortality} = \frac{\text{IF control group} - \text{IF treated group}}{\text{IF control group}} \times 100$$

This calculation is the same as for the Drummond AIT, but larval hatching is not estimated. This would require another four weeks delay in getting a result and the visual estimate of hatching is not an accurate measure. The original intention of including larval hatch was to determine all the effects of an acaricide. This is not necessary for a resistance test.

## The AIT for injectable formulations of MLs

Commercial preparations of 3 MLs (cydectin, ivermectin and abamectin injectables) are dissolved either in ethanol and 2 percent Triton X-100 or in demineralized water. All commercial preparations are at 1 percent ML concentration and are diluted in water to 0.01 percent. The MLs are also diluted in 1 percent ethanol and 0.02 percent Triton X-100 (0.1 ml of ML to 9.9 ml of 1 percent ethanol and 0.02 percent Triton X-100). The injectable MLs diluted in water give a slightly cloudy fluid at the highest concentration of 0.01 percent. The dilutions in 1 percent ethanol and 0.02 percent Triton X-100 give a clearer solution.

AIT is carried out with these preparations using the protocol described for the ML technical material. Results are shown for Avermectin injectable (abamectin 1 percent) and an estimated DD would be 0.008 percent (**Figure 4**).

**Table 4. Complete Adult Immersion Test (AIT) laboratory results report sheet for test of susceptibility of a tick sample to a range of representative acaricide active ingredients**

Property: Date of test:

Owner: Date observing eggs laying:

City: Date observing hatching:

Species of tick: Responsible for test:

| | Engorged female tick | | Egg laying | | | | | % | Efficacy of | % |
|---|---|---|---|---|---|---|---|---|---|---|
| **Active ingredient of product** | **No.** | **Weight (g)** | **Complete** | **Partial** | **Dead** | **Absent** | **Weight (g)** | **Hatchability** | **Reproduction** | **Efficacy** |
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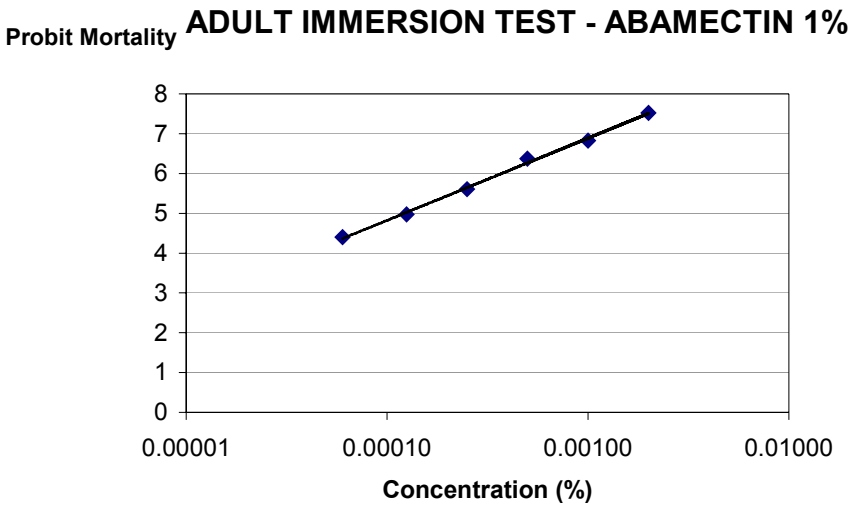


**Figure 4.** Example of results of adult immersion test (AIT) for tick susceptibility to an injectable macrocyclic lactone, abamectin 1%. LC50 = 0.00012%, LC99 = 0.0016%, Slope = 2.06, immersion 30 min, incubation 7 days

**Additional notes**

In any adult tick bioassay some mortality that is not related to treatment with acaricide will occur. Some ticks die and become black prior to or during egg laying. It is suggested that more accurate results could be obtained by not collecting eggs from these ticks and reducing the total weight of females in that batch by the mean weight expected for the dead ticks (e.g. for one dead "black" tick in a group of 10, omit any eggs from this tick and reduce the group weight of ticks by 1/10 of the total weight prior to calculating IF).

## The AIT for technical fluazuron (Acatak)

Relative sensitivity of *B. microplus* strains to Acatak (fluazuron) was described by Graf *et al.* (1994). The methods can be adapted for testing resistance. Fluazuron is prepared in acetone and 2 percent Triton X-100 (solubility is low in ethanol and water).

1. Prepare a solution of 5.0 percent technical fluazuron in 2 percent Triton X-100 in distilled acetone (about maximum saturation).
2. Dilute 1 in 100 with demineralized water to give 0.05 percent Technical Fluazuron in 0.02 percent Triton X-100 in 1 percent acetone.
3. Dilute serially with 0.02 percent Triton X-100 in 1 percent acetone in demineralized water
4. Immerse 20 engorged female ticks in 20 ml of each dilution for one minute.
5. After one minute, pour off the fluid, dry the ticks on tissue paper and incubate them individually at 27 to 28°C and 85 to 95 percent RH in glass tubes.
6. After 6 weeks incubation, estimate the proportion of larvae hatching from eggs visually. **Figure 5** shows a probit analysis of hatching against concentration of

fluazuron for three tick strains. A reasonable discriminating dose (DD) would be 0.02 percent. A longer incubation time could be considered for this protocol. Fluazuron will not inhibit egg laying by the female ticks but affects the ability of larvae to hatch.

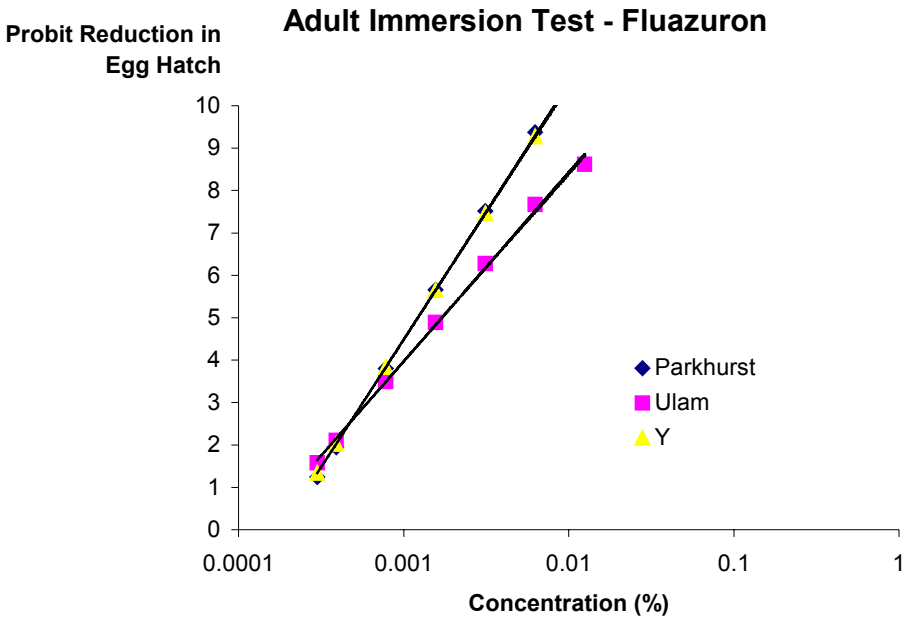


**Figure 5.** Example of interpretation of Adult Immersion Test (AIT) for tick susceptibility to flurazuron (Technical Akatak, Novartis®; Graf *et al.*, 1994).

Y, Yeerongpilly susceptible strain, LC50 = 0.0012%, LC99.9 = 0.004%

Ulam, amitraz resistant strain, LC50 = 0.00165%, LC99.9 = 0.0077%

Parkhurst, SP resistant strain, LC50 = 0.0012%, LC99.9 = 0.0039%) (CSIRO, unpublished)

## 6 EPIDEMIOLOGY AND CONTROL OF TICKS

The successful implementation of rational and sustainable tick control programmes in grazing animals is dependent upon a sound knowledge of the ecology or epidemiology of the parasite as it interacts with the host in specific climatic, management and production environments. In some countries, substantial ecological and epidemiological knowledge bases have been established through extensive studies and field trials. Not all developing countries and those in transition may have such information available, due to a lack of human, economic and infrastructural resources. However, it should now be possible to extrapolate to these from existing information or by using predictive models of cattle tick ecology that have been developed for most ecotypes and management systems.

In most situations, however, efficient and reliable methods for the control of cattle ticks and TBD are based on the use of a chemical treatment, often without a local understanding of

appropriate ecology or epidemiology. Acaricide treatments are commonly used in a *suppressive approach*, applying multiple treatments at regular intervals during the height of infestation. For *Boophilus* spp. ticks, the interval is usually 3 weeks, ensuring that treatment is in effect continuously applied to all ticks in the parasitic phase. Alternatively, application of acaricide is initiated when tick infestations exceed an acceptable level. This is known as a *threshold approach*. Where there is sufficient ecological information and annual population trends enable it, a *strategic approach* to treatment can be used. A strategic approach involves the treatment of specified generations of ticks, usually the first generation in a tick season, even when infestation is at a low level that does little or no damage to the host. The aim is to remove the source of future generations for that year, thereby reducing or eliminating the need for further chemical treatments later in the season.

Suppressive treatments are the most effective in the short term, keeping animals almost tick free, thereby reducing the direct effect of the ticks and the risk of disease transmission. This procedure will, however, select heavily for acaricide resistance in the ticks. It will also leave cattle susceptible to TBD. Threshold treatments may result in some direct production losses, depending on the level of tick infestation permitted before treatments are initiated. There should be a lower probability of outbreaks of TBDs, because acquired immunity to these is maintained due to higher levels of exposure of cattle to ticks infected with haemoparasites. Strategic treatments are more efficient in reducing direct production losses. The effects of each approach on the probability of endemic stability and acaricide resistance result from complex interactions. Overall, the strategic and suppressive approaches might increase the risk of outbreaks of TBDs. Acaricide resistance is fostered by any approach that uses an increased number of acaricide treatments or one in which a higher proportion of the total tick population is treated at any one time.

In addition to the risk of development of acaricide resistance (Davey and George, 1998; Romero Nasayo *et al*., 1997; Jonsson, 1997; Arantes *et al*., 1996; Beugnet *et al*., 1994; Kagaruki, 1991; Henna and Keruma, 1990; Nolan *et al*., 1989; Schnitzerling *et al*., 1989; Laranja *et al*., 1987; Vignon, 1987; Luguru *et al*., 1987; Curtis, 1987; Desquesnes, 1987), chemical control is associated with environmental contamination (Mulbry *et al*., 1996; Karns *et al*., 1995; Shelton and Karns, 1988) and often with high costs (Pegram *et al*., 1993; Spath *et al*., 1990; FAO, 1989a). It is now generally understood that tick control should not only be based on acaricide use, despite the fact that this remains the most efficient and reliable single method. Complementary approaches have been developed and are being researched to enable integrated control strategies against the tick and its haemoparasites. These include vaccines against the ticks and TBDs, grazing strategies and fungal biopesticides. The best strategies for control in any given region rely on application of acaricides with an understanding of local tick ecology (Quijada *et al*., 1997; Martins *et al*., 1995a; Farias *et al*., 1996; Brizuela *et al*., 1996; Saueressig, 1995; Nari and Solari, 1991; Evans, 1992; FAO, 1989b; FAO, 1989c; FAO, 1989d; FAO, 1989e; FAO, 1989f; Baker *et al*., 1989; Bourne *et al*., 1988; Desquesnes and Vignon, 1987; Sutherst, 1986a; Chiomba, 1987; Glass, 1986; Sutherst, 1986b) together with various bio-control methods as appropriate.

## 7 CURRENTLY AVAILABLE CONTROL STRATEGIES

The currently available tools for tick control consist of chemical acaricides relying on treatment with different application methods and/or formulations of acaricides, tick resistant animals, tick vaccines, TBD vaccines and management interventions. The availability of each of these options, their advantages and disadvantages, and the cost benefit of each alternative strategy should be assessed before deciding on a control programme. Ideally, strategies should target the parasitic and free-living phases of the life cycle and the role of the ticks in the transmission of TBDs (babesiosis, anaplasmosis, cowdriosis) should not be neglected. The influence that any of

the cattle tick control strategies might have on the enzootic stability of these diseases must always be included in the decision making process.

## 8 CHEMICAL CONTROL

There are many examples where ticks have been effectively controlled or eradicated solely by the thorough application of efficient acaricides. These products will remain of paramount importance in animal production in much of the world for the foreseeable future. In order to delay the emergence of acaricide resistance, to meet consumer expectations and to prevent environmental contamination, other control procedures must be integrated with acaricides. Acaricides can be applied by several methods, strategies and product formulations. The choice depends on the needs of individual farmers, the availability of human and monetary resources and the economic impact of ticks in the animal production system in question. The recent philosophy of minimising the use of chemical acaricides does not diminish their key role in integrated parasite control systems. Indeed, there are situations, such as where market demand for tender beef has increased, that have forced farmers to return to the use of more tick susceptible, *Bos taurus* breeds of cattle.

An ideal acaricide would be cheap, easily applied, with a strong knock down effect and sufficient residual effect on female ticks to prevent egg laying and to protect cattle from reinfestation by larvae. It should not select for resistance through a prolonged, gradual decay on the animal (i.e. it should have a sharp cut off in efficacy with time). In addition, it should be non-toxic to livestock and humans and have no detectable residues in meat and milk. Unfortunately, such an ideal acaricide has not yet been produced.

It remains to be seen if current levels of cattle production can be achieved without acaricide use in regions where ticks are endemic. Regardless, acaricides will continue to be essential in eradication schemes and where cattle are being transported outside endemic areas.

**Eradication programmes using systematic, suppressive treatments**

The basic concept of eradicating ticks with acaricides is to treat all cattle with acaricides at intervals that are short enough to ensure that all ticks that pass through a parasitic phase are exposed to lethal concentrations of acaricide. These treatments must be continued over a long enough period to ensure that all ticks in the free-living phase will die of exposure or ageing if they do not attach to a host. Experiences with eradication programmes were gathered in the United States (George, 1996; FAO, 1989g; FAO, 1989h) mainly in the first half of this century and more recently, in some areas of Argentina (Signorini and Spath, 1995; Signorini, 1991; FAO, 1989i; FAO, 1989j). The failures, delays and limited success of tick eradication campaigns observed in certain countries, such as Australia, Mexico, Puerto Rico, Uruguay and Cuba, illustrate some of the difficulties involved in using this strategy (FAO, 1989k; FAO, 1989m; FAO, 1989n; FAO, 1989o; Crom *et al*., 1992; George, 1990a; Cordoves *et al*., 1986; Suthern and Combs, 1984).

***Principle:*** Treatments against ticks are applied at short intervals (maximum of 21 days, for *Boophilus* spp.), often using synthetic pyrethroids or amitraz compounds in immersion dips or pour on formulations. The strategy is mainly recommended for eradication programmes.

***Prerequisites:*** When suppressive treatments are intended to achieve eradication of ticks from a defined region, a long-term commitment is essential. This will involve thorough epidemiological surveillance, obligatory periodical acaricide application, effective controls on livestock movement, adequate training for personnel, the active participation and co-operation of farmers, regulatory support from government and adequate and long term guaranteed financial resources. Eradication is also dependent upon sustainable supplies of effective acaricides against which there is little or no resistance. Hence, continuous monitoring of

resistance to acaricides is crucial. In general, eradication is more likely to be achieved in areas that are less favourable for ticks due to ecological and climatic conditions. Once ticks are believed to be eradicated from a region, some form of physical border or effective movement control of livestock must be maintained between the tick free and tick-infested areas. The administration of these borders is expensive and will in many cases be a continuing cost.

***Advantages:*** Although eradication of the cattle tick from a prescribed area is a daunting and expensive task, if achieved and maintained, the long-term benefits generally compensate.

***Disadvantages:*** Cattle produced in areas freed from the ticks may become highly susceptible to TBDs such that if there are any fresh incursions of ticks into the area, outbreaks can be severe. Hence, vaccination programmes for TBDs assume a higher importance. If eradication is not successful, or if there are untoward delays in progress, there is increased selection for acaricide resistance due to the extended intensive exposure to chemicals.

***Epidemiological consequences:*** Risks of acaricide resistance and TBD outbreaks in the final phases of an eradication programme or in the event of reinfestation of previously eradicated areas.

***Possible combination with other strategies:*** If a given area/country does not meet the necessary prerequisites, this strategy should not be promoted.

**Ad hoc or opportunistic treatments**

***Principle:*** When livestock are gathered for general management practices (weaning, dehorning, change of pasture), farmers often implement routine preventive procedures and treatments including tick and worm control. It is usual that farmers decide when the cattle should be treated according to their own estimates of "economic thresholds" for tick infestations, time available, climatic conditions, availability of personnel, acaricide and basic infrastructure.

***Prerequisites:*** No special requirements need to be met, although it is an approach that is better suited to conditions where tick infestations are light to moderate.

***Advantages:*** There is a reduced overall need for gathering animals, with a consequent reduction in labour requirements. Fewer treatments may reduce tick burdens but don't necessarily reduce the number of ticks on cattle below that required to maintain transmission of TBD agents. As a result, the approach might be beneficial in terms of maintaining endemic stability.

***Disadvantages:*** Results are unpredictable when assessed solely from the perspective of cattle tick control and TBD management. Using opportunistic treatments, tick burdens fluctuate and lead to a variation in animal productivity. The effectiveness of this strategy in reducing tick populations is variable, because the intervals between opportunistic treatments are usually too long to prevent the completion of the tick's life cycle.

***Epidemiological consequences:*** The reduction of total tick populations is variable. The natural immunization of calves against TBDs is also unpredictable, but is likely to be better than for calves subjected to suppressive or strategic acaricide treatment regimes.

***Possible combination with other strategies:*** The use of cattle with resistance to tick infestation, the use of vaccines against ticks in conjunction with vaccines against TBDs, together with opportunistic treatments, can provide sufficient control of ticks in specific areas.

**Strategic programmes**

***Principle:*** Strategic control programmes have been developed for *B microplus* primarily in subtropical areas where there is a well-defined tick season, with a relatively synchronous

hatching of larvae in spring from eggs laid in autumn and winter. This is called the spring rise. The basic concept of strategic programmes is to begin treating cattle intensively from the spring rise, at intervals that ensure that all emerging larvae that attach to an animal are exposed to a lethal concentration of acaricide (usually 21 days with short acting acaricides and *B microplus*), and for long enough that all or most of the larvae that were in the free living stage die of other causes (usually 16 to 20 weeks for *B microplus* in subtropical areas). These larvae would constitute the first generation of ticks and reduction in their number has a disproportionate effect on the size of subsequent generations. The total number of acaricide treatments (usually four to six for short acting acaricides and *B microplus*) is based on the degree of innate tick resistance of the cattle used and the local environment (de Araujo, 1994; de Magalhaes and Lima, 1991; Jonsson *et al*., 2000a; Petraccia *et al*., 1988); it should generally span at least the complete generation of ticks on the cattle.

***Prerequisites:*** Knowledge of local ecology and epidemiology.

***Advantages:*** Reduced total number of annual treatments (usually four to six), with consequent savings in economic and human resources. There is some evidence to suggest that strategic programmes treating only a single generation of ticks are associated with a lower probability of acaricide resistance (Jonsson *et al*., 2000a)

***Disadvantages:*** Farmer motivation is usually low because treatments are required before large numbers of ticks are noted on cattle. In some cases, dipping at such times can interfere with late pregnancy and calving. There is a theoretical argument that if a strategic programme were well executed and applied to more than a single generation of ticks, there would be few refugia, resulting in high selection pressure and rapid selection for acaricide resistance.

***Epidemiological consequences:*** This strategy is designed to reduce the contamination of pastures with eggs and larvae of ticks. Consequently it could lead to endemic instability for TBDs.

***Possible combination with other strategies:*** It can be used in combination with resistant animals, tick vaccine and/or rotational grazing to virtually eliminate or eradicate ticks on a local basis. Animals should be protected with TBD vaccines because of low levels of exposure.

**Threshold treatments**

***Principle:*** A predetermined number of engorging female ticks on each animal is used as a threshold above which treatment with acaricides is warranted. The threshold number will depend on the species of tick, the production system and the risk of TBDs. Acaricide treatments can be applied to the whole herd if animals are highly susceptible (or the environment favours high numbers of ticks), or only to individual animals that carry heavy tick burdens, if one is dealing with more tick resistant cattle (or there is an environment where tick survival is more marginal).

***Prerequisites:*** Local studies are needed to establish appropriate threshold levels of infestation according to tick species, their impact, the production system being used, the risk of transmission of tick borne diseases and the nutritional status of animals.

***Advantages:*** Generally, the expense of acaricides is lower because the number of treatments will be lower than in suppressive treatment regimes. Additionally, if only some animals are treated, the selection pressure for resistance is likely to be significantly less because of the presence of a larger proportion of susceptible ticks in the refugia (those ticks escaping any form of chemical treatment).

***Disadvantages:*** There is a need for regular monitoring. Some losses of production could be experienced during the tick season and extra treatments to all of a herd might be necessary until appropriate, safe, threshold levels are established for each specific situation.

***Epidemiological consequences:*** These will be influenced by a number of variables (e.g. weather conditions, resistant status of cattle and type of acaricide used). If only some animals are treated, the possibility of selecting for resistance is reduced because of the presence of a larger, susceptible parasite population in refugia. Exposure to TBDs is likely to be higher, favouring the development of endemic stability.

***Possible combination with other strategies:*** Threshold approaches can be used in combination with resistant animals, tick vaccination and rotational grazing. In small herds, based on the observation of those animals that require repeated treatments, it could be possible for the farmer to cull these most susceptible animals, or to treat them more intensively in isolation.

## 9 APPLICATION METHODS

The choice of acaricide formulation and method of application depends upon acaricide availability and price, the size of the farm and extent of facilities, the production system and the degree to which ticks are seen to be a problem. Small scale farming operations might achieve effective control using spray (Ivancovich *et al*., 1987; Duhnen, 1987), or pour-on formulations. Medium and large farms are more likely to use immersion dips or spray races.

### Immersion dips

Immersion of cattle using a dip tank remains the most efficient and reliable method for routine acaricide applications at the farm level (Davey *et al*., 1997).

***Advantages:*** With this procedure, the animal is completely immersed, all parts of the body being thoroughly saturated by the acaricide solution.

***Disadvantages:*** Problems with maintenance of the correct concentration of the acaricide are common. There can be environmental pollution from the run off liquid when the animals emerge from the dip. The facilities are expensive to build and it is expensive to change from one acaricide to another. Immersion dips are not appropriate for some acaricides such as MLs due to problems with instability.

### Spraying

Application of acaricides to cattle can be carried out using various modes of spray devices, e.g. spray races or corridors, motorized pumps and manual, backpack pumps.

***Advantages:*** The chemical group can easily be changed. No stabilizer is required for amitraz if it is used immediately.

***Disadvantages:*** The risk of environmental pollution is considered to be high. There are frequent problems with blockage of spray nozzles. The cattle are not always completely saturated, especially the axillae and inguinal regions, belly and udder and the insides of the ears. There may be an increased risk of intoxication to the operators. The use of a manual spray is time consuming and tiring to the operator. Its effectiveness depends very much on the operator's skills and the effectiveness of animal restraint.

**Pour on (and spot on)**

The introduction of this method of acaricide application was a remarkable advance in technology for applying acaricides (Petraccia *et al*., 1988; Davey, 1995; Davey *et al*., 1992; Dorn, 1987; Vuotto *et al*., 1991; Vignon, 1988; Sosa and Neuhauser, 1987; Mangold *et al*., 1988). A measured dose of the acaricide based on the weight of the animal is applied to the back-line, from whence it dissipates over the body surface to kill ticks. Some SPs, depending on the residual active period, also provide continuing lethal and repellent protection against attaching ticks for some days after treatment. In the case of ML compounds, application to the skin is followed by absorption and systemic action.

***Advantages:*** Pour-ons and spot-ons are easy to apply. Environmental pollution is reduced. It is a very practical method, especially where no dip tanks are available, or in circumstances where the producer wishes to avoid dipping some of the infested animals (e.g. pregnant cows, just a few animals need to be treated, etc.). New formulations of MLs (Benz and Cox, 1989; Cramer *et al*., 1988a) and novel compounds (Davey *et al*., 1998; Martins *et al*., 1995b; Correa *et al*., 1993) are being introduced onto the market as pour-ons and offer an alternative for the control of SP resistant strains of cattle tick (Martins *et al*., 1995b; Bull *et al*., 1996; Correa *et al*., 1993).

***Disadvantages:*** The higher cost of these new compounds may be an initial limitation for many cattle producers in the developing countries. High concentrations of the applied chemicals are needed for good efficacy. The products tend to have longer persistence in animal tissues.

**Injectable formulations**

This is another practical alternative to avoid dipping or spraying cattle with acaricides. Most of the injectable products currently on the market are MLs (D'Agostino *et al*., 1997; Remington *et al*., 1997; Leite *et al*., 1995; Marques *et al*., 1996; Soerensen *et al*., 1994; Lombardero *et al*., 1995; Sibson, 1994; Muniz *et al*., 1995; Vercruysse, 1993; Gonzales *et al*., 1993; Bridi *et al*., 1992; Maske *et al*., 1992; Silvestri *et al*., 1986; Cramer *et al*., 1988b; Hernandez *et al*., 1986).

***Advantages:*** Ease of administration. Operator safety. Reduced environmental pollution, except possibly in the dung pats where non-target species may be affected. There is a broad spectrum of action against both endo- and ectoparasites. ML compounds also provide an alternative for the control of pyrethroid and amitraz resistant strains of the cattle tick.

**Disadvantages:** Possible residues of some ML products in milk restrict their use in dairy cattle.

## 10 NON CHEMICAL TECHNOLOGY

**Resistant cattle**

Genetically determined resistance of the host to ticks has been demonstrated and *Bos indicus* breeds in general have much higher resistance than European *B taurus* animals (FAO, 1989p; Ralph, 1989). The heritability is high and expression depends on the stimulation of an immune response by feeding ticks. There is substantial individual variation in resistance and a number of external factors may affect its expression, especially season, poor nutrition and stress (de Castro and Newson, 1993; Baker *et al*., 1999; Jonsson *et al*., 2000b; Allen and Uilenberg, 1994; Inokuma *et al*., 1993; Barger, 1989).

***Principle:*** The inclusion of tick-resistant breeds through breeding programmes will increase the average resistance of cattle within a herd. This has been shown to result in lighter infestations and smaller populations of ticks, with a reduced requirement for treatment with

acaricides. The within breed variations in resistance to ticks can be used for improvement, continuously selecting animals for breeding that are consistently infested with the smallest number of ticks. There is currently no strong evidence that resistance against ticks has a negative genetic correlation with production traits (Jonsson *et al*., 2000b).

***Prerequisites:*** This strategy is currently dependent upon the availability of affordable *B. indicus* breeds or cross breeds. Good record keeping and monitoring of tick infestations (or application of artificial infestations) is required for selection of resistance within European breeds of cattle. There is some hope that molecular markers for resistance will be developed to enable selection within breed, without exposure to ticks. *B indicus* cattle have also been shown to have higher resistance to babesiosis and East Coast fever than *B taurus* cattle.

***Advantages:*** Increasing the average host resistance of a herd will reduce the frequency of treatment that is required and reduce the risk of acaricide resistance. Selection programmes for cattle resistant to ticks, based on rapid visual assessment of natural tick loads (George, 1990a), can be introduced on commercial ranches, and also in artificial insemination and cattle distribution centres, without the risk of greatly increasing tick burdens. The successful outcome of such programmes can reduce the need for intensive tick control.

***Disadvantages:*** Interrelationships between resistance and productivity traits (including fertility, behaviour and weight gain) must be clarified to ensure that this approach is economically acceptable. There is a risk of losing other herd characteristics that are profitable for the farmer and strategies based on breeding programmes are relatively slow to implement and to modify.

***Epidemiological consequences:*** There is significant reduction of pasture contamination with eggs and larvae of ticks and increased probability of endemic stability with TBDs.

***Possible combination with other strategies:*** Resistant cattle improve the effectiveness of all other strategies, so can be used in combination with all types of treatment and with vaccination against ticks and tick borne diseases and rotational grazing.

**Vaccines**

The development of the first effective vaccine against *B. microplus* was a great advance in the fight against a serious pest that interferes with world food production. The two commercially available vaccines (TickGARD Plus®, Hoechst, Australia and Gavac™, Heber Biotec, Cuba) are now on the market in a limited number of countries.

***Principle:*** Both vaccines are based on hidden antigens from the gut of the tick that once inoculated into cattle, induce the production of antibodies which, when ingested by the tick, result in damage to the gut, slightly reduced survival, reduced egg production and reduced hatchability of eggs. Consequently, tick populations decline with time, although there is little direct mortality effect on ticks. Current vaccines contain a single antigen, known as Bm86.

***Prerequisites:*** Before recommending the large-scale use of this new important tick control tool, cost benefit analyses must be conducted at the farm level. The full extent of their use and impact on tick populations are still being evaluated, even though much has already been published on the subject (Frisch *et al*., 1999; Jonsson *et al*., 2000c; Garcia Garcia *et al*., 1998; Pruett *et al*., 1999).

***Advantages:*** The frequency of acaricide treatment is reduced, and thus the risk for developing acaricide resistance is also reduced. It is possible that vaccines against ticks can reduce tick burdens but not eliminate exposure to TBDs (Fragoso *et al*., 1998). Its application is harmless to the environment.

***Disadvantages:*** The vaccines that are presently available do not have the knockdown effect of traditional chemical acaricides (Pruett *et al*., 1999). Farmers may initially be disappointed if they treat the cattle and do not see immediate tick deaths, an important marketing constraint. Because there is no natural exposure to the antigens, the present vaccines are not reinforced by tick feeding and have a limited duration of protection. Vaccination is recommended every 10 weeks to 3 months in the tick season. The present vaccines have only been proven to be effective against *B microplus*.

***Epidemiological consequences:*** There is a reduction in the level of egg and larval contamination of pastures and in the risk of developing acaricide resistance. There is also a higher probability of endemic stability with TBDs because the vaccine does not affect tick larvae and nymphs that transmit TBDs.

***Possible combination with other strategies****:* Vaccines against *B microplus* can be used in combination with all types of chemical and non-chemical treatment strategies, such as resistant cattle and rotational grazing.

**Rotation of crops with livestock, and pasture "spelling"**

In many areas of the world, rotation between crops and livestock is a measure that reduces tick populations. Improvements in this procedure, such as introducing only clean or uninfested animals into areas where crops have recently been harvested (e.g. by treating them just before introduction into these areas), will reduce the probability of reinfestation. Rotation of sheep and cattle, with treatment of cattle just before entry into their new paddock, has also proved effective in decreasing tick populations on the pastures. In many regions where cattle and sheep are continuously pastured together, cattle typically need to be treated against ticks. However, where they are pastured alone under similar circumstances, cattle carry significantly higher tick burdens. "Spelling" of pastures, which involves removal of all livestock hosts from them for periods of time that ensure death of most or all free-living ticks, has been successful in reducing or eliminating populations of cattle ticks.

***Principle:*** The tick habitat is modified to be less suitable for tick survival and, due to the absence of the host, the parasitic life cycle is disrupted (Desquesnes and Vignon, 1987). Ecological studies (Quijada *et al*., 1997) have shown that from two months after hatching, the percentage of larvae surviving in pastures decreases significantly. This effect is most marked under adverse environmental conditions (high temperatures, dry, short pasture cover). Larval mortality is due to starvation and desiccation. Rotation or spelling periods are therefore shorter in hotter climates and in summer months.

***Prerequisites:*** Knowledge of tick ecology in a region, suitable pasture, animals and fencing.

***Advantages:*** Reduces frequency of treatment and the risk for developing acaricide resistance. This tool could be used without the risk of greatly increasing tick burdens and reducing the need for intensive tick and TBD control. If reintroductions of ticks from other sources can be prevented, very low tick numbers or local eradication become a possibility. Application of this method is harmless to the environment.

***Disadvantages:*** Application can prove difficult for some farmers who do not have sufficient free paddocks. Studies on the population dynamics *of B. microplus* as free-living stages are important in marginal, mainly temperate areas to determine the geographical locations and seasons in which ticks have difficulties in surviving. A small proportion of free-living eggs and larval stages can often extend for up to 7 or 8 months. These are likely to have very poor or no powers of reinfestation of cattle, and are usually of little practical significance in achieving adequate control of ticks but they do interfere with eradication attempts.

***Epidemiological consequences:*** There will be a gradually reduced level of larval contamination in the pastures.

***Possible combination with other strategies:*** It can be used in combination with all types of treatment strategies, resistant cattle and vaccination.

## 11 CONTROL STRATEGIES UNDER DEVELOPMENT

**Biological control**

The biological agents, which potentially could be used for the control of ticks, include some fungi, e.g. *Metarhyzium* spp. and *Beauveria* spp. (Rijo, 1998; Bittencourt *et al*., 1997; Bittencourt *et al*., 1996a; Bittencourt *et al*., 1996b; Bittencourt *et al*., 1995; Bittencourt *et al*., 1994a; Bittencourt *et al*., 1994b; Bittencourt *et al*., 1994c), bacteria, e.g. *Cedecea lapagei* (Verissimo, 1995; Brum and Teixeria, 1992a; Brum and Teixeria, 1992b), nematodes (Brum and Teixeria, 1992b) and ants (Verissimo, 1995) that attack soil living stages of the ticks. These natural pathogens and predators have not yet been subjected to sufficient field testing or validation and require extensive product development (Brum and Teixeria, 1992b; Labarthe, 1994). Nevertheless, preliminary laboratory bioassay trials against *B. microplus* have shown *Metarhizium anisopliae* to have a high level of virulence against ticks.

Myco insecticides are a potentially cost effective, sustainable, environmentally friendly alternative to chemical acaricides that can be applied using conventional technology, thus making them simple for farmers to use. If field trials are successful, more large-scale testing will be needed, including the determination of non-target effects.

It is known that a number of bird species (oxpeckers, cattle egrets, chickens) may contribute to an overall reduction in tick numbers on livestock and in the environment. Their precise effects on tick burdens need further evaluation before they can be considered as significant tick control measures. Until this is known, it is not possible to recommend such alternatives to producers for adoption and practical use in the field.

**Herbal remedies**

The use of some types of grass or leguminous plants with acaricidal or repellent effects needs greater assessment for possible inclusion in schemes for improving tick control. *Brachiaria brizantha*, *Melinis minutiflora*, *Stylosanthes* spp., neem oil, etc., have been shown to have some larvicidal or repellent effect against the larvae of ticks (Lopez, 1997; de Barros and Evans, 1991; de Barros and Evans, 1989; Wilson and Sutherst, 1990; Wilson *et al*., 1989; Sutherst *et al*., 1988; Wilson and Sutherst, 1986; Sutherst *et al*., 1986). Extracts of some plants are also active against certain tick species (Maske *et al*., 1996; Banerjee, 1997; Stuti Vatsya and Singh, 1997; Sivaramakrishnan *et al*., 1996; Chungsamarnyart *et al*., 1994; Chungsamarnyart and Jansawan, 1996; Williams and Mansingh, 1996; Porter *et al*., 1995; Jansawan *et al*., 1993; Chungsamarnyart and Jiwajinda, 1992; Chungsamarnyart *et al*., 1992; Williams, 1993; Srivastava and Sinha, 1990; Hazzari and Misra, 1989). The feasibility of these species for feeding animals for tick control under field conditions has not yet been adequately studied.

## 12 RESISTANCE MANAGEMENT AND INTEGRATED TICK CONTROL

Measures recommended for tick control should have the potential to reduce parasite populations over successive generations and ultimately reduce or eliminate the need for regular chemical applications (George, 1990b; Honer and Gomes, 1990).

The implementation of successful strategies to minimize the likelihood of resistance emerging in the field to a new or existing efficient acaricide, is critically important in any cattle tick management programme. Although there is a considerable body of theoretical information

on population genetics of resistance in arthropods, and computer simulation studies have been undertaken, there are few reports of experimental studies conducted on ticks in the field. Hence, much of the knowledge about the effects of dose and concentration, frequency and timing of treatments, the use of mixtures and rotation of acaricides, remains hypothetical or axiomatic. Consequently, general agreement has not been reached on optimal recommendations that are certain to delay resistance, although some studies are starting to provide an idea of the relative importance of the various factors.

Difficulties in standardizing methods of control emphasize the need to consider ecological and socio-economic issues on a local basis before decisions are taken on specific strategies for tick control. Control programmes should be designed for long term outcomes, while taking into account the real needs of cattle producers. The economic impacts of ticks and related diseases are appreciated mainly at a general rather than specific level (i.e. the cost structure of ticks to an industry will differ from that on an individual farm).

Whatever the case may be, the thoughtful integration of chemical control methods with non-chemical methods will always be expected to produce better outcomes in terms of tick control and delay of resistance than the use of chemical acaricides alone. The introduction of *B. indicus* and their crosses effectively reduces the tick population by increasing the general level of genetic resistance of the herd to ticks (Angus, 1996). The recent advances in the development of vaccines against ticks (McKenna *et al*., 1998; Beugnet *et al*., 1998) suggest that they are likely to play an increasingly important role in cattle tick control, especially when combined with chemical acaricides. Models or computer simulation to assess the effect of potential control programmes (Beugnet *et al*., 1998; Saueressig and Honer, 1995; de la Vega and Diaz, 1992; McLeod *et al*., 1995; Sutherst, 1993; Honer *et al*., 1993; Nunn *et al*., 1993; White, 1993; Popham and Garris, 1991; de la Vega *et al*., 1988; de la Vega and Diaz, 1986; Mount *et al*., 1991; Sutherst and Maywald, 1987), including their interaction with climate change, are resources that should also be used for the refinement of future programmes.

Many emerging issues would also benefit from careful modelling. These include the effect of long acting MLs and other long residual effect products that are now available, as well as the effects that components of control programmes have on the emergence of acaricide resistance

**Prudent use of acaricides**

A single application of most acaricides will not always eliminate all the ticks on an animal, even assuming correct preparation and application. The surviving ticks are the ones that eventually will contribute to the development of resistance. The risk of this happening increases if the population of susceptible parasites is very low because of climatic effects or because of frequent use of acaricides.

There is clearly no point in using an acaricide to which the parasites are resistant. It is therefore recommended that a test for resistance be made before new control strategies are implemented. In the case where acaricides from one or more groups are still efficient, these drugs should be carefully used in the future to avoid/delay the development of resistance to these drugs. Resistance tests allowed continued use of OP acaricides long after resistance to the first OPs were detected (Nolan and Schnitzerling, 1986).

**Development of resistance**

There is a general conflict between the requirements for a high level of control (or eradication) of ticks and the requirements for delaying the development of acaricide resistance. This is clear from the list of factors below that are likely to accelerate the development of resistance. It should be noted that elements in this list require further scientific validation before being considered as fact.

Factors which accelerate resistance:

- Frequent use of acaricides.
- Treatment of the herd at times of the year when the free-living refugia population is small.
- Use of poor quality acaricides of uncertain concentration.
- Use of acaricides with a prolonged, sub-lethal decay curve.
- Under treatment. Although it is widely believed that under-dosing contributes to the development of resistance, the relationship between dose and selection varies depending on the mode of inheritance of resistance and the dose used in relation to the lethal dose for homozygous susceptible, homozygous resistant and heterozygous ticks.

Ways of slowing down the onset of resistance

- Reduce the frequency of treatments.
- Limit the number of ticks exposed to chemical treatment, e.g. by using a threshold approach.
- Avoid the use of slow release devices at times when pasture contamination with ticks is low.
- Apply high quality products in a correct manner and at the right concentration or dose rate.
- To reduce the need for as many chemical acaricide treatments, apply non-chemical strategies such as:
    - Maximising host resistance by selection within the breed or introduction of *B indicus* genetics.
    - Where available, using vaccines against ticks and TBDs.
    - Introducing quarantine measures for newly purchased or returning animals. Avoiding introduction of resistant ticks.
    - Adopting an education policy backed by strong government support in all tick infested areas, and a continuous monitoring service to diagnose the early emergence of field resistance through *in vitro* tests.

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# MODULE 2. HELMINTHS: ANTHELMINTIC RESISTANCE: DIAGNOSIS, MANAGEMENT AND PREVENTION

## 1 INTRODUCTION

The era of modern anthelmintics started in the middle of the 20th century with the introduction of phenothiazine and piperazine, products that are considered to be the first generation of the broad spectrum drugs. The 2nd generation of truly broad spectrum anthelmintics were released in the 1960s and included the benzimidazoles, the pro-benzimidazoles, the imidazothiazoles and the tetra-hydro-pyrimidines. Following the early success of the introduction of the benzimidazoles, extensive research programmes were initiated during which successful structural modification resulted in the production of a series of benzimidazoles. Most recently, a 3rd generation of broad spectrum anthelmintics, the macrocyclic lactones, emerged in the early nineteen eighties.

In addition, other compounds with a narrower spectrum have also been available on the market. These include substituted salicylanilides, phenols and organophosphates.

Thus the pharmaceutical industry has, during the last 35 years, been able to produce a string of highly effective, broad and narrow spectrum anthelmintics, and veterinarians and livestock producers have used these extensively for parasite control either by drenching or injecting cattle, sheep and goats.

The access to efficient drugs and the ease with which they could be applied, combined with the immense progress made in establishing the epidemiology of the gastro-intestinal nematodes of ruminants, led to a period of relative success in the control of worms, particularly in the livestock production systems of the industrialized countries. However, the false assumption that worm control is easy and can be accomplished by using broad spectrum drugs without an epidemiological database was also being promoted, preventing or delaying the epidemiological studies that are a prerequisite for effective control. Further complicating the situation today and for future parasite control programmes is the fact that all the economically important parasite species of sheep and goats have developed resistance to all four groups of anthelmintics.

## 2 RESISTANCE DEVELOPMENT: HELMINTHS

Resistance is probably an inevitable consequence of the use of anthelmintics, and the history of parasite resistance to anthelmintics starts with the first report on phenothiazine resistance in 1957. It is apparent from the records of the reported resistance to the major anthelmintics presented in **Table 1** that resistance tends to develop only a few years after the introduction of the new drugs. It should also be noted that in most cases, *Haemonchus contortus* was the first nematode to develop resistance against the different anthelmintics. There is substantial evidence that when a parasite has developed resistance to one anthelmintic from a certain group it will usually also be resistant to other products from the same group.

There are several phases in the process of resistance development. Firstly, there is an initial phase of susceptibility where the number of resistant individuals within the parasite population is low. With continued exposure to the same drug group, an intermediate phase then follows in which the frequency of heterozygous resistant individuals within the population increases. Finally, sustained selection pressure results in a resistant phase where homozygous resistant individuals predominate within the population. The speed of this process will depend on how severe the selection pressure is on the parasite population. It is known that this is linked to the frequency of treatment and the fact that widespread and excessive use (8 to 12 times per year) of these drugs in sheep, without considering the epidemiology and ecology of the parasites, has led to the development of resistance of the sheep parasites to drugs from all four chemical groups.

**Table 1. The first reports of anthelmintic resistance in nematodes of sheep to drugs with different modes of action (Coles *et al*., 1994)**

| Year | Country | Drug | Nematode |
|---|---|---|---|
| 1957 | USA | Phenothiazine | H. contortus |
| 1964 | USA | Thiabendazole | *H. contortus* |
| 1968 | USA | OP-compounds | *T. circumcinctus.* |
| 1976 | Australia | Levamisole/Morantel | *H. contortus* |
| 1980 | S. Africa | Rafoxanide | *H. contortus* |
| 1987 | S. Africa | Ivermectin | *H. contortus* |

Under-dosing, which is a common problem, is likely to favour the survival of heterozygous individuals, possibly enhancing the selection pressure for resistance. Persistence and initial efficacy of the drugs were found to be far more important in determining the rate of selection for resistance as drug efficacy declined, than was the selection of resistant third larval stage (L3) parasites (Dobson *et al*., 1996). There is also evidence that strategic treatments have contributed to resistance development, particularly at times when the free-living component of the parasite population has been small.

