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Contract introduction: the problem of anthelmintic resistance.

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Anthelmintic (drench) resistance in New Zealand's ruminant livestock is arguably the biggest single threat to sustainable future pastoral production. Clinical and sub-clinical parasitism impacts not only on animal health, but on animal welfare, and hence the image of pastoral production systems, total production and production efficiency and has a marked impact on the economic bottom line for both farmers and the country as a whole. In 2001 it was estimated for the relatively small NZ deer industry alone (worth $311 million in export earnings) that parasitism cost $12.8 million per annum in production losses due to parasitism and parasite control (Mackintosh and Wilson, 2002).

There are only three broad-spectrum anthelmintic groups available for control of ruminant nematodes. These are Group 1, the benzimidazoles (BZ's or white drenches), Group 2, the imidazothiazoles (levamisole LVS or clear drench) and hydropyrimidines (pyrantel/ morantel) and Group 3, the macrocyclic lactones (avermectins and milbemycins, ML) (Coles *et al.*, 2006). No new anthelmintics with different modes of action are expected on the market in the near future (Coles *et al.*, 2006).

The Landcorp Lecture (van Wyk, 2006) will hopefully make those involved in animal production pull their heads out of the sand and face the facts about anthelmintic resistance. Be prepared for a shock. The problem is now entering the final phase where on some farms no anthelmintics remain with which to control worms whilst maintaining profitable animal production (van Wyk, 2006). Last year in Scotland the first report was published of a sheep farm closing due to failure of the final resort, moxidectin (ML), to adequately control the abomasal parasite *Teladorsagia circumcincta* (Sargison et al, 2005).

In this contract session, van Wyk (2006), Rhodes et al., (2006) and preliminary results from Bates et al., (2006) will outline the current level of anthelmintic resistance both in New Zealand and the World. Pomroy (2006) will address the implications of, and what lessons can be learned from, the results obtained from New Zealands largest and most comprehensive survey of drench resistance undertaken to date (Rhodes et al., 2006).

Clearly action is urgently required to address the problem of drench resistance. Both van Wyk (2006) and Pomroy (2006) will offer some suggestions for the way forward to manage drench resistance. Justification for research into alternative control solutions such as bioactive plants (Hoste et al., 2006) is now enhanced, but these alternatives must be considered as components of a holistic integrated approach towards sustainable control of nematode parasitism, which will probably always rely to some extent on anthelmintics.

A profile of anthelmintic resistance and parasite control practices in New Zealand – results from a 2005 survey

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INTRODUCTION

The development and spread of internal parasite resistance to anthelmintics continues, as evidenced by submissions to animal health laboratories, practice surveys and field observations. The spectre of resistance in parasites of sheep was initially highlighted in New Zealand in 1980 (Kemp and Smith, 1980). Through the 2 decades since then farmers have been encouraged to limit excessive use of anthelmintics, drench-test, develop annual drench-family rotations, adopt preventive-drenching practices, use faecal egg counts and trigger drenching, and quarantine management as tactics for reducing the rate of development of resistance on their farm. However, over the same time period, resistance has gone from being an oddity to being common place. Submissions to animal health laboratories over a decade or more have been tabulated and published giving perhaps our strongest indication of the progress in resistance development. The most

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recent of these reports (McKenna, 1998), which was based on a relatively small number of submissions (all from sheep), detected resistance to benzimidazole, levamisole and benzimidazole + levamisole combinations in 66%, 29% and 16% of cases respectively. Recently we have seen confirmation of resistance to the macrocyclic lactone (avermectin) family of drenches occurring in sheep parasites, which marks a significant milestone in that we now have resistance to all major drench action families. In addition, the recent identification in goats of strains of *Ostertagia circumcincta* and *Trichostrongylus colubriformis,* which were highly resistant to a combination of all 3 action families given at higher than normal dose rates, is a cause of concern, especially as these parasites readily infect sheep.

