HOW TO IMPROVE DRENCHRITE®

RJ Dobson[1], PA Stein[1], JH Craven[2]
[1]CSIRO, Division of Animal Production, McMaster Laboratory, Locked Bag 1, Blacktown 2148, Australia,
[2]Horizon Technology, PO Box 598, Roseville NSW 2064, Australia.

Abstract
Understanding the strengths and weaknesses of *DrenchRite* and how it relates to faecal egg count reduction tests (FECRT) will lead to clearer interpretation of *DrenchRite* results and a better perspective on both tests. In previous studies efficacy was estimated for worms resistant to BZ, LEV and BZ/LEV combinations by FECRT, *DrenchRite* and post mortem worm counts. Correlations of *in vivo* efficacy with FECRT determined by them were: *Trichostrongylus colubriformis* 88%, *Ostertagia circumcincta* 56% and *Haemonchus contortus* 69%. The low correlation for *O. circumcincta* was associated with the variability in estimating species composition when *O. circumcincta* was present at low levels. Correlations of *DrenchRite* with *in vivo* efficacy were: *O. circumcincta* 87%, *T. colubriformis* 82% and *H. contortus* 80%. The correlation between resistance determined by Larval Development Assay (LDA) and FECRT was not high at 61% (averaged over drugs and species). Similar data for Macrocyclic Lactones (ML) resistant worms are not yet available.

Previous studies have shown for some LDA systems that eggs are more likely to be inhibited in development to L3s early (2-3 weeks post infection (PI)) and late (beyond 10 weeks PI) after infection and are most resilient to the effect of drugs at about 7 weeks PI. No systematic changes in *DrenchRite* results were observed over age of worm infection when tested weekly for 2 to 14 week old worms.

Large bowel worms of sheep have not generally been examined under LDA conditions so a study was undertaken to determine the LD50s of susceptible *Oesophagostomum columbianum* (nodule worm) and *Chabertia ovina* (large mouthed bowel worm) on *DrenchRite* plates. LD50s for BZ and LEV were approximately 0.04 and 0.8 uM respectively for both worms. For the ML *O. columbianum* had a much higher LD50 (45 nM) than *C. ovina* (8 nM). In relation to the other common sheep worms, development characteristics of *C. ovina* in LDA is like *H. contortus* while *O. columbianum* is more like *O. circumcincta.* Both large bowel worms were very sensitive to BZ/LEV combinations, with LD50s 4 to 8 fold lower than those observed for *H. contortus* and *O. circumcincta.*

Introduction

In this discussion paper we examine the relationship between post mortem worm counts, FECRT and *DrenchRite* results (1). The stability of *DrenchRite* results in relation to worm age will be reviewed together with some recent results from LDA studies. And the sensitivity of some large bowel parasites which are commonly found in worm egg counts is also described.

FECRT, LDA vs. Post Mortem Worm Counts

Sheep infected with mixtures of drug resistant and susceptible strains of *Haemonchus contortus, Trichostrongylus colubriformis and Ostertagia circumcincta* were treated with a benzimidazole (BZ), levamisole (LEV) or a BZ/LEV combination to determine drug efficacy. Efficacy was estimated by FECRT, LDA and post mortem worm counts (1).

Correlation of *in vivo* efficacy with FECR (averaged over drugs, and expressed as the percentage of the total variance that was explained by the regression analysis) was best for *T. colubriformis* (88%) and worst for *O. circumcincta* (56%) with *H. contortus* intermediate at 69%. The low correlation for *O. circumcincta* was associated with the variability in estimating species composition when *O. circumcincta* was present at low levels. For example, if the true mean contribution of one species is 9% then anything between 3% and 15% can be expected from a random sample, and the range for a mean of 4% is 0%-8%. This kind

of variability in % species composition can yield widely differing results for FECR when the total egg count is high (ie. when *H. contortus* and/or *T. colubriformis* are present in large numbers). The current caution applied to FECRT is that the mean FEC needs to be greater than 200 eggs/gram before trusting a FECRT result. An additional restriction: that a species represent more than 15% of the egg count should be applied before a valid FECRT can be assumed for that species.

