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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

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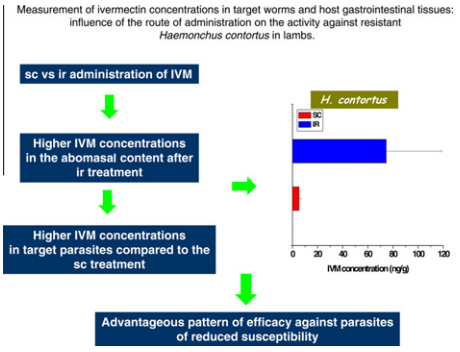
HIGHLIGHTS

- The anthelmintic action depends on the ability of the active drug to reach parasites.
- Ivermectin reaches the parasites from the gastrointestinal contents or from plasma.
- Ivermectin concentrations were higher in the abomasal content after its ir treatment.
- Drug concentrations in *Haemonchus spp* were higher after the ir administration of ivermectin.
- This advantageous pattern of IVM profiles may be relevant against resistant parasites.

GRAPHICAL ABSTRACT



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ABSTRACT

The influence of the administration route on the relationship between efficacy and ivermectin concentration profiles achieved in the bloodstream, the gastrointestinal mucosal tissues/fluid contents and within a target abomasal parasite (*Haemonchus contortus*) was evaluated in lambs. Twenty-six (26) parasitized lambs were assigned into three experimental groups: untreated (control) and ivermectin treated by the subcutaneous and intraruminal route at 0.2 mg/kg. Blood samples were collected between 0 and 15 days post-treatment (plasma disposition study). Four animals from each group were sacrificed at day 3 post-treatment. Mucosa and content samples from abomasum and small intestine and adult specimens of *H. contortus* were collected. Drug concentrations were measured by HPLC. Individual fecal egg counts were evaluated at −1, 3 and 15 days post treatment. Post-mortem examination was done at day 15 post-treatment. Adult nematodes recovered from the digestive tract were counted and identified by species. Ivermectin plasma availability was higher ($P < 0.05$) after the subcutaneous administration (129 ng.d/ml) compared to the intraruminal treatment (58.4 ng.d/ml). However, ivermectin concentrations measured in the gastrointestinal contents were higher in lambs treated by the intraruminal route. The mean ivermectin concentrations achieved (3 days post-treatment) in the abomasal content were 143 ng/g (intraruminal) and 2.53 ng/g (subcutaneous). Ivermectin concentrations were 15-fold higher in *H. contortus* recovered from intraruminally treated lambs. Whereas the subcutaneous administration reduced the number of adult nematodes from 4376 to 1300, the number of adult nematodes after the treatment with ivermectin given by the intraruminal route was 206 ($P < 0.05$). The higher ivermectin

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concentrations achieved in the digestive tract shortly after the intraruminal treatment may account for the observed enhanced efficacy compared to the parenteral administration against parasites of reduced susceptibility.

1. Introduction

Ivermectin (IVM) is a broad-spectrum antiparasitic drug that belongs to the macrocyclic lactones family, widely used to control endo and ectoparasites. IVM is available to be administered to sheep either subcutaneously or orally (McKellar and Benchaoui, 1996). Although this compound exhibits high efficacy against the most important gastrointestinal nematodes in sheep and goats, resistance is becoming a serious problem after many years of intensive use (Kaplan, 2004; Wolstenholme et al., 2004).

The choice of the IVM administration route in sheep is either based on practical management reasons or influenced by the technical marketing of the pharmaceutical companies. In the early days, shortly after the IVM introduction into the market, nematode susceptibility to IVM was high and equivalent efficacy patterns were observed against abomasal parasites following its parenteral and oral administration. A similar pattern was later described for other macrocyclic lactones from both the avermectin (abamectin) and milbemycin (moxidectin) families. After oral treatment with IVM, a slightly improved effect against intestinal nematodes was shown compared to the parenteral treatment (Borgsteede, 1993). However, when the efficacy against IVM-resistant nematodes was assessed, a significant greater pharmacological activity was obtained after the oral administration of both abamectin and moxidectin compared to their subcutaneous injections in lambs (Alka et al., 2004; Gopal et al., 2001). The pharmacological principles behind the observed differences on efficacy against resistant nematodes need to be elucidated. The mechanisms underlying the drug uptake process and their relationship with the exposure of the target parasite to macrocyclic lactones are critical issues to be addressed.