The issue of resistance is not limited to species that infect just sheep and goats. Anthelmintic resistance has generally been regarded as an issue for small ruminants, whereas in cattle nematodes it is a subject that has been given little attention and often dismissed by those interested in parasitology in this host. There have been no surveys of anthelmintic resistance in cattle in New Zealand (or elsewhere in the world) or of parasite control practices in beef cattle in New Zealand. However, resistant parasites have been noted in cattle in several countries, especially in *Cooperia* spp. Resistance to the macrocyclic lactone (ML) anthelmintics has been reported in *Cooperia* spp. in Brazil and Argentina (Anziani al., 2001; Fiel et al., 2001; Anziani et al., 2004) and is present in the UK (Stafford and Coles, 1999) and the USA (Gasbarre et al., 2004).

In New Zealand, a number of reports have been made commencing in the early 1990s of ML resistance in *Cooperia* (West et al., 1994; Vermunt et al., 1995; Watson et al., 1995; Vermunt et al., 1996; Loveridge et al., 2003) to the extent that it was considered to be widespread by the late 1990s. There was some consideration that from the very first introduction of the pour-on formulation of ivermectin for cattle in New Zealand the inefficacy of MLs against this species was already apparent (Bisset et al., 1990), but if so, this was a situation that was unique to this one study compared to others undertaken elsewhere in the world. It is interesting to note that ML resistance has now been reported in *C. oncophora* (Winterrowd et al., 2003)*, C. punctata* (Gasbarre et al., 2004) and *C. pectinata* (Anziani et al., 2001). ML resistance has also been reported in *Trichostrongylus longispicularis* in New Zealand (Loveridge et al., 2003) and *Haemonchus placei* in Brazil (Rangel et al., 2005). There have not, however, been any reports of ML resistance in *Ostertagia ostertagi* despite ML resistance being reported in related species in deer and it being common in closely related species in sheep.

Resistance to benzimidazole (BZ) anthelmintics in parasites of cattle has been reported in several species. In Argentina BZ resistance has been reported to *Cooperia punctata*, *Ostertagia ostertagi* and *Haemonchus placei* (Mejia et al., 2003). In Australia there is one report in *Trichostrongylus axei* (Eagleson and Bowie, 1986). In New Zealand the first reports were in *Cooperia* spp. (Jackson et al., 1987) and later also in *Ostertagia* spp. (Hosking and Watson, 1991). In reviews of laboratory submissions McKenna reported several cases where a failure to reduce egg counts suggested resistance in *Cooperia* principally, but also in *Ostertagia* and *Trichostrongylus* (McKenna, 1991, 1996). It has subsequently been shown that BZ resistance in *Cooperia oncophora* is controlled by similar changes in the B-tubulin gene to other trichostrongylids (Winterrowd et al., 2003).

There have been fewer cases of resistance reported by cattle nematodes to either levamisole or morantel. Evidence of levamisole resistance in *Ostertagia* was presented in Belgium (Geerts et al., 1987) and to morantel in The Netherlands (Borgsteede, 1991). The latter report also indicated the isolate expressed cross resistance to levamisole. However, care needs to be taken when interpreting the efficacy of levamisole against *O. ostertagi* as Prichard (Prichard, 1983) reviewed the overall efficacy as between 85-95% against adult parasites. Morantel resistance has also been reported in *Haemonchus placei* (Yadav and Verma, 1997).

Rapid expansion over the last three decades of intensive bull-beef based cattle systems in New Zealand has seen an increasing reliance on anthelmintics to control gastrointestinal nematodes in this farming system. Although not quantified, there would have also been an increase in anthelmintic use in young cattle in more traditional cow-reared cattle systems. It is not surprising, therefore, that resistant isolates of various species have been reported and with increasing frequency.

This project set out to establish: a New Zealand-wide profile of the prevalence and severity of internal parasite resistance across a sample of breeding ewe flocks; a profile of the prevalence and severity of anthelmintic resistance in trichostrongylid parasites of beef cattle in the North Island; to survey farming and nematode control practices on these farms and then evaluate the occurrence of resistance and its severity with these practices to attempt to identify risk factors.