Correlations of resistance ratio, measured by LDA, with *in vivo* efficacy were more consistent. *O. circumcincta* was best (87%), *T. colubriformis* and *H. contortus* were similar (82% and 80% respectively).

The correlation between resistance determined by LDA and FECRT was not high at 61% (averaged over drugs and species). This indicates that the two tests are measuring slightly different aspects of anthelmintic resistance.

The *DrenchRite* assay, used to estimate drench efficacy, is based on the correlation of LDA resistance ratio and *in vivo* efficacy from the above study (1). The logical approach is to check the validity of a new test against the established one, in this case the FECRT. It is valid to use the FECRT as a guide to the accuracy of the *DrenchRite* estimate of drug efficacy only for *T. colubriformis* where there is a reasonable correlation (77%). For *O. circumcincta* and *H. contortus*, the LDA gives a better estimate of *in vivo* efficacy than the FECRT and for these species the two tests (LDA/FECRT) correlate poorly (53%).

A similar study (to 1) for the Macrocyclic Lactones (ML) has not been conducted because of the lack of a suitable range of ML resistant species and the high cost of undertaking such a study. LDA has been shown to differentiate between known ML resistant and susceptible strains of *O. circumcincta* (2) *and H. contortus* (3). Unpublished data of the authors using *DrenchRite* has demonstrated that ML susceptible and resistant (4) *T. colubriformis* can be distinguished by LDA. With *T. colubriformis*, known susceptible strains are divided into two populations by the ML used in the *DrenchRite* plate (Table 1). Well 1 below is the control well, the and the ML concentration doubles each well to the maximum concentration in well 12.

This data shows between well 5 and 6 about 87% of the susceptible *T. colubriformis* are prevented from developing to L3s, while the remaining 13% of the susceptible *T. colubriformis* are not affected till well 10 (ie. at a 16 fold higher concentration). In a resistant *T. colubriformis* strain (4) no eggs are prevented from developing till well 11 (where about 40% are affected). This division of susceptible *T. colubriformis* into 2 populations is not optimum for field detection of resistance particularly when other worm species are present. Changing the ML drug used in the assay may overcome this problem but this will require continuing research.

Table 1 Percentage of eggs/well inhibited from development to Infective *T. colubriformis* Larvae (% Kill)

Well #	1	2	3	4	5	6
%Kill	0	0	0	0	0	86

Well #	7	8	9	10	11	12
%Kill	90	84	87	100	100	100

Effect of Worm Age on LD50 determined by LDA

Systematic changes in the concentration of drug required to inhibit development of 50% of larvae/eggs present ("LD_{50}") were observed (2) for BZ, LEV and MLs over time after a single infection (ie. with worm age). In their study Amarante *et al* (2) used a liquid medium for the LDA, as described by Hubert and Kerboeuf(5). They demonstrated that eggs are more likely to be inhibited in development to L3s very early and late post infection (PI) (ie. 2-3 weeks PI and beyond 10 weeks PI) and are most resistant to the effect of drugs at about 7 weeks PI.

The authors conducted a similar study with a single batch of *DrenchRite* plates prepared prior to the study and used over a period of 16 weeks. This assay relies on a drug/agar matrix as the development medium as opposed to a liquid medium(2 and 5). One sheep was infected with McMaster susceptible strain of *T. colubriformis*. Two sheep were infected with another susceptible

strain of *T. colubriformis* recently isolated from Armidale NSW. When infections were patent (22 days post infection) *DrenchRite* assays were performed on each sheep and then weekly for 12 weeks except for the McMaster sheep which stopped shedding eggs after the 8th assay.

Table 2 *DrenchRite* Well Number of the LD50 for Susceptible *T. colubriformis* strains in Rows G and H which contain different analogues of a ML

