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Research paper

Field trial assessment of ivermectin pharmacokinetics and efficacy against susceptible and resistant nematode populations in cattle

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ABSTRACT

The study compared the pharmacokinetic (PK) behaviour and anthelmintic efficacy against susceptible and resistant nematodes following subcutaneous (SC) and oral administration of ivermectin (IVM) to cattle. Six commercial farms were involved: Farms 1 and 2 (IVM-susceptible nematode population) and Farms 3, 4, 5 and 6 (IVM-resistant nematode population). On each farm, forty-five calves naturally infected with gastrointestinal (GI) nematodes were randomly allocated into three groups (n = 15): untreated control, IVM SC administration, and IVM oral administration (both at 0.2 mg/kg). PK assessment (plasma and faeces) was performed on Farm 1. Efficacy was determined by Faecal Egg Count Reduction Test. IVM systemic availability upon SC administration (421 ± 70.3 ng·d/mL) was higher ($P < 0.05$) compared to the oral treatment (132 ± 31.3 ng·d/mL). However, higher ($P < 0.05$) faecal IVM concentrations were observed following oral treatment (9896 ± 1931 ng·d/mL) compared to SC administration (4760 ± 924 ng·d/mL). Similar (91–93%) IVM efficacy was observed on Farms 1 and 2 by both routes. Efficacy against resistant nematodes was slightly higher on Farms 3 and 4 after the oral (63 and 82%, respectively) compared to the SC (36 and 68%, respectively) treatment. However, there was complete therapeutic failure (0% efficacy) on Farm 5 and a very low response on Farm 6 (40 and 41% for SC and oral administration, respectively). Although larger faecal concentrations following IVM oral administration may increase drug exposure of GI adult worms, this does not always improve efficacy against resistant nematodes. The potential therapeutic advantages of oral treatments should be cautiously assessed, especially in presence of anthelmintic resistance.

1. Introduction

Parasite control is necessary to ensure adequate production levels in grazing systems of cattle production, given that gastrointestinal (GI) nematodosis is a major cause of economic losses in these systems throughout the world. In Argentina, anthelmintics have been used systematically for many years to control GI parasitism. However, using anthelmintics in this way has not been sustainable because it has led to the current environment of anthelmintic resistance (Kaplan and Vidyashankar, 2012). Results of a study conducted on sixty-two beef farms in Argentina, in which beef production was based on pasture grazing, showed that 95% of the farms presented anthelmintic resistance to either ivermectin (IVM) or ricobendazole (RBZ) (the sulphoxide derivative of albendazole) (Cristel et al., 2017). Resistance to IVM in Argentina has increased from 55% in 2005 (Caracostantogolo et al., 2005) to 94% in 2017 (Cristel et al., 2017). Despite this context, at present, dependence on anthelmintics continues to be high since chemical control is the most practical alternative for parasite control in commercial beef-cattle farms. Therefore, it is essential to search for new strategies to optimize the use of anthelmintics currently available in the veterinary market.

IVM is the most widely used anthelmintic to treat GI nematodes in cattle. It is a broad-spectrum antiparasitic drug that belongs to the macrocyclic lactones family, extensively used in veterinary medicine. IVM is highly effective against adults as well as developing and hypobiotic larvae of most GI nematodes, lungworms (Egerton et al., 1981) and many arthropods in cattle (Campbell et al., 1983). Shortly after the IVM introduction into the veterinary market, when the nematode susceptibility to IVM was high, equivalent efficacies against GI parasites were observed after subcutaneous (SC) and oral administration (Lespine et al., 2005). However, in the current context of anthelmintic resistance, it is essential to search for new strategies to optimize the use of

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IVM, both to minimize the therapeutic failure and to delay the development of resistance. It has been suggested that on farms where resistant nematode populations are present, the oral administration of macrocyclic lactones achieved a greater efficacy compared to the SC injection against GI nematodes in lambs (Gopal et al., 2001; Lloberas et al., 2012). Furthermore, in a recent study performed in horses (Saumell et al., 2017) an excellent (100%) IVM efficacy against small strongyles was observed after its oral administration, while the intramuscular administration resulted in a low efficacy (< 64%). In all cases, the increased parasite drug exposure observed after the oral treatment has been proposed as the reason behind the enhanced efficacy. The administration of anthelmintic drugs by different routes may account for significant differences in the final parasite drug accumulation, since the amount of drug reaching the target parasite is influenced by drug concentration in the tissues/fluids where the parasite is located (Alvarez et al., 2000; Mottier et al., 2006). For that reason, the choice of the IVM administration route should be considered when dealing with IVM-resistant parasites in ruminants. However, the available information about different routes of IVM administration is rather limited in bovine livestock.

