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Failure of ivermectin and eprinomectin to control *Amblyomma parvum* in goats: Characterization of acaricidal activity and drug pharmacokinetic disposition

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Abstract

The therapeutic efficacies of ivermectin (subcutaneous injection) and eprinomectin (topical treatment) given at two different dosage levels to goats naturally infested with *Amblyomma parvum* were assessed. Treatments included subcutaneous injection of ivermectin at 0.2 and 0.4 mg/kg and extra-label pour-on administration of eprinomectin at 0.5 and 1 mg/kg b.w. Ivermectin and eprinomectin failed to control *Amblyomma parvum* on goats. Treatment with ivermectin resulted in a low number of engorged female ticks in relation to untreated control goats and, at the highest dose rate (0.4 mg/kg), the female engorgement weights were significantly lower and the pre-oviposition period significantly longer than those observed in ticks recovered from untreated control goats. The tick efficacy assessment was complemented in a separate group of tick-free goats with a pharmacokinetic characterization of eprinomectin (topically administered at 0.5, 1.0 and 1.5 mg/kg) and ivermectin (subcutaneous treatment given at (0.2 and 0.4 mg/kg) in goats. Heparinized blood samples were taken between 0 and 21 days post-treatment. Higher and more persistent drug plasma concentrations were recovered after the subcutaneous treatment with ivermectin compared to those obtained for eprinomectin topically administered. The understanding of the relationship among the pattern of drug absorption, the kinetic disposition and the resultant clinical efficacy is relevant to improve the poor performance observed for ivermectin and eprinomectin against *A. parvum* on goats.

Keywords: Goats; Ivermectin; Eprinomectin; Pharmacokinetics; *Amblyomma parvum*; Efficacy

1. Introduction

The neotropical tick *Amblyomma parvum* prevails in arid and semiarid conditions of northern Argentina (Guglielmone et al., 1990) where goat herds of low productivity and technical input are common. *A. parvum* is a tick species prone to infest domestic animals in northern Argentina, where infestation of cattle and goats with adult stages of this tick species is rather usual (Nava et al., in

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press). Its life cycle is sustained by subadults feeding on medium size rodents as *Galea musteloides* (Caviidae) and adults on a great variety of hosts but Artiodactyla introduced to the Neotropical region currently constitutes the major food source for this stage of *A. parvum* (Nava et al., 2006). Under these conditions adults of *A. parvum* are frequently found infesting goats during summer, particularly around the eyes, sometimes producing inflammatory conditions in palpebral regions (Guglielmone, 1988).

The avermectins are naturally occurring compounds used against endo and ectoparasites in mammals (Campbell et al., 1983). Ivermectin (IVM), a semi-synthetic derivative of avermectin B1, consists of an 80:20 mixture of the equipotent homologous 22,23 dehydro B1a and B1b (Fisher and Mrozik, 1989). Eprinomectin (EPN) is an avermectin that has been selected after examination of several hundred analogues, to be used as a topical endectocide in cattle. The chemical structure of EPN was chemically manipulated to change the blood–milk partitioning coefficients in lactating dairy animals, which allows its use in dairy cattle with zero-milk withdrawal period (Shoop et al., 1996a). Although EPN is licensed for use in lactating cows, it is currently used worldwide in sheep and goats as an extra-label medication at the same dose rate recommended for its administration in cattle (Dupuy et al., 2001; Hoste et al., 2004). The pharmacokinetic of EPN topically administered was described in cattle (Alvinerie et al., 1999a), goats (Alvinerie et al., 1999b) and sheep (Imperiale et al., 2006). Although a dose rate of 0.5 mg/kg was considered adequate to obtain a good efficacy against endo and ectoparasites in cattle (Shoop et al., 1996b), a lower efficacy was obtained against the dose limiting species in goats (Chartier et al., 1999).

There is no available information on the chemical control of *A. parvum*. Besides, there are not registered parasiticides for use in goats in Argentina as well as in many other countries. Thus, three different experiments were conducted in a 3 years time period to assess the efficacy of IVM and EPN for controlling natural infestations of *A. parvum* in goats. The work was complemented with the evaluation of the pharmacokinetic behaviour of both compounds in goats to broadly understand the relationship between tick control and drug absorption/disposition.

2. Materials and methods

2.1. Efficacy studies

The efficacy studies were carried out in a private property, located in San José de las Salinas, Córdoba, Argentina, using adult female Anglo Nubian goats weighing 40–50 kg body weight, naturally exposed to infestations with *A. parvum*. The goats were maintained in small paddocks from late evening to early morning when they were allowed to fed in natural forests (*Prosopis* sp., *Zizipus mistol*), bushes (*Acacia aroma*, *Celtis spinosa*), climbing plants (*Pilectenium cynanchoides*) and pastures (*Setaria lineata*, *Thrichloris crinita*), and eventually on implanted pastures (*Panicum maximum*), surroundings the paddocks.

The study consisted of two separate efficacy trials with three (3) groups of goats for each trial, using animals with homogeneous tick burdens ($P > 0.05$, test of Kruskal–Wallis) evaluated as total number of females of *A. parvum* per goat on day 0. The first trial was performed with 15 animals that were divided into three groups of five (5) goats each; individuals of one group were treated with a commercial pour-on formulation (0.5%) of EPN (IVOMEC® EPRINEX™ POUR ON, Merial Argentina S.A.) applied on the dorsal midline (from withers to the tail head) at a rate of 1 ml/10 kg of body weight to provide the recommended dose against cattle gastrointestinal nematodes (0.5 mg/kg). Animals in other group were treated with 1.0 mg of EPN per kg (double dose) and the third group was the untreated control. The trial started on 24 February 2005 (day 0) and ended on 24 March (day 28 post-treatment).

