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Research paper

Route of administration affects the efficacy of moxidectin against Ostertagiinae nematodes in farmed red deer (*Cervus elaphus*)

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ABSTRACT

The influence of route of administration on the pharmacokinetics and efficacy of macrocyclic lactone anthelmintics has been a subject of interest due to its potential to influence the development of anthelmintic resistance. For most parasite species studied so far, oral administration results in the highest concentrations of drug in the parasites and the highest efficacy against resistant genotypes. However, a recent study in cattle measured the highest levels of ivermectin in the abomasal *Ostertagia ostertagi* following subcutaneous injection, but it was not possible to correlate these elevated levels with efficacy. Therefore, the current study was initiated to determine whether injectable delivery might be optimal for attaining high efficacy against this important group of parasites.

Three on-farm trials were conducted to measure the efficacy of moxidectin administered by the oral, injectable, and pour-on routes against Ostertagiinae parasites in farmed red deer. Groups of rising 1-year old stags (red or red-wapiti crossbreds) in the 84–104 kg weight range were randomised on liveweight into treatment groups of 6 (1 farm) or 8 (2 farms). Animals were treated to individual liveweight with moxidectin oral (0.2 mg/kg), injectable (0.2 mg/kg), pour-on (0.5 mg/kg) or remained untreated. Twelve days later all animals were euthanised and abomasa recovered for worm count. Adult worms were counted in a 2% aliquot of abomasal washings, and adult and fourth stage larvae in a 10 % aliquot following mucosal incubation in physiological saline. In addition, blood was collected from the same 5 animals in each of the treatment groups on days 0, 1, 2, 3, 5, 7 and 12 after treatment and moxidectin levels in plasma were determined using a mass spectrometer.

The number of Ostertagiinae surviving treatment was significantly different for each of the treatment groups with injectable administration being most effective, oral administration being the next most effective and pour-on administration the least effective. This applied to both adult worms and fourth stage larvae. A similar pattern was seen in the levels of moxidectin in plasma with both the peak value and area under the concentration curve being highest following injectable administration and lowest following pour-on treatment.

Although undertaken in a different host species, the results support the proposition that injectable administration of macrocyclic lactone anthelmintics is likely to be optimal for efficacy against Ostertagiinae parasites and potentially useful in slowing the emergence of resistance in these parasites.

1. Introduction

Nematode parasites constitute a significant production limitation to grazing livestock throughout the world (Charlier et al., 2014). To control infections and minimise potential loses, farmers around the world have become largely dependent on the routine administration of broad-spectrum anthelmintics (Velde et al., 2018). However, the continued effectiveness of these compounds is now threatened by the widespread presence of worm populations resistant to them (Kaplan, 2004; Sutherland and Leathwick, 2011). In response, many different aspects of worm control and anthelmintic use have been investigated in the search for strategies to delay or prevent the further development of resistance (Leathwick and Besier, 2014).

An important topic which has received some attention recently is the impact the route of administration can have on the pharmacokinetics and efficacy against resistant worm genotypes of different anthelmintics (Gopal et al., 2001; Pomroy et al., 2004; Sargison et al., 2009; Leathwick and Miller, 2013; Leathwick et al., 2016). While all anthelmintic products are registered on the basis of efficacy against susceptible worm genotypes, it has become apparent that the concentrations of active drug

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components reaching the tissues of parasite location and found within the parasites themselves can vary significantly depending on how they were administered (Bogan and McKellar, 1988; Gokbulut et al., 2010; Lloberas et al., 2012; Lifschitz et al., 2017). By influencing efficacy against resistant worm genotypes these differences in active drug concentrations can, in turn, influence the rate at which resistance builds up within a worm population (Georghiou and Taylor, 1977; Barnes et al., 1995; Smith et al., 1999; Leathwick and Luo, 2017).

