MOXIDECTIN PHARMACOKINETICS AND RESISTANCE IN DEER

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Abstract

Resistance to Macrocytic Lactone anthelmintics of farmed deer in New Zealand is a reality. This investigation used slaughter trials to establish the status of drench resistance on a Southland farm to available application methods for Moxidectin (injectable, oral and topical) as well as abamectin injection. Evidence of resistance by gastrointestinal parasites (Ostertagia-types) to Moxidectin Pour-On and Abamectin injection was found. The pharmacokinetic study showed moxidectin plasma levels to be highest with moxidectin injection and lowest with moxidectin Pour-On. The continued use of Pour-On by deer farmers will exacerbate the development of anthelmintic resistance on deer farms.

Keywords

Deer, anthelmintic resistance, anthelmintic efficacy, gastrointestinal parasites, Ostertagia, moxidectin Pour-On, moxidectin injection, moxidectin oral, moxidectin pharmacokinetics.

Introduction

Moxidectin has become the most widely used 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. The presence of gastrointestinal parasites resistance to MLs in deer (Hoskin et al 2005) was confirmed by trials on two farms in Southland in 2010 (Lawrence 2010). On these farms the efficacy of Moxidectin Pour-On against Ostertagia-type adults was 70% and 20%, while efficacy against Ostertagia-type larva were 19% and 0%.The Moxidectin injection efficacy against Ostertagia-type adults were 84% and 87%, while efficacy against Ostertagia-type larva were 81% and 82%.

This paper presents a study which extended this earlier work by comparing the efficacy of all formulations of moxidectin plus abamectin injection against GI parasites based on a slaughter trial. The pharmacokinetics of moxidectin in deer has never been determined and forms part of this study.

Background

The aim of the study was to determine the efficacy of Pour-On moxidectin (MOXp),oral moxidectin(MOXo), injectable moxidectin (MOXi) and injectable abamectin (ABAi) against naturally-acquired infection of gastrointestinal (GI) nematodes of finishing deer under field conditions and determine the level, if any of resistance to moxidectin and abamectin. At the same time samples were taken from moxidectin treated deer for a pharmacokinetic study of moxidectin in deer based on formulation and route of administration.

Discussions with management of Landcorp's Mararoa Station in the Te Anau basin occurred in autumn of 2011. There was a desire to determine the resistance status on the farm and to include an evaluation of abamectin. Abamectin had been the most commonly used ML in deer on the farm in recent years. Mararoa is an integrated farming operation running sheep, cattle and deer. 4200 red-type breeding hinds are calved and of these approximately one third are mated to Wapiti terminal sires producing hybrid finishing deer.

Material and methods

Animals

A mob of 250 of the heaviest hybrid weaners (mixed sex) was selected at the start of June for the trial proposed for spring 2011.The mob was wintered on a grass rotation. The last anthelmintic treatment they had was at the beginning of May. A combination of abamectin injection (0.2mg/kg "Genesis" Merial Ancare) and Albendazole/Levamisole oral (10mg/kg albendazole & 7.5mg/kg levamisole HCL "Arrest C" Merial Ancare). The mob was weighed in August and 36 deer selected based on their projected bodyweight being around 100kg by the end of September.

Treatments

The trial had five treatment groups (n=6) comprising control (CON, no anthelmintic), injectable abamectin (ABAi, 0.2mg/kg, "Genesis" Merial Ancare, not licensed for use in deer), injectable moxidectin (MOXi, 0.2mg/kg, "Cydectin", Pfizer, not licensed for use in deer) oral moxidectin (MOXo, 0.2mg/kg, "Cydectin", Pfizer, not licensed for use in deer) and pour-on moxidectin (MOXp, 0.5mg/kg, "Cydectin", Pfizer).

Dose rates were based on individual weights taken immediately prior to administration. Where products used were not licensed for deer the manufacturers recommended dose for cattle or sheep were applied. Administration was by calibrated syringe and separate syringes used for each anthelmintic.

Measurements

At Day-3 the Control group went to the Deer Slaughter Premises (DSP) and 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. Lungs were collected for total lungworm count with dissection technique followed by 12hr floatation.

