Invited article

Route of administration influences the concentration of ivermectin reaching nematode parasites in the gastrointestinal tract of cattle

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ABSTRACT

An animal trial was conducted to measure the concentrations of ivermectin occurring in abomasal and small intestinal contents and mucosa, and in the target parasites (*Ostertagia ostertagi* and *Cooperia oncophora*) following administration by subcutaneous, oral and pour-on routes. Twenty-five steers were infected with ivermectin-resistant isolates of *O. ostertagi* and *C. oncophora* and following patency randomly allocated to 3 treatment groups of 7 and 1 untreated control group of four. On day 0, animals in the treatment groups were administered ivermectin via the oral, injectable or pour-on routes. On days 1, 2, 3, 4, 5, 6 and 8, blood samples were collected from all live animals, one animal from each treatment group was euthanised and the abomasum and small intestine recovered. Control animals were euthanised on each of days 4, 5, 6 and 8. Samples of gastrointestinal tract organs, their contents, mucosa and parasites were collected and assayed for ivermectin concentration using HPLC. The highest plasma concentrations occurred following subcutaneous administration. In the gastrointestinal contents the highest levels occurred following oral administration, although one high value occurred following pour-on administration, which was attributed to self-licking by the treated animal. The lowest GI content levels followed subcutaneous injection. Ivermectin concentrations in the gastrointestinal mucosa were highest following subcutaneous injection. Drug levels in the abomasal parasite *O. ostertagi* were most closely correlated with levels in the abomasal mucosa whereas levels in the intestinal *C. oncophora* were most closely correlated with those in the intestinal contents. Thus, the maximun levels of drug reached *C. oncophora* in the small intestine following oral administration. In contrast, the highest levels of ivermectin in *O. ostertagi* followed subcutaneous injection. Therefore, route of administration is likely to influence the exposure to ivermectin for different parasite species.

1. Introduction

Infection with nematode parasites has the potential to cause significant production losses in grazing cattle (Charlier et al., 2009; 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 this reliance on chemical intervention is now threatened by the widespread and increasing presence of worm populations resistant to these anthelmintics, with resistance in *Cooperia* spp. being very common in some countries (Sutherland and Leathwick, 2011). Importantly, recent years have seen the increasing documentation of resistance to both the benzimidazole and macrocyclic lactone

classes of anthelmintics in the most pathogenic of the cattle parasites *Ostertagia ostertagi* (Demeler et al., 2009; Edmonds et al., 2010; Rendell, 2010; Geurden et al., 2015; Waghorn et al., 2016). In the absence of ongoing development and registration of new classes of anthelmintics, it would seem prudent to devise and implement strategies to prevent or slow the further development of resistance to existing anthelmintic classes (Rose et al., 2015).

While there has been extensive research into anthelmintic resistance and its management in nematodes infecting sheep, there has been much less attention paid in cattle parasites. To an extent, the principles developed for managing resistance in sheep parasites should be transferable to cattle, however, this is by no means certain (Leathwick and Besier, 2014). In addition, there are differences between the way in

which anthelmintics are administered and used in cattle compared to sheep. Perhaps most notable of these is the route by which anthelmintics are administered, with sheep usually being treated via the oral route, whereas in cattle the injectable and topical (pour-on) routes are more usual.

For some years it has been known that anthelmintics administered by different routes can have quite different efficacies against resistant nematodes (Gopal et al., 2001; Pomroy et al., 2004; Sargison et al., 2009; Leathwick and Miller, 2013; Leathwick et al., 2016) and it now appears that this is the result of different concentrations of active reaching the site of action within the target worms in the gastrointestinal tract (Bogan and McKellar, 1988; Gokbulut et al., 2010; Lloberas et al., 2012; Lifschitz et al., 2017). While all products are presumably registered based on evidence of efficacy against susceptible worm genotypes, this differential efficacy against resistant worms has implications for the selection of drug resistance (Georgio and Taylor, 1977; Barnes et al., 1995; Smith et al., 1999; Leathwick and Luo, 2017), i.e. if efficacy against susceptible genotype worms is high, which presumably is the case for products with registration claims for efficacy, then resistance will develop more rapidly if a high proportion of the heterozygous genotypes are able to survive treatment (Smith et al., 1999) and/or there is higher variability in efficacy against resistant genotypes (Leathwick and Luo, 2017). It follows that if delivering the same active by different routes of administration results in substantial differences in the concentration of active reaching the target worms, this is likely to result in meaningful differences in efficacy against resistant genotypes and consequently the development of overtly resistant worm populations.

