Anthelmintic Dose Determination Studies for Levamisole and Oxfendazole against *Ostertagia*-type nematodes in deer

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Abstract

This investigation used a slaughter trial to establish the status of drench resistance on a Southland farm. On the same farm the resistance of *Ostertagia*-type nematodes to moxidectin was determined three years previously. The level of resistance has become more severe in the ensuing three years. The efficacies of oxfendazole at standard dose rate (4.53 mg/kg), oxfendazole at triple dose rate (13.6 mg/kg), levamisole at two and a half times standard dose rate (18.75 mg/kg) and moxidectin injection were determined and compared. The efficacy of oxfendazole at 13.6 mg/kg is significantly better than oxfendazole at 4.53 mg/kg against *Ostertagia*-type nematodes in deer ($p<0.0001$). To avoid further development of anthelmintic resistance on deer farms a triple combination anthelmintic should be used but it should incorporate oxfendazole at 13.6 mg/kg.

Keywords
Deer, anthelmintic resistance, anthelmintic efficacy, anthelmintic dose determination, gastrointestinal parasites, *Ostertagia*, levamisole, oxfendazole, moxidectin injection.

Introduction

Macrocylic Lactone (ML) anthelmintic resistance to gastrointestinal nematodes was first signaled in farmed deer in New Zealand in 2005(Hoskin et al., 2005). Since then slaughter trials on a further seven deer farms has confirmed some degree of *Ostertagia* -type resistance to ML anthelmintics in every case (Lawrence, 2011, Lawrence et al., 2012, Lawrence et al., 2013, Hodgson, 2013, Mackintosh et al., 2013, Leathwick, *pers comm*). Generally the average efficacy of ML Pour Ons was 53 % (n=9), average efficacy of ML orals was 70 % (n=6), and average efficacy of ML injectables was 91 % (n=8).

Moxidectin has become the most widely used ML and in many cases exclusively used anthelmintic on New Zealand deer farms (Castillo-Alcala et al., 2005). Moxidectin Pour-On being the most commonly used formulation and is the moxidectin formulation with the poorest efficacy/most resistance.

Previous studies had shown the combination of moxidectin injection and oral oxfendazole plus levamisole to be an effective combination for treatment of *Ostertagia*-type nematodes resistant to moxidectin .

The deer industry would appear to be in the reverse situation to the sheep industry regarding nematode resistance to anthelmintic families. By 1995 in the sheep industry resistance to either benzimidazole or to levamisole anthelmintics was widespread and common in New Zealand (McKenna, 1995) and the use of ML anthelmintics and their incorporation into combination anthelmintics has delayed further development of resistance. In the deer industry it is likely that ML anthelmintic resistance to

gastrointestinal nematodes is common and widespread and that we need to incorporate benzimidazole and levamisole into anthelmintic combinations ensure the onset of resistance is delayed.
Alternative anthelmintic options have not proven effective in deer. Moxidectin Long Acting and Abamectin/dequantel at the standard sheep dose rate and Monepantel at double the standard sheep dose rate failed to achieve 95 % efficacy against adult *Ostertagia* -types – 81 %, 82 % and 87 % respectively. This poses real restrictions on anthelmintic options available to treat gastrointestinal nematodes in farmed deer in New Zealand. In the sheep industry it has been shown that a single strategic treatment with a new class of anthelmintic could slow the development of resistance to existing classes of anthelmintic (Leathwick and Hoskin, 2009). We do not have that option available in the deer industry.
Levamisole and oxfendazole have been used very little, if at all, on most deer farms in the last decade. The use of levamisole at the standard sheep/cattle dose rate (8 mg/kg), and oxfendazole at the label dose rate for the registered oxfendazole anthelmintics (4.53 mg/kg) were trialed on two farms in 2012 with no history of recent use of these anthelmintics (Lawrence et al., 2013;, Leathwick, *pers comm*). The efficacies shown are not likely to be affected by resistance but reflect the efficacy of that dose rate in deer.
The efficacies of levamisole against *Ostertagia* -type adults were 72 % and 39 % respectively. The efficacies of oxfendazole against *Ostertagia* -type adults were 72 % and 69 % respectively.
No studies have been undertaken to determine the effective dose of levamisole in deer. No trial data could be found to support the label dose rate for the registered oxfendazole anthelmintics for deer. These findings were the basis of prompting this study to identify the effective dose rate of levamisole and oxfendazole to treat gastrointestinal parasites (*Ostertagia*-types) in deer.

