Cervine Triple Combination Anthelmintic Residue Study – moxidectin, oxfendazole and levamisole.

Dave Lawrence

Tikana, 374 Livingstone Road, Browns, R.D.1 Winton, 9781, New Zealand

Abstract
A recommended triple combination anthelmintic for deer (moxidectin injection (0.2 mg/kg) plus oral oxfendazole (18.12 mg/kg) and oral levamisole (15 mg/kg)) is not registered for use in deer and therefore has a default 91day withholding time. A study on 9 deer showed moxidectin and oxfendazole residues at 42days post treatment were clear of MRLs for cervine liver, muscle kidney and fat. Levamisole residues at 42days post treatment were clear of MRLs for cervine liver, muscle and kidney, however, one of the nine deer had a levamisole level greater that the MRL for cervine fat. This result means a 42 day meat WT cannot be recommended.

Keywords
Withholding Time, Triple Combination Anthelmintic, Moxidectin Injection, Oxfendazole oral, Levamisole oral, deer, moxidectin tissue residues, oxfendazole tissue residues, levamisole tissue residues, off label use.

Introduction
The Agricultural Compounds and Veterinary Medicines (ACVM) Act provides for the use of agricultural compounds in food producing animals. Under the Act products are registered for use in specified animals and to meet the requirements of the Act. These agricultural compounds must not result in breaches of domestic food residue standards. Hence products are registered with a Withholding Time (WT) from treatment to slaughter.
The purpose of this trial is not intended to register a product or determine an official label claim WT. The purpose of this study is to provide veterinarians with data to enable them to make informed decisions when prescribing the off label use of a recommended effective triple combination anthelmintic for their deer farmer clients.

Background
There are a small number of anthelmintics registered for use in deer. The existing registered products are unsuitable for use in deer as they all have efficacies that will encourage drench resistance (although they do have commercially acceptable withholding times). Drench resistance in deer has been shown in recent years to be widespread in the industry.
A number of trials undertaken by Leathwick (*pers. comm.*), Mackintosh (Mackintosh et al., 2013) and Lawrence (Lawrence, 2011, Lawrence et al., 2012, Lawrence et al., 2013) have culminated in defining a triple combination that is efficacious and offers the most sustainable outlook for use in deer. The triple combination involves moxidectin injection (0.2 mg/kg) plus oral oxfendazole (18.12 mg/kg) and oral levamisole (15 mg/kg). This is four times the label dose of oxfendazole and twice the label dose of levamisole for sheep and cattle.

Currently this combination has a default 91 day withholding time (WT). The 42 day interval post treatment selected for this study was considered a conservative time regarding the likelihood of residues being present. Furthermore 42 days was considered more practical than the default 91days for farmers to plan treatment pre-slaughter.

Materials and Methods.

Invermay Animal Ethics Committee approval was granted prior to the study commencing (#13390).
Nine wapiti cross rising one year deer were selected on a commercial deer farm in Southland. Selection of animals was based on their being at a desired killable weight at slaughter and they had not received any drenches in the 2 months prior to the trial. All 9 deer were weighed, tagged and treated on 4th November 2014 with the triple combination of moxidectin injection (0.2 mg/kg) and the oral combination of oxfendazole (18.12 mg/kg) and levamisole (15 mg/kg).

The triple combination drench was made up of Cydectin® Injection for Cattle and Sheep (Batch # 17905 exp Feb/2016) administered at a dose rate of 1ml/50kg; and Oxfen C Plus (Batch # 8420 exp 01/17; contains oxfendazole and levamisole) mixed in equals parts with Oxfen C (Batch # 7231 exp 09/2016; contains oxfendazole), administered orally at a dose rate of 1ml/5kg.

The co-operation of MPI was sought prior to the trial and 6 weeks later they responded. "MPI is not in a position to provide approval for the animals to be slaughtered and processed for human or animal consumption as there is no evidence that the combination treatment using moxidectin injection (0.2mg/kg) plus oral oxfendazole (18.12mg/kg) and levamisole (15mg/kg) poses no risk to human or animal health". Thus, the deer were unable to be slaughtered through the local DSP, this despite the fact that this was what the trial was trying to ascertain.

