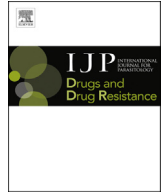




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## Selection for anthelmintic resistant *Teladorsagia circumcincta* in pre-weaned lambs by treating their dams with long-acting moxidectin injection



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### ABSTRACT

Administration of long-acting anthelmintics to pregnant ewes prior to lambing is a common practice in New Zealand. Today, most of these products contain macrocyclic lactone (ML) actives, which because of their lipophilic nature, are detectable in the milk of treated animals and in the plasma of their suckling offspring. This study was conducted to confirm the transfer of ML actives to lambs in the ewe's milk, and to assess whether this could result in selection for ML resistant nematodes in the lamb. Ninety, twin bearing Romney ewes were treated before lambing with a long-acting injectable formulation of moxidectin, a 100-day controlled release capsule (CRC) containing abamectin and albendazole, or remained untreated. After lambing, seven ewes from each treatment group were selected for uniformity of lambing date and, along with their twin lambs, relocated indoors. At intervals, all ewes and lambs were bled, and samples of ewe's milk were collected, for determination of drug concentrations. Commencing 4 weeks after birth all lambs were dosed weekly with 250 infective larvae (L3) of either an ML-susceptible or –resistant isolate of *Teladorsagia circumcincta*. At 12 weeks of age all lambs were slaughtered and their abomasa recovered for worm counts. Moxidectin was detected in the plasma of moxidectin-treated ewes until about 50 days after treatment and in their lambs until about day 60. Abamectin was detected in the plasma of CRC-treated ewes until the last sample on day 80 and in the plasma of their lambs until about day 60. Both actives were detectable in milk of treated ewes until day 80 after treatment. Establishment of resistant L3 was not different between the treatment groups but treatment of ewes with moxidectin reduced establishment of susceptible L3 by 70%, confirming the potential of drug transfer in milk to screen for ML-resistance in the suckling lamb.

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### 1. Introduction

The administration of anthelmintics to adult ewes in the weeks pre- or post-lambing has been a common practice amongst sheep farmers in New Zealand for many years (Brunsdon et al., 1983; Lawrence et al., 2007) and the practice is also common in other countries (Sargison et al., 2012; Dever and Kahn, 2015). The potential of treating ewes at this time to accelerate the development of anthelmintic resistance has been recognized for almost as long (Dash et al., 1985; Michel, 1985). Considering this practice as high risk for selecting anthelmintic resistance in New Zealand was supported by results of a modelling study (Leathwick et al., 1995), a

replicated field trial (Leathwick et al., 2006) and a national survey of resistance and associated management factors (Lawrence et al., 2006). Although another localised survey in New Zealand failed to find an association between long-acting ewe treatments and ivermectin resistance (Hughes et al., 2007) this does not negate the conclusion that treatment of ewes pre-lambing, especially with long-acting anthelmintics, is a high risk practice for the development of resistance (Leathwick and Besier, 2014). Today, farmer's preferred choices for treatment of ewes pre-lambing are long-acting products such as CRC and macrocyclic lactone products with persistent activity.

Two products commonly used as pre-lambing treatments for ewes in New Zealand today are moxidectin injection and a CRC continuously releasing low doses of both abamectin and albendazole over approximately 100 days. The long persistency associated with moxidectin, (Carceles et al., 2001; Imperiale et al., 2004), and

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the continuous release of abamectin by the CRC mean that these actives are present in plasma and tissue in the ewe for many weeks after lambs are born. Like all ML compounds, the lipophilic nature of moxidectin and abamectin results in a proportion of the administered dose being excreted in the milk of lactating animals (Alvinerie et al., 1996; Oukessou et al., 1999; Carceles et al., 2001; Imperiale et al., 2004; Dupuy et al., 2008; Barrera et al., 2013) and being detectable in the plasma of suckling offspring (Bogan and McKellar, 1988; Alvinerie et al., 1996; Cerkvenik-Flajs et al., 2007). This raises the possibility that treatment of ewes pre-lambing with these long-acting products could result in sufficient transfer of active ingredient to the suckling lamb to result in anthelmintic activity, and the potential for subsequent selection for ML-resistant parasites in the lambs (Dever and Kahn, 2015). Here we describe a study which was designed to test firstly for the transfer of moxidectin and abamectin from treated ewes into their lambs, and secondly for the potential of any such transfer to select for ML-resistance in *Teladorsagia circumcincta*.