## 3 RESISTANCE TO ANTHELMINTICS: CURRENT STATUS

The first reports of anthelmintic resistance were made on farms attached to parasitological research establishments where anthelmintics were often used intensively. Because of the seemingly formidable chemotherapeutic arsenal at the disposal of the stockowner, such reports were often considered to be merely parasitological oddities and their potential significance was overlooked. It was not until control failed on a substantial number of farms that relied heavily on intensive anthelmintic treatment to maintain productivity, that the potential implications were fully realized. Since the early 1980s resistance has been detected among the gastro-intestinal nematode parasites of sheep and goats throughout the world, and large scale surveys have shown that the situation is critical in many Latin American countries, South Africa, Australia and New Zealand. Many farms have resistance to at least two of the anthelmintic groups and a substantial number have resistance to all four groups. An extensive literature search of all the main life sciences databases for the period 1993 to 1998 found 142 publications on resistance. These have been analysed to determine the species of parasites in which resistance has been diagnosed and to what drugs. The results are presented in **Table 2**; the reported cases of resistance in sheep and goat helminths to the different anthelmintic groups, from 1993 until the present, are presented in **Table 3**. In sheep, narrow spectrum anthelmintics such as the salicilanides, rafoxanide, organophosphates and thiophanate, could be alternative tools where resistance to the major anthelmintic groups is present.

**Table 2. Reported cases (X) of resistance in helminth parasites of sheep and goat according to class of anthelmintic**

| Parasite | BZ | IMZ | ML | SAL | OP | RA | TH |
|---|---|---|---|---|---|---|---|
| *H. contortus* | X | rare | X | X | X | X | X |
| *Ostertagia* spp. | X | X | X | - | - | - | - |
| *Trichostrongylus* spp. | X | X | X | - | - | - | - |
| *Nematodirus* spp. | X | - | - | - | - | - | - |
| *Fasciola hepatica* | X | - | - | X | - | - | - |

BZ= Benzimidazole; IMZ= Imidazothiazole; ML= Macrocyclic lactones;
SAL=Salicylanilide; OP= Organophosphate; RA= Rafoxanide; TH=Thiophanate

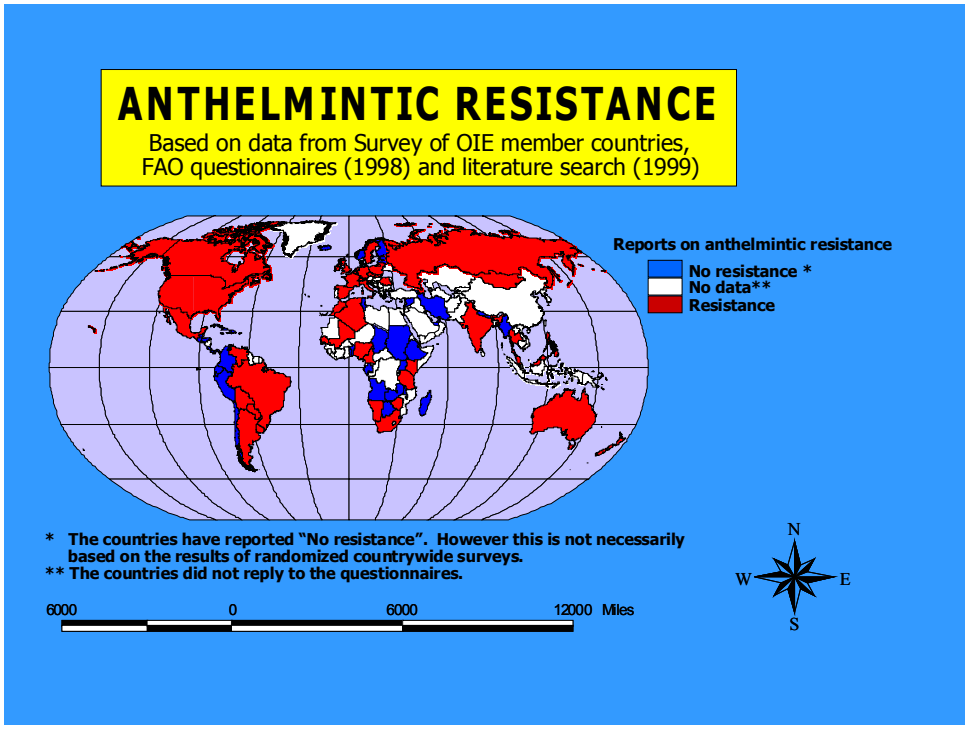
ANTHELMINTIC RESISTANCE
Based on data from Survey of OIE member countries,
FAO questionnaires (1998) and literature search (1999)
Reports on anthelmintic resistance
No resistance *
No data**
Resistance
* The countries have reported "No resistance". However this is not necessarily based on the results of randomized countrywide surveys.
** The countries did not reply to the questionnaires.
6000
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**Table 3. Reported cases of resistance in sheep and goat helminths listed for each anthelmintic group.**

| Drug | Anthelmintic resistance reference |
|---|---|
| Benzimidazole | Coles *et al*., 1998a; Romero *et al*., 1998; Chaudhri *et al*., 1997; Maingi *et al*., 1998; Chartier *et al*., 1998; Waruiru *et al*., 1998; Kerboeuf *et al*., 1997; Campos Ruelas *et al*., 1997; Mukaratirwa *et al*., 1997; Corba *et al*., 1998; Farias *et al*., 1997; Waruiru, 1997a; de Souza *et al*., 1996; Borgsteede *et al*., 1997; Balicka Ramisz *et al*., 1997; Barre *et al*., 1997; Waruiru *et al*., 1997; Waruiru, 1997b; Van Wyk *et al*., 1997; Boersema and Pandey, 1997; Sutherland *et al*., 1997; Singh and Yadav, 1997; Sani and Chandrawathani, 1996; Wanyangu *et al*., 1996; Maingi *et al*., 1997a; Maingi *et al*., 1997b; Borgsteede *et al*., 1996; Chartier *et al*., 1996; Dhirendra Singh *et al*., 1995; Bauer *et al*., 1996; Hong *et al*., 1996; Waruiru *et al*., 1996; Soccol *et al*., 1996; Yadav *et al*., 1996; Mage *et al*., 1994; Eddi *et al*., 1996; Echevarria *et al*., 1996; Maciel *et al*., 1996a; Maciel *et al*., 1996b; Miller and Craig, 1996; Mwamachi *et al*., 1995; Cabaret *et al*., 1995; Yadav *et al*., 1995; Srivastava *et al*., 1995; Watson *et al*., 1993; Waruiru, 1994; Varady *et al*., 1995; McKenna *et al*., 1995; Whelan *et al*., 1995; Charles and Medeiros, 1993; Uppal *et al*., 1995; Kochapakdee *et al*., 1995; Waruiru *et al*., 1994; Burger *et al*., 1994; Borgsteede *et al*., 1995; Dorny *et al*., 1994; Nilsson *et al*., 1994; Rahman, 1994a; Hong *et al*., 1994; Kerboeuf *et al*., 1995; Ndarathi, 1992; Dorny *et al*., 1993; Nilsson *et al*., 1993; Guadarrama *et al*., 1991; Ruelas *et al*., 1990; Yadav and Uppal, 1993; Singh *et al*., 1992; Pandey and Sivaraj, 1994; Overend *et al*., 1994; Nilsson *et al*., 1992; Hunt *et al*., 1994; Chartier and Pors, 1994; Van Wyk *et al*., 1991; Campos Ruelas *et al*., 1992; Varady *et al*., 1994; Rahman, 1994b; Sivaraj *et al*., 1994; Vieira *et al*., 1992; Romero *et al*., 1992; Taylor and Hunt, 1993; Rudby Martin and Nilsson, 1991; Yadav *et al*., 1993; Oosthuizen and Erasmus, 1993; Echevarria *et al*., 1993; Louw and Reinecke, 1993; Gray *et al*., 1993; Corba *et al*., 1993; Uppal *et al*., 1993; Rothwell and Sangster, 1993; Maingi, 1993. |
| Imidathiazole/ Morantel | Coles *et al*., 1998a; Romero *et al*., 1998; Maingi *et al*., 1998; Chartier *et al*., 1998; Waruiru *et al*., 1998; Mukaratirwa *et al*., 1997; Corba *et al*., 1998; Hoekstra *et al*., 1997; Farias *et al*., 1997; Waruiru, 1997a; Sharma, 1996; Waruiru *et al*., 1997; Waruiru, 1997b; Van Wyk *et al*., 1997; Boersema and Pandey, 1997; Singh and Yadav, 1997; Sani and Chandrawathani, 1996; Wanyangu *et al*., 1996; Maingi *et al*., 1997a; Maingi *et al*., 1997b; Hong *et al*., 1996; Soccol *et al*., 1996; Coles and Simkins, 1996; Eddi *et al*., 1996; Echevarria *et al*., 1996; Maciel *et al*., 1996a; Maciel *et al*., 1996b; Miller and Craig, 1996; Mwamachi *et al*., 1995; Yadav *et al*., 1995; Santos *et al*., 1993; Praslicka *et al*., 1995a; Varady *et al*., 1995; McKenna *et al*., 1995; Sangster and Bjorn, 1995; Dorny *et al*., 1994; Hong *et al*., 1994; Chartier and Pors, 1994; Varady *et al*., 1994; Sivaraj *et al*., 1994; Yadav *et al*., 1993; Corba *et al*., 1993; Uppal *et al*., 1993; Rothwell and Sangster, 1993; Maingi, 1993. |
| Macrocyclic lactones | Romero *et al*., 1998; Kotze, 1998; Gill *et al*., 1998a; Gill *et al*., 1998b; Farias *et al*., 1997; Rolfe and Fitzgibbon, 1996; Besier, 1996; Waruiru, 1997a; de Souza *et al*., 1996; Waruiru *et al*., 1997; Sutherland *et al*., 1997; Sani and Chandrawathani, 1996; Maingi *et al*., 1997a; Maingi *et al*., 1997b; Soccol *et al*., 1996; Eddi *et al*., 1996; Echevarria *et al*., 1996; Maciel *et al*., 1996a; Maciel *et al*., 1996b; Miller and Craig, 1996; Watson *et al*., 1996; Mwamachi *et al*., 1995; Le Jambre *et al*., 1995; Watson *et al*., 1993; Varady *et al*., 1995; Charles and Medeiros, 1993; Le Jambre, 1993; Leathwick, 1995; Gill *et al*., 1995; Miller and Barras, 1994; Shoop *et al*., 1993; Le Jambre *et al*., 1999; Van Wyck and Van Schalwyck, 1991; Varady *et al*., 1994; Sivaraj and Pandey, 1994; Sivaraj *et al*., 1994; Vieira *et al*., 1992; Oosthuizen and Erasmus, 1993; Echevarraid *et al*., 1993; Pomroy and Wheelan, 1993; Corba *et al*., 1993; Rothwell and Sangster, 1993. |
| Salicylanilide | Anonymous, 1996; Soccol *et al*., 1996; Echevarria *et al*., 1996; Mwamachi *et al*., 1995; Oosthuizen and Erasmus, 1993; Echevarria *et al*., 1993; Louw and Reinecke, 1993; Rothwell and Sangster, 1993. |
| Organophosphate | Lorenzelli *et al*., 1996; Van Wyk *et al*., 1997; Soccol *et al*., 1996. |
| Rafoxanide | Waruiru *et al*., 1998; Boersema and Pandey, 1997; Dhirendra Singh *et al*., 1996; Van Wyk *et al*., 1991. |
| Thiophanate | Waruiru *et al*., 1996; Yadav *et al*., 1996; Chartier, 1993. |

The problem of anthelmintic resistance in cattle parasites has not been investigated nearly as intensively as it has for small ruminants (McKenna, 1996a). Assessing the situation in cattle, it should be taken into account that in general, cattle parasites are nearly always a sub-clinical problem. It is possible that even efficacy levels of 50 to 70 percent could well hide the adverse effects of parasites on cattle, so the same frequency of resistant worms in a population might be less likely to be detected in cattle as they are in sheep. However, more and more reports are being published and it appears that anthelmintic resistance in cattle parasites is an emerging problem, with the potential of developing to similar proportions as experienced with sheep and goat parasites (Coles *et al*., 1998b). Analyses of the currently available publications are presented in **Tables 4** and **5**.

**Table 4. Reported cases of resistance in helminth parasites of cattle according to class of anthelmintic**

| Parasite | BZ | IMZ | ML |
|---|---|---|---|
| *H. contortus* | X | X | |
| *Ostertagia* spp. | X | X | |
| *Trichostrongylus* spp. | X | X | |
| *Cooperia* spp. | X | X | X |

BZ= Benzimidazole; IMZ= Imidazothiazole; ML=Macrocyclic lactones;

**Table 5. Reported cases of resistance in helminth parasites of cattle listed for each anthelmintic group**

| Drug | Anthelmintic resistance reference |
|---|---|
| Benzimidazole | Bauer *et al*., 1996; McKenna, 1996a; Hosking *et al*., 1996; Vermunt *et al*., 1995; Borgsteede *et al*., 1992. |
| Imidothiazole/Morantel | Vermunt *et al*., 1996; Palmer and Olsen, 1993. |
| Macrocyclic lactones | Coles *et al*., 1998b; Vermunt *et al*., 1996; McKenna, 1996a; Vermunt *et al*., 1995; West *et al*., 1994; Anziani *et al*., 2001. |

In conclusion, the available data from the literature review showed that the vast majority of economically important gastro-intestinal nematodes of sheep and goats, and to a lesser extent cattle, have developed resistance to all of the available anthelmintics.

Resistance to anthelmintics could also become a problem for wildlife. The role of wild ruminants in spreading anthelmintic-resistant nematodes between flocks of sheep or goats has already been confirmed (Praslicka *et al*., 1995b).

Considering the situation, it would be natural to look to the pharmaceutical industry for new drugs (Coles, 1998). We are however led to believe that there are likely to be few forthcoming for the following reasons. The expenses incurred by the companies from the identification of a new chemical until it is tested and marketed are in excess of US$100 million. Thus the companies are looking very carefully at the potential markets before investing, and the industry has most recently focused on the human market and on products for pets.

It is fair to say that parasite control is at a crossroads and it is essential that we:

Preserve and safeguard the drugs we have available and use them wisely.

Realize that the time of easy parasite control based on the use of anthelmintics only is over.

Understand, that in the future we will have to rely on a combination of strategies which will require more work and more monitoring.

Understand that when resistance has been detected, the drugs will have reduced or no effect and the use of the compound, or any from the same family of drugs, is likely to be a waste of resources.

# 4 DIAGNOSIS OF RESISTANCE: AN OVERVIEW OF METHODOLOGIES

With the development and spread of anthelmintic resistance in nematodes of livestock, the need for methods to detect resistance has evolved simultaneously. Different *in vivo* and *in vitro* tests are now available and there is an ongoing effort to refine, standardize and validate these tests. The development of molecular tests (Beech *et al*., 1994) is also progressing and is trying to apply DNA-probe and polymerase chain reaction (PCR) technology.

## Diagnostic methods: *In vivo*

### Faecal egg count reduction test. (FECRT)

This is the most common test to study anthelmintic resistance. This test was originally designed for sheep, but can be used also for cattle, swine and horses.

Modern broad spectrum anthelmintics are highly efficacious, and treatment should normally result in a reduction of faecal egg counts by more than 95 percent. Thus this test provides an estimation of anthelmintic efficacy by comparing faecal egg counts of animals before and ten days after treatment (Gill *et al*., 1998a). For monitoring of normal fluctuation, the treated group is generally compared with non-treated controls.

This test is particularly suitable for field surveys and it has the advantage that the number of groups can be increased if appropriate, to test the efficacy of a range of broad or narrow spectrum anthelmintics at one time.

### The controlled test

In this test, the efficacy of an anthelmintic is determined by comparing parasite populations in groups of treated and non-treated animals. Basically, the procedure compares worm burdens of animals artificially infected with susceptible or suspected resistant isolates of nematodes. The parasitized animals are randomly separated into medicated and non-medicated groups and at a suitable interval after treatment (10 to 15 days), a necropsy is carried out and the parasites are recovered, identified and counted. This test is not extensively used, except in cases of special interest or when confirmation of resistance is required at species level, and for evaluation of the effect on larval stages (Reinecke and Louw, 1994).

In an attempt to reduce the cost and labour required for this test, laboratory animal models have been used and guidelines for evaluating anthelmintic efficacy using the controlled test have been published (Powers *et al*., 1982; Presidente, 1985).

## Diagnostic methods: *In vitro*

Several different *in vitro* tests are available but the majority is almost exclusively used for research purposes. These tests can be used to quantify the level of resistance but they require considerable technical expertise and in some cases, expensive laboratory equipment. Ideally, these tests require mono-specific infections because there can be difficulties in the interpretation of results with field infections, which usually consist of multiple parasite species. The maintenance of standard laboratory strains, both drug susceptible and resistant is necessary for comparative purposes (D'Assonville *et al*., 1996). The main bioassays are listed in **Table 6**.

**Table 6. Bioassays for the diagnosis of anthelmintic resistance**

| 5 | Tests | Diagnosis of resistance to: |
|---|---|---|
| | Egg hatch | Benzimidazoles-Levamisole/Morantel |
| | Larval paralysis | Levamisole/Morantel |
| | Tubulin binding | Benzimidazoles |
| | Larval development | All drugs |
| | Adult development | Benzimidazoles |

Several attempts have been made to establish the sensitivity of the different tests. For example, comparative studies to determine the resistance to thiabendazole and levamisole have used (a) an egg hatch assay, (b) an egg hatch paralysis assay, (c) a larval development assay, (d) a larval paralysis assay, (e) a larval paralysis assay with physostigmine and (f) a larval micro-motility assay.

Of all the available tests, the larval development test is the most sensitive for quantitatively measuring thiabendazole and levamisole resistance. The egg hatch assay is also sensitive and accurate in determining benzimidazole resistance. It was concluded that the other methods were unsuitable for use in field monitoring of resistance (Varady and Corba, 1999).

**The egg hatch assay**

The egg-hatch test has been developed to differentiate between resistant and susceptible strains of gastro-intestinal nematodes for the benzimidazoles and for the levamisoles. It provides an accurate method for assessing the susceptibility of mixed nematode populations, and it is comparatively more rapid and economic to conduct than the FECRT. It is based on the determination of the proportion of eggs that fail to hatch in solutions of increasing drug concentration in relation to the control wells, enabling the user of the test to develop a dose response line plotted against the drug concentration. To obtain meaningful data, eggs for the egg hatch test must be fresh and should be used within three hours of being shed from the host, as sensitivity to some benzimidazoles decreases as embryonation proceeds. The test has only been shown to work on nematode species in which eggs hatch rapidly. Due to difficulties in the interpretation of the results this assay is not widely used for field surveys.

**Larval paralysis and motility assay**

The test is used for levamisole and morantel resistance. This assay discriminates between resistant and susceptible strains of parasites, by estimating the proportion of third stage larvae in tonic paralysis after incubation with a range of levamisole and morantel drug concentrations. It is relatively easy to carry out, stocks of infective larvae are readily obtained and it is reported that there is a fairly good reproducibility of the test, any differences in repeatability being attributed to the age of larvae. However, the interpretation is complicated by the fact that if the anthelmintic is added to the egg suspension too early, the development has not proceeded far enough; if it is added too late the drug has no effect.

A modification of the technique was developed using the micro-motility meter, an instrument for measuring the motility of larval and adult nematodes after incubation with benzimidazole and levamisole. A further modification of the larval paralysis assay has been made in order to apply it for the detection of thiabendazole resistance. Some lack of repeatability in this method has been attributed to the reversibility of paralysis.

**Tubulin binding assay**

This test is based on the mode of action of the drugs. The mechanism of benzimidazole resistance appears to be associated with a reduced affinity of tubulin for the anthelmintics. The test is based on the differential binding of benzimidazoles to tubulin, an intracellular structural protein from susceptible and resistant nematodes. The test involves the incubation of a crude tubulin extract from adult parasites, infective larvae or eggs, with a tritiated benzimidazole until equilibrium is reached. The free, unbound drug in test suspension after incubation is removed using charcoal, and the tubulin-bound label is sampled and counted by liquid scintillation spectrophotometry. Tubulin extracts from resistant parasites bind substantially less strongly than do those from susceptible parasites. The test is claimed to be rapid, robust, highly reproducible and sensitive to minor changes in the resistance status of parasite populations, but it requires relatively large numbers of larvae, making it unsuitable for routine field assays. Moreover, it requires access to expensive laboratory apparatus for high performance liquid chromatography (HPLC) estimations and a source of radiolabelled drug.

**Larval development assay (LDA)**

The larval development tests are the only ones that allow the detection of resistance against all the drugs, irrespective of their mode of action. Several methods have been described, but reproducibility, linearity of the dose-response and susceptibility differ. The LDA is an *in vitro* assay for the detection of resistance to benzimidazole, levamisole, combinations of benzimidazole and levamisole, and avermectin and milbemycin drenches in the major gastrointestinal nematode parasites of sheep, *Haemonchus contortus, Trichostrongylus colubriformis* and *Ostertagia circumcincta.* In this test nematode eggs, isolated from faecal samples submitted by producers, are applied to the wells of a micro-titre plate and larvae hatch and develop to the L3 stage in the presence of anthelmintic. The concentration of anthelmintic required to block development is related to an anticipated *in vivo* efficacy.

**Adult development assay**

The adult development assay for detecting benzimidazole resistance in trichostrongylid nematodes has advanced significantly and *Haemonchus contortus* has been cultured through to the adult egg-laying stages, although this test is mainly for research purposes.

**Research and development of new tests**

Lately, gene probes, allele frequencies, trans-membrane functional analysis, PCR and flow cytometry have been investigated as tools for the determination of anthelmintic resistance. Currently, these procedures are exclusively for research purposes (Kwa *et al*., 1998).

Gene probes have been used to analyse restriction fragment length polymorphism between susceptible isolates and isolates of *Haemonchus* resistant to benzimidazole; levamisole and benzimidazole; or benzimidazole, ivermectin and closantel. A P-glycoprotein gene probe was also isolated from *Onchocerca volvulus* and an *Onchocerca*-specific PCR was developed for detection of resistance strains (Kwa *et al*., 1998).

Analyses of allele frequencies showed significant differences between the unselected and the drug-selected derived strains. In all three drug-selected strains, an apparent selection for the same allele was observed. It is suggested that P-glycoprotein (Pgp) may be involved in resistance to both ivermectin and moxidectin in *H. contortus* (Blackhall *et al*., 1998).

A functional analysis of trans-membrane transport of drugs in drug-resistant helminths was undertaken using a flow cytometry method on two isolates of *Haemonchus contortus* that were susceptible or resistant to benzimidazoles and ivermectin. The results confirm those obtained with biological drug assays, using both anthelmintics and verapamil, which suggest the involvement of

Pgp in drug resistance, and provide a quantitative and effective methodology for the functional study of multi-drug resistance in nematodes (Kerboeuf *et al*., 1999).

A very sensitive PCR test was developed that can detect benzimidazole resistance in the sheep parasite *Haemonchus contortus*. With this assay, the population genetics of benzimidazole susceptible and resistant worms can be studied in more detail under different conditions of selection. This may lead to a better control and a delay in the development of anthelmintic resistance (Roos *et al*., 1994).

Flow cytometry could be applied to the analysis of nematode populations. Forward-scatter emission can be used as a discriminating parameter for egg size. The hatching rate and side-scatter emission have a significantly positive relationship. The rate of resistance to the anthelmintic can be observed as a significant regression on the native green-fluorescence pulses that might reflect the state of oxidation of associated flavin molecules (Kerboeuf *et al*., 1996).

## 5. DETECTION OF ANTHELMINTIC RESISTANCE: PROTOCOLS FOR RECOMMENDED METHODOLOGIES

Considering all the limitations of the various tests, such as difficulties in repeatability, requirement for complex and expensive laboratory procedures and lack of sensitivity, the guidelines will provide the protocol for two methods of detecting anthelmintic resistance, namely the faecal egg count reduction test (FECRT, *in vivo*) and the larval development assay (LDA, *in vitro*). If the FECRT is inconclusive it can be complemented by the efficacy test which will be briefly described.

### Faecal egg count reduction test (FECRT) (*In vivo*)

The FECRT remains the most practical method of determining resistance by nematodes in sheep to anthelmintics.

***Principle:*** The ability of the anthelmintic in question to reduce the concentration of eggs per gram of faeces (EPG) by more than 95 percent, measured ten days after treatment, in comparison with the EPG measured at the time of treatment. Failure to do so is indicative of resistance.

***Prerequisites:*** The required group size is at least ten animals per group per anthelmintic to be tested. In addition, a non-treated control group is required. The pre-treatment EPGs must exceed 150 to 200.

***Advantages:*** It is a simple and robust test that does not require highly trained personnel, expensive resources, sophisticated equipment or facilities, and it can be used for testing anywhere and for any of the anthelmintics routinely used. This procedure can be used for testing in sheep, goats, cattle, horses and pigs.

***Disadvantages:*** Required group size and relatively high pre-treatment EPGs. False positive and negative indications of resistance to levamisole (Grimshaw *et al*., 1996). It is not reliable for the detection of low levels of anthelmintic resistance (McKenna, 1997).

**Protocol for FECRT: sheep and goats**

**1. Selection of animals**

Use young animals, three to six months of age, which have been bred on the farm. Older animals can be used if individual egg counts are above 150 eggs per gram of faeces (EPG). Animals should not have been treated in the previous 8 to 12 weeks. If animals have been recently treated, the test may be conducted on 'pre-selected' worms but will not represent the normal distribution of the parasitic population.

Randomly allocate animals or allocate by ranking of faecal egg counts into control and treatment groups of at least 10 and preferably 15 animals each. Create one group for each of the anthelmintics to be tested. A control (untreated) group should be used to allow for monitoring of natural changes in egg counts during the test period.

**2. Treatment**

Animals are treated with the anthelmintic according to the manufacturer's recommended dose either as (a) the accurate dose in mg/kg (for research purposes) or (b) according to the weight of the heaviest animal in the group (for clinical diagnosis). Anthelmintics should be administered with a syringe or a drench gun that has been previously calibrated.

**3. Sampling procedures**

For pre-screening of animals for sufficient egg counts, a minimum of 5 g (10 to 15 pellets) of faeces should be collected from each animal directly from the rectum. The same procedure should be followed at the post-treatment sampling. Samples must be placed in individually sealed containers and returned rapidly to the laboratory for egg counts. If group mean egg counts are below 150 EPG (limit of sensitivity of McMaster counting technique is 50 EPG), assessment of resistance will not be reliable. Group mean egg counts below 150 EPG can be common in adult sheep.

The post-treatment collection of faecal samples should be 10 to 14 days after treatment. Sampling earlier may give misleading results with a number of anthelmintics.

**4. Processing of samples**

For faecal egg counts, a modified McMaster method should be used.

Weigh 3 g of faeces into a suitable container.

Add 42 ml of water and soak for a few minutes to 1 h, until the faeces are soft.

Homogenise using a laboratory stirrer or place in a shaker jar with about 45 × 8 mm diameter glass beads and shake until all the pellets have been broken up.

Pour through a 100 mesh (0.15 mm aperture), 20 cm diameter sieve into a bowl.

Swirl the liquid and pour 15 ml into a 17 ml centrifuge tube.

Centrifuge for 2 min at about 300 × g (approximately 1500 rev/min on a bench-top centrifuge).

Gently pour or suck off the supernatant.

Agitate the tube to loosen the sediment.

Add suitable flotation fluid to give the same volume as before (15 ml).

Invert the tube five or six times.

Immediately withdraw a sample with a Pasteur pipette.

Fill the chamber of a McMaster slide.

Repeat the process of inversion and fill the second chamber.

At 10 × magnification count all the eggs under the two ruled grids (total volume 0.3 ml).

Multiply the number of eggs by 50 to give the EPG in the faecal sample. For greater sensitivity count all the eggs in each chamber (total volume 1 ml and multiply by 15 to give the EPG).

## 5. Analysis and interpretation of data

Calculate the arithmetic mean, percentage reduction and 95 percent confidence interval. The arithmetic mean is preferable to the geometric mean as: (a) it is easier to calculate; (b) it provides a better estimate of the worm egg output; (c) it is a more conservative measure of anthelmintic activity.

The percentage reduction, FECR % = 100(1-*Xt*/*Xc*)

Where *Xt* is the mean egg count of the treated group at 10-14 days and *Xc* is that of the control group at 10–14 days.

Details of the calculation of the 95 percent confidence intervals are given in an example in **Table 7**. A computer program, *RESO*, is available for this calculation. Resistance is considered to be present if:

(i) the percentage reduction in egg counts is less than 95 percent and

(ii) the 95 percent confidence level is less than 90 percent.

If only one of the two criteria is met, resistance is suspected.

## 6. Factors affecting the result

There might be disagreement in the presentation of the results from calculated FECR depending on whether they are based on the use of the arithmetic mean or the geometric mean for EPG calculations. Similarly, inclusion of pre-treatment EPG or control group EPG in the calculation of FECR percentage could influence the determination of resistance (Maingi *et al*., 1997b). In general it is recommended to use the arithmetic mean, because it will give more conservative results. Where the control group is included in the calculations using geometric means, the percentage efficacy is corrected for changes that occur in this group by the equation:

$$\text{FECR \%} = [1 - (C_1/C_2) \times (T_2/T_1)] \times 100$$

Where *T* and *C* are the geometric means for the treated and control groups, and subscripts 1 and 2 designate the counts before and after treatment, respectively (Presidente, 1985; Hotson *et al*., 1970).

FECRT results may not estimate anthelmintic efficacy accurately because nematode egg output does not always correlate well with worm numbers, and the test only measures the effects on egg production of mature worms. A good correlation was found between faecal egg counts and worm counts of *H. contortus*, and *O. circumcincta* but not for *T. colubriformis*. Egg counts for *Nematodirus* spp. are generally low and bear little relationship to actual worm burdens (Martin, 1985).

## 7. Diagnosis of genus and species present

The FECRT might not provide sufficient information on its own for correct interpretation. The failure of an anthelmintic to effectively reduce egg counts indicates resistance, but as most natural infections include a mixture of species and only one species may be resistant, there is a need to determine the resistant species. Third-stage larvae are therefore cultured from the eggs in faeces from controls and from treated groups separately. If remaining samples are to be cultured for the determination of nematode species, samples should not be stored at 4 ºC as it may affect the hatching of *Haemonchus contortus.* The procedure is as follows:

a. Collect about 50 g of faeces by combining similar size samples from each animal in one treatment group.

b. Break up faeces finely using a spatula. They should be moist and crumbly but not really wet. With wet faeces add vermiculite, crushed charcoal or sterile peat moss.

c. Fill glass culture dishes (e.g. crystallizing dish) with the mixture, covering but not sealing them, and culture for seven days at 22–27ºC.

d. Collect the larvae in a Baermann apparatus or by suspending the mixture in water in muslin, or by standing the mixture in a Petri dish containing water.

e. Treat the larvae with Lugol's iodine and identify 100 larvae. Identification guides are given in the chart below.

The nematode genera that are represented at the time of routine FECRT must be taken into account to reduce the likelihood of being misled when undertaking assessments of farm resistance status (McKenna, 1996b; Hotson *et al*., 1970; Martin, 1985; Taylor, 1992; Kerboeuf, 1994; Dash *et al*., 1985).

A modification of the FECRT has been described in which no pre-treatment samples are taken. The authors argued that statistically, both treatment and control groups are taken from the same population mean and as a result, the pre-treatment sample can discounted.

**Protocol for FECRT: cattle, horses and pigs**

The procedures are the same as those described above for sheep.

**Table 7. Example of FECRT calculations**

| **Data** | **Post treatment egg counts (10–14 days)** | | |
|---|---|---|---|
| | **Control** | **Treatment1** | **Treatment 2** |
| | 525 | 15 | 180 |
| | 450 | 0 | 135 |
| | 270 | 0 | 510 |
| | 540 | 30 | 180 |
| | 90 | 0 | 105 |
| | 765 | 0 | 225 |
| | 120 | 0 | 390 |
| | 945 | 0 | 210 |
| | 465 | 45 | 15 |
| | 225 | 0 | 150 |
| **Calculations** | | | |
| Number in group $n_i$:($N=\Sigma n_j$) | 10 | 10 | 10 |
| Arithmetic mean count $X_i=\Sigma_i X_{ij}/n_I$ | 443 | 9 | 210 |
| Variance of counts $s^2_i =(\Sigma_j X^2_{ij}$ ---$(\Sigma_j X_{ij})^2/n_i)/(n_i-1)$ | 74062 | 260 | 20300 |
| Percent reduction $R=100(1-X_t/X_c)$ | 0 | 98 | 53 |
| Variance of reduction (on log scale $v=[(s^2_t/(n_t X^2_t))]+[(s^2_c/(n_c X^2_c))]$) | | 0.36 | 0.08 |
| Approximate 95 % confidence interval for R 100 = $[(1-(X_t/X_c))\exp(\pm 2.1\sqrt{v})]$ | | | |
| Upper confidence limit $100[1-(X_t/X_c)\exp(-2.1\sqrt{v})]$ | | 99 | 74 |
| Lower confidence limit $100[1-(X_t/X_c)\exp(+2.1\sqrt{v})]$ | | 93 | 13 |

Where $_i$ denotes either the treated ($_t$) or control ($_c$) groups
$_j$ denotes each sheep in the group
$s^2_I$ denotes the variance on the arithmetic scale, calculated as above or:
$s^2_I=\Sigma_j(X_{ij} - X_I)^2/(n_i-1)$

In order to make valid comparison between results of different investigators, the conduct and the interpretation of the procedure require standardization (Waller, 1986; Cawthorne and Cheong, 1984).

### Efficacy test

When results from an FECRT undertaken 10 days post-treatment are not definitive, the efficacy test can be carried out to reach final conclusions. A complete parasitological necropsy should be performed in five animals selected at random from both groups used in the FECRT to determine the worm burden in each test group.

The gastrointestinal tract should be processed for worm recovery according to the standard procedures; the abomasum through to the large intestine (each segment tied off at both ends) and the lungs and complete trachea must be collected from each carcass. All viscera must be processed for worm recovery within one hour of slaughter.

Analysis of the data

The arithmetic mean must be calculated for each nematode species in each group. Efficacy, expressed as a percentage (PE) for each species, will be obtained, using the following formula:

$$PE\ (\%) = [(MC - MT)/MC] \times 100$$

*Where* $MC$ = mean number of worms in the control group.

$MT$ = mean number of worms in the treated group.

Resistance is considered to exist when the PE is below 90 percent.

### *In vitro* tests for anthelmintic resistance

Of all the available *in vitro* tests, the larval development test (LDA) is the most sensitive for the quantitative determination of thiabendazole and levamisole resistance. The egg hatch assay is also sensitive and accurate in determining resistance to benzimidazole, levamisole and macrocyclic lactones. It was concluded that the other *in vitro* methods described in the literature were unsuitable for use in field monitoring of resistance (Varady and Corba, 1999).

### 1. Larval development assay (LDA)

The LDA is an *in vitro* assay for the detection of resistance to benzimidazoles, levamisoles and macrocyclic lactones in nematodes parasites of sheep (Taylor, 1990; Varady *et al*., 1996), horses (Kerboeuf, 1994), pigs (Varady *et al*., 1996) and cattle. There is a commercial version of the LDA available which measures the resistance status of these anthelmintics.

***Principle:*** Nematode eggs are isolated from a faecal sample, placed into wells of a micro-titre plate and allowed to develop through to infective L3 larvae in the presence of a range of concentrations of anthelmintic.

***Advantages:*** The LDA allows simultaneous evaluation of all broad spectrum anthelmintics in a single farm visit with minimal on-farm work.

***Disadvantages:*** The counting procedure to determine the $LD_{50}$ is time consuming. As in other *in vitro* techniques, susceptible and resistant strains of parasites are necessary for comparison. In the case of macrocyclic lactones the test provides only an indication of anthelmintic resistance.

**Protocol**

**1. Field collection of the samples**

At least 10 animals are selected at random from the flock to be tested and no less than 100 g of faeces are collected as a pool in a plastic bag. The test must be carried out on faeces with a mean EPG above 200.

**2. Nematode egg recovery technique**

a. Suspend 20 g of faeces in water for 10 minutes, break up with a pestle and remove organic debris by filtration through a 100-mesh sieve.

b. Collect the filtrate and centrifuge at 2000 rev/min for 10 minutes.

c. Discharge supernatant and agitate the tubes to loose the sediment, then refill the centrifuge tubes with saturated sucrose solution until a meniscus forms above the tube.

d. Add a cover slip and centrifuge again at 1000 rev/min for 5 minutes. Gently pluck the cover slip off the tube and wash off the eggs to another centrifuge tube.

e. Fill the tube with distilled water and centrifuge for another 5 minutes at 1000 rev/min. This last step is to further clean the eggs isolated from the sucrose solution.

f. Remove the supernatant and estimate the eggs per ml and dilute to the required concentration (80 eggs approx. in 20 µl of water) if necessary.

**3. Test procedure**

a. The isolated eggs will be incubated in a micro-titre plate for 7 days at 25 ºC. To prevent dehydration a wet sponge should be introduced under the plate and the system should be covered with a pouch. The water in the wells must be checked every day during incubation.

b. Add 90 µl of fungizone per ml of egg suspension and mix the solution.

c. Dispense 20 µl egg suspension (80 eggs approx.) to each well of the micro-titre plate.

d. Incubate the plate at 25 ºC for 24 hours. After hatching occurs supplement all the wells with 20 µl of growth nutritive medium. Abandon the assay if 60 percent or more of eggs fail to hatch after 48 hours. This may happen if the eggs were exposed to high temperature.

e. After the addition of growth medium, add 10 µl of distilled water to the control wells.

f. Prepare a range of dilutions of anthelmintics with dimethyl sulphoxide (DMSO) 1% and put 10 µl of each drug concentration in the other wells of the plate. Drug concentrations are tested in duplicate.

g. Return the plate to the incubator for another 6 days.

h. At day 7 of incubation, kill all the larvae by adding 10 µl of a dilute solution of Lugol´s iodine and examine the plate under a binocular microscope. For every anthelmintic row, count the L3 in each well till they are reduced to 50 percent of the average number of L3 in the control wells.

**4. Results**

The results are expressed as the concentration of each drug that inhibits to 50 percent, the development of larvae to infective third stage in relation to control wells ($LC_{50}$). Resistance factors (RF) can be determined for comparison with known reference isolates.

# DETECTION OF RESISTANCE IN FLUKES

Resistance of *Fasciola hepatica* to common flukicides should be detected as follows:

- the faecal egg count reduction test (FECRT);
- efficacy trials (with or without previous isolation of the resistant strain).

**1. The faecal egg count reduction test (FECRT)**

Six to ten animals should be allocated into two groups. Group 1 should be treated with the drug to be tested, while group 2 will be considered as an untreated control. If enough infected animals are available, a third group should be treated with a drug of known efficacy. Since *F. hepatica* is frequently resistant to triclabendazole, the third group should be treated with closantel.

Individual faecal samples should be collected on the day of treatment (day 0) and at least seven days later (day 7). It should be noted that it is valid to do the trial with faecal samples taken between day 7 and day 21 post-treatment.

The percentage efficacy in terms of reduction of the egg counts is determined using the following formula:

$$PE\ (\%) = [(MC - MT)/MC] \times 100$$

Where, $MC$ = mean egg count on day 0

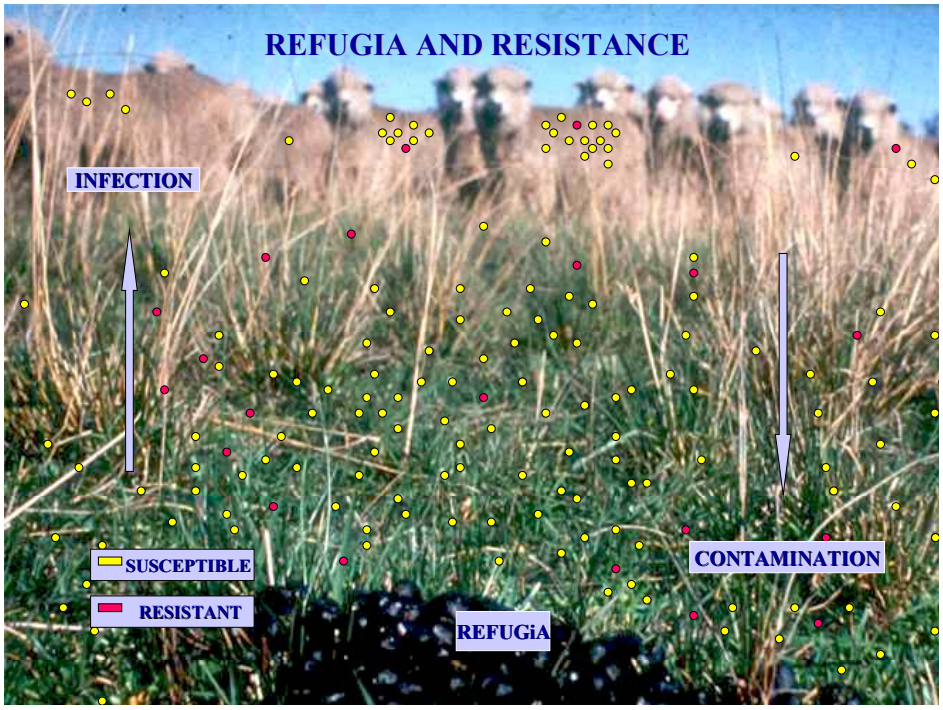
$MT$ = mean egg count on day 7, 10 or 21

Resistance is considered to exist when the PE is below 90%.

Due to extreme EPG values, calculation of the geometric mean is recommended over the arithmetic mean.

## 6. EPIDEMIOLOGY AND CONTROL OF GASTRO-INTESTINAL NEMATODES

The most important single requirement for the successful implementation of rational and sustainable helminth parasite control programmes in grazing animals, is a sound knowledge of the epidemiology of the parasite as it interacts with the host in a specific climatic, management and production environment (Barger *et al*., 1999) The epidemiological knowledge base has been established through extensive studies and field trials in many developed countries, and mostly in the context of industrialized livestock production. This is not the case for the majority of developing countries and countries in transition, and if information is available it rarely covers the diversity of their production systems. The reasons for this are often the obvious lack of human, economic and infra-structural resources. However, it is also often wrongly assumed that the epidemiological work conducted in one climatic region or production system can be extrapolated to another, or that the availability of modern broad spectrum anthelmintics eliminates the need for epidemiological knowledge.




In the absence of appropriate epidemiological knowledge the approach by which anthelmintics are administered is limited to two.

The livestock owner can choose to treat in a suppressive manner at intervals close to or at the end of the pre-patent period, or if drugs with residual effect are used, coincident with the length of persistence of the drug.

Treat whenever clinical signs of infection appear (curative approach).

The first option is the most effective at reducing the parasite populations and production losses in the short term, but this approach will select heavily for drug resistance in the parasites. The second option is associated with considerable risk of uncontrolled production losses and possible clinical disease, but will select less strongly for resistance.

Thus a prerequisite for the development and implementation of successful sustainable parasite control programmes is epidemiological knowledge.

## 7 CURRENTLY AVAILABLE CONTROL STRATEGIES

The currently available tools for gastro-intestinal nematode control consist of chemical and non-chemical technologies. The chemical technology relies entirely on treatment with different formulations of anthelmintics used in different control strategies according to whether epidemiological knowledge is absent or available. The non-chemical technology is based on, among other things, pasture and breeding management and nutritional interventions.

## 8 CHEMICAL TECHNOLOGY

During the last 35 years the pharmaceutical industry has produced a succession of highly effective, broad spectrum anthelmintics, and veterinarians and livestock producers have come to expect that worm control is easy, either by drenching or injecting cattle, sheep and goats with these products. This has made helminth control easy but has not fostered conservative use of the products. The following are strategies for the use of chemical anthelmintics.

**Suppressive (systematic) treatments**

This is a strategy that has been widely applied, particularly for parasites of small ruminants in the tropics and sub-tropics, where epidemiological knowledge is limited or absent. Without this knowledge owners of sheep and goats have been forced to treat regularly to keep their animals alive.

***Principle:*** Regular treatments at intervals at or near the length of the pre-patent period of the parasite, or if drugs with residual effect are used, the length of the effective persistence of the drug, whichever is greater.

***Prerequisites:*** Availability of the chosen drugs at affordable prices.

***Advantages:*** This approach is very effective in the short term in minimising parasite populations and production losses.

***Disadvantages:*** Numerous examples from the field and modelling have clearly demonstrated that this strategy selects inexorably for drug resistance in the parasites. It is also not necessarily cost effective.

***Epidemiological consequences:*** This strategy will initially lead to reduced contamination of pastures with parasite eggs and a subsequent lower challenge with infective larvae. However, resistance develops quickly because of the small refugia (parasites not exposed to the chemical agent) and consequent high selection rate. As resistance develops, the parasite epidemiology will change and control is lost.