The current study was undertaken to measure the concentrations of ivermectin reaching different tissues and target worm species in cattle which had been treated either orally, topically or by injection. A better understanding of the pharmaco-kinetic differences between routes of administration should lead to more effective use of anthelmintics in order to delay the development of resistance.

2. Materials and methods

2.1. Animals

Twenty-five autumn born Hereford x Friesian steers, with a mean liveweight of 208 kg (range 175–259 kg), were purchased at the beginning of summer (November 2017). On arrival at the farm they were treated with the manufacturer's recommended dose of an oral combination anthelmintic containing abamectin, oxfendazole and levamisole (Matrix mini-dose, Merial New Zealand limited) to remove any existing worm burden. Efficacy was confirmed by post-treatment faecal nematode egg counts (FEC). The cattle were subsequently grazed, as a single mob, on pastures infested with parasites (*O. ostertagi* and *Cooperia oncophora*) previously demonstrated to be resistant to ivermectin. The farm had previously been tested by faecal egg count reduction test which showed reduced efficacy of ivermectin (71%), albendazole (85%) and levamisole (63%) against *Ostertagia* spp. (Waghorn et al., 2016) and ivermectin (49%) and albendazole (89%) against *Cooperia* spp.

2.2. Parasites and infection

Thirteen and 14 days after anthelmintic treatment the animals were infected with ivermectin-resistant isolates of *O. ostertagi* and *C. oncophora*. The parasite strains had previously been recovered from the farm on which the study was conducted, and cycled once through calves housed indoors, to produce enough infective stage larvae (L3) to infect the animals in the trial. Each animal received an artificial challenge of approximately 15 000 *Cooperia* L3 and 7600 *Ostertagi* L3 administered orally in a small volume of water, as two equal doses on consecutive days. Thus, the parasites to which the animals were naturally exposed when grazing and with which they were artificially challenged, were essentially the same, which allowed for the animals to remain on pasture

throughout the duration of the trial.

2.3. Design and sampling

Infections were monitored every 2–3 days by FEC from day 21 post infection and once fully patent (day 26 post infection, designated as Day −2) all animals were ranked by FEC and liveweight and randomised into three treatment groups of seven animals and an untreated control group of four animals. On day 0, animals were weighed and anthelmintic treatments administered to individual liveweight at the manufacturer's recommended dose rates using graduated syringes. The treatments were: 1. Ivermectin oral (Ivomec liquid for sheep and goats, Merial, Auckland New Zealand) at 0.2 mg/kg liveweight. 2. Ivermectin injection (Noromectin for cattle, sheep and pigs, Norbrook laboratories Ltd, Northern Ireland) at 0.2 mg/kg liveweight. 3. Ivermectin Pour-On (Noromectin pour-on for cattle and deer, Norbrook laboratories Ltd, Northern Ireland) at 0.5 mg/kg.

Following treatment, and for the remainder of the trial period, the pour-on treated animals were confined to individual cells (18 × 10 m) within the paddock. The cells were double electric fenced with a minimum 1-m gap between cells, to prevent animals licking one another. Each steer was also neck braced, to try and prevent animals licking themselves. The oral, injectable and control animals continued to be run as a single mob in the same paddock.

Fresh samples of faeces to determine FEC were taken from all animals on day −2 for randomisation then from all live animals on days 0, 4, 5, 6 and 8 post treatment. FEC was performed using a modified McMaster method (Lyndal-Murphy, 1993) in which each egg counted is equivalent to 25 eggs/g wet faeces.

Blood samples were collected from the neck of all animals alive on days 0, 1, 2, 3, 4, 5, 6 and 8 post treatment, using tubes containing EDTA, to determine plasma concentrations of ivermectin. Blood samples were centrifuged at 2000 g for 20 min with the plasma being recovered into labelled vials and frozen at −20 °C until analysed by HPLC.

2.4. Collection of tissues and parasites

One animal from each of the anthelmintic treatment groups was euthanised, by captive bolt followed by exsanguination, on days 1, 2, 3, 4, 5, 6 and 8 post-treatment. From day 4–8, one control animal was also euthanised each day. The abomasum and the entire small intestine from each animal were tied off and relocated to the laboratory for adult parasite extraction and sample collection.