For the majority of parasite species investigated thus far, oral administration of macrocyclic lactone (ML) anthelmintics has been found to result in higher efficacy against resistant worms than either injectable or pour-on formulations (Bogan and McKellar, 1988; Gopal et al., 2001; Pomroy et al., 2004; Leathwick and Miller, 2013; Lloberas et al., 2012; Leathwick et al., 2016). However, a recent study in cattle (Leathwick et al., 2020) found that against the abomasal parasite *Ostertagia ostertagi*, the concentrations of ivermectin present in adult worms were highest following subcutaneous administration. This suggests that the injectable route may be optimal for achieving high efficacy against resistant genotypes of this worm species. In order to recover sufficient worms for analysis it was necessary for these authors to use highly ML-resistant *O. ostertagi*, so it was not possible in their experiment to measure efficacy resulting from the different treatments. Hence, it was not possible to correlate the higher drug concentrations found in adult *O. ostertagi* following injectable administration with efficacy of the different treatments.

A complex of Ostertagiinae species including *Ostertagia leptospicularis*, *Spiculopteragia spiculoptera*, *Spiculopteragia asymmetrica* and, on occasions, *Ostertagia ostertagi* have been shown to be important parasites of farmed red deer and wapiti (elk) (*Cervus elaphus*) in New Zealand (Mackintosh and Wilson, 2003). While in general the Ostertagiinae are highly susceptible to macrocyclic lactone anthelmintics (Egerton et al., 1981), numerous studies have suggested that deer metabolise and/or excrete anthelmintics more rapidly than sheep and cattle, and consequently higher dose rates may be required to achieve consistent efficacy in deer (Mackintosh et al., 1985; Watson and Manley, 1985; Andrews and Lancaster, 1988; Connan, 1991; Andrews et al., 1993; Waldrup et al., 1997). Indeed, numerous studies have demonstrated suboptimal efficacy of ML anthelmintics against these parasites on deer farms in New Zealand (Hoskin et al., 2005; Hodgson, 2013; Mackintosh et al., 2014), a result which has been largely attributed to anthelmintic resistance. Regardless of the cause, this reduced efficacy presents an opportunity to investigate the influence of routes of administration on the efficacy of ML anthelmintics against Ostertagiinae parasites in this host. The current study, therefore, set out to compare the efficacy of the ML moxidectin administered by the oral, injectable, and pour-on routes against abomasal parasites of red and red-wapiti-crossbred deer.

2. Materials and methods

Anthelmintic efficacy against abomasal nematodes was evaluated in controlled anthelmintic efficacy studies (Johansen, 1989) on three commercial deer farms in New Zealand between 2010 and 2013. Two farms (designated M and S) were in the southern South Island near Te Anau, while the third (designated R) was in the central North Island near Taupo. All three farms were mixed enterprise (deer, sheep, and cattle) and farmed more than 1000 breeding red-deer hinds. The study design was the same for all three trials.

2.1. Animals

On each farm an even line of rising 1-year-old stags (of similar age and weight) was selected for study enrolment. These were of an age and weight (mean across all farms of 92 kg, range 84−104 kg) that would allow slaughter of the untreated animals in a commercial abattoir (with collection of abomasa) to minimise cost. On two farms these were red deer while on the third they were red-wapiti crossbreds.

2.2. Prior to trial commencement

A short time (3–5 days) before each trial was scheduled to begin, six animals from the herd which contained the trial animals were sent to a commercial abattoir and the abomasa recovered for worm count. These were transferred immediately to the laboratory, the contents washed out, and the number of worms present enumerated. An average total worm count of 1000 was deemed necessary for the trial to commence. This approach was necessary because faecal nematode egg counts in deer of this age are unreliable as an indicator of worm burden (Hodgson, 2013).

2.3. Trial design

For one trial 24 animals were randomised on liveweight into four groups of six, while for the other two trials 32 animals were randomised into four treatment groups of eight animals each. On Day 0, all allocated animals were weighed before they were treated with either moxidectin oral (Cydectin oral drench for sheep, Zoetis NZ Ltd) at 0.2 mg/kg liveweight, moxidectin injection (Cydectin injection for cattle and sheep, Zoetis NZ Ltd) at 0.2 mg/kg, moxidectin pour-on (Cydectin Pour-On for cattle and deer, Zoetis NZ Ltd) at 0.5 mg/kg, or left untreated.

