Anthelmintics and Preserving their Effectiveness

L.F. Le Jambre

Abstract

Parasite control for the past thirty years has depended on anthelmintics. Now, however, anthelmintic resistance to the benzimidazole and the levamisole group of chemicals has reached a critical stage in much of the pastoral regions of the world. The introduction of the avermectin-milbemycin group of anthelmintics has provided some opportunity for continued reliance on chemical control but already avermectin resistance has been reported from many regions. It is agreed by parasitologists that anthelmintics will remain a part of parasite control strategies in the foreseeable future but that they should be integrated with other control measures such as selective breeding and grazing management. Consequently, it is vital that the present arsenal of anthelmintics remain effective. Important tools in combating the spread of anthelmintic resistance are methods of detecting anthelmintic resistance while the genes that cause it are still at a low frequency in the parasite population. At present, monitoring anthelmintic resistance relies almost entirely on the Faecal Egg Count Reductions Test (FECRT). The FECRT has a number of shortcomings including the need to visit a farm on at least two occasions, and the requirement for a relatively large number of animals. Furthermore, the technique is relatively insensitive. A larval development assay (LDA), developed by CSIRO, is being marketed by Horizon Technology. The LDA requires only one farm visit and a simple microscope scanning of the proportion of nematode eggs developing in a range of anthelmintics to provide a direct correlation between in vitro and in vivo drug efficacy. This technique will be of great benefit to those responsible for managing anthelmintic resistance in the field.

Parasite control for the past thirty years has rested firmly on the shoulders of anthelmintics. Now, however, this modern day Atlas is showing distinct signs of weariness. Anthelmintic resistance to benzimidazole and levamisole chemicals has reached serious proportions in much of the pastoral regions of the world. The introduction of the avermectin-milbemycin group of anthelmintics has prolonged the reign of anthelmintic control but already avermectin resistance has been reported from many regions. Studies indicate that the frequency of resistance genes in unselected parasite populations is high and that this group too will succumb unless immediate action is taken to delay the development of resistance.

There are several reasons why it is imprudent to rely on new anthelmintics to replace those whose effectiveness has been lost due to resistance. First is the escalating increase in the already high cost of development. The growing sophistication of analytical techniques for the detection of drug residues along with the use of new techniques for monitoring toxicity, teratogenic effects and extension of testing to a wide range of species to assess environmental safety all add to an increasing cost of development. To illustrate this point, the cost of development of a new drug was estimated to be US$30 million in 1985 (Hotson 1985) while today the cost is in the vicinity of US$230 million (McKellar 1994).
There is a second, less obvious reason that could slow the development of new anthelmintics which relates to identifying market need. Anthelmintics make up only 3% of world-wide sales of animal health products (Hotson 1985) but their relative importance in the mix of products is greater in the Southern Hemisphere which has the majority of pastoral regions. Head offices of the pharmaceutical companies are in the Northern Hemisphere where intensive animal production systems predominate. Therefore, what may seem of great importance from our perspective is not quite as important from another.

Shifting to intensive production systems similar to those in the Northern Hemisphere is not the answer as ruminants will remain an important means of converting low quality forages to meat and fibre. Therefore, to ensure that parasite control remains effective, it will be necessary to develop an integrated approach in which anthelmintics play an important but not exclusive role.

**Anthelmintics**

There are a limited number of highly efficient anthelmintics available for the control of round worm parasites although, looking at the vast array of commercial formulations available, one would be excused for thinking otherwise. This is not the case. Most of the apparent variety is related to the fact that there are different chemicals within a group that have a similar mode of action. Thus, it is important to be aware that there is side resistance within similar mode-of-action anthelmintics, so that once resistance has developed to one chemical, resistance can be expected to others within the same mode-of-action group. For this reason it is of benefit to describe briefly the major mode-of-action groups of anthelmintics currently available.

**Benzimidazoles**

Thiabendazole (TBZ) was introduced in the early 1960s and ushered in a new era in anthelmintics, being much more efficient and less toxic than previous compounds. TBZ quickly became accepted as the market leader for treatment of parasitic disease in sheep, cattle and horses. Through the use of TBZ to suppress parasitic infections it became possible for the first time to see clearly the effects of parasites on production under field conditions.

It was known that TBZ was rapidly absorbed, hydroxylated to the 5-hydroxy compound, conjugated as the glucuronide or sulphate and excreted mainly in the urine (Tocco et al. 1964). Consequently, pharmaceutical companies expended considerable effort to find chemical groupings that would block the 5-position, preventing hydroxylation and excretion, in order to prolong the time the drug remained in the body, thereby increasing efficiency. This research led to a range of substituted benzimidazoles (BZs) being discovered with the 2-thiazolyl of TBZ replaced by 2-methyl carbamate and a range of aliphatic and aromatic side chains at the 5-position. The nature of the side chain in the 5-position greatly influences the rate of excretion and the efficiency and spectrum of activity.

Prichard et al. (1978) reported that the tertiary BZs (fenbendazole, albendazole and oxfendazole) reach maximum blood levels in sheep 15 to 24 hours after dosing compared to the 4 to 6 hours taken by thiabendazole, oxibendazole and parbendazole. The BZs act by attaching to tubulin dimers, preventing their polymerisation to microtubules and also by causing the disassembly of existing cytoplasmic microtuble structures (Borgers et al. 1975). These actions reduce the absorption of nutrient and must be maintained for some time to allow irreversible metabolic changes to occur, otherwise the parasite can survive by reducing its energy demands (Le Jambre 1985). Thus the tertiary BZs which remain in the host's body for longer periods have a broader spectrum of activity and include lung worms and tapeworms in their therapeutic range.

**Levamisole and morantel**

Levamisole (LVS) and morantel affect the nematode’s nervous system. They are absorbed by the host almost immediately and causes a rapid paralysis in those parasites which are exposed. Once paralysed, the nematodes are swept out of the host along with the ingesta. Both compounds are active against the major intestinal parasites of sheep and cattle with the exception of inhibited *Ostertagia ostertagi*. It is possible that the larvae in the gastric crypts may be paralysed but not removed by the host. Thus when the drug is excreted, the worms are still present in their normal habitat and recover. Levamisole is also active against lung worms.

**Avermectins**

The avermectins (AVMs) are a family of 16-membered macrocyclic lactones isolated from *Streptomyces avermitilis* and are potent nematocides and insecticides (Campbell et al. 1983). Structurally, the AVMs belong to the milbemycin class of macrocyclic (Takiguchi et al. 1980). Within the AVM/milbemycin class, five analogues are commercially available for the treatment of parasitic nematodes in animals: ivermectin (IVM), avermectin
B1, doramectin, moxidectin and milbemycin A4-5-oxime. Ivermectin is also used for the treatment of infections of the filarial parasite, Onchocerca volvulus in humans (Campbell 1991). A number of potential sites of action for the AVMs have been identified in studies undertaken in a variety of species and test systems. Today, it is thought that AVM interacts with glutamate gated chloride channels (Arena et al. 1992). IVM is a potent inhibitor of the motility and development of the freelifing stages of trichostrongylid nematode parasites.

Substituted salicylanilides and phenols

These compounds are either trematocidal or cestocidal and at normal dose rates some are highly effective against Haemonchus contortus in sheep. These compounds act by uncoupling oxidative phosphorylation resulting in energy depletion. They are all relatively toxic and are detoxified by the host binding the absorbed drug to plasma protein where it is available to blood sucking parasites such as H. contortus and Fasciola hepatica. Closantel, one of the substituted salicylanilides, as a single oral dose exerts a suppressive anthelmintic effect against H. contortus for 30 days after treatment and a significant effect at 60 days (Hall et al. 1981). If used at the commencement of a period when H. contortus eggs have a high probability of developing to infective larvae, closantel can reduce the contamination of pastures for long periods. Closantel is used in this fashion in the 'Wormkill' program on the Northern Tablelands of NSW.

Organophosphates

The organophosphates act by inhibiting acetyl cholinesterase, thus allowing a build-up of acetylcholine and continual stimulation of the nerve ending which results in a spastic paralysis. The worms are then removed by the normal peristaltic action of the intestines. The main anthelmintic in this group is naftalofos (naphthalophos) and was first introduced as a broad spectrum compound in sheep. At a dose rate of 50 mg/kg, it has a high efficacy against H. contortus, moderate efficacy against O. circumcincta and Trichostrongylus spp., but little efficacy against the large bowel parasites. Naftalofos also lacks activity against immature stages. The moderate broad spectrum activity of this anthelmintic often leads to a revival of interest in it as an anthelmintic of last resort when resistance to the other broad spectrum anthelmintics has rendered them ineffective.

Anthelmintic Treatment of Sheep and Goats

Sheep and goats share the same species of gastrointestinal parasites and the same mode-of-action groups of anthelmintics are used to treat both hosts. However, the pharmacokinetic disposition of these anthelmintics are not the same in sheep and goats. Following single oral dose therapy of BZ, there was a lower systemic availability of the drug in goats than in sheep, possibly owing to a more rapid hepatic clearance in goats (Bogan et al. 1987; Short et al. 1987; Hennessy et al. 1993a,b). Knox et al. (1995) found a similar difference between sheep and goats with fenbendazole (FBZ) administered either as an intraruminal infusion or formulated into a ureasmolasses feed supplement block (UMB). These authors concluded that if FBZ were to be formulated into a UMB as a means of controlling parasites then the target dose rates for goats should be 0.75 mg/kg compared with 0.5 mg/kg in sheep. Hennessy et al. (1993c) reported that closantel was also less effective in goats than in sheep. These workers reported that the half-life of the expulsion phase of closantel was 4 days in goats compared with 14 days in sheep. It appeared that the sustained action of closantel would be greatly reduced in goats due to their more rapid clearance of the drug.

It is apparent from these studies that due to the rapid clearance of anthelmintics by goats, it is necessary to increase the dose rate in this species in order to obtain a similar efficacy as the same chemical in sheep. Thus, the cost of parasite control by anthelmintics in goats is greater than in sheep. The pharmacokinetic difference between sheep and goats presents manufacturers with a conundrum: if they follow the sheep dose, the efficacy that they can claim in goats is low, but if they aim at achieving the same efficacy level as in sheep, the cost of treatment is higher. Consequently, pharmaceutical companies often do not claim an efficacy for their drug in goats. This strategy relieves them of the cost of registering their chemical for goats.

However, goats still require anthelmintic treatment to control internal parasites and when pharmaceutical companies do not provide a dose rate, farmers are likely to use the same dose to treat both sheep and goats. The consequence is that the lower efficacy will result in the heterozygote resistant parasites being selected. It is clear that dosing goats with the sheep dose of anthelmintics is similar in consequence to underdosing with the effect of allowing the heterozygote resistant worms to survive. There appears to be some truth in the derisive claim by
some sheep farmers that resistance develops first in goats and then spreads into the sheep flocks.

Current Status of Anthelmintic Resistance

There are many recent reviews of the present status of resistance throughout the world (e.g. Waller, 1996). Table 1 provides a breakdown of resistance by countries within continents. It becomes clear from Table 1 that there is no area where there are small ruminants entirely free of reports of resistance. Also apparent is a Hemisphere bias with the Southern Hemisphere containing the world's hot spots of resistance. As noted above, it is the Southern Hemisphere that accounts for most of the anthelmintic sales. Consequently, the rest of the world would be wise not to become complacent as the obvious conclusion is: where anthelmintics are used most, then that is where resistance is likely to develop first.

Resistance monitoring

Aims of monitoring

Effective resistance monitoring is a key requirement in managing anthelmintic resistance and needs to be sensitive to small changes in gene frequencies. Effective monitoring enables agricultural advisers to detect resistance at an early stage when it is still possible to limit its development. The aims of resistance monitoring are:

- confirm cause of control failure;
- measure and identify resistance genotypes;
- determine changes in distribution and severity of resistance;
- provide early warning of resistance problems;
- make recommendations for pesticides least affected by resistance;
- measure biological characteristics of genotypes under field conditions;
- test effectiveness of resistance management tactics.

Table 1. Prevalence of anthelmintic resistance in sheep nematodes.

<table>
<thead>
<tr>
<th>Continent</th>
<th>Country</th>
<th>No. of flocks tested</th>
<th>Prevalence (%) of resistance to:</th>
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<td></td>
<td>BZ</td>
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<tr>
<td>Africa</td>
<td>Kenya</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>South Africa</td>
<td>96</td>
<td>36</td>
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<td>60</td>
<td>81–95</td>
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<td></td>
<td>Thailand</td>
<td>39</td>
<td>15–47</td>
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References cited in Table 1.

<sup>1</sup>Mwamachi et al. 1995  <sup>2</sup>Sivaraj et al. 1994  <sup>3</sup>Coles et al. 1994
<sup>3</sup>Van Wyk 1990  <sup>4</sup>Kochapakdee et al., 1995  <sup>5</sup>Le Jambre 1994
<sup>5</sup>Dorny et al. 1994  <sup>6</sup>Overend et al. 1994  <sup>6</sup>Waller et al. 1996

BZ = benzimidazoles  LVS = levamisole  COM =  IVM = ivermectin
Techniques for monitoring anthelmintic effectiveness

Anthelmintic resistance can be detected by a variety of in vivo and in vitro techniques. The in vivo techniques can be characterised as time-consuming and expensive, producing data that reflect the high degree of between-animal variation and the pharmacodynamics of the anthelmintic in the host. Nevertheless, the drench efficacy test where animals are first treated with anthelmintic, subsequently slaughtered and their worm burdens counted, is the standard against which all assays must be validated.