Extensive information describing the IVM plasma disposition kinetics in sheep is available (Barber et al., 2003; Imperiale et al., 2004; Lifschitz et al., 2010). The characterization of the plasma concentration profiles of the macrocyclic lactones has provided useful information to a better understanding of the pharmacological features of these antiparasitic compounds. However, the *in vivo* concentrations required to kill the different nematodes species in the gastrointestinal tract remain unknown. In this context, the evaluation of the avermectin/milbemycin concentration profiles reached in the tissues of parasite location as well as within target nematodes, may greatly contribute to the comprehension of the time course of action for these compounds. The current work aimed to assess the influence of the administration route on the relationship between efficacy and IVM concentration profiles achieved in the bloodstream, the gastrointestinal mucosal tissues/fluid contents and within a target abomasal parasite (*Haemonchus contortus*) in lambs naturally infected with resistant nematodes.

2. Material and methods

2.1. Animals

Twenty-six Romney Marsh lambs (27.2 ± 4.48 kg), naturally infected with resistant gastrointestinal (GI) nematodes were involved in this trial. The selected farm is a sheep experimental unit with a parasite control program based on the intensive use of anthelmintics over the years, where IVM failure to control nematodes was previously corroborated (Entrocasso et al., 2008; Lifschitz et al., 2010). The selection of the animals was among 50 lambs based on worm egg per gram counts (epg). On day −1 all lambs were checked for epg, ear tagged and the individual body weights were recorded. Experimental animals had an average of 2893 ± 1143 epg counts ranging from 1500 to 4980. All lambs with counts of at least 1000 epg were ranked by fecal egg counts, blocked into groups of three, and within a block lambs were randomly assigned to an experimental group. Animals were allocated in a paddock and fed on a lucerne/white and red clover pasture 20 days before starting the clinical efficacy study and during the experiment. 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 087/02) of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina (http://www.vet.unicen.edu.ar).

2.2. Experimental design, treatments and samplings

Experimental lambs were assigned into three (3) experimental groups. Group A ($n = 6$) remained as untreated control. Animals in Group B ($n = 10$) received IVM (Ivomec®, Merial Argentina) (200 μg/kg, subcutaneously) (sc IVM). Animals in Group C ($n = 10$) received IVM (Ivomec®Oral, Merial Uruguay) (200 μg/kg, intraruminally) (ir IVM). The intraruminal route was selected instead of the oral administration to avoid the closure of the esophageal groove and to minimize the variability. To study the distribution of IVM to target tissues and parasites four animals from groups B and C were sacrificed at day 3 post-administration and samples of blood, abomasal and small intestine (cranial jejunum) contents and mucosal tissue were taken following the procedures described in Lifschitz et al., 2000. From the abomasum of each animal the total mass of *H. contortus* was recovered to measure the drug concentration in the parasites.

To characterize the efficacy and the plasma disposition of IVM after its administration by both routes, fecal samples were collected from all the lambs in each experimental group at days −1 and 15 post-treatment in order to estimate the epg counts. Jugular blood samples (7 ml) were collected into heparinized vacutainer tubes prior to and at 0, 3, 6, 9 h and 1, 2, 3, 5, 7, 9, 12 and 15 days post-treatment. Blood samples were centrifuged at 2000*g* for 20 min and the recovered plasma was kept in labeled vials. Plasma, tissues and gastrointestinal contents samples were stored at −20 °C until analyzed by high performance liquid chromatography (HPLC). Additionally, at 15 days post-treatment, all the animals in each experimental group were sacrificed by captive bolt gun and rapidly exsanguinated. Abomasum and different gut sections were identified and isolated (small and large intestine) and the content analyzed to record the different parasite stages following the World Association for the Advancement of Veterinary Parasitology guidelines (Wood et al., 1995).