The second trial involved eighteen (18) goats divided into three (3) experimental groups of six (6) individuals each. Goats of one group were treated with 0.2 mg/kg of a commercial injectable (subcutaneous) IVM formulation (IVOMEC®, Merial Argentina S.A.) to provide the recommended dose against cattle and sheep gastrointestinal nematodes. Animals in other group received 0.4 mg/kg of IVM, while the remainder goats formed the untreated control group. The trial started on 15 February 2006 (day 0) and ended on 15 March (day 28 post-treatment).

The *A. parvum* female ticks attached to each goat were counted on days 1, 2, 3, 7, 14, 21 and 28 post-treatment. Two operators searched for female ticks for a period of 10 min per goat. Whenever available engorged female ticks were manually collected from the experimental goats up to day 7 post-treatment, where it was expected to reach the maximum effects of IVM and EPN. The ticks were weighted and maintained in darkness at 27 ± 1 °C, 83–86% of relative humidity to evaluate the pre-oviposition and minimum egg incubation periods. The reproductive efficiency index (REI) that expresses the number of eggs laid per mg of body weight (Drummond and Whetstone, 1970), and the fertility efficiency index (FEI) expressed as the number

of hatched larvae per mg of body weight (Aguirre et al., 2005) were also estimated. To achieve this aim, the larvae and unhatched eggs for each eggs mass laid by a female tick were counted as described by Guglielmone et al. (1989).

Drug treatment efficacies were evaluated by comparison of female tick burdens (Kruskal–Wallis test), while biological parameters of engorged female ticks were analyzed by using chi-square distribution, proportion test and ANOVA when applicable.

2.2. Pharmacokinetic study

2.2.1. Experimental animals, treatments and sampling

The pharmacokinetic study was carried out in the Campo Anexo, Agencia Extensión Regional Dean Funes, Instituto Nacional de Tecnología Agropecuaria, Dean Funes, Córdoba, Argentina which is free of *A. parvum*. This complementary kinetic disposition evaluation was designed after observing a poor and differential therapeutic response for EPN and IVM. The study started on 12 February and ended on 5 March 2007. It was conducted in Anglo Nubian adult female goats, weighing 33–45 kg. The goats were grazed in natural forests (bushes, some climbing plants and pastures) as above described for the animals used in the efficacy trials. However, the animals were housed separately in roofed small pens and grazed separately after treatment to avoid liking-related cross-contamination after topical treatment. The general body condition was similar among all the goats (infected and uninfected) used in the different trials. Five (5) groups of five goats each from a herd free of ticks were treated as follows: animals in Groups 1–3 received EPN by topical (pour-on) treatment at the rates of 0.5 mg/kg (Group 1), 1.0 mg/kg (Group 2) or 1.5 mg/kg (Group 3). Goats in the remainder groups were subcutaneously treated with IVM at either 0.2 mg/kg (Group 4) or 0.4 mg/kg (Group 5). The mean body weights (kg) (±S.D.) for each treated group in the pharmacokinetic trial were as follow: 38.8 ± 4.95, 38.3 ± 4.86, 38.4 ± 4.63, 39.0 ± 5.21 and 38.7 ± 5.04 ($P = 0.999$).

Heparinized blood samples were obtained from each goat at 0, 4, 8 h and at 1, 2, 3, 4, 5, 7, 9, 11, 15 and 21 days post-treatment. All samples were centrifuged 10 min at 3500 rpm and the plasma obtained frozen at −20 °C until processing for HPLC analysis.

2.2.2. Analytical procedures

2.2.2.1. Chemical extraction and derivatization. The extraction of IVM (22,23 dehydro-avermectin B1a), from spiked and experimental plasma samples was carried out following the technique first described by Alvinerie et al. (1993) slightly modified by Lifschitz et al. (1999). EPN was analyzed following the technique described by Imperiale et al. (2006). Basically, 1 ml-aliquot of plasma sample was combined with 10 ng of the internal standard compound (abamectin) and then mixed with 1 ml of acetonitrile–water (4:1). After mixing for 20 min, the solvent-sample mixture was centrifuged at 2000 *g* during 15 min. The supernatant was manually transferred into a tube that was then placed on the appropriate rack of an Aspec XL sample processor (Gilson, Villiers Le Bel, France). The supernatant was injected to a Supelclean LC_{18} cartridge (Supelco, Bellfonte, PA, USA), previously conditioned by passing 2 ml methanol and 2 ml deionized water. The cartridge was flushed with 1 ml of water and 1 ml of water/methanol (4:1). The compounds were eluted with 1.5 ml of methanol and concentrated to dryness under a stream of nitrogen. The dry residue of IVM and EPN was derivatized as previously described by De Montigny et al. (1990) and Danaher et al. (2001), respectively.