The undiluted abomasal content was emptied through the omasoabomasal opening into a graduated container without dilution. The abomasum was squeezed and contents pushed out through the omasoabomasal opening into the container. Under continuous mixing 5 × 2 ml subsamples were taken for ivermectin (HPLC) analysis. The abomasum was then opened along the narrow fold and the surface and folds of the abomasal wall were rinsed into the same container with physiological saline at 37 °C. Finger-tips were used to gently remove adult worms and debris. The contents were then made up to a known volume, and under continuous mixing, two 1% aliquots were collected for worm counts. Warm agar was added to the remaining contents to a final concentration of 1% and the adult *O. ostertagi* rinsed into collection containers with physiological saline at 37 °C as they migrated out of the solidified contents (Van Wyk and Gerber, 1978). Parasites were individually picked out of the collection containers, rinsed and stored in water in a 2 ml Eppendorf vial at −20 °C prior to being assayed for ivermectin.

The washed abomasum was then laid flat on the bench and scraped lengthwise along the internal mid-line with a glass microscope slide to remove the mucosa, which was then subsampled into 2 × 2 ml Eppendorf vials and stored at −20 °C.

The 1st meter of the cranial jejunum was double clipped and removed from the rest of the small intestine. The contents were

extracted, before the organ was opened and thoroughly rinsed with physiological saline at 37 °C, using finger-tips to remove adult worms and debris. It was then laid flat on the bench and the internal surface scraped with a glass slide to remove the mucosa, which was then subsampled and placed into 2 × 2 ml Eppendorf vials. The extraction and rinsing procedure were repeated with the remaining portions of small intestine and 2 × 1% samples were collected for worm counts prior to the addition of agar to the remaining contents in order to recover adult *C. oncophora* as described above.

All samples were stored at −20 °C prior to analysis. Prior to HPLC assay adult worm samples were thawed, placed onto blotting paper where water was removed by vacuum suction and absorption. The adult worms were then picked off the paper and weighed in a tared Eppendorf vial.

2.5. Analytical methods

Ivermectin was extracted from plasma, gastrointestinal contents and mucosa following the technique described by Lifschitz et al. (2000). Weighed samples of parasites (0.02–1.10 g), gastrointestinal contents (0.25 g) and mucosa (0.25 g) were spiked with 10 ng abamectin as an internal standard. Samples were extracted with acetonitrile, using a TissueLyser II (Qiagen, MA USA) and a sonicator to disrupt the cell membranes. The supernatant was removed after centrifugation. Parasite extracts were dried under a nitrogen gas stream.

Water was added to the gastrointestinal intestinal content and mucosa extracts to achieve a 50:50 v/v acetonitrile: water mix, before being transferred to a preconditioned (2.5 ml methanol followed by 2.5 ml water) SPE cartridge (Strata C18-E, 3 ml/100 mg, Phenomenex, New Zealand).

An aliquot of 500 μl plasma, was spiked with 10 ng internal standard and left standing for 30 min at room temperature. Acetonitrile (900 μl) was added and vortexed for 5 min, before 400 μl of water was added, the solution vortexed again, and then centrifuged at 16 300 g for 10 min. As above, the supernatant was transferred to a preconditioned SPE cartridge. 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 avermectin compounds with 1.5 ml methanol. The 1.5 ml methanol eluent was placed into a 2 ml auto-sampler vial and dried under nitrogen at 30 °C.

Dried parasite and gastrointestinal intestinal samples were derivatized (Alvinerie et al., 1995) with the dry residue dissolved in 100 μl of freshly prepared N-methyl imidazole solution in acetonitrile (1:1 v/v) before adding 150 μl trifluoroacetic anhydride solution in acetonitrile (1:2 v/v). The solutions were briefly mixed to complete the derivatization (<30 s). The sample was transferred into a 250 μl glass insert and placed in an auto-sampler vial.

Analyses were performed using an UltiMate 3000 UHPLC Systems (ThermoFisher Scientific, Auckland, NZ). Samples were injected (10 μl) onto a reverse phase HPLC column (Luna Omega 1.6 μm Polar- C18 100 A Column 50 × 2.1 mm Phenomenex, New Zealand) held at 35 °C. Isocratic elution was performed with a flowrate of 450 μl/min, solvent A = methanol: acetonitrile (40:56 v/v) (LiChroSolv grade, Merck, New Zealand), solvent B = 0.2% acetic acid in water at 6.5% B and a runtime of 5 min. Detection was by fluorescence, with the excitation and emission wavelengths at 365 and 475 nm respectively. Calibration curves were constructed for mucosas, gastrointestinal contents and parasites in the range of 1–1000 ng/g. The linear regression lines for both anthelmintics showed correlation coefficients >0.99. The precision of the analytical procedures obtained after HPLC analysis showed a coefficient of variation between 6.95 and 14%.

