Veterinary Parasitology 266 (2019) 73–79

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Pharmacokinetic profile and anthelmintic efficacy of moxidectin administered by different doses and routes to feedlot calves

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ARTICLE INFO

Keywords:
Moxidectin
Resistance
Cattle
Pharmacokinetics
Efficacy
Feedlot

ABSTRACT

We evaluated the comparative plasma disposition kinetics and efficacy of moxidectin (MXD), administered by the intraruminal (IR) or subcutaneous (SC) route at two different dosage levels (0.2 and 1 mg/kg) in feedlot calves. Additionally, the efficacy was compared to an ivermectin (IVM, SC administration) treated group. This study was divided into two separate studies, the "Pharmacokinetic (PK) study" and the "Efficacy study". The "PK study" involved 24 calves free of gastrointestinal nematodes (GIN), which were allocated into 4 groups (n = 6) and treated with MXD by either the SC or the IR route at the therapeutic ($MXD_{SC0.2}$, $MXD_{IR0.2}$, respectively) or at fivefold the therapeutic dose ($MXD_{SC1.0}$, $MXD_{IR1.0}$, respectively). Blood samples were collected from 3 h up to 14 days post-treatment. MXD concentrations in plasma samples were analyzed by HPLC. The "Efficacy study" included 125 calves naturally infected with GIN, which were allocated into five experimental groups (n = 25 each); the same four MXD-treated groups described for the "PK study", and an additional group treated by the SC route with IVM ($IVM_{SC0.2}$). The efficacy of IVM given at its therapeutic dose and the different MXD groups at the therapeutic and fivefold the therapeutic dose was calculated by analysis of the individual efficacy using the package eggCounts-2.1-1' on the R software environment, version 3.5.0 (R Core Team, 2018). Daily weight gain (DWG) was also measured over the first 47 days of the fattening cycle. Independently of the administration route, MXD peak plasma concentration (C_{max}) and area under the concentration-time curve (AUC) were higher in groups treated with the higher dose (1.0 mg/kg), whereas a longer time to reach C_{max} (T_{max}) was observed after the IR treatments. The observed MXD efficacies were 85% ($MXD_{SC0.2}$), 94% ($MXD_{SC1.0}$), 84% ($MXD_{IR0.2}$) and 99% ($MXD_{IR1.0}$), at day +27. At day +27, all MXD-treated groups showed higher efficacies than the group having received IVM (45%). The post-treatment *Cooperia* spp. L_3 counts were particularly low in the groups $MXD_{SC1.0}$ and $MXD_{IR1.0}$. All of the groups treated with MXD showed better DWG than the $IVM_{SC0.2}$ group (P = 0.01). Dose and administration route modifications effectively improved the anthelmintic and productive performance of MXD. A high dose of MXD improved the control of IVM-resistant GIN in feedlot calves. However, this practice must be taken with caution, since MXD resistance could rapidly emerge, especially in grazing cattle.

1. Introduction

Infections with gastrointestinal nematodes (GIN) can lead to productive losses and negatively impact the product quality of affected calves (Kaplan, 2004). Furthermore, anthelmintic resistance (AR) of GIN to macrocyclic lactones (ML) is an increasingly widespread trend that limits livestock industry worldwide (Kaplan and Vidyashankar, 2012). In Argentina, 1.6 million animals per year are fattened (finished) in feedlot systems (Arelovich et al., 2011). Drugs from the ML group, mainly ivermectin (IVM), have been widely adopted in feedlots due to their broad-spectrum activity against nematodes and arthropods (Nessel et al., 1989). Unfortunately, the frequent use of IVM, particularly in cattle grazing on *Rhipicephalus microplus* habitats, has led to an increase in the prevalence of GIN resistance (Fiel et al., 2005; Cristel et al., 2017). Although GIN outbreaks are not frequently reported in feedlot cattle (Coles, 2002), AR is an increasingly serious concern in this

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https://doi.org/10.1016/j.vetpar.2018.12.016
Received 4 July 2018; Received in revised form 26 December 2018; Accepted 29 December 2018

productive system, mostly through subclinical losses. Up to 8.3% weight loss was observed in cattle parasitized with ML-resistant GIN after IVM treatment compared to calves free of GIN, thus leading to an extension of the fattening cycle (Fazzio et al., 2012, 2014). This problem has motivated the search for alternatives, including the development of new active compounds, the use of drug combinations, and the study of alternate doses and administration routes (reviewed by Lanusse et al., 2018). Several strategies have been proposed to improve the performance of ML and avoid post-treatment residual nematode burdens.