## 2. Materials and methods

### 2.1. Experimental animals and design

Ninety mixed-age Romney ewes were selected on the basis of ultrasound pregnancy scanning as being mated in the first cycle and carrying twin lambs. In the weeks prior to the start of the study the ewes were preconditioned to a pelleted diet while still grazing on pasture, in order to facilitate their transfer indoors. Ten days prior to the expected date of first lamb drop (Day 0) the ewes were randomised into three groups of 30 based on liveweight (mean of 80.5 kg). Group 1 animals were then administered 1 mg/kg moxidectin by subcutaneous injection of a 2% solution (Cydectin long acting injection for sheep, Zoetis New Zealand Ltd) at the base of the ear, Group 2 animals were administered a CRC releasing 160 mg of abamectin, 4.62 g albendazole, 24 mg selenium and 120 mg cobalt over approximately 100 days (Bionic, Merial NZ Ltd, Auckland New Zealand), while Group 3 animals remained untreated.

All ewes were then set-stocked for lambing, with all lambs being tagged at birth so they could be identified to their mother, and records were kept on which ewes gave birth each day. Seventeen days after treatment 21 ewes, 7 from each treatment group, were selected for uniformity of lambing day ( $\pm 2$  days from the mean lambing date) and along with their twin lambs relocated indoors. The 21 trial ewes were treated with albendazole at 4.75 mg/kg and levamisole at 7.5 mg/kg to remove any existing worm burdens and the effectiveness of this treatment was subsequently confirmed by faecal nematode egg count (FEC).

Animals were housed in a series of pens on rubber matted flooring over concrete. Each ewe and her lambs were fed twice daily an allowance of 2.6 kg DM/day of a pelleted feed designed specifically for lactating sheep (17.1% crude protein, 10.4 MJ ME/kg DM) and 0.375 kg DM of a commercial baylage product (Fibre Pro). Water was available ad lib throughout the experiment.

### 2.2. Sample collection and dosing of lambs

Both ewes and lambs were bled at intervals from the jugular vein in order to measure the drug concentrations in plasma over time (Table 1). Samples (approximately 7 ml) were collected into heparinized vacutainer tubes, centrifuged at 2000 g for 20 min and plasma pipetted into labelled vials and stored at  $-20^{\circ}\text{C}$  until analysis. At intervals, milk samples were collected from the ewes for drug concentration analysis. These were collected by hand milking ewes (that had briefly been separated from their lambs), into a clean 50 ml container with an equal volume being taken from

**Table 1**

Summary of the sampling/dosing regimes for ewes and lambs in the trial where L = measure liveweight, B = bleed from jugular vein, M = milk sample, P = dose with 250 L3 of *Teladorsagia circumcincta*, F = sample for faecal nematode egg count, and S = slaughter for recovery of abomasa.

Days post mean lambing date	Days post treatment	Ewes	Lambs
	0	B	
0	10		
7	17	B	
14	24	L	L
17	27	BM	LB
29	39	BLM	LBP
35	45	L	LP
43	53	LBM	LBP
49	59	L	LP
56	66	LBM	LBPF
63	73	L	LPF
70	80	LBM	LBPF
74	84		BF
78	88		BF
80	90		S
84	94		S

each half of the udder. Two subsamples (2 ml) were stored frozen at  $-20^{\circ}\text{C}$  until analysis.

Between four and 10 weeks after birth all lambs were dosed weekly with 250 infective stage larvae (L3) of *T. circumcincta*. One lamb from each pair was dosed with L3 of a known drug susceptible isolate (Wallaceville-11-susceptible) and the other was given L3 from a known ML-resistant isolate (SOL-ivm-resistant). The latter, was isolated from sheep on a commercial farm in 1999, when the efficacy of ivermectin, abamectin and moxidectin against this isolate was 42, 96 and  $>99\%$ , respectively (Leathwick et al., 2000). Since then, it has been maintained in the laboratory by passage in lambs without further selection with anthelmintics. Towards the end of the study lambs were sampled for FEC to determine whether infections had established and on days 80 and 84 post lambing all lambs were euthanized and the abomasa collected for worm counts. The same numbers of lambs from each treatment group were euthanized at each kill date.

