Efficacy of Levamisole, Moxidectin oral, Moxidectin injectable and Monepantel against *Ostertagia*-type nematodes in deer

DW Lawrence[a], JT MacGibbon[b], PC Mason[c]

[a] *Tikana, 374 Livingstone Road, Browns, R.D.1 Winton, 9781, New Zealand*
[b] *Northern Southland Veterinary Services P.O.Box 22,Te Anau, 9672, New Zealand*
[c] *Mason Consulting, 317 Dunns Crossing Road, RD 8, Christchurch 7678, New Zealand*

Abstract

This investigation used a slaughter trial to establish the status of drench resistance on a Southland farm. The efficacies of oral and injectable formulations of moxidectin were compared. Efficacies of levamisole, oxfendazole and monepantel were determined. Evidence of resistance by gastrointestinal parasites (*Ostertagia*-types) to moxidectin oral was found. Titration studies are required to determine the correct dose rate in deer of levamisole for gastrointestinal nematodes. The label dose rate for the registered oxfendazole anthelmintics warrants review. Monepantel at double the sheep dose rate is not effective on gastrointestinal nematodes of deer. To avoid further development of anthelmintic resistance on deer farms a triple combination anthelmintic should be used. The Macrocyclic Lactone component should be injectable.

Keywords
Deer, anthelmintic resistance, anthelmintic efficacy, gastrointestinal parasites, *Ostertagia*, levamisole, moxidectin injection, moxidectin oral, monepantel.

Introduction

Macrocylic Lactone (ML) anthelmintic resistance on New Zealand deer farms to gastrointestinal nematodes continues to be of concern. All known investigations to date to determine deer farm status have confirmed some degree of resistance (Hoskin et al 2005, Lawrence 2011, Lawrence et al 2012, Hodgson pers comm, Mackintosh et al 2013, Leathwick pers comm). 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 the moxidectin formulation with the poorest efficacy/most resistance. Recent efficacy of Moxidectin Pour-On against *Ostertagia*-type adults has ranged from 94% to 19%, while efficacy against *Ostertagia*-type larvae has ranged from 71% to 0%.

Moxidectin injection and Moxidectin oral have consistently provided higher efficacies against both mature and larval *Ostertagia*-type nematodes than Moxidectin Pour On. Pharmacokinetic studies of plasma levels of moxidectin produce different results depending on route of administration help to explain this (Lawrence et al 2012, Mackintosh et al 2013). There is a suggestion however that the efficacy of the injectable formulation of moxidectin may be superior to the oral formulation.

Previous studies had shown the combination of moxidectin injection and oral oxfendazole plus levamisole to be an effective combination for treatment of *Ostertagia*-type resistant nematodes. The role of levamisole is somewhat controversial, as based on studies in deer three decades ago, the use of levamisole has been advised against strongly. There has been little or no levamisole used on New Zealand deer farms over that time.

This paper presents a study which is an extension of earlier work. The efficacy of levamisole against gastrointestinal nematodes of farmed deer and determining if injectable moxidectin was more effective than the oral formulation were the primary objectives of the study.

Background

Discussions were held in autumn 2012 with a farmer in the Te Anau basin who had a desire to determine the resistance status on the farm as the history suggested an issue may be present. The 270 hectare farm is an integrated farming operation running cattle and deer. The deer are a breeding/finishing operation with around 800 hybrid weaners finished each year (a mix of Wapiti, Eastern and Red deer genetics).

The property has been run as a deer farm for 10 years. For the first six years moxidectin pour on was the principal anthelmintic used. At six years there was perceived poor response to the pour on and moxidectin injection was used. In the last two years moxidectin injection has been used in combination with oral oxfendazole and levamisole. This study was undertaken on rising 1 year old finishing deer with a naturally-acquired infection of gastrointestinal (GI) nematodes. Under these commercial field conditions the study had four facets.

