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Research paper

The route of administration drastically affects ivermectin activity against small strongyles in horses

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ABSTRACT

The goal of the current study was to evaluate the comparative efficacy of ivermectin (IVM) against small strongyles (cyathostomins) following its oral and intramuscular (IM) administration, in naturally parasitized horses. The parasitological data were complemented with the assessment of the plasma disposition kinetics of IVM. The trial included two different experiments. In experiment I, 40 horses naturally infected with small strongyles were randomly allocated into four experimental groups ($n = 10$) and treated with IVM (0.2 mg/kg) as follows: IVM oral paste, animals were orally treated with Eqvalan® (IVM 1.87% paste, as the reference formulation) by the oral route; IVM oral solution, animals were orally treated with Remonta® (IVM 2% solution, as a test formulation); IVM IM solution, animals were IM treated with the test product (Remonta® IVM 2% solution); and control, animals were kept without treatment as untreated controls. In experiment II, 24 horses naturally parasitized with small strongyles were randomly allocated into the same four experimental groups ($n = 6$) described for experiment I. Faecal samples were individually collected directly from the rectum of each horse prior (day −1) and at 7 and 15 (Experiment I) or 7, 15 and 21 (Experiment II) days after-treatment, to assess the eggs per gram (epg) counts and estimate the efficacy of the treatments. Additionally, the comparative plasma disposition kinetics of IVM in treated animals was assessed in experiment II. In both experiments, an excellent (100%) IVM efficacy was observed after its oral administration (test and reference formulations). However, the IM administration of IVM resulted in a low efficacy (36–64%). Similar IVM plasma concentration was observed after its oral administration as a paste or as a solution. The higher IVM plasma profiles observed after the IM administration accounted for an enhanced systemic availability. The improved IVM efficacy observed against adult cyathostomins after its oral administration can be explained by an enhanced drug exposure of the worms located at the lumen of the large intestine. These findings may have a direct impact on the practical use of macrocyclic lactones in horses.

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1. Introduction

The effectiveness of macrocyclic lactones against the most economically important nematode parasites of adult horses has made them the gold standard for control programs of equine parasites. Small strongyles (cyathostomins) represent a heterogeneous group of intestinal nematodes that affect horses and other equids (Soulsby, 1987). Cyathostomins are the most prevalent gastrointestinal nematodes of adult horses in Argentina (Fusé and Saumell, 2002; Fusé et al., 2013), as well as in other parts of the world (Matthews et al., 2004). Most cyathostomin infections may develop without clinical signs. In naturally parasitized horses, adult worms are found in the large intestine, mainly in the cecum and ventral colon (Ogbourne, 1976; Cirak et al., 1996; Steinbach et al., 2006), and a larger number of parasites are found as encysted larvae in the mucosa and submucosa of the large intestine. The massive emergence of the encysted cyathostomin larvae from the large intestinal wall has been associated with diarrhea, severe inflammation of the mucosal wall, ventral edema, colitis and weight loss

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(Murphy and Love, 1997). At this stage, cyathostominosis can be fatal in up to 50% of cases (Love et al., 1999). Resistance of cyathostomins to anthelmintic drugs, mainly pyrantel and benzimidazole anthelmintics, is widespread (Kaplan, 2004; Traversa et al., 2007). However, resistance to macrocyclic lactones is beginning to emerge in cyathostomins. A reduction of the egg reappearance period has been proposed as an early indicator of a shift in nematode population sensitivity towards anthelmintic resistance (Borgsteede et al., 1993; Sangster, 1999; von Samson-Himmelstjerna et al., 2007; Edward and Hoffmann, 2008; Molento et al., 2008; Lyons et al., 2008; Traversa et al., 2009; Kaplan and Vidyashankar, 2012; Canever et al., 2013). Furthermore, there is evidence that the observed shortened egg reappearance periods are due to resistance in luminal L4-stages of equine cyathostomins (Lyons et al., 2009, 2010; Lyons and Tolliver, 2013).

As a chemical group, macrocyclic lactones include several molecules active against endo- and ecto-parasites. Ivermectin (IVM), the first macrocyclic lactone introduced as an antiparasitic drug in 1981, is effective against endo- and ecto-parasites in a wide variety of hosts, including cattle, sheep, goats, dogs, pigs and horses, as well as domesticated wild animals (Campbell et al., 1989). IVM was the first macrocyclic lactone approved in horses as a broad spectrum antiparasitic compound (Campbell et al., 1989). The first commercial formulations include a paste designed for oral administration (Eqvalan Paste, IVM 1.87%, Merck and Co., Inc., Rahway, NJ), a solution which may be administered by stomach tube (nasogastric intubation) or as an oral drench (Eqvalan Liquid, IVM 1%) and a micellar formulation for intramuscular (IM) use (Eqvalan Injectable, IVM 2%). In all cases, the recommended dose of IVM was 0.2 mg/kg. In the early days, shortly after the introduction of IVM into the market, nematode susceptibility was high and equivalent efficacy patterns were observed against gastrointestinal parasites after parenteral or oral treatment in horses (Campbell et al., 1989). In fact, most of the early studies on the efficacy of IVM in horses used IM injection of the drug (Campbell et al., 1989). These trials demonstrated that the efficacy of parenterally and orally administered IVM in horses was almost identical (Campbell et al., 1989). For example, the reduction of small strongyles egg counts after the administration of IVM either by the oral or IM route was 100% at day 14 post-treatment and 90% at day 60 post-treatment (Baker et al., 1984). Similarly, no significant differences in the mean strongyle egg counts were observed after the topical and oral administration of IVM to horses (Gokbulut et al., 2010). In controlled trials, IVM efficacy was compared after the administration of the paste and injectable preparations. Against adult *Cyathostomum* spp and unidentified species of small strongyles the efficacy of both formulations was higher than 99% (Wallace and Duncan, unpublished data cited by Campbell et al., 1989). Thus, the activity of the paste and the injectable formulations seemed to be similar in those trials. However, the manufacture and distribution of Eqvalan Injectable was cancelled due to the adverse reactions related to the contamination of the inoculation site with bacteria (*Clostridium* sp.) and anaphylactic reactions (Campbell et al., 1989).