2.6. Statistical and pharmacokinetic analysis

The correlation between drug availability parameters in the different gastrointestinal contents and mucosa and nematodes was calculated

with GraphPad Prism. Pharmacokinetic parameters that reflect the drug availability were determined using non-compartmental analysis. The plasma concentrations versus time curves obtained after each treatment were fitted with the PK Solutions 2.0 (Ashland, Ohio, US) computer software. The peak concentration (C_{max}) was read from the plotted concentration-time curve. The area under the concentration-time curves (AUC) were calculated by the trapezoidal rule (Gibaldi and Perrier, 1982).

Worm counts collected between days 4–8 were compared by one-way ANOVA, with anthelmintic treatment as the independent variable and worm count as the dependent variable.

3. Results

Ivermectin was measured in plasma, gastrointestinal tissues and in pooled samples of parasites of all treated animals from 1 to 8 days post-treatment. Plasma concentrations were higher after the subcutaneous administration than the oral and pour-on treatments (Fig. 1).

In the abomasum, the highest ivermectin concentrations were measured within the abomasal content after the oral (989 ng/g) and pour-on treatments (288 ng/g). In contrast, the peak concentration was only 9.9 ng/g after SC administration (Table 1, Fig. 2, Table 2 supplemental). Similarly, in the small intestine, the highest ivermectin concentrations were found in the intestinal content after pour-on and oral administration (93 and 91 ng/g, respectively), although the high pour-on value likely reflects oral ingestion of product by that animal. As in the abomasum, the lowest levels occurred after SC administration (Table 1, Fig. 3).

Drug concentrations in the mucosal tissues (both abomasum and small intestine) following oral and pour-on administration tended to be lower than in the gastrointestinal contents (Figs. 2 and 3). However, following SC administration, the values tended to be higher in the mucosa than in the contents (Table 1).

Following oral administration, the ivermectin concentration showed an early peak and then decreased rapidly in the different gastrointestinal sites and target parasites. In contrast, following SC and topical administration levels showed a more sustained concentration profile (Figs. 2 and 3). These differences impacted on the total drug exposure, measured as AUC (Table 1), for the different routes of administration, with the oral treatment often showing a lower AUC despite having the highest peak value (Figs. 2 and 3).

Despite the ivermectin AUC in the abomasal content being >20-fold-higher after the oral and topical administration, the exposure in *Ostertagia* spp. was higher after the SC treatment and was most strongly associated with the drug concentrations in the abomasal mucosa (Table 1, Fig. 4A). In contrast, the exposure of *C. oncophora* was most closely correlated with the ivermectin concentration in the intestinal content (Fig. 4B). Thus, the two parasite species appear to be accessing
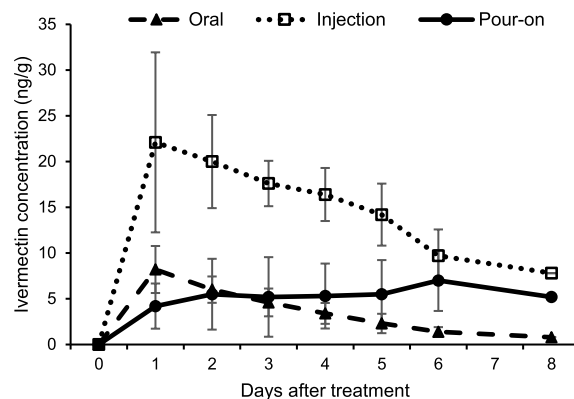

Fig. 1. Mean (±1 SD) concentration of ivermectin in the plasma of calves following treatment via the oral, subcutaneous and pour-on routes.

Table 1

Drug disposition expressed as observed peak concentration (Cmax) and area under the concentration vs time curve (AUC_{0-8d}) for ivermectin in plasma, gastrointestinal contents, mucosa and nematodes after its administration by the oral, subcutaneous and pour-on route to cattle.