1. Determine the farm status for GI nematode resistance to Moxidectin.
2. To determine the efficacy of levamisole against GI nematodes
3. To compare the efficacy of oral moxidectin(MOXo)and injectable moxidectin (MOXi)
4. Evaluate monepantel (ZOLo) as a possible anthelmintic option for deer

Material and methods

Animals

In 2012 all weaners were wintered on brassica crop with their last anthelmintic treatment given immediately prior to the move onto brassicas. A combination of moxidectin injection (0.2mg/kg "Cydectin ® Injection for Cattle and Sheep" Zoetis) and oxfendazole/levamisole oral (4.53mg/kg oxfendazole & 8mg/kg levamisole HCL "Scanda ®" Coopers). The mob was weighed in September and 70 mixed sex deer selected with a range in bodyweight from 80 – 95kg. These 70 were tagged and run separately from the main mob.

Treatments

In mid-October 42 deer out of the 70 were randomly allocated (based on weight) into the 7 trial groups of (n=6). The trial groups contained 36 male and 6 female deer. The trial comprised a control (CON, no anthelmintic) and six treatment groups; injectable moxidectin (MOXi, 0.2mg/kg, "Cydectin", Zoetis, not registered for use in deer) oral moxidectin (MOXo, 0.2mg/kg, "Cydectin", Zoetis, not registered for use in deer), oral oxfendazole (OXo, 4.53mg/kg, "Bomatak ® C", Bayer, registered for use in deer), oral levamisole (LEVo, 7.5mg/kg, "Levicare", Merial, not registered for use in deer),triple combination of injectable moxidectin (MOXi, 0.2mg/kg, "Cydectin", Zoetis, and oral oxfendazole/levamisole (OXLEVo, 4.53mg/kg oxfendazole & 8mg/kg levamisole HCL, "Scanda", Coopers, not registered for use in deer)and oral monepantel (ZOLo, 5mg/kg, "Zolvix ® ", Novartis, not registered for use in deer).

Dose rates were based on individual weights taken immediately prior to administration. Where products used were not registered for deer the manufacturers recommended dose for cattle or sheep were applied. The exception to this was monepantel (ZOLo) where the dose given was double the sheep dose rate. Administration was by calibrated syringe (to the nearest 0.1ml) and separate syringes used for each anthelmintic.

Measurements

At Day-3 the Control group had faecal samples collected for faecal larval culture(FLC) prior to dispatch to the Deer Slaughter Premises (DSP). At the DSP abomasa were collected for abomasal washing and abomasal incubation. Total worm counts (with a minimum 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 deer in the treatment groups were weighed and the MOXi, MOXo, OXo, LEVo, MOXi + OXLEVo and ZOLo groups were treated.

At Day 12 all 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. All other groups were treated with anthelmintics not registered for deer and the default withholding time of 91days (the exception being 49 days for MOXi (Lawrence 2011)) meant they were all necropsied on-farm. Abomasa were collected from all groups for a 2% aliquot count of abomasal washings and a 10% aliquot count following abomasal incubation. Speciation was done on all treatment groups. Parasitology work-up followed the WAAVP procedures for evaluating the efficacy of anthelmintics in ruminants (Wood et al 1995). Speciation of *Ostertagia*-type nematodes followed Lichtenfels & Hoberg (1993) and Dróżdż (1995).

Results

The 42 trial deer averaged 92kg at time of slaughter (range 77-122kg).

The predominant worm type present in all 6 CON deer was *Ostertagia*-type and interestingly there were no *Cooperia* present. The large intestinal parasite *Oesophagostomum* was present in 3 of the six deer.