### 2.3. Parasitology

FEC was determined using a modified McMaster method where one egg counted equates to 17 eggs per g fresh faeces. Lambs were euthanized by percussive stunning and exsanguination, and their abomasa recovered. Abomasa were opened and repeatedly washed before 10% aliquots of the washings were passed over a 38  $\mu\text{m}$  sieve and the number of nematodes recovered was enumerated.

### 2.4. Determination of moxidectin levels in plasma

The levels of moxidectin in plasma were determined using the method of Hughes et al. (2013). Briefly, 100  $\mu\text{l}$  of thawed plasma was placed in a micro-centrifuge tube and 400  $\mu\text{l}$  of cold acetonitrile added to precipitate the protein. The sample was briefly vortexed and then centrifuged for 5 min at 12,000 rpm. A 200  $\mu\text{l}$  aliquot of the supernatant was transferred to an auto-sampler vial for subsequent analysis using a liquid chromatography-triple quadrupole mass spectrometry (LC-MS) system (TSQ Access Max, Thermo, New Zealand). A 5  $\mu\text{l}$  aliquot was injected onto a reverse-phase column (SB-C8, 50  $\times$  2.1 mm, 1.9  $\mu\text{m}$  particle size, Agilent Technologies, New Zealand) held at 25  $^{\circ}\text{C}$ . Gradient elution with the following HPLC solvents was performed with a flow rate of 600  $\mu\text{l}/\text{min}$ ; solvent A = 0.1% formic acid in de-ionised water; solvent B = 0.1% formic acid in acetonitrile (LiChroSolve grade, Merck, New

Zealand). The gradient started at 40% B, increased to 95% B over 4 min, then returned to 40% B over 30 s and re-equilibrated for 30 s, for a total run time of 5 min.

Mass spectra were acquired using a heated electrospray ionisation probe at 400 °C in positive ionisation mode, with the mass spectrometer programmed to perform a selected reaction monitoring experiment for 640.3 *m/z* as the parent ion and 498.3 *m/z* and 528.3 *m/z* as the product ions.

### 2.5. Determination of moxidectin levels in milk

The moxidectin was extracted from milk with an approach similar to [Alvinerie et al. \(1995\)](#), using solid phase extraction (SPE). Protein was precipitated by adding 1 ml of 4:1 acetonitrile:water (v/v) to 1 ml of milk and sonicating the resulting mixture for 10 min. The sample was then centrifuged for 5 min at 14,000 rpm, and supernatant decanted and diluted with 2 ml of water. Diluted supernatant was then loaded onto a preconditioned (2.5 ml methanol followed by 2.5 ml water) SPE cartridge (Strata C18-E, 3 ml/100 mg, Phenomenex, New Zealand). The cartridge was washed with 1 ml of water followed by 1 ml of 3:1 water:methanol (v/v) and then vacuum dried for 5 min, before eluting the moxidectin with 1.5 ml methanol. The methanol eluent was then evaporated to dryness under nitrogen, re-constituted with 0.5 ml acetonitrile and transferred into a 2 ml auto-sampler vial for analysis.

The concentration of moxidectin in milk was measured using the LC-MS procedure described above for plasma. Moxidectin recovery from the milk was performed by spiking five 1 ml volumes of milk were with either 10 or 40 ng of moxidectin. The spiked milk was then thoroughly mixed and subjected to the above extraction and analysis. The mean recovery was 70.7% ± 2.7%, similar to the recoveries achieved by [Alvinerie et al. \(1995\)](#).