In this context, the main goal of the current study was to evaluate the comparative PK behaviour and anthelmintic efficacy against susceptible and resistant nematodes observed after IVM administration by the SC and oral routes to cattle.

2. Material and methods

2.1. Field trials

The current study was conducted on six cattle commercial farms located in the Buenos Aires Province, Argentina. In this way, the study included two farms with predominance of an IVM-susceptible nematode population (Farms 1 and 2) and four farms with an IVM-resistant nematode population (Farms 3, 4, 5 and 6). Farms were selected according to previous reports of susceptibility/resistance to IVM. The resistance status on each farm was previously determined by the faecal egg count reduction test (FECRT). All the farms included in the study had grazing systems of meat production representative of the Argentina bovine production.

2.2. Animals

Forty-five (45) male Aberdeen Angus calves aged 9–11 months old and naturally infected with GI nematodes either susceptible or resistant to IVM were involved in this trial. On day -1, all calves were checked for worm egg per gram (EPG) counts, ear tagged and the individual body weights were recorded prior to treatment. The animals were selected based on EPG counts. Only animals with at least 180 EPG on day -1 were included in the study. Experimental animals had an average of 477 ± 125 EPG counts.

All the animals had free access to water. Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy (act 12/2013) of the Facultad de Cs. Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina.

2.3. Treatments

On each farm, all parasitized animals (n = 45) were ranked according to EPG counts and then assigned into three groups of 15 animals each: Untreated control: animals not receiving anthelmintic treatment; IVMsc: animals were treated with IVM (Ivomec®, 1% solution, Merial, Argentina) by the SC route at a dose of 0.2 mg/kg; IVMoral: animals were treated with IVM (Ivomec®, 1% solution, Merial, Argentina) by the oral route at the same dose used for the SC treatment. In both treatments, the same IVM formulation was used since there is no specific commercial formulation of IVM in Argentina intended for oral administration in cattle. The mean EPG at day -1 were similar ($P > 0.05$) across all groups.

2.4. PK trial

The PK trial was carried out on Farm 1. Eight randomly selected animals from each treated groups (IVMsc or IVMoral) were used in the PK trial. Blood samples (10 mL) were taken from the jugular vein in heparinised Vacutainer® tubes (Becton Dickinson, NJ, USA) prior to treatments and at 3, 6, 9, 12, 16 and 24 h and 2, 3, 4, 7, 9, 12 and 25 days post-treatment. Additionally, faecal samples were individually collected directly from the rectum in plastic tubes at 1, 2, 3, 4, 7, 9, 12 and 25 days post-treatment. Plasma was separated by centrifugation at 3000 *g* for 15 min and placed into plastic tubes. Both samples (plasma and faeces) were frozen at −20 °C until analysis by High Performance Liquid Chromatography (HPLC).

2.5. Anthelmintic efficacy trial: faecal egg count reduction test and coprocultures

Faecal samples were individually collected from the rectum of each calf pre-treatment (day -1) and on day 15 post-treatment. EPG counts were performed by a modified McMaster technique with a sensitivity of 10 EPG (Roberts and O'sullivan, 1950). Additionally, the genera and species of the nematodes recovered from parasitized calves were determined by the identification of the third-stage larvae (L3) recovered from faecal cultures obtained from each experimental group (MAFF, 1986). L3 were collected by the Baermann technique and 100 larvae were differentiated from each sample. Thus, the relative participation of each genus per experimental group was determined.

The anthelmintic efficacy of the different treatments was assessed by the FECRT, calculated according to the formula recommended by the WAAVP (Coles et al., 1992):

FECRT (%) = 100 x (1-[T2/ (C2)]

where T2 is the arithmetic mean EPG count in the treated group at 15 days post-treatment, and C2 is the arithmetic mean EPG count in the control group at 15 days post-treatment. The 95% confidence intervals were calculated as reported by Coles et al., (1992). In addition, efficacy against different genera was calculated by partitioning the mean faecal egg count of the control group and each treatment group, by the proportion of L3 of each genus in the corresponding coproculture (McKenna, 1990).

2.6. Analytical procedures

The extraction of IVM from spiked and experimental plasma/faecal samples was carried out following the technique earlier described by Alvinerie et al. (1993) and adapted by Lifschitz et al. (1999). IVM concentrations were determined by HPLC using a Shimadzu 10 A-HPLC system with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan). There was no interference of endogenous compounds in the chromatographic determinations. Calibration curves were prepared in the range between 0.2–5 ng/mL and 5-100 ng/mL-g and 1-5 µg/g. The limit of quantification (LOQ) was established at 0.2 ng/mL-g. The linear regression lines for IVM showed correlation coefficients of 0.999 for plasma and 0.982 for faeces.