Background

A farm in central southland was chosen in 2013 for the study. The same farm had undergone slaughter trials in 2010 to determine the status of the farm. At that time moxidectin in a Pour On formulation had 71.2 % efficacy against *Ostertagia*-type adults and moxidectin as an injectable formulation had 83.5 % efficacy against *Ostertagia*-type adults. The farm is an integrated farming operation running sheep and deer with 110hectares that are deer -fenced accommodating 400 hinds in a breeder/finisher operation using Wapiti terminal sires over red-type base hinds. There is also a small velveting herd.
This study was run under commercial field conditions using rising one year old (R1) finishing deer with a naturally-acquired infection of gastrointestinal (GI) nematodes. The study had three objectives:-

1. Determination of the efficacy of moxidectin against *Ostertagia*-type gastrointestinal parasites on a farm whose previous resistance to Moxidectin in the treatment of such parasites was quantified in 2010.
2. Determination of the appropriate dose rate of Oxfendazole for deer against *Ostertagia*-type nematodes.
3. Determination of the appropriate dose rate of Levamisole for deer against *Ostertagia* -type nematodes.

Material and methods

Animals

In 2013 all weaners were wintered on grass. They were weighed in the first week of August and 60 stag fawns/R1 were selected based on the likelihood of achieving a 60 kg carcass weight by late October. Their last anthelmintic treatment was given at the time of weighing in August - a combination of moxidectin injection (0.2 mg/kg "Cydectin ® Injection for Cattle and Sheep" Zoetis) and oxfendazole/levamisole oral (4.53 mg/kg oxfendazole and 8 mg/kg levamisole HCL "Scanda ®" Coopers).

Treatments

In mid-October six stags were randomly selected to be sent to slaughter. These were the Indicative Control Group (CON_8 , no treatment). Abomasa from the CON_8 were collected at the slaughter plant and sent to the laboratory to determine if we had adequate levels of parasitism to start the trial.

Later in October the mob was randomly split into five groups of 10 weaners

1. Control (CON_{10}, no anthelmintic)
2. Oxfendazole, oral oxfendazole (OXo, 4.53mg/kg, "Oxfen C", Merial, registered for use in deer), standard dose
3. Oxfendazole, oral oxfendazole (OX3, 13.6mg/kg, "Oxfen C", Merial, not registered for use in deer at this dose rate), treble the standard dose
4. Levamisole, oral levamisole (LEVo, 18.75mg/kg, "Aviverm", Jaychem, not registered for use in deer),2.5 times the standard dose
5. Moxidectin, injectable moxidectin (MOXi, 0.2mg/kg, "Cydectin", Zoetis, not registered for use in deer)

Dose rates were based on individual weights taken immediately prior to administration. Administration was by calibrated syringe (to the nearest 0.1ml) and separate syringes used for each anthelmintic. Where products used were not registered for deer or at dose rates not registered for deer, the deer were slaughtered on farm. All other deer were slaughtered at Silver Fern Farms, Kennington Deer Slaughter Premises (DSP).

Measurements

At Day -8 the CON_8 group was sent to the DSP. At the DSP abomasa were collected for abomasal washing and abomasal incubation. Adult worm counts (with a 2 % aliquot) were made and notified as soon as possible to allow treatment to proceed. Abomasal incubation counts and speciation were subsequently performed.