Moxidectin (MXD) is a milbemycin ML with a mechanism of action and anthelmintic spectrum similar IVM (Prichard et al., 2012). However, some pharmacodynamic and pharmacokinetic differences have been documented, mainly attributed to its higher lipophilicity, which leads to a better efficacy profile (Prichard et al., 2012; Lloberas et al., 2013). Several strategies have been assessed to improve ML efficacy against resistant GIN, such as using the oral versus the subcutaneous (SC) route (Gopal et al., 2001; Lespine et al., 2005; Lloberas et al., 2012; Leathwick and Miller, 2013; Leathwick et al., 2016; Saumell et al., 2017; Canton et al., 2018) and increasing the IVM dosage levels (Alvarez et al., 2015; Lloberas et al., 2015), among others. On the other hand, a small number of reports deal with the use of alternative administration routes or increased dosage regimens to control resistant GIN in cattle. Leathwick and Miller (2013) found a significantly higher efficacy following oral treatment with MXD (91.1%) than following its SC injection (55.5%) or pour-on administration (51.3%). Similar results have recently been reported in cattle (Leathwick et al., 2016; Canton et al., 2018). However, although Fazzio et al. (2016) reported that fecal egg count reduction (FECR) and daily weight gain (DWG) were increased in MXD-treated compared to untreated control cattle, efficacy remained under 90%, which is considered the threshold for an adequate GIN control (Coles et al., 1992).

The impact of increasing the dose of MXD on its systemic exposure and resultant efficacy against IVM-resistant nematodes in cattle remains unclear. In this context, the goal of the current study was to compare the pharmacokinetic (PK) behavior and anthelmintic efficacy of MXD administered by different routes and at different doses to feedlot cattle naturally infected with IVM-resistant GIN.

2. Materials and methods

All of the experimental procedures were approved by the Institutional Animal Care and Use Committee (CICUAL, after its Spanish acronym), Faculty of Veterinary Sciences, National University of La Plata, Argentina (Protocol N º 56-6-16 P). Any unusual behavior such as depression, ataxia or prostration, was recorded as a potential sign of toxic effects induced by treatment.

2.1. Experimental design

This study was divided into two separate studies as follows:

2.1.1. PK study

The animal phase of this study was carried out during May 2016, at the Instituto Tecnológico Chascomús (INTECH), National University of San Martín (USAM), CONICET, Argentina. Twenty-four GIN-free (measured by individual fecal egg counts), crossbred *Bos indicus* x *Bos taurus* female calves (196 ± 22 kg) were randomly assigned to four groups (n = 6): $MXD_{SC0.2}$, animals were treated with MXD (Cydectin alfa®, Fort Dodge, Argentina) by the SC route at its therapeutic dose (0.2 mg/kg); $MXD_{SC1.0}$, animals were treated with MXD by the SC route at five times the therapeutic dose (1.0 mg/kg); $MXD_{IR0.5}$, animals were treated with MXD by the intraruminal (IR) route at its therapeutic dose (0.2 mg/kg); and $MXD_{IR1.0}$, animals were treated with MXD by the IR route at five times the therapeutic dose (1.0 mg/kg). Blood samples were taken from the jugular vein and collected in EDTA K2 tubes at times 0 (immediately before treatment), 3, 5, and 10 h, and 1, 3, 6, 10, and 14 days post-treatment. Samples were centrifuged (15 min at 2000 xg) and the plasma thus obtained was frozen at -20 °C until analysis by HPLC.

2.1.2. Efficacy study

The study was carried out from June to August 2015 in a commercial feedlot farm located in Buenos Aires, Argentina (-34.7968 S, -58.9002 W). Crossbred *Bos indicus* x *Bos taurus* female calves (n = 174) naturally infected with GIN resistant to IVM were involved in this study. In previous studies (Fazzio et al., 2012, 2014, 2016; Galvan et al., 2016), the parasite burdens´ composition was dominated by the genera *Cooperia* and *Haemochus*, regardless of the sampling season. The animals arrived from an extensive commercial farm in Esquina, Corrientes, Argentina (-30.0173 S, -59.5496 W), which is located 900 km north of Buenos Aires and where ML AR had been previously demonstrated (Fazzio et al., 2014; 2016). After arrival (day -5), calves remained in pens with free access to hay and water. The trial started at day -3. Sanitary treatments included a single dose of clostridial polyvalent vaccine (Policlostrigen®, Biogenesis-Bagó, Argentina), a broad-spectrum injectable antibiotic (Tilmicosin, Maxityl®, Biogenesis-Bagó, Argentina), and ear tagging. The calves were individually sampled for feces and the individual weights (196 ± 22 kg) were registered. From day 0 (treatment) to the end of the study, the animals´ diet was based on corn grain, sunflower meal, wheat bran, and the addition of vitamin and mineral supplements. Protein and fiber accounted for 15% and 25%, respectively, of the calves' diet for the first 27 days, and then gradually changed to a finishing diet containing 12% protein and 8% fiber.