2.2.2.2. Chromatographic conditions. IVM and EPN concentrations were determined by high performance liquid chromatography (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 (Phenomenex, 5 μm, 4.6 mm × 250 mm). The mobile phase for IVM was acetic acid 0.2% in water/methanol/acetonitrile (3.8/40/56.2) at a flow rate of 1.5 ml/min at 30 °C. A mobile phase of de-ionized water, methanol, triethylamine, phosphoric acid, and acetonitrile (6:25:0.2:0.2:68.6) pumped at a flow rate of 1.0 ml/min was used for EPN analysis. Both molecules were detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), reading at 365 nm (excitation) and 475 nm (emission wavelength). IVM and EPN concentrations were determined by the internal standard method using the Class LC 10 Software version 1.2 (Shimadzu Corporation, Kyoto, Japan) on an IBM compatible AT computer. The peak area ratios were considered to calculate the IVM and EPN 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 and EPN was performed before starting analysis of the experimental samples obtained during the pharmacokinetic trial. Calibration curves in the range between 0.1–50 ng/ml and 0.1–20 ng/ml were prepared for each compound. Calibration curves were established using least squares linear regression analysis and correlation coefficients (r) and coefficient of variations (CV) calculated. Linearity was established to determine the IVM and EPN concentrations/detector responses relationship. Percentages of IVM and EPN recovery from plasma were obtained in the range between 0.1 and 50 ng/ml and 0.1 and 20 ng/ml, respectively. The inter-assay precision of the extraction and chromatography procedures was estimated by processing replicate aliquots ($n = 6$) of pooled plasma samples containing known IVM and EPN concentrations (between 0.1 and 20 ng/ml) on different working days. The limit of quantification was established as the lowest concentration measured with a recovery higher than 70% and a CV <20%. Concentration values below the quantification limit were not considered for the kinetic analysis of experimental data.

2.2.3. Pharmacokinetic and statistical analyses

Pharmacokinetic parameters of IVM and EPN were determined using a model-independent method. The peak concentration (C_{max}) and the time to peak concentration (T_{max}) were read from the plotted concentration-time curve for each individual animal. The absorption half-life ($T_{1/2}$ ab) was calculated as ln 2/K_{ab}, where K_{ab} represents the first order absorption rate constant (h^{-1}). The K_{ab} was determined applying the method of residuals to the first portion of the plasma concentration–time curve. The area under the concentration–time curves from time 0 to the last measurable concentration (AUC_{0-last}) was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982). Statistical moment theory was applied to calculate the mean residence time (MRT) for both drugs as follows:

$$MRT = \frac{AUMC_{0-last}}{AUC_{0-last}}$$

where AUC is as defined previously, and AUMC is the area under the curve of the product of time and drug concentration vs. time from zero to infinity (Gibaldi and Perrier, 1982). Mean pharmacokinetic parameters are reported as mean ± S.D. Mean parameters obtained after the administrations of the different formulations were statistically compared by analysis of variance (ANOVA) or Student t-test. A non-parametric test (Kruskal–Wallis or Mann–Whitney test) was used where significantly differences among standard deviations were observed. A value of $P < 0.05$ was considered significant.

3. Results

The evolution of the infestation with *A. parvum* female ticks on goats treated either with IVM or EPN is shown in Fig. 1, where a sharp decreased on tick burdens at day 7 post-treatment is evident for all groups of goats (see Section 4). No obvious effect for IVM or EPN treatments was noticed and tick distribution amongst groups was not statistically significant ($P > 0.05$). Nevertheless, goats treated with IVM produced lower number of engorged ticks than untreated controls. A total of 26 engorged female ticks were obtained from days 1 to 7 in the control and IVM treated animals. Five (19.2%), 5 (19.2%) and 16 (61.6%) ticks were collected from goats subcutaneously

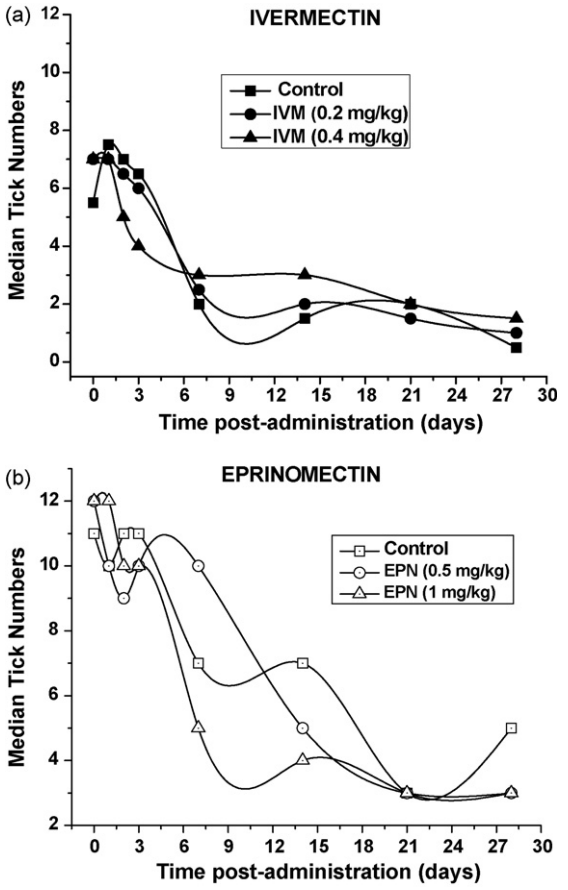

Fig. 1. Median numbers of *Amblyomma parvum* female ticks on goats treated with 0.2 or 0.4 mg/kg of injectable ivermectin and control (a), and treated with 0.5 or 1 mg/kg of eprinomectin pour-on and control (b).