The most common means of detecting resistance is the Faecal Egg Count Reduction Test (FECRT). This technique requires that egg counts be made on sheep with infections of the parasites in question, then the animals be drenched with the anthelmintics to be tested. Ten days following the drench, faecal egg counts are done on the animals again. Based on the reduction in egg counts an estimate of the anthelmintic's effectiveness is determined. Larval differentiations made from the eggs collected ten days after drenching determine which species of parasites are resistant.

In vitro assays have the advantage of being less time-consuming and by removal of the host variation from the calculations of anthelmintic effect have greatly improved precision of resistance estimates. In vitro techniques can either employ the intact parasite or preparations of parasite tissue. Whole organism assays monitor the effects of an anthelmintic on a normal physiological process such as egg hatch, larval development or motility.

In vitro assays employing whole worms, or worm eggs, were previously restricted to a narrow field of application within specific anthelmintic classes since these methods require the use of a technique in which the drugs show discernible action. Thus, the action of levamisole on motility constitutes the basis of a paralysis assay but as BZs and other classes do not exhibit such a strong action on motility, they cannot be assayed in this way. The dependence of the current in vitro techniques on the pharmacodynamics of specific anthelmintic classes has limited their widespread adoption for the diagnosis of resistance. The necessity of using three or four separate in vitro techniques to examine anthelmintic resistance in unknown isolates is far more daunting than either of the in vivo techniques where no assumptions regarding how the drug acts on parasites are necessary and trends can be established despite the poor quality of in vivo experimental data.

DrenchRite™

This lack of practical in vitro assays prompted consideration of the development processes in free-living stages for assay development. Lacey et al. (1990) developed a larval development assay (LDA) in which nematode eggs are added to an agar matrix containing the drug and developed through to infective L₃ larvae. Standardisation of the LDA demonstrated that currently used anthelmintic classes gave excellent dose-response data for the inhibition of egg to L₃ larval development in Trichostrongylus colubriformis, H. contortus and O. circumcincta.

A commercial in vitro assay called DrenchRite was developed from Lacey's LDA for the detection of resistance to BZ, LVS, BZ/ LVS combinations and IVM in the gastrointestinal nematode parasites of sheep and goats, H. contortus, T. colubriformis and O. circumcincta. In the DrenchRite assay, the nematode eggs are placed into the wells of a microtitre plate and hatched larvae develop to the L₃ stage in the presence of anthelmintic. The concentration of anthelmintic required to block development is related to an anticipated in vivo efficacy. The DrenchRite assay plate contains a single lane for the control and eleven lanes which contain increasing concentrations of a drug from each drench class. The plate is colour coded: green for susceptible, orange for weak to intermediate resistance and red for highly resistant. Parasite eggs isolated from faecal samples submitted by producers are applied to wells of the assay plate. After hatching, the first stage larvae are fed to sustain development through to the infective L₃ stage over the next five days. In the presence of the drenches, development of susceptible larvae is blocked. By scanning the plate under the microscope the number of the well in which development is blocked in half the larvae present is determined for each drench.

If the larvae are resistant to a drench, the well in which development in half the larvae is blocked will move from the green to the orange and subsequently to the red region of the plate for that drench, depending on the level of resistance. The well numbers so determined for each drench class are then used to estimate drench efficacy from a supplied table. For the avermectin/milbemycins where resistance in the field is very rare at present, the well number is used in a slightly different way. Rather than estimating efficacy, the presence of L₃ in wells containing avermectin are an indicator that a small number of worms in the population are resistant to the drug. This assay provides a repeatable method of detecting resistance and estimating anthelmintic efficacy. Incorporating the DrenchRite assay into a monitoring program would allow comparisons of
resistance levels between regions and would provide a sensitive method of detecting changes in those levels.

**Resistance Management Tactics**

**Models**

Models are especially useful in comparing the impact of management tactics on the development of resistance. A good introduction to the use of models to simulate gastrointestinal nematode control systems can be obtained from Barnes et al. (1995). However, at least a general idea of how each resistance management tactic works and where it can be most effective can be obtained from the simple principle of discrimination between genotypes. In the least complicated case, that of a single gene encoding resistance, the genotypes would be: RR, RS and SS. Selection can only occur at concentrations that kill at least some susceptible homozygotes but allow at least some resistant homozygotes to survive. Tactics that reduce this discrimination, particularly the discrimination between susceptible homozygotes and resistant heterozygotes (the most common carriers for resistance genes early in a resistance episode), will generally slow the rate of resistance evolution. Once the genetic nature of resistance is recognised, it is easy to see the benefits of a sensitive assay. It is obvious that resistance management tactics are most effective when implemented at low resistance gene frequencies. For example, it may be possible to increase the dose rate of an anthelmintic or to increase the persistence of an anthelmintic to a level that renders the heterozygote recessive. This would tend to slow the development of resistance as homozygous resistant individuals are very rare when a new chemical is first introduced. Once the frequency of the resistance gene increases, heterozygotes become more common and the increased selection applied by increased dose rate will then tend to increase the rate at which the resistance spreads in the population.

**Refuges**

Allowing refuges for escape of susceptibles has often been proposed for conserving susceptible genotypes in insect populations. Recently, it has been proposed for nematode parasites by Barnes et al. (1995). Basically, this would entail not drenching a portion of the flock, perhaps 20% of the flock that appeared the most fit. Such a strategy would allow the unselected worms in these sheep to continue to produce offspring and dilute the effects of offspring of resistant worms from the treated 80% of the flock. Needless to say, this strategy would work best while the proportion of resistant worms was low.

**Changing anthelmintic groups**

Switching to anthelmintics from a group with a different mode of action when resistance has been discovered is another tactic. This could include consideration of more expensive or even less effective anthelmintics. These disadvantages could be offset by the possibility of salvaging a more desirable anthelmintic for future use. In Australia, a switch to organophosphates has been recommended, based on this principle.

**Aim to make heterozygote recessive**

One means of making the heterozygote recessive is to increase the dose rate. The disadvantages of this simple approach are that cost of treatment increases and host toxicity problems may be encountered. There are other means which include switching to anthelmintics with the same mode of action but with increased persistency, if available. These anthelmintics are often second and third generation chemicals such as the substituted BZs among the BZs and the milbemycins among the macrocyclic lactones. There are other methods of increasing the persistence of anthelmintics. Prichard et al. (1978) infused BZ into the animals and their results indicated that persistence was an important determinant of the efficacy and spectrum of BZ anthelmintics. These authors speculated that increased persistency may also improve the efficiency of other anthelmintics. An increased efficacy through increased persistence was also achieved against BZ resistant strains of parasites by Le Jambre et al. (1981) by incorporating the BZ into a sustained release device and these authors suggested that by incorporating earlier, less-persistent anthelmintics into sustained release devices their useful life could be extended. Anthelmintics also can be incorporated into feed-supplement blocks to allow self-medication over extended periods of time. The self-medication technique works especially well when used in tethered husbandry systems. Ali and Hennessy (1993) demonstrated that the persistence of anthelmintics could be increased by slowing down the flow of digesta through the host and that this resulted in a significant increase in efficacy. A practical means of slowing down the flow of digesta is to temporarily restrict feed intake of the animals before treatment with the anthelmintic.

When reports of ivermectin resistance in goat and sheep parasites started to appear, a dispute developed around the apparent susceptibility of these parasites to moxidectin (Shoop et al. 1993; Kieran 1994; Rothwell and Rolfe 1994). Kieran (1994) attributed these findings to either a greater potency of
moxidectin or to a different mode of action compared with ivermectin. Now that many strains have been described with resistance to both ivermectin and moxidectin (Leathwick 1995; Rolfe et al. 1994; Le Jambre et al. 1995) it appears that the greater potency of moxidectin can be attributed to its greater persistence in the host (Afzal et al. 1994). The situation is analogous to the introduction of the second generation BZs which had a greater persistency in the host and a greater efficacy against TBZ resistant nematodes. Unfortunately, this benefit of the second generation BZs was transitory and it is likely to be the same with the macrocyclic lactones.

Once it became clear that moxidectin’s greater efficacy was likely to be due to its greater persistency another debate developed over whether the selection for resistance in parasites exposed to the gradual decline in efficacy in a persistent anthelmintic was more effective in selecting for resistance than that of a short-acting anthelmintic. This debate was given further emphasis when Le Jambre et al. (1995) reported that avermectin resistance in H. contortus was inherited as a dominant trait. Dominant traits respond to selection faster at low gene frequencies than do incomplete dominants or recessives such as BZ resistance. Furthermore, increasing the dose to make the heterozygote recessive is not an option when dealing with dominant resistance genes.

The response of a dominant resistance gene under selection by a persistent anthelmintic was investigated using a simulation model (Dobson et al. 1996). In this model efficacy against incoming infective larvae (L3) was assumed to decline or remain high over the period of drug persistence (3 days to 4 weeks) thus allowing the estimation of the relative importance of selecting resistant L3s on the development of resistance in the worm population. These factors were also examined against a background of initial efficacy levels, against adults, and mode of inheritance. The outcome of the simulations was that persistence and initial efficacy were found to be far more important in determining the rate of selection for resistance on adults and larvae in the host at the time of treatment than was selection of incoming resistant L3 as drug efficacy declined.

Combinations

Using formulations that contain two anthelmintics, each with a different mode of action has also proved successful in extending the life of the combined chemicals. The genetic principle behind this strategy is that resistant genes are rare in the population so that the odds of a single worm having both rare genes is the product of the frequency of each gene in

Non-chemical control

Of course, the most effective means of managing resistance and of conserving susceptibility genes in the parasite population is to use methods of parasite control that do not rely on chemicals. The three most promising techniques in this area are described in the papers by Woolastion, Barger and Waller in these Proceedings.

Resistance in Cattle Parasites

Previously, cattle producers could be excused for thinking that anthelmintic resistance was exclusively a problem for sheep and goat farmers and that one of the tangible benefits of specialising in large ruminants was freedom from worry about which drench to use to control parasites. How can this be so, with cattle and buffalo sharing many of genera of parasites with small ruminants? Le Jambre (1979) has shown that the cattle parasite H. placei can express the BZ resistance gene from H. contortus. Likewise, H. placei can also express the ivermectin resistance gene from H. contortus (Le Jambre, unpublished). This evidence indicates that there is no antagonism between resistance and the essential features in a cattle parasite’s genome. There are two likely explanations for the sparse reports of anthelmintic resistance in the gastrointestinal parasites of large ruminants. First, in many temperate regions of the world drenching cattle is an infrequent activity and consequently there is little selection for resistance. However, suppression of parasites, particularly in young cattle, has increased, coinciding with the development of the controlled release devices (CRDs). Following the lead of CRDs, manufacturers of injectable and oral formulations have begun to recommend that their products be used with increased frequency to emulate the same control levels. The result, sales promotions aimed at total suppression, may have resulted in the few cases of resistance in cattle parasites reported in Europe and Australasia, for example, the first reports of resistance to ivermectin in bovine Cooperia spp. (Vermunt et al. 1995).
The second reason, as pointed out by Waller (1996), is that there have been no surveys of resistance in those regions of the world where intensive treatment of young cattle is commonplace. For example, anthelmintic resistance has been recorded in cattle parasites in southern Brazil (Pinheiro and Echevarria, 1990) but there has been no survey to estimate the proportion of properties where resistance occurs. It may be in the cattle producing regions of South America that resistance in cattle parasites will be first recognized as a serious problem. If there is a benefit to early detection of anthelmintic resistance in small ruminant parasites, then it is of even greater benefit for cattle. Because there is little anthelmintic resistance in cattle parasites at present, the option to change anthelmintics or combine anthelmintics with different modes of action has a greater chance of success.

Conclusion

The problem of anthelmintic resistance should be recognised throughout the world as the greatest threat to the grazing industry. The solutions will not be found in any single approach. Farmers in countries where resistance is rare should realise that the cause of this rarity is most likely to be infrequent use of anthelmintics rather than some intrinsic lack of resistance genes in the parasites. It is fortunate that a large proportion of susceptible genes are in the indigenous parasites. These susceptible genes should be seen as a most valuable resource and a strenuous effort should be made to conserve them (Wood and Bishop 1981). One of the most valuable weapons in the battle to conserve susceptibility in nematode populations is the ability to detect resistance while it is still at a low level.

References


Worm Control of Livestock — the Biological Alternative

Peter J. Waller

Abstract

Biological control, by the use of nematophagous fungi, is now emerging as the most promising non-chemotherapeutic control option of nematode parasites of livestock. Practical methods of fungal deployment are now being developed, such as feed supplements, feed blocks and controlled release devices. Biological control has many obvious advantages. For example, it is applicable to the range of nematode parasite species for all classes of livestock. It will provide the opportunity for livestock producers to capitalise on the increasing demands by consumers for chemical-free livestock products. Also, it is difficult to envisage the development of resistance mechanisms by nematodes to biological control, which now is an enormous threat to the future of chemotherapy.

LIVESTOCK in the tropical/subtropical regions of the world experience much greater ravages from internal parasitic disease than those in the more temperate climes. Here, the limiting ecological factor influencing the severity of parasitism is rainfall, as temperatures are almost always favourable for the development and survival of the free-living stages. Therefore, apart from the arid areas in the tropics/subtropics, where animals are typically allowed to browse or graze over extensive areas and worms are rarely a problem, rainfall, both total amount and seasonal distribution, determines the severity and occurrence of clinical parasitic disease in livestock.

Farmers have by experience come to recognise when problems are likely to occur, and if they can afford it, treat their animals with anthelmintics. In many instances lengthy periods of high rainfall require regular and frequent treatment. This has resulted in the widespread emergence and rapid escalation in the problem of anthelmintic resistance. There are now large areas in the tropical/subtropical regions of the world where grazing of sheep and goats is becoming unsustainable because of the existence of high levels of resistance to virtually all available anthelmintic groups (Waller et al. 1995).