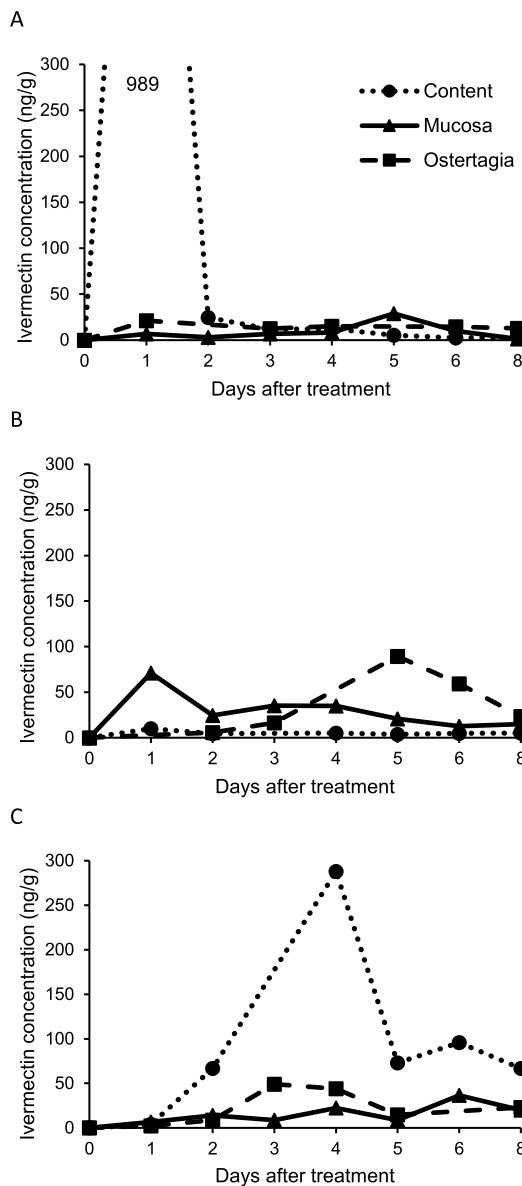| Ivermectin kinetic parameter values						
	Cmax (ng/g; ml)			AUC_{0-8d} (ng.d/g; ml)		
	Oral	Injection	Pour-on	Oral	Injection	Pour-on
Plasma	8.2	22.1	7.0	40	149	47
Abomasal Content	989	9.89	288	981	40	822
Abomasal Mucosa	29.0	71.0	36.5	84	223	129
Ostertagia spp.	21.2	89.4	49.1	77	256	137
Intestinal Content	91.1	35.3	92.8	120	116	215
Intestinal Mucosa	22.8	38.0	17.9	63	163	85
Cooperia spp.	40.1	35.1	78.5	62	140	175

Fig. 2. Ivermectin concentration (ng/g) in mucosa, content of the abomasum and in *Ostertagia ostertagi* following administration by the oral (A), injectable (B) or topical (C) routes.
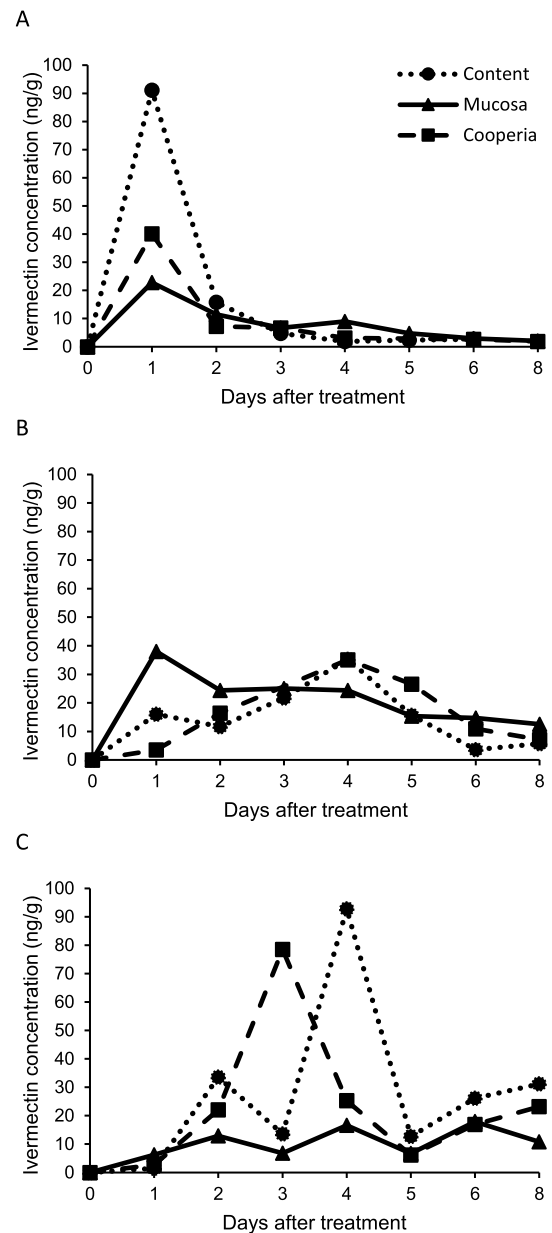

Fig. 3. Ivermectin concentration (ng/g) in mucosa, content of the small intestine and in *Cooperia oncophora* following administration by the oral (A), injectable (B) or topical (C) routes.

their drug concentrations primarily via different pathways, *O. ostertagi* via the drug levels in the mucosa, and *C. oncophora* via those in the intestinal content.

