

Turning






the Worm

Issue 12 – December 2003 – ISSN 1442-8466

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In this issue:

-  Methods of detecting anthelmintic resistance – Kathryn Kemper (Pages 1-4)
-  Clarification-Drench Resistance in Cattle Nematodes: Persistent Protection Periods for ML Drenches in Cattle Nematodes – Gareth Hutchinson (Pages 4-5)
-  Contents - last issue (Page 4)

Best wishes to all for 2004!

Welcome to this issue of TTW. The main purpose of this informal newsletter is to share information with those particularly interested in the management of endoparasites of farmed animals, including sheep, goats and cattle.

Methods of Detecting Anthelmintic Resistance

Kathryn Kemper - UNE

Around November this year, Kathryn Kemper submitted a BRurSci honours thesis entitled "Detection and Management of Anthelmintic Resistance at the University of New England's Kirby Research Station" (Armidale, NSW). Her supervisor commented that the findings were interesting, not only on the resistance status at Kirby, but on various

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ways of detecting resistance and a comparison of the results they give.

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Anthelmintic resistance is endemic in the Australian Sheep industry and threatens its future sustainability. Identification and ongoing

problem. Testing for anthelmintic resistance is generally done by a faecal egg count reduction test (FECRT) in sheep or a lab-based larval development assay (LDA). The FECRT involves monitoring of faecal worm egg count (FEC) following administration of recommended doses of the anthelmintics being tested. The LDA involves submission of a pooled faecal sample from which worm eggs are extracted and cultured in the presence of increasing concentrations of the anthelmintics under test to identify the concentrations which inhibit development. There are several formulae available for calculating FECR, each requiring a different set of measurements. The recommended time for measuring FEC following administration of anthelmintic also varies between 7 and 14 days. The objective of this project was to compare a

range of detection methods and at the same time to investigate the anthelmintic resistance status of the UNE research farm "Kirby". The value of duplicating faecal egg counts was also investigated.

Table 1. The percent faecal egg count reduction (FECR) for the 3 different methods in *Haemonchus contortus* for the treatment groups of benzimidazole (BZ), levamisole (LEV), combination BZ/LEV, ivermectin at ½ and full recommended dose rates, naphthalophos (NAP) and closantel at the treatment to sampling intervals of 7 and 12 days (inv.).

Trmt	Inv.	Percent FECR		
		Method 1	Method 2	Method 3
BZ	7	64.0*	61.9*	63.3
LEV	7	100.0	100.0	100.0
BZ/LEV	7	100.0	100.0	100.0
½ IVM	7	89.3	88.6	89.1
Full IVM	7	98.8*	98.9*	98.8
NAP	7	99.6	99.6	99.6
Closantel	7	-3.4	-40.5	-5.4
BZ	12	-2.7*	-8.6*	24.9
LEV	12	98.8	98.7	99.1
BZ/LEV	12	99.9	99.9	99.9
½ IVM	12	76.8	75.4	76.7
Full IVM	12	93.3*	93.5*	95.1
NAP	12	99.9	99.9	99.9
Closantel	12	-37.4	-86.7	-0.5

*Significant difference between treatment to sampling intervals of 7 and 12 days.

Method 1 compared an initial and final mean FEC from the treated animals. Calculation **Method 2** compared the initial FEC from a control group and the final FEC from treated animals while **method 3** compared an initial and final mean FEC from treated animals when the reduction was adjusted according to the change in the control animals.

Wethers (n=169) from the University of New England's 'Kirby' research station were subjected to a faecal egg count reduction test (FECRT) with anthelmintic treatment groups of benzimidazole (BZ), levamisole (LEV), combination BZ/LEV, ivermectin (IVM) at full and half dose rates, naphthalophos (NAP) and closantel. Half dose ivermectin treatment was included to detect emerging ivermectin resistance as the recommended dose rate exceeds the lethal concentration for susceptible nematodes. Pre-treatment faecal samples were utilized for individual duplicate faecal egg counts (FECs), pooled treatment larval differentiation and submitted for a pooled population

monitoring of anthelmintic resistance status are key steps in the successful management of the

in vitro DrenchRite® larval development assay (LDA). The LDA was conducted by NSW Agriculture's Regional Veterinary Laboratory at

Woodbridge Road Menangle NSW. Faecal samples taken at 7 and 12 days post-treatment were used for duplicate FECs and pooled treatment larval differentiation.