Table1; Faecal Larval Culture(FLC)

Worm Type	Control Group average
Haemonchus	-
Ostertagia-type	69%
Trichostrongylus	11%
Cooperia	-
Oesophagostomum/Chabertia	20%
Nematodirus	-

The CON group slaughtered on the 16th October had an average of 1495 *Ostertagia*-type adults (range 250 – 2650). Based on this result, the trial proceeded. All treated deer were slaughtered on the 31st

October. The anthelmintics had the following efficacy against the adult *Ostertagia*-type nematodes: MOXi 100%, MOXo 97.9%, OXo 71.8%, LEVo 71.7%, MOXi + OXLEVo 100% and ZOLo 86.6% (Table 2).

Table 2: Group mean total worm counts for adult and immature *Ostertagia*-types. Anthelmintic treatment group efficacy against *Ostertagia*-type adults.

	Oster-type adults	Oster-type larva
Control	1495	17
MOXi % efficacy	0 100%	67
MOXo % efficacy	32 97.9%	173
OXo	422 71.8%	173
LEVo	423 71.7%	118
MOXi + OXLEVo	0 100%	14.5
ZOLo	200 86.6%	14

The numbers of *Ostertagia*-type larvae present in the Control group was very low and as a result no conclusions can be drawn as to efficacy of the various anthelmintics against larvae from this trial. *Trichostrongylus* nematodes were present in the CON but in low numbers (average 23). All treatment groups were clear except the LEVo group (average 2).

Statistical analysis of the trial data based on arithmetic means showed all treatments had a significant treatment effect compared to the CON group but no difference between anthelmintics. The geometric mean did reveal some differences between treatments. Of particular interest is a significant difference between MOXi and MOXo.

Table 3: Statistical analysis of trial data

Treatment group	No. positive	Range	Arithmetic mean (efficacy)	Geometric mean (efficacy)
1: Control	6/6	260-2650	1495.0^{b} (N/A)	1081.6^{d} (N/A)
2: MOXi	0/6	0-0	0.0^{a} (100%)	0.0^{a} (100%)
3: MOXo	3/6	0-120	31.7^{a} (97.9%)	5.6^{b} (99.5%)
4: OXo	6/6	120-570	421.7^{a} (71.8%)	377.3^{cd} (65.1%)
5: LEVo	6/6	130-610	423.3^{a} (71.7%)	379.0^{cd} (65.0%)
6: MOXi + OXLEVo	0/6	0-0	0.0^{a} (100%)	0.0^{a} (100%)
7: ZOLo	6/6	80-360	200.0^{a} (86.6%)	166.5^{c} (84.6%)

$^{a\,b\,c\,d}$ 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 4. *Ostertagia leptospicularis* (O.l) 67% and *Spiculopteragia asymmetrica* (S.a.) 26% were the

predominant Ostertagia-type species present. Present but in lower numbers were *Spiculopteragia spiculoptera* (S.s) 5% and *Ostertagia ostertagi* (O.o) 2%

Table 4: Mean worm count and anthelmintic efficiency by *Ostertagia*-type species

	O.o	O.l	S.a	S.s
Control	30	1002	389	75
MOXi	0	0	0	0
% efficacy	100%	100%	100%	100%
MOXo	0	0	32	0
% efficacy	100%	100%	91.8%	100%
OXo	0	333	42	42
% efficacy	100%	66.8%	44%	44%
LEVo	0	186	152	85
% efficacy	100%	81.4%	60.9%	0%

The efficacy of MOXi was 100% against each worm species identified. The efficacy of MOXo against each worm species identified was 100% except for *Spiculopteragia asymmetrica* (S.a.) where it was 91.8%.

Discussion

The FLC from the CON deer gives us an indication only of GI nematodes present. The correlation between FEC and total worm burden in rising yearling deer is at best reasonable in autumn and poor in spring (Mackintosh et al 2011) Hence care must be taken in assuming the nematode species identified in the FLC and their proportions actually reflect the GI nematode burden. The full GI nematode recovery at slaughter (Mackintosh et al 2013)gives a true breakdown *Ostertagia*- type 92%, *Oesophagostomum* 5%, *Trichostrongylus* 3%, *Haemonchus, Nematodirus* or *Cooperia* nil or negligible on that farm. The absence of *Cooperia* on both farms is reassuring in light of the cattle industry *Cooperia* resistance issue with ML anthelmintics.