At Day 0 heparinised blood samples were taken from 5 deer in each of the MOXi, the MOXo and the MOXp groups. Then the ABAi, the MOXi, the MOXo and the MOXp groups were treated

At Days 1, 2, 3, 5, 7, and 12, heparinised blood samples were taken from the same 5 deer in each of the MOXi, the MOXo and the MOXp groups

At Day 12 the MOXp group was slaughtered at the DSP and the ABAi, MOXi and MOXo groups were necropsied on-farm. Abomasa were collected from all groups for a 2% aliquot count of abomasal washings and 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). The heparinised blood was cooled to 4 degrees C after collection and within 6 hours centrifuged. Plasma was drawn off each sample and frozen. At the end of trial the frozen plasma (bagged by group) was couriered to AgResearch Palmerston North for moxidectin assay

Results

Gastrointestinal (Abomasal) parasites

A CON group was killed on 3rd October. All six deer had GI worms present with a group mean of 1092 (range 220 - 1800) Ostertagia-type adults. Based on concerns with the range and mean a decision was made to delay the trial a fortnight. The CON group killed on 17th October had a mean of 1647 Ostertagia-type adults (range 1280 – 2100). Based on this result, the trial proceeded. Worm count data are presented in Table 1, and a summary of mean counts and efficacy is presented in Table 2.

Table 1: Individual animal and group mean data for ostertagia-type adult and immature and *Trychostronylus axei* adult worm total counts. Control deer were slaughtered on Day 0, while other groups were slaughtered Day 12 after anthelminitic treatment.

Group and ID	Oster-type adults	*T. axei* adults	Oster-type larva
Control			
1	1370	20	639
2	2080	60	1532
3	1280	10	2429
4	2100	130	5333
5	1280	640	2908
6	1770	100	1175
Mean	**1647**	**160**	**2336**
ABAi			
1	0	0	369
2	0	0	0
3	0	0	34
4	10	0	67
5	0	0	87
6	0	0	297
Mean	**1.7**	**0**	**142**
MOXi			
1	0	0	0

2	0	0	0
3	0	0	41
4	0	0	0
5	0	0	31
6	0	0	0
Mean	**0**	**0**	**12**
MOXo			
1	0	0	0
2	0	0	0
3	0	0	82
4	0	0	167
5	0	0	248
6	0	0	85
Mean	**0**	**0**	**97**
MOXp			
1	860	60	62
2	250	0	311
3	360	0	1191
4	260	0	359
5	110	0	44
6	200	0	2062
Mean	**340**	**10**	**672**

Table 2: Summary of mean gastrointestinal worm counts and anthelminitic efficacy

	Oster-type adults	**T.axei adults**	**Oster-type larva**
Control	**1647**	**160**	**2336**
Abamectin Inj	**1.7**	**0**	**142**
% efficacy	**99.9%**	**100%**	**93.9%**
Moxi Inj	**0**	**0**	**12**
% efficacy	**100%**	**100%**	**99.5%**
Moxi Oral	**0**	**0**	**97**
% efficacy	**100%**	**100%**	**95.8%**
Moxi PourOn	**340**	**10**	**672**
% efficacy	**79.4%**	**93.8%**	**71.2%**

Efficacy against adult Ostertagia-types was 99.9% for ABAi, 100% for MOXi, 100% for MOXo and 79.4% for MOXp. Efficacy against immature forms was 93.9% for ABAi, 99.5% for MOXi, 95.8% for MOXo and 71.2% for MOXp. Efficacy against adult T. axei was 100% with ABAi, MOXi and MOXo but 93.8% with MOXp

Identification and mean number of each species of abomasal worm identified are presented in Table 3. *Ostertagia leptospicularis* (O.l) species has a similar morph *Ostertagia kolchida* which has been included in the numbers for O.l. The predominant Ostertagia-type species were *Ostertagia leptospicularis* and *Spiculopteragia asymmetrica*.

Table 3: Speciation and mean number of worms recovered at slaughter from control and Moxidectin Pour-On treated groups

Parasite	**Control**	**MOXp**
	N (%)	**N (%)**
Ostertagia circumcincta	**0 (0)**	**0 (0)**
Ostertagia trifurcata	**0 (0)**	**0 (0)**
***Ostertagia ostertagi* (O.o)**	**49 (3)**	**0 (0)**
***Ostertagia leptospicularis* (O.l)**	**732 (44.5)**	**306 (90)**
***Spiculopteragia asymetrica* (S.a)**	**833 (50.5)**	**34 (10)**
***Spiculopteragia spiculoptera* (S.s)**	**33 (2)**	**0 (0)**
Total	**1647 (100)**	**340 (100)**

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

Group	***O. ostertagi***	***O. leptospicularis***	***S. asymmetrica***	***S. spiculopteragias***
Control	**49**	**732**	**833**	**33**
Moxi Pour-On	**0**	**306**	**34**	**0**
% efficacy	**100%**	**58.5%**	**95.9%**	**100%**

Efficacy of MOXp against each worm species identified is presented in Table 4. MOXi and MOXo showed 100% efficacy against all of the Ostertagia-type species present. MOXp had a 58.5% efficacy against *Ostertagia leptospicularis* and 95.9% against *Spiculopteragia asymetrica*. MOXp was 100% effective against *Ostertagia ostertagi* and *Spiculopteragia spiculoptera*

Statistical analysis
Statistical analysis of GI nematode data was performed at AgResearch, Invermay.