Table 1
Means and standard deviations of engorgement weight (EW) (mg), pre-oviposition (POP) and minimum incubation egg (MIEP) periods (days), reproductive efficiency (REI)[1] and fertility efficiency (FEI)[2] indexes for *Amblyomma parvum* female ticks collected from two independent trials using goats treated with 0.2 and 0.4 mg injectable ivermectin/kg body weight and controls (C), and goats treated with 0.5 and 1.0 mg of eprinomectin pour-on/kg body weight and controls

Ivermectin trial		Eprinomectin trial	
EW C ($n = 16$)	190.2 ± 44.03 a[3]	EW C ($n = 6$)	212.3 ± 72.16 a
EW 0.2 mg ($n = 5$)	158.2 ± 99.99 ab	EW 0.5 mg ($n = 6$)	159.0 ± 51.05 a
EW 0.4 mg ($n = 5$)	65.6 ± 29.35 b	EW 1.0 mg ($n = 5$)	209.0 ± 21.37 a
POP C ($n = 16$)	6.2 ± 0.91 a	POP C ($n = 6$)	5.0 ± 0.00 a
POP 0.2 mg ($n = 4$)	7.3 ± 0.50 a	POP 0.5 mg ($n = 6$)	5.2 ± 0.41 a
POP 0.4 mg ($n = 4$)	9.5 ± 1.73 b	POP 1.0 mg ($n = 5$)	5.2 ± 0.45 a
MIEP C ($n = 16$)	34.8 ± 0.77 a	MIEP C ($n = 6$)	32.0 ± 1.10 a
MIEP 0.2 mg ($n = 4$)	36.5 ± 2.65 a	MIEP 0.5 mg ($n = 6$)	31.3 ± 1.75 a
MIEP 0.4 mg ($n = 4$)	37.0 ± 3.46 a	MIEP 1.0 mg ($n = 5$)	32.0 ± 1.22 a
REI C ($n = 16$)	2.9 ± 1.06 a	REI C ($n = 6$)	5.8 ± 0.67 a
REI 0.2 mg ($n = 5$)	1.4 ± 1.10 a	REI 0.5 mg ($n = 6$)	6.9 ± 1.59 a
REI 0.4 mg ($n = 5$)	2.7 ± 2.04 a	REI 1.0 mg ($n = 5$)	5.8 ± 1.69 a
FEI C ($n = 16$)	2.8 ± 1.07 a	FEI C ($n = 6$)	5.6 ± 0.69 a
FEI 0.2 mg ($n = 5$)	1.2 ± 1.02 a	FEI 0.5 mg ($n = 6$)	6.6 ± 1.40 a
FEI 0.4 mg ($n = 5$)	2.4 ± 1.97 a	FEI 1.0 mg ($n = 5$)	5.6 ± 1.46 a

[1] REI: reproductive efficiency index that expresses the number of eggs laid per mg of body weight (Drummond and Whetstone, 1970).
[2] FEI: fertility efficiency index that expresses the number of hatched larvae per mg of body weight (Aguirre et al., 2005).
[3] ANOVA and Scheffe's test for mean comparison if $P < 0.05$, values followed for different (a-b) letters are statistically significant ($P < 0.05$).

treated with IVM at 0.2, 0.4 mg/kg and from untreated controls, respectively ($P < 0.05$). Table 1 shows the biological values for the engorged female ticks collected during the IVM trial. The female engorgement weights of ticks recovered from goat treated with 0.4 mg/kg were significantly lower and the pre-oviposition period significantly longer ($P < 0.05$) than those observed in ticks recovered from untreated control goats. No significant effect was observed in ticks collected from goats treated with 0.2 mg/kg.

Seventeen engorged female ticks were obtained from days 1 to 7 after the topical administration of EPN. Six (35.3%), 5 (29.4%) and 6 (35.3%) ticks were recovered from goats treated with 0.5 mg/kg, 1.0 mg/kg of EPN pour-on and untreated controls, respectively ($P > 0.05$). No significant effects ($P > 0.05$) were determined for biological parameters obtained from engorged female ticks on the EPN treated groups (Table 1).

The methodology used to quantify IVM and EPN in goat plasma was validated following well-established analytical standards. The linear regression lines showed correlation coefficients $\geq$0.99. The mean recoveries of IVM and EPN from plasma were 75% and 77%, respectively. The inter-assay precision of the analytical procedures obtained after HPLC analysis of IVM and EPN spiked standards on different working days, showed a CV between 3.5% and 9.5%. The limit of quantification for both compounds was established at 0.1 ng/ml.

IVM was recovered from the bloodstream between 4 min and 15 days post-treatment. EPN was detected in plasma between 4 h and 7 (treatments at 0.5 and 1 mg/kg) and 9 days (treatment at 1.5 mg/kg) post-administration. Low EPN plasma concentrations were obtained after its pour-on administration to goats. The plasma concentration profiles obtained after topical

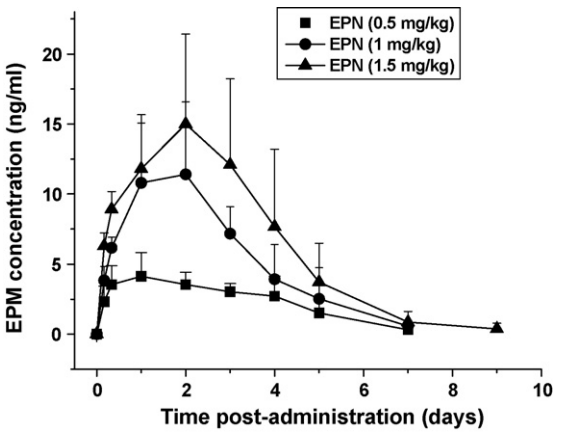

Fig. 2. Mean eprinomectin (EPN) plasma concentration ($n = 5$) obtained after its topical administration at 0.5, 1 and 1.5 mg/kg to goats.