The deer were humanely slaughtered on farm 42 days post treatment (16th December) and samples collected. Duplicate samples (A and B sets) of liver, muscle, kidney and fat were collected from each of the 9 deer. The number of deer sampled was based on the guide provided in the ACVM Registration Standard And Guideline For Determination Of A Residue Withholding Period For Veterinary Medicines), Table A1.4, minimum number of data points for single time point assessed WHP.All samples were frozen and the "A" set couriered to Hill Laboratories, Hamilton the following day.

Results

The results of the tissue analysis are presented in the complete original Hill Laboratories Analysis Report below. Lab Number 1366414.
The limit of detection for moxidectin, oxfendazole and levamisole is 0.005mg/kg.
The level of moxidectin, oxfendazole and levamisole in liver, muscle and kidney samples from all nine deer were below the limit of detection.
The Maximum Residue Limits (MRL) are listed in The New Zealand Mandatory Food Standard Table of MRLs or The Meat Residue Regulations Notice 1996 or any succeeding Notice or Specification.

The MRL of Moxidectin in Deer fat is 0.5mg/kg

In three of the nine deer moxidectin residue levels were recorded in the fat, the other six being less than the limit of detection. These levels of 0.009mg/kg, 0.008mg/kg and 0.005mg/kg were respectively 55 times, 62.5 times and 100 times less than the MRL.

The MRL of oxfendazole in Deer fat is 0.05mg/kg
In one of the nine deer oxfendazole residue level were recorded in the fat, the other eight being less than the limit of detection. The level of 0.012mg/kg was 4 times less than the MRL.

The MRL of levamisole in Deer fat is 0.01mg/kg
In two of the nine deer levamisole residue levels were recorded in the fat, the other seven being less than the limit of detection. The level of 0.005mg/kg was 2 times less than the MRL while the level of 0.025mg/kg is 2.5 times greater than the MRL.

The one positive residue result of the 108 samples tested was from the animal identified as Red 153. A repeat test for levamisole on the original fat sample of Red 153 was requested and the “B” fat sample from the same animal couriered to Hill Laboratories for analysis.

The results of these subsequent tissue analyses are presented in the complete original Hill Laboratories Analysis Report below. Lab Number 1378609.
The repeat analysis of the “A” fat sample from Red 153 was 0.34mg/kg and is 3.4 times greater than the MRL.
Analysis of the “B” fat sample from Red 153 was 0.25mg/kg and is 2.5 times greater than the MRL.

Hill Laboratories
BETTER TESTING BETTER RESULTS

R J Hill Laboratories Limited
1 Clyde Street
Private Bag 3205
Hamilton 3240, New Zealand

Tel +64 7 858 2000
Fax +64 7 858 2001
Email mail@hill-labs.co.nz
Web www.hill-labs.co.nz

ANALYSIS REPORT

Client:	Dave Lawrence	**Lab No:**	1366414	SDSPv1
Contact:	Dave Lawrence	**Date Registered:**	18-Dec-2014	
	374 Livingstone Road	**Date Reported:**	09-Jan-2015	
	RD 1	**Quote No:**	65969	
	WINTON 9781	**Order No:**		
		Client Reference:		
		Submitted By:	Dave Lawrence	

Analysis Results

Sample Name:	Lab Number	Fenbendazole mg/kg	Fenbendazole sulfone mg/kg	Levamisole mg/kg	Moxidectin mg/kg	Oxfendazole mg/kg
Red 153 muscle	1366414.1	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 154 muscle	1366414.2	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 155 muscle	1366414.3	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 157 muscle	1366414.4	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 159 muscle	1366414.5	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 167 muscle	1366414.6	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 168 muscle	1366414.7	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 171 muscle	1366414.8	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 173 muscle	1366414.9	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 153 liver	1366414.10	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 154 liver	1366414.11	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 155 liver	1366414.12	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 157 liver	1366414.13	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 159 liver	1366414.14	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 167 liver	1366414.15	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 168 liver	1366414.16	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 171 liver	1366414.17	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 173 liver	1366414.18	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 153 kidney	1366414.19	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 154 kidney	1366414.20	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 155 kidney	1366414.21	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 157 kidney	1366414.22	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 159 kidney	1366414.23	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 167 kidney	1366414.24	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 168 kidney	1366414.25	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 171 kidney	1366414.26	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 173 kidney	1366414.27	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 153 fat	1366414.28	< 0.005	< 0.005	0.025	< 0.005	0.012
Red 154 fat	1366414.29	< 0.005	< 0.005	< 0.005	0.005	< 0.005
Red 155 fat	1366414.30	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 157 fat	1366414.31	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 159 fat	1366414.32	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 167 fat	1366414.33	< 0.005	< 0.005	0.005	< 0.005	< 0.005
Red 168 fat	1366414.34	< 0.005	< 0.005	< 0.005	0.009	< 0.005
Red 171 fat	1366414.35	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Red 173 fat	1366414.36	< 0.005	< 0.005	< 0.005	0.008	< 0.005