At Day 0 the 50 stags were weighed, tagged and randomly allocated into five groups of 10 animals. The four treatment groups, OXo,OX3, LEVo and MOXi had anthelmintic administered. Two animals in the LEVo group were treated first and observed for 30minutes for symptoms of levamisole toxicity before the remaining 8 deer in that group were treated. Periodic observation continued for 12hours with the LEVo group.

At Day 10 CON_{10}, OXo,OX3 and LEVo groups were slaughtered. The OXo group was the only group treated with an anthelmintic licensed for use in deer. The meat withholding time for OXo of 10days

allowed this group to be sent to the DSP along with the CON_{10} group. The LEVo group was treated with an anthelmintic not registered for deer and the OX3 group with a dose rates above label and so in both cases the default withholding time of 91days applied. The 20 deer in the OX3, LEVo groups were all necropsied on-farm.
At Day 12 the MOXi group was slaughtered on farm.
The split in slaughter dates was for logistical reasons. Timing of collection and processing capacity dictated this.
Abomasa were collected from all groups for a 2% minimum aliquot count of abomasal washings and a 10% minimum aliquot count following abomasal incubation. Speciation was done on all treatment groups. Parasitology work-up followed the World Association for the Advancement of Veterinary Parasitology (WAAVP) procedures for evaluating the efficacy of anthelmintics in ruminants (Wood et al., 1995). Speciation of *Ostertagia*-type nematodes followed Lichtenfels and Hoberg (1993) and Dróżdż (1995).

Results

At Day 0 the 50 trial deer averaged 110.5kg (range 91-126kg).
At day of slaughter (day 10 and day 12) the 50 trial deer averaged 112.8kg (range 92-131kg). There was no significant change in liveweight for any group.
There were no *Haemonchus* or *Trichostrongylus* nematodes encountered in any of the abomasal washings or abomasal incubations. Total numbers of *Ostertagia*-type adults and *Ostertagia*-type larvae LL4 (late L4) are a combination of those found in both abomasal washings and abomasal incubation washings. *Ostertagia*-type larvae EL4 (early L4) were only found in the abomasal incubations.

Table 1: CON_8 (Indicative Control Group) total worm counts for adult and immature *Ostertagia*-types.

TAG	Adults	LL4	EL4
Yellow 1	5140	50	3877
Yellow 2	11770	65	3338
Yellow 3	5005	5	2302
Yellow 4	5540	0	4163
Yellow 5	7660	60	1577
Yellow 6	2675	80	3554
Mean	**6298**	**43**	**3135**

The trigger level to continue the trial was regarded as a mean >1000 adults and nematodes present in all animals. In the CON_8 adult *Ostertagia*-types were present in the abomasal washings of all six deer – ranging from 2100 to 10650 with a mean of 5242. Due to the time involved with abomasal incubation a decision to proceed or delay the trial was made on receipt of adult *Ostertagia*-type count in the abomasal washings alone.
Levamisole was administered to two of the 10 LEVo group and they were observed for symptoms of levamisole toxicity. Signs of Levamisole toxicity in the host animal are largely an extension of its antiparasitic effect, ie, cholinergic-type signs of salivation, muscle tremors, ataxia, urination, defecation, and collapse. No symptoms were observed and the rest of the LEVo group deer were treated along with the remaining treatment groups. Subsequent periodic observations of the LEVo group in the 12 hours post treatment revealed no symptoms or adverse behaviour.

Table 2: Control (CON_{10}) and treatment group (OXo, OX3, LEVo and MOXi) total worm counts for adult and immature *Ostertagia*-types.