Table 2
Comparative mean (±S.D.) ($n = 5$) kinetic parameters for eprinomectin (EPN) obtained after its topical administration at 0.5, 1 and 1.5 mg/kg to goats

Kinetic parameters	EPN (0.5 mg/kg)	EPN (1 mg/kg)	EPN (1.5 mg/kg)
$T_{1/2}$ ab (days)	0.44 ± 0.19^a	0.36 ± 0.36^a	0.58 ± 0.14^a
T_{max} (days)	1.80 ± 1.30^a	1.60 ± 0.55^a	2.00 ± 0.71^a
C_{max} (ng/ml)	5.0 ± 0.58^a	13.1 ± 5.03^b	16.2 ± 6.06^b
AUC_{0-last} (ng day/ml)	16.5 ± 2.81^a	39.1 ± 15.3^b	57.3 ± 24.6^b
$T_{1/2}$ el (days)	1.98 ± 1.39^a	1.09 ± 0.36^a	1.06 ± 0.38^a
MRT (days)	2.48 ± 0.30^a	2.31 ± 0.26^a	2.58 ± 0.15^a
ClB/F (l kg/day)	30.1 ± 6.43^a	27.9 ± 10.4^a	25.6 ± 6.60^a

Within a row, mean kinetic parameters lacking a common superscript letter are significantly different at $P < 0.05$.
$T_{1/2}$ ab: absorption half-life. T_{max}: time to peak plasma concentration. C_{max}: peak plasma concentration. $T_{1/2}$ dist: distribution half-life. $AUC_{(0-last)}$: area under the concentration–time curve from time 0 to the last time with a measurable concentration. $T_{1/2}$ el: elimination half-life. MRT: mean residence time. ClB/F: total body clearance, which represents its true value divided by the bioavailability (F).

EPN administration at 1 and 1.5 mg/kg showed a linear pharmacokinetic behaviour. The EPN plasma concentration profiles measured after the administration of the drug at different dose levels are shown in Fig. 2. Marked differences were obtained for the C_{max} and AUC values after the administration of EPN at different dose rates. However, the MRT and $T_{1/2}$ el values were similar for the three EPN treated groups. The main plasma pharmacokinetic parameters obtained for EPN after the treatment at the different dose rates are summarized in Table 2.

Higher and more persistent drug concentrations were obtained after the subcutaneous administration of IVM at both levels of dose rate compared to those obtained after the topical treatment with EPN. The plasma concentrations profiles of IVM are shown in Fig. 3. The C_{max} obtained for IVM after the administration of both dose rates were 18.6 ng/ml (0.2 mg/kg) and 41.7 ng/ml (0.4 mg/kg). The MRT and $T_{1/2}$ el values were similar between both treatments. The main plasma pharmacokinetic parameters obtained for IVM after the treatment at the different dose rates are summarized in Table 3. The relative availability of EPN considering the administration of IVM at 0.4 mg/kg as the 100% was estimated. The relative availability obtained for EPN after the administration of the different dose rates were between 9% and 10%. There was a direct relationship between the availability of IVM and some of the biological values for the engorged female ticks. The Fig. 4 shows the relationship between the engorgement weight of ticks and the IVM and EPN availability. The effect of IVM availability on the pre-oviposition periods is shown in Fig. 5.

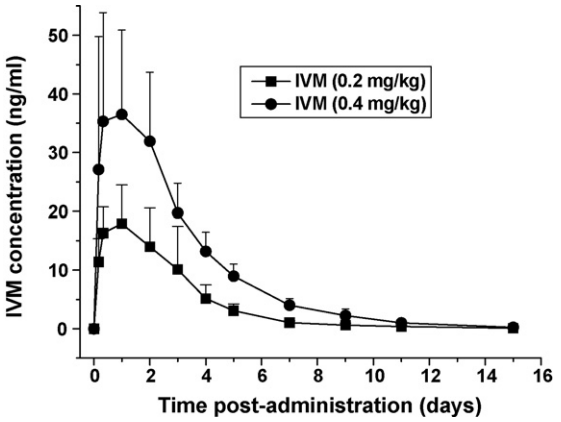

Fig. 3. Mean ivermectin (IVM) plasma concentration ($n = 5$) obtained after its subcutaneous administration at 0.2, and 0.4 mg/kg to goats.