Immediate and drastic measures are needed in an effort to contain this problem. The search for alternatives to chemotherapy for the control of ruminant parasites has been directed towards developing worm vaccines, selection of livestock with natural resistance to parasites and, relatively recently, at the biological control of the free-living stages of parasites on pasture.

Biological Control

Biological control is not assumed to be a substitute for chemotherapy, where the expectation, if not the reality, is that parasites may be virtually eradicated by the frequent use of drugs with efficacies approaching 100%. The ultimate aim of any biological control scheme is to prevent clinical disease and production loss by reducing the exposure of susceptible hosts to pathogenic levels of infection.

Separating hosts from their faeces is the simplest, cheapest and most effective form of biological control of parasitic diseases. The classic example of success by these means is in human helminth control whereby the simple expedient of using pit toilets has led to dramatic reductions in trematode and nematode infections of people in the developing world. In the same respect, the practice of dung collection for use as fuel could, in the broadest sense, be regarded as a form of biological control. However, in areas where this is a common practice, it is restricted to bovine dung, and severe undernutrition or malnutrition

1CSIRO Division of Animal Production, Pastoral Research Laboratory, Armidale, NSW, Australia
rather than parasites are generally the major causes of losses in animal productivity.

Dung dispersal, or destruction, can occur in other ways and in regions of the world where parasites are of greater significance. A variety of birds rely heavily on coprophagous invertebrates as a food source and to seek these, they tear dung pats apart (McCracken 1993). But it is their invertebrate prey, notably dung beetles and earthworms, that are capable of rapid and often complete dung removal and thus are indirectly responsible for significant reductions in the number of free-living stages of parasites (Wailer and Faedo 1996). However, such dung dispersal activity is notoriously labile, being dependent on ideal weather conditions, therefore little opportunity exists to exploit these organisms in attempts to achieve cost-effective and reliable biological control of nematode parasites.

Another example of indirect biological control applies to trematodes. In contrast to nematodes, trematodes and cestodes require intermediate hosts to complete their life cycle. Control of the snail intermediate hosts by foraging flocks of ducks has been shown to be of practical value in the control of Fasciola gigantica infections of ruminants raised in rice producing areas of Southeast Asia. Here the benefit is twofold: not only do the ducks seek the snails as a food source, but the free living stages of Echinostoma revolutum, which is a common trematode parasite of ducks, compete with F. gigantica to utilise snails as intermediate hosts (Partoutomo et al. 1995).

A number of organisms have been identified that exploit the free-living stages of parasites as a food source. These include microarthropods, protozoa, predacious nematodes, viruses, bacteria and fungi (Waller and Faedo 1996). Although all are of intrinsic interest, it is from the latter two groups of organisms that breakthroughs in biological control are likely to emerge.

Bacteria

Many species of bacteria are associated with the cuticle, body cavity and gut of nematodes and some of these are pathogenic. Bacillus penetrans has been shown to be a promising candidate for the control of parasitic nematodes of plants. It produces highly resistant spores which attach to the cuticle and then invade the nematode host. This bacterium is highly host-specific, which is both a good and a bad thing. It is good from the standpoint that only the target nematode pest will be affected, but bad insofar as the search for the specific B. penetrans pathogen for each of the whole range of nematode pests would be most laborious, expensive and fruitless in many cases. Another factor that is hampering the exploitation of this organism is the difficulty in synthetic culturing of large quantities of B. penetrans, which is an absolute pre-requisite for commercialisation.

Many bacteria and closely related organisms, the Actinomycetes, produce important secondary metabolites, which include antibiotics, insecticides and anthelmintics. As such they should be regarded as microbial control agents rather than true biological control agents.

Fungi

Fungi that exhibit anti-nematode properties have been known for a long time. They consist of a great variety of species which include nematode-trapping (predacious) fungi, endoparasitic fungi, fungi that parasitise nematode eggs, and fungi that produce metabolites that are toxic to nematodes (Barron 1977). The most important groups of nematophagous fungi are the first two, namely:

Nematode-trapping fungi.

These fungi produce specialised hyphal trapping devices, such as adhesive networks, knobs, and constricting or non-constricting rings. Fungi in this class may also produce nematode chemoattractant and/or chemotoxic substances (Waller and Faedo 1993). Within a short period of time following capture of the nematode, the fungus penetrates the worm and destroys it.

Endoparasitic fungi.

These fungi invade the nematode from adhesive spores that stick on the cuticle, from spores that are ingested by the nematode, or from motile spores in water.

Fungi from these two classes are found in all environments throughout the world, but are particularly abundant in rich agricultural soils. Under laboratory conditions, where fungi are grown as a monoculture on standardised, generally nutrient-poor media and are provided with a nematode prey that cannot escape, results can be spectacularly successful. Total capture and destruction of nematodes can occur within a matter of hours. However, this type of work provides little relevant information as to how these fungi would perform as practical biological control agents against animal parasitic nematodes. Testing needs to be done to determine the limitations and opportunities for parasite control associated with the livestock production systems being considered.
Methods for Selecting Fungi as Biological Control Agents

The most important principle for selecting candidate fungi as putative biological control agents is to obtain isolates from the field in the region, or country, where this work is to be performed. This is important for several reasons.

Firstly, it has been observed that laboratory stocks of fungal isolates lose various attributes, which may include nematophagous capacity, following repeated passage.

Secondly, most countries have stringent requirements regarding the importation and field release of exotic living organisms. These two drawbacks would apply if strains of fungi with known nematophagous activity were obtained from the major fungal collections or repositories in Europe or North America.

Thirdly, most of the current research on biological control is with *Arthrobotrys oligospora* and particularly, *Duddingtonia flagrans* conducted in Denmark and Australia. Local isolates are being used in these studies (Denmark, see Larsen et al. 1991; Australia, see Larsen et al. 1994) which represent the cold to cool temperate regions of the world. These fungal species could well prove to be inappropriate in the humid tropics/subtropics. However, in the broader sense it is possible that fungal species would have evolved to be more suited to any given region, or locality, than those derived from centralised fungal collections, or from laboratories in Denmark or Australia.

The most relevant sites for sampling would be the environments where the fungi are expected to exert their effects, notably fresh faecal deposits, but in intensive animal production systems, animal bedding may also be appropriate.

The reason for restricting the sampling to these sources is simply to save unnecessary labour at a later stage, because in almost all circumstances, fungal deployment will be in ways which require it to survive passage through the gastro-intestinal tract of animals and then to trap nematodes in freshly deposited faeces. Almost certainly, a plethora of nematophagous fungi would be isolated from other sources such as soil, pasture etc. However almost all would fail the most important test of gut survival and thus their isolation (and any other testing) would be a wasted effort.

The use of animals as a stringent screening procedure means that the number of occasions on which isolations can be expected is very few. Therefore if a serious attempt is to be made, a large number of small samples should be collected *per rectum*, from livestock found on a comprehensive range of farms in the region. Suitable procedures have been described by Larsen et al (1994). Following isolation by these means, pen trials should be carried out to confirm the gut survival and nematophagous capabilities of the fungal strains.

Means of Fungal Deployment

Direct application

This could only be considered in the most intensive forms of animal production where animals are closely confined, and of course, where internal parasitism is a problem. Such an example would be the intensive calf-rearing units in the southern islands of Japan where *Strongyloides papillosus* can cause sudden death in massively infected animals in the hot summer months (Taira and Ura 1991). A practical solution to this problem may be the direct application of fungal elements to the bedding. Therefore, the requirement for fungi to survive gut passage is not relevant in this circumstance. All that would be required is for the fungi rapidly to colonise the bedding and to reduce the overwhelming number of *S. papillosus* larvae responsible for the sudden death syndrome, but to allow sufficient numbers to survive to provoke the normal, rapid acquisition of immunity which characteristically occurs against this parasite.

However, apart from similar forms of highly intensive livestock production, it is beyond the bounds of reality to conceive of a practical means of applying fungal material, especially to the grazing environment, to produce reliable and substantial reduction in the free-living stages of parasites.

Supplementary feeding

Danish workers have demonstrated that a daily supplement of barley grains supporting the growth of *D. flagrans* will reduce parasitism and increase productivity in grazing cattle (Gronvold et al. 1993), pigs (Larsen et al. 1995), horses (Nansen et al. 1995) and sheep (Larsen et al. 1996). These results are particularly exciting as they demonstrate that the principle of biological control of nematode parasites using nematophagous fungi is particularly robust, being applicable across the whole spectrum of grazing livestock species. Clearly then, the transfer of this technology to those industries where long-term daily supplementary feeding is a common management procedure would be relatively straightforward. The major impediment would be the need to scale-up production to satisfy the commercial requirements for the fungal grain supplementary feed option for biological control of nematode parasites.
**Feed blocks**

Block administration, developed mainly for mineral supplementation and to a lesser extent for anthelmintic medication, is now undergoing a resurgence of interest as a means of low-cost nutrient supplementation of livestock in the developing world. These blocks can be manufactured using simple, low-cost technology and generally incorporate surplus plant by-products as the nutrient source. These by-products may well prove to be suitable growing substrates for locally isolated strains of nematophagous fungi. A range of block formulations containing *D. flagrans* chlamydospores have been tested and the results are very encouraging (Waller and Knox, unpublished data). These blocks have also been shown to have a shelf life of at least six months. Fungal blocks could prove to be an important control option in the humid tropics and sub-tropics where tethered husbandry and night housing with stall feeding are common animal management practices and where anthelmintic resistance is a serious problem.

**Controlled release devices**

Intra-ruminal sustained or controlled release devices are a modern advance in anthelmintic medication. Although the unit costs of these devices are high, they allow great flexibility in animal management insofar as they provide protection against parasite infection for an extended period of time. Rather than using anthelmintic compounds, devices containing fungal spores could provide this extended prophylactic effect. The objective would be to develop a device which would release sufficient spores for an extended period (60 days or more) to result in a substantial reduction in the number of infective larvae which succeed in migrating to pasture over the same time period. These devices could be administered at epidemiologically critical times to reduce seasonal peaks in larval numbers but would allow sufficient larvae to escape and thus provoke the development of naturally acquired immunity in grazing livestock.

Investigations have shown that chlamydospores of *D. flagrans* can withstand tableting pressures required for manufacture of these devices. The devices have a good shelf life and can release optimum concentrations of spores for effective parasite control in vivo (Waller and Ellis, unpublished data). Further work is required to test the time/release profiles of fungal chlamydospores in these prototype devices and to verify the long-term in situ viability of spores in devices administered to livestock. Although it is premature to speculate as to whether commercially attractive, fungal controlled release devices will be developed, they have an enormous potential market as a non-chemotherapeutic, environmentally benign form of parasite control to all the grazing livestock industries throughout the world.

**Conclusion**

Significant recent advances in the development of practical means of deploying nematophagous fungi as biocontrol agents of nematode parasites for a range of livestock species, clearly indicate that this may be the first non-chemical alternative to parasite control, other than selective breeding. This will not be before time. Resistance to anthelmintics is now rampant in nematode parasites of small ruminants in many countries in the tropical/sub-tropical zone. As more countries reach this chemotherapeutic endpoint, abandonment or complete restructuring of their small ruminant industries will be the inevitable and tragic consequence. Biological control may be one way of delaying this doomsday scenario.

**References**


Increasing Resistance by Selection

R.R. Woolaston

Abstract

Resistance to nematode parasites can be improved by selection, but only recently have efforts been made to include resistance as a trait in commercial livestock breeding programs such as those currently operating in Australia and New Zealand. The steps required parallel those used for improving other traits. Three approaches have been used: breeding for resistance (reduced parasite numbers, as determined by faecal worm egg count), resilience (production during parasitism), or number of treatments required during parasitism. It is necessary, but difficult, to assess the economic benefits of improving resistance relative to other traits, because the impact of parasites varies widely depending on the production environment and on the availability, effectiveness and sustainability of alternative control measures. Selection for resistance usually requires that animals are exposed to parasites so that the effect of the host on parasite numbers can be assessed. In the longer term, it is desirable that selection criteria for all major diseases be developed that will be informative in healthy animals. Molecular genetic markers offer promise, but simple genetic markers have so far been as elusive as physiological traits to predict resistance in undiseased animals. Useful genetic markers should eventually be found and techniques for combining these with phenotypic information need to be developed.

RESISTANCE of small ruminants to nematode parasites can be improved by selection, but only recently have efforts been made to include the trait in commercial livestock breeding programs such as those currently operating in Australia (Nemesis, Anon. 1994) and New Zealand (WormFEC™, McEwan 1994). Southern and Southeast Asia encompasses a wide range of ecological zones in which there are numerous indigenous breeds of goats and sheep. Many of these have evolved in areas with high levels of nematode parasite challenge. As production systems intensify, it is likely that between-breed variation will be a useful resource to be exploited in genetic improvement programs designed to enhance resistance. Aspects of between-breed variation have been discussed by Baker (these Proceedings) and this author will concentrate mainly on matters related to within-breed selection for worm resistance in sheep. Discussion will be mainly based on a large body of data collected from the Australian Merino, augmented with data collected from tropical sheep and goats in Fiji, and with research conducted in New Zealand.