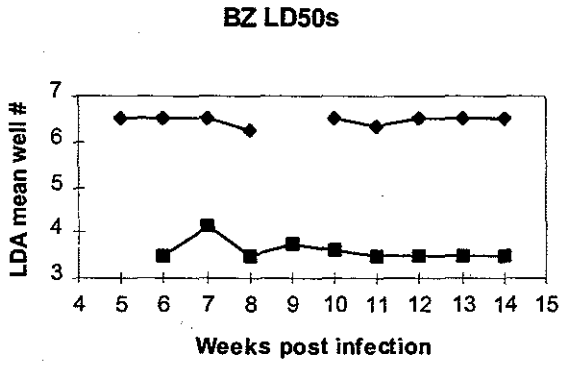
Assay #	1	2	3	4	5	6	7	8	9	10	11	12
Row G												
McM	6.5	6	6	4.5	3.5	5.5	5.5	5				
Ar #1	5.5	4.5	5.5	4.5	5.5	5.5	5.5	5.5	6	4.5	5.5	5.5
Ar #2	4.5	4.5	5.5	4.5	5.5	5.5	5.5	5.5	6.5	5	6	5.5
Row H												
McM	6.5	6.5	6	5.5	4.5	5	6	6.5				
Ar #1	5.5	5.5	5.5	5.5	5	5.5	6.5	6	5.5	5.5	7	6.5
Ar #2	5.5	5.5	6.5	6.5	5	6.5	6.5	9.5	8.5	5.5	6	7

Note: assay #1 was conducted 22 days post infection and the following assays weekly thereafter.

For BZ virtually no variation LD_{50}s was observed, for 35 of the 36 assays the LD50 occurred between well 4 and 5 (ie.4.5), and was 3.5 for the McMaster isolate on the 5th assay. For LEV, one of the sheep infected with the Armidale strain (Ar #1) consistently had an LD50 at well 4.5, the departures from this were at assays 6, 8, 10 and 12 when the LD50 was 5.5, 5.5, 5.5 and 4 respectively. The other sheep infected with the Armidale strain (Ar #2) consistently showed an LD50 for LEV at well 5.5 departures being at assays 4, 5, 10 and 12 with LD50s at wells 4.5, 5, 5 and 4.5, respectively. The McMaster strain(McM) fluctuated between 5.5 and 4.5 for LEV in no consistent way. For BZ/LEV combinations the LD50 was found at well 5.5 for a little over 2/3 of the assays, the remaining 10 assays ranged between well 6 and 4.5 in no consistent way. Table 2 shows well numbers for ML LD50s for both worm strains and both analogues of ML used in the *DrenchRite* plate, and again no consistent trend emerges.

A similar study to the above was conducted with BZ and ML resistant strains of *H. contortus* but included additional ML only LDA plates so the two ML compounds (rows G and H) could be replicated. The susceptible McMaster *H. contortus* (McM Hc) strain had a longer pre-patent period (about 4 weeks) than the other strains tested (2-3 weeks), and at 5 weeks McM Hc still had a low egg count. The BZ resistant (DAP Hc) strain was isolated at McMaster Laboratory from local sheep, this strain is also partially resistant to ML. The other strain used (only in ML LDA assays) was ML resistant (CAVRS Hc) and was isolated at Armidale NSW (6). Figure 1 shows the mean well number at each sampling time where the BZ-LD_{50} occurred. Where the data is missing no *DrenchRite* plate was set up for that strain at that sampling time. Data points are means of 2 or 4 replicates. It is clear that little fluctuation occurred in BZ LD_{50}s for either strain over the course of the infection.

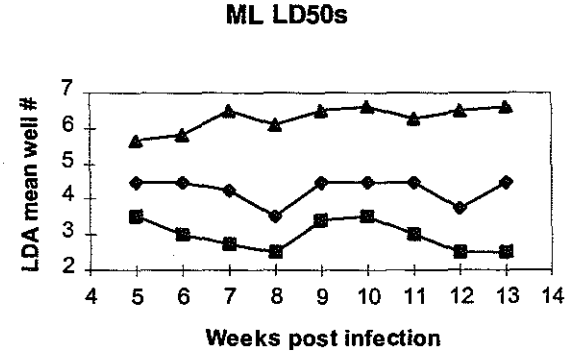
Figure 1: *DrenchRite* well number in which BZ LD_{50} occurs for McM Hc (susceptible ■) and DAP Hc (resistant ◆) strains over 14 weeks of infection.



McM Hc and DAP Hc strains were susceptible to LEV and BZ/LEV combinations (data not shown) and only random fluctuations in LD50 for these drugs were observed over time. Figure 2 shows the well number in which the ML LD50s (row H) occurred for CAVRS Hc, DAP Hc and McM Hc (data points are means of 6, 2, and 4 replicates respectively).