GROUP	Adults	LL4	EL4
CON_{10} 1	1940	10	1397
CON_{10} 2	9240	20	1867
CON_{10} 3	2490	10	636
CON_{10} 4	2680	70	1051
CON_{10} 5	1960	50	1385
CON_{10} 6	3880	10	1860
CON_{10} 7	2360	0	1297
CON_{10} 8	1620	10	350
CON_{10} 9	1040	125	1855
CON_{10} 10	7490	100	1169
Mean	**3470**	**41**	**1287**
OXo 1	1180	110	156
OXo 2	770	20	89
OXo 3	1390	140	0
OXo 4	1470	160	65
OXo 5	900	100	0
OXo 6	3560	120	0
OXo 7	1600	260	0
OXo 8	1160	140	104
OXo 9	2260	150	0
OXo 10	1610	250	98
Mean	**1590**	**145**	**51**
OX3 1	640	80	0
OX3 2	1100	260	0
OX3 3	1090	120	0
OX3 4	460	220	0
OX3 5	260	20	91
OX3 6	120	20	0
OX3 7	190	120	0
OX3 8	80	80	0
OX3 9	420	180	0
OX3 10	280	260	0
Mean	**464**	**136**	**9**
LEVo 1	1980	100	239
LEVo 2	2550	10	125
LEVo 3	1850	10	1780
LEVo 4	1070	210	192
LEVo 5	770	70	0
LEVo 6	2970	200	754
LEVo 7	2580	50	362
LEVo 8	2590	50	1997
LEVo 9	3530	110	221
LEVo 10	1000	0	226

Mean	**2089**	**81**	**590**
MOXi 1	1150	120	149
MOXi 2	1020	80	210
MOXi 3	2453	100	0
MOXi 4	2977	133	0
MOXi 5	1503	67	299
MOXi 6	1087	33	0
MOXi 7	1457	100	71
MOXi 8	2033	210	0
MOXi 9	2073	53	280
MOXi 10	1567	0	83
Mean	**1732**	**90**	**109**

Table 3: Group mean total worm counts for adult and immature *Ostertagia*-types. Anthelmintic treatment group efficacy against *Ostertagia*-types.

	Adults	LL4	EL4
CON_{10}	3470	41	1287
OXo % efficacy	1590 **54%**	145 N/A	51 **96%**
OX3 % efficacy	464 **87%**	136 N/A	9 **99%**
LEVo % efficacy	2089 **40%**	81 N/A	590 **54%**
MOXi % efficacy	1732 **50%**	90 N/A	109 **92%**

The CON_{10} group and treated groups OXo, OX3 and LEVo were slaughtered on the 4th November and the MOXi treated group on the 6th November. The anthelmintics had the following efficacy against the adult *Ostertagia*-type nematodes: OX3 87 %, Oxo 54 %, MOXi 50 % and LEVo 40 % (Table 3). Anthelmintic efficacy against the EL4 *Ostertagia*-type nematodes was OX3 99 %, Oxo 96 %, MOXi 92 % and LEVo 54 % (Table 3).

The numbers of *Ostertagia*-type LL4 present in the Control group was very low and as a result no valid efficacies for the various anthelmintics against LL4 can be calculated.

Statistical analysis of the trial data for *Ostertagia*-type adults (Table 4) based on arithmetic means showed all treatments had a significant treatment effect compared to the CON_{10} group. OX3 showed a significant difference to LEVo and MOXi . Based on the geometric mean OXo and OX3 had a significant treatment effect compared to the CON_{10} group and OX3 showed a significant difference to all other treatments.

Table 4: Statistical analysis of total *Ostertagia*-type adult worm counts

Treatment group	No. positive	Range	Arithmetic mean (efficacy)	Geometric mean (efficacy)
CON	10/10	1040-9240	3470.0^{a} (N/A)	2775.3^{a} (N/A)
OXo	10/10	770-3560	1590.0^{bc} (54.2%)	1446.8^{b} (47.9%)

OX3	10/10	80-1100	464.0^{c} (86.6%)	338.8^{c} (87.8%)
LEVo	10/10	770-3530	2089.0^{b} (39.8%)	1875.9^{ab} (32.4%)
MOXi	10/10	1020-2977	1732.0^{b} (50.1%)	1632.8^{ab} (41.2%)

$^{a\,b\,c}$ Means in the same column not sharing a common superscript are significantly different at the 5% level.