Moxidectin Pour On has been the formulation of moxidectin which has consistently shown resistance by *Ostertagia*-type nematodes on all deer farms evaluated to date (n=6). With this knowledge and the desire to evaluate additional treatment groups, budget restraints meant that Moxidectin Pour On was not included as a treatment option in this trial.

One of the main objectives of this trial was to determine if there was a significant difference in efficacy between MOXo and MOXi. Previous trials in deer were suggestive of this (Lawrence et al 2012). In contrast it has been shown in sheep (Gopal et al 2001) that oral moxidectin was more effective than injectable moxidectin against ivomec resistant GI nematodes (*Trichostrongylus colubriformis).* A recent cattle study in New Zealand also found oral moxidectin(91.1%) to be more effective than injectable moxidectin (55.5%)against GI nematodes(Leathwick 2013) This cattle study was based on Faecal Egg Count Reduction Test (FECRT) and not slaughter trials. The expectation with this deer trial was that there would be significant *Ostertagia*-type nematode resistance present as this was needed to show a clear difference between oral and injectable efficacies. Despite there not being significant resistance efficacy of MOXi and MOXo. The Invermay deer farm (Mackintosh et al 2013) transpired to have

extensive ML resistance and confirmed beyond doubt the superior place of MOXi for deer.In that study the efficacy of MOXi against *Ostertagia*-type adults was 79% but only 31% efficacy achieved with MOXo.

The efficacy achieved with OXo (71.8%) against adult *Ostertagia*-types using the deer/cattle dose rate 4.53mg/kg is interesting. OXo is available as an anthelmintic registered for deer and while that means there is a label dose rate for use in deer , there is no supporting published efficacy data. As minimal benzimidazole anthelmintic had been used on this farm it raises the question of whether the correct dose of oxfendazole in deer was used. In a review of deer anthelmintics (Charleston 2001) it was stated that "the published information on efficacy of anthelmintics currently used ranges from barely adequate to non-existent". Nothing has changed since then and there is no published data on efficacy of oxfendazole against GI nematodes. Some work was done with the less potent benzimidazole, albendazole. In a slaughter trial the efficacy of albendazole against adult *Ostertagia*-types ranged from 88%-99% (Waldrup et al 1997). Our result is supported by another recent slaughter trial where also using the 4.53mg/kg dose rate, an efficacy of 69% for oxfendazole was shown. (Leathwick pers comm)

Levamisole has for 30 years not been used in the deer industry. In the early 1980's lungworm was considered the most significant parasite of farmed deer. All studies regarding levamisole as an anthelmintic for use in deer focused on its efficacy against lungworm (Mason 1982, Mackintosh et al 1984).These studies consistently showed levamisole had poor efficacy against lungworm and were supported by experience of practice veterinarians around New Zealand. There are no records of efficacy trials for levamisole against GI nematodes. The assumption was it would have little value due to the indication that deer metabolise levamisole and other anthelmintics more quickly than sheep and cattle. The standard sheep dose rate we used of 7.5mg/kg of levamisole produced an efficacy against adult *Ostertagia*-types of 71.7%. This was much greater than expected and was shown to be not significantly different to oxfendazole.

Clearly both oxfendazole and levamisole have a role to play in combination anthelmintics for deer. The concern is that at these dose rates we are effectively underdosing. Toxicity at higher dose rates has not been evaluated in deer and caution should be exercised particularly with levamisole. Signs of intoxication occur in goats at 16mg/kg (Pomroy pers comm) and higher doses are fatal for goats. A dose rate of 12mg/kg was used in a trial to measure serum levamisole levels in deer (Mason 1982) with no reported side effects. Anecdotally a number of farmers have been using a double dose of "Scanda" which in effect is 16mg/kg of levamisole with no untoward effects reported. Further toxicity and efficacy studies are required.

