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Research paper

Eprinomectin accumulation in *Rhipicephalus* (*Boophilus*) *microplus*: Pharmacokinetic and efficacy assessment

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ABSTRACT

Eprinomectin (EPM) is a macrocyclic lactone used against endo-ectoparasites without withdrawal time in milk and meat after its pour-on administration at 0.5 mg/kg. Previous experiments evaluated the efficacy of EPM against *Rhipicephalus* (*Boophilus*) *microplus* in cattle. This study assessed EPM efficacy against *R. (B.) microplus* after topical administration at two dose rates and investigated the relationship between EPM systemic exposure in the host and drug concentrations accumulated in ticks recovered from treated animals. A standardized pharmaco-parasitological study was performed in two phases. In phase 1 eighteen Braford cattle naturally infected with *R.* (*B.*) *microplus* were divided into three experimental groups with a similar level of infestation (Kruskal–Wallis test, $P > 0.05$): control group and treated groups with EPM pour-on (1 and 1.5 mg/kg). Samples of heparinized blood and ticks at different life stages were taken between 0 and 21 days (d) post-administration to measure EPM concentrations by HPLC. The efficacy trial (phase 2) included eighteen Braford calves naturally infected with *R.* (*B.*) *microplus* divided into control group and 1 mg/kg and 1.5 mg/kg EPM treated groups. Female ticks (4.5–8 mm) on cattle were counted between 1 and 23 days post-treatment to evaluate the efficacy of EPM. The reproductive efficiency index (REI) and the fertility efficiency index (FEI) were evaluated. Plasma concentrations of EPM showed a linear relationship with the level of dose rate administered. Peak plasma concentrations were within a range between 13.8 and 90 ng/ml, which guarantee milk drug concentrations below the maximum residues level. High EPM concentrations were detected in ticks. EPM concentrations in *R.* (*B.*) microplus were correlated to plasma concentrations between 1.25 days and 21 days post-administration (r 0.84; $P < 0.05$). EPM efficacy calculated using the Henderson–Tilton formula was 98.9% and 99.1% (7 days post-administration) and 100% (23 days post-administration) after EPM treatment at 1 and 1.5 mg/kg, respectively. EPM administered at 1.5 mg/kg also showed a significantly higher deleterious effect on tick fertility as measured by FEI ($P < 0.01$). Therefore, treatment with EPM may be useful for controlling ticks in cattle, particularly in dairy production systems.

1. Introduction

The cattle tick *Rhipicephalus (Boophilus) microplus* has an economic impact on animal production because it reduces weight gain, milk yield and the value of animal skin (Spath et al., 1994; Jongejan and Uilenberg, 2004). In addition *R.* (*B*) *microplus* acts as a vector by transmitting pathogens that cause babesiosis and anaplasmosis (Peter et al., 2005).

Macrocyclic lactones (MLs) are broad-spectrum antiparasitic drugs widely used to control endo and ectoparasites. Several trials confirmed the efficacy of MLs in the control of *R.* (*B*) *microplus* (Davey et al., 2005; George and Davey 2004). Long-acting formulations of MLs were introduced in several countries to extend the protection period against tick re-infestation. However, the increasing problems of tick resistance to MLs (Martins and Furlong, 2001; Perez-Cogollo et al., 2010) and residue accumulation in meat and milk led to the search for strategic applications of these products (Nava et al., 2015). The lack of compliance with the required with-

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drawal times is a relevant issue accounting for the controversy on the use of long-acting formulations in cattle in different countries.

Eprinomectin (EPM) was selected for use as topical endectocide after examination of several hundred avermectin analogues. Manipulation of the chemical structure of EPM changed the blood–milk partitioning coefficients in lactating dairy animals, allowing the use in dairy cattle with zero-milk withdrawal period (Shoop et al., 1996). The EPM efficacy against *R. (B) microplus* in cattle was evaluated in a double application treatment regime with a 4-day interval between treatments with EPM (0.5 mg/kg) (Davey and George, 2002) and in a single administration of EPM at 1 mg/kg (Aguirre et al., 2005). Additional information is necessary to understand the activity of EPM against *R.* (*B*) *microplus* and to validate its use as a feasible option in control programs. This study assessed EPM efficacy against *R.* (*B.*) *microplus* after topical administration at two different dose rates and investigated the relationship between EPM systemic concentrations in the host and in the ticks recovered from treated animals.