The "Remonta y Veterinaria" section of the Argentine Army manufactures its own veterinary pharmaceutical products including a propylene glycol-based formulation of IVM prescribed for oral or IM administration to horses (Remonta®, IVM 2%,). For many years, this product has been used for parasite control in horses by IM route without reports of adverse reactions and/or reduced efficacy. However, in 2010, after the IVM administration by the IM route, nematode egg counts were observed at 10 days post-treatment, and anthelmintic resistance and/or poor quality of the formulation was suspected. The goal of the current study was to evaluate the efficacy of IVM against small strongyles in naturally parasitized horses following treatment with the "reference" formulation (Eqvalan® Paste, IVM 1.87%) by the oral route and the test preparation (Remonta® solution) administered by the oral and IM route). Additionally, the plasma disposition kinetics of IVM in horses after the different IVM treatments was assessed.

2. Material and methods

2.1. Animals

The animal phase of the current experimental work was carried out in two different years (2010 and 2012) in the farm "Haras General Lavalle". This farm belongs to the Argentine Army and is located in Tandil, Buenos Aires province, Argentina. The farm had approximately 1000 grazing horses destined for sports activities (polo and/or horse riding). The area of Tandil has rainfall of 1.2 L/m^2 per year and an annual mean temperature of 16 °C, which represents ideal conditions for the development of nematode populations. The horses were maintained over a surface of 670 ha of implanted and natural pastures. Since most of the animals were grazing on pasture all year, nematode infection was common. The parasite control program at the "Haras General Lavalle" was based on the treatment with IVM, which has been in use for many years (five to six treatments a year). Two-year-old "Silla Argentino" horses (Argentine Saddlebred) naturally infected with gastrointestinal nematodes were involved in the two experiments described here. All the animals had a similar weight, body condition and parasite burden. Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina (FCV-UNCPBA, 2016).

2.2. Experimental design

2.2.1. Experiment I

Experiment I was performed in May 2010 (autumn season in Argentina). Forty animals were selected from a herd of 250 horses based on egg per gram (epg) counts (≥100 epg). The horses were weighed, identified with their specific record cards and kept on a grazing pasture with free access to water throughout the experiment. Horses (496 ± 53 kg) with an average of 401 ± 248 epg (trial day −1) ranging from 100 to 1140 epg, were ranked according to increasing epg. Groups of replicates of four animals were formed based on increasing epg, and within each replicate, animals were randomly assigned to each experimental group (n = 10). Experimental animals received the following treatments (trial day 0): **IVM oral paste**, animals were orally treated with Eqvalan® (IVM 1.87% paste, Merial, Argentina, considered the reference formulation); **IVM oral solution**, animals were orally treated with Remonta®, a propylene glycol-based formulation (IVM 2% solution, División de Remonta y Veterinaria, Ejército Argentino, Argentina, considered the test formulation); **IVM IM solution**, animals were intramuscularly treated with the test formulation (Remonta®, IVM 2% solution); and **Control**, animals were kept without treatment as untreated controls. All treatments were performed at the dose rate of 0.2 mg/kg. Faecal samples were individually collected directly from the rectum pre-treatment (day −1) and at 7 and 15 days post-treatment, to assess the epg counts.

2.2.2. Experiment II

Experiment II was performed in April 2012 (autumn season in Argentina). From a herd of 160 horses, 24 animals were selected based on egg counts, weighted and identified with their specific record cards. All the animals were maintained on a grazing pasture during the experiment and had free access to water. Horses (511 ± 26 kg) with an average of 163 ± 41 egg counts (trial day −1)

ranging from 80 to 420 egg counts, were randomly allocated as previously explained, into the same four experimental groups ($n = 6$) described for experiment I. Faecal samples were individually collected directly from the rectum pre-treatment (day −1) and at 7, 15 and 21 days post-treatment to assess the egg counts. Additionally, the comparative plasma disposition kinetics of IVM in treated animals was carried out. Blood samples (5 mL) were taken from the jugular vein using 10 mL heparinised Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ, USA), before administration (time 0) and at 2, 4, and 6 h and 1, 2, 3, 5, 7, 10, 15 and 21 days after the oral or IM administration of IVM. Plasma was separated by centrifugation at 2000 × *g* for 15 min, placed into plastic tubes and frozen at −20 °C until analysis by high performance liquid chromatography (HPLC).