### 2.6. Determination of abamectin levels in plasma and milk

The plasma and milk concentrations of abamectin were determined using high-pressure liquid chromatography (HPLC) with fluorescence detection with modifications of methods described previously ([Alvinerie et al., 1995](#); [Žele et al., 2010](#)). Briefly, 500 µl of plasma or milk was placed in a micro-centrifuge tube, spiked with 500 µl of ivermectin standard (50 ng/ml in acetonitrile) and 500 µl of acetonitrile to precipitate the protein. The plasma sample was thoroughly mixed with a vortex mixer, or in case of milk the sample was sonicated for 1 min to ensure efficient mixing of the internal standard and sample matrices. The plasma or milk samples were spiked with a further 400 µl of water to dilute the acetonitrile content and then centrifuged for 3 min at 12,000 rpm. The supernatant was then transferred to a pre-conditioned C18 SPE cartridge and extracted as for moxidectin (above). The 1.5 ml methanol eluent was placed into a 2 ml auto-sampler vial and dried under a constant stream of nitrogen at 60 °C to prepare for derivitization.

The derivitization procedure was as per [Alvinerie et al. \(1995\)](#), where the dry residue was dissolved in 100 µl of freshly prepared N-methylimidazole solution in acetonitrile (1:1 v/v). To initiate the derivitization, 150 µl trifluoroacetic anhydride solution in acetonitrile (1:2 v/v) was added and after briefly mixing the solutions for completion of derivitization (<30s) the sample was transferred into a 250 µl glass insert and placed back in the auto-sampler vial. Analyses were performed using a HPLC system (Shimadzu LC10ADvp, Shimadzu Oceania, New Zealand) equipped with a fluorescence detector. Samples were injected (10 µl) onto a reverse phase HPLC column (C18 Luna, 150 × 3 mm, 3 µm particle size, Phenomenex, New Zealand) held at 35 °C. Gradient elution with the following HPLC solvents was performed with a flow rate of 500 µl/min; solvent A = 0.2% acetic acid in water; solvent

B = methanol:acetonitrile (40:56 v/v) (LiChroSolv grade, Merck, New Zealand). The gradient started at 80% B, increased to 100% B over 15 min, then held for 9 min at 100% B and returned to 80% B over 1 min before being re-equilibrated for 5 min, for a total run time of 30 min. Detection was by fluorescence, with the excitation and emission wavelengths at 365 and 475 nm respectively.

Abamectin recovery from both the plasma and milk relative to the internal standard ivermectin was performed by spiking five 500 µl volumes of plasma and milk with either 5 or 20 ng of abamectin (equivalent to 10 and 40 ng/ml spikings). The spiked samples were thoroughly mixed and subjected to the above extraction and analysis. The mean recovery of abamectin relative to the internal standard ivermectin was 103.4% ± 4.1% for plasma and 104.0% ± 5.3% for milk.

### 2.7. Statistical analysis

No attempt was made to describe the plasma kinetics of moxidectin and abamectin (e.g. C<sub>max</sub> or AUC), as this was not the purpose of the study and the number of samples collected were inadequate for this purpose. Data are presented as means ± SEM.