On Day 0 prior to treatment and subsequently on Days 1, 2, 3, 5, 7 and 12 after treatment a blood sample was taken from the same five animals from each of the treatment groups for subsequent analysis for moxidectin levels.

On Day 12 the untreated and moxidectin pour-on animals were slaughtered at a commercial abattoir and the abomasums collected prior to the carcasses being processed. Concurrently, the animals treated with moxidectin oral and injection were euthanised on farm, and the carcasses buried after the abomasums had been recovered. This was a legal requirement as these products are not registered for use in deer in New Zealand and so the carcasses could not enter the food chain.

2.4. Parasitology

Processing of abomasa followed WAAVP guidelines (Wood et al., 1995). Abomasa were opened along the longest curvature and the contents were flushed out with tap water over a 38-micron aperture sieve and made up to a fixed volume before 2% aliquots were taken under continuous stirring for worm count. Each abomasum was then incubated for 24 h in physiological saline (0.9 % sodium chloride), before washing over a 38-micron sieve. The number of worms in a 10 % aliquot of this second sample were also enumerated. Male worms for speciation were mounted in lactophenol, cleared in an embedding oven at 56 °C, and differentiated morphologically using Drózdz (1995) and Lichtenfels and Hoberg (1993).

2.5. Pharmacology

Plasma moxidectin levels were quantified using the method of Hughes et al. (2013). Briefly, 100 µL of thawed plasma was deproteinated with 400 µL of ice-cold acetonitrile, vortexed briefly, centrifuged and an aliquot of the supernatant placed in an autosampler vial for analysis using a Thermo TSQ triple-quadrupole LCMS/MS. A 5 µL aliquot of sample was injected onto an Agilent C8 column (50 × 2.1 mm, 1.9 µm particle size) and eluted using formic acid: water: acetonitrile gradient elution over a 5-minute period. The mass spectrometer was operated in positive electrospray mode monitoring the moxidectin transitions 640.3 *m/z* fragmenting to 498.3 *m/z* and 528.3 *m/z*, and the moxidectin peak areas quantified using external standards and the Xcalibur software package (Thermo).

2.6. Statistical analysis

Four response variables were considered for analysis: adult and L4 worm counts, and maximum and area-under-curve (AUC) for plasma concentration. Worm counts (both adult and L4) were transformed by $Log_e(10+count)$, ten being half the smallest count seen – this prevented the zero counts from being outliers. The maximum concentration of moxidectin recorded in plasma was read from the data and the area under the concentration curve until day 7 was calculated using the trapezoid rule. Several missing data points on day 12 prevented the AUC from being calculated over a longer period. Both concentration responses were transformed by square root to equalize variances. All four responses were analysed by ANOVA using a model which included Farm, Treatment, and the Farm x Treatment interaction. Estimated marginal means are presented as back transformed means with 95 % CI. Post-hoc tests used Tukey's adjustment to control for multiple comparisons.

3. Results

3.1. Worm counts

Anthelmintic treatment was significant in influencing the number of adult abomasal worms recovered at necropsy ($p < 0.001$), farm was significant ($p < 0.001$) and the treatment-by-farm interaction was also significant ($p = 0.026$). When averaged across the three farms all four treatment groups had significantly different worm counts. All three treatments significantly reduced worm count compared to control, with worm count in the oral group significantly lower than pour-on, and worm count in the injection group significantly lower than both pour-on and oral (Fig. 1A, Table 1).

The significant farm x treatment interaction appears to reflect a greater reduction in worm count on Farm M compared to the other two farms. While the control counts tended to be lower on this farm, the reduction following treatment (efficacy) also appeared greater. This was most notable for the pour-on treatment which, based on back-transformed means, was 81 % on farm M, 65 % on Farm S and 20 % on farm R. In contrast, the efficacy of the injectable administration approached 100 % on all farms, being 100 %, 99.8 % and 98.8 % on farms M, S and R, respectively (again based on back-transformed means).