2. Materials and methods

Animal procedures and management protocols were carried out according to the Animal Welfare Policy (Academic Council Resolution 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).

2.1. Pharmacokinetic study

Experimental animals were in the Instituto de Investigación Animal del Chaco Semiárido (IIACS, INTA), located in Leales, Tucumán, Argentina. Eighteen 17-month-old Braford calves that were naturally infested with *R.* (*B.*) *microplus* were divided into three homogeneous groups on day 0 (Groups 1, 2 and 3). The three groups showed a similar level of *R.* (*B.*) *microplus* infestation at day 0 (Kruskal–Wallis test, $P > 0.05$) Mean number of ticks was: Group 1: 21.2, Group 2: 21.3 and Group 3: 22.3). Experimental animals were treated with EPM pour-on (IVOMEC® EPRINEX™ POUR ON, Merial Argentina S.A.) at 1 mg/kg (Group 1) and 1.5 mg/kg (Group 2). Group 3 calves were the control group. Mean body weight (kg ± SD) for each Group in the pharmacokinetic trial on day 0 was: Group 1 (242.3 ± 11.4), Group 2 (246.8 ± 12.4) and Group 3 (257.3 ± 8.6). After treatment, animals were kept in tick-free corrals to prevent re-infestation. Therefore, quantification of EPM accumulation in ticks was guaranteed from the beginning of the experiment.

Heparinized blood samples and tick specimens at different life stages were collected at 0, 2, 4, 6, 24, 30 h (h) and 2, 3, 6, 7, 10, 14, 21 days (d) post-administration. Blood samples were centrifuged at 2000 × *g* for 10 min. Semi-engorged ticks were collected from the tailhead, tail, escutcheon and belly of the calves. Between 2 and 4 ticks were collected from each experimental animal at each sampling time. Ticks were rinsed extensively with saline solution. Plasma and tick samples were frozen at −20 °C until processing for HPLC analysis.

2.2. Efficacy study

The efficacy study was performed from 3 March 2015 to 26 March 2015. On day 0, eighteen 17-month-old Braford calves naturally infested with *R.* (*B.*) *microplus* were divided into three homogeneous groups of six animals each according to the level of *R.* (*B.*) *microplus* infestation (Kruskal–Wallis test, $P > 0.05$). Each group comprised six (6) animals of similar level of *R.* (*B.*) *microplus* infestation (Kruskal–Wallis test, $P > 0.05$). Tick infestation at day 0 (mean followed by maximum and minimum in parentheses) was 14 (10–25), 14 (8–34) and 13.2 (7–16) in Groups 1, 2 and 3, respectively. All animals had been grazing on the same pasture for more than 3 weeks prior to day 0, ensuring a similar level of larval and nymphal infestation at the beginning of the efficacy study. The pasture was divided into three 10-ha paddocks by an electric fence in order to maintain the three groups separately. Experimental animals received the same treatments as those described in the pharmacokinetic study. Group 1 calves were treated with EPM at a dose rate of 1 mg/kg of body weight; animals of Group 2 were treated with EPM at 1.5 mg/kg of body weight, whereas animals of Group 3 remained untreated (control group). The drug was applied to the dorsal midline.

R. (*B.*) *microplus* females (4.5–8.0 mm long) were counted on one side of the calves on days 1, 3, 7, 14 and 23 post-treatment. Statistically significant differences in the distributions of *R.* (*B.*) *microplus* numbers among the three groups were determined using the non-parametric Kruskal–Wallis test with a Dunn *post hoc* test (Zar, 1999). Differences were considered significant at $P < 0.01$. The corrected efficacy percentage was calculated with the modified Abbot's formula using the mean number of ticks (Henderson and Tilton, 1955).

The effect of EPM on the reproductive parameters of engorged female ticks was tested. Immediately after tick collection, engorged females were kept at 25 °C and 83–86% relative humidity, with a daily photoperiod of 12 h light–12 h dark. Larvae and unhatched eggs were counted as described by Guglielmone et al. (1989). The reproductive efficiency index [REI = number of eggs laid/weight of the females in mg (Drummond and Whetstone 1970)] and the fertility efficiency index [FEI = number of hatched larvae/weight of the females in mg (Aguirre et al., 2005)] were calculated. The statistical significance of the differences among REI and FEI values obtained for each group was tested using an analysis of variance (ANOVA) followed by a posteriori Tukey test (Zar, 1999). The pre-oviposition period of engorged females (days from tick collection from cattle until beginning of oviposition) and incubation period of eggs (days from the laying of the first egg until first egg hatched) were also calculated (mean ± SD).