Within-breed Genetic Variation in Resistance

Host resistance is heritable and so it is possible to make genetic progress in a breeding program. One of the most-widely used measures of resistance, faecal worm egg count (FEC), has a heritability of 0.2 to 0.3 (Gray et al. 1995). Comparable heritabilities for other traits in the Australian Merino are 0.40 for fleece weight, 0.5 for average fibre diameter, 0.4 for body weight and 0.1 for reproductive rate (Ponzoni 1987). Heritability, however, is not the sole determinant of genetic progress. Variability, as described by variance or coefficient of variation (CV), is equally important. The response to selection, in percentage units, is given by the formula:

\[ \% \text{Response} = \text{heritability } \times \text{selection intensity } \times \text{CV\%} \]

so it is clear that for any given selection intensity, the percentage response in a trait is proportional to the product of that trait's heritability and its variability.
Coefficients of variation in FEC in excess of 100% are common, compared with about 60% in reproductive rate, and 7–15% in other traits of economic importance. Thus FEC is an extremely variable trait and for this reason, relatively rapid genetic progress should be possible when selecting for resistance. This prediction has been born out in single-character selection lines, such as the CSIRO Merino lines selected for divergent response to challenge with *Trichostrongylus colubriformis* (Windon et al. 1987) or those selected for increased or decreased resistance to *Haemonchus contortus* (Woolaston and Piper 1996, Fig. 1).

In another experimental flock run at the University of New England, 60 rams were progeny tested for resistance to *H. contortus* and the distribution of their estimated breeding values for FEC is shown in Figure 2.

![Figure 1](image1.png)

**Figure 1.** Estimated breeding values for *Haemonchus contortus* faecal egg count (back-transformed) in a long-term selection flock (after Woolastron and Baker 1996).

![Figure 2](image2.png)

**Figure 2.** Frequency distribution of deviations of sire group means from the overall mean for four week egg counts, square root transformed (after Albers et al. 1987).
In the University of New England flock, the progeny means for most sires were clustered together (Fig. 2), reflecting a normal distribution, but one sire had extremely resistant progeny. This ram, dubbed the 'Golden Ram', was originally thought to be the carrier of a major gene for resistance to *H. contortus*, but exhaustive investigations have failed to prove this hypothesis (Woolaston et al. 1990, Woolaston, Gray and Piper, unpublished). Another possibility was that the sire came from a separate Merino sub-population to the other sires under test, and that the difference was merely a manifestation of between-flock variation. Extensive tests of a large number of Merino genotypes in experimental resource flocks (Eady et al. 1996) have shown that this is also an unlikely explanation. The total variation in FEC following challenge of weaner sheep with *H. contortus* or *T. colubriformis* can be partitioned into the component sources of variation. Table 1 shows that most of the genetic variation in FEC can actually be found within-flock and that by comparison, variation between major (strains) and minor (between flocks within strains) genetic classifications of the breed is very much smaller. This contrasts with the sources of variation in production traits such as fleece weight, average fibre diameter and body weight, where systematic strain and flock effects are far greater.

### Genetic Comparisons

During collection of the data summarised in Table 1, care was taken to ensure that comparisons were made on animals born and managed together as a single group. This is particularly important when testing for resistance, so that age-acquired resistance is not a confounding effect in between-animal comparisons and so that management history is standardised, as this can have profound and lasting effects on expressions of resistance (Gray et al. 1990). An important inference to be drawn from Table 1 is that within-flock genetic variation in resistance can be much greater than between-flock or between-strain variation, and so adequate sampling is essential in any breed comparison. The simplest way to ensure adequate representation of a breed would therefore be to maximise the number of unrelated sire groups represented.

### Approaches to Selection

Three main approaches have been advocated for improving the host's ability to deal with parasites. The first, selecting for resistance, focuses on maximising the effect of a host on its parasites. The other two approaches, viz. breeding for resilience or reduced number of treatments, aim to minimise the effect of parasites on the host. Each of these three methods has advantages and disadvantages and these have been discussed at some length by Woolaston and Baker (1996). Breeding for resistance is arguably the most practical method, because resistance, as measured by FEC, is more highly heritable than the other two traits and there are fewer practical problems encountered when incorporating the trait into commercial breeding programs.

Some New Zealand workers have suggested that breeding for lower FEC will not necessarily reduce the level of scouring or dag formation in a flock, nor will it necessarily reduce the number of anthelmintic treatments required (Bisset et al. 1994, 1996). These authors advocated using a selection procedure which includes some assessment of the need to treat individual animals for protection against parasites, whether measured by the number of drenches required in a given period or by the age at first treatment. This should have the dual advantages of reducing the need for anthelmintic treatment and also of reducing the prevalence of scouring and dag formation. Both of these measures, however, were quite poorly inherited (estimated heritabilities of 0.06 and 0.03, respectively) but strongly correlated with live-weight gain and dag score. Furthermore, the procedure used to measure the need for treatment appeared to adversely affect the heritability of production traits. Together, these results suggest that selecting for production plus some measure of resilience may be less efficient than selecting for

### Table 1. Sources of variation in faecal egg count (average of *H. contortus* and *T. colubriformis* challenges, from Eady et al. 1996) and production traits (from Mortimer and Atkins 1989).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Faecal egg count</th>
<th>Clean fleece weight</th>
<th>Av. fibre diameter</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between strain</td>
<td>1%</td>
<td>29%</td>
<td>25%</td>
<td>21%</td>
</tr>
<tr>
<td>Between-flock (within strain)</td>
<td>3%</td>
<td>13%</td>
<td>12%</td>
<td>13%</td>
</tr>
<tr>
<td>Within-flock genetic</td>
<td>24%</td>
<td>16%</td>
<td>31%</td>
<td>23%</td>
</tr>
<tr>
<td>Within-flock non-genetic</td>
<td>72%</td>
<td>42%</td>
<td>32%</td>
<td>43%</td>
</tr>
</tbody>
</table>
production in animals that are essentially non-parasitised, except during a brief period when resistance is tested with a FEC. Given the parameters published by Bisset et al. (1994, 1996), it seems likely that a procedure which places selection pressure simultaneously on increasing production while decreasing FEC will lead to direct gains in productivity and resistance. This should give favourable progress in reducing the need for anthelmintic treatments, while at the same time reducing scouring and dag score. Where it is considered necessary, further reductions in the level of scouring and dag formation should be obtainable by including dag score as an additional selection criterion, or perhaps by placing more selection emphasis on body weight.

Selection Criteria for Resistance
Various indicator traits have been investigated as an alternative to FEC:

- Packed cell volume. This trait is particularly useful where the main parasite under investigation is *H. contortus*, and the trait is used widely in tropical Africa as a measure of *H. contortus* infection (R.L. Baker, pers. comm.). It appears to be about as heritable as FEC and is highly correlated with it under *H. contortus* challenge (Woolaston and Piper 1996). The trait, however, is of little use in predicting the level of infection of other important parasites such as *Trichosirostrongylus* spp. or *Ostertagia circumcincta*.

- Lymphocyte responsiveness. An in vitro assay for determining lymphocyte responsiveness to nematode antigens was used to select sheep in lines developed for resistance to *O. circumcincta* (Cummins et al. 1991) but has since been found to be less suitable than FEC in that breeding program.

- Circulating eosinophils. Woolaston et al. (1995) investigated this trait in several sheep populations and concluded that although it was informative in some situations, the trait was not as suitable as FEC as a selection criterion for a breeding program.

- Ovine lymphocyte antigen type. Recent studies have indicated that this trait is of little use for indicating resistance to *H. contortus* and its usefulness for indicating resistance to *T. colubridiformis* also appears to be limited (Woolaston, Gray, Eady and Outteridge, unpublished data).

- Faecal antigens. Workers in Western Australia are currently developing a test which uses worm antigens in faecal samples to estimate the worm species present in the gastrointestinal tract (R.B. Besier, pers. comm.). The test also shows promise as an indicator of comparative numbers of parasite species present in a sheep's gastrointestinal tract. It is unknown at this stage whether the trait will be suitable for routine use in breeding programs, but appropriate investigations are in progress.

- Circulating antibodies. Douch et al. (1995) evaluated a test to quantify worm antibodies circulating in sheep blood, and genetic correlations suggest that the trait can add useful information to a breeding program. The *WormFEC* program in New Zealand (McEwan 1994) can accommodate information from circulating antibodies, when it is available.

- Molecular genetic markers. In the longer term, these offer considerable promise as criteria for identifying animals with genetically superior disease resistance. Once a sufficient number of markers are known, it should be possible to identify resistant animals without the need to expose hosts to the disease under a standard testing protocol. However, it will be some years before markers are able to supplement phenotypic data, and it is not clear at this stage how such markers might best be used in commercial breeding programs.

Incorporating Resistance into Breeding Programs
Procedures currently used in Australia and New Zealand rely on infecting animals with third stage roundworm larvae, either artificially or naturally, then allowing a period to elapse (at least four weeks) so that FECs are sufficiently high to allow discrimination between animals in their resistance status. At this time, faecal samples are obtained and submitted to a laboratory for FEC determination. After FEC data are known, they are transformed statistically (usually using a cube root or logarithmic transformation) and combined in a conventional selection index with data from other traits. Genetic theory predicts that adding another trait to a breeding objective will reduce expected genetic progress in the existing traits. It is therefore wise to provide the breeder with information on the extent to which they are compromising gain in other traits by including resistance in their breeding objective and this is routine practice in both the Australian *Nemesis* program and the New Zealand *WormFEC* program. Figure 3 demonstrates graphically the effect of diverting some selection pressure to improving resistance, using data from a typical Merino flock.
Figure 3. Scatter plot showing worm resistance and productivity in a typical Australian Merino flock. The broken lines delineate the best 30 animals either on production alone, or on a combination of production and resistance.

Figure 4. Estimated breeding values (EBV) for resistance, expressed in standard deviations of cube root transformed faecal egg count (epg). Data are derived from a centralised Merino sire progeny test in Australia. (After Eady 1995).
The extent to which gain in other traits should be compromised in order to improve resistance can only really be determined when the relative economic value of resistance is known. Reliable objective information on the benefits of improving traits such as fleece weight, average fibre diameter, body weight or reproductive rate in specific sheep production systems is readily available (Morris et al. 1982, Ponzoni 1987). However, insufficient information is available at present to quantify accurately the value of a unit change in resistance relative to gains in production traits and there is an urgent need to quantify the economic benefits of improving resistance. Such data are obtainable from suitably designed field studies of resistant and susceptible genotypes.

Other relevant information available to Australian Merino breeders is provided by central test sire evaluation schemes, where a number of sires are progeny-tested over a common group of ewes. Information from several testing programs can be combined, as can information collected over time, by ensuring that sufficient genetic links exist. A wide range of traits are recorded in these schemes and a recent innovation has been to also record FEC. Findings thus far illustrate the wide genetic variation that exists between sires in the Australian Merino population (Fig. 4).

**Conclusion**

Breeding for resistance is possible and several selection lines have demonstrated the progress that is possible in sheep (Baker et al. 1991, Karlsson et al. 1991, Cummins et al. 1991, Windon et al. 1993). In the context of sustainable parasite control systems, the benefits attainable from manipulating host genotype include a reduction in pasture contamination and parasite numbers, a reduction in scouring and associated problems of dag formation and reduced use of anthelmintic chemicals. Genetic theory predicts that these improvements should be attainable while simultaneously improving productivity.

If so desired, relatively rapid genetic improvement can be made in resistance, but maximum gains can only be made by compromising the rates of improvement in other traits. The optimal way of using the available selection pressure to change the individual components of a breeding objective will depend heavily on the relative economic values of those components. Aspects that require further study include the epidemiological value of reducing FEC in a production system, the relative importance of scouring and dag formation particularly in breeds maintained for fibre production, and the physiological cost, if any, associated with maintaining resistance in the presence of various levels of parasite challenge. In assigning a relative economic weight to improved resistance in their own breeding programs, breeders should also consider the importance of parasitism as a production constraint in the target population and also uncertainty surrounding the availability of chemotherapeutic control measures at some future time.

**Acknowledgements**

Much of the research work discussed in this paper has been supported by Australian woolgrowers, most recently through the International Wool Secretariat.

**References**


Characterisation and Utilisation of Sheep and Goat Breeds that are Resistant to Helminths

R.L. Baker

Abstract

There is a large and diverse range of sheep and goat breeds in the world and some of these, particularly the indigenous tropical breeds, appear to have some unique genetic ability to resist or tolerate diseases. This paper reviews the information available on between-breed genetic variation for resistance to helminthiasis (mainly the gastrointestinal (GI) nematodes) and discusses how this genetic variation can be used in breeding programs.

The experimental design used in nearly all the breed comparisons reviewed is inadequate. In particular, the number of animals of each breed evaluated is too small, very few studies take account of variation among sires within breeds, and how the animals are sampled is not stated. However, there are a number of sheep breeds that have been identified as resistant in a number of independent studies and these include the East Africa Red Maasai, the Florida Native, the Barbados Blackbelly and the St. Croix. There is much less evidence for breeds of goats that are resistant to GI nematodes but the indigenous tropical breeds such as the Small East African and West African Dwarf may be somewhat resistant.

Helminths constitute one of the most important animal health constraints to small ruminant production in both the temperate (McLeod 1995) and tropical (Fabiyi 1987) regions. The widespread occurrence of infection with endoparasites, the associated loss of production, the cost of anthelmintics and in some cases high mortality rates of infected animals (particularly in the tropics) are some of the major concerns. There is also growing public concern about environmental issues, particularly the regular and often excessive anthelmintic usage leading to chemical residues in animal products and pastures.

In nearly all regions of the world control of endoparasites in ruminant livestock is currently largely achieved by the use of anthelmintics, although pasture management also plays a role in some temperate regions. In many tropical regions of the world these control methods are limited by the high cost of anthelmintics, their uncertain availability, increasing frequency of drug resistance (in both tropical and temperate regions), and limited scope in many communal pastoral systems for controlled grazing to reduce parasite contamination of pastures. In this situation an attractive, sustainable solution is identification and utilisation of host genetic variation for resistance or tolerance to endoparasites.