2.7. IVM analysis in plasma

Plasma samples (1 mL) were spiked with internal standard (IS) abamectin (ABM). After addition of 1 mL of acetonitrile, samples were mixed for 15 min (multi-tube vortexer, VWR Scientific Products, West Chester, PA, USA) and then centrifuged at 2000 *g* for 15 min (Jouan®,

BR 4i Centrifuge, Saint Herblain, France). The supernatant was recovered and transferred into a Supelclean LC_{18} cartridge (RP-18, 100 mg, Strata®, Phenomenex, CA, USA) previously conditioned. After washing with deionized water (1 mL) and followed by 1 mL water–methanol (4:1 v/v), the cartridges were dried off for 5 min. Finally samples were eluted with methanol (1.5 mL) and concentrated to dryness under a stream of nitrogen. The resuspension was carried out with a solution of N-methylimidazole (Sigma Chemical, St. Louis, MO, USA) in acetonitrile (1:1) (De Montigny et al., 1990). Derivatization was initiated by adding trifluoroacetic anhydride (Sigma Chemical, St Louis, MO, USA) solution in acetonitrile (1:2). Finally, an aliquot of this solution was injected directly into the chromatographic system. The mean recovery percentages for concentrations ranging between 0.2 and 100 ng/mL (n = 6) was 79% with a coefficient of variation (CV) of 10.1%.

2.8. IVM analysis in faecal samples

Faecal sample (0.5 g) was homogenized with water and after spiking with IS, the samples were added to 1 mL of acetonitrile and mixied for 10 min. The batch of tubes containing the mixtures was placed in an ultrasonic bath (Ultrasound Bath, Lab-Line Instrument, Inc., Melrose Park, OL, US) for 10 min and then centrifuged at 2500 *g* for 15 min. The supernatants were recovered and the precipitates obtained from the samples were extracted again with 1 mL of acetonitrile as described above.

The collected supernatants were joined and added an equal part of water, and the samples were injected into a Supelclean LC_{18} cartridge (RP-18, 100 mg, Strata®, Phenomenex, CA, USA), previously conditioned. The samples were washed, eluted, concentrated and resuspended as previously described for plasma samples. Derivatization was initiated by adding trifluoroacetic anhydride (Sigma Chemical, St Louis, MO, USA) solution in acetonitrile (1:2). Finally, an aliquot of this solution was injected directly into the chromatographic system. The mean recovery percentage was 68% with CV of 9.3%.

2.9. Pharmacokinetic analysis of the data

The concentration vs. time curves for IVM administered by SC or oral route were adjusted with the PK Solution 2.0 software (Summit Research Service, CO, USA). The peak concentration (C_{max}) and time to peak concentration (T_{max}) were displayed from the plotted concentration-time curve of each analyte. The area under the plasma concentration-time curve from zero up to the quantification time ($AUC_{0\text{-}LOQ}$) was calculated by means of the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (ß). The elimination ($T_{½el}$) and absorption ($T_{½ab}$) half-lives were calculated as ln2/ß and ln2/k, respectively, where ß represent the terminal slope (h^{-1}) and k is the slope obtained by feathering which represents the first order absorption rate constant. The mean residence time (MRT) was determined as AUMC/AUC (Perrier and Mayersohn, 1982) where AUMC is the area under the curve of the product of time and the plasma drug concentration vs. time from zero to infinity (Gibaldi and Perrier, 1982), and AUC is as defined above.

2.10. Statistical analysis of the data

The PK parameters, concentration data and faecal egg counts are reported as arithmetic mean ± standard deviation (SD). Mean PK parameters for IVM obtained after its SC or oral administration were statistically compared using Student *t*-test. Faecal egg counts were compared between treatment groups by non-parametric Kruskal–Wallis test. A value of $P < 0.05$ was considered statistically significant. The statistical analysis was performed using the Instat 3.0 software (Graph Pad Software, CA, USA).