3. Analytical procedures

3.1. Chemical extraction and derivatization

EPM was extracted from plasma samples following the technique described by Imperiale et al., (2006) and Lifschitz et al., (2008). Briefly, a 0.5 ml-aliquot of plasma sample was combined with 5 ng of the internal standard compound (abamectin) and mixed with 0.5 ml of acetonitrile and 0.125 ml of water. The solvent-sample was mixed for 20 min and then centrifuged at 2000 × *g* for 15 min.

Between 2 and 4 ticks were analyzed in each sample from each experimental animal. Each tick sample was weighed and homogenized together. Ticks samples were homogenized using a Potter-Elvenjem PTFE pestle with 1 ml of acetonitrile and combined with 20 ng of abamectin. Homogenates were mixed for 20 min and then centrifuged at 2000 × *g* for 15 min. The supernatant from plasma and tick samples was then placed on the appropriate rack of an Aspec XL sample processor (Gilson, Villiers Le Bel, France) to perform the solid-phase extraction (Lifschitz et al., 1999). The supernatant was injected onto a C18 cartridge (Strata, Phenomenex, CA, USA)., previously conditioned by passing 2 ml methanol and 2 ml deionized water. The cartridge was flushed with 1 ml of water followed by 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. Each sample was subjected to a derivatization, as described by Danaher et al., (2001). Once the

reaction was completed, an aliquot (100 μl) of each sample was injected directly into the chromatographic system.

3.2. Chromatographic conditions

EPM 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 performed using a C_{18} reverse-phase column (Kromasil, 5 μm, 4.6 mm × 250 mm, Eka Chemicals, NY, USA). A mobile phase of methanol, triethylamine, phosphoric acid, and acetonitrile (60:0.2:0.2:39.6 v/v/v/v) pumped at a flow rate of 1.0 ml/min was used for EPM analysis. EPM was detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), reading at 365 nm (excitation) and 475 nm (emission wavelength). EPM 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. EPM concentrations in spiked (validation) and experimental plasma samples were calculated using the peak area ratios. There was no interference of endogenous compounds in the chromatographic determinations. The solvents (Baker, Phillipsburg, NJ, USA) used during extraction and drug analysis were HPLC grade. Calibration curves were prepared in the range between 0.1–100 ng/ml (plasma) and 0.1–1000 ng/g (ticks) and were established using least squares linear regression analysis. Correlation coefficients (r) and coefficient of variations (CV) were calculated. Linearity was established to determine the EPM concentrations/detector responses relationship. Percentages of EPM recovery from plasma and ticks were >70%. The precision of the extraction and chromatography procedures for plasma and tick samples was estimated by processing replicate aliquots ($n = 4$) of samples containing known EPM concentrations. The method precision measured with the coefficient of variation was between 1.94 and 12.6%. The limit of quantification was established at 0.1 ng/ml (plasma) and 0.1 ng/g (ticks).

4. Pharmacokinetic and statistical analyses

Pharmacokinetics of EPM was determined using a model-independent method. The peak concentration (Cmax) and the time to peak concentration (Tmax) were read from the plotted plasma concentration-time curve for each animal. The terminal (elimination) half-life (T ½ el) was calculated as ln 2/λz, where λz is the elimination rate constant. The area under the concentration-time curves from time zero to the last measurable concentration ($AUC_{0\text{-last}}$) was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982). The statistical moment theory was applied to calculate the mean residence time (MRT) for both drugs as follows:

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

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 ± SD. Mean parameters obtained after the administrations of EPM at different dose rates were statistically compared by Student *t*-test. A non-parametric test (Mann–Whitney test) was used when significant differences among standard deviations were observed. The correlation (Pearson r) between EPM concentration in plasma and ticks was evaluated. All statistical analyses were performed using Instat 3.0 Software (Graph Pad Software, CA, USA). A value of $P < 0.05$ was considered statistically significant.