Statistical analysis of the trial data for *Ostertagia*-type EL4 (Table 5) based on arithmetic means showed all treatments were significantly different compared to the CON_{10} group (worm counts were significantly lower) and that OXo,OX3 and MOXi showed a significant difference to LEVo . Based on the geometric mean, OXo, OX3 and MOXi had a significant treatment effect compared to the CON_{10} group and LEVo. LEVo and MOXi were significantly different to each other and there was also a significant difference between OXo and LEVo.

Table 5: Statistical analysis of total *Ostertagia*-type EL4 worm counts

Treatment group	No. positive	Range	Arithmetic mean (efficacy)	Geometric mean (efficacy)
CON_{10}	10/10 (100%)	350-1867	1286.7^{a} (N/A)	1160.4^{a} (N/A)
OXo	5/10 (50%)	0-156	51.2^{c} (96.0%)	9.0^{bc} (99.2%)
OX3	1/10 (10%)	0-91	9.1^{c} (99.3%)	0.6^{c} (99.9%)
LEVo	9/10 (90%)	0-1997	589.6^{b} (54.2%)	220.7^{a} (81.0%)
MOXi	6/10 (60%)	0-299	109.2^{c} (91.5%)	19.9^{b} (98.3%)

$^{a\,b\,c}$ Means in the same column not sharing a common superscript are significantly different at the 5% level.

Identification and mean number of each species of abomasal nematode identified are presented in Table 6. *Ostertagia leptospicularis* (O. *leptospicularis*) comprised 33 % of the total in the control group. *Spiculopteragia asymmetrica* (S. *asymmetrica*) at 50 % were the predominant Ostertagia-type species present. Present but in lower numbers were *Spiculopteragia spiculoptera* (S.*spiculoptera) at* 17 %.

Table 6: Mean worm count and anthelmintic efficiency by *Ostertagia*-type species

	O. *leptospicularis*	S. *spiculoptera*	S. *asymmetrica*
Control	1145	590	1735
OXo	1113	95	382
% efficacy	3 %	84 %	78 %
OX3	357	0	91
% efficacy	69 %	100 %	95 %
LEVo	689	167	1233
% efficacy	40 %	72 %	29 %
MOXi	381	26	1325
% efficacy	67 %	96 %	24 %

The efficacy against *S. spiculoptera* by OX3 was 100% and that of MOXi was satisfactory at 96%.The efficacy of OX3 against *S. asymmetrica* was also satisfactory at 95%. None of the treatments had desirable efficacies for *O. leptospicularis* .

Discussion

Due to the anticipated efficacies of the treatment groups and the desire to achieve statistically significant difference in treatment between groups, the number of deer per treatment group was increased from the recommended 6 (Wood et al., 1995) to 10. Within constraints of funding and available deer this reduced treatment options.

Levamisole toxicity has been well documented in other livestock. In cattle dose rates of between 24 and 40 mg/kg produce symptoms of toxicity and in goats symptoms occurred at 35 mg/kg (Babish et al., 1990) A dose rate of 18.75 mg/kg used on the deer in this trial produced no symptoms of toxicity. There is anecdotal evidence that no toxic symptoms have been seen in deer effectively given a triple dose of levamisole (22.5 mg/kg). This has occurred in large numbers of weaner deer over multiple farms (Lawrence, *pers comm)*. All deer in this trial were considered clinically healthy animals and caution should be used when administering levamisole at dose rates >7.5 mg/kg to deer in poor condition.