Based on their individual weight and worm egg per gram (EPG) counts, 125 female calves were selected, and randomly allocated into five groups of 25 animals each. Four groups received the same MXD treatments as described for the “PK study” and an additional group was included in the “Efficacy study” ($IVM_{SC0.2}$ group), in which animals were treated with IVM (Ivomec®, Merial, Argentina) at its therapeutic dose (0.2 mg/kg) by the SC route. EPG counts were carried out by the modified McMaster method, where each counted egg represented 10 eggs/g of faeces (Roberts and O'sullivan, 1950). Pooled coprocultures were performed before (day -3) and after (days +14 and +27) treatments, in order to assess the relative contribution of each nematode genus. The third stage larvae (L_3) identification was made following the descriptions of Niec (1968), and Van Wyk et al. (2004). Individual weight was registered on days 0 (treatment), +27 and +47 (post treatment).

2.2. Analytical procedures

2.2.1. Sample extraction

Spiked and experimental plasma samples were extracted to quantify MXD. Plasma aliquots (0.5 mL) with 0.125 mL of water were combined with 0.5 mL of acetonitrile. After shaking for 15 min (Multi-tube Vortexer; VWR Scientific Products, West Chester, PA, USA), samples were centrifuged (15 min at 2000 xg). The supernatant was transferred to a C18 cartridge (100 mg/mL, Strata C18-T, Phenomenex) for solid phase extraction using a vacuum manifold (Baker spe-24 G). The cartridges were previously conditioned with methanol (2 mL) followed by water (2 mL), both HPLC grade. After applying samples to the cartridges, they were sequentially washed with water (1 mL) and methanol/water (1:4) (1 mL), dried with air for 5 min, and eluted with HPLC grade methanol (1.5 mL). The eluted solvent was evaporated to dryness in a vacuum concentrator (Speed-Vac, Savant, Los Angeles, CA, USA). The dry sample was reconstituted in N-methylimidazole/acetonitrile solution (1:1 v/v) (100 µL) and derivatized by adding trifluoroacetic anhydride/acetonitrile solution (1:2 v/v) (150 µL). An aliquot of 100 µL was injected into the chromatographic system.

2.2.2. HPLC quantification

MXD was determined by HPLC (Shimadzu chromatography system, Shimadzu Corp., Kyoto, Japan) with spectrofluorometric detection (Detector RF 10, Shimadzu) following the methodology previously described by Lifschitz et al. (1999). Excitation and emission wavelengths were 365 and 475 nm, respectively. A mobile phase composed of water/methanol/acetonitrile (6:40:54, v/v), and a C18 column (Kromasil 100-5C18, 5 µm, 4.6 × 250 mm) placed in an oven at 30 °C were used. A complete validation of the analytical procedures for the extraction and quantification of MXD in plasma was carried out. The compound was identified by the retention time of pure standard MXD, which was 6.3 min. No interference by endogenous compounds was observed after analysis of blank plasma samples. The linearity of the method was tested by construction of analytical calibration curves with blank plasma samples fortified with MXD (range of calibration: 0.5 − 200 ng/mL). The analyte recovery (extraction efficiency) was determined by comparison of the peak areas from fortified blank plasma samples with the peak areas from equivalent quantities of pure standard. Precision and accuracy (intra- and interday) were determined by analysis of replicates (n = 5) of blank plasma samples fortified with MXD at 0.5, 5, and 50 ng/mL. Precision was expressed as coefficient of variation (% CV). The limit of quantification (LOQ) was determined by the lowest drug concentration (n = 5) on the range of calibration that could be quantified with precision < 20%, an accuracy of ± 20%, and an absolute recovery ≥70%. The analytical calibration curve for MXD in plasma showed a correlation coefficient of 0.998. Mean absolute recovery percentages ranged between 75 and 80%. The interday precision of the method after HPLC analysis of MXD plasma samples showed CV between 8 and 12%. The LOQ was established at 0.5 ng/mL.