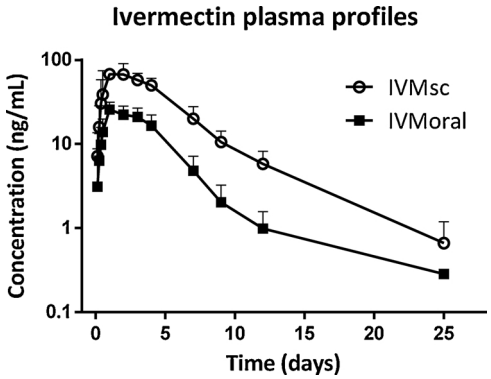

Fig. 1. Comparative mean (± SD) ivermectin (IVM) plasma concentration profiles obtained after its subcutaneous (IVMsc) and oral administration (IVMoral) (0.2 mg/kg) to parasitized calves (n = 8).

3. Results

3.1. Pharmacokinetic study

Fig. 1 presents the mean (± SD) plasma concentration profiles for IVM after its SC and oral administration to parasitized calves. The PK parameters obtained in plasma and faeces after the administration of IVM by both routes are summarizes in Table 1. The IVM plasma disposition kinetics showed significant differences according to the route of administration. Higher IVM plasma concentrations were obtained after the SC treatment, which represents an enhanced systemic availability after this treatment compared to the oral administration. In fact, the $AUC_{0\text{-}LOQ}$ value of IVM obtained after its SC administration (421.0 ± 70.3 ng·d/mL) was significantly higher ($P < 0.05$) than that obtained after the oral treatment (132 ± 31.3 ng·d/mL). Additionally, a higher ($P < 0.05$) peak plasma concentration was observed after the SC (80.6 ± 36.1 ng/mL) compared to the oral (28.7 ± 4.73 ng/mL) IVM administration. No differences in other PK parameters such as T_{max}, $T_{½el}$, $T_{½ab}$ or MRT were observed between the different administration routes.

In contrast to what was observed in plasma, higher IVM faecal concentrations were obtained after the oral treatment (Fig. 2). The greatest differences in the faecal concentrations between both administration routes were detected up-to four days after treatment (Insert of the Fig. 2). In fact, the $AUC_{partial\ (0–4)}$ value of IVM obtained after its oral administration (9170 ± 1985 ng·d/g) was significantly higher ($P < 0.05$) than that obtained after the SC treatment (1527 ± 507 ng·d/g). The comparative PK parameters in faeces obtained after the administration of IVM by both routes are shown in Table 1.

3.2. Parasitological study

Table 2 shows the overall faecal egg counts obtained for all experimental groups (all farms) on day 15 after treatments, including the results of the FECRT and lower and upper confidence limits (95%). On Farms 1 and 2, the FECR for SC and oral treatments were over 90%, demonstrating that the nematode population of these farms was susceptible to IVM.

In contrast, on Farms 3, 4, 5 and 6 the overall efficacies for both routes of administration were below 90% (≤82%), indicating that the animals were infected with IVM-resistant nematodes. Similar (91–93%) anthelmintic efficacy was observed on both farms infected with susceptible nematodes (Farms 1 and 2) after IVM administration by both routes. The IVM efficacy against resistant nematodes populations tended to be higher on

farms 3 and 4 after the oral (63 and 82%, respectively) compared to the SC (36 and 68%, respectively) treatment. However, a similar

Table 1
Plasma and faecal pharmacokinetic parameters (mean ± SD) after ivermectin (IVM) administration by either subcutaneous (SC) or oral routes (0.2 mg/kg) to naturally parasitized calves.

Pharmacokinetic parameters	PLASMA		FAECES	
	IVM SC	IVM ORAL	IVM SC	IVM ORAL
T_{max} (d)	2.12 ± 1.13[a]	1.62 ± 0.92[a]	2.13 ± 0.64[a]	1.38 ± 0.52[b]
C_{max} (ng/mL-g)	80.6 ± 36.1[a]	28.7 ± 4.73[b]	618 ± 270[a]	4866 ± 689[b]
$AUC_{0\text{-}LOQ}$ (ng·d/mL-g)	421 ± 70.3 [a]	132 ± 31.3[b]	4760 ± 924[a]	9896 ± 1931[b]
$AUC_{partial\ (0\text{-}4)}$ (ng·d/mL-g)	n.d.	n.d.	1527 ± 507[a]	9170 ± 1985[b]
$AUC_{0\text{-}\infty}$ (ng·d/mL)	425 ± 68.7 [a]	134 ± 31.1[b]	n.d.	n.d.
MRT (d)	5.10 ± 1.44[a]	4.10 ± 0.54[a]	n.d.	n.d.
$T_{½abs}$ (d)	0.56 ± 0.27[a]	0.39 ± 0.23[a]	n.d.	n.d.
$T_{½el}$ (d)	3.34 ± 0.75[a]	3.44 ± 0.20[a]	n.d.	n.d.