2.3. Analytical procedures

2.3.1. Parasitological techniques

The individual fecal egg counts were performed using the modified McMaster technique (Roberts and O'Sullivan, 1949). The anthelmintic efficacy of the treatments was evaluated by the fecal

egg count reduction test (FECR), calculated according to the formula (Coles et al., 1992):

$$\mathrm{FECR}(\%) = 100^{*}(1 - T/C),$$

where T is the arithmetic mean epg counts in the treated group at 15 days post treatment and C is the arithmetic mean epg counts in the untreated control group at 15 days post treatment. The 95% confidence intervals were calculated as reported by Coles et al., (1992). Direct adult nematode counts of animals from experimental groups were determined 15 days after treatment according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Wood et al., 1995). The efficacy of each anthelmintic treatment was determined by the comparison of worm burdens in treated versus untreated animals. The following equation expresses the percentage of efficacy (% E) of a drug treatment against a given parasite species (S) in a single treatment group (T) when compared with an untreated control (C):

$$\%\mathrm{E} = [(\text{Mean of S in C} - \text{Mean of S in T})/\text{Mean of S in C}]^{*}100$$

The geometric mean was used according to recommendation of Wood et al. (1995).

2.3.2. Pharmacological determinations

2.3.2.1. IVM chemical extraction, derivatization and HPLC analysis. The extraction of IVM from spiked and experimental plasma samples was carried out following the technique first described by Alvinerie et al. (1993) and slightly modified by Lifschitz et al. (2000). Basically, 1 ml aliquot of plasma, 0.5 g of gastrointestinal samples (mucosas and contents) and 50 mg of parasites were combined with 10 ng of the internal standard compound (abamectin) and then mixed with 1 ml of acetonitrile–water (4:1). After the liquid–liquid extraction, the supernatant was then placed on the appropriate rack of an Aspec XL sample processor (Gilson, Villiers Le Bel, France) and the process of solid phase extraction was done. The derivatization was initiated as described by De Montigny et al. (1990). IVM concentrations were determined by HPLC using a Shimadzu 10A HPLC system with autosampler and fluorescence detection (Shimadzu Corporation, Kyoto, Japan) as described by Lifschitz et al. (2000). A complete validation of the analytical procedures used for extraction and quantification of IVM from plasma, gastrointestinal mucosa/contents and parasites was performed before starting the analysis of the experimental samples. The linear regression lines for IVM showed determination coefficients between 0.982 and 0.999. The mean recoveries of IVM were in a range between 84% and 96%. The inter assay precision of the analytical procedures obtained after HPLC analysis of IVM on different working days showed a coefficient of variation (CV) < to 10%. The limit of quantification was established at 0.1 ng/ml (plasma) and 1 ng/g (gastrointestinal mucosa/content and *H. contortus*).

2.4. Pharmacokinetic analysis of the data

The plasma concentrations versus time curves obtained after each treatment in each individual animal were fitted with the PK Solutions 2.0 (Ashland, Ohio, USA) computer software. Pharmacokinetic parameters were determined using a non-compartmental model method. The peak concentration (Cmax) was read from the plotted concentration–time curve in each individual animal. The area under the concentration versus time curves (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 (λz). The terminal (elimination) half-life ($t_{1/2el}$) was calculated as $\ln 2/\lambda z$. IVM plasma concentrations and all the estimated pharmacokinetic parameters are reported as mean ± SD.

2.5. Statistical analysis

Fecal egg and nematode counts (reported as arithmetic mean ± SD) were analyzed by non-parametric ANOVA (Kruskal–Wallis test). Mean pharmacokinetic parameters for IVM were statistically compared using Student's *t*-test. The assumption that the data obtained after treatments have the same variance was assessed. A non-parametric Mann–Whitney test was used where significant differences among standard deviations were observed. The statistical analysis was performed using the Instat 3.0 Software (Graph Pad Software, CA, USA). A value of $P < 0.05$ was considered statistically significant.

3. Results

The low percentage of reduction in the number of eggs in feces observed in the current trial indicates the presence of gastrointestinal (GI) nematodes resistant to IVM. The number of eggs in feces in lambs intraruminally treated (ir IVM) was significantly lower (mean 990 eggs) compared to the untreated control and sc IVM group (mean 2340 eggs) at 3 days post-treatment. However, there were no differences between the groups at 15 days post-administration of IVM. The FECR showed a reduction of 45.5% (sc IVM)

Table 1
Nematode egg counts (arithmetic mean range), reduction percentage of fecal egg counts (FECR), nematode worm counts (geometric mean and range) and efficacy obtained 15 days after ivermectin (IVM) (0.2 mg/kg) administration by the subcutaneous (sc) or intraruminal (ir) route to infected lambs. Nematode worm counts recorded in the untreated control group are also shown.