2.3. Analytical procedures

Parasitological analysis: The epg count was performed by a McMaster technique (MAFF, 1986), with a detection limit of 10 nematode eggs per g of faeces. Three g of faeces were mixed in 57 mL of saturated sodium chloride solution (SG 1.2). The suspension was sieved through a gauze. The filtered suspension was thoroughly mixed before the filling of the INTA McMaster slide chambers (consisting in a four-chambered slide of 0.5 mL each) (Instituto Nacional de Tecnología Agropecuaria, Buenos Aires, Argentina). After 5 min, eggs were counted and the total number was multiplied by 10 to obtain the final epg counts. All samples were processed within four days of collection. The faecal egg count reduction (FECR) was calculated for each group of horses at 7 and 15 (experiment I) or 7, 15 and 21 days post-treatment (experiment II), using the following formula (Coles et al., 1992):

$$\text{FECR}(\%) = 100 \times (1 - Ct/Cc);$$

where *Ct* is the arithmetic mean of epg counts in the treated group at time *t* (7, 15 or 21 days post-treatment, according the experiment) and *Cc* is the arithmetic mean of epg counts in the untreated control group at time *t* (7, 15 or 21 days post-treatment, according the experiment). The 95% confidence intervals (CI) were calculated as reported by Coles et al. (1992).

Faecal cultures (approx. 100 g faeces each group) were performed pre- (trial day −1) and post-treatment with equal amounts of faeces obtained from each animal (approx. 10 g), and then pooled according the experimental group. The faeces were broken up finely and incubated in glass culture dishes for 14 days at 27 °C (MAFF, 1986). After Baermann sedimentation (24 h), one hundred larvae or the number of larvae recovered were examined for strongyle differentiation using the key of MAFF (1986).

HPLC analysis: The extraction of IVM (22,23 dehydro-avermectin B1a), from spiked and experimental plasma samples was carried out following a well established technique (Alvinerie et al., 1993, slightly modified by Lifschitz et al., 1999). IVM concentrations were determined by HPLC using a Shimadzu 10 A HPLC system with autosampler (Shimadzu Corporation, Kyoto, Japan). HPLC analysis was undertaken using a reverse phase C_{18} column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 μm, 4.6 mm × 250 mm) and 0.2% acetic acid in water/methanol/acetonitrile (1.6/60/38.4) mobile phase at a flow rate of 1.5 mL/min at 30 °C. IVM was detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), reading at 365 nm (excitation wavelength) and 475 nm (emission wavelength). IVM concentrations were determined by the internal standard method using the Class LC 10 Software version 1.2 (Shimadzu Corporation, Kyoto, Japan). The peak area ratios were considered to calculate the IVM concentrations in spiked (validation) and experimental plasma samples. There was no interference of endogenous compounds in the chromatographic determinations. The solvents (Baker, Phillipsburg, NJ, USA) used during the extraction and drug analysis were HPLC grade. A complete validation of the analytical procedures used for extraction and quantification of IVM was performed before starting the analysis of the experimental samples obtained during the pharmacokinetic trial. Calibration curves in the range between 0.2–5 ng/mL and 5–50 ng/mL were prepared for each compound. Calibration curves were established using least squares linear regression analysis and correlation coefficients (r) and CV were calculated. Linearity was established to determine the IVM concentrations/detector response relationship. IVM recovery from plasma was obtained for the range between 0.2 and 50 ng/mL. The inter-assay precision of the extraction and chromatography procedures was estimated by processing replicate aliquots ($n = 4$) of pooled horse plasma samples containing known IVM concentrations (0.2, 5 and 50 ng/mL) on different working days. The limit of quantification (LOQ) was defined as the lowest measured concentration with a CV <20%, an accuracy of ±20% and an absolute recovery ≥70%. The LOQ obtained for IVM was 0.2 ng/mL. Values below LOQ were not included in the pharmacokinetic analysis. The linear regression lines for IVM showed correlation coefficients to ≥0.999. Mean absolute recovery percentage for concentrations ranging between 0.2–50 ng/mL were 85%. The inter assay precision of the analytical procedures obtained after HPLC analysis of IVM on different working days showed a CV < 7%.

Pharmacokinetic analysis of the data: Non-compartmental pharmacokinetic analysis for the plasma concentration versus time curves of IVM for each individual animal after the different treatments was conducted using the PK Solution 2.0 software (Summit research services, CO, USA). The peak concentration (Cmax) and time to peak concentration (*T*max) were displayed from the plotted concentration-time curve of IVM. The area under the concentration time-curve (AUC) was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (β). The elimination ($T½$el) and absorption ($T½$ab) half-lives were calculated as $\ln 2/\beta$ and $\ln 2/k$, respectively. 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.

Statistical analysis of the data: Faecal eggs counts (reported as arithmetic mean ± SD) were analysed by nonparametric ANOVA (Kruskal–Wallis test). The pharmacokinetic parameters and concentration data are reported as arithmetic mean ± SD. Parametric (ANOVA + Tuckey) and non parametric (Kruskal–Wallis) tests were used for the statistical comparison of the pharmacokinetic parameters obtained from the different experimental groups. The statistical analysis was performed using the Instat 3.0 Software (Graph Pad Software, San Diego, CA, USA). In all cases, a value of $P < 0.05$ was considered statistically significant.

3. Results

The strongyle egg counts (mean ± SD) and FECR obtained at days −1, 7 and 15 for the different experimental groups involved in experiment I are shown in Table 1. An excellent (100%) efficacy was observed after IVM oral administration, either with IVM oral paste or the Remonta oral solution. However, the parenteral (IM) administration of IVM (IVM IM solution) resulted in a lower FECR (36% at day 15 post-treatment).