4. Discussion

The main therapeutic feature of the antiparasitic avermectin compounds is their high efficacy against

Table 3
Comparative mean (±S.D.) ($n = 5$) kinetic parameters for ivermectin (IVM) obtained after its subcutaneous administration at 0.2, and 0.4 mg/kg to goats

Kinetic parameters	IVM (0.2 mg/kg)	IVM (0.4 mg/kg)
$T_{1/2}$ ab (days)	0.49 ± 0.11^a	0.28 ± 0.30^a
T_{max} (days)	0.87 ± 0.30^a	0.56 ± 0.40^a
C_{max} (ng/ml)	18.6 ± 6.02^a	41.7 ± 18.4^b
AUC_{0-last} (ng day/ml)	61.8 ± 23.7^a	144 ± 35.8^b
$T_{1/2}$ el (days)	1.75 ± 0.64^a	1.88 ± 0.31^a
MRT (days)	2.66 ± 0.33^a	2.98 ± 0.48^a
ClB/F (l kg/day)	3.53 ± 1.08^a	2.93 ± 0.81^a

Within a row, mean kinetic parameters lacking a common superscript letter are significantly different at $P < 0.05$.
$T_{1/2}$ ab: absorption half -life. T_{max}: time to peak plasma concentration. C_{max}: peak plasma concentration. $T_{1/2}$ dist: distribution half-life. $AUC_{(0-last)}$: area under the concentration–time curve from time 0 to the last time with a measurable concentration. $T_{1/2}$ el: elimination half-life. MRT: mean residence time. ClB/F: total body clearance, which represents its true value divided by the bioavailability (F).

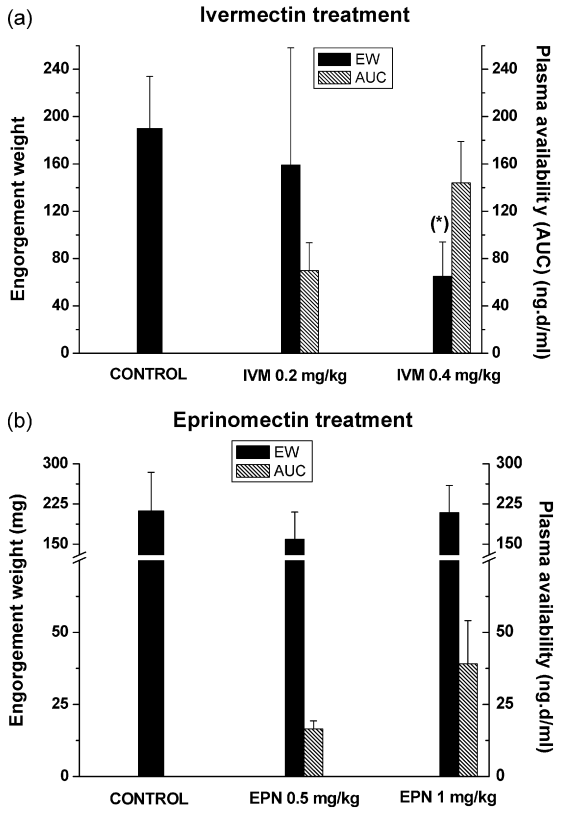

Fig. 4. Relationship between ticks engorgement weight (EW) and plasma availability obtained for ivermectin (IVM) (a) and eprinomectin (b) after their subcutaneous (0.2 and 0.4 mg/kg) and topical (0.5 and 1 mg/kg) administration respectively to goats. *Values significantly different from those obtained in the control and IVM treated (0.2 mg/kg) groups.

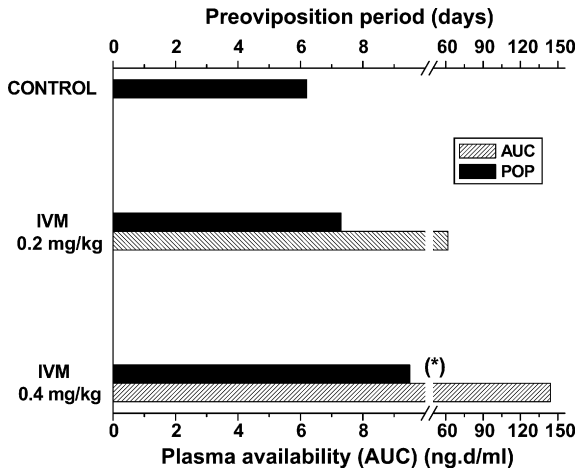

Fig. 5. Relationship between pre-oviposition period (POP) and plasma availability obtained for ivermectin (IVM) after its subcutaneous (0.2 and 0.4 mg/kg) administration to goats. *Values significantly different from those obtained in the control and IVM treated (0.2 mg/kg) groups.

endo and ectoparasites. Although there are available an important body of information on the pharmacological behaviour of the broad antiparasitic spectrum macrocyclic lactones, the concentration to be achieved at the target tissues to inhibit the establishment of different internal and external parasites, remains unknown (Lifschitz et al., 1999, 2000). Surprisingly, the efficacy results obtained after treatment with injectable IVM and topical EPN to control *A. parvum* female ticks on goats were unsatisfactory. Nevertheless, IVM showed capacity to diminish the number of engorged females and at the highest dose (0.4 mg/kg) collected engorged females had a significantly reduced weight but were still able to produce a viable progeny. The lack of any acaricidal effect after the pour administration of EPN was unexpected.

The peak of infestation of *A. parvum* female ticks in the study area occurs in January to February declining into March (Nava et al., in press). Therefore, lower tick number by the end of the study periods was expected but the strong decline on day 7 after treatment was unpredicted. Restrain of goats for experimental reasons reduce their exposure to ticks in the pasture when adult population of *A. parvum* was declining, which may explain, at least part, of the sharp drop of tick numbers on the experimental animals.