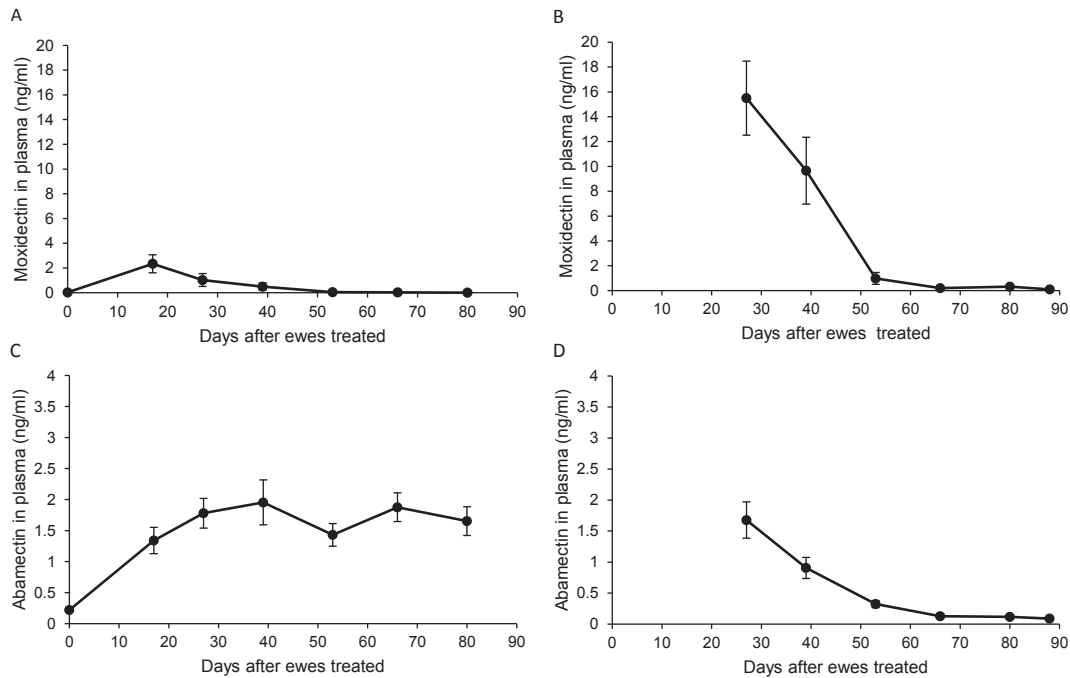
Because of low counts and the number of zero values in FEC data from the lambs, the mean FEC over four sampling occasions was used for analysis. These numbers were transformed by Ln(x + 1) before analysis by 2-way ANOVA using a model which included anthelmintic treatment (untreated, moxidectin or CRC), parasite isolate (ML-resistant or -susceptible) and their interaction. The effect of treatments to the ewes on the worm burdens of their lambs was also assessed by 2-way ANOVA using the same model. Where the F-test was significant, an LSD (5%) was used to compare treatments.

## 3. Results

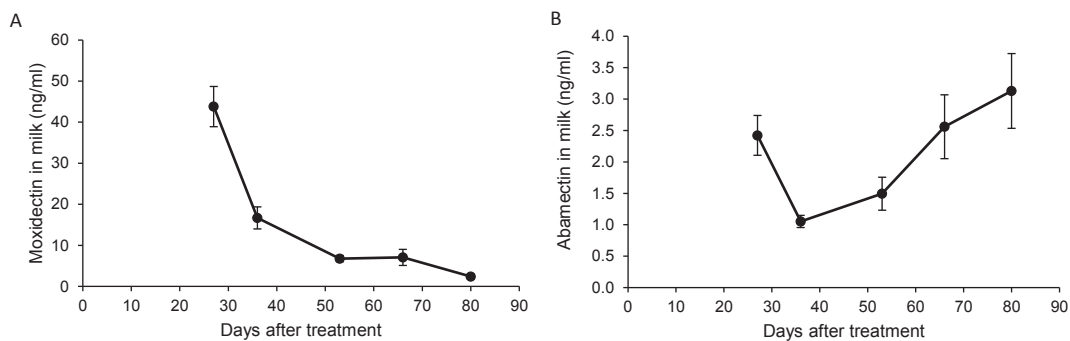
### 3.1. Drug concentrations in plasma and milk

Following treatment, moxidectin was present in all plasma samples from ewes treated with this drug until about day 50 after treatment ([Fig. 1a](#)) and in their lambs until about day 60 after treatment ([Fig. 1b](#)). Although levels approached zero towards the end of the sampling period the measured values were always higher in the lambs than in the ewes ([Fig. 1](#)). Abamectin was present in the plasma of all CRC-treated ewes, except in samples collected before treatment, until the last sampling on day 80 after treatment ([Fig. 1c](#)) and in the plasma of all their lambs until at least day 60 after treatment ([Fig. 1d](#)). Neither drug was detected in the plasma of ewes or lambs in the untreated control group. Seventeen (±2) days after birth the concentration of moxidectin in the plasma of the lambs was >15 times higher than in the ewes treated with this drug (i.e. 15.5 vs 1.0 ng/ml in lambs and ewes, respectively, [Fig. 1a & b](#)). In contrast, 17 days after birth the concentration of abamectin in the plasma of the lambs was similar to that in the ewes (1.78 and 1.68 ng/ml, respectively) and it subsequently declined to levels lower than that in the ewes ([Fig. 1c & d](#)).