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Biological Specimens			
Test	**Method Description**	**Default Detection Limit**	**Sample No**
Fenbendazole	Solvent extraction, SPE cleanup, LC-MS/MS analysis. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	0.005 mg/kg	1-36
Fenbendazole sulfone	Solvent extraction, SPE cleanup, LC-MS/MS analysis. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	0.005 mg/kg	1-36
Levamisole	Solvent extraction, SPE cleanup, LC-MS/MS analysis. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	0.005 mg/kg	1-36
Moxidectin	Solvent extraction, SPE cleanup, LC-MS/MS analysis. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	0.005 mg/kg	1-36
Oxfendazole	Solvent extraction, SPE cleanup, LC-MS/MS analysis. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	0.005 mg/kg	1-36

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

S.R Clay

Shaun Clay BSc
Section Manager - Food and Bioanalytical Division

Hill Laboratories
BETTER TESTING BETTER RESULTS

R J Hill Laboratories Limited
1 Clyde Street
Private Bag 3205
Hamilton 3240, New Zealand

Tel +64 7 858 2000
Fax +64 7 858 2001
Email mail@hill-labs.co.nz
Web www.hill-labs.co.nz

ANALYSIS REPORT

Client:	Dave Lawrence	**Lab No:**	1378609	SPv1
Contact:	Dave Lawrence	**Date Registered:**	29-Jan-2015	
	374 Livingstone Road	**Date Reported:**	03-Feb-2015	
	RD 1	**Quote No:**		
	WINTON 9781	**Order No:**		
		Client Reference:		
		Submitted By:	Dave Lawrence	

Sample Type: Biological Specimens

	Sample Name:	Red 153 Fat (1366414/28)	Red 153 Fat - B Sample			
	Lab Number:	1378609.1	1378609.2			
Levamisole	mg/kg	0.034	0.025	-	-	-

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Biological Specimens

Test	Method Description	Default Detection Limit	Sample No
Levamisole	Solvent extraction, SPE cleanup, LC-MS/MS analysis. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	0.005 mg/kg	1-2

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

S. R. Clay

Shaun Clay BSc
Section Manager - Food and Bioanalytical Division

Discussion

Studies into the efficacy of levamisole against lungworm (Mason 1982, Mackintosh et al., 1984) showed that levamisole was metabolized more rapidly in deer than in cattle. It is widely accepted that levamisole has no residual effect in ruminants. Levamisole is readily absorbed, metabolized mostly in the liver (only 5% of the administered dose is excreted unchanged through urine) and rapidly excreted (90% of the administered dose is excreted in 24hours). Thus, the one tissue result with a residue level above the MRL for cervine fat is very difficult to rationalize.

Normally the default meat withholding period for ruminants given non-depot products is 91 days. However, there are some exceptions to this.
Annex V Standardised WHP Specifications of ACVM Registration Standard And Guideline For Determination Of A Residue Withholding Period For Veterinary Medicines states :-
"Oxfendazole oral formulations at dose rates to not exceed 7.5 mg/kg bw for cattle and 5mg/kg bw for sheep, goats and deer and containing no excipient intended to prolong persistence in the alimentary tract and no other active ingredient(s) except:

- Fenbendazole, its sulphone and febantel at active ingredient inclusion rates within limits as required

by the *ACVM Chemistry Standard;*

- Levamisole at concentrations to not exceed 8.1 mg/kg bw as the base;
- Praziquantel at concentrations to not exceed 7.5 mg/kg bw:

10 days meat WHP for cattle, sheep, goats, deer"

Granted, oxfendazole and levamisole have been given at much higher concentrations than the dose rates expressed above, and moxidectin had been added to the combination, but a levamisole residue level above the MPL for cervine fat is still difficult to explain. Is there something about the metabolism of levamisole that is significantly altered when given in combination with oxfendazole and moxidectin? Or is this simply an aberrant result?