The high drug exposure observed in both the abomasal and small intestinal content (on day 4 post-treatment) after the topical administration confirms that self-licking occurred with this animal. Given that one pour-on treated animal was able to lick itself, the possibility cannot be excluded that other animals were also able to do so, even if to a lesser extent. All data were left in the data set for analysis, but it must be acknowledged that self-licking has likely biased the results and potentially their interpretation.

All animals were positive for FEC after the ivermectin treatment consistent with the resistance status of the parasite isolates (data not shown). The number of adult worms recovered 4–8 days after treatment was not significantly different between any of the treatment groups (p = 0.117 and p = 0.075 for *O. ostertagi* and *C. oncophora*, respectively). The
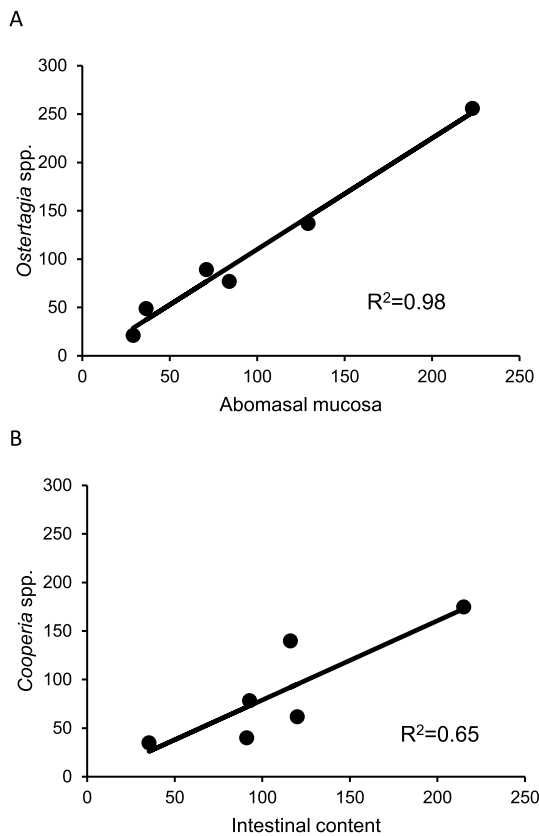
A

B

Fig. 4. Correlation between the concentration (ng/g) of ivermectin measured in parasites and the tissue/content with which they were most closely associated i.e. A) *O. ostertagi* and the abomasal mucosa, and B) *C. oncophora* and the small intestinal contents.

mean efficacy against *O. ostertagi* was 39%, 26% and 59% after the oral, SC and pour-on administration, respectively. Efficacy against *C. oncophora* averaged 76%, 30% and 62% for the oral, injectable and pour-on treatments respectively (Fig. 5).

4. Discussion

It has previously been established, that for anthelmintics in the macrocyclic lactone class both formulation and route of administration substantially influence pharmacokinetics (Albert Lo et al., 1985; Wicks et al., 1993). More recently, the relationship between drug
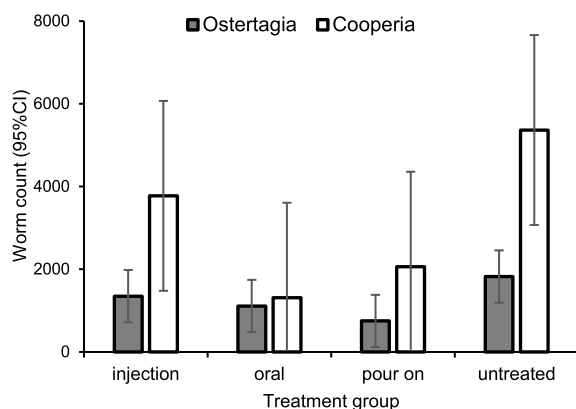

Fig. 5. Worm counts (95% CI) from animals (n = 4) killed 4–8 days after treatment with ivermectin administered by either subcutaneous injection, pour-on or oral administration, or left untreated.

concentration in the tissues of parasite location, within the parasites themselves and the associated efficacy have been investigated (Alvarez et al., 2015; Lloberas et al., 2012; 2015; Lifschitz et al., 2017). These experiments have all been conducted in lambs using *Haemonchus contortus* as the target parasite, the adults of which are relatively large compared with many other gastrointestinal species. To date no experiments have attempted to establish similar relationships in parasites of cattle. Therefore, the purpose of this study was to investigate the concentrations of ivermectin in tissues of parasite location and worm species of cattle following drug administration by different routes. In part, the purpose was also to establish whether it was possible to measure drug concentrations within these parasites, and a method for doing so.