In experiment II, an increase in egg counts between days −1 and 21 was observed in the untreated control group, reflecting the progression of the parasite infection. IVM administered by the oral route as a paste (IVM oral paste) or as a solution (IVM oral solution)

Table 1
Mean nematode egg counts[1] (±SD) and faecal egg count reduction (FECR) after administration of different ivermectin (IVM) formulations: Eqvalan® by the oral route (IVM oral paste) and Remonta® by the oral (IVM oral solution) and intramuscular (IM) (IVM IM solution) route, to parasitized horses (experiment I).

Experimental groups	Egg counts			FECR[1] (CI)			
	Day −1	Day 7	Day 15	Day 7		Day 15	
Control	386 ± 290[a]	346 ± 235[a]	**362 ± 257**[a]	–	–	**–**	–
IVM oral paste	334 ± 93[a]	0[b]	**0**[b]	100%	–	**100%**	–
IVM oral solution	526 ± 336[a]	0[b]	**0**[b]	100%	–	**100%**	**–**
IVM IM solution	358 ± 191[a]	128 ± 80[a]	**232 ± 185**[b]	63%	(80-32)	**36%**	(68-0)

[1] FECRT estimated according to Coles et al. (1992); CI = 95% confidence intervals (upper and lower); nematode egg counts at −1, 5 or 15 days post treatment with different superscript (a or b) are statistically different at $P < 0.05$.

Table 2
Mean nematode egg counts[1] (± SD) and faecal egg count reduction (FECR), after administration of different ivermectin (IVM) formulations: Eqvalan® by the oral route (IVM oral paste) and Remonta® by the oral (IVM oral solution) and intramuscular (IM) (IVM IM solution) route, to parasitized horses (*Experiment II*).

Experimental groups	Egg counts				FECR[1] (CI)					
	Day −1	Day 7	Day 15	Day 21	Day 7		Day 15		Day 21	
Control	160 ± 95[a]	380 ± 307[a]	**653 ± 467**[a]	703 ± 402[a]	–	–	–	–	–	–
IVM oral paste	173 ± 88[a]	0[b]	**0**[b]	0[b]	100%	–	**100%**	–	100%	–
IVM oral solution	170 ± 124[a]	0[b]	**0**[b]	0[b]	100%	–	**100%**	–	100%	–
IVM IM solution	150 ± 42[a]	143 ± 160[a]	**233 ± 262**[a]	433 ± 412[a]	62%	(89-0)	**64%**	(89-0)	38%	(76-0)

[1] FECRT estimated according to Coles et al. (1992); CI = –95% confidence intervals (upper and lower); nematode egg counts at 5, 15 or 21 days post treatment with different superscript (a or b) are statistically different at $P < 0.05$.

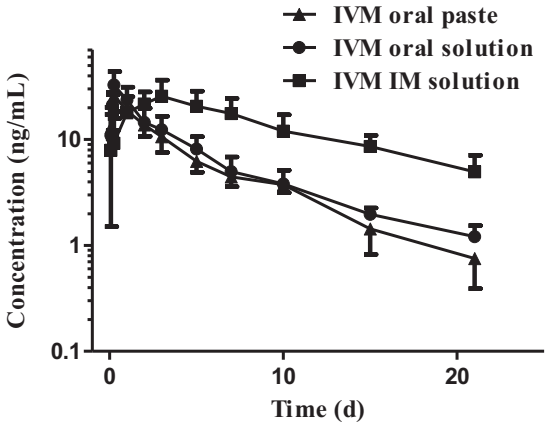

Fig. 1. Comparative mean (±SD) ivermectin (IVM) plasma concentration profiles ($n = 6$) obtained after the administration (0.2 mg/kg) of different IVM formulations to parasitized horses: IVM oral paste (Eqvalan®) and IVM solution (Remonta®) both by the oral route and IVM IM solution (Remonta®) by the intramuscular (IM) route (*Experiment II*).

Table 3
Plasma pharmacokinetic parameters (mean ± SD) for ivermectin (IVM) obtained after the administration (0.2 mg/kg) of different IVM formulations: Eqvalan® by the oral route (IVM oral paste) and Remonta® by the oral (IVM oral solution) and intramuscular (IM) (IVM IM solution) routes.

Pharmacokinetic parameters	Experimental groups		
	IVM oral paste	IVM oral solution	IVM IM solution
*T½*abs (d)	0.10 ± 0.06[a]	0.08 ± 0.02[a]	0.73 ± 0.37[b]
Cmax (ng/mL)	30.1 ± 6.78[a]	33.7 ± 9.61[a]	25.9 ± 10.2[a]
*T*max (d)	0.33 ± 0.33[a]	0.50 ± 0.39[a]	2.80 ± 0.45[b]
AUC_{0-LOQ} (ng d/mL)	108 ± 10.9[a]	128 ± 35.1[a]	276 ± 94.8[b]
$AUC_{0-\infty}$ (ng d/mL)	114 ± 12.7[a]	138 ± 36.6[a,b]	347 ± 91.9[b]
*T½*el (d)	5.48 ± 2.10[a]	6.32 ± 1.18[a]	9.16 ± 4.77[a]
MRT_{0-LOQ} (d)	5.10 ± 0.60[a]	5.20 ± 0.16[a]	8.10 ± 0.84[b]