	Untreated group		sc IVM		ir IVM
Mean epg[1] (range)					
Day −1	2867 (1500–4980)		2930 (1560–4680)		2883 (1560–4280)
Day 15	4803[a] (1800–11220)		2620[a] (840–5400)		2320[a] (480–3720)
FECR (%) (UCL-LCL)			45 (77–0)		52 (79–0)
	Worm counts	Worm counts	Efficacy (%)	Worm counts	Efficacy (%)
Abomasum					
Haemonchus spp.	502[a] (310–980)	597[a] (230–1090)	0	300[a] (50–520)	40.2
Teladorsagia spp.	790[a] (430–1640)	36[b] (0–850)	95.4	3.2[c] (0–10)	99.9
Trichostrongylus axei	322[a] (120–690)	20.6[c] (0–400)	93.6	0.6[c] (0–10)	99.8
Small intestine					
Trichostrongylus colubriformis	1491[a] (370–3230)	96[b] (0–2430)	93.6	1.5[c] (0–20)	99.9
Nematodirus spp.	342[a] (140–650)	7.8[b] (0–1200)	95.7	0.5[c] (0–10)	99.9
Large intestine					
Oesophagostomum spp.	54.3[a] (10–140)	0.7[b] (0–20)	98.8	0[b]	100

[1] Arithmetic mean of eggs per gram of feces; UCL: 95% upper confidence limit; LCL: 95% lower confidence limit. Nematode egg and adult nematode counts at day 15 post-treatment with different superscript are statistically different at $P < 0.05$.

Table 2
Mean (± SD) plasma pharmacokinetic parameters for ivermectin (IVM) ($n = 6$) obtained after its subcutaneous (sc) and intraruminal (ir) administrations (0.2 mg/kg) to lambs.

Kinetic parameters	sc IVM	ir IVM
T 1/2 ab (days)	0.54 ± 0.28	0.33 ± 0.11
Tmax (days)	1.67 ± 0.52	1.33 ± 0.52
Cmax (ng/ml)	22.2 ± 9.03	17.2 ± 7.30
AUC (ng.d/ml)	129 ± 41.5	58.4 ± 13.0 *
MRT (days)	5.65 ± 2.90	3.32 ± 0.39 *
T 1/2 el (days)	3.43 ± 2.23	2.07 ± 0.23 *

T 1/2 ab: absorption half-life. Tmax:time to peak plasma concentration. Cmax:peak plasma concentration. AUC: area under the concentration vs time curve extrapolated to infinity. MRT: mean residence time. T½ el: elimination half-life. (*) Values are statistically different from those obtained after sc administration of IVM at $P < 0.05$.

and 51.7% (ir IVM). After the treatment with IVM, significant differences were found in the number of adult parasites by both administration routes. Whereas the sc administration reduced the number of adult nematodes from 4376 to 1300, the number of adult nematodes was 206 ($P < 0.05$) after the ir IVM treatment. The mean number of the different abomasal and intestinal nematodes was lower after the ir administration of IVM. *H. contortus* showed to be highly resistant to IVM with a mean worm burden reduction of 0% (sc treatment) and 40.5% (ir treatment) compared to the control group. Besides, the efficacy of sc IVM against intestinal nematode parasites such as *Trichostrongylus colubriformis* was <95% showing a low level of resistance. The fecal egg and the adult nematode counts and resultant clinical efficacy obtained after the administration of IVM by both routes are shown 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. The higher IVM plasma profiles observed in the sc IVM group accounted for an enhanced systemic availability. The mean IVM AUC values were 129 (sc) and 58.4 ng.d/ml (ir). A prolonged plasma elimination half-life was obtained after the sc treatment (1.66 fold). The comparative pharmacokinetic parameters obtained after the administration of IVM by both routes are shown in Table 2.