Anthelmintic actives, consistent with their respective treatments, were present in the milk of all treated ewes from the first sample, 27 days after treatment, until the final sample 80 days after treatment ([Fig. 2](#)). For moxidectin, concentration declined from a high initial level (>40 ng/ml) on day 27 to a low concentration on day 80 ([Fig. 2a](#)). In contrast, the concentrations of abamectin in milk from CRC-treated ewes were lower (1–3 ng/ml) and did not show any consistent trend over time ([Fig. 2b](#)).



**Fig. 1.** The concentration of moxidectin (mean  $\pm$  SEM) in the plasma of ewes treated with long-acting moxidectin a) and their lambs b) and the concentration of abamectin in the plasma of ewes treated with a controlled release capsule containing abamectin and albendazole c) and their lambs d). Lambs were born on day 10 ( $\pm 2$ ).



**Fig. 2.** The concentration (mean  $\pm$  SEM) of moxidectin a) and abamectin b) in the milk of ewes treated prior to lambing with either a long-acting formulation of moxidectin or a controlled release capsule containing abamectin and albendazole.

### 3.2. FEC and worm burdens in lambs

Although FEC was low in all lambs there were significant differences between the isolates and between the treatment groups (Fig. 3a). Geometric mean FECs for the susceptible and resistant isolates, averaged across treatments, were 3.8 and 2.0 epg, respectively ( $p = 0.047$ ). Also, GM FEC for the different treatment groups were 6.4, 2.4 and 1.4 epg for the untreated, CRC- and moxidectin-treated groups respectively ( $p = 0.006$ ).

There was a significant difference in infectivity between the two isolates of *T. circumcincta* with worm burdens of the susceptible isolate being significantly greater than those of the resistant isolate in the lambs from untreated ewes (means of 353 and 107 for the susceptible and resistant isolates respectively,  $LSD = 151$ , Fig. 3b). There was no difference in the worm burdens of groups of lambs infected with the resistant isolate indicating that treatment of their dams had no effect on establishment of the resistant L3. However, there was a significant difference between the worm burdens of lambs infected with the susceptible isolate in that lambs from moxidectin-treated ewes had significantly lower burdens than

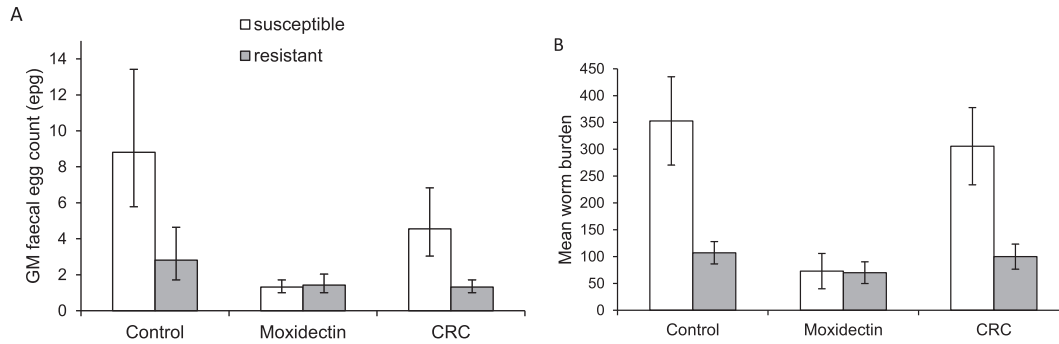
those from the other groups (means of 353, 73 and 306 for the untreated, moxidectin-treated and CRC-treated groups respectively,  $LSD = 151$ , Fig. 3b).

## 4. Discussion

This study had two aims; the first to confirm previous findings regarding the transfer of ML anthelmintics into milk (Carceles et al., 2001; Dupuy et al., 2008; Barrera et al., 2013), and hence to the suckling offspring (Alvinerie et al., 1996), in this case lambs (Bogan and McKellar, 1988; Cerkvenik-Flajs et al., 2007); and the second to test the hypothesis that the presence of transferred drug could result in selection for anthelmintic resistant genotype nematodes in the young lambs.