METHOD

Beef farm selection

A random sample of 62 beef enterprises from the North Island were sampled. There were approximately half that were farming rising yearling bulls through the autumn and winter, and half farming cow-reared rising yearling cattle under moderately intensive grazing management. The latter group involves cow-reared young stock, the introduction of weaners into a grazing system in the autumn at around 6 to 9 months of age, and typically less intensive stocking and grazing systems than occur in bull-beef enterprises.

Sheep farm selection

A random sample of 80 farms (random farms) was drawn from the AgriQuality Agribase (Sanson and Pearson, 1997; Sanson, 2000). Farms were restricted to those with greater than 1000 ewes wintered. Regional bias was minimised by ensuring that the regional distribution of farms in the sample was proportionate to the total number of farms with greater than 1,000 ewes by region. Sheep farms in all regions of New Zealand, with the exception of South Island High Country farms, were included. High country South Island farms were excluded since their management is typically extensive and a separate project was investigating similar issues for this enterprise type.

An additional non-random group of 32 sheep farms was targeted on the additional criterion of having a high probability of macrocyclic-lactone resistance. Veterinarians were invited to refer farms for this group. These are referred to as the purposive group.

Details of the methodology associated with farm selection have previously been reported (Rhodes et al 2005).

Treatments and analysis

A faecal egg count reduction test (FECRT) was conducted for all farms with all using the same protocol. The mean faecal egg count (FEC) to enable commencement of the FECRT was specified as 700 epg and 250 epg, for lambs and cattle respectively. Treatments involved 60 animals on each farm, with 10 lambs and 15 cattle in each treatment. Each animal was dosed by its individual weight with a syringe. All anthelmintics were given by the oral route. For both sheep and cattle one group remained untreated as a control group. Treatment groups and the proprietary names of the treatments employed are listed in Table 1.

Table 1: Treatment active ingredients and proprietary names

Active ingredient	Lambs Trade name	Cattle Trade name
Control	Control	Control
Ivermectin full dose	Ivomec	Erase MPC
Levamisole	Nilverm	Levicare
Albendazole	Valbazen	Valbazen
Ivermectin half dose	Ivomec	Nil
Levamisole + Albendazole	Arrest	Nil

A post-treatment sample was taken 7 to 10 days later. All egg counts were carried out using a modified McMaster technique where 1 egg counted represents 50 and 25 epg for sheep and cattle respectively. Where overall efficacy was determined to be less than 95%, faecal material was bulked by treatment group from all the implicated groups and the untreated control and cultured to provide material for identification of infective larvae. Efficacy was calculated using the formula in Equation 1.

$$100 \times \left(1 - \left(\frac{drug\ post - drench\ mean\ epg}{drug\ pre - drench\ mean\ epg}\right) \times \left(\frac{control\ pre - drench\ mean\ epg}{control\ post - drench\ mean\ epg}\right)\right)$$

If cultures were performed it was possible to calculate the efficacy for each genus using the formula shown in Equation 2

$$1 - \left(\left(\frac{drug\ post - drench\ mean\ epg \times \frac{n\ drug\ test\ larvae}{100}}{drug\ pre - drench\ mean\ epg \times \frac{n\ control\ larvae}{100}}\right) \times \left(\frac{control\ pre - drench\ mean\ epg}{control\ post - drench\ mean\ epg}\right)\right) \times 100$$

Prevalences and proportions are displayed throughout as point estimates with 95% confidence intervals in brackets. Confidence intervals along with point estimates give an indication of the precision of the effect and the uncertainty about the point estimate. If the confidence intervals are 95%, then we can say in general terms that in 95% of replications of the study the interval will include the true value of the point estimate. Confidence intervals were calculated using the formula from EpiSheet© 2002 written by Ken Rothman.