Differences in total worm counts for adult and immature *Ostertagia*-types between CON_8 and CON_{10} provide an interesting insight into the dynamics of this parasite. There was 18 days separating the CON_8 and the CON_{10} . The *Ostertagia*-type larvae are ingested as an L3 and in our trial the EL4 are the earliest larval stage recorded. The drop in EL4 from 3135 to 1287 indicates that the majority of the 3135 EL4 have developed into LL4 and adult *Ostertagia*-types. (The time from L3 ingestion to adult *Ostertagia*-type is normally 10days (Pomroy, *pers comm.*)). This drop also suggests that the incoming nematode challenge on the pasture has dropped over those 18 days. The corresponding drop in *Ostertagia*-type adults from 6298 to 3470 would suggest a rapid turnover of adults. The life expectancy of adult *Ostertagia*-types in the abomasum of deer is not known. In sheep and cattle it is species and/or density dependent. When there is a high challenge on pasture then nematodes in the abomasum live for a shorter time than when the challenge of incoming nematodes is lower. At times of a high turnover of nematodes in the abomasum they may only live for around a month (Mason, *pers comm.*). The dynamics seen in this trial would suggest the adult *Ostertagia*-types may live for less than a month.

One of the objectives of this trial was to look for changes in the moxidectin resistant status of the farm. In 2010 a slaughter trial on the same farm found the efficacy of moxidectin injection to be 83.5 % against adult *Ostertagia*-types (Lawrence 2011). In this trial the efficacy of moxidectin injection against adult *Ostertagia*-types was 67 %. Since the 2010 trial there has been a change in anthelmintic use on the farm. All subsequent anthelmintics administered have been a triple combination of moxidectin injection (0.2 mg/kg "Cydectin ® Injection for Cattle and Sheep" Zoetis) and oxfendazole/levamisole oral (4.53 mg/kg oxfendazole and 8 mg/kg levamisole HCL "Scanda ®" Coopers). There is some evidence from the sheep industry that the resistance status of a farm can be modified or even improved by the judicious use of appropriate anthelmintics. At face value this drop in efficacy of moxidectin would suggest a deterioration in the resistance status of the farm. Unfortunately differences in trial design between the 2010 and 2013 trials do not allow a valid comparison of these figures. In 2010 the control deer were slaughtered 15 days prior the moxidectin injection treated group whereas in 2013 the control deer were slaughtered 2 days prior to the moxidectin injection treated. However the efficacy of moxidectin

injection when analysis is made of the different species of *Ostertagia*-types does allow some valid comparison.

Table 7: Change in population of *Ostertagia*-type species by mean worm count

	O. *leptospicularis*	S. *spiculoptera*	S. *asymmetrica*
2010 Control mean	8522	9429	181
Percentage	47%	52%	1%
2013 Control mean	1145	590	1735
Percentage	33%	17%	50%

Table 8: Change in Moxidectin efficacy by *Ostertagia*-type species

	O. *leptospicularis*	S. *spiculoptera*	S. *asymmetrica*
MOXi 2010			
% efficacy	91%	77%	100%
MOXi 2013			
% efficacy	67%	96%	24%

Table 8 shows a drop in efficacy of Moxidectin injection against both *O. leptospicularis* and *S.asymmetrica*. This is very significant for this farm as these two species account for 83% of the *Ostertagia*-type population.

There has been a large change in the make-up of the *Ostertagia*-type population over three years. In 2010 S. *spiculoptera* made up 52 % of the *Ostertagia*-type population and by 2013 was reduced to 17 %. The individual efficacies of the three anthelmintics used in the intervening three years are higher against S. *spiculoptera than* the other two *Ostertagia*-type species. Of particular interest is the change seen with *S.asymmetrica* . It was only 1% of the *Ostertagia*-type population in 2010 and by 2013 was 50%. This is perhaps not surprising if we consider the individual efficacies against *S.asymmetrica* of the three anthelmintics used OXo 78 %, LEVo 29 % and MOXi 24 %(Table 6). By contrast the make-up of *O.leptospicularis* in the population has dropped from 47 % to 33 % and this is where efficacies against *S. asymmetrica* of the three anthelmintics used were OXo 3 %, LEVo 40 % and MOXi 67 %. This seeming anomaly may well support the fact that when using a combination drench the result is not merely the additive efficacies of the three components.