Cmax: peak plasma concentration; *T*max: time to the Cmax; AUC_{0-LOQ}: area under the plasma concentration vs. time curve from time 0 to the quantification limit; $AUC_{0-\infty}$: area under the concentration vs. time curve extrapolated to infinity; *T½*el: elimination half-life; *T½*abs: absorption half-life; MRT: mean residence time from t_0 to the quantification limit. Pharmacokinetic parameters with different superscript letters ([a] or [b]) among groups are statistically different at $P < 0.05$.

also showed an excellent anthelmintic efficacy (100%) at every evaluated day (Table 2). However, a low efficacy level was observed after the IM administration of IVM (IVM IM solution) at day 15 post-treatment in both, experiment I (efficacy 36%, 95% CI = 68-0) and experiment II (efficacy 64%, 95% CI = 89-0).

The faecal cultures of pooled samples showed in all cases the presence of small strongyles (100%).

The mean (±SD) IVM plasma concentration profiles obtained after the different treatments are shown in Fig. 1. IVM was quantified in plasma between 2 h and 21 days post-treatment in all the experimental animals. Table 3 summarizes the IVM plasma pharmacokinetics parameters obtained for the different experimental groups. A similar plasma exposure was observed after the oral administration of IVM either as a paste or as solution. The IVM AUC_{0-LOQ} were 108.3 ± 10.9 ng d/mL (IVM oral solution) and 128.3 ± 35.1 ng d/mL (IVM oral solution) and the Cmax values were 30.1 ± 6.78 ng/mL and 33.7 ± 9.61 ng/mL, respectively. Furthermore, there were no differences in the absorption and elimination patterns after the oral administration of both formulations. However, higher IVM plasma AUC_{0-LOQ} ($P < 0.05$) was obtained after the IM administration compared to that observed following the oral treatment (Table 1). A longer MRT was observed after IVM IM administration to horses compared to the oral treatment. Whereas AUC_{0-LOQ} values represent 95% (IVM oral paste) and 93% (IVM oral solution) of the $AUC_{0-\infty}$, a lower value (74%) was observed after the IM administration of IVM, which indicates the need of an extended sampling period.

4. Discussion

In the current trial, IVM administered by the oral route as a paste or as a solution resulted highly efficacy (100%) against small strongyles. However, a low efficacy was observed after the IM administration of the solution (IVM IM solution) in both experiments. Previous data also showed that an injectable formulation was 63% effective against adult *Poteriostomum*, while the efficacy of the paste was 100% (Yazwinsky, unpublished data cited by Campbell et al., 1989). This result would be an early indicator of the fact that the oral administration has some advantage over the parenteral administration, at least against small strongyles. The lack of IVM efficacy observed in the current work after the

IM administration cannot be considered as evidence of resistance because the drug administered by the oral route showed an excellent anthelmintic efficacy. However, we consider that the cyathostomin population involved in the current work has a "reduced susceptibility" to IVM, since a lower efficacy was observed after its IM administration. It is important to highlight that the efficacy of parenterally and orally administration of IVM in horses was almost identical after the introduction of IVM into the market (Campbell et al., 1989). A short egg reappearance period is currently used as an early indicator of resistance to macrocyclic lactones in cyathostomins (Nielsen et al., 2014). Unfortunately, we did not measure egg reappearance period in the study population, which could confirm the potential emerging resistance in the parasite population involved in the current work.

Anthelmintic drugs can reach target helminth parasites by either oral ingestion (from the host's blood, and/or gastrointestinal contents), diffusion through the external surface or some combination of both (Thompson et al., 1993; Thompson and Geary, 1995). Although the oral route cannot be ruled out, there is clear evidence that transcuticular diffusion from the surrounding medium is the most important access route for non-nutrient and non-electrolyte substances, including anthelmintics, in nematodes (Ho et al., 1990; Sims et al., 1992; Cross et al., 1998; Alvarez et al., 2001; Lloberas et al., 2012). Thus, 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). Similarly to the situation observed in the current study, treatment by the oral route results in a greater anthelmintic efficacy of macrocyclic lactones against IVM resistant nematodes of sheep (Gopal et al., 2001; Alka et al., 2004) and cattle (Leathwick and Miller, 2013; Canton et al., 2015), compared with the parenteral (subcutaneous) administration. These results can be explained by differences in drug accumulation in the parasite related to the route of administration. In fact, IVM concentrations of 74.4 ng/g and 5.19 ng/g were quantified in adult *Haemonchus contortus* recovered from lambs treated by the intraruminal and subcutaneous routes, respectively (Lloberas et al., 2012). The higher concentrations measured in the abomasal content after the intraruminal administration of IVM accounted for a greater amount of drug being measured in *H. contortus* recovered from treated sheep (Lloberas et al., 2012). These remarkable differences highlight the importance of the in vivo IVM entrance into gastrointestinal nematodes and confirm the relevance of the transcuticular diffusion process. A similar situation may explain the results observed in the current study. After the oral administration of IVM to horses, a higher drug concentration in the intestinal content could be expected in comparison to that achieved after the IM administration. In fact, it has been previously demonstrated that horses treated by the oral route with the macrocyclic lactone doramectin had higher and earlier faecal concentrations than those treated by the IM route (Perez et al., 2010). Since the concentration gradient is one of the major determinants of drug accumulation within a target parasite (Alvarez et al., 2007), the higher IVM concentrations at the parasite' surrounding medium (intestinal fluid), the greater IVM accumulation into the parasites (i.e. adult cyathostomins). When nematode population behaves fully susceptible to IVM, it is expected that differences in parasite drug accumulation related to the route of administration do not affect the anthelmintic efficacy. However, differences in efficacy have been observed when the nematode population has attained reduced drug susceptibility (Gopal et al., 2001; Alka et al., 2004; Leathwick and Miller, 2013; Canton et al., 2015; Leathwick et al., 2016), as apparently occurs with the cyathostomin population involved in the current study. In this case, the oral route results in much higher concentrations of IVM in the gut tissue and in the worms themselves compared to the parenteral administration, and consequently a higher efficacy could be expected. However, further research is needed to measure IVM concentrations in cyathostomins in order to confirm this hypothesis.