Moxidectin was present in both the plasma and milk of all ewes treated with this active, and abamectin was present in the plasma and milk of all ewes treated with a CRC, confirming the results of previous studies which demonstrated the transfer of ML actives into the milk of lactating animals (Carceles et al., 2001; Imperiale et al., 2004; Cerkvenik-Flajs et al., 2007). Further, the





**Fig. 3.** Geometric mean (backtransformed from  $\ln(x + 1)$  scale) faecal nematode egg count ( $\pm$ SEM) (A) and mean worm burden (SEM) (B) in 10–12 week old lambs trickle dosed with either an ML resistant or susceptible isolate of *Teladorsagia circumcincta* and whose mothers were either untreated or treated 10 ( $\pm$ 2) days before birth with a long-acting formulation of moxidectin or a controlled release capsule (CRC) containing abamectin and albendazole.

concentrations of moxidectin in milk were higher than the concentrations in plasma of the moxidectin-treated ewes for every sampling date, a result similar to that of Imperiale et al. (2004) who found moxidectin was present in milk at higher concentrations than in plasma, until at least 35 days after treatment. However, while those authors recorded a mean concentration in milk of 30.3 ng/ml 35 days after treatment with moxidectin at 0.2 mg/kg, in this study a somewhat lower concentration of 16.7 ng/ml was measured 32 days after treatment despite the administered dose rate being 1 mg/kg. Therefore, the levels of moxidectin measured in milk in the current study were lower than might have been expected based on earlier studies. While it is unclear why this occurred, it is possible that the administration of other anthelmintics on day 17 may have had an effect. Interactions between anthelmintic classes, resulting in altered pharmacology, have been recorded (Lanusse et al., 2015) and it is possible that such an interaction, resulting from the administration of albendazole and levamisole when the ewes were moved indoors, may have contributed to the lower than expected levels of moxidectin recorded here. However, moxidectin was measured in the plasma of all lambs whose mothers were treated with this active until they were at least 8 weeks old, confirming the transfer of anthelmintic active to suckling offspring in milk demonstrated previously (Bogan and McKellar, 1988; Carceles et al., 2001; Cerkvenik-Flajs et al., 2007).

The concentrations of abamectin found in the plasma and milk of the CRC-treated ewes was lower than the concentrations of moxidectin found in the equivalent samples from moxidectin-treated ewes, and the levels measured in samples of plasma and milk from sheep treated with abamectin by subcutaneous injection (Cerkvenik-Flajs et al., 2007). This is likely to reflect, at least in part, the different delivery profiles of the CRC from that of subcutaneous injection. While the CRC releases low levels of abamectin (approximately 0.02 mg/kg liveweight per day) continuously over about 100 days, administration by subcutaneous injection delivers a single large dose on administration (Cerkvenik-Flajs et al., 2007). Hence, it would be expected that the moxidectin treatment would result in a high initial peak concentration in both plasma and milk, followed by a protracted period of declining levels as the active is excreted from the animal (Dupuy et al., 2007). In contrast, the slow continuous release of abamectin would be expected to result in a low but more constant level of active in plasma and milk. In both cases, this is what was observed in the current study.

However, it has also been shown that the more lipophilic nature of moxidectin results in a milk to plasma ratio greater than for other ML actives (Oukessou et al., 1999; Imperiale et al., 2004; Cerkvenik-Flajs et al., 2007; Dupuy et al., 2008; Barrera et al., 2013) with a

higher proportion of the total administered dose being recovered from milk (Carceles et al., 2001; Imperiale et al., 2004). This was seen here in the samples taken 27 days after treatment, when the concentration of moxidectin in the ewe's milk and plasma was 43.8 and 1.03 ng/ml, respectively, a milk to plasma ratio of 42.5. In contrast, the equivalent concentrations of abamectin were 1.78 and 2.42 ng/ml for plasma and milk, respectively, a ratio of 1.4. This difference in affinity for milk undoubtedly contributed to the higher concentrations of moxidectin (15.5 ng/ml) found in the plasma of lambs whose mothers were treated with this drug, than the levels of abamectin (1.7 ng/ml) in lambs from ewes treated with a CRC. Our results are, therefore, consistent with earlier studies in that moxidectin concentrations tended to be considerably higher in milk than in plasma (Imperiale et al., 2004), whereas abamectin concentrations in milk and plasma tended to be similar (Cerkvenik-Flajs et al., 2007).