2.2.3. HPLC data analysis

MXD plasma concentrations were expressed as ng/mL. The PK parameters and concentration data are reported as mean ± SD. The PK analysis of the plasma concentration *vs.* time curves for MXD, obtained for each animal after both routes of administration, were carried out using the PK Solution 2.0 software (Summit 10 Research Services, CO, USA). The software performs the analysis using non-compartmental (area) and compartmental (exponential terms) methods without assuming any specific compartmental model. The plasma peak concentrations (C_{max}) and time to peak concentration (T_{max}) were read from concentration-time curves. The regression parameters were used to calculate the presented PK parameters. The elimination ($T_{½el}$) and absorption ($T_{½ab}$) half-lives were calculated as ln2/ß and ln2/k, respectively. The area under the concentration–time curves (AUC) were calculated according to the equations of Gibaldi and Perrier (1982). Statistical moment theory was applied to calculate the mean residence time (MRT) in plasma (Gibaldi and Perrier, 1982).

2.2.4. Parasitological analysis

The fecal eggs count reduction (FECR) was calculated at days +14 and +27 using the model described by Torgerson et al. (2014) (http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/). The RESO analysis software (version 4.0, CSIRO, Australia) was used to calculate the efficacy against different genera among groups at days +14 and +27.

2.2.5. Statistical analysis

The PK parameters and concentration data are reported as arithmetic mean ± SD. PK parameters obtained from the different experimental groups were statistically compared using Student t-test. The statistical analysis of the PK data was performed using the Instat 3.0 Software (Graph Pad Software, San Diego, CA, USA).

A general linear model was used to assess the effect of the different treatments on the DWL throughout the experiment. The dependent variable was the DWG of the period 1 (0 through +27), period 2 (+27 through +47) and total weight gain (0 through +47), while the fixed variable was the treatment group. DWG comparison was analyzed by means of IBM-SPSS (version 22). In all cases a P-value < 0.05 was considered statistically significant.

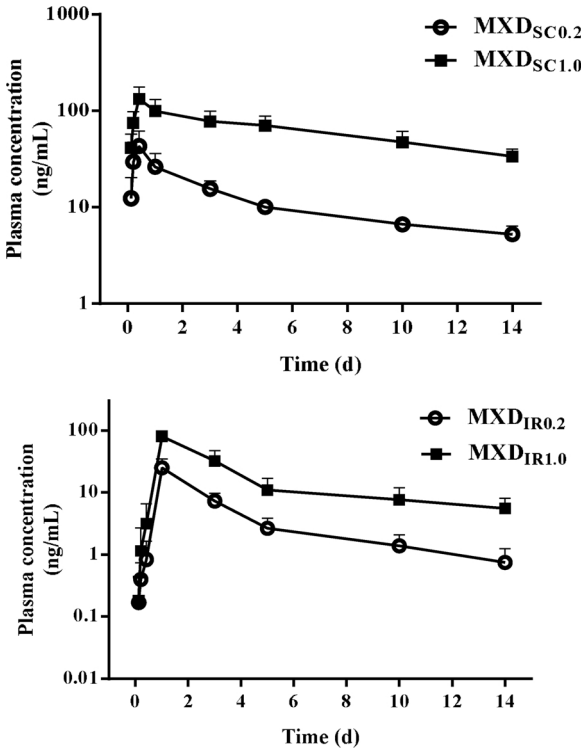

Fig. 1. Comparative mean (± SD) moxidectin (MXD) plasma concentration profiles obtained after subcutaneous (SC) and intraruminal (IR) administration at the therapeutic dose (0.2 mg/kg) ($MXD_{SC0.2}$, $MXD_{IR0.2}$) and at fivefold the therapeutic dose (1 mg/kg) ($MXD_{SC1.0}$, $MXD_{IR1.0}$) to calves (n = 6).

3. Results

3.1. PK study

In all of the experimental groups, plasma concentrations of MXD were quantified at all post-treatment sampling times. The mean (± SD) MXD plasma concentration *vs.* time profiles after its SC and IR administration at two dose levels are shown in Fig. 1. The comparative plasma PK parameters obtained after MXD administration to cattle by both routes and at both doses are shown in Table 1. Comparing the same dose, MXD plasma exposure (expressed as AUC_{0-t}) was higher (P < 0.001) after its SC than after its IR administration (Table 1). A longer plasma elimination half-life was obtained with the SC route, and this behavior was more evident in the group administered the 0.2 mg/kg dose. The sampling time was the same with both administration routes. However, after the SC treatments AUC_{0-t} values represented 65% (0.2 mg/kg dose) and 67% (1.0 mg/kg dose) of the $AUC_{0-\infty}$, which indicates the need for an extended sampling time for this route. MXD reached significantly higher C_{max} (P = 0.001) and AUC (P < 0.001) values after the SC treatment at 1 mg/kg dose than at the therapeutic dose (0.2 mg/kg) (Table 1). However, no statistical differences were observed in normalized AUC (P = 0.723) and C_{max} (P = 0.091) values between doses (SC treatment). Furthermore, no differences were found in T_{max}, $T_{½el}$, $T_{½abs}$ and MRT values between groups treated with MXD by the SC route at 0.2 and 1.0 mg/kg doses.