Much of the recent research on genetic resistance to endoparasites in sheep has concentrated on quantifying within-breed genetic variation and selection of resistant (high responder) and susceptible (low responder) lines of sheep as reviewed by Gray (1991), Gray and Woolaston (1991), Gray et al. (1995) and Woolaston and Gray (1996, these Proceedings). There is a large and diverse range of sheep and goat breeds in the world and some of these, particularly the indigenous tropical breeds, appear to have some unique genetic ability to resist or tolerate diseases. This paper reviews the information available on between-breed genetic variation for resistance to helminthiasis (mainly gastrointestinal nematodes) in small ruminants and discusses how this genetic variation can be used in practical breeding programs.

1International Livestock Research Institute, PO Box 30709, Nairobi, Kenya
Evidence for Breed Variation in Resistance to Endoparasites

There have been many reports since the mid-1930s of variation among breeds of sheep in resistance to GI nematodes, particularly to *Haemonchus contortus*, *Trichostrongylus* spp and *Ostertagia* (Teladorsagia) spp. Gray (1991) reviewed and summarised 23 publications on this subject and this was expanded to 34 publications in a review by Baker et al. (1992). Space does not permit tabulating a summary of these publications in this paper, but this information is available from the author on request. Some of the important conclusions which arise on reviewing these publications are the following:

- Host resistance to *H. contortus* has been most commonly found but there is also evidence for resistance to *Ostertagia* sp and *Trichostrongylus* sp.
- Resistance has been demonstrated both with artificial infection and natural pasture challenge. Usually with natural challenge this involves a number of parasite genera with one or two predominating.
- In nearly every case faecal egg counts (FEC) have been used to measure resistance, but packed cell volume (PCV) and worm counts following necropsy have also been commonly measured. Production traits and mortality rates have been recorded less commonly.
- Resistance has been demonstrated in both lambs and mature animals (ewes, rams and wethers).
- The experimental design used in nearly all these breed comparisons was poor. In particular the number of animals of each breed evaluated (commonly about 5–10) was too small, very few studies took account of variation among sires within breeds, and how the animals were sampled was not stated. Requirements for adequate experimental designs for breed evaluation have been comprehensively reviewed and discussed by Dickerson (1969). How animals are sampled and the family structure (i.e. number of sires and progeny per sire) are critical factors.
- While many of the publications on breed variation for resistance to endoparasites can be criticised in terms of experimental design, it is reassuring to note that some breeds have been identified as resistant in a number of independent studies. This applies particularly to the East African Red Maasai (Preston and Allonby 1978 and 1979; Bain et al. 1993; Baker et al. 1993, 1994a, 1994b), the Florida Native (Loggins et al. 1965; Bradley et al. 1973; Zajac et al. 1988), the Barbados Blackbelly (Yazwinski et al. 1979 and 1981) and the St. Croix (Courtney et al. 1984, 1985a, 1985b; Zajac et al. 1990; Gamble and Zajac 1992; Zajac 1995) and for these breeds it can be concluded that they are relatively resistant to GI nematodes.
- Most of the breeds identified as being relatively resistant are indigenous or 'unimproved' breeds. This presumably reflects the fact that these breeds have been under natural selection for resistance for many centuries with no anthelmintic treatment. In addition to the breeds mentioned above that have been reasonably comprehensively characterised as resistant to GI nematodes there are other interesting breeds which may be resistant. These include the West African Djallonke sheep which may be resistant to endoparasites and trypanosomiasis (Baker 1995); the Sabi sheep in Zimbabwe (McKenzie 1986) and the Garole sheep in India (Ghalsasi et al. 1994). It is worthy of note that the Carribean St. Croix sheep originated from West Africa and are probably related to the Djallonke sheep (Bradford and Fitzhugh 1983).

The evidence for genetic variation for resistance to endoparasites among goat breeds is limited (Baker et al. 1992; Baker 1995) and all of these studies suffer from the same shortcomings in experimental design noted for sheep. As for sheep, it is usually the indigenous goat breeds (e.g. the Small East African and the West African Dwarf) that are more resistant than the imported exotic breeds. It is possible that the mechanisms or level of resistance may be different in sheep and goats, since, as goats are predominantly browsers, they are likely to have been under less intense natural selection for resistance. Indeed, it is usually reported that goats are innately more susceptible to nematode parasites than sheep, but the degree of susceptibility can differ for different parasite species (Gruner 1991). In those areas where browse is freely available it is often observed that the prevalence of endoparasites is higher in sheep than goats. This may not tell us anything about the relative resistance of sheep and goats to endoparasites, but could just reflect different grazing habits. More research on resistance of goats to internal parasites is needed, especially in view of their numbers and importance in many tropical regions.

In an effort to rectify the shortcomings in experimental design observed in most previous breed comparisons, in 1991 the International Livestock Centre for Africa (ILCA — now ILRI) initiated a pan-African research project to investigate more comprehensively both between- and within-breed genetic resistance to GI nematodes in some of the indigenous small ruminant breeds. Currently this research is evaluating the Menz and Horro sheep breeds in Ethiopia; Red Maasai and Dorper sheep and Small East African and Galla goats in Kenya;
Table 1. Least squares means by breed group for weaning weight (WWT, kg) PCV%, GFECa (EPG) and mortality (MORT%).

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<tr>
<td></td>
<td>WWT</td>
<td>PCV</td>
<td>GFEC</td>
</tr>
<tr>
<td>DxD</td>
<td>288</td>
<td>11.1</td>
<td>23.1</td>
</tr>
<tr>
<td>Dx(RMxD)</td>
<td>392</td>
<td>10.9</td>
<td>24.7</td>
</tr>
<tr>
<td>DxRM</td>
<td>97</td>
<td>10.5</td>
<td>25.5</td>
</tr>
<tr>
<td>RMxD</td>
<td>229</td>
<td>10.7</td>
<td>25.2</td>
</tr>
<tr>
<td>RMx(RMxD)</td>
<td>404</td>
<td>10.7</td>
<td>26.1</td>
</tr>
<tr>
<td>RMxRM</td>
<td>154</td>
<td>10.0</td>
<td>27.1</td>
</tr>
<tr>
<td>Total Number</td>
<td>1564</td>
<td>1267</td>
<td>1258</td>
</tr>
<tr>
<td>Overall Mean</td>
<td></td>
<td>10.7</td>
<td>25.3</td>
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aFEC logarithm transformed for analysis and then the anti-log (i.e., the geometric mean-GFEC) presented in this Table.


The sheep research in Kenya is now almost completed with the sixth and last lamb crop having been born in 1996. Some of the results from the first five lamb crops (1991–95) will be presented to illustrate what can be achieved in a more comprehensive breed evaluation experiment.

The study is being carried out at Diani Estate of Baobab Farms, 20 km south of Mombasa in the subhumid coastal region of Kenya. In 1991 Dorper and Red Maasai × Dorper (F1) ewes, and in 1992 and subsequently Dorper, F1 and Red Maasai ewes, were single-sire mated to 12 Dorper and 12 Red Maasai rams each year in a complete diallel to produce the six lamb genotypes shown in Table 1. At least half of both the Dorper and Red Maasai rams used each year were replaced by new rams the next year. Over the period reported here (1991–95) a total of 41 Dorper and 34 Red Maasai rams have been used. The rams were obtained from a wide range of sources and districts to ensure representative samples of each breed.

The ewes were weighed six times during the reproductive cycle: at mating, three months after mating, two weeks before lambing and one, two and three months after lambing. Blood and faeces samples were collected from all ewes at each weighing. Blood was taken to determine packed cell volume (PCV) — a measure of anaemia — and was examined for trypanosomes. Faecal egg counts (FEC) — a measure of endoparasite infestation — were taken and faecal samples, bulked by breed, were cultured and parasite larvae present were identified. Ewes found to have a FEC greater than 4000 eggs per gram (EPG) or a PCV less than 15% at any sampling time were treated with an anthelmintic drug.

Lambs were weighed as close to birth as possible, usually within 24 hours, and then every two weeks up to weaning at three months old. PCV and FEC were recorded on all lambs at one and two months of age. Individual lambs were treated with anthelmintic drugs at these times if they had a FEC greater than 2000 EPG or a PCV less than 20%. All the lambs were treated with an anthelmintic at weaning (90 days of age). They were then grazed on pasture until a monitor group of about 50 lambs, from which samples were taken every week, reached a FEC averaging between 1500 and 2000 EPG. All the lambs were then weighed and faeces and blood samples were taken on two consecutive days. All lambs were then treated with an anthelmintic. This procedure was repeated until the lambs reached one year of age, which usually involved four or five sampling times.

Differences among the breeds and crosses for weaning weight, PCV, FEC and mortality for lambs at weaning (1991–95 data) and at one year of age (1991–94 data) are shown in Table 1. Faecal cultures at these times showed that 66% of the larvae were Haemonchus contortus, 30% Trichostrongylus spp and 4% Oesophagostomum spp. The results in Table 1 show that Red Maasai (RM) lambs are more resistant to endoparasites than Dorpers (D) in the subhumid zone of coastal Kenya. Red Maasai lambs have significantly lower FEC, higher PCV and lower lamb mortality than Dorper lambs. There is also an additive genetic breed effect in the crossbred lambs for resistance (i.e. for FEC, PCV or lamb mortality), but no evidence for heterosis for any of these traits.
The difference among breed groups in mortality is dramatic and post-mortem results show that about 50–60% of the post-weaning mortality is due to haemonchosis. The relative resistance of the Red Maasai lambs confirms earlier reports from research conducted in the Kenya Highlands (Preston and Allonby 1978 and 1979; Bain et al. 1993). In addition, Red Maasai ewes have lower FEC and higher PCV over the lambing-lactation period (the periparturient rise) than Dorper ewes (Baker et al. 1994b).

Based on these results an initial assessment has been made of the flock productivity of Dorper and Red Maasai sheep in this coastal Kenyan site (Table 2). The combined effect of higher lambing rate, lower lamb mortality and similar yearling live weights results in approximately a three-fold increase in the number of yearling sheep for sale and the weight of yearling sheep for sale in a Red Maasai vs a Dorper flock. There is therefore a clear economic advantage for farming the more resistant Red Maasai breed in the sub-humid regions of Kenya.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Dorper</th>
<th>Red Maasai</th>
</tr>
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<tbody>
<tr>
<td>Number of ewes mated</td>
<td>464</td>
<td>219</td>
</tr>
<tr>
<td>Ewes lambing/ewes mated (%)</td>
<td>62</td>
<td>74</td>
</tr>
<tr>
<td>Prolificacy (%)</td>
<td>102</td>
<td>103</td>
</tr>
<tr>
<td>Lamb mortality (Bth-Ylng, %)</td>
<td>63</td>
<td>27</td>
</tr>
<tr>
<td>Yearling live weight (kg)</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>Offtake (1 yr)a</td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td>Number of sheep</td>
<td>264</td>
<td>745</td>
</tr>
<tr>
<td>Live weight (kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aOfftake based on a 100 ewe flock with a 20% replacement rate.

The Dorper Flock is not sustainable at this replacement rate.

In addition to confirming genetic variation in resistance to GI nematodes among breeds and crosses, this study is also providing some reliable evidence of genetic variation within breeds because of the reasonably large number of sires used in the experiment. Heritabilities of both PCV and FEC were small (0.01–0.04) and non-significant in lambs at weaning but were significant at the yearling stage, strongly suggesting that this is an acquired immune response. The heritability of logarithm transformed FEC in 10–12 month old lambs was 0.22±0.07, but higher in Dorper-sired lambs (0.32±0.13) than Red Maasai-sired lambs (0.11±0.07). This suggests that after many centuries under endoparasite challenge that the Red Maasai sheep have become fixed for some of the important genes for resistance.

Virtually all the research on genetic variation to endoparasites in small ruminants has concentrated on the nematode parasites. In many areas of the tropics and temperate regions of the world liver fluke (trematode) infections (Fasciola hepatica and Fasciola gigantica) are also an important constraint to small ruminant production (FAO 1992). While it is well documented that sheep can mount an effective immune response (self-cure) to nematode parasites, it has been amply demonstrated that sheep are unable to acquire resistance to liver flukes (e.g. Haroun and Hillier 1986; Boyce et al. 1987). Possibly for this reason very little research has been undertaken on genetic resistance to liver fluke infections and few studies have been published. Boyce et al. (1987) found significant breed differences in faecal egg counts and fluke counts following experimental infection of five breeds of sheep with F. hepatica. Barbados Blackbelly sheep were the most susceptible to infection while St. Croix and Florida Naive sheep were the most resistant. While none of the breeds demonstrated an ability to resist reinfection with F. hepatica, clear breed differences were detected in response to infection. Wiedosari and Copeman (1990) reported relatively high resistance to F. gigantica in Javanese thin-tailed sheep, although there was no contemporaneous breed comparison. Roberts et al. (1995) compared the resistance to F. gigantica of Javanese thin-tailed sheep with St. Croix sheep and F2 and F3 crosses between these breeds. They concluded that the Javanese thin-tailed sheep were more resistant than St. Croix sheep and that resistance may be controlled by a major gene with incomplete dominance.

Breeding Programs

The main pathways of genetic improvement are choices among breeds or populations, selection within breeds and crossbreeding designed to exploit heterosis and/or combine the merits of different breeds. These genetic improvement options are not incompatible, but once a particular crossbreeding system is chosen and it has stabilised, then any further genetic progress can only be achieved through selection.