***Possible combination with other strategies:*** Suppressive strategies should not be promoted.

**Curative treatments**

Treatment based on clinical diagnosis was a common practice in the past. With the introduction and promotion of strategic treatments, this method went out of use in most of the areas with industrialized livestock production. It was however still used by many small-scale farmers. Due to the problems of anthelmintic resistance, tissue residues and possible negative impact of chemicals in the environment, this strategy is being re-evaluated.

***Principle:*** Animals are treated therapeutically, whenever production losses and/or uncontrolled disease is considered to be significant. The trigger for treatment has been based on different criteria according to knowledge or interest and availability of support services. The triggers include:

Any clinical signs or evidence of sub-clinical disease.

Rise in faecal egg count. Attainment of threshold levels of EPG in groups of monitored animals indicates a need for treatment. The threshold will vary according to the composition of the parasite population, host type and geo-climatic conditions.

Anaemia in sheep and goats identified using systems such as the FAMACHA method (Van Wyk and Van Schalkwyk, 1990) (see below for more details).

In order to best utilize 2. and 3. it is necessary to know the composition of the parasite population on the farm. In cases where treatment has been initiated using 1. or 2. as indicators of infection level, two different approaches have been applied:

Treat all animals in the herd or flock.

Treat only those animals that are perceived to need treatment.

The use of FAMACHA specifically aims to differentiate between the animals in a group that need treatment and those that do not.

***Prerequisites:*** A regularly applied monitoring system such as clinical examination, faecal egg counts or FAMACHA.

***Advantages:*** Reduced expenses for anthelmintics as number of annual treatments will be lower and, if selective treatment is practised, the number of animals treated will be lower. The possibility of selecting for resistance is significantly reduced, and the risk of selection is delayed if only some animals are treated, as this will ensure the presence of a susceptible parasite population. Regular monitoring in the context of improved animal production and health management.

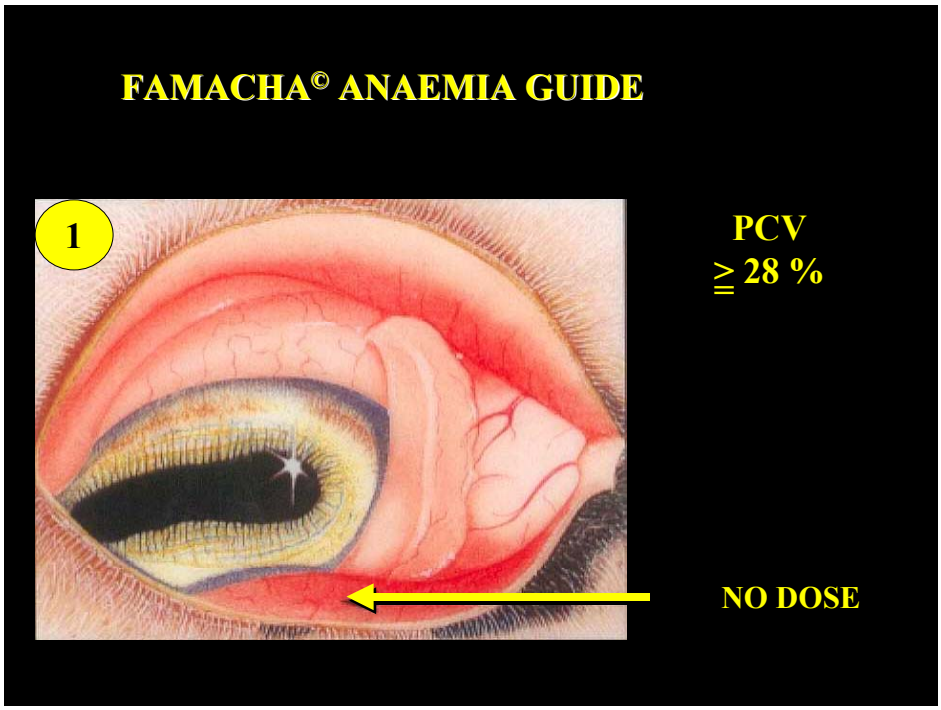
***Disadvantages:*** Regular monitoring needs labour input.

***Epidemiological consequences:*** These will depend on the variables. This strategy may not, however, reduce the overall contamination level and subsequent numbers of infective larvae on pasture. With EPG or FAMACHA monitoring, the sub-clinical effect should be controlled (Van Wyk and Schalkwyk, 1990).

***Possible combination with other strategies:*** No validated, integrated strategies have yet been developed.

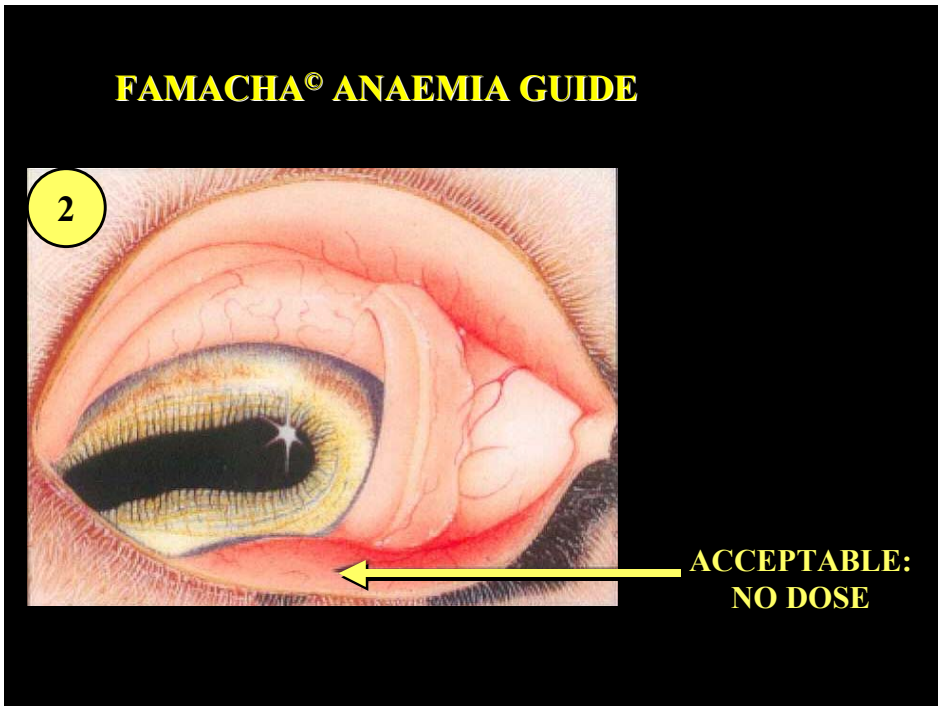
**Monitoring of *Haemonchus* infections using the FAMACHA system**

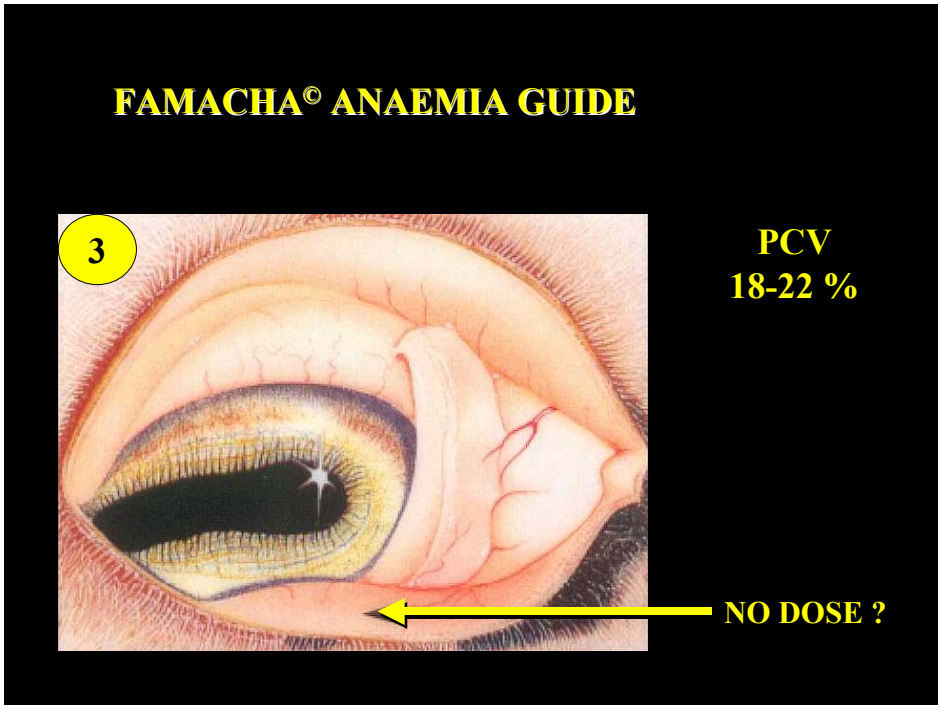
Based on the strong correlation that exists between the coloration of the mucous membranes of the conjunctival sac of sheep, and the degree of anaemia (packet cell volume (PCV)) caused by the blood-sucking parasite *Haemonchus contortus*, a standardized test known as the FAMACHA system has been developed by South African scientists (Van Wyk and Van Schalkwyk, 1990).



***Principle:*** Based on the above-mentioned correlation this assay uses a standardized colour chart showing illustrations of sheep eyes with colour variations from bright pinkish red to almost white. Treatment is recommended when the colour of the mucous membranes of sheep matches a tint that is correlated with anaemia.

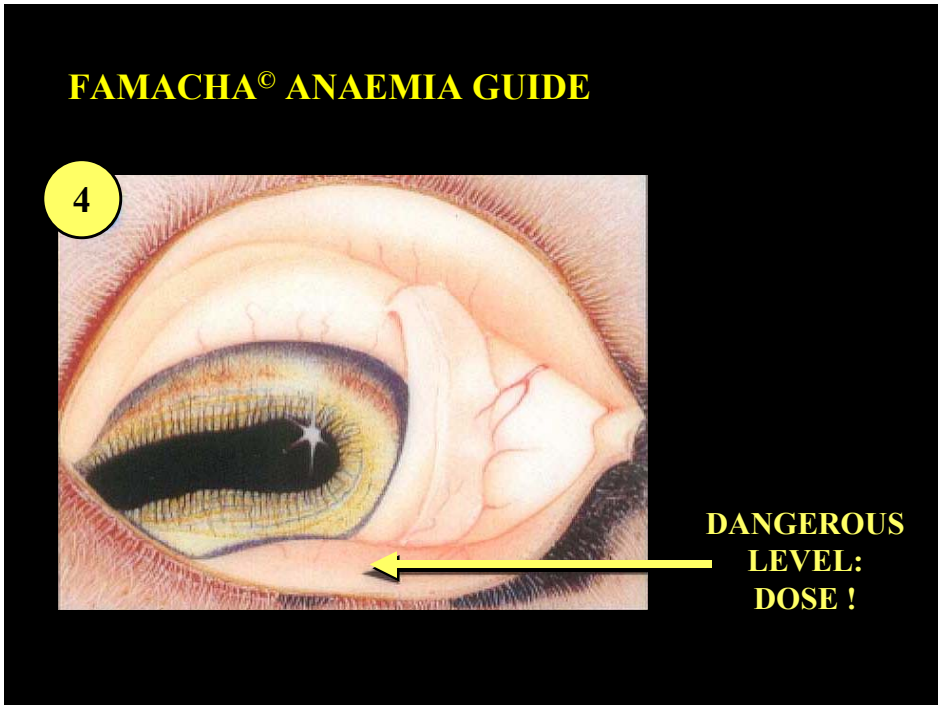
***Advantages:*** The method is easy and cheap to apply for continuous monitoring and it is easily taught to farmers. The use of pictures and signs make it suitable for illiterate sheep owners. There is a substantial reduction in the costs of drenching. A lower rate of selection for anthelmintic resistance is expected.

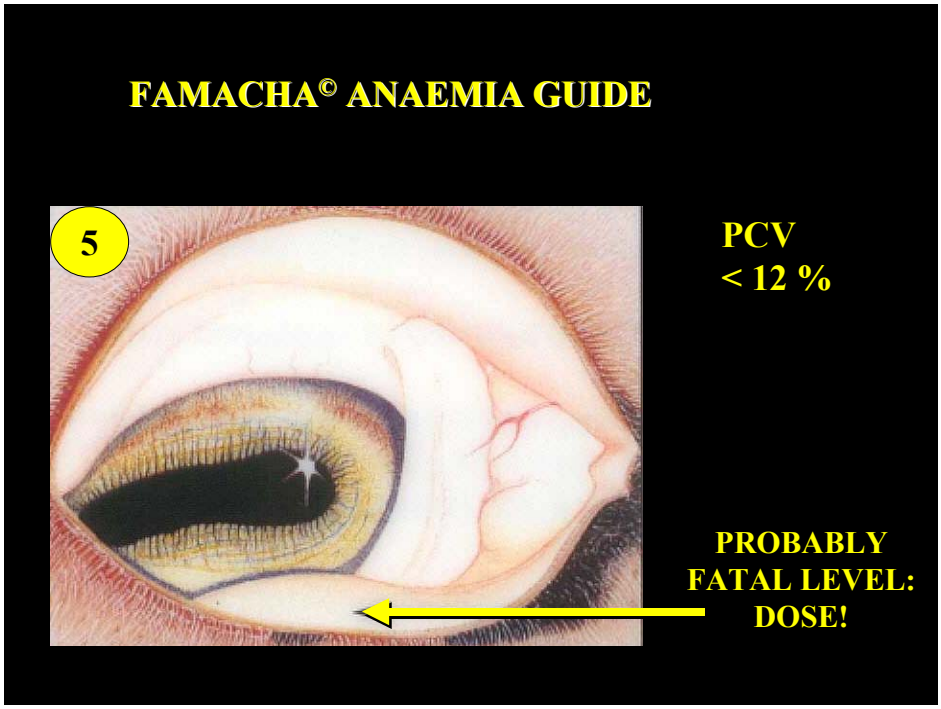





***Disadvantages***: Currently the method only applies to infection with *Haemonchus contortus* and the assay is only validated for sheep.

***Possible combination with other strategies***: Based on records that identify which animals require repeated treatment, it is possible for the sheep owner to cull these and breed from the most resistant animals, increasing the overall resistance of the flock.






## Current strategies based on modified use of anthelmintics

### The "Worm Kill" principle

As a consequence of widespread anthelmintic resistance in sheep parasites in Australia, the "Worm Kill" programme was developed. The main aim of this programme was to reduce the number of treatments, while maintaining effective control of parasites. This was done by the use of a narrow spectrum drug, closantel, in combination with a minimum number of treatments with broad spectrum anthelmintics. Closantel is particularly effective against *Haemonchus* and has a persistent effect for 2 to 3 months.

### Strategic treatments based on epidemiology

Clinical parasitic disease in ruminants usually occurs at or shortly after times of peak larval availability. The timing of peak larval availability on pasture is of crucial importance in understanding the population dynamics of the parasite population, because this is when the largest worm burdens are acquired. It is in order to prevent these seasonal peaks from developing that strategically timed control measures are implemented. Thus treatments are often administered at times when the larval challenge on pasture is low and the majority of the parasite population is in the host. This reduces the pool of susceptible parasites and may increase selection pressure for resistance. Due to the effect of the climate and weather on development, survival and transmission of free-living stages, weather conditions play a dominant role in determining the timing of strategic treatments. Geographical differences in the seasonal availability of infective larvae from the pastures have similarly been a key factor for determining the timing of strategic treatments. In temperate climatic zones, sequential treatments at the beginning of the grazing season, using intervals similar to the pre-patent period or pre-patent period plus the length of the residual effect of the drugs, have been used. Similar sequential treatments have been applied at the beginning of the rainy season in tropical zones, with one treatment added during the dry season when pastures would be almost sterile.

***Principle:*** Strategic treatments are administered, not only for therapeutic purposes to rid the animals of worms, but also as a prophylactic measure to prevent future contamination of pasture and reduce the risk of future re-infection (Barger *et al*., 1999; Barger *et al*., 1994a).

***Prerequisites:*** Knowledge of local epidemiology of relevant parasite species.

***Advantages:*** Proven record of reducing contamination of pastures with parasite eggs, and subsequent challenge of grazing animals with infective larvae. This has resulted in significant increases in productivity.

***Disadvantages:*** Some of the strategies are associated with strong selection pressure for anthelmintic resistance.

***Epidemiological consequences:*** Significant reductions in egg excretion and pasture larval contamination (Barger *et al*., 1994b).

# 9 RECOMMENDATIONS FOR BETTER USE ("SMART USE") OF EXISTING DRUGS

Successful worm treatment relies on effective on-farm management practices and recent studies have provided improved opportunities for maximizing drench action – giving the drench the best chance to work (Ali and Hennessy, 1995; Prichard and Van den Bossche, 1980).

In order to maintain the efficiency of benzimidazoles and avermectin-like drenches (macrocyclic lactones) it is worth remembering a few points. Increasing the efficiency of drenching means:

More worms are removed, leaving healthier animals.

Less pasture contamination.

### 1. Place the drench gun over the tongue

The value of weighing sheep and using the correct dose is reduced if the drench does not go to the right place.

The dose should go wholly into the rumen or first stomach, where it can be slowly released with prolonged exposure in the case of benzimidazoles and macrocyclic lactones, NOT levamisole. Depositing the drench in the front of the mouth can activate the oesophageal groove, allowing the drench to by-pass the rumen and dramatically shorten the time during which there is a sufficiently high concentration of the drug to kill the worms.

Care should be exercised to ensure that no drench is directed into the airways.

### 2. Reduce feed before drenching

Restricting access to feed for 24 hours before drenching slows the flow of gut contents containing the drench from the rumen. Reduced feed intake prolongs drench uptake, extending the effective duration of the killing effect.

Muster animals in the morning and provide little or no feed – especially not fresh green feed – for the rest of the day and overnight. Provide access to water. Drench the following morning. For maximum effect, keep animals off feed for a further six or so hours before returning them to pasture. For better farmer compliance, it may be useful to demonstrate the effect of this advice by comparing a group treated after reduced access to feed, with a group treated traditionally, and to use the reduced faecal egg counts as an indicator of improved drenching efficiency.

Some local conditions (e.g. poor feed availability, drought) will reduce the effectiveness of this application. You should not restrict feed if the sheep are heavily pregnant, severely stressed or in poor condition.

**3. Use only the recommended dose rate**

Recommended doses are designed to persist in the animal for a specific period. In some cases – where resistant worms are present – increased dose rates have been used. Because of the way drenches are removed from the animal, doubling the dose only marginally extends the "killing time". This does not matter with levamisole because its action is related to peak concentration, but it is important with other drenches.

If resistance has reduced the efficiency of drenches other than levamisole, and a higher dose is considered, remember that increased dose rates may breach regulations and require written veterinary permission. Rather than double the dose rate, it is better to administer two single doses at the recommended dose rate, separated by 12 hours. The two separate doses are much more efficient than one double dose. If possible, the use of two separate doses can be combined with reduced feed intake before drenching.

Attention should be paid to the fact that goats metabolize drenches faster than sheep. Ensure that goats always receive a full dose. In some cases it is recommended, particularly if resistance is suspected, to give goats a second or third drench, each 12 hours after the previous dose. Because sheep and goats have the same species of worms, resistant strains will be passed from goats to sheep.

## 10. NON-CHEMICAL TECHNOLOGY

With the increasingly widespread problem of resistance to anti-parasiticides and the increase in consumer pressure for quality animal products without residues, the demand for alternative, non-chemical parasite control interventions will increase. Few of these methods have, however, been sufficiently validated to the point where they can be recommended for general use. It is also likely that they will have to be used in combination with other interventions in order to obtain the desired effect. Among the non-chemical tools available immediately for implementation are various forms of pasture management, breeding management and improved nutrition. Others, namely utilization of herbal remedies, use of copper particles and biological control, still require research, development and validation in different geo-climatic regions under a variety of production systems. They may, however, already be incorporated in integrated control strategies in some circumstances.

### Pasture management

The thorough knowledge of epidemiology, including the seasonal variations in the pattern of larval development and availability on pasture, can form the basis for control of gastro-intestinal nematodes through pasture management. A number of different grazing systems have proved helpful in the control of these parasites.

**Rapid rotational grazing:**

Recently there has been increased interest in using rotational grazing of pastures for the optimization of pasture growth and productivity. This is an excellent tool, from the productivity point of view, as animals will consume a higher proportion of the available forage, which stimulates pasture re-growth (Barger *et al*., 1999).

***Principle:*** This is a grazing management technique involving subdivision of pastures in which each paddock is grazed for a short time and then rested for a relatively much longer time. The major requirement for parasite control would be sufficient resting time for most of the

infective larvae originating from the previous grazing to have died off. This is probably not achievable in temperate climates, given that substantial declines in pasture infectivity may take from 3 to 9 months, depending on the climate and time of the year. It may, however be useful on wet tropical pastures where larval survival times are short. This makes it possible to design a practicable rotation that is short enough to prevent auto-re-infection within a single grazing period, because development from egg to infective larva can take as little as 4 or 5 days. A slightly modified version (strip-grazing) is based on making fresh pasture available for the animals between two movable electric fences, which prevent the animals from going back to previously grazed parts of the pastures.

***Prerequisites:*** Suitable pastures and fencing.

***Advantages:*** Continuous reductions in pasture larvae availability. Reduction in use of chemicals and reduced risk of resistance development. Better pasture utilization.

***Disadvantages:*** Capital investment in fencing and watering facilities. Increased labour requirements.

***Epidemiological consequences:*** Reduced pasture contamination levels creating safer pastures.

***Possible combination with other strategies:*** An anthelmintic treatment at the time of introducing the rotational system. Following validation of biological control strategies these could be used in combination with rotational grazing, creating a parasite control strategy without chemicals.

**Safe pastures**

***Principle:*** The safe pasture concept is based on the fact that the number of larvae in the pasture are reduced over time by resting the pasture during the period when they are normally being re-contaminated (spring, rainy season) or through the growing and harvesting of a crop of hay or silage, followed by a period of re-growth.

***Prerequisites:*** Available and suitable pastures for hay or silage production and/or pastures in crop rotation.

***Advantages:*** Reduction in number of larvae on pasture. Reduction in use of chemicals. Better pasture utilization.

***Disadvantages:*** In order to have a substantial impact on transmission, the period of resting may be too long to be practical for the producers. The approach is only suitable in combined crop and livestock production systems. If combined with the use of anthelmintics (dose and move systems) it may increase the selection pressure for anthelmintic resistance development.

***Epidemiological consequences:*** Reduction of the number of infective larvae on the pasture reduces the worm burden and subsequent contamination levels.

***Possible combination with other strategies:*** The movement of animals to safe pasture has been combined with anthelmintic treatment. The possible increase in selection pressure for anthelmintic resistance should be considered before recommending this strategy.

**Alternate grazing**

***Principle:*** Using alternate grazing for parasite control is based on different age groups of the same species, or different species grazing the pastures in sequence. In cases where different age groups are used it is common practice to graze calves followed by older cattle, taking advantage of higher resistance in the older animals. If the system is based on alternating between species (sheep – cattle) it utilizes the fact that many parasites show little cross-infectivity between adult

cattle and sheep and/or the reduced susceptibility of different host species. It should be kept in mind that cool moist weather prolongs larval survival, and it is likely that alternate grazing systems will be less efficient in controlling parasites in temperate climates compared to tropical and subtropical regions.

***Prerequisites:*** This approach requires that two or more different species are available on the farm, or the operation of a management system where sufficient numbers of different age groups are grazed separately. Monitoring of faecal egg counts is also desirable with this approach.

***Advantages:*** Older animals or a different species of livestock will act as a 'vacuum cleaner' reducing the number of infective larvae on pasture.

***Disadvantages:*** In order for this to significantly reduce the number of infective larvae, the time intervals between returning the same age group or species to the pasture may have to be prolonged, depending on temperature and precipitation, making this system less attractive to the farmer. Some parasite species can survive and reproduce in different hosts. The system requires increased monitoring. It can only be used for different age groups of cattle, NOT for sheep.

Note: The annual variation in pasture production may negatively affect or prevent the use of this system. It is recommended that hay or silage production with subsequent re-growth is considered as a regulatory mechanism for an even feed distribution.

***Epidemiological consequences:*** Reduced level of pasture larval contamination.

***Possible combination with other strategies:*** This can be combined with anthelmintic treatment at the time of introducing another species or age group.

Note: A modification of the alternate grazing management strategy is the use of mixed grazing, where two or more different animal species graze together, resulting in removal of infective larvae by non-susceptible hosts. The effect of this in the context of parasite control is variable, but it may have an additive effect to other measures and it does contribute to better pasture utilization. It cannot be recommended as a stand-alone strategy (Barger *et al*., 1994).

## Supplementary feeding

Gastro-intestinal parasitism of ruminants is a production-related phenomenon, enhanced by chronic malnutrition and under-nutrition, which is particularly common in the developing world. Research has shown that improved nutrition reduces production losses and mortality rates due to worm parasites of livestock. Strategic feed supplementation, particularly to susceptible classes of stock such as young and peri-parturient animals, can have long-term benefits.

***Principle:*** Low-cost mineral and non-protein nitrogen supplements dramatically change the physiology of the rumen. These lead to greater feed intake and increased microbial protein production, resulting in increased protein for digestion and absorption in the small intestine.

***Prerequisites:*** Availability of low-cost mineral and non-protein nitrogen supplements and the technology for the preparation of blocks, pellets and other feed supplement formulations.

***Advantages:*** Productivity is increased and supplemented animals have an increased ability to withstand the effects of parasitism. The feed supplements can include locally produced surplus plant by-products, which will enhance the supply of nutrients without greatly increasing the cost to the farmer.

Disadvantages: Cost.

***Epidemiological consequences:*** The ability of animals to better cope with parasites may result in lower egg output by the worms and a subsequent reduction in pasture infectivity levels.

***Possible combination with other strategies:*** Supplementary feeding can and should be included in any parasite control programme. Several common livestock management procedures in the tropics/subtropics, particularly for small ruminants, lend themselves to the administration of low-cost supplementation of nitrogen and essential minerals by way of feed blocks. There are very simple methods for manufacturing such blocks; and some may prove to be suitable substrates for the growth of locally isolated strains of nematode-destroying fungi.

## Genetically resistant or resilient animals

A substantial amount of evidence is now available to demonstrate that there are between breed and within breed differences in the ability of animals to respond to the challenge of gastro-intestinal nematode infection (Bisset *et al*., 1996; Albers *et al*., 1987). Thus, breeding sheep, cattle and goats that require minimal anthelmintic treatment is an option for managing anthelmintic resistance, as well as providing control of helminth parasites.


In cattle, the relative economic importance and the dependence on anthelmintics are smaller, but the potential for rapid genetic progress is greater due to the opportunities afforded by more widespread use of artificial breeding strategies.

***Definitions:*** Resistant animals have the ability to suppress the establishment and/or subsequent development of a parasitic infection. Resilient animals are animals that can maintain relatively unaffected production despite being subjected to parasite challenge. The heritability of resistance has been documented. The heritability of resilience is substantially lower than that of resistance, and selection progress for this trait could be slow.

***Principle:*** Develop breeding programmes for the selection of resistant or resilient animals, thereby increasing the overall resistance of the flock or herd and reducing the requirement for the use of anthelmintics.

***Prerequisites:*** The introduction of breeding programmes for resistance will require increased monitoring of the animals with regular worm egg counts or use of genetic markers when these become available.

***Breeding strategies:*** The understanding of the reaction of sheep to nematode parasitism has increased significantly during the last 5 to 10 years, but it is not completely clear what is the most appropriate breeding strategy to achieve a flock which requires minimal anthelmintic treatment.

***Advantages:*** Reduced use of anthelmintics. Reduced risk of residues in animal products.

***Disadvantages:*** Requires increased monitoring and record keeping.

***Epidemiological consequences:*** Increased genetic resistance in a herd or flock will reduce the contamination of pastures with parasite eggs, with a subsequent reduction in the number of infective larvae. This may not be the case with resilient animals where the worm burden is not necessarily lower, but resilient animals will be able to cope with the challenge. However, in cases where the flock or herd consists of a mixture of animals of carrying susceptibility, or if susceptible animals follow resilient animals on to pastures, the effect of having resilient animals may not be strong enough to prevent the negative effect of nematodes. This will also be the case at the beginning of breeding programmes for resistant animals.

***Possible combination with other strategies:*** The selection for resistance can be combined with any of the other strategies as required during the process of creating resistant herds and flocks.

## 11 CONTROL STRATEGIES UNDER DEVELOPMENT

### Biological control

Almost all strategies for the control of gastro-intestinal nematodes target the parasitic stages within the animal. In contrast, the biological control of nematode parasites is targeted at the free-living stages on pasture. Current biological control aims to exploit the nematode-destroying properties of certain micro-fungi, particularly *Duddingtonia flagrans*.


***Principle:*** Biological control using nematophagus fungi is a prophylactic measure reducing the infectivity level of pastures. It has no effect on parasites once the grazing animals have acquired them. *Duddingtonia flagrans* produces many resistant, thick-walled resting spores, chlamydospores, which have the ability to successfully pass through the gastrointestinal tract and survive in the faecal material. Upon stimulation they rapidly germinate and spread on and in fresh dung, and capture the infective larvae of most gastrointestinal worm species including *Cooperia, Ostertagia, Haemonchus, Nematodirus and Trichostrongylus* before they can migrate to the pasture.

***Prerequisites:*** Availability of large amounts of chlamydospores, a vehicle to administer the spores and a management system to supply these to the animals.

***Advantages:*** It is anticipated that this will be an inexpensive, sustainable non-chemical control method particularly suited to the practice of housing animals at night. It would be augmented where animals are provided with feed supplementation when housed, so that fungal material could either be co-administered or grown directly on the supplement if it consists of plant by-product material.

***Disadvantages:*** It is not anticipated that this will be a stand-alone control strategy. Currently there is not a standardized product available. Each country will have to produce the fungus and identify suitable means of fungal delivery.

***Possible combination with other strategies*:** The whole philosophy of using a biological control agent against parasitic nematodes, is to reduce the number of infective stages available to be picked up by susceptible, grazing livestock. The reduction in infective stages on herbage will subsequently reduce the build-up of worm burdens in hosts, which otherwise would cause sub-clinical or clinical responses, in particular in young animals. A steadily increasing number of review articles has been published within the last 4 to 5 years on the subject of biological control of helminths, as well as on possibilities for the various elements in integrated control strategies The introduction of micro-fungi as a biological control in an integrated control programme of gastrointestinal nematodes, could be as part of a feed supplement, or incorporated into various

kinds of feed blocks presented to animals. This technology is currently under development and research (Larsen, 1999; Waller, 1999).

## Vaccines

Considerable resources have been and still are being allocated to research into the effector mechanisms of naturally acquired immunity to gastrointestinal helminth infections of sheep (Sutherland *et al*., 1999) and cattle, with the aim of facilitating the development of vaccines. However, the situation is complex, involving a combination of local hypersensitivity, in addition to cell mediated, antibody and inflammatory responses, and is complicated further by the natural unresponsiveness which exists in the young lamb or calf, and in the dam around parturition.

Using the successful development of the irradiated larval vaccine against the bovine lungworm, *Dictyocaulus viviparus* as a model, attempts have been made to produce vaccines against gut parasites in ruminants, but they have all been disappointing.

Likewise, this seemed to be the case with the vaccines based on antigenic fractions of parasitic material. Early attempts to immunize ruminants against gastrointestinal helminths, either with crude worm homogenate antigen or by ectopic infection, met with little or no success. Currently, attempts are being made to direct high titre antibody responses towards potentially susceptible targets on or secreted by the parasite. In the case of blood-feeding species, several target molecules have been identified on the surface of the intestine of the parasites. Because molecules on the luminal surface of the parasite's intestinal cells are not normally recognized by the host during infection, these antigens are classified as "hidden". Several vaccines using "hidden" antigens were developed for *H. contortus* in sheep, and these provided 94 percent protection in relation to EPG and their efficacy reached 90 percent when worm burdens were studied (Newton *et al*., 1995).

Another way to induce protection has been to use "homologous" antigen; that is an antigen first shown to be protective against another helminth species. An example is the glutathione-S-transferases (GST) of *Fasciola hepatica*, which were chosen as candidate vaccine antigens because homologous protein from *Schistosoma mansoni* and *S. japonicum* had been shown to be protective in laboratory animal models of infection. Sheep and cattle immunized with native GSTs isolated from *F. hepatica*, have been protected on average by 49 and 29 percent respectively, although the results from individual trials have been quite variable (Morrison *et al*., 1996).

The expectation was that for any vaccine to be acceptable, it had to compete favourably with modern anthelmintics, not only in terms of cost but also with regards to spectrum and levels of efficacy. Attitudes are now changing, largely due to the revelations of mathematical modelling of the effects of vaccination. Simulation studies have shown that substantial benefits might be obtained even with a vaccine that produces only 60 percent efficiency in 80 percent of the flock. It is argued that a vaccine of moderate efficacy measured in terms of economic benefits, rather than one with the ability to induce sterile immunity, may well be effective in the field. This is achieved by priming the host for progressive development of immune regulation of parasites, and thus reducing the overall rate of parasitic population increase in the flock.

Accepting the potentially lower efficiency associated with the use of the irradiated larval technique and reviewing existing data, it appears that these vaccines can provoke levels of protection similar to molecular worm vaccines. Not only would irradiated larval vaccines undoubtedly be much easier and cheaper to produce, but also their manufacture would not be constrained by commercial protection, as the technology involved in larval irradiation is very much in the public arena.

## Copper particles

Various preparations of herbs, copper, arsenic and other more or less toxic minerals were the only products available for the control of helminths prior to the marketing of the first generation of broad spectrum anthelmintics in the late 1930s. The interest in copper formulations was renewed when the importance of trace elements and mineral deficiency were identified. It was shown that there were great benefits from low-dose depot delivery of copper to the rumen of sheep and cattle grazing on deficient pastures, and also from the equally lengthy protection such boluses gave to sheep against *H. contortus*. This however coincided with the promotion of thiabendazole, the first of the safe, broad-spectrum anthelmintics. Hence the possibilities for control of *H. contortus* using low-dose administration of copper were overlooked.

Stimulated by the widespread resistance to anthelmintics, the interest in using alternative measures including copper is being re-evaluated. Currently the use of copper-oxide wire particles (COWP), delivered by way of a capsule, not only to treat copper deficiency, but also to ameliorate the effects of abomasal parasites is being tested. The capsules dissolve in the rumen, resulting in the passage of particles to the abomasum, where they lodge in mucosal folds and release ionic copper over an extended period of time. Administering 5 g capsules to young sheep resulted in 96 percent and 56 percent reductions respectively of *H. contortus* and *Ostertagia circumcincta* infections. However, there was no effect on the intestinal species, *Trichostrongylus colubriformis*. Further work has shown that the protective effect can last for the entire dissolution period of the particles, which is approximately 3 months.

The apparently prolonged action of COWP against *H. contortus* could prove to be of enormous benefit in restoring some measure of control in those regions of the world where this parasite predominates, and anthelmintic resistance is rampant.

## 12 ANTHELMITIC RESISTANCE MANAGEMENT AND INTEGRATED WORM CONTROL:

The prevention or control of parasite resistance has become a major problem for the livestock industry and in particular for the small ruminant producers who, in many countries, are facing an emergency situation of no anthelmintics with sufficient effect to prevent productivity losses. Thus, there is a growing need to combat the problem of anthelmintic resistance in the parasites of ruminants through the development of integrated parasite control methods.

### Prudent use of anthelmintics

There is obviously no point in continuing to use a drug to which the target parasites are resistant. It is therefore recommended that a test for resistance be made before new control strategies are implemented. If any drugs from one or more groups of anthelmintics are still effective, they should be carefully used in the future to delay the development of resistance to them.

### Development of resistance

There is a general conflict between the requirements for a high level of control of helminths, and the requirements for delaying the development of anthelmintic resistance. This is clear from the list of factors below that are likely to accelerate the development of resistance but it should be noted that elements in this list require further scientific validation before being considered as fact.

Factors likely to accelerate resistance include:

Treatment of the flock at times of the year when the majority of the parasite population is in the host.

Treatment of the whole flock instead of only the animals with the highest parasite burden.

Use of poor quality anthelmintics of uncertain concentration.

Use of anthelmintics with a prolonged, sub-lethal decay curve.

Under treatment. Although it is widely believed that under-dosing contributes to the development of resistance, the relationship between dose and selection varies depending on the mode of inheritance of resistance and the dose used in relation to the lethal dose for each genotype.

Use of slow-release devices at times of the year where the level of pasture contamination is low.

**Prevention of resistance**

Actions likely to prevent resistance:

Reduce the frequency of treatments

Apply the right dosage. Remember to dose according to the heaviest animal. Determine the weight by using a scale.

Buy products from reputable companies. They are normally a little more expensive but this is compensated for by their improved efficiency and subsequent maintenance of or increase in animal productivity.

Treat only animals that need to be treated. This will maintain a population of susceptible parasites on the pastures.

Avoid the use of slow release devices at times when pasture contamination is low.

Introduce quarantine measures for newly purchased or returning animals. Treat with a macrocyclic lactone and/or closantel, and to reduce the risk of introducing resistant nematodes, keep new stock off pasture until all nematode eggs have been passed.

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# MODULE 3. FLIES: INSECTICIDE RESISTANCE: DIAGNOSIS, MANAGEMENT AND PREVENTION

## 1 INTRODUCTION

*Haematobia irritans* (Linnaeus), known as the horn fly, was introduced into the United States from Europe in the 1880s and now occurs in the western hemisphere from Canada in the north to Argentina in the south, and in the eastern hemisphere from Europe to North Africa. A related species, the buffalo fly, *Haematobia irritans exigua* (De Meijere) occurs in Australia and other tropical areas of the Pacific where beef and dairy cattle are raised. The horn fly (*Haematobia irritans* L.) and the buffalo fly (*Haematobia irritans exigua*) are both blood-sucking flies that primarily attack cattle. They will attack a variety of other hosts such as sheep, goats, horses and some forms of wildlife. Following their emergence as adults, these flies can use these alternate hosts as food sources until they can locate a bovine. The adult remains on the host except for short time intervals to oviposit on bovine dung. The life cycle from egg to adult can be as short as 10 days although it is usually about 14 days. In the United States it is not uncommon for 500 flies per head on cows and several thousand per head on bulls. Data collected indicate that counts in Mexico are usually below 300 flies per head, although the counts in Argentina and Australia are more like the higher United States' populations. In the United States, one annual population peak is common in the north with two peaks in the south. The two peak pattern generally applies in Argentina and Australia, especially in the warmer areas, where the peak season is split due to high temperature and reduced moisture. In Mexico the two peaks are not as definitive. Flies are active from late spring to early autumn and in the more tropical areas flies can be present throughout most of the year.

These flies are economic pests causing losses in excess of US$800 million annually in the United States. Campbell (1976) and Kunz *et. al*. (1984) have shown that horn flies can cause a 14 percent decrease in the weight gain of yearling steers feeding on native grass. In addition, the weaning weights of calves from cows not receiving horn fly control were reduced by 5.4 to 6.3 kg/head. In Australian studies, average fly burdens per animal on untreated cattle were 324, 470 and 172 in successive experiments. Based on counts made prior to each insecticide treatment, buffalo fly populations on different herds of steers were reduced by 52, 64 and 77 percent respectively, resulting in corresponding increases in average live weight gain of 15 percent, 11 percent and 4 percent in the respective treated herds (Bean *et al*., 1987). The effect of the treatment on weight gain was statistically significant in the second experiment only. This roughly corresponds to the 200 flies per head threshold proposed in the United States' studies (Kunz *et al*., 1984). Similar data for the Latin American countries where horn flies exist are not available, but producers are assuming that control measures are warranted. This information has led to the development of control tactics for these pests.

## 2 RESISTANCE DEVELOPMENT

Insecticides continue to be the primary means of control for ectoparasites on livestock but the intensive use of these products has resulted in resistance to organochlorines, organophosphates and pyrethroids among populations of *Haematobia irritans* and *H. irritans exigua*.

The underlying process in arthropod resistance to pesticides is genetic selection, an evolutionary process. The selection pressure imposed by insecticides means that more effective control leads to more rapid development of resistance. In most cases, survival of insects following treatment with insecticides is due to genetic differences rather than the escape of survivors from full exposure. The breeding population which survives this initial application is composed of ever-increasing populations of individuals able to resist the compound and to pass this characteristic on to their offspring. Pesticide users often assume that the survivors did not receive a lethal dose, and may react by increasing the pesticide dosage and the frequency of application. This results in further resistance of susceptible pests and an increase of resistant

individuals. When this happens, the next step is to switch to a new product. With the same type of persistent application, resistance to the new chemical evolves in the same way.

When pest organisms are resistant to one or more compounds, resistance to new groups of chemicals which have either similar modes of action or similar metabolic pathways for detoxification is likely to evolve more rapidly. Twenty years ago, there was particular concern that the newest group of insecticides, the pyrethroids, might have a short useful life due to a gene identified as 'kdr'. This gene also played a role in the genetic evolution of resistance to dichlorodiphenyltrichloroethane (DDT) and has since been identified as providing certain insects with protection against pyrethroids (Kunz and Kemp, 1994; Kunz and Schmidt, 1985).

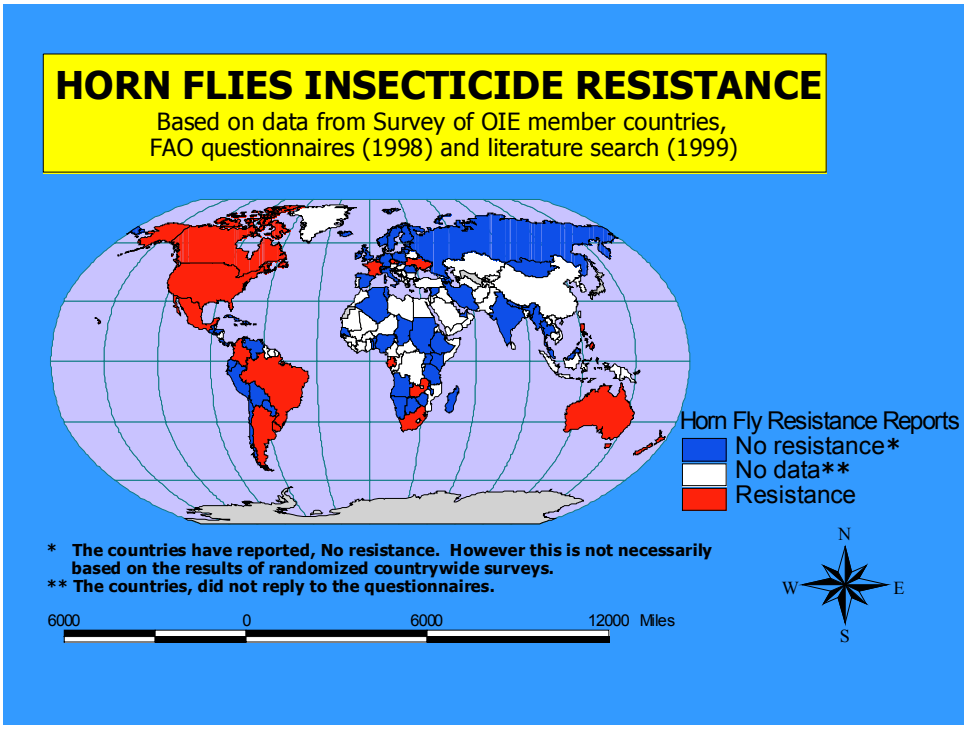
Whatever the cause, the spread of resistance is accelerating while the development of new compounds is slowing. It is increasingly difficult to discover new compounds that are superior to existing insecticides or have different modes of action, and it is increasingly expensive to develop such compounds for production. Many compounds that appear to be effective against certain pests are not brought into production, as cost estimates indicate that they may not return the cost of development to the commercial producer. This reduces the number of potential chemicals that might be used in the fight against resistance. In addition, agricultural pesticide costs have increased due to resistance, as users are compelled to switch to new chemicals (which are generally more expensive), or to increase the frequency of application of pesticides already in use.

With hindsight, the highly selective, continuous dose provided by pyrethroid ear tags is no doubt responsible for pyrethroid resistance. However, intensified use of any compound by any application method that simulates the intensity of application through ear tags, can and will cause the same resistance problem. Pyrethroid resistance also developed in horn flies in Mexico with the use of periodic dips over long periods against *Boophilus* ticks. This is also the probable cause of pyrethroid resistance in flies in Australia and South America. Resistance quickly developed in Argentina because incoming flies had already been heavily exposed to insecticide pressure. This resistance in the horn flies was exacerbated as soon as they arrived in Argentina by the custom of pour-on acaricide use (with insecticide action) for tick control.