To minimize the different variables that may affect drug plasma concentrations, our study was conducted in horses of similar age, weight, body condition and parasite burdens. The rate and extent of absorption were compared after the oral administration of IVM as a paste and as a solution. Similar ($P > 0.05$) IVM plasma pharmacokinetic parameters (Table 1) were observed after the oral administration of both formulations under study. These results suggest a similar extent of absorption and plasma disposition for the reference (Eqvalan®) and test formulation (Remonta®) despite the different composition of their excipients.

The IVM plasma disposition kinetics in horses showed significant differences according to the route of administration, as previously shown by Pérez et al. (2003). Although the plasma disposition kinetics of a different IVM formulation was previously evaluated after the IM route (Pérez et al., 2003), the main pharmacokinetic parameters obtained in the current trial after the oral and IM administration of IVM were similar. The parenteral administration of IVM resulted in a more prolonged period of drug absorption compared to the paste and solution administered orally, which were rapidly absorbed as was reflected in the significantly shorter *T½*ab. After the IM administration (IVM IM solution), a higher plasma concentrations and a delayed Tmax were obtained. The higher IVM plasma profiles observed in the IM treated group accounted for an enhanced systemic availability, which represents an increase between 115 and 155% compared to the oral administration. In addition, a longer MRT was observed after the IM administration of IVM. These results are similar to those previously reported for IVM in sheep (Marriner et al., 1987; Imperiale et al., 2004; Lloberas et al., 2012), in which plasma systemic availability was higher after the parenteral injection compared to the oral treatment. The high degree of association of IVM to the particulate material of digesta and the action of the transmembrane transport protein P-glycoprotein (P-gp) appear as relevant factors modulating the IVM gastrointestinal absorption process, which may also reduce the amounts of drug absorbed after oral administration (Lifschitz et al., 2005).

The greater systemic availability and the longer persistence of IVM concentrations observed after its IM administration to horses have been associated with a longer egg reappearance period (Pérez et al., 2003). However, this potential therapeutic advantage will be lost in the presence of parasites with reduced susceptibility. In these cases, the high concentrations of a lipophilic drug such as IVM in the gastrointestinal tract following its oral administration may be relevant to maintain an acceptable efficacy threshold.

In conclusion, this study demonstrated that the route of IVM administration (oral vs. IM) in horses results in significant differences in the drug systemic availability and in the efficacy against small strongyles. The low IVM efficacy against adult cyathostomins obtained in horses after its IM administration could be explained by a lower drug exposure of the worms located at the lumen of the large intestine, compared to the conventional oral administration.

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References

Alka, Gopal, R.M., Sandhu, K.S., Sidhu, P.K., 2004. Efficacy of abamectin against ivermectin-resistant strain of *Trichostrongylus colubriformis* in sheep. Vet. Parasitol. 121, 277–283.

Alvarez, L., Imperiale, F., Sánchez, S., Murno, G., Lanusse, C., 2000. Uptake of albendazole and albendazole sulphoxide by *Haemonchus contortus* and *Fasciola hepatica* in sheep. Vet. Parasitol. 94, 75–89.

Alvarez, L., Mottier, M.L., Sánchez, S., Lanusse, C., 2001. *Ex vivo* diffusion of albendazole and its sulfoxide metabolite into *Ascaris suum* and *Fasciola hepatica*. Parasitol. Res. 87, 929–934.

Alvarez, L., Mottier, M., Lanusse, C.E., 2007. Drug transfer into target helminth parasites. Trends Parasitol. 23, 97–104.

Alvinerie, M., Sutra, J.F., Galtier, P., 1993. Ivermectin in goat milk after subcutaneous injection. Vet. Res. 24, 417–421.

Baker, N.F., Miller, J.E., Madigan, J.E., Fulton, R., Seward, R.L., 1984. Anthelmintic action of ivermectin, oxibendazole, and pyrantel pamoate against thiabendazole-resistant small strongyles of horses. Equ. Pract. 6, 8–19.

Borgsteede, F.H., Boersma, J.H., Gaasembeek, G.C., van der Burg, W.P., 1993. The reappearance of eggs in faeces of horses after treatment with ivermectin. Vet. Q. 15, 24–26.

Campbell, W., Leaning, W., Seward, R., 1989. Use of ivermectin in horses. In: Campbell, W. (Ed.), Ivermectin and Abamectin. Springer-Verlag, New York, pp. 234–244.

Canever, R.J., Braga, P.R.C., Boeckh, A., Grycajuck, M., Bier, D., Molento, M.B., 2013. Lack of *Cyathostomin* sp. reduction after anthelmintic treatment in horses in Brazil. Vet. Parasitol. 194, 35–39.