It follows then, that the nature of the different actives and their delivery profile in the different products resulted in the different concentration profiles in the plasma of the young lambs i.e. lambs whose mothers were treated with moxidectin showed higher initial levels in plasma than the lambs whose mothers were treated with a CRC. In both cases plasma concentrations declined over time, but in those lambs from moxidectin-treated ewes the rate of decline was faster i.e. from 15.5 ng/ml on day 27 to 0.98 ng/ml on day 52 for lambs from moxidectin-treated ewes, compared to 1.68 ng/ml on day 27 to 0.32 ng/ml on day 52 for lambs from the CRC-treated ewes. A faster decline would be consistent with declining plasma and milk concentrations in the moxidectin-treated ewes relative to the more constant levels in the CRC-treated ewes. Plasma concentrations for both groups of lambs would also be expected to decline as the lambs got older, larger and their milk intake declined.

The second aim of the study was to determine whether this transfer of active to young lambs in the ewe's milk has the potential to confer an advantage to resistant genotype nematodes i.e. to select for anthelmintic resistance. Lambs, from ewes treated with moxidectin, which were trickle-dosed with infective larvae of susceptible genotype *T. circumcincta* had lower worm burdens (by 70%) at slaughter than lambs from the other treatment groups which were dosed with the same isolate. In contrast, all lambs trickle-dosed with the resistant isolate of *T. circumcincta* had similar worm burdens at slaughter. Therefore, pre-lambing treatment of ewes with moxidectin injection resulted in reduced establishment of susceptible, but not resistant, *T. circumcincta* in the lambs as a result of transfer of active in the ewe's milk. Such a disparity did not occur when ewes were treated with a CRC containing abamectin, presumably because the levels of abamectin

reaching the suckling lamb were insufficient to have an effect on establishment of the susceptible genotype L3. This probably reflects the delivery profile of the CRC and less lipophilic nature of abamectin compared with moxidectin, described above, which combine to give lower concentrations of abamectin than moxidectin in the ewe's milk. Therefore, we accept the hypothesis that the presence of moxidectin, transferred in the milk from ewes treated pre-lambing, can result in selection for anthelmintic resistant genotype nematodes in the young lambs. However, we find no evidence to support the equivalent hypothesis with respect to treatment with a CRC containing abamectin.

It has long been recognized that treatment of pregnant ewes in the period immediately before or after lambing has the potential to accelerate the development of anthelmintic resistance (Dash et al., 1985; Michel, 1985). The mechanism for this was perceived to be that an anthelmintic treatment at this time would pre-select for resistance an important source of worm infection in the lambs. In a later modelling study, Leathwick et al. (1995) added that a treatment at this time also effectively eliminated a potentially important source of unselected genotypes i.e. worms in 'refugia' (Van Wyk, 2001). The use of long-acting anthelmintics added an additional potential mechanism for selection of resistance, that of 'tail' selection (le Jambre et al., 1999; Leathwick and Besier, 2014). A large scale replicated field study in New Zealand confirmed the potential of a pre-lambing treatment of ewes with a CRC containing albendazole to accelerate the development of resistance to benzimidazole anthelmintics (Leathwick et al., 2006). Albendazole CRC, such as the ones used in that study, have no withholding periods for meat or milk (Chartier et al., 1996; [http://www.merial.co.nz/Sheep/Products/Pages/extender\\_seco.aspx](http://www.merial.co.nz/Sheep/Products/Pages/extender_seco.aspx)), reflecting the fact that albendazole does not transfer into milk of treated lactating animals to the extent that ML actives do (De Liguoro et al., 1996; Dever and Kahn, 2015), and so the mechanism of resistance selection in that study must have involved only effects against worms in the ewe. Therefore, the process of selection demonstrated here (i.e. selection for resistant worms in the lamb via transfer of drug in the ewe's milk) represents a new, and until recently unconsidered (Dever and Kahn, 2015), pathway by which use of moxidectin injection in lactating ewes could select for resistance. The results of this study, therefore, represent yet another reason why administration of long-acting anthelmintic products to pre-lambing ewes must be considered high-risk for selecting anthelmintic resistance and their use approached with caution.

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