## 3 CURRENT STATUS

Since the introduction of organophosphates (OPs) in the mid 1950s, horn flies have been relatively easy to control. However, as new classes of compounds and new application procedures were developed, horn fly control experienced the same problems that have been encountered with other species (**Tables 1a and 1b**). In the late 1970s the synthetic pyrethroids (SPs) were developed and ear tag technology was used as a means of application in the United States. After about two years of intensive use the first sign of product failure was reported from southern Florida. By 1982 the first resistance to pyrethroids was confirmed. This occurred at about the same time that the first published reports of resistance to pyrethroids appeared, indicating that resistance had been encountered in Australia in 1979 (Kunz and Kemp, 1994). The Australian date indicates that resistance, to the extent that products failed to control flies, existed some three years prior to the United States' report.

After the initial detection of resistance in Florida, resistance continued to develop in other states including California and Hawaii, so that by the early 1990s, resistance was present in almost every cattle producing area of the United States. The fact that resistance was detected in areas far removed from early detection sites indicated that this was a phenomenon independent from other areas, and that resistance was not merely expanding by migration of resistant flies from location to location.




With the expansion of resistance throughout the United States, other countries controlling horn flies and using pyrethroids were taking notice. Resistance was detected in Canada in 1987. Resistance of *H. irritans* to fenvalerate and permethrin was evaluated in Manitoba, Canada, from 1987 to 1989. The number of resistant populations and the intensity of resistance increased during the period. In 1987, resistant flies were observed on 3 of 18 cattle herds sampled. In 1989, resistant flies were found on 10 of 22 herds sampled. Resistance ranged from 1 to 62-fold for fenvalerate and 0.8 to over 100-fold for permethrin. Resistant populations were interspersed among susceptible ones (Mwangala and Galloway, 1993a). Discontinuing the use of pyrethroid-impregnated ear tags for one season was not long enough for substantial reduction in resistance to occur (Mwangala and Galloway, 1993b). In the early 1990s, officials in Mexico became concerned that they too might have horn flies resistant to the pyrethroids. In 1992 populations of horn flies were tested from several locations in northeastern Mexico in the states of Tamaulipas, Veracruz and San Luis Potosi. Resistance levels among field populations ranged from 36 to 199 times at the $LD_{50}$ level, confirming that horn fly control with pyrethroids was unsatisfactory (Kunz *et al.*, 1995). Since the early reports from Australia in 1982, follow up discriminating dose-data indicate that the buffalo fly is resistant to all synthetic pyrethroids tested (cypermethin, deltamethrin, cyhalothrin, flumethrin, cyfluthrin), and that resistance was common (Farnsworth, 1997). In central Queensland, where synthetic pyrethroid resistance in buffalo fly populations is rare, zeta-cypermethrin pour-on gave good control of buffalo fly for 4 weeks. In the far north of Queensland where resistance to synthetic pyrethroids and heavy rain is common, the maximum period of efficacy of zeta-cypermethrin pour-on was reduced to 2 weeks. It is concluded that in areas where there is low resistance to synthetic pyrethroids among buffalo flies, zeta-cypermethrin pour-on can be expected to give good control for 4 weeks (Rothwell *et al.*, 1998). Resistance to all synthetic pyrethroids tested (cypermethrin, deltamethrin, cyhalothrin, flumethrin and cyfluthrin) was common and widespread in coastal zones, but was lower in inland zones. In contrast, there was no resistance to the organophosphate diazinon, and only low levels of resistance to ethion and chlorfenvinfos. Synergism between piperonyl butoxide and cypermethrin was demonstrated (Farnsworth, 1997).

In the mid 1980s, as horn flies moved through Brazil and into Paraguay and Uruguay, researchers in Argentina became concerned when horn flies were first reported to have crossed the borders into their country in 1991. In the 1992/93 season, pyrethroid applications afforded 26 to 28 day control. In 1995, diagnostic studies in Argentina indicated decreased efficiency of

the pyrethroids and that pour-on treatments were unable to protect most cattle seven days after treatment. Studies indicated that resistance ranged from 16.8 to 36.5 percent (Guglielmone and Lanteri, 1999; Sheppard and Torres, 1998; Torres *et al*., 1996; Guglielmone *et al*., 1998). Recently completed studies showed populations resistant to cypermethrin. Resistance to this and other synthetic pyrethroids has also been confirmed in Columbia, Brazil and Uruguay. Recent reports from Chile also indicate resistance to these chemicals.

In the United States the ear tag technology has been indicated as the reason for the rapid build up of resistance. This is also true for the Canadian occurrence. However, the United States and Canada were the only countries using ear tags to any extent, yet pyrethroid resistance was also occurring elsewhere. In Mexico, Australia and South America, ear tags were virtually unknown. However, these countries all had *Boophilus* tick control programmes, which meant that pyrethroid insecticides were being applied periodically and in concentrations that led to fly resistance. In those countries with *Boophilus* tick problems, control efforts were generally aimed at tick rather than fly control. In Mexico and Australia, horn flies were present when ticks were treated, whereas in most of South America the horn fly was not present when pyrethroid treatments of ticks were first started. With the extensive exposure of fly populations to the tick treatment it was inevitable that resistance would result. The prior use of DDT in these countries probably meant that horn flies or buffalo flies were predisposed, and the responsible genetics quickly expressed resistance once treatment was initiated.

So when chemicals are applied in extensive dipping operations, as they are in areas having *Boophilus*, there is a real chance for resistance to build up. Regardless of the application method, resistance can develop as long as the insecticide is applied routinely. Continued, periodic use of insecticides for tick control among Mexican cattle producers maintained indirect selection pressure on horn fly populations, thereby increasing or maintaining resistance. The newer pyrethroids were more toxic and provided some extended control, but their usefulness was short lived. With the high degree of resistance demonstrated in many areas, even the newer generation pyrethroids will no longer be effective.

Cross-resistance of horn flies to other pyrethroids is well documented. With the continued use of pyrethroids against *Boophilus*, prolonged and increased resistance in horn fly populations can be expected. In areas where horn flies and *Boophilus* ticks co-exist, treatment of either one of the species can no longer be accomplished without affecting the other, especially if insecticides effective against both are used.

In summary, following the development of resistance in the United States and Canada, resistance to pyrethroids was recognized in Mexico in 1995 (Kunz *et al*., 1995). In Mexico, pyrethroids were primarily used for tick control in dip vats and as pour-ons but the horn fly was exposed and became resistant. In Argentina, resistance to cypermethrin was confirmed (Guglielmone and Lanteri, 1999; Sheppard and Torres, 1998; Torres *et al*., 1996). The exposure to insecticide was with pour-on formulations that were also being used for tick control. The resistance to pyrethroids documented in Australia were also primarily due to dip vat and pour-ons. Of all the countries now recording resistance, the United States and Canada were the only ones using ear tags and also the only countries not controlling *Boophilus* ticks as the primary pest. This fly-tick combination will play an important role in developing control strategies, especially if the same chemicals are used.

**Table 1a. Reports of existing literature data on resistance of *Haematobia irritans irritans*, *H. irritans exigua* and *Lucilia cuprina***

| Chemical | *H. irritans irritans* | *H. irritans exigua* | *L. cuprina* |
|---|---|---|---|
| **Pyrethroids** | | | |
| permethrin | Kaufman *et al*., 1999; Sheppard, 1994, Steelman *et al*., 1994; Mwangala and Galloway, 1993a; Guglielmone *et al*., 1998; Tozer and Sutherst, 1996; Bean *et al*., 1987; Kunz and Kemp, 1994; Schmidt *et al*., 1985. | Campbell, 1976. | |
| tetramethrin | Baron and Lysyk, 1995. | | |
| resmethrin | Baron and Lysyk, 1995. | | |
| cyhalothrin | Baron and Lysyk, 1995; Tozer and Sutherst, 1996; Cilek and Knapp, 1986. | Farnsworth, 1997; Steelman *et al*., 1997 | |
| cyfluthrin | | Farnsworth, 1997; Steelman *et al*., 1997; Farnsworth, 1997; Campbell, 1976. | |
| deltamethrin | Baron and Lysyk, 1995 | Farnsworth, 1997; Steelman *et al*., 1997; Farnsworth, 1997; Campbell, 1976. | |
| tralomethrin | Baron and Lysyk, 1995 | | |
| cypermethrin | Guglielmone and Lanteri, 1999; Farnsworth *et al*., 1997, Baron and Lysyk, 1995; Cilek and Knapp, 1986. | Farnsworth, 1997; Steelman *et al*., 1997 Farnsworth, 1997; Campbell, 1976. | Levot and Barchia, 1995. |
| flumethrin | | Farnsworth, 1997; Steelman *et al*., 1997 Farnsworth, 1997; Campbell, 1976. | |
| fenvalerate | Scott *et al*., 1997; Szalanski *et al*., 1995; Burg *et al*. 1995; Baron and Lysyk, 1995; Kunz *et al*., 1995; Sheppard, 1994; Mwangala and Galloway, 1993a; Tozer and Sutherst, 1996; Bean *et al*., 1987; Schmidt *et al*., 1985. | | |

**Table 1b. Reports of existing literature data on resistance of *Haematobia irritans irritans*, *H. irritans exigua* and *Lucilia cuprina***

| Chemical | *H. irritans irritans* | *H. irritans exigua* | *L. cuprina* |
|---|---|---|---|
| **Organophosphates** | | | |
| diazinon | Kaufman *et al*., 1999; Steelman *et al*., 1994; McKenzie and Byford, 1993. | | Wilson *et al*., 1996; Kotze *et al*., 1997; Levot *et al*., 1995; Guerrero *et al*., 1998. |
| coumaphos | Sheppard, 1994; McKenzie and Byford, 1993. | | |
| pirimiphos-methyl | McKenzie and Byford, 1993. | | |
| tetrachlorvinphos | McKenzie and Byford, 1993. | | |
| dioxathion | McKenzie and Byford, 1993. | | |
| ethion | | Farnsworth, 1997; | |
| chlorfenvinfos | | Farnsworth, 1997; | Levot *et al*., 1995; Guerrero *et al*., 1998. |
| diflubenzuron | | | Kotze *et al*., 1997. |
| dichlofenthion | | | Levot *et al*., 1995. |
| prometamphos | | | Levot *et al*., 1995; Guerrero *et al*., 1998. |
| **Organochlorines** | | | |
| dieldrin | | | Levot and Barchia, 1995 |
| **Miscellaneous** | | | |
| methoxychlor | McKenzie and Byford, 1993. | | |
| Macrocyclic lactones | | | |
| ivermectin | Kaufman *et al*., 1999; Steelman *et al*., 1994; Byford *et al*., 1999. | | |

# 4 DIAGNOSIS OF RESISTANCE: AN OVERVIEW OF METHODOLOGIES

Diagnostic techniques were quickly developed once resistance was first suspected. The first test was a treated-cloth technique that allowed for very accurate $LD_{50}$ and $LD_{90}$ determinations (Schmidt *et al*., 1985). This led to the development of the filter paper/Petri dish method by Sheppard and Hinkle (1987) which has now been accepted as the standardized test to determine both $LD_{50}$ and $LD_{90}$ probits and the discriminating dose (DD). The filter paper test has been validated in studies conducted in the United States, Canada, Mexico and Argentina. This technique has also been validated in studies in Australia against the buffalo fly (Farnsworth, 1997). This method is quick and easy to use in the field with acceptable repeatability. It does not require the feeding of the test insect during testing and can be used two to three times, making it possible to make multiple observations in the field with minimal time for determinations. Unfortunately the filter paper test is not suitable for organophosphate determinations. Instead, glass treated vials have been used successfully with diazinon as the primary test compound. The treated glass vial technique has been validated in the United States, Mexico and Argentina to determine both the $LD_{50}$ and $LD_{90}$ and DD values.

There is a growing need to monitor field populations of horn flies for resistance to pyrethroid and organophosphate insecticides due to the rapid development of resistant strains. A three-fold increase in the level of resistance can result in product failure (Schmidt *et al*., 1985). Several monitoring techniques have been developed to determine resistance in a field population:

- direct observations of wild populations;
- topical application bioassay;
- exposure to insecticide-treated cloths;
- exposure to insecticide-treated filters;
- exposure to insecticide-treated vials.

**Direct observation of field populations**

Observation of wild populations of horn flies is achieved by counting the flies on a randomly selected sample of cattle (at least 10 percent of the herd) and calculating the fly density of the herd based on observations taken prior to and periodically during an experiment. While an effective method of calculating horn fly populations within a herd, it is not an acceptable way to observe the quantitative differences in insecticide susceptibility. This requires direct exposure of the flies to a known quantity of insecticide. At the point when populations are observed to be increasing, resistance is already occurring.

**Topical application bioassay**

The topical application bioassay is an effective laboratory technique to determine the $LD_{50}$ of horn fly populations (Harris, 1964). This technique applies insecticide solutions to the thoracic dorsum of the horn fly using a microapplicator. Flies are then held in containers with mortalities being observed at specified times. While an effective bioassay, this is a time consuming technique and is not feasible to perform in the field. Flies must be collected and transported to the laboratory for treatment.

**Insecticide-treated cloth bioassay**

The insecticide-treated cloth bioassay was developed by Schmidt *et al* (1985). Unbleached 10 $cm^2$ cotton muslin cloths are laid on foil and treated with 1 ml of acetone solutions of the desired concentrations of insecticides. The acetone is then allowed to evaporate off before the cloths are wrapped in foil and held overnight. Flies are knocked down with $CO_2$ and held on a cold table for counting into plastic specimen cups. Cups are covered with the treated muslin cloths, which are held in place with cardboard lids with a large hole punched in the centre. Flies are fed by putting a 2 $cm^2$ cotton muslin cloth over the opening above which is placed a cotton pad soaked in Gatorade. Mortality counts are taken at specified times. Dose mortality is expressed in $\mu g/cm^2$.

**Insecticide-treated filter bioassay**

The insecticide-treated filter bioassay was developed by Sheppard and Hinkle (1987). No. 1 Whatman filter papers are laid on foil and treated with 1 ml of acetone solutions of the desired concentrations of insecticides. These filters are allowed to dry prior to being wrapped in foil and held overnight. Flies are knocked down with $CO_2$ and held on a cold table for counting into Petri dishes containing treated filters. Mortality counts are taken at specified intervals over a period of 2 hours. Dose mortality is expressed in $\mu g/cm^2$.

**Insecticide-treated glass vial bioassay**

The insecticide-treated glass vial bioassay was developed by Cilek and Knapp (1986). Glass scintillation vials of 20 ml capacity are treated with 0.25 ml of acetone solutions of the desired concentrations of insecticides. These vials are rolled under a fume hood until the acetone evaporates and they are then capped and allowed to cure overnight. Flies are knocked down with $CO_2$ and held on a cold table for counting into treated vials. Vials are loosely capped. Mortality counts are taken at 2 hours. Dose mortality is expressed in $\mu g/cm^2$.

**Requirements for efficient field test kit:**

- ease of use in the field;
- consistency of results;
- sensitivity of tests;
- portability of kit.

**Adaptations for field test kits**

Exposure to treated surfaces more closely mimics insecticide exposure under natural conditions and is more easily translated into a kit that can be taken to the field. The insecticide-treated cloth bioassay, insecticide-treated filter bioassay and insecticide-treated glass vial bioassay are easily adapted for the field. The cups and Petri dishes are adapted for field use by putting a hole in them, which is covered with tape. This hole is used for transferring flies to the container with the use of an aspirator or fly handling cage. Flies can be transferred to the vial by inserting the aspirator or fly handling cage tip into the vial and quickly capping. The cloth bioassay and the filter bioassay can be transported in an insulated ice chest while moving on to another test site, or they can be set up in the shade for 15 minute readings over the course of 2 hours.

A rapid screening procedure based on polymerase chain reaction (PCR) has recently been developed to test individual horn flies (*H. irritans irritans*). This is used to check for the presence of a specific nucleotide substitution in the sodium channel gene sequence that has been associated with pyrethroid resistance. By a systematic optimization of reaction conditions, and judicious choice of PCR primers differing in DNA sequence by a single nucleotide, the authors identified pyrethroid-susceptible or resistant sodium channel alleles in individual flies. Laboratory and field populations (from Texas, USA) were examined by both the PCR assay and conventional filter paper bioassays with the pyrethroid cyhalothrin. These were used to verify that populations containing greater proportions of individuals with the resistant sodium channel allele DNA sequence, also had higher bioassay $LC_{50}$ values. The PCR assay for resistance alleles gave definitive information on the genotype of an individual fly, and detected the presence of

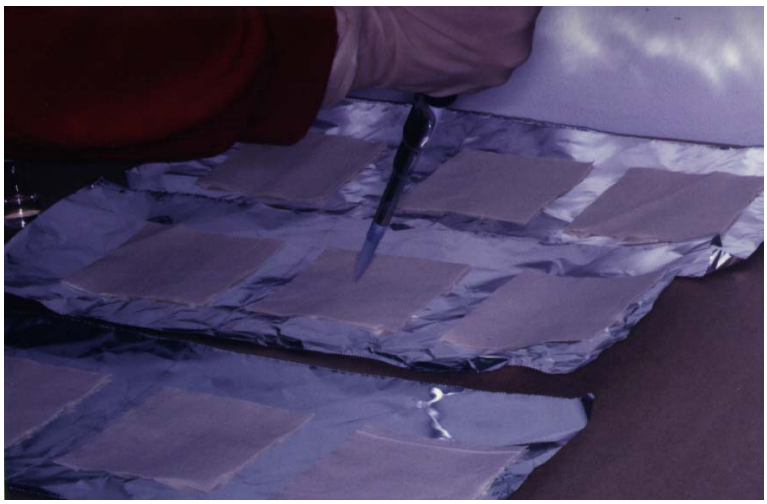
heterozygous individuals that might serve as reservoirs of resistance genes in field populations (Guerrero *et al*., 1998).

## 5 DETECTION OF RESISTANCE: PROTOCOL FOR RECOMMENDED METHODOLOGIES

The following protocols have all been used extensively and have been proven to fit the needs of resistance detection programmes. The procedures, as outlined, have been used to train personnel in a number of countries that are in need of this technology.

### Organophosphate treated cloth bioassay

Using the technique described by Schmidt, *et al*. (1985), unbleached 10 $cm^2$ cotton muslin cloths are laid on aluminium foil and treated with 1 ml of an acetone solution of each concentration of organophosphate (diazinon) applied with an automatic pipette. When the plunger is pressed slowly and the pipette moved from side to side or in a circular motion above the cloth, the liquid flows uniformly over the cloth.



Cloths are treated with a series of dilutions that includes a control (acetone only) and several concentrations of active ingredient, treating three cloths per concentration. The cloths are allowed to dry under the fume hood until the acetone has evaporated. Cloths are then wrapped in aluminium foil and held overnight.

In the laboratory, horn flies are immobilized with $CO_2$ and held on a refrigerated chilling table for counting.

Laboratory reared flies from 72 to 120 hours (3 to 5 days) old are used for testing. In field studies, these are compared to unsexed, mixed age flies collected from cattle. Flies are placed in the test containers with an aspirator.

Twenty five flies are placed in a 200 ml clear plastic specimen cup; the cup is then covered with the treated cloth, which is held in place with the use of a cardboard lid having a 2.5 cm diameter hole cut in the centre.

A 2 $cm^2$ piece of cotton soaked with citrated bovine blood or Gatorade is placed on a second patch of cloth (about 2.5 $cm^2$) on top of the treated cloth. The extra cloth prevents the blood or Gatorade from saturating the treated cloth and perhaps diluting the treatment. It also allows the fly to feed through the cloth as it would feed through the skin of a treated animal.

After 18 to 24 hours at room temperatures of 24 to 27°C, the immobile flies and those unable to crawl on the walls of the cup are considered dead and are counted.

The resulting data from this bioassay are then analyzed with the use of POLO-PC.

**Dilutions for the bioassay are determined as follows:**

A 1 percent stock solution is usually prepared using technical grade diazinon with acetone as a solvent.

The quantity of diazinon required for a particular concentration and area of cloth = concentration (expressed in µg/$cm^2$) × area of the cloth (100 $cm^2$) = *a* µg.

Applying this quantity at the rate of 1 ml/cloth (*a* µg/1 ml) will require (*b*) µg/ml

The amount in µg needed from the stock solution for a final total volume (*c*) of desired solution = (*b*) µg/ml × (*c*) ml = (*d*) µg

Using a 1% solution, (10 µg/µl**) = (*d*) µg/10 µg/µl = (*e*) µl of 1% solution in acetone with (*c*) ml total volume.

** 1% solution yields 10 µg/µl

0.1% solution yields 1 µg/µl

0.01% solution yields 0.1 µg/µl

Example:

Need to prepare 10 ml of treatment with a concentration of 2 µg/$cm^2$.

Quantity of material required = 2 µg/$cm^2$ × 100 $cm^2$ = 200 µg (*a*), which applied at 1 ml/cloth (200 µg/1 ml) = 200 µg/ml

200 µg/ml × 10 ml = 2000 µg

2000 µg/10µg/µl = 200 µl.

200 µl of 1% stock solution + 9.8 ml acetone = 10 ml of 2 µg/$cm^2$ treatment.

For weaker dilutions, it is easier to prepare the stronger dilution and then use serial dilutions to make a less concentrated solution. For example:

Need to prepare a 0.002 µg/cm$^2$ treatment.

First prepare 2 µg/cm$^2$ treatment as above, then dilute with acetone as follows:

1 ml of 2 µg/cm$^2$ + 9 ml of acetone = 10 ml of 0.2 µg/cm$^2$

1 ml of 0.2 µg/cm$^2$ + 9 ml of acetone = 10 ml of 0.02 µg/cm$^2$

1 ml of 0.02 µg/cm$^2$ + 9 ml of acetone = 10 ml of 0.002 µg/cm$^2$.

## Pyrethroid treated filter bioassay

With the technique described by Sheppard and Hinkle (1987), using disposable materials to evaluate horn fly insecticide resistance, 9cm Whatman® #1 qualitative filters are laid on aluminium foil and treated with 1 ml of an acetone solution of each concentration of pyrethroid, applied with an automatic pipette. When the plunger is pressed slowly and the pipette moved from side to side or in a circular motion above the filter, the liquid flows uniformly over the filter.

Filters are treated with a series of dilutions that includes a control (acetone only) and several concentrations of active ingredient, treating three filters per concentration. The filters are allowed to dry under the fume hood until the acetone has evaporated. Filters are then wrapped in aluminium foil and held overnight.

In the laboratory, horn flies are immobilized with $CO_2$ and held on a refrigerated chilling table for counting. Laboratory reared flies from 72 to 120 hours (3 to 5 days) old are used for testing. In field studies, these are compared to unsexed, mixed age flies collected from cattle. Flies are transferred to the Petri dish with an aspirator.

For exposure to the treatment, the treated filters are placed in the lid of an inverted Petri dish and the bottom is used as the lid.

Twenty five flies are placed in each Petri dish and mortalities are recorded every 15 minutes for a 2 hour period. The immobile flies and those unable to crawl are considered dead and are counted.

The resulting data from this bioassay are then analysed with the use of POLO-PC.

Dilutions for the bioassay are determined as follows:

A 1% stock solution is normally prepared using technical grade permethrin with acetone as a solvent.

Concentration desired (expressed in µg/cm$^2$) × area of the filter (63.6 cm$^2$) = (*a*) µg

Applying 1 ml/filter: (*a*) µg/1 ml = (*b*) µg/ml

Total volume of desired solution (*c*) = (*b*) µg/ml × (*c*) ml = (*d*) µg (amount in µg needed from stock solution)

Using a 1% solution, (10 µg/µl**) = (*d*) µg/10 µg/µl = (*e*) µl of 1% solution in acetone with (*c*) ml total volume.

** 1% solution yields 10 µg/µl

0.1% solution yields 1 µg/µl

0.01% solution yields 0.1 µg/µl

Example:

Need to prepare 10 ml of treatment with a dilution of 2 µg/cm$^2$.

2µg/ cm$^2$ × 63.6 cm$^2$ = 127.2 µg

127.2 µg/1 ml = 127.2 µg/ml

127.2 µg/ml × 10 ml = 1272 µg

1272 µg/10 µg/µl = 127.2 µl

127.2 µl of 1% stock solution + 9.87 ml acetone = 10 ml of 2 µg/cm$^2$ treatment

For weaker dilutions, it is easier to prepare the stronger dilution and then use serial dilutions to make a less concentrated solution. For example:

Need to prepare a 0.002 µg/cm$^2$ treatment

First prepare 2 µg/cm$^2$ treatment as above, then dilute with acetone as follows:

1 ml of 2 µg/cm$^2$ + 9 ml of acetone = 10 ml of 0. 2 µg/cm$^2$

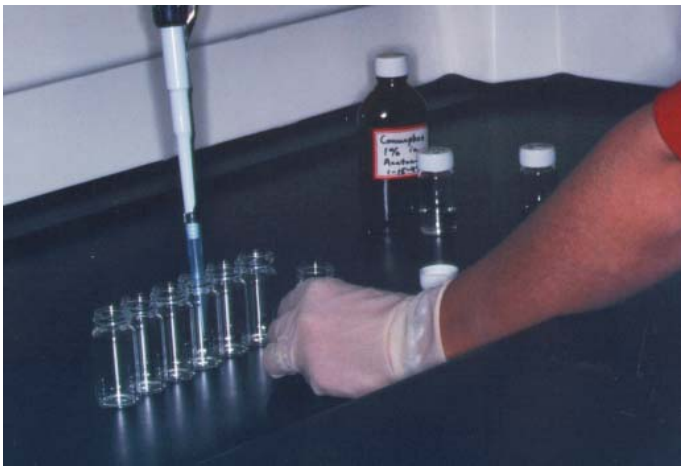
1 ml of 0.2 µg/cm$^2$ + 9 ml of acetone = 10 ml of 0.02 µg/cm$^2$

1 ml of 0.02 µg/cm$^2$ + 9 ml of acetone = 10 ml of 0.002 µg/cm$^2$

## Insecticide-treated glass vial bioassay

This bioassay is based on the technique developed by Cilek and Knapp (1986) to treat glass test tubes.

1. Glass scintillation vials of 20 ml capacity are treated with 250 µl of an acetone solution of each concentration of organophosphate or pyrethroid with an automatic pipette.



2. The vials are rolled under the hood until the acetone evaporates, allowing the chemical to coat the sides and bottom of the vials.

3. Vials are allowed to sit uncovered for one hour and are then tightly capped and stored overnight in a cool temperature and out of direct light.

4. Flies are put into the glass vials with an aspirator and lightly capped. Mortalities are recorded at one and two hour intervals. Dose mortalities are expressed in µg/cm$^2$

**Dilutions for treating vial bioassay**

A 1 percent stock solution is normally prepared using technical grade diazinon or cypermethrin with acetone as a solvent. Dilutions are prepared as follows:

Concentration desired (expressed in µg/cm$^2$) × area of the vial (40 cm$^2$) = (*a*) µg

Applying 0.25ml (250µl)/vial: (*a*) µg /0.25 ml = (*b*) µg/ml

Total volume of desired solution (*c*): (*b*) µg/ml × (*c*) ml = (*d*) µg (amount in µg needed from stock solution)

Using a 1% solution, (10 µg/µl**) = (*d*) µg/10 µg/µl = (e) µl of 1% solution in acetone with (*c*) ml total volume.

** 1% solution yields 10 µg/µl

0.1% solution yields 1 µg/µl

0.01% solution yields 0.1 µg/µl

Example:

You want to make a 0.0281 µg/cm$^2$ dilution to coat 40 cm$^2$ vials using a 1% solution in a total volume of 15 ml.

0.0281 µg/cm$^2$ × 40 cm$^2$ = 1.124 µg

Applying 250 µl (0.25 ml): 1.124 µg /0.25 ml = 4.496 µg/ml

4.496 µg/ml × 15 ml = 67.44 µg

Using a 0.01% solution (0.1 µg/µl): 67.4 µg/0.1 µg/µl = 674 µl of 0.01% solution in 15 ml total volume.

(Add 674 µl of 0.01% solution to 14.326 ml of acetone, which is done by placing 15 ml of acetone in a vial and removing 674 µl prior to adding the stock chemical).

From this, serial dilutions are made using a specified percent dilution.

***Making serial dilutions***:

Use the strongest desired concentration following the preceding actions to formulate this dilution. Then using a predetermined percent dilution, make serial dilutions by adding a given amount of the solution to acetone to mix decreasing concentrations:

Example:

Using the 0.0281 µg/cm$^2$ concentration formulated above, make 20% serial dilutions down to 0.0031 µg/cm$^2$.

Taking 0.0281 µg/cm$^2$ × 0.8 = 0.0225 µg/cm$^2$

0.0225 µg/cm$^2$ × 0.8 = 0.0180 µg/cm$^2$

0.0180 µg/cm$^2$ × 0.8 = 0.0144 µg/cm$^2$

…and so on until you reach the final concentration you desire.

From the 15 ml volume of 0.0281 µg/cm$^2$ made in the preceding section, we take 12 ml and add it to 3 ml of acetone in a second container making our 20% dilution at 0.0225 µg/cm$^2$, continuing in this manner until all the desired dilutions are made.

## 6 EPIDEMIOLOGY AND CONTROL

The epidemiology of insecticide resistance in *Haematobia* populations is basic knowledge necessary to develop population management programmes. Epidemiological surveys to determine levels of resistance and its distribution will indicate to what extent the currently available pesticides can be used. Knowledge about which pesticides remain effective and in which areas, will allow the development of use patterns for the specific insecticides. This database will be needed for both OP and SP chemicals. The diagnostic tests available and discussed elsewhere will be used to develop this information.

Biological and ecological information about the pest will be invaluable. Resources to collect the data may be more limited and difficult to obtain from many of the affected countries, however the scientific literature provides ample information to enable adequate programmes to be developed.

A most important consideration is the economic threshold, values for which are not available for most countries. However, the range of 200 to 300 flies per head can be used until local values can be established. The most difficult problem perhaps will be for a producer to determine the number of flies on his or her animals. It is most important that producers wait until flies are present and not start treatment before the populations increase.

Suppression should be the goal of a chemical control programme. Eradication of a horn or buffalo fly population is not feasible.

# 7 CURRENTLY AVAILABLE CONTROL STRATEGIES

The currently available tools for horn fly control consist of chemical technology, relying on treatments with different application methods and/or formulations of insecticides. These can be used with or without the benefit of local epidemiological knowledge.

Farmers and veterinarians commonly implement treatments against horn fly when high numbers of flies are present. Horn fly is primarily a summer problem. The impact of insecticide on the environment, on horn fly populations and as residues in food is a continuing concern. The dependence on chemicals to control horn fly and other parasites is under continual review.

Three control alternatives are in use:

ad hoc or opportunistic treatments;

threshold treatments;

strategic treatments.

## 1. Ad hoc or opportunistic treatments

***Principle:*** In conjunction with general management practices (weaning, dehorning, change of pasture or paddocks) farmers often implement routine preventive procedures and antiparasitic treatments. On some occasions this will involve using macrocyclic lactones that not only have insecticide effects but also anthelmintic activity as well.

***Prerequisites:*** Regular observation to avoid a sudden, uncontrolled level of infestation.

***Advantages:*** There is a reduced overall need for gathering animals, resulting in a reduced input of human and economic resources. Treatment with macrocyclic lactones (MLs) offers a bonus in that it will also provide some horn fly control.

***Disadvantages:*** Due to the life cycle of the horn fly, a single opportunistic treatment is likely to be inefficient and results are unpredictable when assessed solely from the point of view of immediate fly control. The effectiveness of this strategy in reducing fly populations is questionable as the interval between two opportunistic treatments is often too long to decrease the fly population in any area. The repeated or routine treatment with MLs would be expensive. Any treatment not warranted will only lead to increased chances of resistance.

***Epidemiological consequences:*** The effective, long-term reduction of the overall fly population is unlikely from a single application of a short-acting product.

***Possible combination with other strategies:*** Nothing specific.

## 2. Threshold treatments

***Principle:*** Farmers implement threshold treatments when some animals are infested with a high number of flies and/or whenever the risk of production losses and/or uncontrolled infestations is considered to be substantial. It is usual that farmers decide when animals should be treated according to their own estimates of economic thresholds. Most studies suggest that this is about 200 flies per head in the case of horn or buffalo fly.

***Prerequisites:*** Ability to monitor fly numbers on a regular basis.

***Advantages:*** The decision when to treat the animals is based on an objective parameter (number of flies per head). Therefore, it is relatively easy to implement on a farm if the counting technique is repeatable. The number of treatments will be reduced by applying a threshold level, thus delaying resistance, increasing safety to the host and the producer and reducing the threat of contamination of the environment.

***Disadvantages:*** Regular examination of cattle to implement treatments. There are no sound published data that define good economic thresholds for most areas of the world.

***Epidemiological consequences:*** Fly population is maintained below the economic threshold level.

***Possible combination with other strategies:*** No specific combinations.

### 3. Strategic treatments

***Principle:*** Farmers implement strategic treatments in the early summer, before there is an increase in number of flies, at the peak of fly infestation during mid summer and at the end of the fly season (late summer). Late-season treatment should be initiated before onset of over-wintering (60 days before the end of the fly season) to take full advantage of reducing over-wintering populations.

***Prerequisites:*** All animals should be treated. To avoid the introduction of new flies onto the farm, all recently introduced animals should also be treated.

***Advantages:*** Lower number of treatments. High level of control is possible.

***Disadvantages:*** There is a need for gathering all animals on the farm, resulting in an increased input of human and economic resources. Not effective for small farms where population dynamics of flies on neighbouring farms are as important or more important than those on the farm in question.

***Epidemiological consequences:*** There are likely to be effective reductions of fly populations.

***Possible combination with other strategies:*** No specific measures.

## 8 CHEMICAL CONTROL

Although resistance is present throughout the areas where *Haematobia* is distributed, chemicals remain the primary control tactic. Both the pyrethroids and organophosphates have a useful place in a control programme, provided that adequate epidemiological data are available. MLs are effective but expensive. Use of chemicals should be in conjunction with control strategies discussed subsequently. The continued use of a chemical in an area with resistance to that chemical will cause an even greater problem. The control of horn flies in countries having ticks also presents greater problems, because treating cattle for ticks with the same chemicals as used for horn fly control will increase the probability of resistance. Reduced chemical use should result in safer, environmentally friendly and more economic production.

An ideal insecticide should be economically acceptable, easily applicable and have good efficacy and sufficient residual effect to protect animals from re-infection. It should not select for resistance due to its gradual decay on the animal (i.e. it should have a sharp cut-off in efficacy with time). In addition, it should have a minimal toxicological effect on animals and humans with only minimal residues in meat and milk. Unfortunately, such an ideal insecticide has not yet been produced.

## 9 APPLICATION METHODS

### Plunge dips

Plunge dips remain one of the most efficient and reliable methods for routine insecticide applications at farm level. They are more commonly used in tick-infested areas. Use of plunge dips has been declining in recent years.

***Advantages:*** Animals are completely saturated and all parts of the body have adequate contact with the insecticide solution.

***Disadvantages:*** Problems with maintenance of the correct concentration of the insecticide are common. Elaborate installations for handling of animals are necessary. There can be environmental pollution from the run-off liquid when the animals emerge from the dip. The facilities are expensive to build. They are not appropriate for some insecticides (such as MLs). It is expensive to change insecticides.

**Wash/Spray**

Application of insecticides to cattle can be carried out using various modes of spray device, e.g. spray races or corridors, motorized pumps, backpack manual pumps. Some fly treatments require application to the full body, while others are applied as backline or oversprays.

***Advantages:*** If carried out correctly, animals receive more individual treatment; the amount of the insecticide applied is controlled, the concentration of the acaricide is adequate. The chemical group can easily be changed.

***Disadvantages:*** The animals are not always completely wetted, especially in the lower body parts, insides of the ears, etc. Animals must be appropriately secured during the operation. With the backpack manual pump, it is time-consuming and tiring for the operator. The use of manual spray pumps may well be the simplest method of insecticide application to animals, but not necessarily the most effective. Its effectiveness depends very much upon the operator's skills and the effectiveness of restraining the animals. There is the risk of environmental pollution. There is increased risk of intoxication to the operators and the animals. There are frequent problems with blocking of the spray nozzles. Spray runoff is a possible source of groundwater contamination.

**Pour-on treatments**

A volume of the drug proportional to the weight of the animals is applied along or on the dorsum of the animal. From here it dissipates over its body surface to kill or repel flies and depending on the residual active period of some SPs, also offers continuing lethal and repellent protection against subsequently arriving flies. In the case of ML compounds, the method permits the parasiticide to be absorbed and to act systemically.

***Advantages:*** Pour-ons are easy to apply. There is no need for expensive application equipment and there are fewer dose miscalculations. Environmental pollution is reduced. It is a very practical method, especially where no dip tanks are available, or in circumstances where the producer wishes to avoid dipping some of the infested animals (e.g. pregnant females or when just a few animals need to be treated, etc.). Some of the SP compounds can be applied with this formulation. New formulations of MLs and other compounds are being introduced that employ this method of application and they offer an alternative for the control of pyrethroid resistant strains of cattle fly.

***Disadvantages:*** The higher cost of these new compounds may be an initial limitation for many farmers in the developing countries. High concentrations of the applied chemicals are needed for good efficacy. Possible residues of such products in milk restrict their use in dairy animals. A confinement area (chute) is needed.

**Injectable formulations**

This is another practical alternative to avoid dipping or spraying of animals with insecticides. Most of the injectable products currently on the market are MLs.

***Advantages:*** Easy to treat selected animals, little chance of soil or water contamination. There is reduced environmental pollution, except possibly in the dung pats where non-target species may be affected.

***Disadvantages:*** Possible residues of such products in milk restrict their use in dairy animals. In general terms, these compounds are more expensive than the other alternatives. Conducive to cross resistance with helminth infections.

**Ear tags**

This is another practical alternative to avoid the dipping or spraying of animals with insecticides.

***Advantages:*** Ear tags are easy to apply and provide long-term control. There is no environmental pollution. There is a broad spectrum of action, not only against horn flies but also lice.

***Disadvantage:*** Tags need to be removed from the animal at the expiry date. They are resistance inducing. In general terms, these compounds are more expensive than the other alternatives.

**Dust bags/Oilers/Back-rubbers**

Dust bags containing carbaryl powder and oilers, consisting of a cloth wick drawing from a drum of insecticide in oil solution are a means of self-treatment for cattle. They can be located in strategic positions such as in the laneway to the milking parlour and enable cattle frequent access.

***Advantages:*** These application methods have very low labour requirements.

***Disadvantages:*** Self-treatment means that dosage is not controlled, with wide variation in insecticide concentrations between animals. Aside from resistance management issues, this is a problem for the management of insecticide residues in meat and milk. Use of recycled motor oil presents the problem of contamination with heavy metals and other toxins.

**Larvacides**

Chemicals such as methoprene that are administered orally to cattle and are effective in the faeces against the immature forms of the horn fly.

***Advantage:*** Easy to apply as feed additive. Chemicals can be added to feedstuffs or to salt and mineral stock. These chemicals belong to a different group, thus providing a true alternative product, against which resistance has not been demonstrated. Boluses are expected to provide longer term treatment.

***Disadvantage:*** Head restraints are needed for bolus treatment. If larvicide is combined with adulticide, it could result in double exposure to chemical. Adding chemical to feed means daily feeding to maintain the levels of toxicity necessary for effectiveness. Salt mixes are not effective where animals have access to natural salt deposits, as consumption is erratic and insufficient.

## Other considerations

Fortunately there are no serious disease considerations with the control of *Haematobia*. It is important that fly control programmes be coordinated with tick control activities when the same chemical or chemical group is being used for both species. It has been demonstrated that the treatment for one pest can result in resistance in the other.

## Treatments available for pyrethroid resistant flies

Coumaphos

Diazinon

Ethion

Pyrethroids – some areas where they are still effective with extreme care.

Chlorfenvinphos

Avermectins

Methroprene

Ivermectin pour-ons will provide 21 to 28 days effective treatment. Because of the habits of the fly (movement from animal to-animal) on the host animal, a full treatment dose on 50 percent of the herd repeated 30 days later on the remaining 50 percent will provide almost 60 days control. This results in full treatment of the internal parasites in all animals and doubles the fly control time. This is preferable to a half dose on all animals at a 30-day repeat, which will neither provide internal parasite nor fly control.

## 10 NON-CHEMICAL CONTROL

No non-chemical control methods for *Haematobia* are in common use at present. Parasites and predators against *Haematobia* have not been shown to be effective, although the natural competition occurring during the development of the horn fly in the manure pat accounts for upward of 90 percent reduction when that competition is artificially eliminated. In Australia there is considerable interest in the use of dung beetles for fly control.

A non-chemical tactic that has been used to some extent is fly traps, which have been developed and/or reintroduced. An Australian trap used in Florida, eliminated the need to use insecticides to control this pest on milking dairy cattle (Tozer and Sutherst, 1996). This method has not been used extensively in most horn or buffalo fly areas. There have been past problems with the manufacture and maintenance of these traps. A functional maintenance-free trap offers a practical, environmentally acceptable, safe and sustainable means of fly control. No doubt as chemical methods fail, this technology will be perfected and brought into full use.

Example of a fly trap used for dairy cows when they are gathered for milking

## 11 CONTROL STRATEGIES UNDER DEVELOPMENT

Other technologies have been identified as possible developments but they are high risk, expensive projects that are receiving little research support. Some of these are introduced here.

Recognizing that some animals carry fewer flies, studies have been undertaken to examine some of the physiological characteristics of the host animals to try to determine the cause. With the possibility that fewer effective insecticides will be available and pesticide resistance will increase, these areas of study could play an important role in the future of fly control and resistance management. Animals with high hair density and sebum levels were found to carry lower horn fly populations (Steelman *et al*., 1997). In antibody response studies, horn fly salivary antigen may have an immunomodulating effect on the host (Baron and Lysyk, 1995). Optical density of plasma and electrophoresis of plasma proteins from beef cows were found to be related to *Haematobia irritans* densities, and these parameters may provide a relatively inexpensive physiological "marker" of genetic resistance to horn flies (Tarn *et al*., 1993). Plasma characteristics (optical density, cortisol, and protein pattern) were studied in beef cattle classified (cow-type) as horn fly resistant or susceptible. When the ratio of area percentage for protein bands seven and nine (Mr 74 000 and 54 000, respectively) was determined, cows could be categorized as horn fly resistant or susceptible. It is suggested that a serological marker will need to be tested on a larger population of cattle (Tarn *et al*., 1994). The efficacy of Brahman (Zebu, *Bos indicus*) breeding, used as an alternative tactic to manage insecticide-resistant populations of adult horn flies, was determined. Mean horn fly counts on Brahman cows were significantly lower than on Angus cows. Mean fly counts on Brahman × Angus cows were approximately intermediate between those of the two purebreds. Brahman cross-breeding caused significant reductions in the number of organophosphate resistant flies, which had been equal to or greater than that obtained from continued spraying with OP insecticides. The Brahman × Hereford cows, which have one eighth greater Brahman breeding than the Brangus cows, had fewer horn flies on 48 out of 56 sampling dates, and significantly fewer flies on 37 sampling dates. The effectiveness of Brahman cross-breeding in causing lower numbers of insecticide-resistant horn flies, increased as the percentage of Brahman breeding increased (Steelman *et al*., 1994). The resistance of Brahman-cross cattle to fly infestation has not been widely used so far.

This new information could lead to the breeding of cattle genetically resistant to flies, or to the development of vaccines which would render the animals resistant to flies.

## 12 RESISTANCE MANAGEMENT AND INTEGRATED CONTROL

The frequency of resistance in a pest population is largely a result of selection pressure from pesticide use. Strategies to manage resistance are aimed at reducing this pressure to a minimum while still achieving control. Recommended strategies involve the use of tactics designed to increase the useful life of a pesticide and decrease the interval of time required for a pest to become susceptible once more to a given pesticide (Kunz *et al*., 1994).

Some of the most important issues which impinge on the development and selection of management tactics to prevent, delay or manage resistance to pesticides are as follows:

- biology of the pest, and type of pesticide and application used;
- dynamics of resistance;
- lack of supporting data and field validation of data.