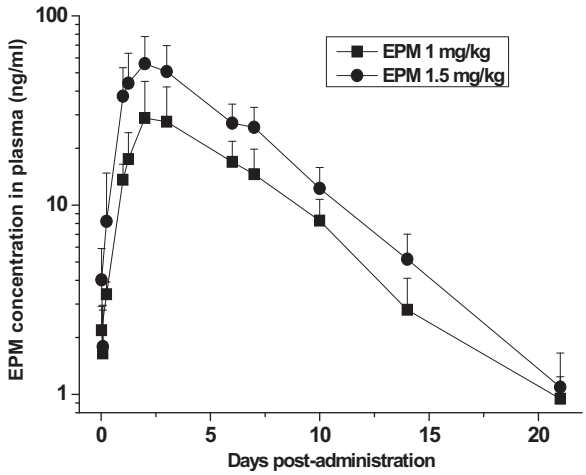

Fig. 1. Mean eprinomectin (EPM) plasma concentrations obtained after topical administration to calves at 1 mg/kg ($n = 6$) and 1.5 mg/kg ($n = 6$).

Table 1
Comparative mean (±SD) ($n = 6$) plasma kinetic parameters for eprinomectin (EPM) obtained after topical administration at 1 and 1.5 mg/kg to calves.

Kinetic parameters	EPM (1 mg/kg)	EPM (1.5 mg/kg)
Tmax (days)	2.3 ± 0.50^a	2.20 ± 0.40^a
Cmax (ng/ml)	30.0 ± 15.8^a	57 ± 20.0^b
$AUC_{0\text{-}21d}$(ng.d/ml)	213 ± 66.5^a	382 ± 99.3^b
T ½ el (days)	3.57 ± 0.50^a	3.13 ± 0.40^a
MRT (days)	6.42 ± 0.70^a	5.70 ± 0.60^a
Normalized Cmax (ng/ml)	30.0 ± 15.8^a	$38.0 \pm 13.7^{a,*}$
Normalized AUC (ng day/ml)	213 ± 66.5^a	$255 \pm 66.2^{a,*}$

Numbers with a different superscript between EPM treatments are significantly different ($P < 0.05$). Tmax: time to peak plasma concentration. Cmax: peak plasma concentration. $AUC_{(0-21)}$: area under the concentration-time curve from time zero to 21 d post-administration. T ½ el: elimination half-life. MRT: mean residence time.
* Cmax and $AUC_{0-21days}$ values were dose-normalized by dividing the observed values by 1.5.

5. Results

The EPM plasma concentration profiles measured after drug administration at different doses are shown in Fig. 1. EPM was detected in the bloodstream between 2 h and 21 days post-treatment in both treated groups. EPM plasma concentrations were related to the administered dose rate. The plasma concentration profiles obtained after topical EPM administration at 1 and 1.5 mg/kg showed a linear pharmacokinetic behavior. The main plasma pharmacokinetic parameters obtained for EPM after treatment at the different dose rates are summarized in Table 1. Cmax and AUC were significantly affected by the dose administered. After normalization by the dose rate, Cmax and AUC did not show significant differences between calves treated with EPM at 1 and 1.5 mg/kg. Plasma MRT and T ½ el values were similar for both EPM treated groups.

Mean EPM concentrations measured in ticks after administration at 1 and 1.5 mg/kg are shown in Fig. 2. Drug concentrations were detected in ticks between 2 h and 21 days post-treatment. Mean EPM concentrations in *R. (B) microplus* were between 3110 and 3.5 ng/g. The highest EPM concentrations in ticks were measured between 2 h and 1.25 days post-administration. Drug concentrations in ticks did not reflect EPM plasma concentrations during this period. However, there was a positive correlation between EPM concentrations in *R.* (*B*) *microplus* and plasma between 1.25 days and 21 days post-administration (r 0.84; $P < 0.05$). Drug concentrations observed in ticks were related to the used dose rate. Significant differences ($P < 0.05$) were observed in