In many sheep or goat production systems in the temperate regions of the world within-breed selection is the only genetic improvement option which can be considered because breed substitution or crossbreeding would have unacceptable effects on the primary breeding goal (e.g. wool production in Merino sheep or mohair production in Angora.
goats). It has been clearly demonstrated that single-trait selection for resistance to GI nematodes is feasible in sheep and the important issue of how to combine selection for endoparasite resistance and production traits in multi-trait selection indexes is being addressed (Woolaston 1994; Gray et al. 1995; Sivarajasingam 1995).

In many tropical small ruminant production systems there is more flexibility to utilise both between- and within-breed genetic variation for endoparasite resistance in breeding programs (Baker et al. 1992; Baker 1995). A large amount of indiscriminate crossing with exotic breeds has occurred in the tropics. This is often done without any attempt to compare the exotic breeds or their crosses with the indigenous breeds, even though in some cases it has been extremely difficult to keep the exotic breeds alive and reproducing. It is now becoming increasingly clear that indigenous livestock that have evolved over centuries in the diverse, often stressful tropical environments, have a range of unique adaptive traits (e.g. disease resistance, heat resistance, ability to cope with poor quality feeds) which enable them to survive and be productive in these environments (Baker and Rege 1994).

Because of the many constraints to developing breeding programs in the tropics (e.g. small flock sizes, communal grazing, year-round breeding, poor market structure) there are clear advantages to creation of nuclei or elite flocks of indigenous small ruminant breeds to facilitate the efficient implementation of genetic improvement programs (Ponzoni 1992) and conservation and utilisation of these unique breeds and populations.

In general, crossing with exotic breeds should be avoided, although planned crossbreeding among indigenous breeds with complementary attributes could have a place in a well-structured industry (e.g. Gatenby et al. 1995; Romjali 1995).

References


Genetic Markers as Selection Criteria

M.J. Callaghan¹ and K.J. Beh¹

Abstract

The abundance of microsatellite sequences available for exploitation as genetic markers will lead eventually to dense genetic maps for a number of livestock species. Evenly spaced markers covering the entire genome selected from these maps can be used in linkage studies to detect markers associated with economically important traits and ultimately to track down the genes responsible. While such approaches are most readily applied to traits influenced by a single major gene, linkage analysis techniques have been applied to discover chromosome segments carrying genes with an effect on quantitative traits such as parasite resistance. However, given the relative sparsity of the sheep genetic map, discovery of the precise genes responsible will require supplementation of linkage methods with more direct approaches. These approaches will involve pinpointing genes by differential analysis of DNA or mRNA. This paper therefore provides a review of current strategies available for detection of genetic markers associated with important traits, with particular emphasis on approaches for detection of suitable candidate genes that can be tested for their effect on parasite resistance by linkage analysis. Genetic marker technology promises significant increases in accuracy when selecting livestock for breeding resulting in increased rates of genetic progress.

PARASITIC helminths cause considerable morbidity and mortality in livestock populations. The nematodes or roundworms are the most numerous, widespread and economically important group of internal parasites affecting sheep in Australia and are estimated to cost the industry $370 million annually (Collins 1992). Included in this estimate are the costs of administration of anthelmintics, the effectiveness of which continues to decrease with time, thus necessitating continual development of new, more expensive compounds.

In addition there is increasing public awareness of the effects of such treatments on the environment. This situation is driving development of alternative strategies for parasite control and in this respect genetic manipulation is seen as a promising option for sustainable control of parasites in livestock. Studies have shown that resistance to nematode infestation in ruminants is at least partly under genetic control with heritability estimates for *Haemonchus contortus* and *Trichostrongylus colubriformis* resistance of $h^2=0.34$ and $h^2=0.41$ respectively (Kloosterman et al. 1992).

It is therefore feasible to select genetically superior animals for parasite resistance using traditional selective breeding methods that have been used for a variety of other characteristics over the past several decades (Haley 1995). The use of genetic marker technology will enable streamlining of the selection process while increasing accuracy and minimising intrusive and time-consuming methods for assessing the resistant phenotype. Genetic marker technology will allow selection of animals for breeding at any age and may ultimately lead to identification of the actual genes responsible for a trait, elucidating their biological role in the phenotype.

Major Genes for Parasite Resistance

Reverse genetics approaches lend themselves most readily to the detection of markers for monogenic traits (i.e., those controlled primarily by the effects of a single locus), especially when the animals under

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¹CSIRO Division of Animal Production, McMaster Laboratory, Locked Bag 1, Blacktown, NSW 2148, Australia.
study come from outbred, half-sibling families commonly found in livestock populations. Unfortunately, like many other traits of economic importance such as growth rate, body composition and wool characteristics, resistance to parasites appears to be controlled by the combined action of many genes modified by various environmental factors, which means that associations will be difficult to detect even with the most powerful methods. However, for many economic traits there may be a few genes that account for a large proportion of the variation observed.

While most current genetic models assume input from an infinite number of genes, there is some evidence for the existence of genes having a major effect on parasite resistance in sheep. This evidence comes from the observations of Whitlock and Madsen (1958) that a Suffolk ram (named Violet) gave rise to offspring more resistant to the anemia associated with *H. contortus* infestation, and of Albers et al. (1987) that the offspring of one Merino sire (the so-called Golden ram) displayed a high level of resistance to *H. contortus* infection. In the latter example, segregation analysis of descendants from the resistant sire failed to reveal the existence of a major gene affecting *H. contortus* resistance in this flock. However, segregation analysis methods lack statistical power since even single gene effects are often confounded by the actions of other genes and by environmental influences.

A more powerful approach to detect major genes is to narrow the search to regions of the chromosome where suspected candidate genes loci reside. Parasite resistance appears to have an immunological basis, since vaccination-challenge experiments (Windon 1990) show that parasite resistance is an acquired response. The central role of the immune system is further shown by additional experiments where removal of the ability to mount an immune response removes the difference between resistant and susceptible animals (Presson et al. 1988). However the plethora of genes encoding immune system components are located at diverse regions of many chromosomes and while some studies have been performed to test some individual obvious candidates such as MHC, IgH and L chain and T-cell receptor genes (Blattman et al. 1993; Blattman and Beh 1994), no associations have yet been found. New methods to detect major genes will be discussed later in this review but currently most searches for genes affecting parasite resistance in sheep are based on whole genome scans using genetic markers and applying linkage analysis to detect co-segregation of markers and parasite resistance in resource families.

### Molecular Basis of Genetic Markers

Genetic markers arise from the polymorphic differences in DNA segments at a particular genomic location between two individuals. The earliest genetic markers were derived from mutations in restriction enzyme sites at a particular locus. On digestion of genomic DNA followed by Southern blotting and hybridisation to a cloned probe, size differences are detectable between individuals (Southern 1975), hence these markers are referred to as restriction fragment length polymorphisms (RFLPs). However because the polymorphisms observed are based on the presence or absence of a restriction site, they are usually diallelic. As mapping tools RFLPs were superseded by the considerably more polymorphic minisatellite probes first described by Wong et al. (1986) which detect variable numbers of tandem repeats (VNTRs) at multiple loci in digested DNA. These polymorphisms manifest also as size variations on a gel but show much greater variation due to the presence of hypervariable repeat sequences. When converted to single locus probes, VNTR motifs show a high level of polymorphism but they are generally restricted to telomeric regions of chromosomes (Royle et al. 1988).

The markers of choice for detecting associations with traits of economic importance are microsatellites which are composed of 1–4 base-pair motifs reiterated up to a hundred times. These simple tandem repeats have been found interspersed throughout the genomes of many eukaryotes (Hamada et al. 1982) and polymorphisms in repeat number are detected following PCR amplification of a short (100–300 base-pairs) region of DNA using specific primers that flank the repeat sequence. Products are radioactively labelled and are sized on polyacrylamide sequencing gels. Beckmann and Soller (1990) estimated that microsatellite repeats occur on average once every 50–100 kb in mammalian genomes which make them ideal markers to use when total genome coverage is important. Moreover, candidate loci showing little or no polymorphism as RFLPs are likely to be located near a microsatellite that could be characterised and used as a polymorphic marker for that gene. With such a high degree of polymorphism, the availability of microsatellite markers means that the extended families common in livestock populations should still be useful for linkage studies without necessarily having been designed for maximum heterozygosity.

In the absence of a restriction site mutation or microsatellite, another source of genetic markers can be derived from the presence of single base changes between alleles that are detectable by numerous
methods. One approach of particular utility, provided that sequence information is available, is to reveal these changes as differences in single stranded conformation (SSC) of PCR-amplified DNA (Hayashi 1991). PCR primers are designed to flank a gene region of several hundred base-pairs that would be expected to show variation between individuals, such as 5' or 3' untranslated sequences or introns. Longer fragments are digested with a suitable restriction enzyme before electrophoresis. Following amplification, the denatured DNA product migrates as single strands through a non-denaturing polyacrylamide gel in a manner dependent on fragment size and on the conformation assumed by the DNA strands, thus enabling detection of a single base mutation by differences in migration.

Recently, a method was developed that enables detection of SSCP's for fragments larger than 1 kb in agarose gels (Monckton and Jeffreys 1994). Mutations may be detected in double stranded DNA by following movement of PCR products through temperature or buffer gradients (Fischer and Lerman 1983). Another source of DNA polymorphisms employs the technique of RAPD (random amplified polymorphic DNA) analysis (Williams et al. 1990). Arbitrary 10-mer primers at low annealing temperatures during PCR yield DNA fingerprints that differ between individuals. Polymorphic bands can be converted to single locus probes if desired. To date, RAPD markers have been of limited use in genome analysis in animal populations.

Discovering Markers Associated with Resistance Genes

Population association studies

Some studies aim to detect linkage disequilibrium between a marker and the gene or trait of interest. The degree of linkage disequilibrium in a population depends on effective population size and the recombination fraction between pairs of loci and may arise not only by chance in small populations but also when two populations are crossed, when a population encounters a bottleneck or through selection that favours particular combinations of alleles at different loci. An obvious prerequisite for such methods is a candidate gene locus or an associated marker. Thus population association studies will not lead to the discovery of new genes and lack statistical power.

Linkage methods

With the potential availability of unlimited genetic markers there has been an upsurge in the use of more powerful linkage methods which make use of genetic and physical maps. Family data for which phenotypic data for the trait of interest and family member genotypes at marker loci are known are used to search for linkage between the gene and typed marker loci.

This technology presents the opportunity to scan the entire genome for marker loci associated with the trait of interest, eventually allowing identification of the actual genes involved. Indeed such studies have led to the isolation of genes responsible for important human genetic diseases such as Duchenne muscular dystrophy (Monaco et al. 1986), cystic fibrosis (Kerem et al. 1989) and familial breast cancer (Miki et al. 1994). With the number of mapped genetic markers on the ovine map at over 240 (Crawford et al. 1994), the use of evenly spaced ovine markers to detect major genes for parasite resistance is now feasible.

The more advanced cattle map has already yielded important linkage information on genes responsible increased milk production (Georges et al. 1993a) and for polledness (Georges et al. 1993b). However, although the road to detection of chromosomal segments containing genes affecting quantitative traits is now open, precise targeting of the gene will be difficult without densely mapped markers in the region of interest. Comparative maps can provide some assistance with generating such markers or even in suggesting possible gene candidates but the process of moving from linked marker to gene is a long and involved one.

In the next section, new techniques are presented that have the potential to assist with identifying key candidate genes from which to develop polymorphic markers thus increasing the chances of developing useful markers, without the need for a dense genetic map. Since they directly identify the genes involved it is possible that these methods will displace linkage studies in future as the method of choice for finding selective genetic markers.

Analysis of differences in expressed genes

Several recently developed techniques making use of the polymerase chain reaction show considerable potential towards enabling more direct isolation of genes responsible for a trait. The best possible selective genetic markers are those most closely linked to the genes responsible for the observed trait or ideally the genes themselves. While mapping experiments provide a good chance of locating the general chromosomal regions containing these genes, identification of the actual gene within this region requires that the region be densely mapped and also requires pedigrees suited to fine scale mapping of the trait in question. Methods for directly identifying
genetic differences between two phenotypes are variations of two distinct procedures: representational difference analysis (RDA), and differential display.

**Representational difference analysis**

RDA was developed to determine and isolate the differences between two genomes (Lisitsyn et al. 1993). It employs the use of a tester genome sample that contains unique sequences to be cloned and a driver sample in excess designed to remove all sequences common to both genomes (Lisitsyn 1995; Figure 1). The technique is performed in two stages, one referred to as representation and the other as enrichment. The purpose of the representation stage is to reduce complexity of the DNA species in the tester and driver samples and this is achieved firstly by a restriction enzyme digest of each sample followed by ligation of an oligonucleotide to the 5' ends of the fragments in each population. PCR is then carried out using the oligonucleotide as primer under conditions favouring amplification of short (0.6 kb) fragments. The resulting modified tester and driver samples are used as the input to the next stage, referred to as enrichment. The first step in this part of the protocol involves ligation of another oligonucleotide adaptor only to the ends of the tester DNA fragments. Tester and driver samples are then mixed, the driver sample being in excess. Denaturation and reassociation causes tester sequences that are present in the driver sample to form heteroduplexes with driver fragments. Subsequent PCR amplification using the oligonucleotide as primer allows enrichment only of reannealed tester homoduplexes. These products represent the differences between tester and driver samples and following digestion with the original restriction enzyme are usually used as the input for further rounds of enrichment using a different oligonucleotide adaptor.