FEC were adjusted according to larval culture results and the analysis completed for the dominate parasite only. This was Barber's pole worm (*Haemonchus contortus*) which comprised 95% of the initial larval differentiation. The data were subjected to three different faecal egg count reduction (FECR) calculation methods, each with treatment to sampling intervals of 7 and 12 days. Calculation **method 1** compared an initial and final mean FEC from the treated animals, calculation **method 2** compared the initial FEC from a control group and the final FEC from treated animals while **method 3** compared an initial and final mean FEC from treated animals when the reduction was adjusted according to the change in the control animals. Confidence intervals were used to determine significant differences between FECR calculation methods and sampling intervals. The value of duplicate FEC was estimated using a nested model analysis of variance to partition the variance in FECs between treatments, animals within treatments and duplicates within animals.

The results of the FECR calculation methods are shown in Table 1. The LDA estimated BZ efficacy at 55%, LEV at 94% and combination BZ/LEV at 92% while the macrocyclic lactone (ML) anthelmintics were considered to be an effective drench. The closantel assay was reported to be inconclusive as the assay "failed to detect any [larval] mortalities". Results from the nested model analysis of variance showed that greater than 90% of the variation in FEC in untreated animals was due to between animal variation.

Calculation methods of FECR did not produce significantly different estimates of faecal egg count reduction. However the method utilizing post-treatment control animals to adjust for changes in the FEC of untreated animals over the period of the experiment (method 3) was concluded to be the most appropriate. This method accounted for immature stages of nematodes exposed to the anthelmintic at the time of treatment but which

were not detectable by the initial FEC. The advantage of this method is greatest when FEC changes (either increases or decreases) in undrenched control animals during the FECRT. Although the additional workload associated with this method may limit its commercial application, it is recommended that post-treatment samples from untreated control animals be utilized to estimate the full nematode population exposed to the anthelmintic.

Significant differences were obtained between the treatment to sampling intervals of 7 and 12 days for the BZ and IVM treatments with greater reduction of FEC at day 7. Contrary to expectations this was not seen with the LEV treatment. The greater FECR at day 7 was concluded to be the result of either ovi-suppression (BZ, possibly IVM) or reduced efficacy against immature larval stages (IVM only). Non-significant differences in LEV were concluded to be due to high efficacy of LEV against late immature stages of *H. contortus*. It is recommended that the treatment to sampling interval remain at 10-14 days for all the anthelmintics examined when estimating efficacy against *H. contortus*. A shorter treatment to sampling interval may be required when estimating efficacy of LEV against *Trichostrongylus* or *Ostertagia* spp. as this anthelmintic shows reduced efficacy against late immature stages of these nematodes.

The FECRT and LDA differed slightly for the prediction of susceptibility of *H. contortus* to the ML anthelmintic class. The LDA did not detect any resistance for ML, while the FECRT showed clear indications of emerging resistance to IVM (a member of the ML anthelmintic class). A similar finding was reported by Kotze *et al.* (2002). Changes to the commercially applied resistance criterion for the ML LDA were recommended. In addition, in the closantel anthelmintic group the LDA was unable to distinguish complete resistance from assay failure. This suggests that control susceptible nematodes need to be included in the assay to eliminate assay failure in the future.

Duplicate counts on faecal samples from the same animal were found to account for less than 7% of

the variation in FEC in untreated animals. As the between-animal variation was found to be the major source of variation, it was recommended that additional resources should be utilized by increasing the number of animals in a FECRT rather than duplicating FECs.

These results have demonstrated that marginal results for anthelmintic resistance should be treated with caution and that each detection method contains its own unique advantages and disadvantages. The FECRT with calculation method 3 proved to be the most appropriate resistance test under examination, with a wide variety of anthelmintic classes able to be assessed. In addition the results have confirmed the prevalence of widespread anthelmintic resistance in *H. contortus* in the New England Tablelands of NSW, but possibly the susceptibility of this resistant nematode to LEV or NAP.

REFERENCE

Kotze, A.C., Dobson, R.J., Tyrrell, K.L. and Stein, P.A. 2002, 'High-level ivermectin resistance in a field isolate of *Haemonchus contortus* associated with a low level of resistance in the larval stage: Implications for resistance detection', *Veterinary Parasitology*, vol. 108, pp. 255-63.