Like *T. colubriformis* the 3 *H. contortus* strains display no consistent systematic change in LD50 over time, although slight divergence in ML LD50 lines by week 7 may be evident (see Figure 2). No similar study has been undertaken using *DrenchRite* plates for *Ostertagia spp.*

Figure 2 *DrenchRite* well number in which ML LD50 occurs for McM Hc (susceptible ■), DAP Hc (tolerant ◆) and CAVRS Hc (resistant ▲) strains over 13 weeks of infection.



Large Bowel Worms

Despite rumours to the contrary, large bowel worms, *Oesophagostomum columbianum* (nodule worm) and *Chabertia ovina* (large mouthed bowel worm) in particular, are still alive and well in the eastern states of Australia, frequently constituting up to 30% of the worm eggs found in sheep faeces. Because the behaviour of these parasites under LDA conditions has not been studied, we examined LD50s over time in *DrenchRite* plates. Susceptible strains of both species were assayed weekly for 5 weeks, each assay was replicated and additional ML assays were conducted each sampling time. *O. columbianum* and *C. ovina* have pre-patent periods of 6 and 10 weeks respectively. When the assays were commenced the *O. columbianum* and *C. ovina* infections (in separate sheep) were 9 and 10 weeks old respectively.

Table 3 *DrenchRite* well number in which the LD50 for Large Bowel Worms occurred

DRUG	BZ	LEV	BZ/LEV	ML
SPECIES				
O. columbianum	3.5	4.0	2.5	6.5
C. ovina	3.5	4.0	2.5	4.0

Development in control wells was normal for *O. columbianum* (ie around 90%+ of eggs dispensed became L3s), however, for *C. ovina* this did not occur till week 12 despite a high egg count (about 500epg) at 10 and 11 weeks PI. The poor development of *C. ovina* immediately after patency could mean that a high proportion of eggs were not fertile. Apart from their relatively high sensitivity to the BZ/LEV combination (*O. circumcincta*, *T. colubriformis* and *H. contortus* have LD50s around well 5) *O. columbianum* and *C. ovina* show similar sensitivities to *O. circumcincta* and *H. contortus* respectively.

Conclusion

The above data on changes in LD50 over period of infection suggest worm age is unlikely to influence *DrenchRite* results. Knowing the sensitivity of the common large bowel parasites will assist the users of *DrenchRite* to determine if the broad spectrum anthelmintics are effective for these parasites. However, the difficulty encountered when developing *C. ovina* to L3s immediately after patency does imply if some young infections have reduced egg viability this

potentially may decrease the sensitivity of a *DrenchRite* assay.

Acknowledgements - We are grateful for the financial support of Horizon Technology.

References

1 Lacey E, Craven JH, Gill JH and Baker PJ (1993) The Larval Development Assay as an Alternative to Faecal Egg Reduction Test for Field Diagnosis of Broad Spectrum Anthelmintic Resistance. *The Australian Society for Parasitology: Program and Abstracts of Papers* Annual scientific meeting held at Heron Island September 28 - October 1, 1993

2 Amarante AFT, Pomroy WE, Charleston WAG, Leathwick DM and Tornero MTT (1997) Evaluation of a Larval Development Assay for the Detection of Anthelmintic Resistance in *Ostertagia circumcincta Int J Parasitol* 27: 305-311

3 Gill JH, Redwin JM, Van Wyk JA and Lacey E (1995) Avermectin Inhibition of Larval Development in *Haemonchus contortus* - Effects of Ivermectin Resistance *Int J Parasitol* 25: 463-470

4 Shoop WL (1993) Ivermectin resistance *Parasitol Today* 9: 154-159

5 Hubert J and Kerboeuf D (1992) A microlarval development test assay for the detection of anthelmintic resistance in sheep nematodes *Vet Rec* 130: 442-446

6 Le Jambre LF, Gill JH, Lenane IJ and Lacey E (1995) Characterisation of an avermectin resistant strain of Australian *Haemonchus contortus. Int J Parasitol* 25: 691-698.