Following the IR administration of MXD, the AUC_{0-t} represented 92% ($MXD_{IR0.2}$) and 81% ($MXD_{IR1.0}$) of the $AUC_{0-\infty}$ for each experimental group, confirming that the 14 days sampling time was an adequate period for estimation of MXD plasma disposition kinetics by this

Table 1
Plasma pharmacokinetic (PK) parameters (mean ± SD) for moxidectin (MXD) obtained after its subcutaneous (SC) or intraruminal (IR) administration to cattle at two different doses: 0.2 and 1 mg/kg.

Pharmacokinetic parameters	SC treatment		IR treatment	
	$MXD_{SC0.2}$	$MXD_{SC1.0}$	$MXD_{IR0.2}$	$MXD_{IR1.0}$
C_{max} (ng/mL)	43.2 ± 18.7	136 ± 47.3*	27.2 ± 8.71	85.3 ± 12.8*,a
T_{max} (d)	0.40 ± 0.00	0.40 ± 0.00 n.d.	1.00 ± 0.00 n.d.	1.00 ± 0.00 n.d.
AUC_{0-t} (ng·d/mL)	164 ± 26.7	881 ± 223*	67.7 ± 24.2[a]	284 ± 67.0*,a
$AUC_{0-\infty}$ (ng·d/mL)	253 ± 44.9	1322 ± 325*	74.3 ± 29.4[a]	349 ± 90.4*,a
$T_{1/2\ el}$ (d)	11.0 ± 4.10	9.18 ± 3.00	5.20 ± 1.21[a]	7.50 ± 2.19*
$T_{½ab}$ (d)	0.12 ± 0.02	0.17 ± 0.07	0.72 ± 0.28[a]	1.13 ± 0.37*,a
MRT (d)	13.8 ± 6.30	13.0 ± 4.04	4.62 ± 1.30[a]	7.91 ± 2.13*,a
Normalized AUC[1]	253 ± 44.9	264 ± 64.9	74.3 ± 29.4	69.9 ± 18.1
Normalized C_{max}[1]	43.2 ± 18.7	27.2 ± 9.47	27.2 ± 8.71	17.1 ± 2.57*

C_{max}: peak plasma concentration; T_{max}: time to the C_{max}; AUC_{0-t}: area under the plasma concentration *vs*. time curve from 0 up to the last sampling time; $AUC_{0-\infty}$: area under the concentration *vs*. time curve extrapolated to infinity; $T_{½el}$: elimination half-life; $T_{½ab}$: absorption half-life; MRT: mean residence time (obtained by non-compartmental analysis of the data).[1]AUC_{0-t} and C_{max} values were dose-normalized dividing the observed value by 5 (dose ratio). *For each treatment (SC or IR), PK parameters statistically different ($P < 0.05$) between doses. [a]For each dose, PK parameters statistically different ($P < 0.05$) between route of administration. n.d.: statistical differences between doses or routes of administration not determined.

route. Taking into account the ratio between doses, the AUC value increased proportionally ($AUC_{1.0}/AUC_{0.2} = 4.8$). In fact, no significant differences were found between normalized AUC values between groups. On the other hand, the C_{max} value also increased, alb not proportionally, and thus normalized C_{max} values were statistically different between groups. A similar T_{max} (1 d) was observed after MXD administration by the IR route at both dose levels. However, significant differences were found for $T_{½el}$ ($P = 0.043$), $T_{½abs}$ ($P = 0.049$) and MRT ($P = 0.007$).

3.2. Efficacy study

None of the animals involved in the current study showed any adverse events like central nervous system toxicity (ataxia, prostration, anorexia, etc.), even at the dose of 1.0 mg/kg. The EPG counts (mean, range) obtained for all experimental groups and the results of the FECR with 95% uncertainty interval (UI) are shown in Table 2. The $IVM_{SC0.2}$ group showed the lowest efficacy, confirming the presence of GIN highly resistant to IVM. A higher efficacy was observed following MXD treatments, with FECR of 85% and 94% (SC administration at 0.2 and 1.0 mg/kg dose, respectively), and 84% and 99% (IR administration at 0.2 and 1.0 mg/kg dose, respectively) at day +27. Interestingly, the SC and IR administration of MXD at the highest dose resulted in an efficacy over 90% at day +27.