The rate at which pesticide resistance develops is extremely variable between species (Kunz and Kemp, 1994). Selection with permethrin, diazinon and ivermectin resulted in development of resistance in generations 21, 31 and 30, respectively (McKenzie and Byford, 1993). Such factors as the rate of reproduction, pest movement and relative fitness of resistant members all contribute to the dynamics of resistance. These elements all influenced the development of resistance in horn flies in the United States. Horn flies can develop from egg to adult in as little as ten days. Generations are therefore short, and numerous per season, especially in the southern horn fly belt (Kunz *et al*., 1994). Further north, the climate induces longer developmental cycles, fewer

generations and slower build-up of resistance. The ability of flies to travel long distances – either by flight or using human transport vehicles – contributes to the spread of resistant genes into susceptible populations. In areas where resistance occurs, the presence of these immigrating resistant individuals must be considered when planning effective resistance management (Sheppard, 1994). Conversely, these flight characteristics can also help susceptible genes move into resistant populations. A difference in the relative fitness of resistant flies has shown that the reproductive potential of resistant house flies is lower than that of susceptible insects. Resistant horn flies pupated significantly less successfully, and their rate of adults produced per 100 eggs laid was significantly less than that for susceptible flies. The relative biotic potential of the resistant strain was 0.57, and it developed more slowly than the susceptible strain. Resurgence of resistant populations may be forestalled by treating with pyrethroids only when economic thresholds are reached, and by seasonal alteration of pyrethroids with insecticides that have different modes of action (Scott *et al*., 1997). In the presence of treatment, susceptible flies may be killed, but reproduction of resistant flies continues at sufficient rates to maintain a pool of resistant genes in the population. Resistance development in a no-pyrethroid-use area indicated that movements by sufficient numbers of horn or buffalo flies can significantly change the RR (Resistance Ratio) (Sheppard and Joyce, 1992).

Resistance is not absolute throughout the range of a pest, and susceptible populations of some pests continue to exist. Furthermore, in an area where resistance has occurred, continued use of a pesticide may be required to control other pests which remain susceptible, such as face flies (*Musca autumnalis* De Geer) in the United States or the bush fly (*Musca vetustissima* Walker) in Australia. This could confound attempts at pest management. The same could also be true in crop production areas. The specific effect of aerial applications (drift) of pesticides on crops is not known with regard to horn fly resistance. It has been shown that aerial application of pesticides to certain crops may cause or increase resistance in mosquitoes (Kunz and Kemp, 1994). Once resistance has developed, low-level exposure resulting from crop applications may be sufficient to maintain the resistance level.

In order to safeguard the utility of the chemicals that are currently available for pest control, rational pest control strategies are needed to manage resistance. These strategies must be designed to both prolong the effectiveness of current pesticides and reduce the environmental impact of their use. Strategies should be based on integrated pest management (IPM) techniques which exploit the biology of the pest being controlled. It remains vital to pursue the development of new chemicals that are effective through new modes of action, although this increases producer costs. However, pests can be expected to evolve strains that are also resistant to various new control agents. It is therefore paramount that practitioners using pesticides apply these materials judiciously to maintain the stock of pest control options in the future.

Although there is significant resistance in horn and buffalo fly populations to the pyrethroids, there is evidence that the organophosphates continue to offer adequate protection in most locations. In Mexico, where coumaphos was extensively used in dip vats prior to pyrethroids, there seems to be little evidence of widespread resistance, albeit in limited studies. Good susceptibility of diazinon is also demonstrated in field trials. This same situation is confirmed in Argentina and Brazil where susceptibility tests show field populations to be fully susceptible. In the United States diazinon remains effective for the most part. There have been some reports of resistance and these are being fully evaluated. Currently, United States Department of Agriculture (USDA) researchers in Texas are evaluating data which indicate that flies from at least one location in Oklahoma may be quite resistant. This population of horn and buffalo flies has been exposed to diazinon ear tags since 1987. It is understood that the control of ear ticks was the primary reason for treatment. Again we appear to have resistance in a species not the primary target and with many more generations per year than the ear tick.

The use of insecticides by cattle producers to control ticks or flies needs be carried out on a more strategic basis. The key to successful management of *Haematobia* populations is ECONOMIC THRESHOLD. Economic threshold levels of infestations rather than a calendar date should dictate when cattle are treated. Cattle should be inspected for ticks and flies. If the pest populations are below economic thresholds, then insecticide treatments should not be made. This could lead to a reduction in the use of chemicals. This will reduce production costs and decrease the environmental and safety concerns for the cattle, the livestock producers and the consumers.

So, as few new compounds are being introduced and labelled for use, the organophosphates are the primary pesticides available for fly control. Despite the fact that there is a shortage of pesticides, there are pest management strategies that can be implemented to aid in the control of horn or buffalo flies.

Since the frequency of resistance is a result of selection pressure, strategies to manage resistance aim to reduce this pressure to the minimum. Recommended strategies involve the use of tactics designed to prolong the useful life of a pesticide.

## Strategies to be used in managing horn and buffalo fly resistance

Strategies can be used singly or in combination with other strategies (Kunz *et al*., 1994).

**Do not treat for horn flies**

This is the harshest of all strategies but the one that would have the greatest influence in reducing resistance levels. Other strategies will allow for periodic treatment to provide relief to cattle.

**Separate mature animals from growing animals, if possible**

There is no evidence to suggest that horn flies significantly affect the mature animal unless it is producing milk for a calf. Flies are a nuisance and might cause the animal to lose some condition, but condition will be regained quickly when the fly season is over. We have no data on the effects of heavy fly populations on the breeding performance of either the dam or sire. An animal in "weight gain" mode should be treated to obtain the most efficient use of its resources.

**Do not treat mature animals without calves**

See comments under "Do not treat for horn flies."

**Delay control tactics until flies exceed an acceptable threshold level**

There are no sound published data that define good economic thresholds for most areas of the world. In areas of the southern United States, population levels of 200 flies/head have generally been considered as the control level. This number may be reduced in more northern areas such as Canada. Pure-breed producers or producers with animals maintained for "show" may require absolute control for short periods of time. A level of 200 flies/head is acceptable on mature range animals not requiring special cosmetic consideration.

**Treat periodically with organophosphate (OP) sprays or dust to reduce early fly population build-ups**

Any periodic treatment providing high initial control, and then a period of no control during build-up of the population, would help to delay or reduce resistance. One or two of these treatments can significantly delay the time until more sustained control procedures are initiated. In areas of moderate fly numbers this may well keep populations below the threshold numbers. If significant resistance to a particular pesticide exists, do not treat with this pesticide at any concentration in any formulation.

**Continue with periodic treatment or apply OP ear tags, dust bags, oilers or sprays**

If possible, periodic spray treatments or the use of dust bags or oilers (also known as back-rubbers) using OP chemicals would be best, but if management practices require, OP ear tags can be applied. If OP tags have been used for two years, the bolus treatment is suggested as the 90 day "height of season" fly control method. This treatment would provide control of any OP resistance that might be occurring in the population. Also, this treatment does not interfere with indigenous or immigrant adult susceptible flies on the host.

**Remove tags late-season before onset of diapause**

Removal of the spent tags will ensure that populations are not receiving sublethal doses, which increase the chance of resistance. Diapause (over-wintering stage) dates will vary and may not be established for some areas.

**Peak-season bolus treatment if available**

The bolus treatment delivers a pesticide that has a mode of action different from either the pyrethroids or organophosphates. Also, the bolus is active against the immature stages and does not interfere with the cross mating of resistant and susceptible adults. The bolus treatment provides control of resistant populations yet allows the full effect of any cross mating, thus reducing the resistance levels in any survivors. The bolus treatment provides two additional chemistries that could well be used in a resistance management programme.

**Late-season treatment**

Late-season treatment should be initiated before the onset of diapause (60 days before end of fly season) to minimise over-wintering populations. If late-season control is required, treat periodically with carbamate or OP sprays or dust, or provide alternative bolus treatment. Alternative bolus treatment (alternative chemistry) will reduce the genes which have developed resistance to OP use. If pyrethroid and/or OP resistance is present, the third chemistry will not reduce the percentage of resistant genes. It will, however, reduce the number of resistant flies going into diapause. The over-wintering fitness of resistant flies is unknown, but a reduction in over-wintering numbers could delay onset of treatment the following spring.

**Eliminate late-season control**

If populations are below the economic threshold - don't treat.

Note: In a non-resistant area for pyrethroids, pyrethroid ear tags can still be used. Even if pyrethroid tags are still effective, serious consideration should be given to either an OP tag or a bolus treatment for the peak fly season.

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# MODULE 4. MITES: ACARICIDE RESISTANCE: DIAGNOSIS, MANAGEMENT AND PREVENTION

## 1 INTRODUCTION

Scabies (mange) has remained for centuries a disease of economic importance affecting animal production and welfare. Most types of mange are forms of allergic dermatitis, characterized by encrustation, alopecia, and pruritus, initiated and maintained by a number of mite species. All the major mange mite species are contained within the orders Astigmata and Prostigmata. The Astigmata are a well-defined group of slow-moving, weakly sclerotised mites, including the medical or veterinary important families Sarcoptidae and Psoroptidae. The Prostigmata include the Trombiculidae (harvest mites), parasitic as larvae but free-living predators in the nymphal and adult stages, and the true mange mite families Cheletoidea (*Psorobia* (*Psorergates*) sp.), Demodicidae (*Demodex* sp.) and Cheyletiellidae (*Cheyletiella* sp.). The latter (being parasites of companion animals) are of no direct significance to livestock production. Worldwide losses from mange mites on livestock production have been estimated to amount to US$14.4 million (Drummond *et al*., 1981).

**Psorobic mange**

Two species of *Psorobia* have been isolated from domestic animals: the benign 'parasite' *P. bos* from cattle and the more important *P. ovis*, occurring in Merino sheep in Australia, South Africa and South America. Most mites are found under the stratum corneum in the superficial layers of the skin of the sides, flanks and thighs, feeding on the exuding fluid. The infested area is dry and scurfy; wool fibres break easily, with the remaining wool coming together as ragged tufts. Irritation causes the sheep to rub and kick the affected area and chew its fleece, resulting in "fleece derangement" and downgrading of the wool clip.

**Demodectic mange**

*Demodex* are easily recognized by their annulate, vermiform ("worm-like") shape, but may be overlooked on account of their small size. *Demodex* inhabit the hair follicles and the sebaceous and meibomian glands of the skin of a number of wild and domesticated mammals, including humans. Different species occur on different hosts, and more than one species may occur on the same host. Two species have been isolated from sheep. *D. aries,* is a benign commensal of the follicles and sebaceous glands of the feet, face, eyelids, ears, prepuce and vulva and *D. ovis* lightly parasitises the hair follicles and sebaceous glands of primary hairs over the entire body, with highest populations occurring on the neck, flanks and shoulders. Infested follicles become distended with mites, mite exuvia, eggs and epithelial cells, forming nodules, and pyogenic bacteria convert these nodules into pustules. Skin with advanced lesions is thick and scaly, alopecic, nodular or pustular. Itching may stimulate kicking, biting and rubbing of the lesions. In general the disease is of low incidence and of little importance (Desch, 1986). On cattle, demodicosis occurs as flat nodules in the skin with a massive enlargement of the sebaceous glands, which contain vast numbers of *Demodex* mites. Demodicosis (*D. bovis*) was recorded as the most common skin defect in Kenyan, Tanzanian and Ugandan cattle hides (Bwangmo, 1969).

**Sarcoptic mange**

Mites in the family Sarcoptidae are obligate parasites, burrowing into the skin of mammals. The itch mite (*Sarcoptes scabiei*) is the cause of scabies in humans and mange in a wide range of domestic and wild mammals throughout the world, generally affecting the sparsely haired parts of the body. The number of species within the genus is still open to debate. Studies of populations of *Sarcoptes* mites from a wide range of hosts have suggested that there is only one type species

(*Sarcoptes scabiei*) with a number of variants infesting a wide range of mammalian hosts (Fain, 1968). Recent investigations based upon molecular analysis of the ITS-2 of the mRNA gene suggest that the genus *Sarcoptes* is monospecific (Zahler *et al*., 1999).

*S. scabiei* var. *suis* is one of the most important skin diseases in pigs worldwide, with reported losses in the United States estimated at US$200 million per annum (Hogg, 1989). Sarcoptic mange is endemic in pig herds throughout the European Union and a number of member states have active eradication programmes (e.g. Belgium, Denmark and Holland) and according to the Swedish Animal Health Service, pigs sold as fatteners must be certified free from sarcoptic mange. In 1991 sarcoptic mange was identified in 27.9 percent of British breeding herds and 67 percent of finishing units (Anonymous, 1989a), with the majority of cases sub-clinical and restricted to the inside of the pinnae. Sarcoptic mange is likely to be present in most herds unless derived from specific pathogen free (SPF) sources (Dobson and Davis, 1992).

*S. scabiei* var. *bovis* affects cattle world-wide with infestations generally located at the base of the tail, the inner thigh, under the neck and the brisket. Although disease is generally sub-clinical in the United Kingdom, generalized infestations can occur (Bates, 1997). In sheep, sarcoptic (head) mange, caused by *S. scabiei* var. *ovis* has been recorded in Europe, Africa, the Middle East, the Balkans, India and South and Central America (Bates, 2000a). *S. scabiei* var. *ovis* is found on the sparsely haired parts of the body, such as the face and ears. Mites burrow into the epidermis and feed on tissue fluids. The burrowing and feeding of the mite causes irritation and consequential scratching, leading to inflammation and exudation to form crusts. Small foci of infection do not appear to affect the health of an animal adversely but can be more serious if the condition spreads. *S. scabiei* can temporarily infest humans (Bates, 2000a).

## Family Psoroptidae

Mites in the family Psoroptidae are oval, non-burrowing mites, parasitic on mammalian skin. Three genera, *Psoroptes*, *Chorioptes* and *Otodectes* are of veterinary importance, although the latter (being a parasite of the ears of carnivores) is of no direct significance to livestock production.

### Chorioptic mange

Mange caused by species of *Chorioptes* is more localized and often asymptomatic and is therefore not as serious as that caused by *Sarcoptes* or *Psoroptes*. Reservoirs are found on the fore and hind pasterns (Baker, 1999) and from these areas mites can spread to the rest of the body.

Two species of *Chorioptes* are recognized: *C. bovis*, infesting cattle, goats, horses, sheep and rabbits and *C. texanus* recorded on goats, reindeer and cattle (Rosen *et al*., 1989; Sweatman, 1957). In cattle, chorioptic mange commonly occurs on the base of the tail, the perineum, and the back of the udder. The hooves may also be affected, resulting in lameness. Heavy infestations can cause loss of condition, which can lead to emaciation and damage to hides (Walker, 1994).

*Chorioptes* infestations of the major breeds of cattle in the United Kingdom (Hereford, Holstein Friesian, Jersey, etc.) are generally asymptomatic, but the mite can be a cause of extensive mange on continental cattle breeds (Limousine, Charolais, etc.), particularly in bulls. This form of mange is an extreme form of allergic dermatitis and the gross symptoms can easily resemble bovine psoroptic mange. Symptoms include a thick crusty scab and intense irritation. The scab itself may affect the efficacy of synthetic pyrethroid pour-on acaricide formulations, by acting as a sponge and absorbing the formulation at the site of application, thus preventing translocation around the body (Bates, 1997).

A low incidence of foot and scrotal mange due to *C. bovis* has been recorded in Australian and New Zealand sheep. In the late 1960s the parasite was found to have infested the pasterns of

sheep in the United Kingdom (Bates, 2000a) and was thought to have been eradicated following 18 years of compulsory dipping for sheep scab (*P. ovis*), but the parasite has recently been recorded as the cause of scrotal mange on Suffolk rams (Sargison *et al*., 2000).

**Psoroptic mange**

Although *Sarcoptes* and *Chorioptes*, and to a lesser extent *Demodex* and *Psorobia*, can be regarded as having significant effects on animal production (particularly *Sarcoptes* on pig production), *Psoroptes ovis* is by far the most damaging and cosmopolitan mange mite.

*P. ovis* are non burrowing, cosmopolitan, obligate ectoparasites, causing a debilitating dermatitis, involving hair or wool loss and a pruritic scab formation. The parasite occurs in all the sheep and cattle rearing countries of the world, although it was eradicated from Australia and New Zealand towards the end of the nineteenth century (**Table 1**). Infestations on sheep (sheep scab) can be a cause of considerable suffering and even mortality within infested flocks. Flock productivity can be severely affected, either directly through reduced lamb crops or downgraded wool or leather, or indirectly through the use of expensive chemical control programmes with their associated withholding periods for meat, milk or fleece. In Argentina, psoroptic mange is the most damaging ectoparasitic disease affecting domestic livestock. In 1989 the estimated annual losses ranged between US$100 million in cattle and US$150 million in sheep (Nuñez, 1989).

**Table 1. Status of sheep scab throughout the world**

| Country | Date eradicated | Date returned |
|---|---|---|
| Argentina | Never eradicated | |
| Australia | 1896 | |
| Austria | Never eradicated | |
| Brazil | Never eradicated | |
| Canada | 1927 | |
| Denmark | 1929 | 1979 |
| France | Never eradicated | |
| Germany | 1948 | 1973 |
| Hungary | 1965 | 1978 |
| India | Never eradicated | |
| Iran | Never eradicated | |
| Lesotho | 1935 | 1973 |
| New Zealand | 1885 | |
| Norway | 1894 | |
| Republic of Ireland | Never eradicated | |
| Saudi Arabia | Never eradicated | |
| South Africa | Never eradicated | |
| Swaziland | Never eradicated | |
| Sweden | 1934 | |
| Uruguay | Never eradicated | |
| United Kingdom | 1953 | 1973 |
| United States of America | 1973 | |

Five species of *Psoroptes* are recognized (Sweatman, 1958): *P. ovis*, a body mite causing mange in sheep, cattle and horses, *P. equi*, a body mite of equids, *P. natalensis* a body mite of cattle and horses, *P. cuniculi* the ear mite of rabbits, goats, horses and sheep and *P. cervinus* an ear mite of bighorn sheep, elk and wapiti. A sixth, invalidated, species is *P. auchinae*, an ear mite of new world camelids. Like the genus *Sarcoptes*, the numbers of species in the genus *Psoroptes* is open to debate, *P. ovis* and *P. cuniculi* may be variants of the same species (Bates, 1999a).

***Psoroptes on rabbits***

Ear canker, caused by *P. cuniculi*, is a common disease of domestic rabbits throughout the world. In Egypt mange (sarcoptic or psoroptic) in rabbits is considered to be second to coccidiosis in importance, with high losses reported (Ezzat, 1955). In Great Britain the parasite is extremely common in pet rabbits and commercial rabbit colonies, either for meat or laboratory rabbit production. Infestations appear to be confined to domestic rabbits. Surveys of ectoparasites of wild rabbits in Great Britain (Bates, 1999b) and Australia (Mykytowycz, 1957; Williams, 1972) have not recorded *P. cuniculi*, but it is not clear whether the Australian surveys included an examination of the ears (Strong and Halliday, 1992). The only report of psoroptic otoacariasis occurring in wild rabbits was in France (Guilhon, 1990). Lesions are usually confined to the ear canal or internal aspects of the pinnae but infestations can spread out of the ear canal to produce extensive, clinical lesions of the entire pinnae, the base of the ears, the cheeks, dewlap and face. Lesions and mites can also be present between the digits of both hind feet (Bates, 1999b). Secondary infections have been recorded, including otitis media and otitis internal, torticollis, ulcerous meningocephalitis accompanied by abscessation of the medulla oblongata region and interference with the central nervous system (Von Ribbeck and Ilchmann, 1969). Changes in the tympanum of infested rabbits attributed to *P. cuniculi* and mites have actually been seen in the immediate vicinity of the brain of an infested rabbit (Von Ribbeck and Ilchmann, 1969).

***Psoroptes on goats***

*P. cuniculi* (synonym *P. caprae*) has been reported in Australia (Roberts, 1952), Bangladesh (Nooruddin and Mondal, 1996), Brazil (Faccini *et al*., 1981), Canada (Lofstedt *et al*., 1994), Fiji (Munro and Munro, 1980), India (Shastri and Deshpand, 1983), Israel (Yeruham *et al*., 1985), Italy (Perrucci *et al*., 1996), New Zealand (Heath *et al*., 1983), South Africa (Shilston, 1915), Sudan (Abu Samra *et al*., 1981), British Isles (Littlejohn, 1968; Bates, 2001), United States (Williams and Williams, 1978) and Zimbabwe (Odiawo and Ogaa, 1987). Most infestations are subclinical, asymptomatic (other than the occasional episode of ear scratching with the hind feet) and are easily overlooked (Bates, 2001; Schillhorn van Veen and Williams, 1980). *P. cuniculi* has been isolated from the external ear canals of feral goats in Australia and New Zealand (Heath, 1979; McKenzie *et al*., 1979; Hein and Cargill, 1981). Ovine psoroptic mange (sheep scab) is not endemic to either Australia or New Zealand and *P. cuniculi* in the ears of goats are not therefore considered a reservoir of infestation.

In meat and dairy goats infestations are usually confined to the ears. Transmission can occur between mother and offspring as early as five days after birth (Heath *et al*., 1989) and is a function of age, with the highest infestation in animals between 6 and 12 months old (Bates, 2001). Infestations are generally confined to the external auditory canal, which can be plugged with thick, brown, laminated scab, close to the tympanic membrane (sometimes completely occluding the canal) although no damage to the tympanic membrane has been observed at post mortem (Williams and Williams, 1978; Odiawo and Ogaa, 1987). The waxy plug deep within the external auditory canal contains *Psoroptes* mites of all stages. Infestations (often classified as *P. caprae*) have also been recorded to involve the entire pinna, or spread to form body lesions, involving the poll, neck, withers, back, abdomen, pasterns and inter-digital spaces (Lofstedt *et al*., 1994; Munro and Munro, 1980; Littlejohn, 1968). *P. cuniculi* has also been shown to be capable

of carrying mycoplasmas (possibly pathogenic) between goats (Cottew and Yeats, 1982; Da Massa, 1990).

In Brazil the prevalence of infestation and number of mites per host were higher in goats than in sheep (Faccini and Costa, 1992). There is a possibility that *P. cuniculi* may not be host specific and may freely transfer between the ears of sheep and goats, given the correct set of circumstances (Williams and Williams, 1978; Sweatman, 1958). There is little evidence for the goat strain of *P. cuniculi* causing clinical sheep scab. Artificial and natural exposure of sheep to *P. cuniculi* infested goats has never resulted in classical sheep scab (Williams and Williams; Heath *et al*., 1989; Sweatman, 1958). This is supported by the fact that *P. cuniculi* is common in domestic and feral goats in Australia, New Zealand and the United States (Roberts, 1952; Heath *et al*., 1983; Williams and Williams, 1978; Schillhorn van Veen and Williams, 1980; Heath, 1979; McKenzie *et al*., 1979; Hein and Cargill, 1981; Heath *et al*., 1989; Cottew and Yeats, 1982; Cook, 1981; Friel and Greiner, 1988) where sheep scab has been eradicated and ovine psoroptic otoacariasis has not been recorded. Evidence for the transfer of scab mites to goats is not so well documented. The hair coat of dairy goat breeds may not be suitable for maintaining the optimal microclimate for mite survival and thus colonization by *Psoroptes* mites. The long fibres of Angora goats may be more conducive to mite survival. *P. cuniculi* is capable of causing serious damage to the skin and hair of angora goats (Graham and Hourrigan, 1977) and considered to be a threat to the Angora fibre industry world-wide. Ivermectin injected subcutaneously (200 mg per kg body weight) is an effective method of control (Bates, 2001; Odiawo and Ogaa, 1987).

***Psoroptes in horses***

Equines can be infested with three species of *Psoroptes*: *P. cuniculi* (*P. hippotis*) infesting the ears and *P. equi* and *P. natalensis* infesting the body (Sweatman, 1958).

*P. cuniculi* has been recorded in Australia (Lucas, 1946; Johnston, 1963; Shaw, 1966; Arundel, 1978; Pascoe, 1980), Great Britain (Gerring and Thomsett, 1980), France (Henry, 1917) and the United States (Montali, 1976). A survey of horses in Queensland showed 20 percent to be infested (Pascoe, 1980). Clinical signs of equine psoroptic otoacariasis may be restricted to ear discharge, but can also include ears held at right angles or giving a lop appearance (Shaw, 1966; Montali, 1976). Ear drooping is usually associated with severe rubbing of the affected ear or ears. Other common symptoms in horses include scratching ears with the hind feet (Shaw, 1966), rubbing the ear base on stalls etc. (Lucas, 1946; Shaw, 1966; Montali, 1976), head shaking (Lucas, 1946; Shaw, 1966; Gerring and Thomsett, 1980; Montali, 1976) and touchiness of poll (Lucas, 1946).

Equine psoroptic mange (*P. equi*) has been recorded in Germany (Diez and Wiesner, 1984), Libya (Gabaj *et al*., 1992), South Africa (Zumpt, 1961), Sudan (Abu Samra *et al*., 1981; Abu Samra *et al*., 1987) and Great Britain (Kirkwood, 1986a). In Great Britain equine mange (psoroptic or sarcoptic) was notifiable, due to its economic effects on the working horse, especially during wartime, but it was deregulated in 1983, due to the decreased agricultural and military importance of the horse, and the successful use of γ BHC washes. Severe outbreaks of equine psoroptic mange occurred in Germany during the Second World War and the disease was still notifiable in both East and West Germany up until 1984 (Dietz and Wiesner, 1984).

***Psoroptes on cattle***

Two species of *Psoroptes* infest cattle: *P. ovis* and *P. natalensis*. *P. ovis* (the sheep scab mite) has been recorded in Argentina (Nuñez, 1989), Czechoslovakia, (Sevcikova *et al*., 1987), Belgium (Losson, 1996), India (Gill *et al*., 1989), Italy (Genchi *et al*., 1995), Libya (Gabaj *et al*., 1992) and the United States (Hourrigan, 1979). *P. natalensis* has been recorded infesting cattle in Brazil (Sweatman, 1958), France ( Sweatman, 1958), India (Shastri and Ghafoor, 1974), New Zealand

(Sweatman, 1958), South Africa (Hirst, 1922), South America (Rocha *et al*., 1952), Uruguay (Sweatman, 1958) and Great Britain (Bates, 1999b).

Bovine psoroptic mange begins as moist plaques of hair over the withers, followed by intense pruritus with active rubbing against fixed equipment, leading to loss of hair, serum exudation, ulceration and bleeding. Eventually, thickened, scabby lesions, oozing blood and serum, progress over the withers and tail-head, before extending along the back and down the flanks and legs (Linklater and Gillespie, 1984). Pyoderma is common due to secondary bacterial infections. Psoroptic mange can be life threatening to calves under one year old but deaths rarely occur in older animals, although infested cattle are predisposed to pulmonary infections and may die (Losson, 1996). Like sheep scab, bovine psoroptic mange is considered a winter disease, but clinical outbreaks are sometimes observed in July or August (Losson, 1996; Hirst, 1922). Heavy infestations are readily detected, but light infestations are difficult to discern, especially during the early stages of disease, when lesions are very small (Bates, 1997; Fisher *et al*., 1986). Mixed infestations with *Chorioptes bovis* or *Sarcoptes scabiei* var. *bovis* are common, complicating control measures (Losson, 1996). Cattle mange can spread rapidly within confined situations of a feedlot but transmission at pasture is slower, especially in the summer when there is no close body contact and mites are in the ("alleged") quiescent phase (Meleney and Christy, 1978).

In Great Britain, 61.4 percent of bovine mange is caused by *C. bovis* and 30.0 percent by *S. scabiei* var. *bovis*. The remaining 8.6 percent of cases were due to isolated outbreaks of *Psoroptes* spp. imported from mainland Europe (Bates, 1997). The current low prevalence of bovine mange in Great Britain may be associated with treatment for other ectoparasites, e.g. compulsory treatment for warble fly (*Hypoderma* sp.), initially using systemic organophosphates and latterly ivermectin-based formulations. In addition, the current increase in the use of endectocides (doramectin, ivermectin, moxidectin etc.), either as anthelmintics or ectoparasiticides, may have contributed to the current low prevalence (Bates, 1997).

Bovine psoroptic mange is present on mainland Europe. In Belgium an estimated 400 000 cattle are treated each year (Pouplard *et al*., 1990). Belgian White and Blue cattle (BWB) represent around 50 percent of the Belgian national herd and are highly susceptible to *Psoroptes,* with infestations being generalized and chronic (Losson, 1996). In general, beef breeds are more susceptible and dairy breeds (e.g. Holstein) are more resistant. Bovine psoroptic mange was once notifiable in the United States and is still considered to be a major parasite of cattle.

Bovine psoroptic mange has been incriminated as the cause of a defect ("white spot") in leather, although conclusive evidence is lacking (George *et al*., 1986).

***Psoroptes on sheep (sheep scab)***

Sheep scab (*Psoroptes ovis*) is a form of allergic dermatitis initiated by allergens contained in the mite secretory or excretory products (Bates, 1981). *P. ovis* exploits the allergic reaction: the heat and humidity produced by the inflammation forming the micro-climate needed for mite survival and the leakage of serous exudate forming the basis of the mite's nutrition (Bates, 1981). In this inflamed condition skin breakages occur, mainly as a result of host scratching but also through small haemorrhages caused by the abrasive action of the mite's mouthparts. These skin breakages result in increased leakage of serum, with accompanying scab formation and skin thickening (Raffert and Gray, 1987).

Sheep scab can have profound effects on the health, welfare and economics of infested flocks through the effects of ram fertility (uncomfortable or interrupted mating), weak or still born lambs (through nutritional stress as a result of the constant irritation), reduced lamb growth and death of breeding stock (through debility and exhaustion, dehydration, secondary bacterial infections or hypothermia). The yield and quality of by-products such as leather and fleece are also adversely affected.

In 1986 scab was reported in at least 149 countries throughout the world (**Table 1**), with the disease still notifiable in many (Kirkwood, 1986b). Although eradicated from Australia, New Zealand and the United States, scab is considered to be a serious threat to the sheep industries of Europe, South America and southern Africa. Some Member States of the European Union have Government implemented control or eradication schemes, other states treat the disease as it occurs, having no national policy. There is a possibility therefore, that new strains of *Psoroptes ovis* could be transported throughout the Member States, particularly as a result of the Single European Market.

## 2 RESISTANCE DEVELOPMENT

As with other parasites, resistance to acaricides in populations of mites results from the selection of individuals with lower inherent susceptibility by exposure to acaricides. It is likely that genes that confer resistance are already present at very low levels in the parasite population before the introduction of a new acaricide. Although resistance develops slowly initially, once identified, it quickly becomes a problem. The rate at which a resistant allele becomes established in the population and the time it takes for the control of the parasite population to be lost is dependent on many factors. These include: frequency of the original mutation in the population before treatment, mode of inheritance of the resistant allele, frequency of acaricide treatment, and the proportion of a population that is not exposed to the acaricide.

Mites are obligate parasites with only small populations in refugia, so a resistant allele can become established very quickly in the population. However, the small population in refugia does enable much more efficient control of mange.

A practical definition of resistance to a given product is: “decreased susceptibility of an ectoparasite to an insecticide (or acaricide) at concentrations on or above a defined threshold”. The defined threshold concentration being the dose stipulated by the manufacturer for its use, printed on the product label (e.g. the maintenance concentration for plunge dips). Basically this means that if all the instructions are followed to the full and the product is still ineffective (following controlled investigations), the parasite can be considered to be resistant to that product (Bates, 1998). Another definition must also be considered, that of “tolerance”. Tolerance can be described as “a decreased susceptibility to an insecticide or acaricide at concentrations below a defined threshold” (usually shown by *in vitro* studies). In practical terms this can be interpreted as: “if all the manufacturer’s instructions are followed to the full and the product is still effective”. Progressive tolerance can lead to resistance.

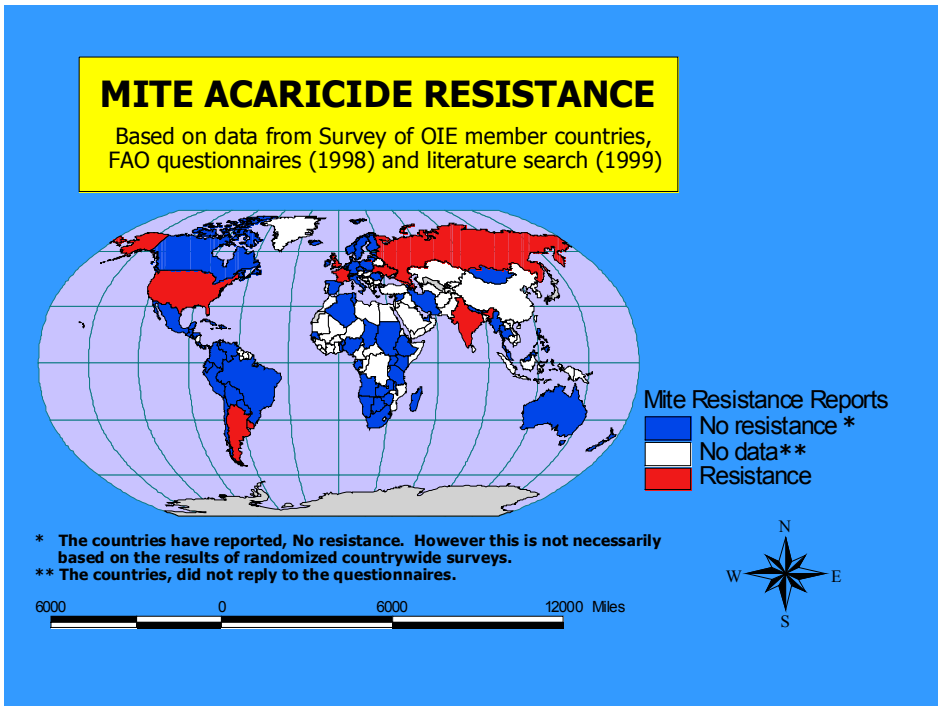
## 3 CURRENT STATUS

### Sheep scab

Scab was eradicated from Norway in 1894 and Sweden in 1934 (Kirkwood, 1986b) and both countries continue to remain free from disease. Scab was eradicated from Denmark in 1929 (Henriksen, 1979) and continued to be free from infestation for more than 50 years until infested sheep in several flocks were confirmed in the late 1970s (Henriksen, 1979), presumably from Germany. Periodic infestations have since been recorded (Henriksen *et al*., 1995).

The recent history of sheep scab in Germany is not dissimilar to that of the United Kingdom. Scab was almost eradicated from the Federal Republic of Germany (FDR) in 1948 by plunge dipping in γ BHC, but notification requirements were not being strictly observed and infestations resurged in 1973 (Liebisch *et al*., 1978; Meerman, 1978). Whereas in the past the majority of German sheep were regularly dipped at least once a year, a certain degree of negligence developed while scab was at a very low prevalence. Many flocks were dipped every two years or even after longer intervals. A further problem was the restrictions imposed on γ BHC. A considerable proportion of the increase in sheep numbers in the FDR since 1967 (5 percent per

annum) took place on the dyke farms of the North Sea coast. In addition to the increasing demand for mutton, a further incentive to increase flock size was the additional European Commission (EC) money for sheep kept in outer dyke areas. The resultant higher stocking density, close contact and scarcity of forage were the main factors conducive to the spread of sheep scab. Another factor was the communal sheep farms (common grazing) set up in North Friesland when sheep were kept on dyke pastures in the summer (Meerman, 1978). Scab was deregulated in 1991 and responsibility for control was left exclusively to the farmer (Worbes, 1995). The expansion and liberalization of the livestock trade following the re-unification of Germany, experimentation with new breeds and the introduction of the Single Market in 1993 saw a considerable number of new outbreaks (Worbes, 1995).




Scab was notifiable in France, although the law was generally never complied with (Autef and Girard, 1987). Despite national awareness campaigns there was a lack of strictness among farmers in deciding to carry out treatment or in choosing a correct method, product or regular prophylaxis (Autef and Girard, 1987). This lack of strictness led to poor results following treatment and an increased frequency of "accidents" with considerable mortality leading to court cases, and blame put on the method or the product (Autef and Girard, 1987), although laxity has been incriminated as the main cause of failure in France (Autef and Girard, 1987). Sheep scab was deregulated in France in March 1995 (Personne, *personal communication*.), but it still remains compulsory to treat infested flocks. Scab is generally found in the areas of the country among flocks grazed exclusively outdoors and in the south of the country where transhumance is practised (several flocks gathered together in the period May to September to graze the mountains). There are local control programmes, where it is compulsory for all sheep in these areas to be treated with the help of the local veterinary services. These programmes involve 20 percent of the national flock and have shown promising results (Personne, *personal communication*).

The history of scab control in Ireland mirrors that of the rest of the British Isles, only there was no period of temporary eradication. Sheep scab is notifiable in the Republic of Ireland, but compulsory dipping was abolished in 1994. The onus or responsibility to notify scab is now with the flock owner or veterinary surgeon. While scab was eradicated from the rest of the United Kingdom, the disease was still prevalent in Northern Ireland. Scab was recorded in 272 Irish flocks at the time of removing compulsory dipping in 1993 (O'Brien, 1992). Like the majority of Europe, γ BHC was withdrawn from the Irish market in 1985 due to meat residues and environmental concerns (O'Brien, 1996).

In Great Britain scab was made notifiable in 1869, and in 1948 the organochlorine γ BHC (lindane) was approved as a single dip, but the continued use of the old double dipping formulations was allowed (Page, 1969; Spence, 1951). γ BHC dips, together with rigid official enforcement, good sheep husbandry and restricted animal movement were responsible for eradication in 1952. It was postulated that eradication was achieved, not because every sheep had been dipped, but because every infested sheep had been dipped (Kirkwood, 1986a). The continued presence of chewing lice (*Bovicola ovis*) proved this. Scab was re-introduced to Great Britain in 1973 and γ BHC based dips continued to be used until 1984, when they were voluntarily withdrawn following pressure from Europe over possible residues in lamb exported from Britain (Henderson, 1991). Organophosphate formulations containing diazinon or propetamphos eventually replaced γ BHC in the fight against scab. Since the re-introduction of scab in 1973, there were a variety of policy measures aimed at eradicating the disease for a second time (Bates, 1999b). Without Government control scab could cost the United Kingdom sheep industry £600 million over thirty years (Kirkwood, 1986a). The cost to the Government for State veterinary input was estimated to be £12 million per annum together with £2.1 million for Local Authorities to enforce Government policy. Together with this expense the question was asked, "why should scab remain notifiable?": it is easily controlled, it is not zoonotic, the compulsory use of organophosphorus (OP) based dip formulations may be an unnecessary health risk, synthetic pyrethroid (SP) based dip formulations may have a severe ecological impact, adequate animal health legislation is in position and the EU makes no mention of sheep scab. Consequently scab was deregulated in June 1992 (Anonymous, 1992). Reasons for failure to eradicate scab were identical to France, Germany and Ireland. In addition, flocks were not inspected regularly, owners were not aware of the symptoms of scab or unwilling to report disease, flocks were not completely gathered and those that were, were not dipped correctly. The growing antipathy to OP dips may also have been important (Bates, 1999b). Since compulsory annual dipping was abandoned in Britain (and the Republic of Ireland), the problem of sheep scab has received much attention (O'Brien, 1996). The infrastructure involved in these schemes, and which accompanied the relevant treatment regime, has mainly disappeared. It is now impossible to quantify the extent of spread. However it is unquestionable that there has been an increase geographically and numerically in outbreaks of the disease (O'Brien, 1996).

In Brazil scab was under control for almost 20 years, but there was a resurgence in 1976 (Kirkwood, 1986b). It is now endemic in southern Brazil and is still a notifiable disease in Argentina and Uruguay. The use of γ BHC virtually eradicated scab from Argentina by 1960 (Nuñez, 1977). Unfortunately eradication was slowed down with the development of resistant strains of *P. ovis* in 1962 (Ault *et al*., 1962). The introduction of diazinon followed with initially spectacular results, but in 1965 the efficacy of diazinon dips in the provinces of south eastern Buenos Aires was in doubt, and diazinon resistance was first recorded in 1966 (Rosa and Lukovich, 1970). The development and maintenance of resistance was observed to be directly related to the standard of animal husbandry and the relative importance given to sheep production (Nuñez, 1977). In the Province of Patagonia, where sheep were the main enterprise, the standard of husbandry was high and scab resistance was rare. In the provinces of south eastern Buenos Aires, Entre Rios and Corrientes, where sheep production was secondary to other agriculture, the

standards of husbandry were low and resistance was a significant problem (Nuñez, 1977). Falling world wool prices have forced drastic reductions in the Argentinean national flock (44 million in 1980 to 9 million in 2001) and have virtually eradicated (resistant) sheep scab in areas where mixed farming was predominant. Sheep farming is still profitable in Patagonia, where large flocks predominate and scab continues to be a problem (Olaechea, *personal communication*). Similarly the number of sheep in Uruguay has dropped to 14 million and sheep have lost their importance. In some areas farmers are unwilling to invest in disease control and these areas continue to be foci of infestation. Uruguay abandoned compulsory plunge dipping in 1997 (Bonino, Mari and Mederos, *personal communication*).

Scab has been a problem in the Republic of South Africa since the 17th century and has increased in prevalence since 1973 (Van Heerden, 1977). The disease was almost eradicated in the 1930s using lime-sulphur dips. Sporadic outbreaks between 1940 and 1966 were mostly due to unlawful sheep movements across borders, and sheep migrating from neighbouring states. Since only sporadic outbreaks occurred in South Africa for almost 30 years up to 1967, few farmers, stock inspectors and veterinarians had experience with the disease. The main market for South African wool is Germany, which, like most European Union States, is concerned about high levels of insecticide (Erasmus, *personal communication*). In 1992 the South African authorities observed the outcome of scab deregulation in the United Kingdom, contemplating mutual deregulation. At present scab is still notifiable and an increasing problem, spreading from the former tribal homelands (where common grazing is practised) and the independent territories of Lesotho and Swaziland. Since the “new government”, there has been a breakdown in official control policy and a severe reduction in extension services (Louw, *personal communication*). It is compulsory under the Diseases of Animals Act to treat all ectoparasite infestations and to have a permanent dip tank. An unsuccessful campaign was launched by the South African Government in 1990, but scab is now left for the farmer to control. Local husbandry methods make control difficult and eradication seems impossible. Scab was eradicated from Lesotho in 1935 but returned in 1973 (Kirkwood, 1986b).

## 4 DIAGNOSIS OF RESISTANCE

Acaricide resistance is mainly detected through field experience, after failure of a particular treatment. It must be understood that in the case of mites, proper treatment and control measures can only be implemented in conjunction with an accurate diagnosis. Failure of treatment is often difficult to detect as mites can survive on the host without showing any sign of their presence (Bates, 2000b).

There are five different methods of identifying populations of *Psoroptes* resistant to contact acaricides (organochlorines (OCs), organophosphates (OPs) or synthetic pyrethroids (SPs)), three *in vitro* methods and two *in vivo* methods. The three *in vitro* methods could also be adapted for *Chorioptes* mites. The first is based on the use of "tea bags" where mites are exposed to different drug concentrations. The bioassays principally assess efficacy by determining $LC_{50}$ (or $LC_{90}$/$LC_{95}$) values in order to calculate a resistance factor (RF). The $LC_{50}$ (or $LC_{90}$ or $LC_{95}$) is the concentration of acaricide that is lethal to 50 percent (or 90 or 95%) of mites after correction for non-specific mortality in the negative (solvent) control. It is also important to note that when deciding suitable concentrations, dip bath concentrations of OC or OP acaricides do not equate to fleece levels (the dip bath concentration of diazinon may be 400 ppm but the levels of diazinon in the fleece may be 4000 ppm (Bates, personal communication).

# 5 DETECTION OF RESISTANCE: PROTOCOLS FOR RECOMMENDED METHODOLOGIES

## 1. Tea bag dipping *in vitro* test

The original method (Wright and Riner, 1979) has been adapted and used routinely at Onderstepoort, South Africa. A fusion of the two protocols is described below.

Filter paper or heat sealable rice paper envelopes ("tea bags") (2.0 to 5.0 cm long × 2.0 to 6.0 cm wide) are prepared. Three sides of each envelope are sealed.

Suspected acaricide-resistant mites are collected directly from donor animals, using a vacuum pump device or a mounted needle.

Under a dissecting microscope, 20 to 30 normal adult female mites are selected and introduced into the "tea bags" (immature mites are prone to escape) and the open side is sealed with a "bulldog clip".

A suitable range of dilutions of the test acaricide are prepared (**Table 2**) and maintained (in 100 ml aliquots) in aluminium foil dishes (to prevent contamination).

The envelopes are then held in forceps and dipped in appropriate concentrations of the test acaricide, for between 20 and 30 seconds, with constant stirring.

To prevent depletion of the acaricide, only one tea bag is immersed in each acaricide dilution. Replicates are also prepared.

In each case, untreated controls without exposure to acaricide are also prepared.

A separate run should also be carried out using an acaricide-susceptible population of *P. ovis*.