Canton, C., Ceballos, L., Moreno, L., Fiel, C., Domínguez, P., Bernat, G., Lanusse, C., Alvarez, L., 2015. Evaluation of ivermectin oral and injectable formulations against susceptible and resistant gastrointestinal nematodes in cattle. In: Proceedings of the 25th International Conference of the World Association for the Advancement of Veterinary Parasitology, Liverpool, UK.

Cirak, V.Y., Hermosilla, C., Bauer, C., 1996. Study on the gastrointestinal parasite fauna of ponies in northern Germany. Appl. Parasitol. 37, 239–244.

Coles, G.C., Bauer, F.H.M., Borgsteede, S., Geerst, T.R., Klei, T.R., Taylor, M.A., Waller, P.J., World association for the advancement of veterinary parasitology (W.A.A.V.P.), 1992. Methods for detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44.

Cross, H., Renz, A., Trees, A., 1998. *In vitro* uptake of ivermectin by adult male *Onchocerca ochengi*. Ann. Trop. Med. Parasitol. 92, 711–720.

Edward, C.L., Hoffmann, A.A., 2008. Ivermectin resistance in horse in Australia. Vet. Rec. 162, 56–57.

FCV-UNCPBA, 2016. http://www.vet.unicen.edu.ar/index.php/facultad/comite-bienestar-animal.

Fusé, L.A., Saumell, C.A., 2002. Epidemiología y control de endoparasitosis en potrancas criollas. Rev. Med. Vet. 83, 154–158.

Fusé, L.A., Saumell, C.A., Iglesias, L., 2013. Variación estacional del parasitismo interno en equinos: fenómeno de hipobiosis de los pequeños estrongilos (Cyathostominae) en Tandil, Buenos Aires, Argentina. Rev. Med. Vet. (B. Aires) 94, 62–72.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics, 2nd edn. Marcel Dekker, NewYork, pp. 145–198.

Gokbulut, C., Cirak, V.Y., Senlik, B., Aksit, D., Durmaz, M., McKellar, Q.A., 2010. Comparative plasma disposition, bioavailability and efficacy of ivermectin following oral and pour-on administrations in horses. Vet. Parasitol. 170, 120–126.

Gopal, R.M., West, D.M., Pomroy, W.E., 2001. The difference in efficacy of ivermectin oral, moxidectin oral and moxidectin injectable formulations against an ivermectin-resistant strain of *Trichostrongylus colubriformis* in sheep. N. Z. Vet. J. 49, 133–137.

Ho, N., Geary, T., Raub, T., Barsuhn, C., Thompson, D., 1990. Biophysical transport properties of the cuticle of *Ascaris suum*. Mol. Biochem. Parasitol. 41, 153–165.

Imperiale, F.A., Busetti, M.R., Suárez, V.H., Lanusse, C.E., 2004. Milk excretion of ivermectin and moxidectin in dairy sheep: assessment of drug residues during cheese elaboration and ripening period. J. Agric. Food Chem. 52, 6205–6211.

Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol. 20, 477–481.

Kaplan, R.M., Vidyashankar, A.N., 2012. An inconvenient truth: global worming and anthelmintic resistance. Vet. Parasitol. 186, 70–78.

Leathwick, D.M., Miller, C.M., 2013. Efficacy of oral, injectable and pour-on formulations of moxidectin against gastrointestinal nematodes in cattle in New Zealand. Vet. Parasitol. 191, 293–300.

Leathwick, D.M., Miller, C.M., Sauermann, C.W., Candy, P.M., Ganesh, S., Fraser, K., Waghorn, T.S., 2016. The efficacy and plasma profiles of abamectin plus levamisole combination anthelmintics administered as oral and pour-on formulations to cattle. Vet. Parasitol. 227, 85–92.

Lifschitz, A., Virkel, G., Pis, A., Imperiale, F., Sanchez, S., Alvarez, L., Kujanek, R., Lanusse, C., 1999. Ivermectin disposition kinetics after subcutaneous and intramuscular administration of an oil-based formulation to cattle. Vet. Parasitol. 86, 203–215.

Lifschitz, A., Virkel, G., Ballent, M., Sallovitz, J., Pis, A., Lanusse, C., 2005. Moxidectin and ivermectin metabolic stability in sheep ruminal and abomasal contents. J. Vet. Pharmacol. Ther. 28, 408–411.

Lloberas, M., Alvarez, L., Entrocasso, C., Virkel, G., Lanusse, C., Lifschitz, A., 2012. Measurement of ivermectin concentrations in target worms and host gastrointestinal tissues: influence of the route of administration on the activity against resistant *Haemonchus contortus* in lambs. Exp. Parasitol. 131, 304–309.

Love, S., Murphy, D., Mellor, D., 1999. Pathogenicity of cyathostomin infection. Vet. Parasitol. 85, 113–121.

Lyons, E.T., Tolliver, S.C., Ionita, M., Lewellen, A., Collins, S.S., 2008. Field studies indicating reduced activity of ivermectin on small strongyles in horses on a farm in central Kentucky. Parasitol. Res. 103, 209–215.

Lyons, E.T., Tolliver, S.C., Collins, S.S., 2009. Probable reason why small strongyle EPG counts are returning "early" after ivermectin treatment of horses on a farm in Central Kentucky. Parasitol. Res. 104, 569–574.