T_{max}: time to peak concentration; C_{max}: peak concentration; $AUC_{0\text{-}LOQ}$: area under the concentration vs. time curve from 0 to the limit of quantification; $ABC_{partial\ (0–4)}$: area under the concentration vs. time curve from 0 to 4 days post-treatment; $AUC_{0\text{-}\infty}$: area under the concentration-time curve extrapolated to infinity; **MRT**: mean residence time; $T_{½abs}$: absorption half-life; $T_{½el}$: elimination half-life. n.d.: not determined.
Pharmacokinetic parameters with different superscript are statistically different at $P < 0.05$.

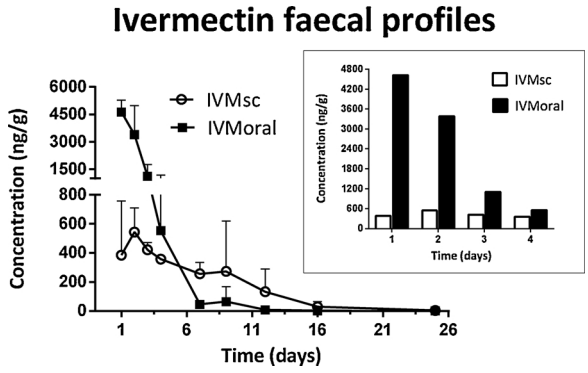

Fig. 2. Comparative mean (± SD) ivermectin (IVM) faecal concentration profiles obtained after its subcutaneous (IVMsc) and oral administration (IVMoral) (0.2 mg/kg) to parasitized calves (n = 8). The insert shows the IVM faecal concentrations up-to 4 days post-treatment.

nematode control after the administration of IVM by both routes was obtained on Farm 5 and 6, where animals were infected with IVM-resistant nematodes. While there was a complete therapeutic failure (0% efficacy) for both routes on Farm 5, a very low response to both treatments was observed at farm 6 (40 and 41% for SC and oral administration, respectively). However, no significant differences in the overall efficacy were found between the SC and oral treatments, neither in susceptible nor in resistant scenarios ($P > 0.05$).

The anthelmintic efficacies against *Cooperia* spp., *Haemonchus* spp., *Ostertagia* spp., *Oesophagostomum* spp. and *Trichostrongylus* spp. for the different treatments are shown in Table 3. Although both routes of administration achieved an adequate overall efficacy in the susceptible scenarios (Farms 1 and 2), the IVM treatment did not show effective control against all the GI nematode. In fact, on these farms (1 and 2) IVM administered by SC and oral route failed to control *Cooperia* spp. (FECR below 90% for both treatments). Regarding resistant scenarios, on farms where the overall efficacy was higher for the oral administration of IVM (Farms 3 and 4), *Cooperia* spp. was the only genus resistant to IVM (< 90% FECR for both routes). Instead, on the farms where the total FECR were similar for SC and oral treatments (Farms 5 and 6), IVM failed to control *Cooperia* spp. and *Haemonchus* spp., indicating IVM resistance in both genera. On Farm 6, differences were observed between both routes of administration against IVM-resistant *Haemonchus* spp., showing efficacies of 0% and 76% for the SC and oral treatment, respectively. IVM administered by both routes achieved an effective control against *Ostertagia* spp., *Oesophagostomum* spp. and *Trichostrongylus* spp. in all susceptible/resistance scenarios considered in the current study.

Table 2
Nematode egg per gram counts (EPG, arithmetic mean) and reduction percentages of faecal egg counts (FECR) (undifferentiated) with its lower and upper confidence intervals 95%, after the subcutaneous (SC) and oral administration of ivermectin (IVM, 0.2 mg/kg) to naturally parasitized calves.