Unfortunately, despite efforts to prevent it, it appears that some animals were able to lick the topically applied product off their backs resulting in oral ingestion of at least part of the administered dose. After parenteral (pour-on) treatment, the active secretion of macrocyclic lactones from the bloodstream to the abomasal lumen is of little relevance. For example, the concentrations of doramectin in the abomasal content after pour-on administration was 38-fold lower when animals were prevented from licking, compared to free licking calves (Sallovitz et al., 2005). Similarly, even after the SC administration of ivermectin to sheep at ten times (2 mg/kg) the therapeutic dose, concentrations in the abomasal content remained low (Bogan and Mckellar, 1988). Therefore, after either SC or pour-on administration the ivermectin concentrations in the abomasal contents would be expected to be low. The fact that, in this study, a small number of samples showed high levels of drug in the gastrointestinal content is a clear indication of oral ingestion of ivermectin.

The implications of licking by pour-on treated animals has received considerable attention in the scientific literature (Laffont et al., 2003; Bousquet-Mélou et al., 2004; 2011; Sallovitz et al., 2005). The wide variation in effective dose absorbed by treated animals when licking is allowed to occur can significantly influence both efficacy (Bousquet-Mélou et al., 2011) and potentially the development of anthelmintic resistance (Leathwick and Lou, 2017). The purpose of this study was to compare the concentrations of ivermectin reaching tissues in the GI tract and the worms themselves as a result of different administration routes. To that end every effort was made to prevent licking.

As shown in earlier studies (Oksanen et al., 1995; Lespine et al., 2005; Lloberas er al., 2012; Leathwick and Miller, 2013; Macintosh et al., 2014; Cocquyt et al., 2016; Canton et al., 2018; Fazzio et al., 2019), the plasma concentrations of ML anthelmintics are generally higher following subcutaneous administration than following oral or pour-on administration (Fig. 1; Table 1). In the current study, the summary statistics for levels in plasma in the oral and pour-on treated animals were similar (Table 1; Fig. 1) despite the pour-on being administered at 2.5 times the dose rate of the oral. However, this is in contrast with other studies comparing oral and pour-on administration of ML products to cattle, which showed significantly higher levels in plasma following oral administration (Wen et al., 2010; Leathwick and Miller, 2013; Leathwick et al., 2016). A similar pattern has also been shown in other host species (Gokbulut et al., 2010; Mackintosh et al., 2014). Therefore, as outlined above, the findings in this study are consistent with oral ingestion of product by at least some of the pour-on treated animals.

IVM concentrations in cattle tissues of parasite localization were previously evaluated after SC administration (Lifschitz et al., 2000). That study was carried out up to 48 days post-IVM administration, which makes the AUC incomparable with the current work. However, the Cmax, values obtained were similar to those observed in the present experiment. In the liquid content of the abomasum, the highest concentrations of ivermectin were recorded following oral administration with the subcutaneous route producing the lowest values. However, subcutaneous administration resulted in the highest levels in the abomasal mucosa, and also in the *O. ostertagi* parasites in the abomasum (Table 1). The level of ivermectin within these parasites was

significantly correlated with the levels in the mucosa, rather than the levels in the content. This indicates, therefore, that the often close association of *O. ostertagi* with the abomasal mucosa (Sutherland and Scott, 2010) means that their exposure to drug concentration is most strongly associated with the levels in this tissue. The data, therefore, also indicates that the highest levels of ivermectin in these target worms was achieved via the injectable route. This finding is in contrast with studies on another abomasal parasite (*H. contortus*) in sheep where concentrations of ivermectin in the worms, and the resultant efficacy, was correlated with drug levels in the abomasal contents, rather than in the abomasal mucosa (Lloberas et al., 2012; 2015).