Whereas similar IVM concentrations were measured in abomasal and intestinal mucosal tissues (between 38.6 and 65.6 ng/g) after treatment by both routes, higher IVM concentrations were recovered in the gastrointestinal contents, particularly in abomasum, after the ir treatment. The mean IVM concentrations achieved (3 days post-treatment) in the abomasal content were 143 ng/g (ir IVM) and 2.53 ng/g (sc IVM). The levels of IVM in the intestinal contents were 120 ng/g (ir IVM) and 55.6 ng/g (sc IVM). The IVM plasma concentration profiles and the relationship (ratio) between IVM concentrations achieved in the abomasal and intestinal contents/ mucosal tissues are shown in Fig. 1. Interestingly, marked differences were observed in IVM concentrations measured within *H. contortus* recovered from lambs treated by the sc and ir routes. The IVM concentrations in *H. contortus* were 74.4 ng/g (ir) and 5.19 (sc). The relationship between IVM concentrations measured in plasma, abomasal contents and *H. contortus* is shown in Fig. 2.

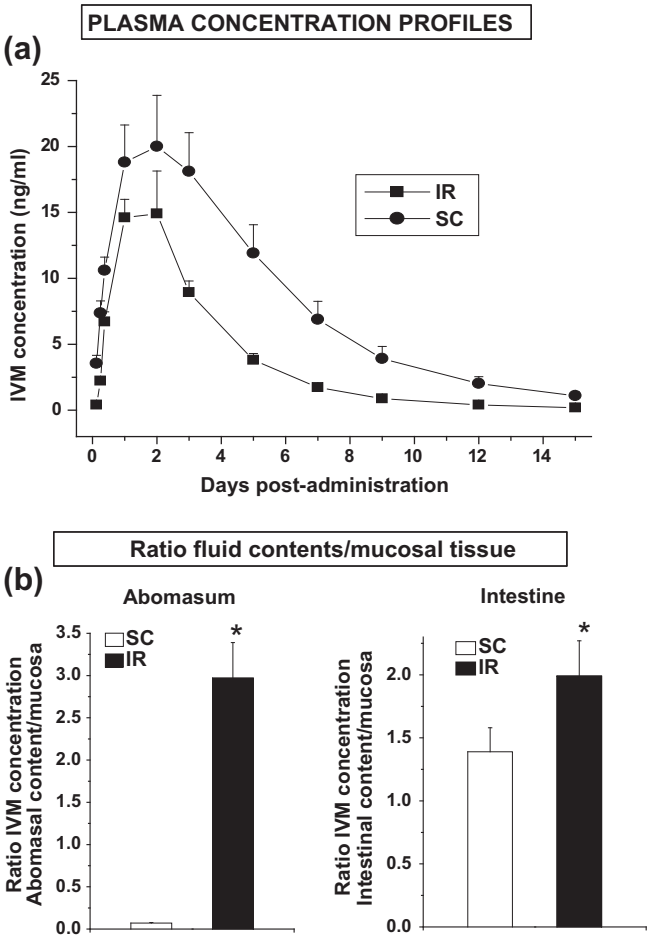

Fig. 1. Mean (± SD) ($n = 6$) plasma concentrations (a) and mean concentration ratios (± SD) ($n = 4$) of ivermectin (IVM) measured in the abomasal fluid content and mucosal tissue and in the content and mucosa of the small intestine (b) obtained after its subcutaneous (sc) and intaruminal (ir) administration (0.2 mg/kg) to lambs. (*) Values are statistically different from those obtained after sc administration of IVM at $P < 0.05$.

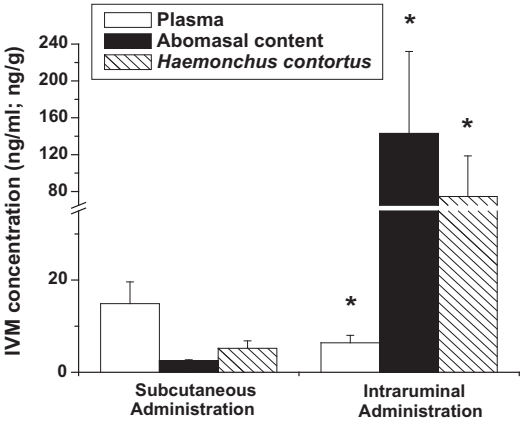

Fig. 2. Mean (± SD) ($n = 4$) ivermectin concentrations measured in plasma, abomasal content and adult *H. contortus* at day 3 after its subcutaneous (sc) and intaruminal (ir) administration (0.2 mg/kg) to lambs. (*) Values are statistically different from those obtained after sc administration of IVM at $P < 0.05$.