The pharmacokinetic information obtained for IVM and EPN in goats in the current work is in agreement to well-established kinetic features for these macrocyclic lactone compounds. The availability of IVM in goats administered subcutaneously at 0.2 mg/kg was similar (AUC: 57 ng day/ml) to the value obtained by Alvinerie et al. (1993) (AUC: 59.9 ng day/ml). However, a faster IVM absorption process with a higher C_{max} value and shorter mean residence time were observed in the current trial compared to those previously reported (Alvinerie et al., 1993). The evaluation of the kinetic features obtained for EPN after its topical administration at three different dosage levels in goats contributed with some interesting information. The main EPN pharmacokinetic parameters in goats after its administration at the lowest assayed dose rate (0.5 mg/kg) were in agreement with data already available (Dupuy et al., 2001), where low plasma availability (16 ng day/ml) and a rather low peak concentration (5 ng/ml) in the bloodstream were clear indicators of a restricted percutaneous absorption after the topical application. The dose rate increment of IVM (from 0.2 to 0.4 mg/kg) and EPN (from 0.5 mg to 1 mg/kg and to 1.5 mg/kg) assessed in the current experiment trial did not prolong the persistence of either IVM or EPN plasma concentrations. A linear relationship between the

concentration achieved in the bloodstream and the dose of IVM (McKellar and Benchaoui, 1996) and EPN (Dupuy et al., 2001) has been described. In the current trial, the dose-dependent pharmacokinetic parameters such as C_{max} and AUC were significantly greater after the treatment with IVM and EPN at the highest dose rates, whereas, no changes were observed in the time of residence of the drug in the bloodstream (MRT) after treatments with both compounds at the different dose rates.

The poor efficacy of IVM and EPN against *A. parvum* may be explained with pharmacokinetic information generated in the current trial. The duration of effective levels of IVM in the bloodstream was described as a relevant issue in the treatment of tick infestation (Jackson, 1989). A high efficacy against *Boophilus microplus* was reported in cattle treated with EPN pour-on both at 0.5 and 1 mg/kg (Aguirre et al., 2005). The EPN plasma availability (expressed as AUC) in cattle topically treated at 0.5 mg/kg was 241 ng day/ml (Alvinerie et al., 1999a). However, the systemic availability of EPN administered at the highest dose used (1.5 mg/kg) in goats in the trial reported here was only 57.3 ng day/ml, which may clearly explain the lack of any acaricidal effect. In other words, the limited percutaneous absorption of EPN in goats did not allowed to reach systemically available drug concentrations capable to kill the *A. parvum* female ticks even at the highest dosage level. Differences between skin morphology/physiology between cattle and goats may have influenced the complex process of absorption occurring after the pour-on administration of EPN, which could reduce the amount of drug passing through the skin layers to reach the systemic circulation. The increment on the EPN dose rate from 0.5 to 1 mg/kg improved the efficacy against the intestinal nematode *Trichostrongylus colubriformis* in goats (Chartier et al., 1999; Chartier and Pors, 2004) and the tick *B. microplus* (Aguirre et al., 2005) in cattle. However, it seems unlikely to have the same effect against *A. parvum* in goats, where any effect was observed although the drug concentration profiles were twice higher after doubling the administered dose rate.

The classical IVM formulation (propylene glycol/glycerol formal 60:40) used at 200 μg/kg, was approved as an aid in the control of *B. microplus* in cattle. The enhanced plasma AUCs and prolonged persistence of IVM concentrations in the bloodstream obtained after the administration of the highly concentrated (3.15%) long-acting IVM preparations (Lifschitz et al., 2007), assure a high acaricidal effect in cattle. In the current trial, IVM showed an improved activity against *A parvum* compared to EPN, particularly when was administered at 0.4 mg/kg (see Figs. 4 and 5). Considering that the systemic availability of EPN at the three assayed dosages was between 9% and 10% of that obtained for IVM, the enhanced clinical performance against *A. parvum* after the IVM treatment may be based on an advantageous absorption process reached after the injectable compared the EPN topical treatment.

There is no available information on the minimum therapeutic levels required to kill different endo-ectoparasites in ruminants. In an attempt to relate the tick efficacy and the IVM plasma concentrations, Jackson (1989) used a model with experimental tick infestations in rabbits. The critical plasma AUC value required to induce the 95% tick mortality was 350 ng day/ml. Assuming the difference existing among tick genera and among ruminant species, this data showed that the plasma availability obtained in the current trial in goats for both IVM and EPN (even at the highest dose rates) was insufficient to obtain an adequate efficacy against *A parvum*. The integration between parasitological and pharmacological information is relevant to improve our understanding of the relationship between pharmacokinetics and efficacy of this type of compounds against endo and ectoparasites and to optimize their practical use under field conditions.

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References

Aguirre, D.H., Gaido, A.B., Cafrune, M.M., Castelli, M.E., Mangold, A.J., Guglielmone, A.A., 2005. Eprinomectin pour-on for control of *Boophilus microplus* (Canestrini) ticks (Acari: Ixodidae) on cattle. Vet. Parasitol. 20, 157–163.

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

Alvinerie, M., Sutra, J.F., Galtier, P., Mage, C., 1999a. Pharmacokinetics of eprinomectin in plasma and milk following topical administration to lactating dairy cattle. Res. Vet. Sci. 67, 229–232.

Alvinerie, M., Lacoste, E., Sutra, J.F., Chartier, C., 1999b. Some pharmacokinetic parameters of eprinomectin in goats following pour-on administration. Vet. Res. Commun. 23, 449–455.