The numbers of *Ostertagia*-type larvae present in the CON group in the current trial was very low. Consequently no conclusions can be drawn as to the efficacy of any of the anthelmintics from this trial against larvae. The only explanation is that the treated groups had significant *Ostertagia*-type larval challenge in the 15days between the slaughter dates of the CON and treatment groups. What is significant is there were larvae present in all treatment groups. Clearly none of the treatments achieved 100% efficacy against *Ostertagia*-type larvae. The trend seen in previous trials is that anthelmintic efficacies against *Ostertagia*-type larvae are normally lower than efficacies achieved against *Ostertagia*-type adults.
Reference to the potential use of the new anthelmintic, Monepantel (Pomeroy 2011) was the basis for using the double sheep dose in our trial. It is acknowledged there is a likely issue with efficacy against lungworm with Monepantel. Our slaughter trial focused only on GI nematodes where an unsatisfactory efficacy of 86.6% was achieved against adult *Ostertagia*-types with this double dose. A triple dose of Monepantel appeared to be required for control of GI nematodes in llamas (Dadak et al 2013). It is

possible that a greater than double dose would efficacious in deer but the expense of this anthelmintic makes it cost prohibitive.

There were four *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). *Ostertagia ostertagi* is an important cattle nematode previously only observed in white-tail deer in New Zealand (McKenna 1997) but made up 2% of the *Ostertagia*-type species present in these deer and 3% on another Te Anau deer farm (Lawrence et al 2012). It is worthy of note that the sheep *Ostertagia*-type nematode (*Teladorsagia)* was not present on this farm and in fact has never been identified in farmed deer in New Zealand to date.
On this farm Moxidectin Pour On was not evaluated and all of the *Ostertagia*-type species were well controlled by MOXi. *Spiculopteragia asymmetrica* was the species showing the least efficacy to MOXo on this farm. Technically resistance to an anthelmintic can only be claimed if the dose rate to provide efficacy has been determined. The dose rate in deer for MOXo has not been determined and therefore we cannot claim *Spiculopteragia asymmetrica* is resistant on this farm. Interpretation of the efficacies of OXo and LEVo is not appropriate in light of previous discussion regarding the need to determine/re-evaluate the correct dose of these anthelmintics in deer.
Previous reports on New Zealand deer farms indicated Ostertagia-type species exhibiting resistance to Macrocyclic Lactone anthelmintics. *Ostertagia leptospicularis* was resistant to Moxidectin Pour On (Lawrence et al 2012), *Ostertagia leptospicularis* and *Spiculopteragia spiculoptera* to Moxidectin Pour On and MOXi (Lawrence 2011), and *Ostertagia leptospicularis* resistant to Moxidectin Pour On and *Ostertagia leptospicularis, Spiculopteragia spiculoptera* and *Spiculopteragia asymmetrica* to Ivermectin oral (Hoskin et al 2005).

The management of drench resistance in the sheep industry is a useful tool for deer veterinarians to learn from. The use of combination anthelmintics has been an accepted method of delaying the onset of anthelmintic resistance development (Leathwick et al 2011). Previous studies in deer have shown that combination anthelmintic treatment has been effective in the face of resistance (Lawrence 2011).

Conclusion

- This farm has *Ostertagia*-type resistance/efficacy <95% to oral moxidectin
- Levamisole does have an effect against gastrointestinal parasites in deer
- Injectable Moxidectin is more effective than oral Moxidectin in deer
- To avoid drench resistance developing on deer farms a triple combination should be used and it should be Moxidectin Inj plus an oral combination of oxfendazole/albendazole and levamisole.
- Zolvix does not currently provide an alternative drench option for deer
- Further titration studies are required to determine the correct dose rate in deer for levamisole.
- The label dose rate for oxfendazole needs to be evaluated.

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