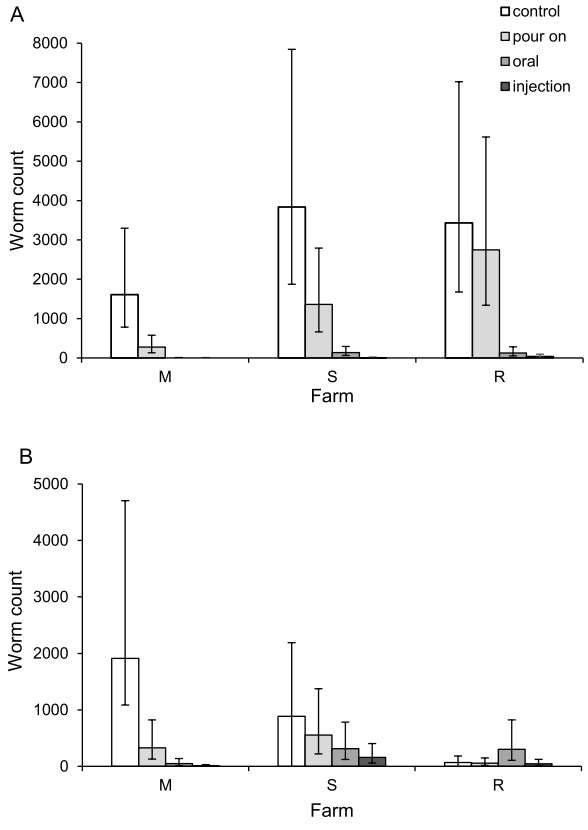

Fig. 1. Back-transformed mean worm counts (±95 % CI) for A) adult Ostertagiinae worms and B) total L4 worm count in deer that were either untreated or treated 12 days previously with moxidectin administered by different routes.

Table 1
Back-transformed mean worm counts (adult and L4), maximum recorded concentration of moxidectin in plasma (Cmax) and area under the plasma concentration curve (AUC) between days 0-7.

Treatment	Adult worms	L4 worms	Cmax (ng/mL)	AUC (ng/mL/day)
Untreated	2768 A[1]	504 A		
Pour-on	1019 B	221 AB	1.2 A	3.5 A
Oral	48 C	173 B	8.0 B	16.7 B
Injection	10 D	43 C	43.4 C	95.8 C

[1] Means within a column which have a letter in common are not significantly different.

There were significant differences in L4 worm counts, with Treatment, Farm and the Treatment x Farm interaction all being significant ($p < 0.001$). Averaged across the three farms, treatment with moxidectin pour-on did not reduce the L4 count significantly compared to the untreated control whereas the oral and injectable treatments did so. Also, the oral treatment was not significantly different to the pour-on treatment, whereas the injectable treatment resulted in L4 counts significantly lower than all other groups (Fig. 1B, Table 1).

There was a significant difference between farms in L4 counts with Farm S having overall higher counts than the other two, although this effect also reflects the greater number of L4 present after treatment (i.e. it is averaged across all the treatment groups). If only the untreated animals are considered, Farm R had fewer L4 present than the other two farms, which were not significantly different from each other. This can be seen as contributing to the significant Farm x Treatment interaction (Fig. 1B) where farm R had low numbers of L4 in the untreated group with higher values in the oral treatment group – this pattern was unlike the other two farms (Fig. 1B) resulting in the significant interaction.

