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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 circumcinta*. 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.

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 lipophylic 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 (±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 °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 each half of the udder. Two subsamples (2 ml) were stored frozen at −20 °C until analysis.

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 circumcinta*, 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

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 μm 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 μl of thawed plasma was placed in a micro-centrifuge tube and 400 μl 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 μl 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 μl aliquot was injected onto a reverse-phase column (SB-C8, 50 × 2.1 mm, 1.9 μm particle size, Agilent Technologies, New Zealand) held at 25 °C. Gradient elution with the following HPLC solvents was performed with a flow rate of 600 μl/min; solvent A = 0.1% formic acid in de-ionised water; solvent B = 0.1% formic acid in acetonitrile (LiChroSolvo grade, Merck, New