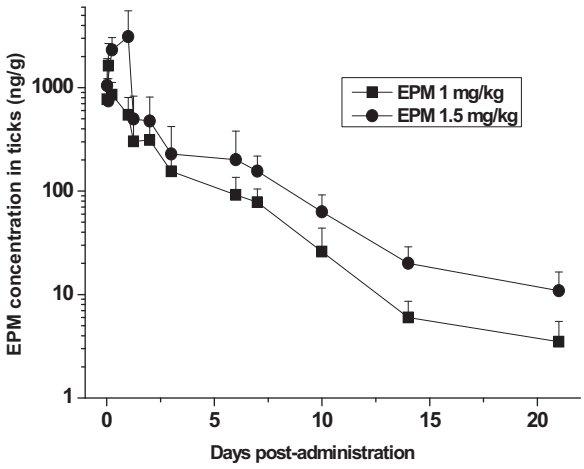

Fig. 2. Mean eprinomectin (EPM) concentrations measured in *Rhipicephalus* (*Boophilus*) *microplus* recovered from calves treated topically with 1 mg/kg ($n = 6$) and 1.5 mg/kg ($n = 6$). Each value represents the mean of six determinations of EPM concentrations. Between 2 and 4 ticks recovered from individual infested calves were analyzed in each determination.

drug exposure in ticks expressed as AUC between 1.25 days and 21 days post-administration of EPM at both dose rates. The AUC in ticks was 1212 ± 474 ng day/g (1 mg/kg) and 2502 ± 782 ng day/g (1.5 mg/kg). Drug exposure in ticks normalized by dose rate was not significantly different after the administration of EPM at 1 and 1.5 mg/kg. The relationship between EPM exposure in *R.* (*B*) *microplus* and in plasma was analyzed using the ratio between the AUC measured in ticks and plasma, which were 6.51 (EPM at 1 mg/kg) and 7.18 (EPM at 1.5 mg/kg) between 1.25 and 21 days.

There was an obvious effect on *R.* (*B*) *microplus* after EPM treatments (Table 2).

The effect of both EPM dose rates on ticks became evident by day 7 post-treatment, when the efficacy percentage was close to or higher than 90% in the treated groups (Table 2). The number of ticks observed in Group 3(control group) was significantly ($P < 0.01$) higher than the number of ticks recovered from the Groups 1 and 2 (treated groups) from day 3 post-treatment until the end of the trial (Table 2). Engorged female ticks were collected from calves of the control group all throughout the trial, but not in the treated groups, where they were only found at day 1 post-treatment. Therefore, REI and FEI were compared only for day 1 post-treatment. REI values for Groups 1, 2 and 3 were 8.31, 8.70 and 12.1, respectively (ANOVA, $P < 0.01$). EPM also showed a significant effect on tick fertility as measured by FEI. Values of FEI obtained for Group 1 (7.94), Group 2 (2.61) and Group 3 (11.7) were statistically different from one another (ANOVA, $P < 0.01$). The pre-oviposition periods of the engorged females belonging to the Groups 1, 2 and 3 were 4.0 ± 0.58 days, 5.0 ± 1.0 days and 4.8 ± 0.37 days, respectively ($P > 0.05$) The values of incubation period of eggs were 25.6 ± 0.92 days in Group 1, 26.0 ± 0.0 days in Group 2 and 25.8 ± 0.20 in Group 3 ($P > 0.05$)

6. Discussion

MLs are available in the veterinary pharmaceutical market as several preparations to be administered to cattle by the subcutaneous and topical routes. EPM was launched to be used by the pour-on administration at 0.5 mg/kg. Although the EPM plasma kinetic behavior in cattle was previously studied, the current trial evaluated for the first time the drug concentrations of EPM attained in ticks after topical administration to naturally infected calves.

In this study, EPM was administered to calves at either 1 or 1.5 mg/kg. A linear relationship between blood concentrations and EPM dose was observed. The normalization of the systemic availability by the dose rate did not show significant differences (Table 1). The dose rate increment (from 1.0 mg/kg to 1.5 mg/kg) assessed in this study did not prolong the persistence of EPM plasma concentrations. The plasma concentration profiles of EPM measured in the current trial were in agreement with those observed in a pharmacokinetic trial performed in cattle after administration at 0.5 mg/kg (Wen et al., 2010)