4. Discussion

As it was previously reported, the IVM plasma systemic availability was higher after the sc injection compared to the ir treatment (Marriner et al., 1987; Imperiale et al., 2004). The higher IVM plasma concentrations obtained after its sc administration support the clinical indication to use this route to control ectoparasites. Besides, the longer mean residence time and elimination half-life observed for IVM after its sc administration account for the persistent antiparasitic activity (over 10 days) against *H. contortus* (Borgsteede, 1993), which is in contrast to the shorter persistence of action obtained after the oral/intraruminal administration. Traditionally, the administration of IVM by the oral or sc routes showed similar

efficacy against *H. contortus* and slightly higher efficacy against intestinal endoparasites (Borgsteede, 1993). However, after the ir administration of IVM to lambs infected with resistant parasites the efficacy was higher compared to that obtained after the sc treatment (Table 1). *H. contortus* was fully resistant to IVM after the sc administration (0% efficacy) and the activity increased to 40.5% after the ir administration of the drug. Similar results were obtained with other macrocyclic lactones such as abamectin and moxidectin (Gopal et al., 2001; Alka et al., 2004) where the highest efficacy against resistant *T. colubriformis* was obtained after the oral administration of the antiparasitic drugs to sheep.

The anthelmintic action depends on the ability of the active drug to reach its specific receptor within the target parasite. Thus, drug entry and accumulation in target helminths are important issues to obtain an optimal clinical efficacy (Alvarez et al., 2007). It seems that the transcuticular diffusion is the main route of access for different substances in nematodes and the drug lipophilicity is the major determinant of the rate of transfer across the nematode cuticle (Thompson et al., 1993). Lipophilic drugs such as IVM, may reach the target parasite from the gastrointestinal contents (transcuticular route) or from plasma (oral ingestion) if the nematode (*H. contortus*) feeds on host blood. Although similar concentration profiles were measured in the abomasal mucosa after treatment by both routes, markedly lower IVM concentrations were recovered in the abomasal contents after its sc injection. The active secretion of IVM (and other macrocyclic lactones such as doramectin) from the bloodstream to the abomasal lumen is of little relevance (Hennessy et al., 2000) as opposed to that observed at the small intestine level. Consistently, the sc administration of IVM to sheep at ten times (2 mg/kg) the therapeutic dose resulted in very low concentrations in the abomasal content (Bogan and Mckellar, 1988). The amount of drug reaching the target parasite is influenced by the drug concentration in the tissue where the parasite is located (Lifschitz et al., 2000). In the current trial, the higher concentrations observed in the abomasal content after the ir administration of IVM, accounted for the greater amount of drug in *H. contortus* (Fig. 2). These enhanced IVM concentrations may explain the lower number of adult *H. contortus* recovered after the ir treatment.

Similarly, higher IVM concentrations were observed in the small intestinal content after the ir treatment. Nevertheless, the magnitude of differences in drug concentrations between the two administration routes was smaller than that described in the abomasum. The biliary elimination of macrocyclic lactones in ruminants (Bogan and McKellar, 1988; Hennessy et al., 2000; Lifschitz et al., 2000) and the *P*-glycoprotein-mediated intestinal secretion (Laffont et al., 2002; Ballent et al., 2006, 2007) determine that a large amount of IVM reaches the intestinal luminal content after the sc administration. However, the higher drug concentrations measured in the intestinal content during the first 2–3 days after the oral/IR administration may be crucial to the efficacy against resistant parasites located in the intestine, such as *T. colubriformis*.

In vivo drug concentrations required to inhibit parasite establishment or larval development have not been determined yet. These *in vivo* trials are necessary to complement the available data obtained under *in vitro/ex vivo* assays. The level of drug concentrations attained in the systemic circulation and gastrointestinal tract tissues after the sc and oral/ir administration of IVM account for an equivalent efficacy against susceptible gastrointestinal nematodes. However, the high concentrations of drug detected in the gastrointestinal tract during the first 2–3 days after the oral/ir treatment may have a relevant effect on the resistant nematodes, being of great importance to induce the pharmacological action at the target site. The increment of drug exposure may be a strategy to kill heterozygous resistant parasites present in the earliest phases of development of resistance. This type of pharmaco-parasitological integrated information should be considered to optimize the efficacy of widely used drugs (such as IVM) but also of the new compounds recently introduced into the veterinary pharmaceutical market.