3.2. Moxidectin in plasma

The maximum recorded concentration of moxidectin in plasma was different for each of the routes of administration ($p < 0.001$), being

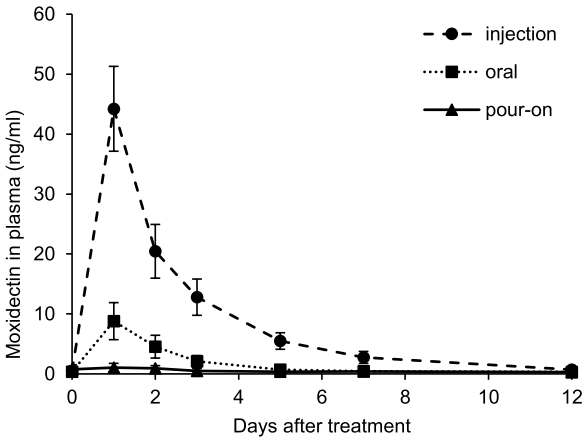

Fig. 2. Mean (±95 % CI) concentrations (ng/mL) of moxidectin in plasma following treatment of deer by either the injectable, oral, or pour-on routes. Note that each line represents the mean of 15 animals except for the day 12 mean for injection which is missing three data points.

highest following administration by injection and lowest following pour-on treatment (Fig. 2; Table 1). Similarly, the AUC up to day 7 was significantly different for each of the routes of administration, again being highest for the injection and lowest for the pour-on (Fig. 2, Table 1).

3.3. Worm species

Four species of Ostertagiinae parasites were recorded, with *Ostertagia leptospicularis* and *Spiculopteragia asymmetrica* being most prevalent (Table 2). In general, there was no consistent shift in species prevalence following anthelmintic treatment which might indicate a lower susceptibility of any species compared to the others (i.e. where adult worms were recovered following treatment these were in similar proportions to the untreated animals on each farm). The possible exception is that the only species present following treatment with moxidectin injection was *O. leptospicularis* (Table 2). However, it should be noted that the number of adult male worms recovered after the injection and oral treatments was low making, the proportions possibly somewhat unreliable.

A small number of *Trichostrongylus axei* were also recovered from the untreated animals on two farms (means of 27 and 35 on Farms M and S, respectively). No *T. axei* were recovered following treatment with moxidectin oral or injection, but worms were recovered following pour-on treatment. Reductions due to pour-on treatment were 94 % and 82 % for farms M and S, respectively.

4. Discussion

The purpose of this study was to compare the efficacy of moxidectin administered by different routes against the complex of Ostertagiinae nematodes found in farmed deer. The rationale for this comparison was a study conducted in cattle which indicated that the highest concentrations of another ML anthelmintic, ivermectin, were recorded in the abomasal *O. ostertagi* following administration by the injectable route (Leathwick et al., 2020). If it can be shown that injectable administration of MLs consistently delivers the highest concentrations of active to these worms, and this results in higher efficacy against resistant worm genotypes, then administration by injection would be indicated as the optimal route of administration to delay the development of resistance in these important parasites.

Studies in sheep and cattle have indicated that for most worm species the highest concentrations of ML reaching the tissues of parasite location and the worms themselves, and consequently achieving the highest efficacy against resistant genotypes, are recorded following oral administration (Bogan and McKellar, 1988; Gopal et al., 2001; Pomroy et al., 2004; Leathwick and Miller, 2013; Lloberas et al., 2012; Leathwick et al., 2016). Thus, the study showing that ivermectin concentrations in *O. ostertagi* in cattle were highest after subcutaneous administration contrasts with these earlier studies (Leathwick et al., 2020). A small number of previous studies indicated that injectable MLs may achieve higher efficacy at equivalent dose rates than orals and/or pour-ons against parasites in the Ostertagiinae (Egerton et al., 1981; Oksanen et al., 1993; Lawrence et al., 2013; Mackintosh et al., 2014). The current study provides further evidence that for this important group of parasites injectable MLs are likely to be optimal for achieving high efficacy against resistant genotypes. In the current set of trials moxidectin administered subcutaneously was significantly more effective against both adult and L4 Ostertagiinae nematodes than the other routes of administration, even though the pour-on is given at 2.5 times the oral/injectable dose rate. Further, while less effective than the injection, moxidectin oral was significantly more effective against adult nematodes than the pour-on administration.