Determination of the appropriate dose rate of Oxfendazole for deer against *Ostertagia*-type nematodes was another objective of this trial. It has been shown that deer metabolise and excrete oxfendazole much more rapidly than sheep (Watson and Manley, 1985) and so it is not surprising that oxfendazole at the sheep dose rate of 4.53 mg/kg produces unsatisfactory results in deer. There are several oxfendazole based anthelmintics registered for use in deer. They all use a label dose rate of 4.5 mg/kg but there are no published trials to support the efficacy of this dose rate against GI nematodes in deer (Charleston 2001). In recent slaughter trials oxfendazole (4.5 mg/kg) efficacy against Ostertagia-type adults was 72 % (Lawrence et al., 2013) and 69 % (Leathwick, *pers comm*). In this trial the efficacy of oxfendazole at 4.5 mg/kg was 54 % against *Ostertagia*-type adults. Triple the standard dose of oxfendazole (13.6 mg/kg) had an efficacy of 87 % against *Ostertagia*-type adults. The biometric evaluation indicates a significant difference between oxfendazole at 4.53 mg/kg and 13.6 mg/kg. Comparing the Oxfendazole standard dose and oxfendazole triple dose, the means and efficacy percentages are similar for arithmetic and geometric means. When it comes to the P-values, there is clear significance using geometric means ($p<0.0001$) but a near miss using arithmetic means ($p=0.0504$). This does not mean that the two sets of results are incompatible, just a reflection that the analysis on

the log scale reduces a lot of the "noise". Any outlying figures within a data set are accounted for using the geometric means.
The significantly better efficacy of oxfendazole at 13.6 mg/kg at 87 % still falls short of an ideal efficacy of >95% and so we cannot claim to have determined the correct dose of oxfendazole for deer. We can however say with confidence that oxfendazole at 4.53 mg/kg is under- dosing and as such the continued use of that dose rate will significantly shorten the effective useful life of oxfendazole in deer. If used as a single active anthelmintic treatment for deer then it is likely to be very short - a matter of years. To optimise the life of the only anthelmintic known to be effective in deer when resistance is present – a triple combination – then the oxfendazole component must be at a dose rate of 13.6 mg/kg or higher.

The third objective of this trial was to determine the appropriate dose rate of Levamisole for use in deer against *Ostertagia* -type nematodes. Previous studies regarding levamisole as an anthelmintic for use in deer focused on its efficacy against lungworm (Mason 1982, Mackintosh et al., 1984). These studies showed that levamisole was metabolized more rapidly in deer than in cattle. They consistently showed that levamisole had poor efficacy against lungworm and for three decades levamisole has not been used as an anthelmintic in deer. Recently levamisole at the standard sheep dose rate of 7.5 mg/kg produced an efficacy against adult *Ostertagia*-types of 71.7 % (Lawrence et al., 2013) and 39 % (Leathwick, *pers comm*). In this trial levamisole at 18.75 mg/kg had an efficacy of 40 % against adult *Ostertagia*-types. Funding and animal constraints did not allow us to have a standard 7.5 mg/kg levamisole treated group in this trial and so we cannot say that a 2.5 times dose of levamisole is no more effective than a standard dose. However the pharmacokinetics of levamisole and concerns with toxicity would make it unwise to think that a greater than 2.5 times levamisole dose would be either safe or achieve anywhere near the desired >95% efficacy.

The efficacy results against the *Ostertagia*-type larva are interesting and overall present a different picture to previous slaughter trial studies in deer (Lawrence, 2011, Lawrence et al., 2012). In these previous studies there was a general trend that efficacy against *Ostertagia*-type larva was lower than efficacies against adult *Ostertagia*-types. These results against *Ostertagia*-type larva were all higher for each anthelmintic treatment. The scale of descending efficacy remained the same for the all anthelmintics against *Ostertagia*-type adults and *Ostertagia*-type larva.