Only *Cooperia* spp. and *Haemonchus* spp. were recovered from coprocultures at days +14 and +27. The results are shown in Fig. 2. FECR values (95% UI) for *Cooperia* spp. and *Haemonchus* spp. on days +14 and +27 post treatment are shown in Table 3. A shift was seen in coprocultures´ composition after MXD treatments, regardless of the anthelmintic dose and administration route. This shift was mainly associated to a higher efficacy against *Cooperia* spp. compared to that observed against *Haemonchus* spp. The mean body weight (± SD) and DWGs measured after different treatments are shown in Table 4. DWGs during the second fattening period in groups treated with MXD were significantly higher ($P = 0.01$) than in the group having received IVM.

4. Discussion

Nematode outbreaks are a health concern in grazing calves (Waller, 2003; Kaplan and Vidyashankar, 2012; George et al., 2017), but they are not usually described in feedlot cattle (Coles, 2002). However, in these production systems, the nematodes that survive the anthelmintic treatment are responsible for weight losses due to the lack of drug efficacy (Stromberg et al., 2012; Fazzio et al., 2014, 2016). In this context, this study assessed the use of high doses of MXD in calves harboring IVM-resistant GIN in a feedlot system.

Although some degree of cross-resistance between IVM and MXD can be expected, resistance to IVM and MXD is not identical (Prichard et al., 2012). In general terms, when the susceptibility of a nematode species to IVM decreases, MXD retains its efficacy (Prichard et al., 2012). This was demonstrated in sheep, in which the efficacy against *H. contortus* was 0% for IVM and > 95% for MXD (Lloberas et al., 2015).

In this study, the mean MXD plasma concentration profiles at both dose levels (Fig. 1) were higher after SC than after IR administration, as expected from previous PK reports involving the use of ML in different animal species (Marriner et al., 1987; Perez et al., 2003; Gokbulut et al., 2007; Lloberas et al., 2012; Leathwick and Miller, 2013; Saumell et al., 2017; Canton et al., 2018). This was corroborated by a significantly higher ($P < 0.001$) MXD systemic availability (estimated as AUC_{0-t}) after the SC administration compared to that found after the IR treatment at both doses (Table 1). High ML adsorption to ruminal particulate digesta explains the lowest drug bioavailability observed after oral/

Table 2
Initial (day -3) and post-treatment (day +14 and +27) egg per gram (EPG) counts, percentage of fecal eggs count reduction (FECR), with their 95% lower and upper uncertainty intervals, and mean individual FECR, after different subcutaneous (SC) and intraruminal (IR) treatments administered to naturally infected feedlot calves: ivermectin (IVM) at 0.2 mg/kg and moxidectin (MXD) at 0.2 and 1 mg/kg.

Group	Mean EPG (max-min)			FECR (95% UI)	
	Day -3	Day+14	Day +27	Day +14	Day +27
$IVM_{SC0.2}$	350 (160-1300)	240 (0-1180)	190 (0-460)	46% (25%-68%)a	45% (23%-63%)a
$MXD_{SC0.2}$	730 (160-2640)	95 (0-360)	90 (0-200)	88% (79%-94%)b	85% (76%-90%)b
$MXD_{SC1.0}$	530 (160-1860)	50 (0-180)	30 (0-80)	91% (83%-96%)b	94% (90%-96%)c
$MXD_{IR0.2}$	580 (160-1080)	120 (0-520)	60 (0-240)	71% (50%-86%)a	84% (75%-86%)b
$MXD_{IR1.0}$	530 (160-2720)	40 (0-140)	10 (0-40)	92% (84%-98%)b	99% (97%-100%)c

UI: uncertainty interval. Different letters in a column indicate significant differences ($P < 0.05$).