The information on plasma concentration profiles is useful to explain the comparative efficacy and persistence of the antiparasitic activity of the MLs. However, the characterization of drug concentration profiles in the gastrointestinal location of parasites as well as in nematodes provides a better understanding of the therapeutic and preventive efficacies observed for these drugs (Lifschitz et al., 2000; Lloberas et al., 2012). The same concept may be applied to ectoparasites. Ticks and other ectoparasites are exposed to systemic chemical agents during feeding and the nature of their food source and feeding duration may have a relevant effect on drug uptake and efficacy (Jackson, 1989). As EPM is topically administered, drug uptake by ticks may be a combination of feeding habits and the contact of tick with the drug depot on the skin surface. The present study evaluated the *in vivo* drug uptake (accumulation) by ticks collected from EPM-treated calves at two dose rates. The EPM concentrations measured in collected ticks were directly related to the dose received by the treated infected calves. Higher EPM concentrations were recovered in ticks collected from calves treated with 1.5 mg/kg, the highest dose (Fig. 2).

The degree of association between EPM availability in the host bloodstream and that achieved in the collected ticks was evaluated. EPM tick concentrations did not reflect the plasma concentrations between treatment and 1.25 days post-administration. The uptake of EPM by contact after topical administration may explain the high EPM concentrations measured in ticks during this period. The distribution through the skin of MLs topically administered to cattle was confirmed for moxidectin. High moxidectin concentrations were measured in dermal layers from the skin of thigh, face and rib cage, which are different skin areas from the administration sites (Sallovitz et al., 2003). However, there was a high correlation between EPM concentrations recovered in plasma and that measured within ticks between 1.25 days and 21 days post-treatment. Therefore, the drug concentrations measured in ticks may reflect the EPM concentrations in plasma during this period, suggesting that EPM may be ingested by blood feeding. The estimated ratio between drug availability measured in ticks and plasma between 1.25 days and 21 days post administration supports this hypothesis. Those similar AUC ratios indicate the correlation between the increment in EPM concentrations in ticks recovered from calves treated with 1.5 mg/kg and the enhanced drug concentrations measured in plasma of the calves receiving the highest dose rate.

The efficacy of EPM against *R. (B) microplus* was previously evaluated. While a single application of EPM at 0.5 mg/kg provided a high efficacy, the application of two treatments at an interval of 4 days resulted in improved control (Davey and George, 2002). To assess the effect of EPM against ticks at a higher dose than that recommended against lung and gastrointestinal nematodes (0.5 mg/kg), Aguirre et al., (2005) evaluated EPM efficacy against *R.* (*B*) *microplus* administered at 1 mg/kg to cattle. Although the authors did not observed a significant change in the efficacy related to the administered dose rate, a great adverse effect on the weight and fertility of female ticks recovered from calves treated with a single dose of 1 mg/kg of EPM was identified (Aguirre et al., 2005). Similar activity results were obtained in the current trial after the administration of EPM at 1 and 1.5 mg/kg. A high efficacy against *R. (B) microplus* was obtained after EPM administration at both dose rates, which reached a 100% on day 23 post-administration. While there were not significant differences in the number of ticks recovered alive among the administered doses, a lower FEI was obtained

Table 2
Mean number (min–max) of *Rhipicephalus* (*Boophilus*) *microplus* females 4.5–8.0 mm in length of the groups treated with eprinomectin (EPM) at 1 and 1.5 mg/kg and control untreated calves. The efficacy percentage (EP) is also indicated.

Days post-treatment	Mean number of ticks (min–max) EPM 1 mg/kg	EP (%) EPM 1 mg/kg	Mean number of 1.5 mg/kg ticks (min–max) EPM	EP (%) EPM 1.5 mg/kg	Mean number of control ticks (min–max)
0	14 (10–25)[a]	NA	14 (8–34)[a]	NA	13.2 (7–16)[a]
1	9.8 (6–16)[a]	19.9	8.5 (5–11)[a]	30.5	11.5 (3–17)[a]
3	6 (2–10)[a]	58.2	2 (0–3)[b]	86.7	13.5 (6–20)[c]
7	0.6 (0–2)[a]	98.9	0.5 (0–2)[a]	99.1	55.8 (15–136)[b]
10	8.6 (5–17)[a]	89.6	4.2 (3–11)[a]	95.0	78 (25–126)[b]
14	2.8 (0–8)[a]	92.3	1.5 (0–3)[a]	95.9	34.3 (9–45)[b]
23	0	100	0	100	4 (2–9)

Kruskal–Wallis test. Numbers with a different superscript among experimental groups are significantly different ($P < 0.01$). NA: not applicable. The statistical comparison is among columns within the same row.

after EPM administration at 1.5 mg/kg. This greater adverse effect on the target parasite agrees with the enhanced EPM concentrations accumulated within the ticks recovered from calves treated with 1.5 mg/kg.