There were three *Ostertagia*-type nematode species identified in the deer on this farm. The two *Spiculopteragia* species of *Ostertagia*-type nematodes (*S. spiculoptera* and *S. asymmetrica*) present are host specific to deer. *Ostertagia leptospicularis* is a deer species but it has been reported in both sheep and cattle in New Zealand (McKenna, 1997). It is worthy of note that despite the sheep being integrated with the deer on this farm, the sheep *Ostertagia*-type nematode (*Teladorsagia)* was not present in the deer and in fact has never been identified in farmed deer in New Zealand to date.
Previous reports on New Zealand deer farms indicated *Ostertagia*-type species exhibiting resistance to Macrocyclic Lactone anthelmintics. *O. leptospicularis* was resistant to Moxidectin Pour On (Lawrence et al., 2012), *O. leptospicularis* and *S. spiculoptera* to Moxidectin Pour On and MOXi (Lawrence, 2011), and *O. leptospicularis* resistant to Moxidectin Pour On and *O.leptospicularis, S.spiculoptera* and *S.asymmetrica* to Ivermectin oral (Hoskin et al., 2005). Technically resistance to an anthelmintic can only be claimed if the dose rate to provide efficacy has been determined. Hence in this trial it can only be suggested that *O. leptospicularis* and *S. asymmetrica* exhibit resistance to Moxidectin injection.

In the sheep industry the use of combination anthelmintics has been an accepted method of delaying the onset of anthelmintic resistance development (Leathwick et al., 2011). In the deer industry the only anthelmintic that has been shown to be effective in the face of resistance to *Ostertagia*-type nematodes

was a triple combination (moxidectin injection (0.2 mg/kg "Cydectin ® Injection for Cattle and Sheep" Zoetis) and oxfendazole/levamisole oral (4.53 mg/kg oxfendazole and 8 mg/kg levamisole HCL "Scanda ®" Coopers)(Lawrence, 2011)). This trial indicates that we need to modify the make-up of this combination to optimise its useful life. The oxfendazole should be incorporated at 13.6 mg/kg or higher.

No one treatment option used in this trial was effective in controlling all three *Ostertagia*-type species present on this farm (Table 6). Further, no one anthelmintic compensated for the deficiencies of another anthelmintic. This places the farm in the precarious situation of being totally reliant on the fact that a combination anthelmintic contains an "X factor" over and above the additive effects of its individual components. There is some suggestion from the sheep industry that this exists where with benzimidazole /levamisole combinations, it was found that compared to the effects of either drug alone, significantly greater efficacy was obtained using combinations (Anderson et al., 1991a, Anderson et al., 1991b Overand et al., 1994 and Mc Kenna et al., 1996). For all its shortcomings, maybe the historical synergistic role that levamisole has laid claim to in past chemical combinations might be valid for triple anthelmintic combinations in deer.

Observations and Recommendations

- This farm has *Ostertagia*-type resistance to moxidectin in the injectable formulation (0.2mg/kg "Cydectin ® Injection for Cattle and Sheep" Zoetis)
- The level of resistance is greater than 3 years ago despite the use of a triple combination shown to be effective 3 years ago.
- Oxfendazole in anthelmintics combinations for deer should be at least 13.6 mg/kg
- Levamisole does have an effect against gastrointestinal parasites in deer and while a 2.5 times standard dose rate had an efficacy of 40 % it did not produce any safety concerns.

The use of combination anthelmintics is one of three strategies being advocated to manage anthelmintic resistance in New Zealand (Leathwick et al., 2009). The other two are vitally important for a sustainable deer industry. High-risk drenching and stock-management practices must be minimised and farms must maintain a refugia for anthelmintic susceptible worm genotypes.

Acknowledgements

This trial was funded by DEEResearch with assistance and advice from Bill Pomroy (Massey), Colin Mackintosh and Dave Leathwick (AgResearch), Victoria Chapman and Andrew Hodge (Zoetis). Anthelmintics were provided by Merial (OxfenC), Jaychem (Aviverm) and Zoetis (Cydectin Injection).All parasitology was carried out by Paul Mason. Thanks to Silver Fern Farms Kennington and most importantly to farmers John and Bruce Hamilton for their co-operation and provision of deer for the trial.

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