Delivery route is known to influence systemic availability of ML (Hennessy and Alvinerie, 2002) as seen here in the plasma profiles. However, plasma concentrations do not always reflect those found in the tissues of parasite location or in the worms themselves (Bogan and McKellar, 1988; Lloberas et al., 2012; Leathwick et al., 2020). The highest concentrations of ML in abomasal and intestinal fluids follow oral administration (Lloberas et al., 2012), due to the affinity for MLs to bind to particulate matter which reduces absorption from the gut (Lifschitz et al., 2017). After subcutaneous administration, low to negligible quantities of moxidectin occur in abomasal particulate or fluid. However, passage across the abomasal wall is indicated because high concentrations of ivermectin (Bogan and McKellar, 1988; Leathwick et al., 2020) and moxidectin (Lifschitz et al., 1999) were detected in abomasal mucosa after subcutaneous administration to sheep and cattle. Thus, it appears that the close association of the Ostertagiinae with the abomasal mucosa (Sutherland and Scott, 2010) brings them into contact with the high levels of ML resulting from subcutaneous administration (Leathwick et al., 2020).

As the skin is a barrier which limits the amount of substances reaching the bloodstream, the plasma concentrations of any given substance achieved exclusively by transdermal absorption will be lower than those administered by oral or subcutaneous routes (Lifschitz et al., 2017). Thus, following topical administration (in the absence of licking behaviour), ML are slowly released from the skin to the systemic circulation resulting in a lower availability in plasma and in the target tissues compared to the other administration routes. This can clearly be seen in these trials where both plasma concentrations and efficacy were significantly lower following pour-on administration.

Further, the Cmax for moxidectin in plasma of cattle following pour-on administration is reported at 1.0–2.3 ng/ml (Sallovitz et al., 2000; Leathwick and Miller, 2013)), compared with the 0.4–1.2 ng/ml reported here and previously by Mackintosh et al. (2014) from red deer. It must be acknowledged that our sampling intervals were less frequent than those often used in pharmacology studies (e.g. Lloberas et al., 2012) so the estimated Cmax may not be as accurate as in other studies. Alternatively, it may be that the skin/hair structure and wallowing behaviours of deer are sufficiently different from cattle to influence uptake. In contrast, the Cmax in plasma following subcutaneous administration of moxidectin in cattle is 18.3–39.4 ng/ml (Hennessey and Alvinerie, 2002) compared to a mean of 43.4 ng/mL recorded here and 71.8 ng/mL reported by Mackintosh et al. (2014). The distribution and elimination processes of ML, particularly moxidectin, are known to be strongly influenced by body fat content (Hennessy and Alvinerie, 2002) which may contribute to these differences. Thus, a comparison of the literature pertaining to cattle and deer appears to indicate lower systemic availability of moxidectin in deer following pour-on administration, but a possibly higher availability following subcutaneous

Table 2
The percentages of adult male worms identified in worm counts from deer which were either untreated with anthelmintic or treated 12 days earlier with moxidectin administered by different routes.

		Percentage of male worms			
Farm	Treatment	O.l	O.o	S.s	S.a
M	Untreated	44	3	2	50
	Injection	–	–	–	–
	Oral	–	–	–	–
	Pour-on	90	0	0	10
S	Untreated	66	1	15	19
	Injection	100	0	0	0
	Oral	77	0	15	8
	Pour-on	65	2	22	12
R	Untreated	58	0	5	37
	Injection	–	–	–	–
	Oral	79	4	0	17
	Pour-on	61	0	5	33

O.l = *Ostertagia leptospicularis.*
O.o = *Ostertagia ostertagi.*
S.s = *Spiculopteragia spiculoptera.*
S.a = *Spiculopteragia asymmetrica.*
- = no male worms recovered.

administration.