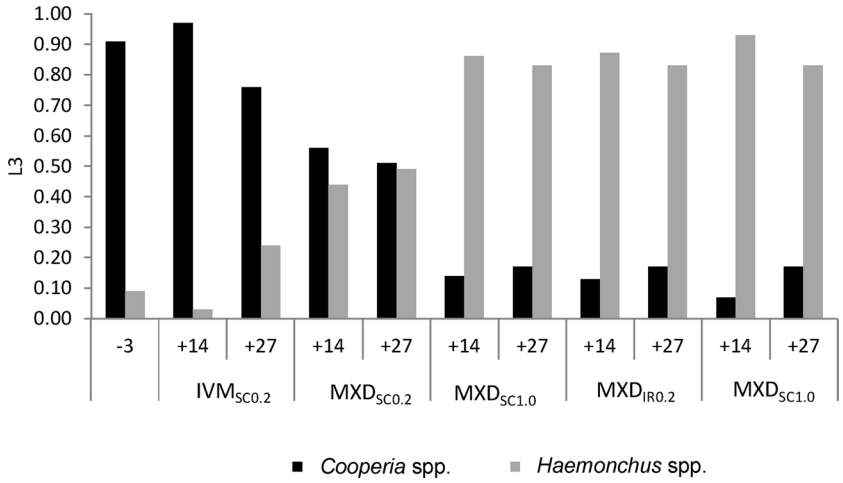

Fig. 2. Percentages of *Cooperia* spp. and *Haemochus* spp. third stage larvae (L_3) recovered from pooled fecal cultures at days +14 and +27, after subcutaneous (SC) and intraruminal (IR) treatments administered to naturally infected feedlot calves: ivermectin (IVM) at 0.2 mg/kg and moxidectin (MXD) at 0.2 and 1 mg/kg.

Table 3
Faecal egg counts reduction (FECR) for *Cooperia* spp. and *Haemonchus* spp. (based on egg counts partitioned by genera using the proportion of each genus recovered as larvae from faecal larval cultures), on days +14 and +27 after different subcutaneous (SC) and intraruminal (IR) treatments administered to naturally infected feedlot calves: ivermectin (IVM) at 0.2 mg/kg and moxidectin (MXD) at 0.2 and 1 mg/kg.

	FECR (95% UI)			
	Day +14		Day + 27	
Group	*Cooperia*	*Haemonchus*	*Cooperia*	*Haemonchus*
$IVM_{SC0.2}$	51% (16%-71%)	85% (74%-91%)	67% (44%-80%)	0% (0%-37%)
$MXD_{SC0.2}$	92% (85%-96%)	39% (0%-69%)	92% (87%-95%)	45% (8%-66%)
$MXD_{SC1.0}$	99% (97%-99%)	13% (0%-55%)	99% (98%-99%)	50% (16%-70%)
$MXD_{IR0.2}$	96% (92%-98%)	0% (0%- 0%)	98% (96%-99%)	0% (0%-20%)
$MXD_{IR1.0}$	99% (99%-100%)	16% (0%-61%)	100% (99%-100%)	87% (61%-95%)

UI: uncertainty interval.

Table 4
Initial Weight* (kg, mean ± SD) at day -3, average daily weight gain (DWG, kg) and mixed model effects after different subcutaneous (SC) and intraruminal (IR) treatments administered to naturally infected feedlot calves: ivermectin (IVM) at 0.2 mg/kg and moxidectin (MXD) at 0.2 and 1 mg/kg.

	LSM					SEM	*p* value
	SC treatment			IR treatment			
	$IVM_{SC0.2}$	$MXD_{SC0.2}$	$MXD_{SC1.0}$	$MXD_{IR0.2}$	$MXD_{IR1.0}$		
Weight*	128 (15)	128 (19)	127 (16)	126 (17)	127 (15)	1.5	0.98
DWG1	0.64	0.77	0.73	0.72	0.70	63	0.69
DWG2	0.77^{a}	0.91^{ab}	1.01^{b}	1.10^{b}	1.12^{b}	65.3	0.01
DWG*t*	0.69	0.82	0.84	0.87	0.86	49.4	0.065

DWG1 = average daily weight gain from 0 through 27 days of study (period 1).
DWG2 = average daily weight gain from 27 through 47 days of study (period 2).
DWG*t* = average daily weight gain throughout all of the study period (0–47 days).
LSM = least squared means; SEM = standard error of the mean (highest value reported).
a,b: Different letters within rows mean $P < 0.05$.

IR administration (Ali and Hennessy, 1996; Lifschitz et al., 2005; Canton et al., 2018). Additionally, after MXD administration, the dose-related parameters AUC and C_{max} increased with the dose increment, independently of the route (Table 1), as reported for IVM in sheep (Alvarez et al., 2015).

Compared to the SC route, the oral/IR administration of ML seems to be more effective. This was clearly evidenced in sheep and goats, in which IVM administration by oral or SC routes showed a similar efficacy against susceptible populations of *H. contortus* (Barnes et al., 2001; Sutherland et al., 2002; Lespine et al., 2005), and to some extent a higher efficacy for the SC route against intestinal endoparasites (Borgsteede, 1993). Similarly, 93% (SC treatment) and 92% (oral treatment) efficacies against susceptible GIN in cattle have been reported (Canton et al., 2018). However, the efficacy after IR IVM treatment was higher compared to that obtained after the SC treatment in lambs infected with resistant parasites (Lloberas et al., 2012; Alvarez et al., 2015). While an *H. contortus* population behaved as completely resistant to IVM after its SC administration (0%) in one of these studies, the efficacy increased to 41% after the IR treatment (Lloberas et al., 2012).