Anthelmintic efficacy is expressed either as the percentage reduction in faecal egg count (Equation 1) or the percentage reduction in larvae (Equation 2). As reported by Leathwick et al (2001), the generally adopted diagnostic definition of anthelmintic resistance in New Zealand, and that of the World Association for the Advancement for Veterinary Parasitology, is a failure to reduce faecal nematode egg counts (FECs) by at least 95%.

There are inevitably errors associated with the processes of treatment administration, faecal sampling, and faecal egg and larvae counting which should be acknowledged when reporting and interpreting FECRTs and larval culture tests. Accordingly, throughout this report, results have been grouped in the following categories shown in Table 2.

Table 2: Categorisation of the level of reduction in faecal egg count and number of larvae

Level of Reduction	Status
>95% reduction	Susceptible
<95% reduction	Ineffective – standard definition for presence of resistance
90-95% reduction	Suspicious
<90% reduction	Ineffective

RESULTS

Prevalence of anthelmintic resistance – beef farms

The FECRT was undertaken on 62 farms for which samples were submitted.

For the 62 participating farms, the prevalence of <95% efficacy of ivermectin was 92% (82, 96) and for one or more of the three anthelmintics tested was 93% (84, 97). Levamisole had <95% efficacy on 8% (3, 18) of farms, but inefficacy to either levamisole or albendazole was detected on 76% (64, 85) of farms. Only 4 farms showed >95% efficacy for all anthelmintics tested.

Inefficacy with <95% reduction in epg's for all anthelmintics tested was detected on 5 farms, 8% (4, 18); for ivermectin and albendazole on 45 farms, 74% (62, 83); for ivermectin and levamisole on 5 farms, 8% (4, 18); and for albendazole and levamisole on 5 farms, 8% (3, 18).

The prevalence of ivermectin resistance was very high in *Cooperia,* but very low in *Trichostrongylus*. There were a small number of cases where the results suggested ivermectin resistance in *Ostertagia* and these are being retested to confirm these earlier results. For albendazole the level resistance in *Cooperia* was also very high, moderate to high for *Ostertagia* and low for *Trichostrongylus*. For levamisole there were no cases of resistance in *Cooperia* or *Trichostrongylus* and only a few in *Ostertagia.*

Management practices – beef farms

Management practices were able to be assessed from completed questionnaires from 59 of the 62 farms that submitted samples for analysis.

Farmers indicated that the median number of treatments given to beef calves between September and March was 2.3, with a median of 3 treatments for beef weaners between April and September. The number of treatments ranged from 0 to 6 for both periods. The median number of occasions for September to September was 5 (range 1 to 12). The 75th percentile was 8, indicating that one in four farmers used anthelmintics on 8 to 12 occasions during that period

Some form of grazing of sheep or deer, or cattle older than 18 months on pastures in between their use for grazing beef for finishing or stores between weaning and 12 months of age was practiced by most farmers. However, for the majority, this was an occasional practice.

For the majority of farms, cattle grazing was limited to only a portion of the total farm area – on about 1/2 of the farms cattle grazed less than ½ the farm area throughout a 12-month period, and on only about a quarter of farms did cattle graze greater than ¾ of the farm area.

Anthelmintic usage over the past 4 seasons predominantly featured MLs , with 59% and 5% of farmers either using this family as a single action treatment or in combination with levamisole respectively.

Cattle older than 12 months of age also feature in farmer's parasite management practices, with only 5% indicating they did not treat older cattle in the previous season. By contrast, older cattle were treated on one, two, three or more than three occasions by 22%, 29%, 12% and 29% of farmers.

Prevalence of anthelmintic resistance – sheep farms

Across the 80 farms in the random selection group, resistance to albendazole was found on 41% (31, 52); to half-dose ivermectin on 36% (27, 47); to full-dose ivermectin on 25% (17, 35); to levamisole on 24% (16, 34), and to combination albendazole-levamisole treatment on 8% (3, 15).

Results across all 112 farms indicated that the month of testing was associated with changes in proportions of nematode genera in the untreated control animals. The proportion of *Ostertagia* present in the controls declined over the period January to May, while *Trichostrongylus* increased as a proportion of the total nematode mix over the same period. The proportions of *Cooperia*, *Haemonchus* and *Nematodirus* remained relatively constant throughout.