Lyons, E.T., Tolliver, S.C., Kuzmina, T.A., Collins, S.S., 2010. Critical tests evaluating efficacy of moxidectin against small strongyles in horses from a herd for which reduced activity had been found in field tests in Central Kentucky. Parasitol. Res. 107, 1495–1498.

Lyons, E.T., Tolliver, S.C., 2013. Further indication of lowered activity of ivermectin on immature small strongyles in the intestinal lumen of horses on a farm in Central Kentucky. Parasitol. Res. 112, 889–891.

Marriner, S.E., McKinnon, I., Bogan, J.A., 1987. The pharmacokinetics of ivermectin after oral and subcutaneous administration to sheep and horses. J. Vet. Pharmacol. Ther. er. 10, 175–179.

Matthews, J.B., Hodgkinson, J.E., Dowdall, S.M.J., 2004. Recent development in research into the Cyathostominae and *Anaplocephala perfoliata*. Vet. Res. 35, 1469–1477.

MAFF (Ministry of Agriculture Fisheries and Food), London, England 1986. Manual of Veterinary Parasitological Laboratory Techniques.

Molento, M.B., Antunes, J., Bentes, R.N., Coles, G.C., 2008. Anthelmintic resistant nematodes in Brazilian horses. Vet. Rec. 162, 384–385.

Mottier, M.L., Alvarez, L., Ceballos, L., Lanusse, C., 2006. Drug transport mechanisms in helminth parasites: passive diffusion of benzimidazole anthelmintics. Exp. Parasitol. 113, 49–57.

Murphy, D., Love, S., 1997. The pathogenesic effects of experimental cyathostome infections in ponies. Vet. Parasitol. 70, 99–110.

Nielsen, M.K., Reinemeyer, C.R., Donecker, J.M., Leathwick, D.M., Marchiondo, A.A., Kaplan, R.M., 2014. Anthelmintic resistance in equine parasites—current evidence and knowledge gaps. Vet. Parasitol. 204, 55–63.

Ogbourne, C.P., 1976. The prevalence, relative abundance and site distribution of nematodes of subfamily cyathostominae in horses killed in Britain. J. Helminthol. 50, 203–214.

Pérez, R., Godoy, C., Palma, C., Cabezas, I., Muñoz, L., Rubilar, L., Arboix, M., Alvinerie, M., 2003. Plasma profiles of ivermectin in horses following oral or intramuscular administration. J. Vet. Med. A: Physiol. Pathol. Clin. Med. 50, 297–302.

Perez, R., Godoy, C., Muñoz, C., Palma, L., Arboix, M., Alvinerie, M., 2010. Plasma disposition and fecal elimination of doramectin after oral or intramuscular administration in horses. Vet. Parasitol. 170, 112–119.

Perrier, D., Mayersohn, M., 1982. Non-compartmental determination of the steady-state volume of distribution for any mode of administration. J. Pharm. Sci. 71, 372–373.

Sangster, N.C., 1999. Pharmacology of anthelmintic resistance in Cyathostomes: will it occur with the avermectin/milbemycins? Vet. Parasitol. 85, 199–201.

Sims, S., Magas, L., Barsuhn, C., Ho, N., Geary, T., Thompson, D., 1992. Mechanisms of microenvironmental pH regulation in the cuticle of *Ascaris suum*. Mol. Biochem. Parasitol. 53, 135–148.

Soulsby, E., 1987. Parasitología y enfermedades parasitarias, 7ma Edición. Ed. Interamericana, México, D.F., México, pp. 177–182.

Steinbach, T., Bauer, C., Sasse, H., Baumgärtner, W., Rey-Moreno, C., Hermosilla, C., Damriyasa, I., Zahner, H., 2006. Small strongyle infection: consequences of larvicidal treatment of horses with fenbendazole and moxidectin. Vet. Parasitol. 139, 115–131.

Thompson, D., Ho, N., Sims, S., Geary, T., 1993. Mechanistic approaches to quantitate anthelmintic absorption by gastrointestinal nematodes. Parasitol. Today 9, 31–35.

Thompson, D., Geary, T., 1995. The structure and function of helminth surfaces. In: Marr, J., Muller, M. (Eds.), Biochemistry and Molecular Biology of Parasites. Academic Press Ltd., London, pp. 203–232.

Traversa, D., Klei, T.R., Iorio, R., Paoletti, B., Lia, R.P., Otranto, D., Sparagano, O.A.E., 2007. Occurrence of anthelmintic resistant equine cyathostome populations in central and southern Italy. Prev. Vet. Med. 82, 314–320.

Traversa, D., von Samson-Himmelstjerna, G., Demeler, J., Milillo, P., Schurmann, S., Barnes, H., Otranto, D., Perrucci, S., di Regalbono, A.F., Beraldo, P., Boeckh, A., Cobb, R., 2009. Anthelmintic resistance in cyathostomin populations from horse yards in Italy, United Kingdom and Germany. Parasit. Vectors 2 (Suppl. 2), S2.

von Samson-Himmelstjerna, G., Fritzen, B., Demeler, J., Schurmann, S., Rohn, K., Schnieder, T., Epe, C., 2007. Cases of reduced cyathostomin egg-reappearance period and failure of *Parascaris equorum* egg count reduction following ivermectin treatment as well as survey on pyrantel efficacy on German horse farms. Vet. Parasitol. 144, 74–80.