Envelopes are hung up to dry (at room temperature) and examined after 24 hours (Wright and Riner, 1979), or incubated in an unsealed humidity chamber (85% relative humidity (RH)) at 26°C and examined after 3 hours.

Envelopes are opened and mite mortality recorded under a dissecting microscope.

Mortality assessments are made as follows: live mites = normal movement. Dead/dying mites = immobile or unable to walk normally.

## 2. Slide immersion *in vitro* bioassay

A slide technique has been published for the house dust mite (*Dermatophagoides* spp.) (Mollett, 1995), although it has not been validated for *Psoroptes* (*Chorioptes or Sarcoptes*).

Twenty five adult female mites are fixed (ventral side up) onto double sided adhesive tape attached to a 2.5 × 7.5 cm glass microscope slide.

Slides are immersed in the diluted acaricide (**Table 2**) and gently agitated for 15 seconds.

The slides are then removed, and rested on one edge on filter paper at room temperature for at least 15 seconds.

They are then transferred to a humidity chamber at 25°C and 75 percent RH and mortality assessed after 48 hours.

## 3. Micro-titre immersion *in vitro* assays

An *in vitro* micro-titre immersion bioassay has been used in the United Kingdom since the late 1980s to screen candidate acaricides and evaluate possible acaricide resistance. A similar assay has been used in Hungary to compare the efficacy of pyrethroids against a deltamethrin resistant population of *P. cuniculi* (Pap *et al*., 1997).

Place ten active, adult female or male/female nymphal *P. ovis* into designated wells of labelled, plastic micro-titre plate using a mounted needle. Each well represents an acaricide dilution.

Using a Gilson automatic pipette, quickly transfer 150 ml of the respective acaricide dilution (**Table 2**) into the wells containing the live mites.

Cover the plate with disposable (Titertek) adhesive plate covers to prevent evaporation and the effects of solvent vaporization.

Incubate at room temperature (20°C to 25°C) for 24 hours.

Examine each well for two minutes using a dissecting microscope and record the numbers of live and dead mites.

This method has been employed at the Veterinary Laboratories Agency (VLA) (Weybridge, United Kingdom) since 1988, in parallel with animal (*in vivo*) studies. Controlled dipping of sheep infested with flumethrin resistant populations of *P. ovis* were ineffective at 33.0, 44.0 and 66.0 mg/l flumethrin, and flumethrin sensitive populations were eradicated from infested sheep at all dilutions assessed. A lethal dose of above 66.0 mg/l was therefore indicated for the resistant isolates. In comparison, micro-titre immersion assays using a formulated flumethrin dip wash demonstrated an $LC_{90}$ of 31.72 mg/l for the flumethrin sensitive population and $LC_{90}$s of 78.85 and 87.79 mg/l for the flumethrin resistant populations. These results corresponded well with the *in vivo* assays. The assays initially used analytical grade flumethrin and were flawed with many technical problems. The main problem being that analytical flumethrin was extremely difficult to dissolve in any solvent that was not itself highly toxic to the mites. A commercial dip formulation containing flumethrin, and diluted in distilled water, was therefore used. The *in vitro* test using formulated flumethrin is therefore an accurate and inexpensive method of assessing for pyrethroid resistance in the sheep scab mite.

## 4. *In vivo* "cell test"

Healthy, full fleeced sheep, without previous treatment for ectoparasites and free of external parasites, are prepared as follows:

The wool along the dorsal area is cut off with scissors in order to expose the skin. The skin is then gently clean shaved using a scalpel blade. The animal is then rested for 24hrs.

The bottom halves of 5 cm diameter aluminium (“pill-box”) cells are then glued to the skin using Superglue® (one cell per dilution of acaricide).

Twenty five to 30 adult female mites are introduced into each cell at day 0, and the lid secured.

At day 7, the presence of mites or a lesion in each cell is confirmed.

At day 14, each cell is exposed to a particular concentration of an acaricide for 30 seconds, and the lid replaced.

The cells are then examined for live mites and resolution of the lesion at three day intervals for a total of 21 days.

**Table 2. Recommended dilutions**

| Acaricide | Dilution (mg/l) | | | | |
|---|---|---|---|---|---|
| γ BHC | 50 | 100 | 200 | 400 | 800 |
| Diazinon | 25 | 50 | 100 | 200 | 400 |
| Cypermethrin | 50 | 75 | 100 | 125 | 150 |
| Flumethrin | 20 | 40 | 60 | 80 | 100 |

## 5. *In vivo* "control test"

Groups of healthy, full fleeced sheep, without previous treatment for ectoparasites and free of external parasites, are challenged with 25 to 30 adult female *P. ovis* obtained from donor animals.

Infestations are allowed to progress for 42 days, with a check to confirm active colonization after 7 days.

After this time, mite counts (*in situ*) and lesion measurements are recorded and the animals are treated with the product with suspect resistance, strictly according to the manufacturers recommendations.

For plunge dipping, the correct volume of water must be added to the dip bath (using a water meter) and the required volume of acaricide concentrate accurately measured and added to the water. The wash must then be thoroughly mixed for not less than five minutes. Dip wash samples must be taken after mixing and after the sheep have been dipped and the concentration of acaricide confirmed by chemical analysis (e.g. gas liquid chromatography (GLC)).

For injections and pour-ons, the syringe or pour-on/spray-on gun must be calibrated, as must all weighing equipment where acaricide administration is according to body weight.

All sheep must be examined for live mites and resolution of disease 7, 14, 28 and 56 days after treatment.

There are currently no published methods to investigate resistance in *Psoroptes* to ingested acaricides (e.g. doramectin, ivermectin or moxidectin), however methods of investigating resistance in *Sarcoptes* have been published (Brimer *et al*., 1993; Brimer *et al*., 1995). These tests are based upon the migrational ability of *Sarcoptes* mites on the surface of agar gels containing acaricide. Mite activity is expressed as a migration index (MI) and compared to a known standard. Good responses were recorded for the organophosphates (OPs) parathion, phosmet and phoxim (Brimer *et al*., 1993) and for ivermectin (Brimer *et al*., 1995). The test is accurate, sensitive and easy to carry out, but like all acaricide resistance assays, requires accurate determination of the acaricide concentration in the substrate (i.e. gel).

## Standardization and interpretation of bioassays

Dilutions must be: made up on the day of assay using volumetric glassware, tightly stoppered and used within 3 hours of dilution.

Concentration of acaricide in the dilutions must be verified by chemical assay (e.g. GLC).

Mites must be used within 3 hours of collection.

If possible, bioassays should be carried out in parallel with a known sensitive or resistant isolate.

Untreated (solvent) controls must be included for each isolate of mite.

Mites will be recorded as alive if they have total mobility or active movement of two or more limbs: they will be recorded as dead if less than two limbs show active movement.

$LC_{50}$ and $LC_{90}$ values are calculated from the corrected mean percent mortality (i.e. data corrected for the non-specific mortality in the negative (solvent) controls) by linear regression using a programmable calculator.

Data corrected for the non-specific mortality in the negative (solvent) controls (mean corrected percentage mortality) are calculated using the formula:

$$(M_p - M_s)/(100 - M_p) \times 100$$

Where $M_p$ = mean test product mortality (%)

$M_s$ = mean solvent mortality (%)

Data collected from the bioassay will be valid until a mean mortality of 30 percent or above is recorded in the test product solvent control. Once a mean mortality of 30 percent or above is recorded in the test product solvent control the bioassay will be terminated.

### New techniques under development: enzyme assays

Techniques have been developed measuring the amount of carboxylesterase $E_4$, an enzyme known to cause resistance to a wide range of insecticides in the peach-potato aphid (*Myzus persicae*) (Devonshire and Moores, 1984). A total esterase activity using the whole homogenate of a single aphid gives a quantitative measure of activity (Devonshire, 1975). This is preferable when investigating very resistant aphid populations because $E_4$ contributes virtually all the activity. In slightly resistant populations other esterases, common to all variants, make a large contribution and can obscure the smaller differences in the amount of $E_4$ between resistant and susceptible aphids. In this case electrophoretic analysis is preferable as it allows isolated $E_4$ to be estimated from the intensity of the stained band on the gel (Baker, 1977; Blackman *et al*., 1977). Although the activity of $E_4$ has been quantified in gels by spectrophotometry (Blackman *et al*., 1977) this is not practicable on a large scale. These techniques may be of use investigating acaricide resistance in mange mites.

Glutathione-S-transferases (GSTs) have been identified in 24 insect species as a polymorphic protein occurring in up to eight isoenzymes in some cases (Baker *et al*., 1994; Yu, 1996). GSTs are used by insects and mites to metabolize xenobiotics in the body (Capua *et al*., 1991; Ibrahim and Ottea, 1995) and elevated levels of GSTs have been shown to confer insecticide resistance (Yu, 1996; Ibrahim and Ottea, 1995; Prapanthadera *et al*., 1995; Bond and Bradley, 1997; Hemmingway *et al*., 1997) in a wide variety of medical, veterinary and agricultural pests. At present there are no published techniques for quantifying the amounts of GSTs in parasitic mites.

## 6 EPIDEMIOLOGY

### The epidemiology of sheep scab

The prevalence of sheep presenting scab lesions within infested flocks can vary between 7.8 and 60.0 percent in large flocks, and the prevalence of sub-clinical lesions (i.e. lesion areas below 100 $cm^2$ or 2.5% body cover) can be between 10.0 and 90.0 percent. Sub-clinical disease is generally asymptomatic; symptoms if they do occur include occasional episodes of restlessness, rubbing against fence posts etc., soiled and stained areas of wool (particularly on the shoulders), head tossing and deranged or tagged fleece. Differential diagnosis can be problematic as these symptoms are also indicators of the presence of other ectoparasites (e.g. chorioptic mange, chewing lice *(Bovicola ovis*), blowfly myiasis (*Lucilia* spp.), keds (*Melophagus ovinus*), biting

flies, or even scrapie. It is of paramount importance that the cause(s) of flock irritation are identified. Administration of an inappropriate control strategy may select for acaricide resistance.

The sheep chewing louse (*B. ovis*) is a common parasite of sheep on common grazing uplands of the United Kingdom. Sheep with pre-disposing infestation of chewing lice will not accept challenges of sheep scab mites, whereas sheep with active scab can be colonized by lice following natural exposure. The exact nature of this inter-species exclusion is unknown, but the skin changes initiated by lice feeding/excretion may render it unfavourable for mite colonization. Lice, on the other hand, may actively feed on the scab lesion (Bates, 1999c), particularly after administration of an endectocide has eradicated *P. ovis.*

In the later stages of *Psoroptes* infestations, rubbing and head tossing become more evident and areas of wool loss appear together with open, bleeding wounds. Sheep rapidly lose condition and epileptiform seizures may be evident (Bygrave *et al*., 1993). Numbers of infested sheep within the flock can vary from one or two in the early days of infestation, to the whole flock as the disease takes hold (depending on the immune status of each individual sheep). Throughout the flock there will be animals with non-established lesions (that will eventually die out) and young sub-clinical lesions, together with animals with obvious extensive disease. All sheep should be considered to be infested and the whole flock should be treated for scab. One missed sheep could re-infect the whole flock.

The transmission of scab is through direct contact between sheep or indirectly, through contact with residual mites in tags of wool or scab attached to fencing, etc. Although *Psoroptes* spp. mites are obligate parasites, they are still capable of surviving off the host for 15 to 16 days (O'Brien *et al*., 1994a), before succumbing to starvation and desiccation. An infestation can be initiated by only one egg laying female or hundreds of mites, depending on the mite burdens on other infested sheep or in the environment, together with the relative period of contact. Infestations can spread rapidly through lowland flocks with restricted grazing but may be slower through extensively grazed hill flocks, that are thinly spread over common grazing and infrequently mustered (Kirkwood, 1986a). Scab outbreaks in Britain originated from lateral spread from contiguous flocks, strays etc. (33.9%), movement of sheep via market (22.3%), direct sheep movements (15.9%) and persistent infestations on unenclosed land (1.0%). Although this direct transmission was the predominant method, an element of indirect transmission is present in all outbreaks, i.e. via mites deposited at marts, in livestock lorries etc. Although the origins of the outbreaks were fully explained in over 73 percent of cases, the origins of infestation remained obscure in 18.5 percent of flocks and disease recrudesced in 0.7 percent of flocks (Bates, 2000b). The development of acaricide resistant strains of *P. ovis* during this period was not suspected.

Sheep scab is a winter disease, with the majority of cases in the Northern Hemisphere occurring between September and April, although a significant number of cases do occur in the summer months, particularly on animals still full fleeced (lambs, hogs etc.) and on "ridges" of longer fleece on poorly shorn sheep. These sheep can subsequently infest ewes with an adequate fleece length. Sheep scab mites were once thought to migrate to the "cryptic sites" (the ears, the infra-orbital fossae, the inguinal pouches and the crutch) in order to survive the summer ("latent phase" or "suppressed scab") (Downing, 1936; Spence, 1949). The migration of *P. ovis* to the cryptic sites is not in dispute, but the intentional seasonality of the migration is open to question. *P ovis* can be found in the cryptic sites of sheep with extensive disease, and then more often in the winter than the summer (Kirkwood, 1986a). Mites have been recorded in only 7 percent of sheep with detectable infestations in one or more cryptic sites during the summer compared to mites over-summering on the broad body surfaces of 32 percent of sheep examined (Roberts *et al*., 1971).

Two species of *Psoroptes* have been recorded to infest sheep: *P. cuniculi* infesting the ears and *P. ovis* infesting the body (Sweatman, 1958). In Great Britain *P. cuniculi* has been recorded

within tubes of scab situated within the last centimetre of the external auditory canal (EAC), next to the tympanic membrane, from sheep with no recent history of scab (Bates, 1996a; Bates, 1996b). Symptoms of psoroptic otoacariasis differ between lambs and adults. In adults the symptoms ranged from the asymptomatic to aural haematomata, violent head shaking and ear rubbing, leading to excoriation and wounding of the ear and ear base. In lambs symptoms include plaques of scab (often bloody) on the external ear cleft, excoriation of the ear base, ear scratching with the hind feet and inflammation of the external aspects of the horizontal canal. In all cases the internal pinnae are clear of the typical psoroptic scabs characteristic of mites in the cryptic phase. *P. cuniculi* ear mites are morphologically identical to the sheep scab mite (*P. ovis*), but do not initiate clinical scab on transfer to scab naive sheep and are not therefore reservoirs of infestation.

On the other hand *P. ovis* were observed in the EACs of 38.6 percent of infested sheep presenting lesion areas from 20.9 to 100.0 percent body cover (Bates, 1999b). Although the incidence of *P. ovis* otoacariasis increased as the leading lesion edge approached the ears, 20.0 percent of sheep were infested in the EAC when the leading edge was as far away as the mid-back. In studies investigating the temporal progression of sheep scab it was demonstrated that *P. ovis* migrated to the EAC as early as 28 days following artificial challenge, with the leading edge 28.0 cm from the base of the ears (Bates, 1999b). Unlike *P. cuniculi*, *P. ovis* isolated from the EAC can be infective to the bodies of sheep. Acquired resistance to scab may have a direct effect on the growth of sheep scab lesions originating, if aural *P. ovis*, (or residual body mites in the regressed or cryptic phase of infestation) are to re-infest their previously infested host (Bates, 2000c). Colonization would be more successful on scab naïve hosts.

Long periods of latency and a sudden increase in vigour and pathogenicity of a mite strain could account for unexplained outbreaks of disease (Roberts and Meleney, 1971). Distinct populations of *P. ovis* were identified in the United States, varying in population reduction in the summer, tolerance to OP acaricides, survival off the host and relative rate of spread through cattle herds (Roberts and Meleney, 1971). Similar studies have been carried out in Great Britain (Bates, 1999d). All the geographical isolates of *P. ovis* which were compared produced a progressive lesion, characteristic of sheep scab, but the extent of the lesion produced with time varied considerably between the isolates (Bates, 1999d). Some created slow chronic infestations while others produced fast acute infestations over the same time period.

## 7 CURRENTLY AVAILABLE CONTROL STRATEGIES

The decision as to which method of scab control to use depends on government policy, the size of the flock, the age of the sheep, whether the sheep are pregnant or lactating, the use to which they will be put (meat, wool, milk or breeding), the availability of labour and facilities (handling pens, dip baths, etc.), weather, geography and the presence of other parasites (nematodes worms, lice, ticks, keds and blow flies) (Bates, 1993). The acaricides currently used for the control of sheep scab (and cattle and pig mange) are shown in **Table 3.**

The currently available tools for mange control consist of chemical technology, relying on treatments with different application methods and/or formulations of acaricides. These can be used with or without the benefit of local epidemiological knowledge.

Farmers and veterinarians implement treatments against mange most commonly when the disease is evident. According to the epidemiology, mange is primarily a winter disease, with the majority of cases in the Northern Hemisphere occurring between September and April, and in the Southern Hemisphere between April and July. Highly effective treatments such as those given during the “cryptic phase” using macrocyclic lactones, are a very good strategy for eradicating mange because they eliminate the source of infection for the next season.

**Table 3. Acaricides used for the control of sarcoptic, chorioptic and psoroptic mange**

| Acaricide | Application | Mite genus | Host |
|---|---|---|---|
| Abamectin | Injection | Psoroptes, Sarcoptes,. | Cattle (National Office of Animal Health, 2000) |
| Amitraz | Sprays/washes | Sarcoptes | Pigs (National Office of Animal Health, 2000) |
| | | Sarcoptes, Chorioptes, Psoroptes | Cattle (Curtis, 1985) |
| | Pour-on | Sarcoptes | Pigs (National Office of Animal Health, 2000) |
| | Plunge Dip | Psoroptes | Sheep (Muñoz Cobenas *et al*., 1978) |
| γ BHC | Wash/Spray | *Sarcoptes* | Pigs (Tucker and Cutler, 1982) |
| | | Sarcoptes, Chorioptes, Psoroptes | Cattle (Schwardt, 1949; Lancaster and Meisch, 1986) |
| | Plunge Dip | Sarcoptes, Chorioptes, Psoroptes | Sheep (Spence, 1951) |
| Coumaphos | Plunge Dip | *Psoroptes* | Sheep (Lancaster and Meisch, 1986) |
| Deltamethrin | Plunge Dip | *Psoroptes* | Sheep (Personne, personal communication) |
| | Shower Dip | | |
| | Jetting | | |
| Diazinon | Plunge Dip | *Psoroptes* | Sheep (Kirkwood and Quick, 1981) |
| Doramectin | Injection | *Sarcoptes* | Pigs (National Office of Animal Health, 2000), |
| | Pour-on | Sarcoptes, Psoroptes | Cattle (National Office of Animal Health, 2000) |
| | | Sarcoptes, Chorioptes, Psoroptes | Sheep (Bates *et al*., 1995; McKenzie, 1997) |
| | | | Cattle (National Office of Animal Health, 2000) |
| Eprinomectin | Pour-on | Sarcoptes, Chorioptes. Psoroptes | Cattle (National Office of Animal Health, 2000) |
| Fenvalerate | Plunge Dip | Psoroptes | Sheep (Personne, personal commmunication) |
| | Shower Dip | | |
| | Jetting | | |
| Flumethrin | Pour-on | Psoroptes | Cattle (Losson and Lonneaux, 1992) |
| | Plunge Dip | Psoroptes | Sheep (Kirkwood and Bates, 1987) |
| Moxidectin | Pour-on | Sarcoptes, Chorioptes, Psoroptes | Cattle (National Office of Animal Health, 2000) |
| | Injection | Sarcoptes, Psoroptes | Cattle (National Office of Animal Health, 2000) Sheep (Parker *et al*., 1999) |
| High-cis Cypermethrin | Plunge Dip | Psoroptes | Sheep (O'Brien *et al*., 1997) |
| Ivermectin | Injection | Sarcoptes | Pigs (National Office of Animal Health, 2000) |
| | | Sarcoptes, Psoroptes | Cattle (National Office of Animal Health, 2000), Sheep (O'Brien *et al*., 1993) |
| | | Sarcoptes | Pigs (National Office of Animal Health, 2000) |
| | In-feed | Sarcoptes, Chorioptes Psoroptes | |
| | Pour-on | Sarcoptes, Chorioptes, Psoroptes | Cattle (National Office of Animal Health, 2000) |
| | Bolus | | Cattle (National Office of Animal Health, 2000) Sheep (Bridi *et al*., 1998) |
| Permethrin | Pour-on | *Chorioptes* | Cattle (National Office of Animal Health, 2000) |
| Phosmet | Pour-on | *Sarcoptes* | Pigs (National Office of Animal Health, 2000), |
| | | Sarcoptes, Chorioptes, Psoroptes | Cattle (National Office of Animal Health, 2000) (Meleney and Roberts, 1979) |
| Phoxim | Spray | Psoroptes | Cattle (Hourrigan, 1979) |
| | Plunge Dip | Sarcoptes, Chorioptes, Psoroptes | Sheep (Muñoz Cobenas *et al*., 1978; Meerman, 1978) |
| Propetamphos | Plunge Dip | Psoroptes | Sheep (Kirkwood and Quick, 1982) |

The impact of acaricides on the environment and as residues in food is a continuing concern. The dependence on chemicals to control scab mange and other parasites is under continual review.

Four control alternatives are used:

1. Suppressive (systematic or eradication) treatments.
2. Ad hoc or opportunistic treatments.
3. Curative treatments.
4. Strategic treatments.

### 1. Suppressive treatments towards eradication

Suppressive treatments are carried out for eradication purposes.

***Principle:*** Treatments against mange are applied at short intervals (twice every 9-12 days) to eliminate completely all the parasitic stages. All animal introductions must be treated immediately upon arrival in order to avoid the spread of new mites onto the farm.

***Prerequisites:*** These include a sustainable supply of inexpensive acaricides and the necessary infrastructure and animal health and production services. In cases where the strategy is used for the eradication of mites, the following would also be required. A long-term commitment that involves thorough epidemiological surveillance, obligatory periodic acaricide application, adoption of quarantine procedures, adequate training for personnel and the active participation and co-operation of farmers, legal support of the respective governments, and adequate, long-term guaranteed financial resources. Extensive knowledge of biological aspects of the mites and the participation of adequately trained personnel are further essential structures for successfully achieving such a goal. Such campaigns are ultimately dependent upon the continued chemical efficacy of the products employed, or their successful substitution. Ongoing monitoring of acaricide efficiency is therefore crucial. Once eradication is complete, there is usually some form of physical border to be maintained between the mange-free and the mange-infested areas. Administration of such borders will be an expensive and long-term requirement.

***Advantages:*** Although eradication of mange from a prescribed area remains a daunting and expensive task, if achieved and maintained, the long-term benefits are generally compensatory.

***Disadvantages:*** Delays in the process of mange eradication could lead to acaricide resistance due to the extended intensive exposure to chemicals.

***Epidemiological consequences:*** Risks of acaricide resistance.

***Possible combination with other strategies:*** Highly effective treatments given during the "cryptic phase" using macrocyclic lactones, are an effective strategy for eradicating mange, because they eliminate the source of infection for the next season. All oncoming stock should be quarantined for at least three weeks, and observed for signs of infestation. If a given area or country does not meet the necessary prerequisites, eradication should not be promoted.

### 2. Ad hoc or opportunistic treatments

***Principle:*** In connection with general management practices (weaning, dehorning, change of pasture or paddocks) farmers often implement routine preventive procedures and antiparasitic treatments. On some occasions they may use macrocyclic lactones that will have acaricidal effects as well as anthelmintic activity. It is usual that farmers decide when the animals should be treated according to their own estimates of "economic thresholds" for mite infestation, time available, climatic conditions, availability of personnel, acaricide and basic infrastructure.

***Prerequisites:*** No special requirements need to be met.

***Advantages:*** There is a reduced overall need for gathering animals, resulting in a reduced input of human and economic resources.

***Disadvantages:*** Due to the biological cycle of mites, only one opportunistic treatment may be inefficient and results are unpredictable when assessed solely from the perspective of immediate mange control. The effectiveness of this strategy in reducing mite populations is questionable, as the interval between two opportunistic treatments is often too long (>10 days) for the acaricide to prevent the completion of the mite's parasitic development on the host.

***Epidemiological consequences:*** The effective reduction of overall mite population is doubtful. Reinfection from mite eggs not affected by the treatment, could maintain the disease on the farm.

***Possible combination with other strategies:*** Regular clinical observation to avoid the sudden spread of the disease.

**3. Curative treatments**

***Principle:*** Farmers implement curative treatments when some animals are presenting clinical signs of mange and/or whenever the risk of production losses and/or uncontrolled disease is considered to be significant.

***Prerequisites:*** The presence of a regularly applied monitoring system such as clinical examination.

No other special requirements need to be met.

***Advantages:*** Only those animals with clinical signs are treated. There is a reduced overall need for gathering all animals on the farm, resulting in a reduced input of human and economic resources.

***Disadvantages:*** The approach is usually inefficient in the long term because the disease is maintained on the farm. Reinfestation will occur. Due to the biological cycle of mites, only one curative treatment may be inefficient and results are unpredictable when assessed solely from the point of view of immediate mange control.

***Epidemiological consequences:*** The effective reduction of overall mite population is doubtful. Reinfestation from mite eggs not affected by the treatment can maintain the disease on the farm.

***Possible combination with other strategies:*** Treated animals should be observed for signs of clinical evolution and if feasible, kept isolated from the remaining animals.

**4. Strategic treatments**

***Principle:*** Farmers implement strategic treatments in the early autumn, before there is an increase in the number of mange outbreaks, and during summer when animals are suffering from just subclinical mite infestations.

***Prerequisites:*** All animals should be treated. All introductions should be observed clinically, to avoid the introduction of infested animals in the farm.

***Advantages:*** Because animals are infected by a low number of mites, early autumn treatments remove the mite population and there will be no mange outbreaks during this season. Removing mange during the summer season means that no further mite infestation will appear in the next season.

***Disadvantages:*** There is a need for gathering all animals on the farm, resulting in an increased input of human and economic resources.

***Epidemiological consequences:*** There will be effective control of mange on the farm.

***Possible combination with other strategies:*** All introductions should be treated in order to avoid introduction of "new" mites onto the farm.

## 8 CHEMICAL CONTROL

**Inorganic compounds**

The early control of scab was based upon plunge dipping in one of four active ingredients: tar acid/tar oil dips, arsenic dips, lime-sulphur dips and tobacco dips, all of these requiring a second dip within 14 days to kill emerging eggs (Spence, 1951).

**Organochlorine (OC) compounds**

The next group of insecticides to be developed were the organochlorines and cyclodienes, e.g. γ BHC, aldrin and dieldrin. The mode of action of OCs was not clearly understood, but they were known to destroy the delicate balance of sodium and potassium within the cell, thus preventing normal transmission of nerve impulses. From 1945 onwards, plunge dip formulations were developed containing γ BHC. These were effective at a single dipping, eradicating scab and lice, together with sufficient chemical remaining on the fleece and skin to kill hatching parasites for a considerable number of weeks after dipping (Spence, 1951). Dichlorodiphenyltrichloroethane (DDT) or dieldrin were not effective against *P. ovis* (Nuñez, 1977), although they were highly effective against lice and blowfly (*Lucilia* sp.) larvae. Dieldrin and DDT were withdrawn from the United Kingdom market in the late 1960s, primarily on environmental grounds. Up to the mid 1980s, plunge dipping in γ BHC wash was the major acaricide in the war against scab world-wide, and continued to be used in the United Kingdom until 1984, when it was voluntarily withdrawn due to residues in meat (Nuñez, 1977). Strains of *P. ovis* resistant to γ BHC were reported in Argentina in 1962 (Ault *et al*., 1962), hampering scab control (Nuñez, 1977). During the eighteen years of compulsory use against sheep scab in the United Kingdom, no cases of γ BHC resistance were recorded. Eventually scab control changed to the use of organophosphate based formulations (Kirkwood, 1986a).

**Organophosphate compounds**

The organophosphate based plunge or shower dip formulations were the next generation of insecticides to appear on the market. OPs act by inhibiting cholinesterase (ChE) enzymes and by preventing the removal of acetylcholinesterase (ACh). The latter "jams the circuit" through its accumulation and interferes with the neuromuscular junction. Diazinon was approved for scab control in the United Kingdom in 1981 (Kirkwood and Quick, 1981), although it had been licensed for blowfly and lice control since the early 1970s. Propetamphos was approved for scab, lice and blowfly control in the United Kingdom in 1982 (Kirkwood and Quick, 1982). In France plunge dipping is considered effective for large flocks. Full fleeced sheep are immersed for 60 seconds and shorn sheep for 30 seconds, with the head pushed under twice. In the United Kingdom and Ireland all sheep are immersed for 60 seconds regardless of fleece length.

OP dip formulations began to be incriminated in post-dipping illness in stock owners and contractors (Anonymous, 1989b). Consequently, safer insecticides were investigated for their efficacy against scab, lice and blowfly strike.

**Synthetic pyrethroids (SPs)**

In 1987 the first non OP dip, containing the synthetic pyrethroid flumethrin, was licensed in the United Kingdom for scab and lice control (Kirkwood and Bates, 1987a), and ten years later high-

cis cypermethrin (HCC) was licensed for the British market (O'Brien *et al*., 1997). Flumethrin is not licensed for scab control in France or the Republic of Ireland. SPs have the advantage of excellent selectivity and high toxicity to arthropods and relative safety to mammals. SPs affect the neuronal membrane, modifying the sodium channels, probably impeding protein conformational changes at the lipid-protein interface, in a manner similar to OC compounds. SPs have remarkable similarities with DDT. Both DDT and SPs have two types of insecticide effect, a) initial rapid knockdown (Kd), rendering the insect motionless and b) a subsequent lethal effect. Development of resistance to DDT by pests around the world was thought by many to foreshadow a similar fate for the SPs (Miller, 1988). The biggest advantage of SPs is that they can also be formulated as pour-ons, revolutionizing louse control on sheep (and cattle), particularly in Australia.

During the years of compulsory dipping in the United Kingdom there was no suspicion of OC, OP or SP resistance. There was in fact little chance of resistance developing in either obligate parasite, *Psoroptes* or lice, due to the "overkill" nature of compulsory dipping, which also included the supervised "double dipping" of confirmed scab infested flocks, and the strict government control of insecticides on the market. Dip formulations containing diazinon, propetamphos or flumethrin were "approved" before the deregulation of sheep scab in 1992. Approval was granted only if they cured the disease and provided protection from re-infestation for at least three weeks on shorn and unshorn sheep. Approval of intermittent replenishment dips was based upon the lowest concentration likely to occur under field conditions (i.e. the maintenance level) (Kirkwood and Bates, 1987b), and was set well below the initial make up concentration and well above the minimum lethal concentration. The maintenance level for non-stripping dips (e.g. flumethrin) is close to the make up concentration, but in stripping dip formulations (e.g. OP) the make-up concentration is considerably higher. The maintenance concentrations for flumethrin, diazinon or propetamphos were 44 ppm, 100 ppm and 125 ppm respectively. The initial make up concentration and the maintenance concentration in the non-stripping flumethrin dip are more or less equal.

OP (propetamphos) and SP (flumethrin) resistant strains of *P. ovis* emerged in Great Britain after deregulation, and to dip formulations that were scab approved during the eradication campaign. Deregulation also removed the requirement for the approval of dip formulations and supervised dipping, resulting in the potential for ineffective treatment. In 1994 two populations of *P. ovis*, from two geographically isolated areas (southwest England and northern Scotland) were found to be resistant to a flumethrin based dip at the recommended use rate of 44 ppm, and also at the stronger rate of 66 ppm used for tick control (Syng *et al*., 1995). Following the identification of these two isolates, a further two flumethrin resistant strains were identified in 1995, both originating from northeast England (Clarke *et al*., 1996). All these isolates were confirmed resistant after extensive laboratory dippings at the VLA (Weybridge). These were the first confirmed cases of acaricide resistance in *P. ovis* in Europe. Further populations have subsequently been shown to be resistant to flumethrin by field investigations and the problem appears to be widespread. In the winter of 1995, a strain of *P. ovis* isolated from northern Scotland, was confirmed resistant to the OP propetamphos after controlled dipping at the VLA (Weybridge) (Bates, 1998; Clarke *et al*., 1996).

Amitraz has been shown to be effective against *Psoroptes* (*Sarcoptes* and *Chorioptes*) (Curtis, 1985; Muñoz Cobenas *et al*., 1978) but dip formulations are not currently licensed in the United Kingdom for scab control, although they are licensed in South America, South Africa and mainland Europe. Although effective against scab, they are very expensive and only used as "OP resistance breakers" and the dip wash has to be stabilized in the dip bath using calcium hydroxide. Cheaper generic amitraz products are now available. The OP sebacil (phoxim) is

licensed in Europe (not the United Kingdom or the Republic of Ireland) for scab control (Meerman, 1978; Worbes, 1995).

*Psoroptes* mites are known to colonize deep within the ear canals of sheep, and dip wash containing a blue dye did not penetrate the ear canal completely (Bates, personal communication). Mites in the ears could therefore survive dipping and their exposure to sublethal concentrations of acaricide could select for resistance.

SP plunge dips are, in general, more effective than pour-on formulations. This is not only because of their acaricidal effect, but also because they physically wash the scab lesion. Since the deregulation of scab in 1992, stockowners (particularly those suffering from the toxic effects of OPs) were no longer obliged to use plunge dips and were confronted with a wider choice of products for the control of ectoparasites. SP pour-ons are not effective against sheep scab (Bates, 1993) and their routine use in the United Kingdom for the control of lice, ticks, blowfly or headfly, could have induced resistance to SP dips, or even augmented existing SP tolerance within a population (Bates, 1998). The belly and legs cannot be reached by pour-on formulations, thus increasing the probability of mite survival.

**Systemic injections**

Macrocyclic lactones (MLs) are fermentation products of soil micro-organisms and have been chemically modified to produce the avermectins (ivermectin and doramectin) and the milbemycins (moxidectin), with greater potency and broader spectrum anti-parasitic activity than their fermentation precursors (abamectin and nemadectin, respectively). The first endectocide to be licensed for scab control was ivermectin (derived from *Streptomyces avermitilis*), with two subcutaneous injections given seven days apart (Bates and Groves, 1991; O'Brien *et al*., 1993; Soll *et al*., 1992). Unfortunately it offered little or no residual protection against re-infestation, therefore sheep must not be returned to infested pens/pastures for at least 17 days. In September 1997 another avermectin, doramectin, was licensed for scab control and was curative after a single intramuscular injection (Bates *et al*., 1995; McKenzie, 1997). Noticeable failures to doramectin have been recorded in France, where it is administered as a single subcutaneous injection at 200 μg/kg (Personne, personal communication ). In the United Kingdom it is administered as an intra-muscular injection at the higher rate of 300 μg/kg. Although the recommendations for doramectin in Europe only require one injection, two injections are required in Argentina. Studies in neighbouring Uruguay have demonstrated that a single, intra-muscular injection of doramectin at 200 or 300 μg/kg was 100 percent effective in controlling artificial infestations of sheep scab (Cardozo *et al*., 2000).

Single or double subcutaneous injections of the milbemycin, moxidectin (derived from *Streptomyces cyaneogriseus*) have been shown to cure scab and to provide residual protection against reinfestation for 28 days (O'Brien *et al*., 1994b; O'Brien *et al*., 1996; Williams and Parker, 1996; Parker *et al*., 1999). Moxidectin does not possess the disaccharide side chain (present in all the avermectins) and has unique side groups: a methoxine group and a dimethylbutenyl group. These subtle differences in molecular structure give rise to markedly different pharmacokinetics and potency properties of moxidectin compared to the avermectins. MLs are referred to as "endectocides" being effective against both internal (endo-) parasites and external (ecto-) parasites. Their main advantage over plunge dipping is that they are quicker and safer to use, cause less stress to the sheep (including pregnant ewes), do not require any special handling facilities and fixed equipment (i.e. dip baths) and there are not the same environmental concerns over the disposal of spent products (Bates, 1993). They also have the added advantage that they are effective broad-spectrum anthelmintics. Their main disadvantage is their relatively narrow range of efficacy against ectoparasites, and alternative compounds may be required for the control of lice, ticks and blowfly. They also require a relatively long meat withdrawal period

(Bates, 1993). Because sheep scab is a form of allergic dermatitis, sheep can suffer irritation for some time after eradication of *P. ovis* by MLs (Bates and Groves, 1991).

In Argentina the availability of over 40 relatively cheap, generic, ivermectin based products has led to injection surpassing plunge dipping in popularity, with grave concerns regarding the selection for ivermectin resistance. Many farmers have now broken up their dipping facilities, relying solely on the use of endectocides. In the United Kingdom and South Africa there is an increasing problem with chewing lice (*Bovicola ovis*), through the sole use of endectocides for scab control.

Long acting formulations of ivermectin, effective after single injection (at 300mg/kg), are licensed in Argentina (but not currently in the United Kingdom). Ivermectin is still detectable 30 to 35 days after treatment, but withdrawal periods are less of a problem for wool growers.

Ivermectin has also been studied as an intra-ruminal controlled release capsule (CRC). Complete eradication of *P. ovis* was achieved within 28 days of administration with protection against re-infestation for 21 to 28 days (Bridi *et al*., 1998).

In the United Kingdom single subcutaneous injections of ivermectin (200 μg/kg) failed to eradicate artificial infestations of a moderately virulent population of *P. ovis*. Mite numbers were reduced by 52 percent within 24 hours, 90 percent within 10 days and 96 percent within 20 days, but live mites were still detectable 86 days after treatment (Bates and Groves, 1991). The numbers of surviving mites correlated directly with the mite burden at the time of treatment (Bates and Groves, 1991). Moulting (pharate) mites cannot feed, consequently they may only ingest sub-lethal concentrations of acaricide once they are active. Potential for this evasive strategy therefore increases proportionally with mite population at the time of treatment (i.e. the virulence of the population). Differences in the efficacy of single injections of ivermectin with respect to mite virulence were thus observed (Bates, 1994). Low virulence populations (characterized by low mite numbers) can be almost eradicated after a single injection, yet significant numbers of mites survive within high virulence populations (characterized by high mite populations). Double injections however eradicated all populations of sheep scab mite (National Office of Animal Health, 2000).

Oral drenching with ivermectin produced a 48 percent drop in mite numbers within 24 hours of treatment, but there was little further decline and no relationship between the initial mite burden and the extent of control (Bates and Groves, 1991). The apparent inefficacy of oral ivermectin may have significant effects on the epidemiology of sheep scab by extending the sub-clinical phase, or selecting for resistance to other endectocides administered by injection (e.g. doramectin, ivermectin or moxidectin).

## 9 APPLICATION METHODS

The choice of formulation and method of application of the acaricide naturally depends on the size of the farm and the management system. Small-scale farming operations facing mange problems might achieve control by using spray or pour-on formulations. Medium and large farms with more facilities and equipment, might use immersion dips or injectable formulations. In-feed preparation for pigs and boluses for cattle, are other alternatives. An ideal acaricide should be economically acceptable, easily applicable and should have good efficacy with sufficient residual effect to protect animals from re-infection. It should not select for resistance due to its gradual decay on the animal (i.e. it should have a sharp cut-off in efficacy with time). In addition, it should have a minimal toxicological effect on animals and man, with only minimal residues in meat and milk. Unfortunately, such an ideal acaricide has not yet been produced.

**Plunge dips**

Plunge dips remain one of the most efficient and reliable methods for routine acaricide applications at farm level.

***Advantages:*** With this procedure, the animals is completely wetted, all parts of the body having adequate contact with the acaricide solution.

***Disadvantages:*** Problems with maintenance of the correct concentration of the acaricide are common. Elaborate installations for handling of animals are necessary. There can be environmental pollution from the run-off liquid when the animals emerge from the dip. The facilities are expensive to build. They are not appropriate for some acaricides (such as MLs) for stability and other reasons.

**Wash/Spray**

Application of acaricide to sheep and cattle can be carried out using various modes of spray devices, e.g. spray races or corridors, motorized pumps, backpack manual pumps.

***Advantages:*** If carried out correctly, animals receive more individual treatment; the amount of the acaricide applied is controlled and the concentration of the acaricide is adequate. Spraying is also generally less expensive per head than dipping, and the chemical group can easily be changed. No stabilizer is required for amitraz if it is used immediately.

***Disadvantages:*** The animals are not always completely wetted, especially in the lower body parts, insides of the ears, etc. Animals must be appropriately secured during the operation. With the backpack manual pump, it is time-consuming and fatiguing for the operator. The use of manual spray pumps may well be the simplest method of acaricide application to animals, but not necessarily the most effective. Its success depends very much on the operator's skills and the effectiveness of restraining the animals. There is the risk of environmental pollution. There is increased risk of intoxication to the operators. There are frequent problems with blocking of the spray nozzles.

**Pour-ons**

The introduction of this method of acaricide application was a remarkable advance in technology for applying acaricides. A volume of the acaricide proportional to the weight of the animals is applied along/on the dorsum of the animal, from where it dissipates over its body surface to kill infesting mites. In the case of some SPs (depending on their residually active period), they could also offer continuing lethal and repellent protection against subsequently arriving mites. In the case of ML compounds, the method permits the parasiticide to be absorbed and to act systemically.

***Advantages:*** Acaricides are easy to apply. Environmental pollution is reduced. It is a very practical method, especially where no dip tanks are available, or in circumstances when the producer wishes to avoid dipping some of the infested animals (e.g. pregnant females, just a few animals need to be treated, etc.). Some of the SP compounds can be applied with this formulation. New formulations of MLs and other compounds employing this method of application are being introduced onto the market and offer an alternative for the control of pyrethroid resistant strains of sheep and cattle mite.

***Disadvantages:*** The higher cost of these new compounds may be an initial limitation for many farmers in developing countries. High concentrations of the applied chemicals are needed for good efficacy. There are currently no pour-on formulations available for the control of sheep scab.

**Injectable formulations**

This is another practical alternative to avoid the dipping or spraying of animals with acaricides. Most of the injectable products currently on the market are the MLs.

***Advantages:*** There is reduced environmental pollution, except possibly in the dung pats where non-target species may be affected. There is a broad spectrum of action (against endo and ectoparasites). They also provide alternative acaricides for the control of pyrethroid and amitraz-resistant strains.

***Disadvantages:*** Possible residues of such products in milk restrict their use in dairy animals. In general terms, these compounds are more expensive than the other alternatives.

## 10 NON-CHEMICAL CONTROL

Alternative control strategies including vaccines and biological control are unlikely to be widely available in the near future and even when they are, they will be integrated with chemotherapy (Hennessey and Andrew, 1997).

## 11 CONTROL STRATEGIES UNDER DEVELOPMENT

**Action by national agricultural departments**

To assure the success of any control programme, resistance testing is necessary before deciding which pesticide should be used and resistance monitoring needs to be continued during the campaign (Thullner, 1997). The frequency of resistance monitoring activities will depend on the parasite's generation interval (Thullner, 1997).

Regional or national veterinary authorities, local veterinary surgeons, agricultural extension services and farmers' groups should be informed if resistance has been identified in their localities. This will allow the situation to be monitored and if required, the correct alternative treatments to be prescribed.

The laboratory carrying out or responsible for resistance testing should be recognized by the regional or national authority concerned. The same laboratory should be responsible for resistance monitoring (Thullner, 1997).

A standard methodology for resistance diagnosis should be defined and agreed upon by all parties involved. A definitive standard operating procedure (SOP) should be prepared that is compliant with an agreed quality scheme (e.g. Good Laboratory Practice (GLP)).

This quality scheme should include a definition of the susceptible reference isolate (Thullner, 1997) and should be low cost and unsophisticated, but validated at regular intervals by a central laboratory using more sophisticated methods.

The maintenance of a susceptible reference strain is required (Thullner, 1997) and should be maintained according to an agreed SOP at the recognized laboratories.

The risk of cross-resistance needs to be covered within the context of efficacy testing. This requires the nomination of resistant strains (Thullner, 1997), again maintained according to an agreed SOP at the recognized laboratories.

A scheme for monitoring resistance development after product registration should be defined.

**Action by research institutes.**

In addition to reliable resistance testing and monitoring, there should also be investigations into resistance mechanisms and their development. This will allow resistance risk analyses to be carried out in the future (Thullner, 1997).

Research efforts must concentrate on the better use of existing insecticide technology (optimizing treatment times, understanding the resistance status of the target pest, etc.) (Levot, 2000).

Investigations into the host specificity of ectoparasites and possible refugia from treatment is necessary.

**Action by regulatory authorities**

The cornerstone for sustainable pesticide resistance management (PRM) is the consideration of the resistance issue in pesticide registration requirements. This should cover proper pesticide use, resistance diagnosis and monitoring and preventative measures (Thullner, 1997).