On the 80 random selection group farms, results of larval culture of treatments where the FECRT was <95% the level of resistance to ivermectin was very high in *Ostertagia* and very low or not identified in the other genera. For levamisole, there was also a high prevalence of resistance in *Ostertagia*, a moderate level of resistance in *Trichostrongylus* and *Nematodirus* and a low prevalence in *Cooperia* and *Haemonchus*. For albendazole, the level of resistance was high or very high for *Cooperia, Ostertagia, Trichostrongylus* and *Nematodirus,* but for *Haemonchus* was low. For the combination of albendazole and levamisole, the level of resistance was low for all genera.

Management practices – sheep farms

About half of farmers either often, or always, return lambs back to the same pasture after weaning. Very few farmers indicated they always weaned lambs onto pasture not previously grazed by lambs and this only increased slightly for those who were able to wean lambs onto paddocks not grazed that season by lambing ewes.

In the period from weaning until one year of age, incorporating a level of ewe or cattle grazing before lambs returned to graze pasture was practiced by more than half of the farmers, however the practice appeared to be quite informal. Grazing with cattle was slightly more common than ewe grazing.

Farmers are influenced in their lamb drenching decisions by a range of factors, some indicative of longer-term planning, while others are in response to direct paddock observation of indirect indicators of apparent stock performance. Those factors mentioned by at least a third of farmers were: to follow a 5-6 drench programme every 3-4 weeks regardless; monitor FEC and drench on high counts; drench on signs of scouring/ illthrift; regularly during period of high risk.

On average, lambs received 5.5 drenches by 12 months of age. The number of drenches given to 2-tooth ewes between 1 year of age and mating averaged 1.5. Anthelmintic treatment of mixed-age ewes was a common practice and only 8 (11%) did not drench ewes between 1st July 2003 and 30th June 2004, 36 (49%) drenched ewes once, and 30 (41%) 2 to 3 times (this would give an approximate mean = 1.5 ewe drenches). About half of farmers never drenched before mating with the remainder either regularly drenching or occasionally drenching at this time. Most farmers either "always" or "sometimes" drenched before lambing whilst few drenched at tailing.

Quarantine drenching was stated to be always practiced by about two-thirds of farmers while others either never or only sometimes did so. The predominantly used quarantine treatment was a single-active ML, with about one third using a double combination treatment. Few farmers used a triple combination as their quarantine treatment.

DISCUSSION

Beef farms

The FECRT identified a high prevalence of inefficacy for both ivermectin and albendazole but was low for levamisole. It is of concern that only 4 farms showed >95% efficacy to all anthelmintics tested and although the sample size for that group is too small for statistical analysis , further investigations of circumstances on those farms are warranted to try and explain their freedom from resistance. There was a very high prevalence of inefficacy of ivermectin and albendazole for *Cooperia*. Fortunately levamisole was generally efficacious against *Cooperia* which currently provides the only effective treatment against this genus on most farms. Reports of levamisole resistance to *Cooperia* have not yet been formally recorded, but must be considered likely in the foreseeable future, especially as this chemical is now being widely used.

Resistance in multiple parasite genera to albendazole on the same farm was demonstrated on about one in every two farms for *Ostertagia* and *Cooperia*, and one in five farms for *Trichostrongylus* and *Cooperia*. Again this shows we have reached a stage where benzimidazoles are of limited use as a single active on most beef farms. Ivermectin and levamisole inefficacy in *Ostertagia* appears to be an emerging issue. *Ostertagia* was the genus resistant to levamisole on all four farms that were evaluated by culture for this drug and inefficacy of ivermectin to *Ostertagia* was suggested on a small number of farms – Further work is underway to confirm these findings.