The level of ML concentration required at the site of parasite location to inhibit endo- and ectoparasite establishment or development has not been accurately determined. However, based on the characterization of drug concentrations attained in target tissues, some estimation has been proposed for gastrointestinal nematodes (Lifschitz et al., 2000). Sustained ML concentrations in blood are required to achieve high efficacy against ticks. The slow ivermectin action in ticks seems to be related to a delayed drug accumulation process caused by a progressive drug-induced paralysis of feeding mechanism (Jackson 1989). The critical amount of drug necessary to obtain an optimal efficacy was estimated in rabbits experimentally infested with *Rhipicephalus appendiculatus*. The systemic availability necessary to induce >95% larval tick mortality was 350 ng/ml over five days (Jackson 1989). A pharmacokinetic-pharmacodynamic approach to estimate the minimal ivermectin active levels against *R.* (*B*) *microplus* was performed in cattle (Miller et al., 1999; Davey et al., 2010). A drug level of 8 ng/ml in blood was established as a threshold for tick control. In fact, a 99% control could be obtained only when ticks were exposed to the threshold concentration during the complete period of tick development (21–27 days), which accounts for the importance of the length of drug exposure above the minimal plasma concentration (8 ng/ml) (Davey et al., 2010). In the current trial, EPM concentrations were above 8 ng/ml for 10 days (1 mg/kg) and 12 days (1.5 mg/kg) post-treatment. The mean tick exposure to EPM (measured as the AUCs of the accumulated drug) during this 10–12 days period ranged between 1930 and 4637 ng day/g. Thus, the EPM concentration levels attained in the bloodstream and, as a consequence, in target ticks, after the administration at 1 and 1.5 mg/kg, are sufficient to obtain a good efficacy pattern. It has been reported that EPM may have a good effect on tick females nearing detachment from the host (Davey and George, 2002). Beyond day 5 post-administration, EPM activity is mainly focused against larval, nymphs and young ticks, which are already on the calves (Aguirre et al., 2005). However, although EPM was administered at a higher dose rate than the recommended for gastrointestinal nematodes, the achieved plasma concentrations after the 10–12 days post-treatment were below those suggested as the minimal lethal concentrations for ticks, which may account for larval re-infestation occurring in treated animals under field conditions

The ML long-acting formulations supply persistent plasma concentrations that may prevent *R.* (*B*) *microplus* re-infestation. However, the high level of milk/tissue residues due to the administration of these formulations promoted their strategic applications to reduce the number of treatments (Nava et al., 2015). Accordingly, alternative drugs with shorter withdrawal time may be useful for tick control programmes. The minor changes introduced on the EPM chemical structure account for low milk/plasma partitioning. Due to this outstanding feature, EPM is administered to dairy cattle without a required milk withdrawal period. The EPM plasma and milk concentrations are in equilibrium and the ratio of milk to plasma concentrations is constant during an extended post-treatment period (Alvinerie et al., 1999). Although the current trial was performed in beef cattle, it is possible to speculate about milk EPM concentrations using the previously proposed partitioning ratio. Previous studies showed that the Cmax milk/Cmax plasma ratio was between 0.12 and 0.14. If the ratio of 0.14 is applied to the plasma Cmax obtained in the current trial, the Cmax in milk would fall between 1.9 ng/ml and 13 ng/ml. These data suggest that milk concentrations of EPM would be below the permitted maximum residue limit (20 ng/ml) after topical administration at both 1 and 1.5 mg/kg.

In conclusion, a high efficacy was observed after topical administration of EPM at 1 and 1.5 mg/kg to calves naturally infected with *R.* (*B*) *microplus*. Tick exposure to the drug was in agreement with the systemic bioavailability of the antiparasitic molecule. The use of EPM dose rates higher than the recommended one (0.5 mg/kg) does not seem to affect the residual concentrations in milk and a zero day withdrawal period could be maintained. Therefore, the scientific data reported here contribute to consider EPM as a valid treatment to be used in strategic programs for tick control in cattle, and are particularly relevant for applications on dairy cattle production systems.

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