Farm	Nematode population	EPG Counts (range)						FECRT[1] (CI)	
		Day -1			Day 15			IVMsc	IVMoral
		CONTROL	IVMsc	IVMoral	CONTROL	IVMsc	IVMoral		
FARM 1	IVM-SUSCEPTIBLE	**485**[a] (220-1060)	**508**[a] (260-1480)	**483**[a] (220-1200)	**277**[a] (120-680)	**20**[b] (0-100)	**24**[b] (0-100)	**93%** (81-97)	**91%** (74-97)
FARM 2	IVM-SUSCEPTIBLE	**405**[a] (180-1280)	**377**[a] (180-1440)	**523**[a] (200-1040)	**324**[a] (60-760)	**23**[a] (0-140)	**28**[b] (0-140)	**93%** (81-97)	**92%** (78-97)
FARM 3	IVM-RESISTANT	**357**[a] (220-700)	**345**[a] (220-740)	**363**[a] (180-780)	**251**[a] (80-1200)	**159**[a] (20-540)	**94**[a] (0-220)	**36%** (0-71)	**63%** (25-81)
FARM 4	IVM-RESISTANT	**477**[a] (180-1200)	**351**[a] (180-660)	**333**[a] (180-780)	**343**[a] (80-1060)	**111**[ab] (0-320)	**65**[b] (0-160)	**68%** (31-85)	**82%** (64-91)
FARM 5	IVM-RESISTANT	**590**[a] (180-1320)	**643**[a] (180-2200)	**619**[a] (180-1920)	**500**[a] (80-1460)	**509**[a] (0-1380)	**499**[a] (20-1440)	**0%** (0-46)	**0.3%** (0-42)
FARM 6	IVM-RESISTANT	**435**[a] (180-1060)	**797**[a] (220-1660)	**503**[a] (180-1600)	**561**[a] (260-1500)	**337**[a] (0-1400)	**330**[a] (180-1000)	**40%** (0-73)	**41%** (2-65)

CI: lower and upper confidence intervals. EPG counts with different superscript letters are statistically different ($P < 0.05$). FECRT (%) for all treatments, $P > 0.05$.
[1] FECRT estimated according to Coles et al., (1992).

Table 3
Reduction percentages of faecal egg counts (FECRT) for *Cooperia, Haemonchus*, *Ostertagia*, *Oesophagostomum* and *Trichostongylus* spp. (based on egg counts partitioned to genera using the proportion of each genus recovered as larvae from faecal larval cultures) after the subcutaneous (SC) and oral administration of ivermectin (IVM, 0.2 mg/kg) to naturally parasitized calves.

Genus - Treatment	FECRT[a] Day 15					
	IVM-SUSCEPTIBLE NEMATODE POPULATION		IVM-RESISTANT NEMATODE POPULATION			
	FARM 1	FARM 2	FARM 3	FARM 4	FARM 5	FARM 6
***Cooperia* spp.**	78%	85%	0%	56%	0%	47%
IVMsc	74%	83%	36%	74%	0%	23%
IVMoral						
***Haemonchus* spp.**	100%	100%	94%	100%	0%	0%
IVMsc	100%	100%	97%	100%	5%	76%
IVMoral						
***Ostertagia* spp.**	100%	100%	100%	100%	100%	97%
IVMsc	100%	91%	100%	100%	100%	91%
IVMoral						
***Oesophagost.* spp.**	100%	100%	100%	100%	100%	–
IVMsc	100%	100%	100%	100%	100%	–
IVMoral						
***Trichostrong.* spp.**	–	–	100%	–	–	–
IVMsc	–	–	100%	–	–	–
IVMoral						

[a] FECR estimated according to Coles et al., (1992).

4. Discussion

The main goal of the current study was to evaluate the comparative PK behaviour and anthelmintic efficacy against susceptible and resistant nematodes for IVM administered by two different routes to cattle: oral or SC injection. At present, dependence on anthelmintics continues to be high since chemical control is the most practical alternative for parasite control on commercial beef-cattle farms. The lack of development of new molecules leads to search for new strategies to optimize the use of anthelmintics currently available in the veterinary market. In this sense, different strategies have been proposed to manage anthelmintic resistance and delay its development. For example, drug combination of two or more anthelmintic compounds (Geary et al., 2012) or different alternatives to increase GI nematodes exposure to the drug such as the choice of the route of administration (Gopal et al., 2001; Lespine et al., 2005; Lloberas et al., 2012). In general, the routes by which anthelmintics are administered are influenced by practical management reasons or by the technical marketing of the pharmaceutical companies (McKellar and Gokbulut, 2012), in such a way that sheep and goats are generally treated orally, but cattle by either parenteral or pour-on (topical) (Leathwick and Besier, 2014). However, the election of the route of administration is a pharmacological tool that could determine a higher parasite-drug exposure, and thus, a higher anthelmintic efficacy. The potency of an anthelmintic depends both on its affinity for a specific receptor (site of action) and on the kinetic properties that facilitate achievement of effective drug concentrations at the site of action (Lanusse et al., 2014).