Genetically directed RDA (GDRDA) is a slight modification of RDA designed to isolate markers in a region of interest that differs between two lines or strains (Lisitsyn et al. 1994). It will detect deleted genes, amplified gene copy number and additional genes and works best for monogenic traits. While near-isogenic lines are ideally suited to such an approach, it may be adapted for use with 2-generation crosses which are more realistic for experiments involving livestock. This is achieved by obtaining a driver by pooling DNAs of several F2 individuals from an F1 intercross where the F2 individuals selected are homozygous for the recessive allele at the locus of interest. In the case of a dominant or co-dominant trait, the tester DNA comes from the parental ‘mutant’ strain and the driver from pooled F2 DNAs from ‘normal’ homozygous recessive individuals. When applied to a recessively determined trait, the tester DNA comes from wild-type individuals and the driver from pooled F2 DNAs from individuals showing a mutant phenotype, i.e. homozygous recessive. The pooling of driver DNAs ensures that variation at other loci is randomised, selecting only at the locus of interest. While best suited to monogenic traits and therefore not very useful in detecting markers for genes determining complex traits such as parasite resistance, such a technique could possibly be applied to fine mapping of a disease locus once flanking markers are identified.

Hubank and Schatz (1994) described cDNA RDA as an adaption of RDA to monitor gene expression. The representation stage is replaced by using mRNA to synthesise cDNA as this reduces genomic complexity to 1–2%. Further, digestion with a restriction enzyme having a 4-base recognition sequence ensures that tester and driver samples will contain short sequences easily amplified by PCR. Studying differences in mRNA populations means studying differences in gene expression thus allowing detection not only of mutant versus normal phenotypes but differences between activated cells from those in a resting state.

**Differential display analysis of mRNA**

The major factor that distinguishes differential display (DD) (Liang and Pardee, 1992) from RDA approaches is that difference products are detected by concurrent gel electrophoresis of products from two samples rather than by a subtractive process. Like cDNA RDA, differential display was developed to analyse differences in gene expression between different tissues, treatments or developmental stages, but has the added advantage of allowing comparison of more than two mRNA populations.

The differential display methodology evolved from another similar technique referred to as RNA arbitrarily primed PCR (RAP-PCR) which employs either one or a pair of arbitrary primers of approximately 20 bases in length to amplify all mRNAs by reverse transcription and second strand synthesis at low stringency (Welsh et al. 1992). PCR at higher stringency follows using the same primer or primer pair. DD-PCR was designed as a more systematic approach to detecting differentially transcribed mRNAs (Liang and Pardee 1992; Figure 2). The first step employs a series of anchored oligo-dT primers (T\textsubscript{1}M or T\textsubscript{1}MN, M; A,G,C; N, any base) to separately reverse transcribe mRNA species (Liang et al. 1994). The resulting cDNA populations are
Figure 1. Diagram outlining the steps involved in representational difference analysis of DNA. Following restriction endonuclease digestion of both tester and driver DNA samples, only the tester sample is ligated to oligonucleotide adaptors. After mixing tester and driver DNAs, denaturation and reassociation yield three types of duplexes but only tester target sequences are amplifiable by subsequent PCR. Another round of the process can be carried out with ligation of a new adaptor sequence to the amplified fragments. (Adapted from Lisitsyn, 1995.)
amplified by further PCR using the anchored primer coupled with a range of random 10-mer primers (which may incorporate a restriction enzyme site) as well as radioactively labelled nucleotides. The products from each pair of identically primed PCR reactions, using different mRNA starting samples, are compared by polyacrylamide gel electrophoresis and the difference products excised from the gel and amplified further by PCR for cloning, sequencing and identification.

Knowledge of the immunological basis of parasite resistance can be used in the design of differential display experiments that aim to determine differences in gene expression. In the search for genes conferring parasite resistance, cloning and sequencing of the difference products between responding and non-responding tissues or individuals for example may lead to identification of novel candidate genes for marker development. These markers can then be tested for association with resistance phenotype in appropriate families.

Application of Genetic Markers

For any genetic marker to be useful it must be cheap and give the sheep breeder a reliable measure of the relative genetic merit of his breeding stock. Breeders must able to provide a sample from each animal for the preparation of genetic material and this is currently in the form of a small amount of blood but could in the future be satisfied by a single wool follicle. Presumably this will impact on cost as well the time and manipulations required for genotyping which is currently being minimised by implementation of semi-automated genotyping using fluorescently labelled nucleotides or primers in the PCR reaction, allowing typing of multiple marker loci per gel lane.

Incorporating Markers into Breeding Programs

Markers may be used within a breed or commercial line in a marker assisted selection program. The most important aspect of markers is the increased accuracy of selection obtained using genotypic as opposed to phenotypic information (Schested and Mao 1992; Smith 1967). In addition, several constraints exist when developing breeding programs that include genetic markers as selection criteria. The genetic markers used to select animals for a particular trait must be close enough to the gene responsible (1–2 cM) so that associations are not eroded by genetic recombination. Some native breeds such as

Figure 2. Outline of the process of differential display PCR analysis of messenger RNA using single-base anchored oligo-dT primers for first strand synthesis. Each oligo-dT primer leads to amplification of a subset of mRNAs when coupled with a 10-mer of arbitrary sequence for second strand synthesis and subsequent PCR amplification. Difference products between the mRNAs of two tissues or treatments, X and Y, are visualised by concurrent polyacrylamide gel electrophoresis.
Red Masai, Florida native and Scottish Blackface show superior resistance to nematodes, a range of breeds must be tested for linkage between relevant marker loci and the corresponding genes responsible for the desirable traits. Before markers are used it is important to check for adverse correlations with other production characteristics. For example, a negative correlation was detected between cow fertility and resistance to worms (Mackinnon et al. 1990). Other studies suggested that selection of cattle (Mackinnon et al. 1991) or sheep (Riffkin and Dobson, 1979) for production characters has been at the expense of resistance to worms. However, the converse is not necessarily true in that in sheep, for example, no differences in production potential were found between genetically resistant and unselected or susceptible sheep (Windon and Dineen 1984; Albers and Burgess 1988). Moreover, selection for resistance to one species of nematode generally leads to greater resistance against other nematodes (Windon 1990; Barger 1989). In most selection indices designed to achieve practical breeding objectives, it is likely that production traits will be given most economic weight thus reducing the intensity of selection for worm resistance. In this case it has been suggested that selection against susceptibility may be easier to integrate into a selection index than intense selection for resistance (Kloosterman et al. 1992).

Another use of marker technology is introgression of genes from native breeds into more productive breeds. The combining of alleles from different breeds of pigs using marker assisted introgression where the aim is to fix the favourable allele in commercial populations with as little as possible of the remainder of the genome of the inferior breed has been discussed by Visscher and Haley (1995). Introgression of one or a few major resistance genes from one breed to another is costly, time consuming and causes disruption to normal breeding programs and therefore would probably only be carried out if the effects of a major gene were large enough to make it worthwhile.

Marker technology can be used to follow the fate of transgenes introduced into a population. Once particular genes are identified, transgenesis becomes an option but at this stage this option carries with it the burdens of being intrusive and somewhat unpredictable in terms of acceptance and effectiveness. Where between-breed crosses are uncommon, selection within a single breed for naturally occurring variation should be implemented with relative ease.

In conclusion, within the next few years genetic markers will become available and will enable more efficient methods for incorporation of traits such as parasite resistance into practical breeding programs. Markers will be identified as a result of linkage analysis or special resource families. Increasing use will be made of candidate gene approach based on genes identified by differential analysis of mRNA expression to identify functional markers within actual genes responsible for the trait.

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OTHER CONTRIBUTIONS
The Anthelmintic Effects of Bithionol Sulfoxide against Sheep Experimentally Infected with *Fasciola gigantica*

Tasanee Chompoochan¹, Piyanoot Prasitirat¹, Suree Thammasart¹, Suwannee Nithiuthai² and Noriyuki Taira³

**Abstract**

The treatment of *Fasciola gigantica* infection with bithionol sulfoxide was investigated in sheep that had been experimentally infected with 500 metacercariae. Ten sheep were divided into three groups: Group 1 (4 sheep) were given a single dose of bithionol sulfoxide 80 mg/kg orally at week 22 after infection; Group 2 (3 sheep) were untreated positive controls; and Group 3 (3 sheep) were untreated negative controls. All animals were examined for fasciola eggs per gram (EPG) of faeces at weekly intervals for a period of 28 weeks. The EPG during weeks 22–23 were examined daily. The results revealed that no eggs were found in the faeces of all treated sheep within one week of treatment. Hence, bithionol sulfoxide was highly effective against mature *F. gigantica* in sheep.

Alterations in serum SGPT, SGOT, copper, iron, total white cell count, total red cell count, MCV, MCHC and live weight were noted.

**Fasciolosis** is a particularly important parasitic disease affecting cattle, buffalo, sheep and occasionally goats (Loehr 1982; Reid et al. 1973; Suksaithachana et al. 1993). In Thailand, it is caused by the tropical large liver fluke, *Fasciola gigantica* whose life cycle involves a fresh water intermediate host snail, *Lymnea rubiginosa* (Chompoochan et al. 1976). *Fasciola* infection in livestock is responsible for considerable economic losses in the country because of reduced growth rates, decreased performance, susceptibility to other infections and an increased percentage of liver condemnations in slaughter animals. The prevalence of liver fluke infections varies considerably between the different animal species and different geographic locations with a range of 0% to 85% (Sritijakarn et al. 1988). To minimise such infections, an optimum treatment and appropriate fasciolosis control program is necessary.

Levacid® (Lek Ljubljana, Yugoslavia), a bolus preparation of bithionol sulfoxide, has been recently introduced by the Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand. As a country project (The Farmer Self-help Program), this drug has been offered as the drug of choice against fasciolosis in ruminants. Prior to the start of the control program, the study was designed to assess the effectiveness and safety of bithionol sulfoxide against *F. gigantica* experimentally infected sheep.

**Materials and Methods**

**Experimental animals**

The 10 one-year-old merino sheep used in this study were reared and maintained in parasite-free concrete pens throughout the experiment. The animals were divided into three groups.

- **group 1** (4 sheep) *F. gigantica* infected animals treated with 80 mg/kg bithionol sulfoxide (Levacid®);
- **group 2** (3 sheep) *F. gigantica* infected, untreated animals;
- **group 3** (3 sheep) non-infected control animals.

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¹National Institute of Animal Health, Bangkok 10900, Thailand
²Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand
³National Institute of Animal Health, Kannondai, Tsukuba, Ibaraki, 305 Japan
Animals in Group 1 and Group 2, were orally infected with 500 F. gigantica metacercariae in a gelatin capsule. Group 3, animals were not infected and served as controls for the experiment.

**Metcercariae**

Metcercariae of F. gigantica were obtained from laboratory reared Lymnea ollula intermediate host snails which had been exposed to miracidia of bovine origin.

**Anthelmintic treatment**

Levacid® was administered orally during week 22 post-infection, to animals in Group 1, at a dose of 80 mg/kg body weight. Animals in Groups 2 and 3 did not receive any anthelmintic treatment.

**Faecal examinations**

Throughout the experiment, faecal samples were collected from all sheep and examined by the beads technique (Taira et al. 1985, Chompoochan et al. 1990) in order to count F. gigantica eggs, before and after treatment, at 1–2 week intervals, for 28 weeks. During days 0–14 post treatment, EPG counts were carried out daily.

**Blood and serum determinations**

Blood samples were collected biweekly from the jugular vein of each animal and divided into two parts, one with EDTA added was used to determine haematological changes (Wbc, Rbc, Hb and PCV). Total Wbc, Rbc, MCV and MCHC determinations were done by a standard laboratory procedures using an automatic cell counter. Haemoglobin was quantified by a haemoglobinometer and PCV by the use of a microcapillary tube technique.

The second sample was placed into vials without anticoagulant for the preparation of sera. These were used to determine total protein, albumin, iron, copper and serum enzymes (SGOT, SGPT). Total serum protein was determined by a hand refractometer; albumin by the Bromocresol Green method; iron and copper by an Atomic Absorption Spectrophotometer and SGOT and SGPT by the Reitman Franken's method.

**Results and Discussion**

All sheep in Groups 1 and 2 which were experimentally infected with 500 metacercariae (MC) of F. gigantica showed egg outputs in their faeces 11–13 weeks post infection. The peak EPG in Group 1 was found during week 22, with a mean EPG of 494 and in Group 2 during week 22 (1244 EPG). At week 22, each sheep in Group 1 was treated with Levacid® and faecal samples were examined daily for 2 weeks afterwards. The results (Fig. 1) showed that fluke eggs were absent on day 7, post treatment, whereas in the infected control animals the EPG still appeared up to the end of the experiment. Two infected control sheep (Group 2) died during weeks 20 and 22. Numerous flukes (131 and 111 adult flukes) were found in the bile ducts and gall bladder. In all treated animals, no clinical side-effects were observed.

Figure 2 shows that sheep in the Levacid-treated group had an antibody titre throughout the experiment which was similar to the non-medicated infected control group. This result was in contrast to studies by other investigators who found that sheep and cattle showed a rapid decrease in antibody titres after treatment with anthelmintics (Brujinling 1978; Kendall et al. 1978).

In this study serum enzyme levels of SGPT and SGOT were highly variable across samplings and no difference between treatment groups was observed. Other studies have found that SGOT level increased slightly eight weeks post infection and then returned to pre-infection levels (Reid et al. 1973).