In the small intestine, the highest levels of ivermectin in the mucosa were again measured following SC administration. However, in the intestinal content, the observed Cmax was equally high for the oral and pour-on treatments, while the AUC was highest following pour-on administration. The AUC for ivermectin in the parasites themselves (*C. oncophora*) was highest for the pour-on and lowest for the oral. In contrast to the results for *O. ostertagi*, the concentrations of ivermectin in *C. oncophora* were most closely correlated with that of the intestinal content. The data suggests, therefore, that efficacy against *Cooperia* is likely to be associated with routes of administration that result in high concentrations of drug in the intestinal contents, rather than the mucosa. A relationship between drug concentrations in the worms and that in the intestinal contents has been hypothesised previously based on the greater efficacy of orally administered MLs over other routes of administration (Leathwick and Miller, 2013; Leathwick et al., 2016; Saumell et al., 2017; Canton et al., 2018), assuming an absence of licking. This conclusion is supported by data from registration trials for ivermectin (Benz et al., 1989) in which efficacy against adult *C. concophora* is listed as 95%, >99% and 96% for ivermectin administered respectively at 0.2 mg/kg by injection, 0.2 mg/kg orally and 0.5 mg/kg topically. As these parasites can be assumed to not be resistant to the anthelmintic, these efficacy values support the view that *Cooperia* spp are dose limiting parasites for IVM in cattle (Benz et al., 1989) and that oral administration is achieving greater exposure of the worms to the drug than the other routes. Obviously, when the nematode susceptibility is reduced, the different drug exposure has a relevant impact on the efficacy, as seen in this study (76% and 30% after the oral and SC administration respectively).

Efficacy measurements, in the current study, failed to demonstrate any significant reduction in worm count by any of the treatments. This clearly demonstrates the highly resistant nature of the parasites used. The use of highly resistant parasites was a necessary requirement of the study to ensure enough adult worms would be recovered following treatment to allow for the measurement of drug levels in them. Unfortunately, this means that no differences in efficacy were established to compare with the drug levels in the worms as a result of different routes of administration.

The primary route of accumulation of ML actives in parasites appears to be via trans-cuticular diffusion (Geary et al., 1995; Ho et al., 1990; Lifschitz et al., 2017). Since concentration gradient is a major determinant of drug accumulation in the worms (Alvarez et al., 2007), it follows that differences in drug concentrations within the worms, and the resultant differences in efficacy, reflect the levels of drug in the tissues surrounding the parasites (Alvarez et al., 2000; Mottier et al., 2006; Lloberas et al., 2012). Based on the greater efficacy of oral delivery over SC and pour-on administration against many parasites (Gopal et al., 2001; Pomroy et al., 2004; Leathwick and Miller, 2013; Saumell et al., 2017; Canton et al., 2018), and the higher ML concentrations measured in the intestinal fluids (Lloberas et al., 2012) it has been assumed that most parasites accumulate drug from the intestinal content (Lifschitz et al., 2017). However, in this study it appears that for *O. ostertagi* this is not the case. The high correlation between ivermectin levels in the abomasal mucosa and within the worms, and the higher levels recorded in both mucosa and parasites following SC administration, indicate the superiority of SC injection for delivery of high doses to *O. ostertagi*. This result is consistent with efficacy studies in deer (Macintosh et al., 2014) in which injectable MLs had higher efficacy against *Ostertagia*-type parasites than oral or pour-on administration.

The current work does come with certain limitations. No-one had previously measured drug concentrations in these worm species, and so initially it was not certain that we would be able to collect a large enough volume of parasites from each animal to measure drug levels. Further, the sacrifice of cattle for sampling at multiple time points post-treatment coupled with the analysis of three administration routes and an untreated control necessitated, for cost and logistical reasons, a low number of experimental animals per sampling point. This obviously limited the accuracy of parameter estimation which may have affected the calculation of exposure, particularly after the administration by the routes with less persistence in the body, such as the oral route. Besides, the estimation of nematode exposure using the whole parasite does not take into account the different body structures in which there could be different drug concentrations given the action of efflux transporters. Despite these limitations the correlation of drug levels in the worms with that in different tissues provides valuable information regarding the concentrations of IVM in the tissues of parasite location and the nematodes themselves. Now that methods have been established, further work to validate the importance of routes of administration on efficacy against these important parasites are warranted.

In summary, this is the first attempt to measure concentrations of any ML in gastrointestinal parasites of cattle. Results are consistent with earlier studies in that administration by SC injection resulted in the highest levels of ivermectin in plasma and the abomasal and intestinal mucosa, while oral administration resulted in the highest concentrations in abomasal and intestinal contents. However, in this study pour-on administration also produced high concentrations in the gastrointestinal contents, a result likely due to oral ingestion of product by at least some animals. As with studies in sheep, ivermectin concentrations in *C. oncophora* were most closely associated with drug levels in the intestinal content. In contrast, drug levels in *O. ostertagi* were highly correlated with concentrations in the abomasal mucosa. Hence, the results suggest that while for most parasite species, oral administration of ML anthelmintics will deliver the highest exposure of parasites to the drug, for *O. ostertagi* SC injection is likely to be the preferred option for achieving maximum drug exposure.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

Appendix A. Supplementary data

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