It is not clear to what extent the reduced efficacies measured in these trials constitute evidence of ML-resistance in the worm populations. Of the anthelmintics tested, moxidectin pour-on is the only product registered in New Zealand for use in deer, and it was by far the least effective in all trials despite the higher application rate. Oral and injectable formulations are not registered and so data supporting efficacy expectations at these dose rates do not exist (Charleston, 2001; Hoskin et al., 2005). Early trials showing high efficacy of ML against gastrointestinal nematodes in deer were mostly conducted with animals housed indoors (Mackintosh et al., 1985, 1993, 1997; Waldrup et al., 1998) whereas subsequent studies conducted outdoors have almost universally shown reduced efficacy of most ML (Hoskin et al., 2005; Mackintosh et al., 2014; Hodgson, 2013). Numerous factors are known to influence pharmacokinetic behaviour of ML anthelmintics (Hennessy and Alvinerie, 2002) including feed intake (duration of drug-digesta passage) (Ali and Hennessey, 1996), breed of deer (Waldrup et al., 1998) and for pour-on administration, the weather (Oksanen et al., 1995; Sargent et al., 2009). Taylor et al. (1992) demonstrated increased availability, as estimated by areas under the plasma concentration-time curves, of ivermectin and fenbendazole when animals were housed on a diet of hay and concentrates compared to grazing pastures outdoors. Whether a difference between indoor and outdoor trials is contributing to the observed efficacies remains to be determined.

An obvious weakness in this study is an inability to consider each parasite species independently for calculating efficacy. Post-treatment worm counts were often low, and sometimes zero, either in all the animals within a treatment group, or in a proportion of the treated animals within a group. Hence, the number of male adult worms recovered for identification were also small and were considered too inconsistent for meaningful analysis or calculation of efficacy at the species level. The data presented in Table 2 are from pooled samples collected from 6 to 8 animals within each treatment group. While generalising reduction in worm count across different species is less than ideal, in this case the efficacy differences were highly significant, such that some confidence can be held that the differences between routes of administration are valid.

In other host species the Ostertagiinae are highly susceptible to macrocyclic lactone anthelmintics (Egerton et al., 1981), but in deer numerous studies have suggested that deer metabolise and/or excrete anthelmintics more rapidly than sheep and cattle, and consequently that higher dose rates may be required to achieve consistent efficacy (Mackintosh et al., 1985; Watson and Manley, 1985; Andrews and Lancaster, 1988; Connan, 1991; Andrews et al., 1993; Waldrup et al., 1997). Given this, simple extrapolation of sheep/cattle dose rates to deer may constitute marginal or even inadequate dose rates in some situations. The possibility that this could be a contributing factor to the reduced efficacies recorded here is supported by the fact that multiple species of parasites, including *T. axei* in some cases, were often present after treatment, and the relative proportions of these did not differ obviously from those in the untreated animals i.e. it would seem unlikely that resistance would occur simultaneously in multiple parasite species on all three farms.

Regardless of whether these parasites can be considered as genetically resistance to moxidectin or not, the results provide compelling evidence for higher concentrations of moxidectin being delivered to the site of parasite location (in this case the abomasal mucosa) following subcutaneous administration. The differences in efficacy between different routes of administration were consistent across trials and were often large. When combined with earlier studies, these results support the conclusion reached by Leathwick et al. (2020) that for the abomasal Ostertagiinae, across a range of host species, administration of ML anthelmintic via the subcutaneous route is likely to be optimal for achieving efficacy against resistant worm genotypes and therefore in slowing the development of anthelmintic resistance.

5. Conclusion

The results of this study support the hypothesis that the highest concentrations of macrocyclic lactone anthelmintics will be found in the abomasal Ostertagiinae following administration by the injectable route. This is likely to result in higher efficacy against resistant worm genotypes and may be useful in slowing the emergence of resistance in these parasites.

CRediT authorship contribution statement

The contributions of the authors were;

Dave Leathwick: study design, analysis and interpretation, manuscript preparation. **Paul Mason:** Parasitology, worm extraction, counts, identification. **Karl Fraser:** Plasma analysis, chemistry. **Chris Miller:** Field trials. **Peter Green:** statistical analysis

Declaration of Competing Interest

The authors report no declarations of interest.

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