Approximately one farm in five demonstrated resistance to multiple parasite genera; *Cooperia* and *Trichostrongylus* to albendazole and *Cooperia* and *Ostertagia* to ivermectin. This represents another indication of a growing issue for control of cattle parasites. It is significant that resistance to multiple anthelmintic families was apparently demonstrated by both *Cooperia* and *Ostertagia* against both ivermectin and albendazole on one farm.

An interesting observation was that despite all farmers having a planned programme, there was still heavy reliance on signs of parasitism such as scouring or dirty hocks, and poor growth rates or condition scores, and intuitive 'gut feeling' for deciding when to treat for internal parasites.

Although scales were used routinely by most farmers, the survey did not enquire as to whether growth rates were routinely calculated and used as an indicator of when to treat. The median number of treatments given between September and March was two and between April and September was three which implies regular treatment is the norm. The range of 0 to six for both periods indicated wide variation in the frequency of treatments and indicates that some farmers are using a high number of treatments at intervals that must be close to less than the pre-patent period for these parasites.

The median number of treatments administered to young cattle from September to September was five and ranged from one to 12. The wide range partly reflects the differences in age of entry on to the farm with some farms only rearing animals either purchased or weaned at about four to six months of age. However, regardless of the effect that age of entry to a farm might have on the overall number of treatments, the high frequency of treatments (eight to 12) on one in four farms would be expected to produce a correspondingly high pressure for selection of resistant parasites.

The proportion of the anthelmintics with extended action that were employed is not known, but all ML treatments would have been with products with persistent activity indicating that the true exposure of parasites to anthelmintic is somewhat higher than these number of treatments suggests. MLs were the treatments of choice in July to September and April to June, but there was comparable use of oral combination products during October to March, when cattle are small and amenable to oral drenching. ML anthelmintics or their combinations with other action families were currently, and for the past five years, used more than benzimidazoles and levamisole and benzimidazole-levamisole combinations. All but four farms had used macrocyclic-lactones at some time during the past five years. The study did not enquire as to reasons for use of particular action families but six of the 25 farmers who indicated they had changed families in the year of the study did so because they were concerned that the drugs were not working.

It was interesting that 24 farmers ranked annual or frequent action family changes as the most important strategies for prevention or control of drench resistance in cattle while 25 other farmers considered them to be useless for that purpose. Treating regularly every four to six weeks to keep worm populations suppressed was given the highest ranking as a strategy for prevention or control of resistance. Clearly there is confusion about the issues of parasite control versus sustainability of anthelmintic efficacy. Other practices that ranked high were quarantine drenching, incorporating sheep or older cattle in the grazing management and treating only when cattle were showing signs of parasitism. However, opinions were divided on the value of almost all methods, and markedly so for treating in response to onset of clinical signs or poor thrift, using generous doses of drench, regular treatments every four to six weeks, and changing action families. Speculation here about the reasons for the divided opinions on many issues is not likely to be productive. The important finding is simply that opinions were divided on many issues.

Sheep farms

The results from this study are disturbing as the levels of ML resistance recorded were considerably higher than anticipated. While 29 of 80 randomly selected farms had efficacy levels >95% for all anthelmintic treatments tested, it should be kept in mind that FECRT results of greater than 95% efficacy give no cause for complacency on the farms where they occur, as the FECRT does not give a direct measure of the prevalence of resistant alleles. It is generally considered that the prevalence of resistant alleles will be high when the FECRT is less than 95%. On the other hand, the fact that 29 of the random selection farms did have efficacy levels greater than 95% is still encouraging. The relatively higher efficacy of combination drenches probably reflects the distribution of resistance across worm species and the chance that an individual worm will be resistant to two anthelmintic action groups.

The number of drenches given does not appear to have changed significantly over the last 25 years. This is surprising because the widespread use of anthelmintics with more persistent activity should logically be expected to have led to a reduced reliance on drenching. In fact the reverse is true; the widespread use of long-acting products means that sheep parasites are in all probability exposed to more anthelmintic than ever before (Leathwick, 2004). It is possible that farmers have unknowingly reacted to emerging resistance by drenching more often.

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