A scheme for monitoring resistance development after product registration should be defined.

The diagnosis of resistance in non-target species through intensive use of a product should be a consideration at registration.

Consideration should be given to the method of product application. The easier this is, the more effective the product is likely to be in the field.

The actual regulations for the registration of veterinary products should be considered. In the United Kingdom prior to 1992, all dips etc. requiring scab approval had to be 100 percent effective against sheep scab. This also conferred 100 percent efficacy against chewing lice. After the deregulation of scab in 1992, the European Commission stipulated in 1974 that only 95 to 98 percent efficacy was required for the licensing of ectoparasiticides. What happens to the two to five percent of the parasites not susceptible to the treatment?

Methods of testing the efficacy of ectoparasiticides for registration should be standardized using an agreed SOP.

**Action by the farmer**

Strategies should be based on Integrated Pest Management (IPM) techniques, exploiting the biology of the pest, reducing selection pressure to a minimum, increasing the useful life of a pesticide and decreasing the interval of time required for a parasite to become susceptible once more to a given pesticide (National Research Council, 1986).

Insecticides should only be used if absolutely necessary and an annual “blanket treatment” of the whole flock should be avoided.

Rotation, alternation or sequences of different classes of parasiticide or different modes and sites of action to control the same parasite are accepted as valid strategies to avoid resistance (National Research Council, 1986). Do not use an SP dip if an SP pour-on is routinely used for the control of other ectoparasites.

Where there is no refuge for the population exposed to the insecticide there is a high selection pressure. This is extremely important in the case of permanent parasites such as mites or lice.

Treatment of uninfested animals is undesirable.

Reduce the use of insecticides.

All oncoming stock should be quarantined for at least three weeks (21 days), observed for signs of infestation and only treated if an ectoparasite has been diagnosed. If an ectoparasite is suspected, a veterinary surgeon should be consulted to advise correct treatment.

If ectoparasites are suspected, the parasite should be professionally identified to ensure that only a product licensed for the control of that parasite is administered, and administered correctly.

Quarantined animals should not be mixed with the main flock until treatment is complete and the parasite eradicated.

In an area where resistance has occurred, continued use of a pesticide may be required to control other parasites which remain susceptible. This could confound attempts at parasite management. In the United Kingdom, SP pour-ons have in the past been used for the control of ticks (*Ixodes ricinus*) in upland grazing (also where scab and lice are currently a serious problem). Use of macrocyclic lactones as anthelmintics (employing injections, oral dosing or slow release boluses) may select for resistance in ectoparasites. The use of OP or SP plunge dip formulations administered through shower dips or jetting races may also select for resistance.

Using doses that are less than 100 percent effective may reduce the threat of resistance if low levels of the parasite can be tolerated (Kunz and Kemp, 1994), i.e. meat producing sheep and chewing lice.

The excessive use of parasiticides for short-term gains may be the worst possible practice in the long term (Kunz and Kemp, 1994).

Fewer or less frequent applications, which reduce the selection pressure over time, would decrease the rate and probability of resistance development (Kunz and Kemp, 1994).

Apply existing products effectively and according to the manufacturer's instructions and using "Good Treatment Practice" (Bates, 1999e). Attention must be paid to the maintenance of plunge dips and showers. The capacity of the dip bath or sump must be accurately calculated and recorded, as should any drop in volume in relation to replenishment. Attention should be applied to top ups and replenishment.

Not all insecticides or their methods of application are effective against all ectoparasites (i.e. broad spectrum). The parasite infesting the flock must be professionally identified and the correct, licensed treatment administered (and administered correctly). The routine use of SP pour-ons for the control of lice, ticks, blowfly or headfly, could have induced resistance to SP dips, or even augmented existing SP tolerance within a population.

Ectoparasites have relatively short generation times, producing relatively large numbers of offspring per generation. The product label instructions must be carried out. If the label states two treatments, then two treatments must be administered. The first treatment will only kill the active stages of parasite present on the sheep at the time of treatment. The second treatment will kill any eggs that have hatched since the first treatment.

Once the sheep are mixed with the main flock the buildings/paddocks housing the infested sheep must be thoroughly cleaned and disinfested with a suitable insecticide. All litter and discarded wool must be collected and burnt or deposited out of sheep contact. No sheep should be housed/grazed in the disinfested area for at least 21 days.

## 12 RESISTANCE MANAGEMENT AND INTEGRATED CONTROL

The development of resistance to current chemical classes of insecticide/acaricides presents an undeniable threat to the long-term viability of the animal health industry (Hennessey and Andrew, 1997). The significant cost of research and development of new therapeutics for food producing

animals, together with the small market share of animal health products is a positive disincentive for drug development. The chemical actives currently available are all that we are likely to have for the foreseeable future, and they must be used more effectively (Hennessey and Andrew, 1997). Insecticides available to producers will probably be "lost" at a greater rate than the registration of new compounds (Levot, 2000). If concerns over residues mean that consideration is given to deregistration or further regulation of pesticide use, producers must be provided with alternative control strategies (Levot, 2000). Rational pest control strategies are needed to manage resistance, not only to prolong the effectiveness of current pesticides, but also to reduce the environmental impact of these substances (Kunz and Kemp, 1994).

Although efforts to establish integrated pest management (IPM) are increasing, control and eradication campaigns still depend largely or totally on pesticides, and can therefore be jeopardized by pesticide resistance (Thullner, 1997). Pesticide resistance triggers a chain reaction which, through deteriorated efficacy, leads to more residues and finally becomes an obstacle to world trade, particularly when maximum residue levels (MRLs) are exceeded (Thullner, 1997). It is often assumed that survivors do not receive a lethal dose and farmers may react by increasing the dosage or frequency of application, resulting in further resistance of susceptible parasites and an increase in susceptible individuals (Kunz and Kemp, 1994). This irrational countermeasure can lead to increased residues in meat, milk, wool or hides, together with the environmental impact of processing the latter. When this happens the next step is to switch to a new product, and with the same type of persistent application, resistance to the new chemical evolves in the same way (Kunz and Kemp, 1994).

**Pesticide safety issues**

Pesticide safety is multifactorial and includes: consumer safety (meat/milk residues); operator safety (human poisoning); environmental safety (eco-toxicity) and safety to the target host species.

Operator safety is of paramount importance in pesticide approval in the United Kingdom. Repeat exposure to low levels of OP may lead to delayed toxicity. Genetic differences may contribute to differences in individual toxicity. In 1993 the purchase of OP dips was restricted to those holding a ‘Certificate of Competence’. The use of SP dips in the United Kingdom increased in parallel with their misuse and, together with the loss of disease investigation and supervision of dipping with the deregulation of sheep scab, the first cases of SP resistance began to appear.

In 1997 there was an upsurge in water pollution incidents (mainly with SP) associated with sheep dipping, particularly in Wales and the north of England. In 1998 the Certificate of Competence Scheme was extended to include SP dips. Farmers wishing to dispose of sheep dip onto land that might lead to a direct pollution incident also had to apply for a licence. Farmers then turned to the endectocides for scab control.

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# MODULE 5. LICE: INSECTICIDE RESISTANCE: DIAGNOSIS, MANAGEMENT AND PREVENTION

## 1 INTRODUCTION

Lice (Phthiraptera) are wingless, dorso-ventrally flattened, permanent ectoparasites of birds and mammals. Over 3000 species have been described, mainly parasites of birds, and are divided into four readily recognizable groups (Anoplura, Rhynchophthirina, Amblycera and Ischnocera) (Kettle, 1995). Simplistically the Anoplura (and Rhynchophthirina) are blood-sucking lice and the Amblycera and Ischnocera (collectively known as the Mallophaga ("wool eaters")) are chewing lice (erroneously called "biting lice"), feeding on skin debris and hair. Lice infest a wide range of domestic livestock, including pigs, cattle, goats and sheep (**Table 1**) and cause a chronic dermatitis (pediculosis), characterized by constant irritation, itching, rubbing and tagging and biting of the hair or fleece. Lice are closely adapted to their hosts and completely dependent upon them for survival.

**Swine lice**

The blood sucking pig louse (*Haematopinus suis*) is the largest louse occurring on domestic livestock, with females 5 mm long. The entire life cycle, from egg to egg takes 29 to 33 days (Lancaster and Meisch, 1986). Pigs are the only hosts and in heavy infestations a pig may be covered with hundreds of lice. Lice frequent the folds of the skin on the neck and jowl, inside the ears, the base of the ears, inside the legs, flanks, and in smaller numbers on the back (Lancaster and Meisch, 1986). Lice attack pigs of all ages, feeding as often as four times per day, with associated constant irritation (Lancaster and Meisch, 1986). As sows farrow, the piglets are quickly infested, resulting in unthrifty growth and production. *H. suis* has been estimated to cause a 2 percent reduction in weight, translating to US$154.4 million per annum in the United States (Drummond *et al*., 1981). Pig lice can be completely eradicated if proper attention is paid to the thoroughness of application and the control of re-infestation. All stock (boars, sows and piglets) should be treated, even those not presenting lice. Care must be taken to treat the ears, both inside and out. All stock coming on to a farm (oncoming stock) must be quarantined and treated, before mixing with the home herd.

**Cattle lice**

Domestic cattle (*Bos taurus*) are the primary hosts of one species of chewing louse (*Bovicola bovis*) and three species of sucking louse (the long nosed louse, *Linognathus vituli*; the little blue louse, *Solenopotes capillatus* and the short-nosed ox louse, *Haematopinus eurysternus*) all of which are cosmopolitan ectoparasites found on cattle throughout the world. *B. bovis* is the most common species in the United Kingdom (71.4 percent of cases), *L. vituli* and *S. capillatus* comprising 26.1 percent and *H. eurysternus* comprising only 2.5 percent of cases. Zebu cattle (*Bos indicus*) are considered the type hosts of the cattle tail-louse (*H. quadripertusus*), although it has also been recorded on *B. taurus* x *B. indicus* hybrids. *H. quadripertusus* has been recorded in the southern United States, the Panama Canal zone, Puerto Rico, Costa Rica, Mexico, Venezuela, sub-Saharan Africa, Madagascar, India, Sri Lanka, Malaysia, Taiwan, Seychelles and Australia (Meleney and Kim, 1974). *H. tuberculatus* (the buffalo louse) is another bovine anopluran louse, principally an ectoparasite of water *buffalo (Bubalas bubalis*) and as such has been recorded where buffalo have been introduced and domesticated (Egypt, the Philippines, Australia, Madagascar, China and Myanmar) (Lancaster and Meisch, 1986). It has also been found on cattle in close association with buffalo (Lancaster and Meisch, 1986).

**Table 1. Anopluran and mallophagan lice affecting domestic livestock**

| Host | Anoplura (sucking) | Insecticide resistance |
|---|---|---|
| Cattle | *Haematopinus eurysternus* | BHC (FAO, 1991), DDT (FAO, 1991) |
| | *Haematopinus quadripertusus* | |
| | *Haematopinus tuberculatus* | |
| | *Linognathus vituli* | |
| | *Solenopotes capillatus.* | |
| | *Bovicola bovis* | |
| Goats | *Linognathus stenopsis* | DDT (FAO, 1991) |
| | *Bovicola caprae* | |
| Angora Goats | *Linognathus africanus* | BHC, aldrin, dieldrin (FAO, 1991) |
| | *Bovicola limbata* | |
| Horses | *Haematopinus equi* | |
| | *Bovicola equi* | |
| Sheep | *Linognathus ovillus* | |
| | *Linognathus pedalis* | |
| | *Linognathus africanus* | |
| | *Bovicola peregrina* | |
| | *Bovicola ovis* | Cypermethrin (Boray *et al*., 1988; Levot and Hughes, 1990; Johnson *et al*., 1992; Levot *et al*., 1995) |
| | | Deltamethrin (Levot *et al*., 1995; Bates, 2001) |
| | | Cyhalothrin (Levot *et al*., 1995) |
| | | Alphacypermethrin (Johnson *et al*., 1992; Levot *et al*., 1995) |
| | | Diazinon (Levot, 1994) |
| | | BHC (Barr and Hamilton, 1965) |
| | | Aldrin (Barr and Hamilton, 1965) |
| | | Dieldrin (Barr and Hamilton, 1965) |
| Pigs | *Haematopinus suis* | Dichlorvos (Muellar and Bulow, 1988) |

The presence and feeding of lice cause irritation, with cattle reacting by rubbing and scratching, resulting in patchy hair loss, sores and untidy appearance. Damage through sucking lice occurs through blood-loss and in serious cases, anaemia, abortion and death. Since records often indicate the presence of two or more species within a herd at a given time, the particular species is relatively less important than the total number of lice on the animal (Lancaster and Meisch, 1986). The actual prevalence of cattle lice is grossly underestimated. A postal survey carried out in the United Kingdom revealed that 50 percent of farmers thought their herds were infested, yet subsequent farm visits suggested this was an underestimate (Milnes, personal

communication). Examination of 470 hides at abattoirs during the winter revealed 377 (80.2 percent) positive for lice (Milnes, personal communication).

Lice can significantly affect hide and leather quality but reports on their effects on live weight gain are equivocal. The type of leather damage is specific, resulting in discrete areas of grain loss termed light spot or fleck. Ectoparasites accounted for 70 to 90 percent of damage to hides in the United Kingdom (costing the bovine leather industry £20 million per year), with lice accounting for 40 to 60 percent of damage (British Leather Confederation, personal communication). The economic impact of lice on cattle production is not well recognized, primarily as their effect on leather is not a direct concern for the producer.

Several workers (Gibney *et al*., 1982; Tweddle *et al*., 1977; Oormazdi and Baker, 1980; Chalmers and Charleston, 1981) demonstrated no significant weight gains resulting from the treatment of low to moderate infestations. Other researchers, although agreeing that cattle in poor condition tend to carry more lice than well nourished cattle, identified no effect on normal growth rates when adequate feed was available (Cummins and Graham, 1982).

Other researchers report that reduced feed intake and reduced weight gain are common sequelae to lice infestations that can have a profound impact on productivity. It has been recorded that dairy heifers were prone to develop severe infestations, retarding their growth, resulting in less production potential when they became producing cows (Matthysse, 1946). Nutritional status of the host may influence the degree of lousiness, with undernourished calves presenting heavier louse burdens (Cummins and Tweddle, 1977; Cummins and Graham, 1982). It is generally conceded that young animals are more susceptible. Calves infested in the autumn do not gain weight at normal rates during the winter and remain stunted until spring. An additional daily weight gain of 250 g has been recorded for treated calves with mixed species infestations compared to untreated controls (Kamyszek and Tratwal, 1977). Estimated losses in the United States (including control costs) have been cited as between US$126.3 million and US$130 million (Drummond *et al*., 1981; Chalmers and Charleston, 1981; Meyer and Koop, 1987), with an estimated loss of 30.9 kg per head in weight for 12 percent of the cattle slaughtered (Drummond *et al*., 1981).

The indirect effects of lice are not generally recognized. Irritation causes the animal to rub and scratch against any available object, causing physical damage to the skin and the resulting leather. Another less direct area of financial loss is the damage to fencing, buildings and equipment through excessive rubbing and scratching. There may also be an association between lice and the ringworm fungus, *Trichophyton verrucosum* (Kamyszek and Tratwal, 1977).

Like all lice, cattle louse infestations are seasonal, with peak numbers in the autumn and winter. This seasonality is influenced by the density of hair coat, condition of the hair, nutritional state, crowding, exposure and other stresses. Infestations begin to decrease with the shedding of the winter coat, with numerous eggs attached to the hair. Exposure to sunlight, improvement in nutrition through new grass and release from winter crowding all contribute. Lice survive the summer on "carrier" animals, who are sufficiently "different" in some way that allow louse populations (sometimes heavy) to be maintained throughout the year. A single carrier animal in the herd will re-infest the entire group when environmental conditions become viable. In the United Kingdom 35 percent of hides examined at an abattoir in August were found to have lice (Milnes, personal communication).

Consequently without any definite economic incentive, the need to treat cattle for lice has been questioned (Bailey *et al*., 1984). Specific treatments for lice are uncommon throughout the world, however products aimed at other parasites (e.g. *Hypoderma* sp., *Boophilus* sp. and *Haematobia* sp.) have had an effect on cattle lice populations, ranging from eradication or

reduction to sub-clinical levels. In the Republic of Ireland during the national campaign for the eradication of *Hypoderma* sp., using organophosphate (OP) pour-ons annually, there was a significant reduction in the prevalence of lice compared to Northern Ireland where only warble-infested cattle were treated. Withdrawal of compulsory treatment in the Republic in 1975 resulted in an increase in louse infestations (Oormazdi and Baker, 1977).

Proper timing is essential and the choice of insecticide makes it possible to control other ectoparasites, thus reducing overall control costs.

**Goat lice**

Domestic goats can be infested with the blood sucking lice, *Linognathus africanus* and *L. stenopsis* and the chewing species, *Bovicola (Damalinia) caprae* (Kettle, 1995). Fibre producing angora goats can be infested with two species of chewing louse: the red louse, *Bovicola limbata* and the less common *B. crassipes*. *B. limbata* is an important parasite of angora goats in Britain (Bates *et al*., 2001), Argentina (Olaechea, personal communication) and South Africa (Fourie, personal communication). In Britain their control relies on spring and autumn plunge dipping in OP or synthetic pyrethroid (SP) (Bates *et al*., 2001).

The host specificity of goat and sheep chewing lice is open to question with cross-infestations reported, but these are unlikely to be common occurrences (Hallam, 1985; O'Callaghan *et al*., 1988). Small numbers of live Angora lice (*B. limbata*) were observed on Saanen dairy goats within four months of exposure to infested Angoras, but these did not establish permanent colonies and were not observed once the goat was isolated from further exposure (Bates *et al*., 2001). *B. limbata* were never observed on similarly exposed sheep (Bates *et al*., 2001).

Malathion, chlorfenvinphos and cypermethrin pour-ons have been shown to be effective against lice on dairy goats (Taylor *et al*., 1984; Himonas and Liakos, 1989). A water-based deltamethrin formulation has been registered for goats (and sheep) in Australia (Levot, 2000). In South Africa the insect growth regulators (IGRs) triflumuron and diflubenzuron are effective against *B. limbata*.

Insecticidal treatments are generally more effective immediately after shearing (Medley and Drummond, 1963; Chamberlain and Hopkins, 1971) and goats may need several treatments between shearing, at approximately six monthly intervals (Darrow, 1983). Kids can be infested within two days of birth; another critical time for treatment is therefore before kidding (Fivaz *et al*., 1990). Showers and jetting races are becoming popular in Britain for the control of blowfly and lice on sheep (Bates, 1999a) and have been used for controlling Angora goat ectoparasites (Wilson *et al*., 1978), but the wetting of animals with high volume sprays in cold, windy weather may also predispose them to pneumonia.

**Sheep lice**

Lice probably occur in all sheep producing countries, but with the exception of wool producing Australia and New Zealand, attract little attention. This is reflected in the numbers of scientific publications on lice originating from these two countries over the last fifty years.

Three species of louse commonly infest sheep: the chewing louse *Bovicola ovis* (formerly *Damalinia ovis*) and the two blood sucking lice *Linognathus ovillus* (the face louse) and *L. pedalis* (the foot louse). In South Africa sheep can also be infested with *L. africanus* and *B. peregrina*) (Fourie and Horak, 2000).

The face louse or blue body louse (*L. ovillus*) has been recorded in Australia, France, New Zealand, the United States, the United Kingdom and probably all other sheep rearing countries. *L.*

*ovillus* can be found on both the haired and woolled areas of the face. As populations increase, infestations can spread over the woolled skin of the entire body. Dense accumulations of *L. ovillus* on the face can discolour white hair or wool to a definite grey. In Tasmania, *L. ovillus* has been observed more frequently in recent years, presumably due to the popularity of pour-on treatments for body lice (*B. ovis*), which have no claimed effect against face lice (Butler, 1986). The foot louse, *L. pedalis* is morphologically similar to *L. ovillus* and occurs in Africa, Australia, the United States and South America. In the United Kingdom the foot louse may have succumbed to the eighteen years of annual compulsory dipping against sheep scab (*Psoroptes ovis*), and has not been recorded for at least twenty years (Bates, 2000). *L. pedalis* inhabits the haired skin between the hooves and knees and hocks, usually forming stationary clusters (often reaching several hundred insects per square centimetre). Heavy infestations may spread onto the woolled areas of the abdomen and scrotum. Adaptation to woolless areas of the sheep limbs allows *L. pedalis* to survive low environmental temperatures for twice as long as *L. ovillus.* Lambs can be infested with *L. pedalis* within 48 hours of birth. Heavy infestations cause foot stamping and biting and can bring about lameness.

The chewing (or body) louse *B. ovis* is a small, pale to red/brown insect with a broad head and chewing mouthparts, feeding on epithelial scales, wool fibres and skin debris. *B. ovis* favours areas close to the skin, especially on the withers, sides and flanks. *B ovis* is a permanent ectoparasite, but its bionomics are greatly influenced by climate. Eggs are individually cemented to wool fibres and hatch after 1 to 2 weeks. The three nymphal stages live for 1 to 3 weeks with the total time from egg to egg being 3 to 5 weeks. Adults can live for up to a month on the host (laying approximately 30 eggs (Kettle, 1995). Recent laboratory studies have shown that adults and nymphs can survive off the host for 11.7 and 24.1 days respectively. If provided with raw wool, lice survived longer (29 days for nymphs). In shearing sheds in winter and early spring, lice survived for up to 14 and 16 days respectively (Morcombe *et al*., 1994). Transfer occurs when sheep are closely herded or penned together and in the close contact between mother and young within the first few hours of birth.

The control of chewing lice in the United Kingdom has in the past been an adjunct to the autumn compulsory scab *(Psoroptes ovis)* dip and consequently they were almost eradicated from the mainland. Pockets of infestation remained on some Scottish islands and isolated areas of Dartmoor, the Lake District and the Pennines. There has been a recent increase in the prevalence of *B. ovis* since the lifting of compulsory dipping in 1992 and lice have now become prevalent on nearly all hill-grazing in the United Kingdom (Bates, 1999b). In 1997 Uruguay abandoned compulsory dipping for the control of lice and scab. Since then the prevalence of *B. ovis* has increased and many stockowners have returned to dipping in diazinon (Mari, personal communication). Lice are also a significant problem in Argentinean Patagonia and to a lesser extent the Pampas and Mesopotamia (Bulman, personal communication)

**Economic effects**

Irritation caused by modest infestations of *B. ovis* is enough to cause scratching and rubbing, causing damage to fleece and hides but light infestations have less impact. *B. ovis* can be a significant problem for wool sheep breeds and in Australia the annual costs and losses to wool growers has been estimated to be above AU$160 million (McKenzie and Whitten, 1984). Lice have little significance on relatively woolless, indigenous, meat breeds e.g. Dorper in South Africa (Fourie and Horak, 2000) and Santa Ines and Morada Nova breeds in Brazil (Madeira *et al*., 2000). Irritation can lead to increased skin secretion and fleece yolk (wool grease and suint) (Kettle, 1985). Fleece damage, through rubbing and biting, can lead to cotting and increased carding losses due to knotted (neps) and short, broken fibres (noils) (Kettle, 1985). The economic effects of lice vary with sheep breed. Controlled studies on Romney cross sheep in New Zealand

have shown no statistically significant effects of lice on the weights of greasy or scoured fleece, although in one three year study, the washed yield from infested fleeces was lower by a mean of 2.6 percent (Kettle, 1985). There is a high correlation between louse numbers and percentage loss when wool is scoured. Infested fleeces receive lower visual grades (Kettle, 1985) and colorimetric comparisons of core samples from lousy sheep were significantly less bright and, in most cases, yellowed in colour: both features lowering wool quality (Kettle, 1985). Controlled studies on Australian Merinos however, have shown more marked effects, with significant reductions in greasy and scoured fleece weights and scoured yield, and significant increases in carding losses (Kettle, 1985). Studies in Queensland, Australia, demonstrated that the greasy and clean fleece weights from treated sheep were significantly higher than the untreated controls (Niven and Pritchard, 1985). Sheep that were treated repeatedly with cypermethrin produced significantly more wool and less cast fleece than controls. Differences in wool value between treated animals and untreated controls ranged between AUS$0.45 to AUS$ 3.19 per sheep (Niven and Pritchard, 1985). Similarly, studies in the northeast of England demonstrated that infested sheep treated with a propetamphos pour-on produced 34 percent more wool than untreated controls and the wool from the treated sheep was of better quality (Ormerod and Henderson, 1986).

Clearly the economic significance of lice to the farmer is dependent upon the system used to determine the base price paid for wool and on the price differentials applied to lower grades of wool (Kettle, 1985). The economic significance of lice therefore, depends on their effects on grading, which directly affects prices. Wool grading is based on many parameters including:

- fibre diameter, length and strength;
- colour and brightness;
- bulking capacity;
- presence or absence of staining or cotting;
- the amount of extraneous vegetable or mineral matter (Kettle, 1985),

all of which can be affected by lice infestations. As is the case for cattle, the effects of lice infestations on live weight gain in sheep are equivocal. Controlled studies in New Zealand and Australia failed to show any adverse effects (Niven and Pritchard, 1985; Kettle and Lukies, 1982), but studies in the United Kingdom demonstrated a mean 18 percent live weight gain in treated sheep compared to untreated (Ormerod and Henderson, 1986). Lice are therefore of less importance where wool is not the primary product.

*B. ovis* can also affect the quality of hides and processed leather. In the United Kingdom ovine ectoparasites (lice, scab and blowfly Myiasis) cost the ovine leather industry £15 to 20 million per year (British Leather Confederation, personal communication). Immediate hypersensitivity to *B ovis* secretory and excretory products can result in a nodular skin defect ("cockle"), down grading the value of the leather. Cockle is detected after depilation, but usually first noted on the pickled pelt or tanned stage of processing (Heath *et al*., 1995). On sheep where lice were removed through treatment or shearing, cockle lesions either disappeared or regressed on pickled pelts (Heath *et al*., 1995).

An added cost for the producer is that of voluntary/compulsory control. In New Zealand the cost of the legally required annual dipping has been estimated to cost approximately NZ$7.5 million per annum for labour and materials alone (Kettle, 1985).

## 2 RESISTANCE DEVELOPMENT

The resistance of lice to insecticides is an inherited phenomenon. It results from exposure of populations of lice to chemical insecticides and survival and reproduction of lice that are less affected by the insecticide. The higher reproductive rate of lice that have heritable resistance factors and the resulting increase in the proportion of the population of lice that carry genes for these factors is known as selection.

Resistance to a given insecticide can be described as a reduction in susceptibility of a parasite to the insecticide when it is used at the recommended concentration and according to all of the recommendations for its use.

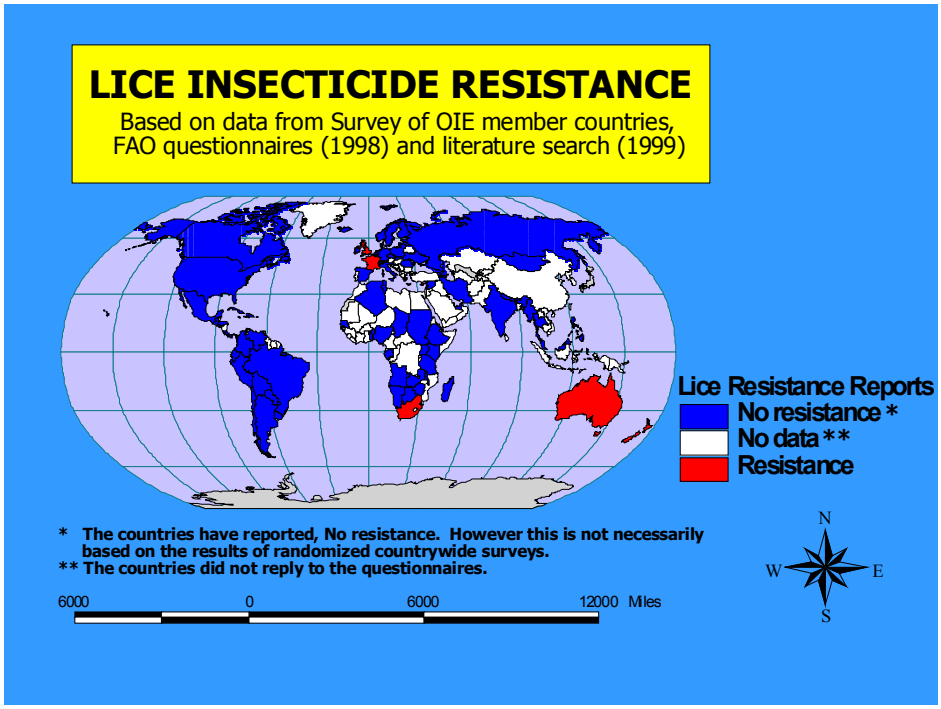
In most cases, it is likely that genes that confer resistance are already present at very low levels in the louse population before the introduction of a new insecticide. The rate at which a resistant allele becomes established in the population and the time it takes for the control of lice to break down is dependent upon many factors. These include the frequency of the original mutation in the population before treatment, the mode of inheritance of the resistant allele (dominant, co-dominant or recessive), the frequency of insecticide treatment, the concentration gradient of the insecticide, and the proportion of the total parasite population that is not exposed to the insecticide (refugia).

Although the frequency of resistant genes initially only increases slowly, by the time declining efficiency of treatment is noticed, the rate of increasing frequency of resistance genes is usually high. In the initial phase, the frequency of heterozygous resistant individuals (single allele mutation) within the population is low and the rate of increase in the frequency of the resistant allele is low. In the next, emerging phase, given continued exposure to a drug, the frequency of heterozygous resistant individuals within the population increases. Finally, the sustained selection pressure results in increasing numbers of homozygous resistant individuals, which ultimately predominate in the population.

As obligate parasites, opportunities for refugia of lice tend to be more limited than in parasites with an extended free-living phase.

## 3 CURRENT STATUS

SP pour-on products were released in 1981 but failure to control *B. ovis* was first reported in the Australian louse population in 1985 and subsequently confirmed experimentally (Boray *et al*., 1988). Although products containing cypermethrin were the subject of most early complaints, claims of failure from all SP pour-ons (including those applied to long wool) and SP plunge dips were also received. Most complaints could be traced to inappropriate applications by farmers, but in an increasing number of cases, resistance was implicated (Levot *et al*., 1995). Highest resistance factors (RF) at this time were only a factor of 26, but this was sufficient to prevent pour-ons from working effectively. Strains of lice with reduced susceptibility to SPs have now been reported in most states of Australia (O'Sullivan, 1988; De Cheneet *et al*., 1989; Johnson *et al*., 1988). By 1991 a population from Hartley, NSW was found to have a resistance factor of 642 to cypermethrin, with side-resistance conferred to other SPs (cypermethrin, deltamethrin, cyhalothrin and alpha-cypermethrin) (Levot *et al*., 1995).




An *in vitro* treated surface technique measuring the response of 30 populations of *B. ovis* from New South Wales (NSW) and Western Australia to cypermethrin recorded a wide variation in $LC_{50}$ and $LC_{95}$. Half the populations were considered to be pyrethroid susceptible, based on 100 percent mortality at 5 ppm (or less) to cypermethrin. This suggested that factors other than pyrethroid resistance were responsible for inefficient lice control. Lice surviving after exposure to 5 ppm or greater were considered provisionally to be resistant. When these individuals predominate, the proportion of lice killed by pour-on treatments is insufficient to prevent detectable infestations being present soon after treatment (Levot and Hughes, 1990). The frequency distribution of $LC_{50}$ and $LC_{95}$ were normally distributed and it was evident that the number of louse strains whose responses fell within this normal distribution were sufficient to reduce the effectiveness of backline treatments (Levot and Hughes, 1990). It was suggested that there were registered treatments that were incapable of eradicating some populations whose responses were at the top end of the normal range (Levot *et al*., 1995). Such high-level resistant populations were the Hartley NSW strain (Levot, 2000) and further high-level resistant populations from Victoria (Keys *et al*., 1993) and South Australia (James *et al*., 1993) using similar treated surface *in vitro* techniques.

Controlled *in-vivo* pen studies demonstrated that SP pour-on treatments using either cypermethrin or alpha-cypermethrin, significantly reduced louse populations but failed to eliminate infestation in 54 percent of lice strains with resistance factors greater than 4 (Johnson *et al*., 1992). One strain reported in NSW with a resistance factor of 98, was not eradicated by dipping in SP at the currently recommended rates (Levot, 1992).

SP resistance was reported in New Zealand in 1994 and low to moderate resistance to high cis cypermethrin was demonstrated using a treated surface (contact) bioassay, with resistance factors ranging from 1.0 to 12.4 recorded (James *et al*., 1993).

The principal effect in the field from high resistance factors is a reduction in the effectiveness of backline treatments applied after shearing, and of longwool treatments. Whereas strains of lice

with low $LC_{50}$ values can be eradicated by backline treatments after shearing, eradication is much less likely when more resistant strains are present (Johnson *et al*., 1988; Johnson *et al*., 1989). The effectiveness of long wool SP treatments can be dramatically reduced when resistant strains of lice are present, and often little or no reduction in lice is observed (Johnson *et al*., 1988; Johnson *et al*., 1989).

It is futile to change to another SP product if SP failure is confirmed. Until a diazinon spray-on was registered in Australia in 1994, the only OP products were aqueous dips (Levot, 2000). There was concern about the reasons why some producers changed from SP to OP products, possibly applying the same mistakes to a new product (Levot, 2000).

With an increase in the use of OP products, there was some concern over OP resistance. A toxicological survey of 28 field populations of *B. ovis* in Australia (mainly NSW) identified one strain (from Orange in central NSW) whose response to diazinon was recognizably lower than the normally distributed responses of the other strains, with an RF (at $LC_{50}$) of about 9 (Levot, 1994). Resistance to diazinon correlated positively with resistance to coumaphos, but not to propetamphos. Diazinon could therefore be recommended for the control of SP resistant *B. ovis* and an SP or propetamphos be recommended to control a diazinon resistant population (Levot, 1994).

Confirmation of SP resistance in the United Kingdom was only a matter of time. The identification of the 'kdr" gene, playing a role in the genetic evolution of resistance to DDT has since been found to provide certain insects with protection against pyrethroids (Denholm and Rowland, 1992). The intensive use of γ BHC plunge dip formulations in the United Kingdom between 1945 and 1953 and between 1973 and 1984 for the compulsory treatment of sheep scab, and the popularity of γ BHC, DDT and dieldrin between 1953 and 1972 through plunge dips, spray races or showers for the control of blowfly and lice, may have already selected for resistance. Resistance to plunge dips containing γ BHC, aldrin and dieldrin developed in populations of lice in northern England in the mid 1960s (Barr and Hamilton, 1965; Page *et al*., 1965).

In a pilot study in the United Kingdom, four populations of *B. ovis* were assessed for sensitivity to deltamethrin, flumethrin and high cis cypermethrin using a treated surface (contact) bioassay (adapted from protocols supplied by Gary Levot, E.M.A.I, New South Wales, Australia) (58). Results demonstrated a deltamethrin $LC_{90}$ for a Devon isolate to be 26.42 mg/l, compared to 13.63, 5.63 and 2.53 mg/l for isolates from Ceredigion, Dumfries and Galloway and Northumberland. Unfortunately the lack of controlled, reliable, field data (i.e. verification or authentication of both treatments and the outcome of the second treatment) rendered it impossible to confirm insecticide resistance (Bates, 2001). In March 2000, another flock, in Renfrewshire, Scotland, was suspected of being infested with an SP resistant population of *B. ovis*. Bioassay results demonstrated a deltamethrin $LC_{90}$ of 35.77 mg/l (an RF of 14.1), greater than the Devon (an RF of 10.4) isolate. Laboratory data and reliable field data thus indicated possible resistance to deltamethrin (Bates, 2001).

**Swine lice**

At present insecticide resistance in *H. suis* is rare, with the only populations resistant to dichlorvos reported in Germany (Muellar and Bulow, 1988).

**Cattle lice**

Insecticides for the control of cattle lice are shown in **Table 2**. Initially, control was achieved through OP compounds applied as sprays, dips or washes. Self-application methods such as dust bags and back rubbers, used principally for horn fly (*Haematobia* sp.), were also used to reduce

louse infestations. Pour-on formulations of OPs, and later SPs, replaced sprays and dusts because of their ease of use and *Hypoderma* sp. control. The next generation of insecticides were the macrocyclic lactones (MLs) administered by subcutaneous injection. Injections were only effective against sucking lice and were marketed simply as "an aid in the control of chewing lice" (National Office of Animal Health, 2000). The current generation of MLs, administered as pour-ons are effective against both sucking and chewing lice. All the current insecticides remain effective, although resistance to previous organochlorine or cyclodiene treatments (γ BHC and DDT) have been reported in *H. eurysternus* and *L. vituli* in Canada and the United States (FAO, 1991).

**Table 2. Insecticides effective against cattle lice**

| Insecticide | Concentration | Application method |
|---|---|---|
| Abermectin | 1.0% | Injection (National Office of Animal Health, 2000)[1] |
| Alphacypermethrin | 1.5% | Pour-on (National Office of Animal Health, 2000) |
| Chloryriphos | 50% | Pour-on (Kettle and Lukies, 1979; Kettle and Watson, 1981; Jones and Johnson, 1984) |
| Coumaphos | | Spray (Lancaster and Meisch, 1986)<br>Pour-on (Jones and Johnson, 1984) |
| Crufomate | 32% | Pour-on (Meyer and Carey, 1977) |
| Crotoxyphos - dichlorvos | nr | Spray (Lancaster and Meisch, 1986) |
| Deltamethrin | 1.0% | Spot-on (National Office of Animal Health, 2000) |
| Doramectin | 0.5% | Pour-on (National Office of Animal Health, 2000)[1] |
| | 1.0% | Injection (National Office of Animal Health, 2000)[1] |
| Dichlorvos | 2.5% | Pour-on (Majewski *et al*., 1976) |
| Dioxathion | nr | Spray (Lancaster and Meisch, 1986) |
| Eprinomectin | 0.5% | Pour-on (National Office of Animal Health, 2000) |
| Famphur | 0.04% | Spray (Klement'eva, 1979)<br>Pour-on (Jones and Johnson, 1984) |
| Fenthion | 7.5% or 20% | Pour-on (Jones and Johnson, 1984) |
| Fenvalerate | 10% | Spray (National Office of Animal Health, 2000) |
| Fipronil | | Pour-on |
| Ivermectin | 0.5% | Pour-on (National Office of Animal Health, 2000) |
| | 1.0% | Injection (National Office of Animal Health, 2000)[1]<br>Bolus (National Office of Animal Health, 2000)[1] |
| Malathion | nr | Spray (Lancaster and Meisch, 1986) |
| Methoxychlor | nr | Spray (Lancaster and Meisch, 1986) |
| Moxidectin | 0.5% | Pour-on (National Office of Animal Health, 2000) |
| | 1.0% | Injection (National Office of Animal Health, 2000)[1] |
| Permethrin | | Spray (Lancaster and Meisch, 1986) |
| | 4.0% | Pour-on (National Office of Animal Health, 2000) |
| Phosmet | 20% | Pour-on (Kettle and Watson, 1981) |
| Temephos | nr | Spray (Biriukova, 1979)<br>Pour-on (Kettle and Lukies, 1979) |
| Tetrachlorvinphos | nr | Spray (Lancaster and Meisch, 1986) |
| Tetrachlorvinphos-dichlorvos | nr | Spray (Lancaster and Meisch, 1986)[1] |
| Trichlorfon | nr | Pour-on (Jones and Johnson, 1984) |

nr not recorded
[1] Sucking lice only

**Goat lice**

Observations in Britain have suggested that SP pour-ons offer only temporary control against chewing lice (Stubbs, personal communication), and apparent SP resistance to *Bovicola* lice (species not designated) has been reported in two angora herds treated with a 2.5 percent cypermethrin pour-on, (Coleshaw *et al*., 1002). Further studies identified the parasite as *B. limbata* (Bates *et al*., 2001) and the result of *in vivo* and *in vitro* studies indicated that cypermethrin pour-ons do not eradicate *B. limbata* from full-fleeced angora goats (Bates *et al*., 2001). The fact that *B. limbata* and *B. caprae* were equally susceptible to cypermethrin suggests that the failure to control was not necessarily due to insecticide resistance (Bates *et al*., 2001). The pharmacokinetics of pour-on formulations may be different on goats with short hair as opposed to wool, and also on goats with long fibre (e.g. angora). Inefficacy may therefore be a case of product failure, and not insecticide resistance as was previously recorded (Bates *et al*., 2001).

Organochlorine resistance has been reported in *Linognathus africanus* (BHC, aldrin and dieldrin) and *L. stenopsis* (DDT) in South Africa (FAO, 1991).

**Sheep lice**

*B. ovis* is a common ectoparasite of sheep in Australia, with a distinct increase in prevalence recorded (Morcombe *et al*., 1994), strongly correlated with changes in the Wool Market Price Indicator and the failure to eradicate lice from flocks. These failures were partly a consequence of the reduced use of insecticidal treatments, the development of SP resistance and an increase in the transmission of lice between flocks (Morcombe *et al*., 1994).

## 4 DIAGNOSIS OF RESISTANCE. AN OVERVIEW OF METHODOLOGIES

When farmers experience reduced efficiency in their treatments against lice, losses in animal production can result and suitable methods to identify and monitor the situation are needed. The test must be capable of identifying resistance at an early stage of its emergence. A second requirement is that the test should be capable of covering a wide range of chemical groups including the most recently developed active ingredients. The ideal diagnostic test should also be relatively simple and inexpensive in terms of materials, drugs, lice and animal supplies. It should provide a rapid and reliable answer, it should be cheap and easy to perform and be appropriately designed for feasible use as a standard method in different laboratories within and between different countries. Currently, the available *in vitro* tests include the use of a fibre substrate that allows lice to move freely on insecticide impregnated cloth in a laboratory bioassay, or on cotton squares impregnated with insecticides in a field trial test. *In vivo* trial involves groups of animals with patent infestations of insecticide resistant lice either purchased from places where control failures had occurred or artificially infested with suspect resistant lice.

A bioassay suitable for the determination of dose response of *B. ovis* to avermectins has been developed (Levot, 1995). The inclusion of wool/skin substrate resulted in 90 percent survival of controls over the 48 h test period. Ivermectin and abamectin were highly effective against *B. ovis*, and similar responses of pyrethroid susceptible and resistant strains indicated that there was no cross resistance to ivermectin (Levot, 1995).

## 5 DETECTION OF RESISTANCE: PROTOCOLS FOR RECOMMENDED METHODOLOGIES

### 1. Laboratory *in vitro* treated surface (contact) bioassay.

A self-dosing, *in vitro* treated surface (contact) bioassay utilizing a fibre substrate that allows lice to move freely has been developed in Australia (Levot and Hughes, 1990).

Lice are removed from donor sheep using a vacuum pump.

Using micro-pipettes and volumetric glassware, a range of insecticide dilutions is prepared in acetone in the following ranges:

For SPs, 10 mg/l, 5 mg/l, 2.5 mg/l and 1.25 mg/l.

For OPs, 20 mg/l, 10 mg/l, 5 mg/l, 2.5 mg/l and 1.25 mg/l.

Two 60 × 60 mm cloth (25 ± 5 threads/cm) squares are prepared for each insecticide dilution and labelled (in pencil) with the relevant dilution.

Starting in the centre of each cloth, 1.0 ml of each dilution is pipetted onto each cloth rectangle and allowed to dry at room temperature for 24 hours.

Cloths impregnated with acetone are prepared in a similar manner.

Impregnated cloths are then inserted (using forceps) into labelled glass tubes.

Ten live lice are placed into each tube and the tubes sealed and incubated (in darkness) at 34°C for 16 hours.

If the test insecticide is an OP, the ideal relative humidity is 100 percent, achieved by having a wide, open container of distilled water in the incubation box containing the tubes. For an SP insecticide the ideal relative humidity is 70 to 80 percent, achieved using a saturated solution of NaCl instead of water.

Lice are removed from the tubes and the relative numbers of dead, knocked down or live lice recorded. Dead lice are immobile and showing signs of desiccation.

"Knocked down" lice show feeble and uncoordinated mobility with curling abdomens while live lice walk away normally.

If there is more than 30 percent mortality in the controls (lice placed in the acetone only tubes), the test should be repeated.

Results are analysed by probit regression (Bany *et al*., 1995b) and the $LC_{50}$ and $LC_{95}$ calculated.

Survival of one or more louse at 5 mg/l or greater is taken as an indication of resistance.