Campbell, W.C., Fisher, M.H., Stapley, E.O., Albers-Schoenberg, G., Jacob, T.A., 1983. Ivermectin: a potent new antiparasitic agent. Science 221, 823–828.

Chartier, C., Pors, I., 2004. Duration of activity of topical eprinomectin against experimental infections with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* in goats. Vet. Parasitol. 10, 415–419.

Chartier, C., Etter, E., Pors, I., Alvinerie, M., 1999. Activity of eprinomectin in goats against experimental infections with *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Vet. Rec. 23, 99–100.

Danaher, M., O'Keeffe, M., Glennon, J.D., Howells, L., 2001. Development and optimisation of an improved derivatisation procedure for the determination of avermectins and milbemycins in bovine liver. Analyst 126, 576–580.

De Montigny, P., Shim, J.S., Pivnichny, J.V., 1990. Liquid chromatographic determination of ivermectin in animal plasma with trifluoroacetic anhydride and *N*-methylimidazole as the derivatization reagent. J. Pharm. Biomed. Anal. 8, 507–511.

Drummond, R.O., Whetstone, T.M., 1970. Oviposition of the Gulf Coast tick. J. Econ. Entomol. 63, 1547–1551.

Dupuy, J., Chartier, C., Sutra, J.F., Alvinerie, M., 2001. Eprinomectin in dairy goats: dose influence on plasma levels and excretion in milk. Parasitol. Res. 87, 294–298.

Fisher, M., Mrozik, H., 1989. Chemistry. In: Campbell, W. (Ed.), Ivermectin and Abamectin. Springer-Verlag, New York, USA, pp. 1–23.

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

Guglielmone, A.A., 1988. Distribución geográfica e importancia relativa de las especies de garrapatas que atacan a los bovinos en la Argentina excepto el Boophilus microplus. Mem. 4a Reun. Anu. Asoc. Arg. Vet. Lab. Diag., Tandil, Buenos Aires, 2–3 dic., pp. 35–39.

Guglielmone, A.A., Mangold, A.J., Aguirre, D.H., Gaido, A.B., Olsen, A.A.de., 1989. The effect of infection by *Babesia* sp. on some biological parameters of engorged females of *Boophilus microplus*. Folia Parasitol. 36, 1–6.

Guglielmone, A.A., Mangold, A.J., Aguirre, D.H., Gaido, A.B., 1990. Ecological aspects of four species of ticks found on cattle in Salta, northwest Argentina. Vet. Parasitol. 35, 93–101.

Hoste, H., Lespine, A., Lemercier, P., Alvinerie, M., Jacquiet, P., Dorchies, P., 2004. Efficacy of eprinomectin pour-on against gastrointestinal nematodes and the nasal bot fly (Oestrus ovis) in sheep. Vet. Rec. 154, 782–785.

Imperiale, F., Pis, A., Sallovitz, J., Lisfchitz, A., Busetti, M., Suárez, V., Lanusse, C., 2006. Pattern of eprinomectin milk excretion in dairy sheep unaffected by lactation stage: comparative residual profiles in dairy products. J. Food Prot. 69, 2424–2429.

Jackson, H., 1989. Ivermectin as a systemic insecticide. Parasitol. Today 5, 146–155.

Lifschitz, A., Virkel, G., Pis, A., Imperiale, F., Sánchez, 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., Sallovitz, J., Sutra, J.F., Galtier, P., Alvinerie, M., Lanusse, C., 2000. Comparative distribution of ivermectin and doramectin to tissues of parasite location in cattle. Vet. Parasitol. 87, 327–338.

Lifschitz, A., Virkel, G., Ballent, M., Sallovitz, J., Imperiale, F., Pis, A., Lanusse, C., 2007. Ivermectin (3.15%) long-acting formulations in cattle: absorption pattern and pharmacokinetic considerations. Vet. Parasitol. 147, 303–310.

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

Nava, S., Mangold, A.J., Guglielmone, A.A., 2006. The natural hosts for larvae and nymphs of *Amblyomma neumanni* and *Amblyomma parvum* (Acari: Ixodidae). Exp. Appl. Acarol. 40, 123–131.

Nava, S., Szabó, M.P.J., Mangold, A.J., Guglielmone, A.A., in press. Distribution, hosts, 16rDNA sequences and phylogenetic position of the Neotropical tick *Amblyomma parvum* (Acari: Ixodidae). Ann. Trop. Med. Parasitol.

Shoop, W.L., Egerton, J.R., Eary, C.H., Haines, H.W., Michael, B.F., Mrozik, H., Eskola, P., Fisher, M.H., Slayton, L., Ostlind, D.A., Skelly, B.J., Fulton, R.K., Barth, D., Costa, S., Gregory, L.M., Campbell, W.C., Seward, R.L., Turner, M.J., 1996a. Eprinomectin: a novel avermectin for use as a topical endectocide for cattle. Int. J. Parasitol. 26, 1237–1242.

Shoop, W.L., DeMontigny, P., Fink, D.W., Williams, J.B., Egerton, J.R., Mrozik, H., Fisher, M.H., Skelly, B.J., Turner, M.J., 1996b. Efficacy in sheep and pharmacokinetics in cattle that led to the selection of eprinomectin as a topical endectocide for cattle. Int. J. Parasitol. 26, 1227–1235.