Analysis of variance with Log (EL4 Recount value-1)
Means: Control = 7.54; ABAi = 3.98; MOXi = 1.2; MOXo = 3.25; MOXp = 5.71

P values (significance of differences between means)

	MOXi	MOXo	MOXp	ABAi
CON	<0.001	<0.001	=0.103	<0.003
MOXi		=0.069	<0.001	=0.016

MOXo			=0.032	=0.5
MOXp				=0.12

MOXi, MOXo and ABAi (but not MOXp) are all significantly better than CON. MOXi was significantly better than ABAi (p=0.016). MOXi was nearly significantly better than MOXo (p=0.069).

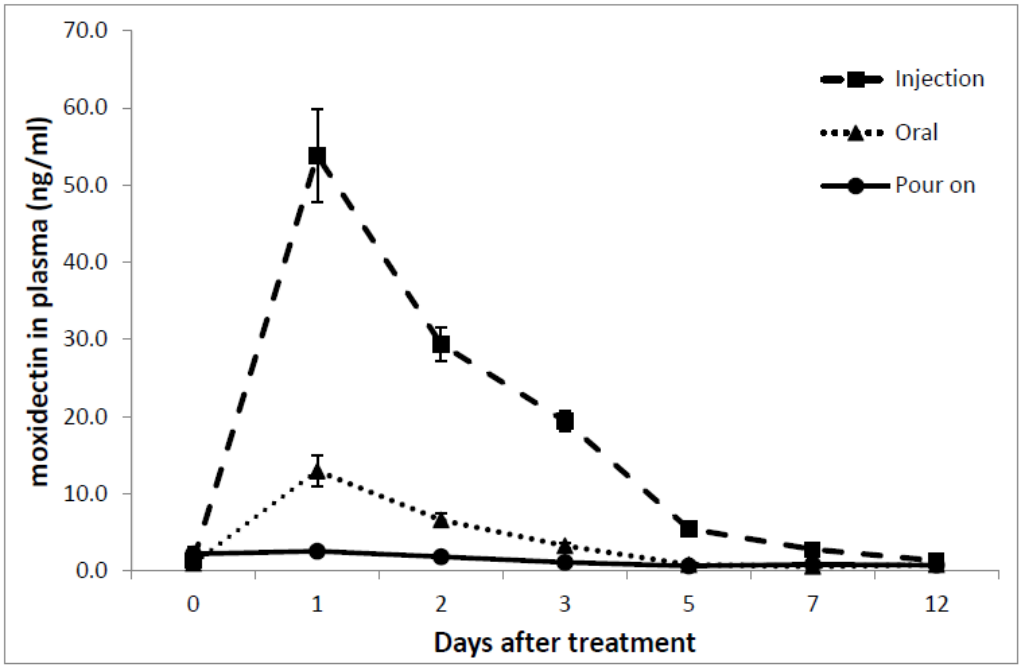
Lungworm

Numbers of lungworm present in the lungs from any of the animals in the CON group were insignificant. Based on this no further lungs were collected from any of the treated groups.

Moxidectin Pharmacokinetics

Peak mean plasma levels of moxidectin for MOXi, MOXo and MOXp were recorded at the time of first sampling 24hrs after treatment. Peak mean plasma levels of moxidectin were highest for MOXi which was 4.5 fold greater than for MOXo and 26 fold greater for than MOXp. Plasma levels declined over time consistent with peak levels attained. Decline was fastest with MOXp. Plasma levels of MOXi 7days after treatment were comparable to MOXp peak levels 1 day after treatment.

Figure 1. Mean Moxidectin plasma levels post treatment with injection, oral and Pour-On formulations



Discussion.