From a practical point of view, the administration route determines the pharmacokinetics of macrocyclic lactones (Lifschitz et al., 2017). In fact, previous PK studies performed in different animal species such as sheep (Marriner et al., 1987; Lloberas et al., 2012), goats (Gokbulut et al., 2007), horses (Pérez et al., 2003; Saumell et al., 2017) and dogs (Gokbulut et al., 2006), have shown that the plasma disposition of IVM is affected by the route of administration. Regarding commercial anthelmintic formulations, while in the current study cattle were treated orally with the injectable formulation of IVM, Leathwick and Miller (2013) used an oral formulation indicated for small ruminants to treat cattle orally. In both cases an off-label use of the commercial formulations was necessary because there are no IVM or MXD oral products registered for use in cattle neither in New Zealand nor in Argentina. Although the off-label administration was only used for experimental purposes, it is expected that the use of an IVM injectable formulation by the oral route would not have a significant impact on its pharmacokinetics and clinical efficacy. In fact, in a similar study carried out in horses (Saumell et al., 2017), a similar extent of absorption, plasma disposition and elimination pattern were observed after the oral administration of IVM either as a paste (Eqvalan®, Merial, Argentina, used as reference formulation) or as a solution (Remonta®, División de Remonta y Veterinaria, Ejército Argentino, Argentina, considered the test formulation) despite the different composition of their excipients/vehicles. However, off-label use of any anthelmintic should not be encouraged for using on commercial cattle farms.

The IVM systemic availability was higher after the SC administration compared to that measured after the oral treatment (Table 1), as it was previously described in the above mentioned animal species. Although published information about both routes of administration in cattle is scarce, similarly to what was observed in the present study, Leathwick and Miller (2013) found plasma levels of moxidectin (MXD) were significantly higher after SC injection compared with oral treatment in cattle. The higher IVM peak plasma concentration observed in the SC treated group and a similar elimination half-life observed between both experimental groups accounted for an enhanced systemic availability after the parenteral administration, which represents an increase of 218% compared to the oral administration. The high association of IVM to the particulate material of digesta appears as a relevant factor modulating the IVM gastrointestinal absorption process, which may also reduce the amounts of drug absorbed after oral administration (Lifschitz et al., 2005). This may be a likely explanation for the lower systemic availability of IVM administered orally, compared to the SC administration. In contrast to what was observed in plasma, markedly higher IVM concentration profiles were observed in faeces collected from orally treated calves (Fig. 2). The greatest differences in the faecal concentrations between both routes were detected up-to four days after treatment, given that after this period the faecal concentrations tend to be similar for both SC and oral administrations (Insert of the Fig. 2). The larger IVM concentrations excreted in faeces reflect the highest concentration achieved at the GI level after the oral administration compared to the SC injection. These results are in agreement with those previously reported for IVM in sheep (Lloberas et al., 2012), in which enhanced abomasal/intestinal IVM concentrations were recovered from orally treated sheep compared to the SC treatment. These greater concentrations measured in the abomasal content after the oral

administration explain the highest amount of IVM in *H. contortus* recovered from sheep treated by the same route (Lloberas et al., 2012). Thus, accumulation of the drug in "target" parasites is directly related to the concentration of the anthelmintic present in the environment where the nematodes are located, for example the GI tract. In this sense, the anthelmintic action depends on the ability of the active drug to reach specific receptors within the GI parasites (Lanusse et al., 2015). Moreover, it seems that the transcuticular diffusion is the main route of access for different anthelmintics, including IVM in gastrointestinal nematodes, as is reported in the literature (Geary et al., 1995; Ho et al., 1990). Thus, drug entry and accumulation into target helminths are important issues to optimize anthelmintic efficacy (Alvarez et al., 2007). The IVM concentrations achieved at the GI level after the oral treatment may have accounted for a greater exposure of the GI located adult worms and, in this way, for a higher IVM accumulation within GI nematodes. Hence, the route of administration-related tendency to improved efficacy should be considered particularly when dealing with IVM-resistant worms in cattle.

The results of the PK assessment are related to the findings of the parasitological study. Shortly after the IVM introduction into the veterinary market, when the nematode susceptibility to IVM was high, equivalent efficacies against GI parasites were observed after SC and oral administration in sheep/goats (Lespine et al., 2005). Similarly, on Farms 1 and 2 of the current study, anthelmintic efficacies of 93% (SC) and 91–92% (oral) were obtained; confirming that both routes of administration achieved similar efficacy against susceptible GI nematodes.