The incubation time is important. 16 hours exposure is the optimum, despite maximum louse responses within 2–4 hours (Levot, 2000). Mortality after 16 hours is particularly useful when slow acting OPs are to be assessed (Levot, 2000).

## 2. Field *in vitro* treated surface (contact) bioassay.

A "Field Lice Test Kit" has been developed at the Elizabeth Macarthur Agricultural Institute, NSW, Australia (Levot, personal communication).

Lice are removed from donor sheep using a vacuum pump.

Test kits consist of 5 ml specimen tubes containing 6 × 6 cm cotton squares impregnated with 1ml solutions of 0, 0.5, 1.25, 2.5, 5.0 and 10.0 mg/l pyrethroid in acetone.

Ten live, active lice are gently placed into each tube, using an insecticide free camel hair brush.

The lice are kept on the treated surface in warm conditions (35°C) for 30 min (a hot water bottle inside a "cool-box" is OK).

Lice are inspected on each surface after 30 minutes, using a magnifying glass or microscope. The condition of the controls (0 mg/l solution) is recorded first. If they are not moving freely the test is discarded.

After this incubation time lice will not be dead at any of the concentrations assessed but they will be "knocked down."

If there are any lice that behave normally at 500 or 100 mg/l the strain is likely to be highly resistant to SPs and pyrethroid dips or pour-ons will not be effective.

If at 20 mg/l all (or most) of the lice are unaffected, the strain is resistant and an SP dip or pour-on could not be guaranteed to work.

If at 5 mg/l the majority of lice are unaffected, the strain is moderately resistant. SP dips should be effective but pour-ons may work but cannot be guaranteed.

If at 5 mg/l the majority of lice are affected, the strain is quite susceptible and an SP pour-on should be expected to be effective.

The results of this test should only be used as a guide. The lice must not be stressed by environmental conditions or by recent insecticide treatments. Stressed lice may be affected by lower concentrations and give false susceptibility readings. Validation by full laboratory bioassay is required.

## 3. Pen trials

Groups of sheep with obvious infestations of insecticide resistant *B. ovis* purchased from flocks where control failures had occurred or groups artificially infested with suspect resistant lice should be used in the studies. In the latter *B. ovis* is transferred either mechanically or through natural contact with infested sheep. All sheep should have two to five lice per parting before treatment.

Where shorn sheep are required, a snow comb should be used, leaving 1 to 2 cm of wool to maintain the louse population. After shearing, the animals should be left undisturbed for 2 to 3 days before treatment.

Prior to treatment study groups of not less than seven sheep should be allocated which are equally weighted for mean louse counts and counts for the groups should be similarly ranked.

Relevant animals should then be treated with the product under suspicion of resistance, strictly according to the manufacturer’s recommendations.

For plunge dipping, the correct volume of water must be added to the dip bath (using a water meter) and the required volume of insecticide concentrate accurately measured and added to the water. The wash must then be thoroughly mixed for not less than five minutes. Dip wash samples must be taken after mixing and after the sheep have been dipped and the concentration of insecticide confirmed by chemical analysis (e.g. gas liquid chromatography (GLC)).

For pour-ons, the gun must be calibrated, as must all weighing equipment where insecticide is administered according to body weight.

Lice counts must be made before and after treatment, weekly up to 8 weeks after treatment and monthly thereafter for up to 18 weeks.

Lice are counted on ten evenly spaced partings on each side of the sheep. If no lice are found, a further 20 partings should be examined.

The arithmetic mean louse count is calculated for each treated and control group and the percentage reductions in mean louse counts determined using the Henderson-Tilton formula:-

$$R(\%) = 100 \times \frac{(1 - Ta \times Cb)}{Ca \times Tb}$$

Where:

Ta = mean post-treatment count on treated sheep
Tb = mean pre-treatment count on treated sheep
Ca = mean post-treatment count on control sheep
Cb = mean pre-treatment count on control sheep

## 6 EPIDEMIOLOGY

**Factors affecting louse populations**

Studies in the United Kingdom showed the prevalence of *B. ovis* within flocks to vary, with the majority of sheep (42.3%) carrying light infestations. Medium or heavy infestations accounting for 22.0 percent and 16.7 percent of sheep respectively. In two flocks significant numbers of sheep (19.1%) were observed to be apparently uninfested, despite light to heavy infestations on contact sheep within the flock (Bates, 2001).

**Seasonality**

Lice populations are seasonal, building up during the autumn, reaching a peak in winter, declining in spring and remaining low throughout the summer (Kettle, 1985). In the United Kingdom the majority of cases occur between January and April, although infested sheep have been recorded as late as June (midsummer) (Bates, 2001). Chewing lice have a low intrinsic rate of increase and spread slowly among sheep (Murray and Gordon, 1969; Cleland *et al*., 1989). Mortalities caused by external factors such as excessively hot or wet weather or management practices (e.g. shearing) can be reflected in louse populations for six months or more (Murray, 1963). Heavy rain resulting in saturated fleeces in the autumn can also reduce louse populations and limit the subsequent winter infestation (Kettle and Lukies, 1982).

As for cattle, young sheep appear to be more susceptible than adults (Bates, 2001; James *et al*., 1998), with the burdens on lambs reaching densities more than three times those on the ewes, even though the lambs were infested for a much shorter period (James *et al*., 1998).

Populations of lice are influenced by fleece length, with high populations observed on sheep with long fleeces (Niven and Pritchard, 1985; Bates, 2001). Suitable fleece fibres and skin temperatures are required for infestations to establish and progress. The normal temperature of sheep skin is 37.5°C, the temperature at which peak *B. ovis* oviposition occurs. In areas of low temperature (e.g. legs and tail) oviposition is inhibited. At a fleece thickness of 3.0 to 10.0 cm most eggs are laid within 6 mm of the skin surface. Even when fleece is 10.0 cm deep few eggs are laid more than 12 mm from the skin surface. In fleeces where the temperature ranges from 38°C at the skin surface to 15°C near the tip of the fleece, 69 percent of the mobile population (nymphs and adults) are within 6 mm of the skin surface and only 15% more than 12 mm away. When the tip of the fleece is shaded and warmed, adults and third stage nymphs come to the surface of the fleece. It is under these conditions that *B. ovis* spreads within a closely herded flock. Thus lice spread quickly within flocks in hot climates (e.g. Australia) and more slowly in

more temperate climates (e.g. the United Kingdom). Populations of *B. ovis* are limited by a number of factors including shearing, when 30 to 50 percent of the population can be lost. During the winter when lice populations thrive, the numbers on a sheep can increase from 400 to 4 000 by the spring.

Heavy infestations of lice are associated with young or old animals in poor health and /or maintained in unhygienic conditions. Populations of lice are influenced by the body condition of the sheep, the lower the body condition score the higher the population of lice (Bates, 2001). It is not certain whether louse infestations bring down the condition of the animal or if the lice exploit an animal already out of condition due to concomitant infections or bad husbandry. The fact that no significant differences were observed between the body weights (or the lamb percentages and lamb weights at weaning) between louse infested sheep and louse free sheep over a four year period is evidence to support the latter (Kettle and Lukies, 1982). Concomitant infections/infestations bringing the body condition down may increase an individual sheep's susceptibility to lice. Anecdotal observations in the United Kingdom have shown a possible relationship between liver fluke (*Fasciola hepatica*) infection and high louse counts (Bates, 2001). Observations in Australia demonstrated that the most prolific source of lice on a particular property was a crippled, bottle-fed lamb and a group of ewes diagnosed with ovine progressive pneumonia (James *et al*., 1998). *B. ovis* populations have been observed to increase during the winter on sheep on a low plane of nutrition (Scott, 1952). Thus it has been postulated that the presence and/or numbers of chewing lice can be a significant indicator of underlying welfare problems within a flock (Bates, 2001).

The clinical signs of chewing lice can be confused with sheep scab and thus possible resistance problems may result if the ectoparasite is not professionally identified and the correct treatment applied (Bates, 1999b). Sheep can present mixed infestations of *Psoroptes ovis* and chewing lice and unlike sheep scab infestations, sheep can carry louse burdens throughout their lives. The use of systemic endectocides (doramectin, ivermectin or moxidectin) will eradicate scab mites but will not resolve the lesion immediately. If chewing lice are present, their populations will be knocked down temporarily, only to recover in higher numbers, using the unresolved scab lesion as a food source (Bates, 1999b; Bates, 2001).

## 7 CURRENTLY AVAILABLE CONTROL STRATEGIES

The development of resistance to current chemical classes of insecticide presents an undeniable threat to the long-term viability of the animal health industry (Finney, 1971). Alternative control strategies including vaccines, biological control and breeding of parasite resistance are unlikely to be widely available in the near future and even then, they will be integrated with chemotherapy (Finney, 1971). The significant cost of research and development of new therapeutics for food producing animals, together with the small market share of animal health products, is a positive disincentive for drug development. The chemical actives that are currently available are all that we are likely to have for the foreseeable future and they must be used more effectively (Hennessey and Andrew, 1997). Insecticides available to producers will probably be "lost" at a greater rate than the registration of new compounds (Levot, 2000). If concerns over residues mean that consideration is given to deregistration, or further regulation of pesticide use, producers must be provided with alternative control strategies (Denholm and Roland, 1992). Rational pest control strategies are needed to manage resistance, not only to prolong the effectiveness of current pesticides but reduce the environmental impact of these substances (Hennessey and Andrew, 1997).

The underlying process in arthropod resistance to pesticides is genetic selection, an evolutionary process. Lice are obligate parasites, with no free-living phase, and the spread of

genes between populations could be expected to occur slowly (James *et al*., 1993). Thus the skewed distribution of resistance between populations could be a reflection of selection occurring in only some populations of lice and the relatively small rate of inter-mixing of different populations (James *et al*., 1993). The $LC_{50}$s of the high virulence strains (Levot, 1992) are well outside these distributions, suggesting the emergence of a category of resistance with a different genetic basis to that previously observed (James *et al*., 1993). Resistance is mostly controlled by a single gene (Morcombe and Young, 1993) and the widespread use of insecticides acting to concentrate the rare individuals carrying this gene. Thus, after a period of selection, the frequency of the resistant form in the population will become sufficiently high to be noticed. If the continued use of the insecticide occurs after this point, the frequency of the resistant individuals will increase until they dominate the population and as a result, an altered response to a treatment occurs. This suggests that resistance in a population is the inevitable outcome of the widespread use of insecticide. The selection pressure imposed by insecticides means that more effective control leads to more rapid development of resistance (Hennessey and Andrew, 1997). In most cases, survival following treatment is due to genetic differences rather than escape from full exposure (Hennessey and Andrew, 1997).

Inefficient application of insecticide has been implicated in the development of resistance in sheep lice (Boray *et al*., 1988). In the period July 1988 to June 1990 insecticide was applied as a backline treatment on 62 percent of Western Australian flocks, with 38 percent treated by shower dipping (Milani, 1954). In 34.7 percent of the flocks that were infested, treatment did not eradicate lice (Milani, 1954). There were no differences in the proportion of consecutive infestations between flocks treated with cypermethrin, deltamethrin or alphamethrin, applied as backline treatments. Among flocks treated in a shower dip, 68.4 percent using coumaphos, 37.8 percent using diazinon and 41.5 percent using cyhalothrin had infestations following treatment (Milani, 1954).

Although efforts to establish integrated pest management (IPM) are increasing, control and eradication campaigns still depend largely or totally on pesticides, and can therefore be jeopardized by pesticide resistance (Thullner, 1997). Pesticide resistance triggers a chain reaction, which, through deteriorated efficacy, leads to more residues and finally becomes an obstacle to world trade, particularly when maximum residue levels (MRLs) are exceeded (Thullner, 1997). It is often assumed that survivors do not receive a lethal dose and farmers may react by increasing the dosage or frequency of application, resulting in further resistance of susceptible parasites and an increase in susceptible individuals (Hennessey and Andrew, 1997). This irrational countermeasure can lead to increased residues in meat, milk, wool or hides, together with the environmental impact of processing the latter. When this happens, the next step is to switch to a new product, and with the same type of persistent application, resistance to the new chemical evolves in the same way (Hennessey and Andrew, 1997).

The currently available tools for lice control consist of chemical technology, relying on treatments with different application methods and/or formulations of chemical drugs. These can be used with or without the benefit of local epidemiology knowledge.

Three control alternatives are in used:

1. Ad hoc or opportunistic treatments.
2. Curative treatments.
3. Strategic treatments.

### 1. Ad hoc or opportunistic treatments

***Principle:*** In connection with general management practices (weaning, dehorning, change of pasture or paddocks) farmers often implement routine preventive procedures and antiparasitic treatments, sometimes using macrocyclic lactones that will not only have insecticide effects but also anthelmintic activity. It is usual that farmers decide when the animals should be treated according to their own estimates of economic thresholds for lice infestation, time available, climatic conditions, availability of personnel, drugs, and basic infrastructure.

***Prerequisites:*** No special requirements need to be met.

***Advantages:*** There is a reduced overall need for gathering animals, resulting in a reduced input of human and economic resources.

***Disadvantages:*** A single opportunistic treatment may be inefficient and results are unpredictable when assessed solely from the point of view of immediate lice control.

***Epidemiological consequences:*** The effective reduction of the overall lice population is doubtful. Re-infestation could maintain a population on the farm.

***Possible combination with other strategies:*** Regular observation to avoid the sudden spread of lice amongst the animal population.

### 2. Curative treatments

***Principle:*** Farmers implement curative treatments when some animals are presenting high numbers of lice.

***Prerequisites:*** No special requirements need to be met.

***Advantages:*** Only those animals with clinical signs are treated. There is a reduced overall need for gathering all animals on the farm, resulting in a reduced input of human and economic resources.

***Disadvantages:*** The approach is usually inefficient in the long term because the disease is maintained on the farm. Re-infestations will occur. A single curative treatment may be inefficient and results are unpredictable when assessed solely from the point of view of immediate lice control.

***Epidemiological consequences:*** An effective reduction of the overall lice population is doubtful. Re-infestation from lice eggs not affected by the treatment could maintain infestation on the farm.

***Possible combination with other strategies:*** Affected treated animals should be observed for clinical resolution and if feasible, isolated from the remaining animals.

### 3. Strategic treatments

***Principle:*** Farmers implement strategic treatments in the early autumn, before there is an increase in the number of lice, and during summer when there is a low burden of lice affecting the animals.

***Prerequisites:*** All animals should be treated.

***Advantages:*** Because animals are infected by a low number of lice, treatment removes the lice population. No further lice infestation will therefore appear next season.

***Disadvantages:*** There is need for gathering all animals on the farm, resulting in an increased input of human and economic resources.

***Epidemiological consequences:*** There will be an effective reduction in the overall lice population.

***Possible combination with other strategies:*** All introduced stock should be treated in order to avoid the introduction of "new" lice onto the farm.

## 8 CHEMICAL CONTROL

Sheep lice (and other ectoparasites) can be controlled by a number of insecticides from a wide number of chemical groups and applied by a variety of methods (**Table 3**).

**Table 3. Insecticides effective against sheep lice**

| Insecticide | Application method | Resistance |
|---|---|---|
| Aldrin | Plunge Dip | (Barr and Hamiliton, 1965; Page *et al*., 1965) |
| Alpha-cypermethrin | Plunge/Shower Dip (Heath *et al*., 1992) | (Levot, 1994; Levot *et al*., 1995) |
| Amitraz | Plunge Dip (National Office of Animal Health, 2000) | |
| BHC | Plunge Dip | (Barr and Hamilton, 1965; Page *et al*., 1965) |
| Chlorfenvinphos | Shower Dip (Higgs *et al*., 1994) | |
| Coumaphos | Shower Dip (Higgs *et al*., 1994) | |
| Cypermethrin | Shower Dip (Hall, 1978) | |
| | **Pour-on (Henderson and McPhee, 1983; Heath and Bishop, 1988)** | (Boray *et al*., 1988; Levot and Hughes, 1990; Johnson *et al*., 1992; Levot *et al*., 1995) |
| | Ear Tags (James *et al*., 1989) | |
| Cyhalothrin | Shower Dip (Rundle and Forsyth, 1984) | |
| | Jetting (Rundle and Forsyth, 1984) | |
| | Wool Tip Spray (Wilkinson, 1985) | |
| | Pour-on (Liebisch and Beder, 1988) | (Levot *et al*., 1995) |
| Cyfluthrin | Pour-on (Liebisch and Beder, 1988) | |
| Deltamethrin | Pour-on (Kettle *et al*., 1982) | (Levot *et al*., 1995; Bates, 2001) |
| Diflubenzuron | Pour-on (Levot *et al*., 1995) | |
| Diazinon | Plunge Dip (National Office of Animal Health, 2000) | (Levot, 1994) |
| | Shower Dip (Higgs *et al*., 1994) | |
| | Wool Tip Spray ( Wilkinson, 1985) | |
| | Pour-on (Heath and Bishop, 1988) | |
| Dieldrin | Plunge Dip | (Barr and Hamiliton, 1965; Page *et al*., 1965) |
| Flumethrin | Plunge Dip (Kirkwood and Bates, 1987a) | |
| | Pour-on (Liebisch and Beder, 1988) | |
| High cis cypermethrin | Pour-on (Heath *et al*., 1992) | |
| | Plunge Dip (National Office of Animal Health, 2000) | |
| Phoxim | Plunge Dip (Hopkins and Lindsey, 1982) | |
| Propetamphos | Plunge Dip (Higgs *et al*., 1994) | |
| | Pour-on (Ormerod and Henderson, 1986) | |
| Triflumuron | Pour-on (Levot *et al*., 1995) | |

**Inorganic compounds**

Plunge dipping in wash containing arsenic/sulphur, lime/sulphur, rotenone or cresolic acid were the mainstay for ectoparasite control well into the 1940s. However none of these formulations were 100 percent effective in controlling ectoparasites. In addition, they had no ovicidal effect and were not residual in the fleece in significant concentrations to kill parasites emerging from the egg. Consequently, a second treatment was required after 10 to 14 days. Arsenic was widely used in Australia until prohibited in 1987 (Levot, 2000).

**Organochlorine (OC) compounds**

The next group of insecticides to be developed were the organochlorines (OCs) and cyclodienes, e.g. lindane (γ BHC), DDT, aldrin and dieldrin. OCs were known to destroy the delicate balance of sodium and potassium within the cell, thus preventing normal transmission of nerve impulses. From 1945 onwards plunge dip formulations were developed containing γ BHC. These were effective at a single dipping, eradicating lice and other ectoparasites, and for a considerable number of weeks after dipping sufficient chemical remained on the fleece and skin to kill hatching parasites. DDT and dieldrin were not effective against the scab mite (*Psoroptes ovis*) (Henderson, 1991), although they were highly effective against lice and blowfly (*Lucilia* sp.) larvae. Dieldrin and DDT were withdrawn from the United Kingdom market in the late 1960s, primarily on environmental grounds. OCs were commonly used, but banned in Australia in 1957 (Levot, 2000). Up to the mid 1980s plunge or shower dipping in γ BHC wash was the major acaricide/insecticide treatment in the war against lice and sheep scab throughout the world. It continued to be used in the United Kingdom until 1984, when it was voluntarily withdrawn due to residues in meat (Henderson, 1991).

**Organophosphate (OP) compounds**

The organophosphate plunge or shower dip formulations, containing diazinon, chlorfenvinphos or propetamphos, were the next generation of insecticides to appear on the market. OPs act by inhibiting cholinesterase (ChE) enzymes. Thus preventing the removal of ACh, "jamming the circuit" through its accumulation and interference with the neuromuscular junction.

**Synthetic (SP) pyrethroids**

Recently OP dip formulations in the United Kingdom have been incriminated in post dipping illness in stock owners and contractors (Anonymous, 1989). Consequently safer insecticides are being investigated for their efficacy against sheep ectoparasites. In 1987 the first non-OP dip, containing the synthetic pyrethroid, flumethrin, was licensed in the United Kingdom for scab and lice control (Kirkwood and Bates, 1987a). SPs have the advantage of having excellent selectivity and high toxicity to arthropods and relative safety to mammals. SPs affect the neuron membrane, modifying the sodium channels, probably impeding protein conformational changes at the lipid-protein interface, in a manner similar to OC compounds. SPs have remarkable similarities to DDT. Both DDT and SPs have two types of insecticidal effect, a) initial rapid knockdown (Kd), rendering the insect motionless and b) a subsequent lethal effect. Development of resistance to DDT by pests around the world was thought by many to foreshadow a similar fate for the SPs (Miller, 1988). This has occurred in the United States in the case of resistance in the cattle horn fly (*Haematobia irritans*) to ear tags impregnated with the SPs pyrethrin or fenvalerate (Quisenberry *et al*., 1984) which has conferred side resistance to all other SPs. *H irritans* had also previously been reported resistant to DDT. The biggest advantage of SPs is that they can in addition be formulated as pour-ons, revolutionizing louse control on sheep (and cattle), particularly in Australia.

**Macrocyclic lactones**

Macrocyclic lactones (MLs) are fermentation products of soil micro-organisms and have been chemically modified to produce the avermectins (ivermectin and doramectin) and the milbemycins (moxidectin), with greater potency and broader spectrum anti-parasitic activity than their fermentation precursors (abamectin and nemadectin, respectively). The first endectocide to be licensed for scab control was ivermectin (derived from *Streptomyces avermitilis*) followed by doramectin and the milbemycin, moxidectin (derived from *Streptomyces cyaneogriseus).* Moxidectin does not possess the disaccharide side chain (present in all the avermectins) and has unique side groups: a methoxine group and a dimethylbutenyl group. These subtle differences in molecular structure give rise to markedly different pharmacokinetics and potency properties of moxidectin compared to the avermectins. MLs are referred to as endectocides, being effective against both internal (endo-) parasites and external (ecto-) parasites. Their main advantage over plunge dipping is that they are quicker and safer to use, cause less stress to the sheep (including pregnant ewes), do not require any special handling facilities and fixed equipment (i.e. dip baths) and there are not the same environmental concerns over the disposal of spent products (Bates, 1993). They also have the added advantage that they are effective broad-spectrum anthelmintics. Their main disadvantage is their relatively narrow range of efficacy against ectoparasites, and alternative compounds may be required for the control of lice, ticks and blowfly. They also require relatively long meat withdrawal periods (Bates, 1993).

In Argentina the availability of over 40 relatively cheaper, generic, ivermectin based products has meant that they have surpassed plunge dipping in popularity, and this has caused grave concerns regarding selection for ivermectin resistance. Many farmers have now broken up their dipping facilities, relying solely on the use of endectocides. In the United Kingdom and South Africa there is an increasing problem with *B. ovis*, because of the sole use of endectocides for scab control.

**The control of sucking lice on sheep**

Cyhalothrin plunge dips are effective against *L. ovillus* and *L. pedalis,* but it is necessary to treat the predilection sites twice within a three-week period (Rundle and Forsyth, 1984). *Linognathus* spp. are blood feeders; consequently the macrocyclic lactones (ivermectin, doramectin, moxidectin) and closantel (Butler, 1986) are effective when administered either by injection or oral drench.

**The control of chewing lice on sheep**

*B. ovis* infestations were notifiable in Australia, with State Departments of Agriculture having authority to quarantine infested flocks but, after 30 years of regulated control (through the use of OP and arsenical dips) 20–30 percent of flocks in NSW were still infested (Levot, 2000). Not all licensed products achieved acceptable control. The comparative efficacy of 13 products registered in New Zealand was assessed on long wool sheep infested with *B. ovis* (Higgs *et al*., 1994). With the exception of one pour-on formulation of cypermethrin, all products were able to achieve a significant reduction in louse burdens. Only the OPs chlorfenvinphos, coumaphos and propetamphos, and the SP alphamethrin, proved capable of "eradicating" lice and preventing their re-establishment (Higgs *et al*., 1994). In Australia infested sheep are treated at, or soon after shearing by the application of a contact insecticide (Levot *et al*., 1995). Long wool treatments are also effective, enabling wool producers to treat infested sheep without shearing and prevent wool damage during the period prior to the next shearing. In Argentina, treatment is also administered directly after shearing in the late spring/early summer and the timing is largely dependent on the movement of the shearing gangs from the north to the south of the country (Bulman, *personal*

*communication*). Weather is also an issue, with late snow making farmers reluctant to shear until the weather has improved.

**Saturation treatments (plunge and shower dipping)**

Plunge dipping sheep using swim-through dip baths of insecticide has been the most effective method of sheep ectoparasite control throughout the world. Topical spraying in shower dips has been used for lice (and blowfly) control in Australia since the 1950s and is regaining popularity as SPs lose their efficacy against chewing lice (Sinclair, 1995). The problems with plunge dipping are multifactorial. To the operator it is time consuming and labour intensive and OP formulations have been incriminated in post dipping illness (Bates, 1993). To the environment, residues in the fleece and the disposal of large volumes of used dip wash pose considerable problems (Bates, 1993). There is also the need to re-muster and treat sheep 10 to 14 days after shearing. Shower dips rely on complex machinery (showers and jetters) that may never have been appropriate for the task, together with poorly understood management of insecticide strength during dipping (Levot *et al*., 1995; Kirkwood and Bates, 1987b). OPs are still very effective against lice, but control is hampered by inadequate application via plunge or shower dips (Levot *et al*., 1995). The efficacy of the six shower dip chemicals most frequently used for the control of *B. ovis* in Western Australia (emulsifiable concentrate (EC) formulations of alphamethrin, cyhalothrin, diazinon or diazinon plus piperonyl butoxide and rotenone) was demonstrated by their eradication. In contrast, wettable powder (WP) formulations containing coumaphos or magnesium fluorosilicate did not eradicate lice. Examination of the fleece 20 minutes after showering showed that WPs penetrated the fleece less effectively than ECs. It was concluded that the degree of wetting attained at dipping was an important factor in achieving eradication of sheep lice (Higgs *et al*., 1994). Showering is a useful technique for short wool sheep provided they are immersed for periods of four to ten minutes (Sinclair, 1995).

**Spraying longwool sheep**

*B. ovis* was eradicated from Merino sheep in Western Australia by spraying insecticide(s) onto the tip of long wool over the sides and back in small volumes at high concentrations (Wilkinson, 1985). If a practical method of applying the insecticide to the tip of the fleece could be developed, this technique would provide an effective method for treating sheep with long wool (Wilkinson, 1985).

Ivermectin applied to longwool sheep by hand jetting has been shown to control *B. ovis*, including pyrethroid resistant strains (Cramer *et al*., 1983).

**Pour-on (backline) treatments**

Innovative pour-on (backline) formulations, containing the SP deltamethrin, revolutionized louse control on sheep, particularly in Australia. Within a few years, pour-ons had gained 70 percent of the market share due to their simplicity and low labour costs, with off-shear sheep treated immediately and returned to pasture (Levot, 2000). Pour-ons containing deltamethrin (Kettle *et al*., 1982; Bayvel *et al*., 1981) and cypermethrin (Henderson and McPhee, 1983; MacQuillan *et al*., 1983) are now widely used in Australia, New Zealand and the United Kingdom for louse (tick, blowfly and headfly (*Hydrotea irritans*)) control, with the addition of alphacypermethrin pour-ons available in Australia only.

Pour-ons deliver a measured dose of insecticide concentrate along the backline of the sheep (Bayvel *et al*., 1981). The insecticide then travels in the emulsion layer that coats the wool fibres and the surface of the skin (Pitman and Rostas, 1981). The concentration of the insecticide decreases with distance from the area of application on the dorsal mid line (Kettle *et al*., 1982; Bates, 1993). Pour-on insecticides take time to diffuse around the body and to attain lethal

concentrations for lice (Kettle *et al*., 1982; Jenkinson *et al*., 1986). If the movement is inefficient for any reason, sub-lethal concentrations of insecticide will occur on some areas of skin. The efficacy of pour-ons depends upon fleece length. Deltamethrin formulations applied to off-shear sheep kill lice within 24 hours and protect against re-infestation for at least 10 weeks, but when applied to sheep 3 weeks after shearing, all lice were killed within 2 to 7 days and protection lasted for 15 weeks (Kettle *et al*., 1982). This difference has been attributed to the viscous nature of the fleece yolk, its increased volume, longer wool staple (Sinclair, 1977) and poorer fleece quality, i.e. high suint and low lipid concentrations (Kettle *et al*., 1982). Although results suggested high efficacy, residue data indicated that the spread of insecticide was much less even than with plunge dipping (Kettle *et al*., 1982). Some movement of an aqueous formulation of alpha-cypermethrin from the back to the lower body can occur after 24 hours, but despite careful application a wide variation in concentration can occur (Johnson *et al*., 1996). The majority of insecticide remains close to the site of application (Bates, 1993; Johnson *et al*., 1996) and near the tip of the wool staple (Johnson *et al*., 1996). A concentration gradient results, with the pyrethroid becoming less concentrated as it moves to the ventral surfaces (Bates, 1993). There are significant differences in insecticide concentration between the tip, middle and base portions of the wool staple from the back and lower flank (Johnson *et al*., 1996). Pour-ons are also relatively slow to work, 4 to 7 weeks being required before a high degree of control is obtained (Henderson and McPhee, 1983; Heath and Bishop, 1988). In Australia it is recommended to administer louse control treatments to sheep off-shears (i.e. within 30 days of shearing), when louse populations are at their lowest (Levot *et al*., 1995).

German studies assessing the efficacy of flumethrin (1%), cypermethrin (1%), cyfluthrin (1%) and cyhalothrin (2%) backline treatments against *B. ovis* demonstrated variations in efficacy with respect to louse numbers and sheep breed (Liebisch and Beder, 1988). Light to moderate infestations were cleared after 7 days, but in heavy initial infestations and in Merino sheep, newly emerged lice were found after 21 days and a second treatment was required (Liebisch and Beder, 1988). With light to moderate infestations, no lice were found after 42 days.

Cypermethrin based pour-on formulations with high cis:trans isomer ratios (80:20) achieved a higher level of control (97%–100% control from 4 to 16 weeks after application) compared to cypermethrin formulations (85% control on sheep 4 weeks after application) (Heath *et al*., 1982). It was suggested that high-cis cypermethrin may provide a better level of control in longwool sheep than cypermethrin by compensating for the diluent effect of lipid (Heath *et al*., 1982).

It is futile to change to another SP product if SP failure is confirmed. Until a diazinon pour-on was registered in Australia in 1994 the only OP products were aqueous dips (Levot, 2000). OP based pour-on formulations are being developed. In New Zealand a diazinon based pour-on rapidly removed *B. ovis* from moderately infested newly shorn sheep, with some sheep protected from re-infestation for 8–10 weeks (Heath and Bishop, 1988). In the United Kingdom a propetamphos based pour-on gave more than 99 percent control of *B. ovis* and protected against re-infestation for 4 months (Ormerod and Henderson, 1986).

## 9 APPLICATION METHODS

The choice of formulation and method of application of the insecticides naturally depends on the size of the farm and the management system. An ideal insecticide should be economically acceptable, easily applied and should have good efficacy with sufficient residual effect to protect animals from re-infection. It should not select for resistance due to its gradual decay on the animal (i.e. it should have a sharp cut-off in efficacy with time). In addition, it should have minimal toxicological effects on animals and people, with only minimal residues in meat and milk. Unfortunately, such an insecticide has not yet been produced.

**Plunge dips**

Plunge dips remain one of the most efficient and reliable methods for routine insecticide applications at farm level.

***Advantages:*** With this procedure, the animals are completely wetted, all parts of the body having adequate contact with the insecticide solution.

***Disadvantages:*** Problems with maintenance of the correct concentration of the drug are common. Elaborate installations for handling of animals are necessary. There can be environmental pollution from the run-off liquid when the animals emerge from the dip. The facilities are expensive to build. They are not appropriate for some drugs (such as MLs) for stability and other reasons.

**Wash/Spray**

Application of insecticide to sheep and cattle can be carried out using various modes of spray devices, e.g. spray races or corridors, motorized pumps, backpack manual pumps.

***Advantages:*** If carried out correctly, the amount of the drug applied is controlled and the concentration of the insecticide is adequate. Spraying is also generally less expensive per head than dipping, and the chemical group can easily be changed.

***Disadvantages:*** The animals are not always completely wetted, especially in the lower body parts, insides of the ears, etc. Animals must be appropriately secured during the operation. With the backpack manual pump, it is time-consuming and fatiguing to the operator. The use of manual spray pumps may well be the simplest method of acaricide application to animals, but not necessarily the most effective. Its effectiveness depends very much on the operator's skills and the effectiveness of restraining the animals. There is a relatively high risk of environmental pollution and intoxication of the operators. There are frequent problems with blocking of the spray nozzles.

**Pour-on**

The introduction of this method of insecticide application was a remarkable advance in technology for applying insecticide treatments. A volume of the drug proportional to the weight of the animal is applied along its dorsum, from where it dissipates over the animal's body surface to kill infesting lice. In the case of some SPs, depending on their residual active period, it may also offer continuing lethal and repellent protection against subsequently arriving lice. In the case of ML compounds, the method permits the parasiticide to be absorbed and to act systemically.

***Advantages:*** Pour-ons are easy to apply. Environmental pollution is reduced. It is a very practical method, especially where no dip tanks are available, or in circumstances when the producer wishes to avoid dipping some of the infested animals (e.g. pregnant animals, or when just a few animals need to be treated, etc.)

***Disadvantages:*** The higher cost of these new compounds may be an initial limitation for many farmers in developing countries. High concentrations of the applied chemicals are needed for good efficacy.

**Ear tags**

This is another practical alternative to avoid the dipping or spraying of animals with insecticides.

***Advantages:*** There is reduced environmental pollution. There is a broad spectrum of action against not only lice but also horn flies.

***Disadvantages:*** In general terms, these compounds are more expensive than the other alternatives.

**Injectable formulations**

This is another practical alternative to avoid the dipping or spraying of animals with acaricides. Most of the injectable products currently on the market are of the ML family.

***Advantages:*** There is reduced environmental pollution, except possibly in the dung pats where non-target species may be affected. There is a broad spectrum of action (against endo and ectoparasites). They also provide alternative acaricides for the control of pyrethroid and amitraz-resistant strains.

***Disadvantages:*** Possible residues of such products in milk restrict their use in dairy animals. In general terms, these compounds are more expensive than the other alternatives. MLs are not effective against chewing lice (*Bovicola* spp.).

## 10 NON-CHEMICAL CONTROL

**Control by good husbandry**

A number of non-chemical, husbandry treatments for flocks have been assessed in New Zealand to maintain an Organic Products Standard (OPS) (Pinnock, 1994). Shearing accounted for a 35.7 to 66.3 percent reduction in mean louse counts. Wetting, either with water or water with added detergent accounted for a 26.9 to 35.3 percent reduction in lice. The combined effects of shearing and wetting, as opposed to shearing alone, were statistically significant on two out of three farms 32 to 35 days post treatment. The effects persisted for the duration of the study (between 48 and 52 days), at which point shearing and wetting with detergent provided 95.3 to 99.6 percent control of lice (Pinnock, 1994).

The increasing environmental concerns about the persistence of chemical residues in wool has stimulated interest in biological control of lice by *Bacillus thuringiensis* (Levot *et al*., 1995; Rugg and Thompson, 1993). Natural plant products like azadirachtin (neem) and pyrethrum have been shown to be effective but loss of persistence is a major drawback (Pinnock, 1994). Investigations into the immunology of *B. ovis* infestations of sheep in Australia (Heath *et al*., 1995) and in New Zealand (Eisemann *et al*., 1994; Pfeffer *et al*., 1994; Bany *et al*., 1995a) are the first steps in the development of a possible vaccine.

## 11 CONTROL STRATEGIES UNDER DEVELOPMENT

Low level resistance to SPs was completely suppressed *in vitro* with the addition of the monooxygenase inhibitor piperonyl butoxide (PBO) (Levot, 1994). Experimental formulations containing cypermethrin:PBO at a ratio of 1:5 reduced the highly resistant Hartley strain by 95 percent for the first six weeks and by 80 percent over 10 weeks (Levot, 1994). Mixtures of existing formulations of PBO and cypermethrin were poorly suited because sheep dips at cypermethrin:PBO ratios of up to 1:10 did not offer any advantage over cypermethrin alone (Levot, 1994). A novel formulation of cypermethrin:PBO (5:1) containing a wetting agent provided a much greater degree of control in small scale field trials using sheep infested with highly pyrethroid resistant lice. Although impressive, this improvement in efficacy was not sufficient to make the mixture a realistic proposition for controlling highly resistant lice (Levot, 1994).

Today SPs have only a small share of the market. Apart from the loss of efficacy, persistent residues in the wool of treated sheep jeopardizes their use. Insect growth regulators (IGRs) like

triflumuron and diflubenzuron have captured the Australian market, significantly improving the prospects for resistance management, but OPs are still widely used (Levot *et al*., 1995).

Polymer matrix ear tags containing 8.5 percent w/w cypermethrin reduced *B. ovis* numbers on longwool sheep by 89 percent and 85 percent when measured 16 and 38 weeks after application respectively (James *et al*., 1989). Further studies demonstrated that 8.5 percent cypermethrin ear tags were comparable to cypermethrin pour-on in their capacity to reduce infestation (James *et al*., 1989).

*In vitro* bioassays have shown that ivermectin and abamectin were highly effective against *B. ovis*, and similar responses of pyrethroid susceptible and resistant strains indicated that there was no cross resistance to ivermectin (Levot, 1995).

## 12 RESISTANCE MANAGEMENT AND INTEGRATED CONTROL

**Action by national agricultural departments**

To ensure the success of any control programme, resistance testing is necessary before deciding which pesticide should be used, and resistance monitoring needs to be continued during the campaign (Morcombe and Young, 1993). The frequency of resistance monitoring activities will depend on the parasite's generation interval (Morcombe and Young, 1993).

Regional or national veterinary authorities, local veterinary surgeons, agricultural extension services and farmers' groups should be informed if resistance has been identified in their localities., This will allow the situation to be monitored and the correct alternative treatments to be prescribed if needed.

The laboratory carrying out or responsible for resistance testing should be recognized by the regional or national authority concerned. The same laboratory should be responsible for resistance monitoring (Morcombe and Young, 1993).

A standard methodology for resistance diagnosis should be defined and agreed by all parties involved. A definitive standard operating procedure (SOP) should be prepared compliant with an agreed quality scheme (e.g. Good Laboratory Practice (GLP)).

This should include a definition of the susceptible reference isolate (Morcombe and Young, 1993) and should be low cost and unsophisticated, but validated at regular intervals by a central laboratory using more sophisticated methods.

The maintenance of a susceptible reference strain is required (Morcombe and Young, 1993) and should be maintained according to an agreed SOP at the recognized laboratories.

The risk of cross-resistance needs to be covered within the context of efficacy testing. This requires the nomination of resistant strains (Morcombe and Young, 1993), again maintained according to an agreed SOP at the recognized laboratories.

The adoption of quality assurance schemes (e.g. Cattle Care in Australia) where producers take reasonable steps to improve hide quality through lice eradication programmes, integrating herd management and insecticidal treatment, are now being promoted but have not gained wide acceptance (Levot, 2000).

**Action by research institutes**

In addition to reliable resistance testing and monitoring, there should also be investigations into resistance mechanisms and development so that resistance risk analysis can be carried out in the future (Morcombe and Young, 1993).

Research efforts must concentrate on the better use of existing insecticide technology (optimizing treatment times, understanding the resistance status of the target pest, etc.) (Levot, 1993).

Investigations into the host specificity of ectoparasites and possible refugia from treatment is necessary.

To reduce the cost of maintaining susceptible or resistant strains of lice, the cryo-preservation of parasites should be investigated.

**Action by regulatory authorities**

The cornerstone for sustainable pesticide resistance management (PRM) is the consideration of the resistance issue in pesticide registration requirements, covering proper pesticide use, resistance diagnosis and monitoring and preventative measures (Morcombe and Young, 1993).

A scheme for monitoring resistance development after product registration should be defined.

The diagnosis of resistance in non-target species through intensive use of a product should be a consideration at registration.

Consideration should be given to the method of product application. The easier the method of application, the more it is likely to be effective in the field.

**Action by the farmer**

Strategies should be based on Integrated Pest Management (IPM) techniques, exploiting the biology of the pest, reducing selection pressure to a minimum, increasing the useful life of a pesticide and decreasing the interval of time required for a parasite to become susceptible once more to a given pesticide (Thullner, 1997).

Reduce the use of insecticides in the flock/herd. Insecticides should only be used if absolutely necessary and an annual "blanket treatment" of the whole flock should be avoided. Treatment of un-infested animals is undesirable.

Rotation, alternation or sequences of different classes of parasiticide or different modes and sites of action to control the same parasite are accepted as valid strategies to avoid resistance (Thullner, 1997). Do not use an SP dip if an SP pour-on is routinely used for the control of other ectoparasites.

Where there is no refuge for the population exposed to the insecticide there is a high selection pressure. This is extremely important in the case of permanent parasites such as lice or scab mites.

All oncoming stock should be quarantined for at least three weeks (21 days), and observed for signs of infestation. If an ectoparasite is suspected, a veterinary surgeon should be consulted who will advise the correct treatment. Failure to do so may necessitate the re-treatment of the whole flock/herd at a later date.

If ectoparasites are suspected, have the parasite professionally identified. Ensure that only a product licensed for the control of that parasite is administered and administered correctly.

Do not mix with the main flock until treatment is complete and the parasite eradicated.

In an area where resistance has occurred, continued use of a pesticide may be required to control other parasites that remain susceptible. In the United Kingdom for example, SP pour-ons have in the past been used for the control of ticks (*Ixodes ricinus*) in upland grazing (also where *B. ovis* is currently a serious problem). Such practices could confound attempts at parasite

management. Use of macrocyclic lactones as anthelmintics (using injections, oral dosing or slow release boluses) may also select for resistance in ectoparasites.

Variations in dose or rate of application may delay or minimize resistance by preserving a sufficient population of susceptible individuals (alleles); this is done by using low rates of a given pesticide, so as not to select against heterozygotes where resistance is recessive (Thullner, 1997). Using doses less than 100 percent effective may reduce the threat of resistance if low levels of the parasite can be tolerated (Hennessey and Andrew, 1997), e.g. meat producing sheep and chewing lice.

The excessive use of parasiticides for short-term gains may be the worst possible practice in the long term (Hennessey and Andrew, 1997).

Fewer applications, which reduce the selection pressure over time, would decrease the rate and probability of resistance development (Hennessey and Andrew, 1997).

The simultaneous use of two or more parasiticides with different mechanisms of action or different target site effects can be an important strategy in maintaining a low level of resistance. However if the parasite population is already resistant to a particular insecticide, this insecticide (even at an increased dosage) would be ineffective as part of a mixture (Hennessey and Andrew, 1997). Use of mixtures must begin before resistance to one component occurs, each component must have similar decay rates and components must have different modes and sites of action, or different resistance mechanisms (Hennessey and Andrew, 1997). Mixing chemicals can sometimes potentiate, rather than merely give additive effects, thus delaying or preventing resistance (Hennessey and Andrew, 1997).

Apply existing products effectively and according to the manufacturers instructions. Taking care to apply topical insecticide formulations directly along the backline immediately after shearing will maximize the even diffusion of active ingredients around sheep flanks, thereby contacting lice inhabiting sites remote from the point of drug application (Finney, 1971). Research efforts must concentrate on the better use of existing insecticide technology (optimizing treatment times, understanding the resistance status of the target pest, etc.) (Denholm and Rowland, 1992). Saturation dipping must be carried out to “Good Dipping Practice” (National Research Council, 1986).

Not all insecticides or their methods of application are effective against all ectoparasites (i.e. broad spectrum). The parasite infesting the flock must be professionally identified and the correct, licensed treatment administered (and administered correctly). The routine use of SP pour-ons for the control of lice, ticks, blowfly or headfly, could have induced resistance to SP dips or even augmented existing SP tolerance within a population.

The selection for insecticide resistance in *L. cuprina* may have begun when blowfly populations were exposed to insecticidal residues in fleece following treatment for other ectoparasites (Bates, 1999c). It is unwise therefore to treat a flock with an SP dip if SP pour-ons are routinely used.

Ectoparasites have relatively short generation times producing relatively large numbers of offspring per generation. The product label instructions must be followed closely. If the label states two treatments, then two treatments must be administered. The first treatment will only kill active stages of parasites present on the sheep at the time of treatment. The second treatment will kill any eggs that have hatched since the first treatment

In eradication programmes, once the sheep are mixed with the main flock the buildings/paddocks housing the infested sheep must be thoroughly cleaned and disinfested with a

suitable insecticide. All litter and discarded wool must be collected and burnt or deposited out of sheep contact. No sheep can be housed/grazed in the disinfested area for at least 21 days.

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