In the last issue (Issue 12, May 2003)

- ✚ Anthelmintic resistance in the UK – Coles. Pages 1-3.
- ✚ Cattle Nematodes and Resistance with particular reference to MLs – Hutchinson. Pages 4-8.
- ✚ Macrocyclic lactone resistance in Australian sheep nematodes – Barger. Pages 8-13.
- ✚ Bob Coverdale & Keith Ellis – (Grand) Masters. Page 13
- ✚ New drenches – NoDrench and

The information contained in this publication is based on knowledge and understanding at the time of writing (December 2003). However, because of advances in knowledge, users are reminded of the need to ensure that information upon which they rely is up to date and to check currency of the information with the appropriate officer of New South Wales Department of Agriculture or the user's independent adviser.

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Clarification

Drench Resistance in Cattle Nematodes: Persistent Protection Periods for ML Drenches in Cattle Nematodes.

The following note relates to an article by Gareth Hutchinson in the last issue of TTW (Issue 12, May 2003). This and other recent issues are available on-line at <www.agric.nsw.gov.au/reader/sheep-internal>.- Ed.

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Readers should note that there is a wide variation between and even within ML drenches registered for use in cattle to protect against different species of incoming infective larvae. For example, moxidectin has no claim for persistent effects against *Cooperia* species, but up to 42 days (depending on formulation) for some other gastrointestinal and pulmonary nematodes.

Caution should therefore be taken in interpreting faecal egg counts as an indicator of failure of anthelmintics, or the onset of resistance when the species of worms are not identified or if the time since treatment is near the prepatent period."

See table – next page.

Table. Persistent Protection Periods (Days) of Selected Cattle Macrocylic Lactone (ML) Anthelmintics against Common Nematodes[@]

Active	Abamectin ^{\$}	Abamectin ^{\$}	Doramectin [#]	Doramectin [#]	Eprino- mectin	Ivermectin [#]	Ivermectin [#]	Ivermectin + Clorsulon [#]	Moxidectin	Moxidectin
Constituent concentration	5 mg/mL	10 mg/mL	5 mg/mL	10 mg/mL	5 mg/mL	5 g/L	10 mg/mL	10 mg/ mL	5 g/L	10 g/L
Route	Pour-on	Injection	Pour-on	Injection	Pour-on	Pour-on	Injection	Injection	Pour-on	Injection
Species										
<i>Ostertagia ostertagi</i>	Up to 14	Up to 7	Up to 35	Up to 21	Up to 28	Up to 21	Up to 7	Up to 7	42	Not less than 21
<i>Haemonchus placei</i>	N/c	Up to 7	Up to 35	Up to 21	21	Up to 14	N/c	N/c	28	Up to 14
<i>Trichostrongylus axei</i>	N/c	N/c	Up to 35	Up to 21	21	Up to 14	N/c	N/c	28	Up to 14
<i>Cooperia oncophora</i>	N/c	N/c	Up to 21	Up to 14	Up to 28	Up to 28	N/c	N/c	N/c	N/c
<i>Cooperia pectinata /punctata</i>	N/c	N/c	Up to 35	N/c	Up to 28	Up to 28 (<i>C.punctata</i>) N/c (<i>C.pectinata</i>)	N/c	N/c	N/c	N/c
<i>Cooperia</i> spp (not speciated)	N/c	Up to 7	N/c	N/c	Up to 28	Up to 14	Up to 7	Up to 7	N/c	N/c
<i>Nematodirus helvetianus</i>	N/c	N/c	N/c	N/c	Up to 28	Up to 28 (immatures only)	N/c	N/c	21	N/c
<i>Dictyocaulus viviparus</i>	Up to 28	Up to 14	Up to 28	Up to 28	28	N/c	Up to 14	14	42	Not less than 35
<i>Oesophagostomum radiatum</i>	N/c	Up to 7	Up to 28	Up to 21	Up to 28	Up to 21	N/c	N/c	42	N/c
<i>Bunostomum phlebotomum</i>	N/c	N/c	Up to 28	Up to 21	N/c	N/c	N/c	N/c	42	N/c

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[@] Source: InfoPest (Queensland DPI) accessed via <http://www.apvma.gov.au/> as at 19 May 2003 unless otherwise indicated.

^{*} Source: Ivomec[®] Eprinex[™] (eprinomectin) Technical manual, Meril IVEP-97-016

^{\$} Source: Virbamec[®] Technical Manual, Virbac (Australia) Pty. Limited

[#] Source: MIMS IVS Annual 2002, Australian edition

N/c: No claim