It may be that levamisole when given in combination with moxidectin and oxfendazole to deer is metabolised differently to other species. Differences to sheep have been observed. A residue study by Hurst (*pers. comm.*),used a single time point assessment at 14 days post treatment with Trimox which is a triple oral combination of moxidectin (0.2 mg/kg), albendazole (4.76 mg/kg) and levamisole (8 mg/kg). In sheep, residue levels of levamisole in fat at 14 days were all below the limit of detection whereas the levamisole residue levels exceeded the MRL for deer fat in all 8 deer sampled. In sheep levamisole levels in kidney, muscle and liver at 14 days were detected but fell well below the MRLs. By comparison most of the deer levamisole residue levels in kidney, muscle and liver exceeded the MRL.

A totally confounding result in the Trimox residue study in deer was the presence of levamisole at levels above the MRL for fat, kidney and muscle samples in an untreated control animal. Is the assay for levamisole detecting something other than levamisole when applied to deer tissues?

Taken as they are, these results do not support a veterinary recommendation to administer the triple combination used in this study 42days away from slaughter. The default WT of 91days applies.

Using a triple combination that contains all three actives is best practice to delay the development of resistance. This is even more crucial in deer than in other livestock as there are no alternatives available (Lawrence, 2011, Lawrence et al., 2013). So although excluding levamisole from a cervine combination is not ideal, it may offer a practical solution for deer farmers wanting to treat finishing stock in the spring. Based on the residue data presented, veterinarians may consider prescribing a combination of Cydectin® Injection - moxidectin injection (0.2 mg/kg) plus oral Oxfen C - oxfendazole (18.12 mg/kg). However, could it be that this latter combination without levamisole may also cause moxidectin or oxfendazole to behave differently to that seen in this study?

Acknowledgements

Elk and Wapiti Society of New Zealand, Southland Branch NZDFA, Marlborough Branch NZDFA, DEEResearch, Zoetis (Victoria Chapman), Merial Ancare (Justin Hurst), Hill Laboratories Hamilton and deer farmer Brian Russell

References

MPI, New Zealand Food Safety Authority, ISBN 0-478-07709-2, 39 ACVM 03/03. ACVM Registration Standard And Guideline For Determination Of A Residue Withholding Period For Veterinary Medicines

Lawrence DW. Moxidectin drug residue trial. *Proceedings for the Deer Branch of the New Zealand Veterinary Association* 28, 95-97, 2011

Lawrence DW. Cervine Anthelmintics – The Bubble Has Burst *Proceedings for the Deer Branch of the New Zealand Veterinary Association* 28, 87-92, 2011

Lawrence DW, **MacGibbon JT, Mason PC.** Moxidectin pharmacokinetics and resistance in deer. *Proceedings for the Deer Branch of the New Zealand Veterinary Association* 29, 41-45, 2012

Lawrence DW, **MacGibbon JT, Mason PC.** Efficacy of Levamisole, Moxidectin oral, Moxidectin injectable and Monepantel against *Ostertagia*-type nematodes in deer. *Proceedings of the New Zealand Veterinary Association Conference* 301, 233-240, 2013

Mackintosh CG, Mason PC, Bowie JY, Beatson NS. Anthelmintics against lungworm (Dictyocaulis viviparous) in red deer (Cervus elaphus). *Proceedings for the Deer Branch of the New Zealand Veterinary Association* 1, 69-77, 1984

Mackintosh CG, Cowie C, Johnstone P, Fraser K, Mason PC. Anthelmintic Resistance to macrocyclic lactones after 30 years of use on an Otago deer farm. *Proceedings of the New Zealand Veterinary Association Conference* 301, 227-231, 2013

Mason PC. Project Report AH 306 – Serum levamisole levels in deer following treatment with Nilverm. *Surveillance* 9/3, 11, 1982