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References

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

Alvarez, L.I., Mottier, M.L., 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.

Ballent, M., Lifschitz, A., Virkel, G., Sallovitz, J., Lanusse, C., 2006. Modulation of the *P*-glycoprotein-mediated intestinal secretion of ivermectin: in vitro and in vivo assessments. Drug Metab. Dispos. 34, 457–463.

Ballent, M., Lifschitz, A., Virkel, G., Sallovitz, J., Lanusse, C., 2007. Involvement of *P*-glycoprotein on ivermectin kinetic behaviour in sheep: itraconazole-mediated changes on gastrointestinal disposition. J. Vet. Pharmacol. Ther. 30, 242–248.

Barber, S., Bowles, V., Lespine, A., Alvinerie, M., 2003. The comparative serum disposition kinetics of subcutaneous administration of doramectin, ivermectin and moxidectin in the Australian merino sheep. J. Vet. Pharmacol. Ther. 26, 343–348.

Bogan, J., McKellar, Q., 1988. The pharmacodynamics of ivermectin in sheep and cattle. J. Vet. Pharmacol. Ther. 11, 260–268.

Borgsteede, F., 1993. The efficacy and persistent anthelmintic effect of ivermectin in sheep. Vet. Parasitol. 50, 117–124.

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

De Montigny, P., Shim, J., Pivinichny, J., 1990. Liquid chromatographic determination of ivermectin with trifluoro-acetic anhydride and *N*-methylimidazole as the derivatization reagent. J. Pharm. Biomed. Anal. 8, 507–511.

Entrocasso, C., Alvarez, L., Manazza, J., Lifschitz, A., Borda, B., Virkel, G., Mottier, L., Lanusse, C., 2008. Clinical efficacy assessment of the albendazole–ivermectin combination in lambs parasitized with resistant nematodes. Vet. Parasitol. 155, 249–256.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics 2nd Edition Revised and Expanded. Marcel Dekker, Inc., New York, USA.

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

Hennessy, D.R., Page, S.W., Gottschall, D., 2000. The behaviour of doramectin in the gastrointestinal tract, its secretion in bile and pharmacokinetic disposition in the peripheral circulation after oral and intravenous administration to sheep. J. Vet. Pharmacol. Ther. 23, 203–213.

Imperiale, F., Lifschitz, A., Sallovitz, J., Virkel, G., Lanusse, C., 2004. Comparative depletion of ivermectin and moxidectin milk residues in dairy sheep after oral and subcutaneous administration. J. Dairy Res. 71, 427–433.

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

Laffont, C.M., Toutain, P.L., Alvinerie, M., Bousquet-Melou, A., 2002. Intestinal secretion is a major route for parent ivermectin elimination in the rat. Drug Metab. Dispos. 30, 626–630.

Lifschitz, A., Virkel, G., Sallovitz, J., Galtier, P., Alvinerie, M., Lanusse, C., 2000. Comparative distribution of ivermectin and doramectin toparasite location tissues in cattle. Vet. Parasitol. 87, 327–338.

Lifschitz, A., Entrocasso, C., Alvarez, L., Lloberas, M., Ballent, M., Manazza, G., Virkel, G., Borda, B., Lanusse, C., 2010. Interference with *P*-glycoprotein improves ivermectin activity against adult resistant nematodes in sheep. Vet. Parasitol. 172, 291–298.

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. 1, 175–179.

McKellar, Q., Benchaoui, H., 1996. Avermectins and milbemycins. J. Vet. Pharmacol. Ther. 19, 331–351.

Roberts, F., O'Sullivan, P., 1949. Methods for egg counts and larval cultures for strongyles infecting the gastrointestinal tract of cattle. Aust. J. Agr. Resour. 1, 99–103.

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

Wood, I.B., Amaral, N.K., Bairden, K., Duncan, J.L., Kassai, T., Malone, J.B., Pankavich, J.A., Reinecke, R.K., Slocombe, O., Taylor, S.M., Vercruysse, J., 1995. World association for the advancement of veterinary parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Vet. Parasitol. 58, 181–213.

Wolstenholme, A., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G., Sangster, V., 2004. Drug resistance in veterinary parasites. Trends Parasitol. 20, 469–476.