The incidence of anthelmintic resistance to GI nematodes in farmed deer in New Zealand continues to grow. On Mararoa "Cydectin Injection", "Cydectin Oral" and "Genesis Injection" were all ≥99.9% effective against adult Ostertagia-types. The efficacy of "Genesis Injection" against immature Ostertagia-types was less than 95% but whether this is indicative of resistance is unclear because the efficay was still high (93.9%) and there is no established efficacy data for this product at this dose rate against susceptible parasites in this host species.. The efficacy against both adult and immature Ostertagia-types by "Cydectin Pour On" was unsatisfactory. Given that moxidectin was registered for use in deer on the basis of being effective this result suggests a level of resistance to moxidectin Pour On is present on Mararoa. However, a firm diagnosis of resistance is difficult given that the two other formulations of moxidectin were highly effective.

There were four Ostertagia-type nematode species identified in the deer on Mararoa. 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). Evidence suggests it has the potential to be a serious pathogen in cattle grazing with deer (Swanson et al 2007). *Ostertagia ostertagi* is an important cattle nematode previously only observed in white-tail deer in New Zealand (McKenna 1997) but made up 3% of the Ostertagia-type species present in these deer.

All of the Ostertagia-type species were well controlled by ABAi, MOXi or MOXo but *Ostertagia leptospicularis* was very poorly controlled by MOXp which showed only a

58.5% efficacy. However MOXp was 100% effective against *Ostertagia ostertagi* and *Spiculopteragia spiculoptera*.

Previous reports indicated Ostertagia-type species exhibiting resistance to Macrocytic Lactone anthelmintics were Ostertagia leptospicularis and Spiculopteragia spiculoptera to MOXp and MOXi (Lawrence 2010), and Ostertagia leptospicularis resistant to MOXp and Ostertagia leptospicularis, Spiculopteragia spiculoptera and Spiculopteragia asymmetrica to Ivermetin oral (Hoskin et al 2005).

It is likely that resistance to any one anthelmintic is not universal across all farms.

The insignificant lungworm counts in CON animals did not allow efficacy evaluation against this parasite to be undertaken in this trial. The low numbers of lungworm in this age group appear typical for spring in the lower South Island. Lungworm challenge to farmed weaner deer is clinically more of an issue in the late summer and autumn.

The moxidectin plasma levels in deer vary considerably based on method of application. This variation probably helps explain the moxidectin efficacy results recorded in recent trials in deer. It is clear from this study that the concentrations of moxidectin circulating in plasma are far lower following pour-on administration than after administration by either the oral or injectible routes. Given the differences in efficacy this suggests significant differences in the concentration of moxidectin reaching the target worms. Clearly the concentrations of drug achieved following oral and injectable administration were sufficient to achieve 100% removal of the adult worms whilst levels reached following pour-on administration were not. This suggests that in the early stages of resistance development, the use of pour-ons will allow resistant genotypes worms to survive when use of other routes of administration will not.

The extent of GI nematode resistance to Macrocytic Lactone anthelmintics is difficult to quantify due to the need to perform slaughter trials to evaluate farm status. This is further compounded due to the limited range of anthelmintics licensed for use in deer. The result is most animals used in slaughter trials to determine farm resistance status are not accepted for human consumption because slaughter falls within the default withholding time (90 days) and therefore data comes with a cost/loss price tag of approximately $500 per animal (current market prices). The number of farms in New Zealand with known anthelmintic resistant status is small but significantly no farm has yet been identified which has not got an issue. Mararoa continues the trend with Moxidectin Pour-On resistance and a question raised regarding Abamectin resistance. While this is the first recorded instance of abamectin resistance in deer in New Zealand it is not surprising as abamectin is a less potent ML than moxidectin. The potency of Macrocytic Lactone anthelmintics varies and is greatest in moxidectin > abamectin > doramectin > eprinomectin > ivermectin.

The unlicensed formulations of moxidectin are consistently showing better efficacies than the licensed moxidectin Pour-On and this appears to be linked to pharmacokinetics. Withholding times following the use of unlicensed anthelmintics is a major concern. The

reality is that the deer industry does not command a large enough market share for the pharmaceutical industry to be interested in spending the required funds to get registration for product use in deer. The default 90-day withholding period for an unlicensed product is now able to be modified to 49days for Moxidectin Injection (Lawrence 2010).

The statistical analysis indicating moxidectin injection almost being significantly better than moxidectin oral is interesting. The moxidectin plasma profile (see Figure 1) may help explain this but further investigation is warranted and planned on this subject.

Drench resistance management 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. Previous studies in deer have shown that combination anthelmintic treatment has been effective in the face of resistance (Lawrence 2010). Caution with treatment of fawns must be exercised around the commonly held perception that lungworm are the significant parasite in late summer/autumn and that GI parasites are of concern in late autumn and spring.
There is plenty of evidence that GI nematodes are present in fawns from January onwards (Audige et al 1998, Druijf et al 2011, Mwendwa 2007). Consequently a combination drench is best practice from the first anthelmintic treatment.