Although the scenario of resistance may differ between host species, the efficacy of ML against GIN after different administration routes in cattle could be compared to that observed in sheep. In fact, Pomroy et al. (2004) reported a higher efficacy of IVM or MXD against ML-resistant *C. oncophora* after their oral administration than after the SC injection. Furthermore, the FECR was significantly greater after the oral administration of MXD (91%) than following injectable (56%) or pour-on (51%) treatments (Leathwick and Miller, 2013). Contrary to what was expected, the efficacy after IR administration was not better than following the SC treatment in this study, since the FECR after MXD administered at 0.2 mg/kg by the IR route was lower (71%) than that observed after the SC route (88%) at day +14. Efficacies for both groups became almost identical at day +27 (84% and 85%, respectively). Nevertheless, when highly resistant nematodes are present, IVM treatments have shown to be inefficacious after both oral and SC treatments (Galvan et al., 2016; Canton et al., 2018).

The overall low efficacy levels observed after IVM treatment indicates the presence of highly resistant GIN, since FECR after the IVM treatment was only 51% (day +14) and 46% (day +27). The efficacy achieved by increasing the MXD dose observed in the current study was similar to that observed in sheep naturally (Lloberas et al., 2015) or artificially infected with IVM-resistant GIN (Alvarez et al., 2015). However, a lack of efficacy of IVM even at a fivefold dose in feedlot calves has been recently reported (Galvan et al., 2016). The low efficacy of high IVM doses has been associated to PK/pharmacodynamic

differences compared to MXD. Since P-Glycoprotein expression is enhanced in IVM-resistant GIN (Xu et al., 1998; Dicker et al., 2011; Williamson et al., 2011; Demeler et al., 2013; Janssen et al., 2013), the differential affinity for this transporter protein could explain some of the observed differences in efficacy between IVM and MXD (Table 2). Similar to what had been previously reported by Fazzio et al. (2016), the efficacy at day +27 increased from 45% (IVM) to 85% (MXD) after the SC administration at a dose of 0.2 mg/kg. Unfortunately, although an improved efficacy was observed after MXD treatment at its therapeutic dose, the efficacy was far from that expected for ML (85% and 84% after the SC or IR administration, respectively) at day +27. When the MXD dose was increased fivefold, the anthelmintic efficacies were 94% (SC treatment) and 99% (IR treatment) at day + 27.

Considering all parasite genera, the observed results indicate that the MXD dose increment led to a better overall efficacy, and that genus-specific efficacy was over 90% against *Cooperia* spp (Table 3, Fig. 2). Nevertheless, MXD efficacy against *Haemonchus* spp. was poor (FECR ≤ 50%), except for the $MXD_{IR1.0}$ group (FECR = 87%). However, these results must be taken cautiously, since the low number of L3 recovered following treatments may lead to an underestimation of the efficacy against different genera.

Due to the massive use of IVM in beef herds, *Cooperia* spp. has become the prevailing nematode genus, leading to production losses (Candy et al., 2018). Several studies have reported DWG increments ranging from 7% to 50% in dewormed cattle compared to calves experimentally inoculated with *C. oncophora, C. pectinata* and *C. punctata* (Herlich, 1965; Armour et al., 1987; Stromberg et al., 2012). It must be noted that *Cooperia* spp. were found in 100% of the Argentinean farms with resistance to IVM (Cristel et al., 2017), with important productive losses (Fazzio et al., 2011, 2012; 2014; 2016). Compared to the IVM-treated animals, all of the groups treated with MXD showed higher DWG during the second fattening period (days +27 to +47). In this study, the group with the lowest DWG was the $IVM_{SC0.2}$, which was also the group in which *Cooperia* spp was the most prevalent nematode. As seen in other clinical trials, *Cooperia* spp. seems to be associated to subclinical weight loss in feedlot cattle (Stromberg et al., 2012).

The high efficacy observed after the administration of a high dose of MXD against IVM-resistant GIN improved the efficacy against *Cooperia* and the DWG in feedlot calves. However, this practice should not be recommended in grazing cattle, where surviving parasites may pass eggs to the pasture thus accelerating the development of MXD resistance.

Conflict of interest

The authors declare no conflict of interest which could potentially bias the results shown in this study.

Funding

This study was partially supported by UNLP research grant V257 and Agencia Nacional de Promoción Científica y Técnica (ANPCyT) (PICT 2012-1342), both from Argentina.

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