Mean values of serum protein and serum albumin are shown in Figures 3 and 4 with a slight increase and fall. These alterations may be due to an increase in the serum gamma globulins of the animals (Reid, et al. 1973). Serum copper levels of all three groups of animals were similar throughout the experiment. Serum iron in the Levacid-treated group (Fig. 5) showed a declining value during week 18, post-infection and a return to normal soon after treatment. In the animals in the Fasciola gigantica infected control group, the serum iron level began to decline rapidly 15 weeks after infection and remained low until the end of the experiment. The effect of F. gigantica on the iron level in the infected animals was presumably due to three basic disturbances to the red blood cell metabolism (Reid et al. 1973) i.e. an increased rate of red cell destruction, a compensatory increase in red cell products and a continuous drain on iron stores, resulting from the blood sucking activities of the liver flukes. The total red cell count, MCV, MCHC and total white cell count results revealed that all values were in the normal range, and that no marked differences of any values were demonstrated in the three groups of animals.

In Figure 6, the live weight gain of animals was evaluated between weeks 0, weeks 22 and weeks 32 post-infection. Weight reductions were found in Group 2 animals. Sheep in Group 1 treated animals showed a slight weight gain from week 32 but this was significantly less than the control group.
Figure 1. Mean EPG of *F. gigantica* in sheep after treatment with Levacid®.

Figure 2. Mean Ab titre of *F. gigantica* in sheep after treatment with Levacid®.
Figure 3. Mean serum total protein of *F. gigantica* in sheep after treatment with Levacid®.

Figure 4. Mean serum albumin of *F. gigantica* in sheep after treatment with Levacid®.
Figure 5. Mean serum iron of *F. gigantica* in sheep after treatment with Levacid®.

Figure 6. Live weight of sheep after treatment with Levacid®.
Acknowledgments

The authors gratefully thank Dr. K. Nontapatamadul, F.E. Zuellig Co. Ltd. for partial support of this project.

References


DISCUSSIONS
AND
OUTCOMES
Sustainable Parasite Control Workshop

Following the presentation of papers, workshop sessions were held. The rationale was to generate a sound analysis of the main constraints in small ruminant production systems and then to determine what inputs in research, extension and training were required to change the existing parasite control programs into sustainable parasite control programs.

The format of the workshop sessions was for delegates to separate into groups of between seven and nine members and to discuss a topic for up to one hour. Then the groups reassembled for a general session where each group’s deliberations were presented to the general session.

Although the group discussions were informal, each group had a ‘Leader’ and a ‘Scribe’. The Leaders’ duties were to generate and moderate the discussion and, together with the Scribe, ensure that the views of the group were expressed during the general discussion. The Scribes’ duties were to make notes of the discussion and list the major points which arose.

The two topics to be considered in the first round of discussions were:

1. For sheep and goats separately, describe the important production systems within Asia;
2. What are the constraints on these systems? Rank parasitism as an animal health constraint.

The results of this discussion are summarised in Table 1. The general agreement was that for the purpose of describing production systems, sheep and goats were equivalent.

In the second session, the groups were asked to consider what inputs (research/extension/training) were needed to turn parasite control into sustainable parasite control. The results of their deliberations are summarised in Tables 2 and 3. It was assumed that adequate funding for research and extension would be provided by national and international agencies and that control options being researched were economically viable.

### Table 1. Major animal production systems and constraints on production within those systems.

<table>
<thead>
<tr>
<th>Production system</th>
<th>Major constraints</th>
<th>Parasitism as an animal health priority</th>
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<tbody>
<tr>
<td>Smallholder</td>
<td></td>
<td></td>
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<tr>
<td>Cut and carry</td>
<td>1. Nutrition/health</td>
<td>HIGH</td>
</tr>
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<td></td>
<td>2. Information, education and extension</td>
<td></td>
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<tr>
<td></td>
<td>3. Inappropriate genotype and infectious disease</td>
<td></td>
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<tr>
<td>Tethered grazing</td>
<td></td>
<td></td>
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<tr>
<td>Landless</td>
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<tr>
<td>Integrated systems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plantation</td>
<td>As for the smallholder system</td>
<td>HIGH</td>
</tr>
<tr>
<td>Other crops</td>
<td>Input costs have greater relative importance</td>
<td></td>
</tr>
<tr>
<td>Transhumance (inc. omadic)</td>
<td>1. Nutrition and epidemic disease</td>
<td>LOW</td>
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Critique of Research Priorities

<table>
<thead>
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<th>Table 2. Research priorities identified by workshop groups</th>
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<tr>
<td>Research priorities</td>
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<tr>
<td>Identification of resistant genotypes</td>
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<td>+ + +</td>
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<tr>
<td>Nutrition/parasite interactions</td>
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<tr>
<td>+ + +</td>
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<tr>
<td>Anthelmintic use and resistance monitoring</td>
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<tr>
<td>Epidemiology</td>
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<td>+ +</td>
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<tr>
<td>Integrated strategies for control</td>
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<td>+ +</td>
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<tr>
<td>Grazing management</td>
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<tr>
<td>+</td>
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<tr>
<td>Biological control</td>
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</table>

1. The need to rapidly develop parasite resistant strains of sheep and goats through selective breeding using recent developments in quantitative and molecular genetics was identified as the top research priority. Before selective breeding of goats for increased resistance was undertaken, it was recognised that further research would be required on the extent of within and between breed genetic variation of resistance to parasites in goats. It was suggested that a scheme be developed to enable genetic comparisons across breeds and between countries. This may involve the establishment of a ‘reference flock’ in one of the collaborating countries.

2. Determine the effects of improved nutrition on the small ruminant’s ability to resist parasitic infection. Research would concentrate on the nutritional approaches to increasing productivity which are technologically appropriate for the target livestock production systems (i.e., low cost supplements, use of agricultural residues). The importance of adequate nutrition in the phenotypic expression of improved resistance genotypes also needs to be assessed.

3. Two types of monitoring were seen by the participants as beneficial. The first was monitoring anthelmintic resistance levels in nematode parasites so that management decisions could be made to prevent control failures. The larval development assay could be a useful means of providing a standard resistance monitoring system. The second type of monitoring was in regard to reports that have indicated that in some countries anthelmintics were found to be diluted before being sold to farmers or veterinarians. As a further step in ensuring that animals are treated with the correct anthelmintic dosage, a national government centre should be nominated to test commercial anthelmintic formulations at the point of sale for accurate labelling.

4. Ensure that adequate knowledge is gathered on the epidemiology of internal parasites of small ruminants in the participating countries. This information would enable the design of integrated control programs (ICP) for testing in each of the target production systems. Such programs would aim to minimise anthelmintic use while taking full advantage of benefits obtainable from grazing management and nutritional strategies designed to reduce the parasite burden. As alternative control options are advanced these could also be included into ICP when available.

Critique of Education and Training Priorities

<table>
<thead>
<tr>
<th>Table 3. Extension training priorities identified by workshop groups.</th>
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<tbody>
<tr>
<td>Extension training priorities</td>
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<tr>
<td>Regional short course training programs for scientists, administrators and extension staff</td>
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<tr>
<td>+ + +</td>
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<tr>
<td>Improved extension/farmer/researcher linkages through improved networks</td>
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<tr>
<td>+ +</td>
</tr>
<tr>
<td>Integration of Animal Health and Production</td>
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<tr>
<td>Extension</td>
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<tr>
<td>+</td>
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<tr>
<td>Farmer training</td>
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Workshop participants recognised the importance of increasing the capability of national staff through training. This could be brought about by a series of initiatives aimed at scientists, extension workers and technicians. These measures included:

(1) **Short courses**

Technical and extension staff employed by each participating country should receive short-term training in the principles of parasite control, anthelmintic resistance diagnoses and genetic selection. This could be achieved by giving selected staff the opportunity to attend in-country training courses conducted by local and overseas experts. Training of technical staff was identified by all workshop groups as a priority need.

(2) **Scientific staff**

The majority of scientists involved in parasite control research have been trained in a specific discipline and experience some difficulties in coping with the multidisciplinary nature of SPC research. It was recommended that greater training opportunities in broad-based programs be made available to country scientists.
Critique of Communication/Networking Recommendation

The need to improve the effectiveness of regional research and development activities through networking was seen as the first priority in communication. It was envisaged that initially the network should function on two levels.

The first level would be a moderated newsgroup to link institutions and researchers involved in helminth control in ruminants in the tropics. This newsgroup should be established immediately and would serve to keep scientists involved in the 'Sustainable Parasite Control Workshop' in contact, and to facilitate the development of a project to implement sustainable parasite control. It was recommended that the newsgroup would be open to all with an interest in sustainable parasite control in ruminants in the tropics. In order to keep all workshop delegates informed, those without email links would receive paper transcripts of the newsgroup discussions.

The second level would be a paper-based system responsible for distributing reports of regional meetings to participants and relevant authorities, for production of information circulars for each country and distribution to other sectors and development agencies, and for publishing a regional newsletter twice each year and distributed to all animal health and production research and extension workers in the region. As the computer links improved in the region, it was envisaged that the newsgroup and paper-based newsletter would evolve into a single information system contactable through the worldwide web.

The group and general discussions on the final day centred on methods of implementing SPC in countries of South and Southeast Asia. Delegates agreed that SPC should be urgently implemented. It was recognised that not all countries represented at the workshop could contribute at the same level for implementing a multidisciplinary strategy of sustainable parasite control. Identifying the strengths and weaknesses of country institutions in their ability to contribute was not possible in the time allocated for the workshop. It was therefore the specific recommendation that a consortium be established with national representation, representatives from international agencies with interest in collaborative research and development in the region and with CSIRO and UNE representatives.

Initially, the consortium will consist of one member from each country and the institutional representatives. The initial task of the country representatives will be either to obtain approval to speak on behalf of their country’s priorities or to enlist an alternative representative from their country who can. The delegates decided on the following terms of reference.

**Consortium — terms of reference**

- Identify group strengths and weaknesses.
- Identify research needs.
- Identify training needs.
- Consider new proposals.
- Identify resources.

**Specific recommendations arising from the workshop and to be acted on immediately**

- Disseminate workshop outcomes.
- Establish a network.
- Establish a consortium.
LIST OF PARTICIPANTS
M. Raisul Alam  
Department of General Animal Science  
Bangladesh Agricultural University  
Mymensingh 2202, BANGLADESH  
Telephone: 880-91-5695-97 Ext: 274 (off)  
880-91-3971 (Res)  
Facsimile: 880-91-3804 or 880-2-888688

M. Anwar  
Animal Sciences Division  
Pakistan Agricultural Research Council  
Plot No. 20, G-5/1, Post Box 1031  
Islamabad 44000, PAKISTAN  
Telephone: 92-51-9209416  
Facsimile: 92-51-9202968

Sjamsul Bahri  
Balitvet, PO Box 52  
Bogor, West Java, INDONESIA  
Telephone: 62-251-331048  
Facsimile: 62-251-336425

R.L. Baker  
International Livestock Research Institute  
PO Box 30709, Nairobi, KENYA  
Telephone: 254-2-630743  
Facsimile: 254-2-631499  
E-mail: L.BAKER@CGNET.COM

Ian Barger  
CSIRO Division of Animal Production  
Pastoral Research Laboratory  
Private Mail Bag  
Armidale NSW 2350 AUSTRALIA  
Telephone: 61-67-76 1430  
Facsimile: 61-67-76 1333  
E-mail: ibarger@chiswick.anprod.csiro.au

J.S.F. Barker  
Department of Animal Sciences  
University of New England  
Armidale NSW 2351 AUSTRALIA  
Telephone: 61-67-73 2226  
Facsimile: 61-67-73 3275  
E-mail: sbarker@metz.une.edu.au

Leo P. Batubara  
Research Assessment Institute of Agriculture Technology (RAIAT)  
PO Box 1  
Galang, North Sumatra, INDONESIA

Aron Batubara  
Research Assessment Institute of Agriculture Technology (RAIAT)  
PO Box 1  
Galang, North Sumatra, INDONESIA  
Facsimile: 62-61-958 013

Kenneth J. Beh  
CSIRO Division of Animal Production  
Locked Bag 1, Mail Centre,  
Blacktown NSW 2148 AUSTRALIA  
Telephone: 61-2-8402941  
E-mail: beh@syd.dah.csiro.au

Beriajaya  
Balitvet, PO Box 52  
Bogor, West Java, INDONESIA  
Telephone: 62-251-331048  
Facsimile: 62-251-336425  
E-mail: para@server.indo.net.id

Sri Budiati  
Directorate General of Livestock Services  
Directorate of Breeding Development  
Jl Salemba Raya 16  
Jakarta 10130, INDONESIA  
Telephone: 62-21-3143637  
Facsimile: 62-21-3143637

Ian H. Carmichael  
VETLAB  
Primary Industries South Australia  
Box 1671 GPO Adelaide SA 5001 AUSTRALIA  
Telephone: 61-8-2077922  
Facsimile: 61-8-2077924  
E-mail: carmichael.ian@pl.sa.govoffice

D.B. Copeman  
Dept. Biomedical and Tropical Veterinary Sciences  
James Cook University  
Townsville QLD 4811 AUSTRALIA  
Telephone: 61-77-814 838  
Facsimile: 61-77-791526  
E-mail: Bruce.Copeman@jcu.edu.au

John Copland  
ACIAR  
PO Box 1571, CANBERRA ACT 2601  
Telephone: 61-6-217 0500  
Facsimile: 61-6-217 0501  
E-mail: copland@aciar.gov.au

Tasanee Chompoochan  
NAHPI, Kasetsart University  
Bangkok, Bangkok, THAILAND 10900  
Telephone: 66-2-579 8908

William D. Dar  
Philippines Council for Agriculture  
Forestry and Natural Resources Research and Development (PCARRD)  
PO Box 425, Los Baños, Laguna, PHILIPPINES  
Telephone: 63-94-5360014 to 5360020  
Facsimile: 63-94-536-0016 to 536-0132