Moxidectin plasma levels have never been measured before in deer. Highest peak plasma levels were obtained with MOXi and are around 60% more than those recorded in cattle (Lifschitz et al 1999a). In cattle the peak plasma level occurs 8 hours post-treatment (Lanusse et al 1997) and so actual peak levels in deer may well be greater than our peak recorded at 24hrs post treatment. Peak plasma levels for MOXp appear similar to those recorded in cattle (Lifschitz et al 1999a).

Conclusion

Although moxidectin plasma levels were not recorded in deer 20 years ago there is no reason to believe they would have been any different to those we have now. However, the MOXp efficacies identified in recent years are very different to what was recorded then. Moxidectin Pour-On was shown to have 100% efficacy against mature and immature lungworm, adult Ostertagia-type nematodes, *Trichostrogylus* sp. and 99.8% efficacy against early L4 Ostertagia-type nematodes (Mackintosh et al 1993). Similar efficacies were reported by Waldrup et al (1998).

Clearly the poor plasma availability of moxidectin following application as a Pour-On has contributed to the growing development of moxidectin resistance in farmed deer in New Zealand. To combat further development of resistance, the deer industry must stop using Pour-On anthelmintics and embrace the use of effective combination anthelmintics.

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References

Audige LJM, Wilson PR, Morris RS. Asurvey of internal parasites and parasite control on North Island deer farms. New Zealand Veterinary Journal 46, 203-215,1998

Castillo-Alcala F, Wilson PR, Pomroy WE. Anthelmintic use in deer: preliminary survey results. Proceedings for the Deer Branch of the New Zealand Veterinary Association 22, 17-20, 2005

Dróżdż J. Polymorphism in the Ostertagiinae Lopez-Neryra, 1947 and comments on the systematics of these nematodes. Systematic Parasitology 32, 91-99, 1995

Druijf JM, Wilson PR, Pomroy WJ. Do farmed red deer hinds display a post-parturient faecal egg and larval count rise? Proceedings for the Deer Branch of the New Zealand Veterinary Association 28, 75-80, 2011

Hoskin SO, Pomroy WE, Wilson PR, Ondris M, Mason P. The efficacy of oral ivermectin, pour-on ivermectin and pour-on moxidectin against naturally acquired infections of lungworm and gastrointestinal parasites in young farmed deer. Proceedings for the Deer Branch of the New Zealand Veterinary Association 22, 21-25, 2005

Lanusse C,. Lifschitz A., Virkel G., Alvarez L., Sanchez S., Sutra J.F., Galtier P., Alvinerie M., Journal of Veterinary Pharmacology and Therapeutics, vol. 20, no. 2, pp. 91-99, 1997

Lichtenfels JR, Hoberg EP. The systematics of nematodes that cause ostertagiasis in domestic and wild ruminants in North America: an update and a key to species. Veterinary Parasitology 46, 33-53, 1993

Lifschitz A., Virkel G., Imperiale F., Sutra J.F., Galtier P., Lanusse C., Alvinerie M., Journal of Veterinary Pharmacology and Therapeutics, vol. 22, 266-273, 1999

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

Mackintosh CG, Waldrup K, Labes R, Taylor M. Efficacy of ivermectin injection and moxidectin pour-on formulations in young red deer (Cervus elaphus). Proceedings for the Deer Branch of the New Zealand Veterinary Association 10, 143-150, 1993

McKenna PB. Checklist of helminthparasites of terrestrial mammals in New Zealand. New Zealand Journal of Zoology 24, 277-90, 1997.

Mwendwa JM. Investigating summer and autumn endoparasitism in farmed red deer, effects of weaning date, anthelmintic treatment and forage species. PhD Study. Massey University, Palmerston North, New Zealand, 2007

Swanson J, Hoskin SO, Wilson PR, Pomroy WE. Shared Parasites of deer, sheep, and cattle. Proceedings for the Deer Branch of the New Zealand Veterinary Association 24, 26-28, 2007

Waldrup KA, Mackintosh CG, Duffy MS, Labes RE, Johnstone PD, Taylor MJ, Murphy AW. The efficacy of a pour-on formulation of moxidectin in young red and wapiti-hybrid deer. New Zealand Veterinary Journal. 46 182-185,1998

Wood IB, Amaral NK, Bairden K, Duncan JL, Kassai T *et al.* World Association for the Advancement of Veterinary Parasitology (WAAVP) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Veterinary Parasitology 58 181-213, 1995