However, a different picture was observed when a resistant nematode population was involved. As shown in Table 2, the oral administration improved the FECR of IVM on Farms 3 and 4. Although published information about both routes of administration in cattle is limited, Leathwick and Miller (2013) reported a significantly higher efficacy after oral treatment of MXD (91.1%) than after its SC injection (55.5%) or pour-on administration (51.3%). Furthermore, in this study oral administration of MXD resulted in higher overall efficacy and significantly less variability in efficacy than when the same anthelmintic was administered as a SC injection or as a pour-on treatment. Similar efficacy trends were observed after comparison of ABM administered by oral and topical routes to cattle, with efficacies of 83% and 35%, respectively, supporting the advantage of the oral route (Leathwick et al., 2016). Overall, all these studies in cattle have shown that oral administration of macrocyclic lactones resulted in higher anthelmintic efficacy and significantly less variability in efficacy than when the same active was administered as a SC or as a pour-on treatments. However, not all of the results following oral treatments against resistant parasites are promising, given that on Farms 5 and 6 of the current study anthelmintic efficacies did not improve after the oral administration of IVM. Similar results were reported in feedlot cattle, where IVM administered by SC and oral route obtained equivalent therapeutic response (FECR of 40% and 45%, respectively) against resistant GI nematodes (Galvan et al., 2016).

In the face of the variability in the efficacies presented above, it should be considered that GI parasitism in cattle always involves different parasite genera. In this sense, although the total anthelmintic efficacies were above 90% on Farms 1 and 2, *Cooperia* spp. resistant to IVM were found on both farms (Table 3). The efficacies against *Cooperia* spp. were below 90% after both SC and oral treatments. In fact, the results of the anthelmintic efficacies against *Cooperia* spp. demonstrated that this genus was always present in the cases of IVM resistance. Similarly, Cristel et al. (2017) found resistant *Cooperia* spp. in the 100% of the farms where resistant to IVM were present. Since *Cooperia* spp. is a dose limiting species for IVM, this is the genus in which IVM resistance would be first expected (Lanusse et al., 2013). Comparing farms with predominance of an IVM-resistant nematode population included in the current trial, differences were found in the resistant parasite genera involved. In fact, on Farms 3 and 4, in which the total efficacy improved after the oral treatment, *Cooperia* spp. was the only genus resistant to IVM. Likewise, other studies in cattle also reported an enhanced efficacy for the oral administration of IVM/MXD against resistant *Cooperia* spp. (Leathwick and Miller, 2013; Pomroy et al., 2004). Instead, on farms where both routes of administration obtained similar nematode control against resistant parasites (Farms 5 and 6), IVM failed to control *Cooperia* spp. and *Haemonchus* spp., indicating IVM resistance in both genera. Again, these results are in agreement with the findings of Galvan et al. (2016), who did not find an increase in the efficacy of IVM after its oral administration against resistant *Cooperia* spp. and *Haemonchus* spp. Similar studies in sheep reported variable results since Lloberas et al. (2012) found a greater efficacy of IVM against resistant *H. contortus* after the oral treatment, but Barnes et al. (2001) did not observe an enhanced efficacy after using the same route of administration against resistant *H. contortus*. Regarding *Ostertagia* spp., similarly to previous studies (Galvan et al., 2016; Leathwick and Miller, 2013), IVM administered by both SC and oral routes achieve a high efficacy in all the scenarios evaluated in the present study. In agreement with these results, Cristel et al. (2017) did not find *Ostertagia* spp. resistant to IVM in Argentina. However, in other regions of the world such as New Zealand, there are reports of *Ostertagia* spp. resistant to IVM in cattle (Waghorn et al., 2016). For this reason, it is essential to rationally use IVM to control *Ostertagia* spp. in Argentina, in order to avoid arriving at the same anthelmintic resistance situation reported for *Cooperia* spp. and *Haemonchus* spp.

Overall, oral administration of IVM may obtain a greater efficacy against GI nematodes compared to SC treatment in cattle. The larger IVM concentrations achieved at the GI level (measured as drug excreted in faeces) after the oral administration compared to the SC injection, may have accounted for a greater exposure of the GI located adult worms. The results obtained in the present study demonstrated that IVM concentrations at the GI level are relevant to anthelmintic accumulation within GI nematodes. In this sense, Leathwick and Luo (2017) demonstrated that routes of administration resulting in lower parasite drug exposure are more likely to select for anthelmintic resistance. Therefore, most of the studies in cattle and sheep indicate that oral administration may be the most efficient at achieving greater exposure of GI parasites, lower variable concentrations of active reaching the GI worms, higher anthelmintic efficacy and hence lower selection for anthelmintic resistance. However, both previous studies and the findings of the current trial showed that the oral administration of IVM does not always have an impact on the anthelmintic efficacy. The potential therapeutic advantages of the oral treatments should be cautiously assessed, especially considering the presence of anthelmintic resistance and the involved nematode population. The results of the current trial confirm that understanding the resistance status on individual farms is essential before applying new strategies to optimize the parasite control on commercial cattle farms.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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