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Original Article

Bioequivalence of two novel formulations of ivermectin 1% combined with fluazuron 12.5% for subcutaneous administration in cattle

[Bioequivalencia de dos nuevas formulaciones de ivermectina al 1% combinada con fluazurón al 12,5% para administración subcutánea en bovinos]

Diego M. Robaina, Silvana Alvariza, Gonzalo Suárez*

Departamento de Clínicas y Hospital Veterinario, Unidad de Farmacología y Terapéutica, Facultad de Veterinaria, Universidad de la República. Alberto Lasplaces 1620, Montevideo, Uruguay.
*E-mail: gsuarez@fvet.edu.uy; suarezveirano@gmail.com

Abstract

Context: Ivermectin (IVM) and fluazuron are present as novel combinations to control ticks. Differences in formulations account for changes to the plasma kinetics and could change exposure of target parasites to active drugs.

Aims: To evaluate bioequivalence for ivermectin administered by two novel formulations combined with fluazuron.

Methods: Twelve male Holstein calves were randomized into two groups (n=6), receiving a single subcutaneous dose of a novel formulation (A or B) of ivermectin combined with fluazuron (ivermectin 1% 0.2 mg/kg + fluazuron 12.5% 2.5 mg/kg). Blood samples were taken until 34 days after dosing. Non-compartmental analysis was applied for bioequivalence assessment. Compartmental analysis was carried out and model acceptance was validated using the visual predictive check graphics.

Results: Noncompartmental analysis shows that both formulations behaved similarly with C_{max} ratio of 0.982 (CI_{90}: 0.861 - 1.12, ANOVA test, p=0.814) and AUC_{0-t} ratio of 1.01 (CI_{90}: 0.97 - 1.05, ANOVA, p=0.586) but at different T_{max} (1.4 ± 0.5 and 2.9 ± 1.2 days (Mann-Whitney U test, p=0.027) for A and B, respectively). The pharmacokinetic model has two compartments, linear elimination with first order absorption and formulation as covariable for the absorption rate.

Conclusions: We conclude that the two novel formulations combined of IVM 1% with fluazuron 12.5%, are bioequivalent for administration of IVM in cattle. This shows that carrying out bioequivalence studies are of great importance for understanding the potential interchangeability between formulations available in the pharmaceutical market.

Keywords: bioavailability; ectoparasites; pharmacometrics; ticks.

Resumen

Contexto: Ivermectina y fluazurón están presentes como combinaciones novedosas para controlar las garrapatas. Diferencias en las formulaciones explican los cambios en la cinética plasmática y modificarían la exposición de los parásitos objetivo a las drogas.

Objetivos: Evaluar la bioequivalencia de la ivermectina administrada mediante dos nuevas formulaciones combinadas con fluazurón.

Métodos: Se asignaron al azar doce terneros machos Holstein a dos grupos (n=6), que recibieron una única dosis subcutánea de una nueva formulación (A o B) de ivermectina combinada con fluazurón (ivermectina 1% 0,2 mg/kg + fluazurón 12,5% 2,5 mg/kg). Se tomaron muestras de sangre hasta 34 días post dosificación. Se aplicaron análisis no-compartimental para evaluar la bioequivalencia. Se realizó análisis compartimentado y se validó la aceptación del modelo mediante gráficos de controles visuales predictivos.

Resultados: El análisis no-compartimental muestra que ambas formulaciones se comportaron de forma similar con relación C_{max} de 0,982 (CI_{90}: 0,861 - 1,12, prueba ANOVA, p=0,814) y relación AUC_{0-t} de 1,01 (CI_{90}: 0,97 - 1,05, ANOVA, p=0,586), pero con diferentes T_{max} (1,4 ± 0,5 y 2,9 ± 1,2 días (prueba U de Mann-Whitney, p=0,027) para A y B, respectivamente). El modelo farmacocinético tiene dos compartimentos, eliminación lineal, absorción de primer orden y la formulación como covariable para la tasa de absorción.

Conclusiones: Concluimos que las dos formulaciones combinadas de ivermectina 1% con fluazurón 12,5%, son bioequivalentes para ivermectina en bovinos. Esto demuestra que los estudios de bioequivalencia son de gran importancia para comprender la intercambiabilidad entre formulaciones disponibles en el mercado.

Palabras Clave: biodisponibilidad; ectoparasitos; farmacometría; garrapatas.

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INTRODUCTION

Pharmacokinetic profile of a given drug differ according to administration route, formulation and animal species. After subcutaneous (s.c.) administration the absorption characteristics, and hence the pharmacokinetics of ivermectin are markedly influenced by drug formulation (Jackson, 1989). After parenteral administration, the deposition in s.c. tissue favors a slow absorption from the injection site and provides prolonged sustained levels of drug in the bloodstream. As demonstrated by Lo et al. (1985), the characteristics of absorption of ivermectin (IVM) drugs are markedly influenced by the drug formulation. In non-aqueous (60% propylene glycol and 40% glycerol formal) injectable preparation (IVOMEC, cattle, Merck, Sharp and Dohme), IVM is absorbed relatively slowly from the site of injection. An injectable aqueous solution of IVM allows faster absorption and higher peak plasma concentration of the drug (Lo et al., 1985). However, after s.c. administration of IVM to cattle, elimination half-life has been reported to be three times longer (8 - 13 days) when given in a non-aqueous vehicle than in an aqueous one (Lo et al., 1985). This result indicates the influence of the absorption kinetics on the disposition of parenterally administered IVM. The mean residence time has been shown to be markedly longer following parenteral administration of lVM in cattle (6.54 days) (Toutain et al., 1988), compared with the i.v. administration (2.8 days) of the compound in the same formulation (Wilkinson et al., 1985).

Slight differences between formulations can provoke changes in drug pharmacokinetics and thus different exposure of the target parasites to drug concentrations. This has been confirmed by the extended residence times of IVM in plasma and target tissues and the prolonged persistence of its anthelmintic activity, following the administration of a novel oil-based (IVM 1%) formulation to cattle, compared to the standard innovator preparation (Lifschitz et al., 2004). The duration of effective levels of ivermectin in the blood is particularly important in the treatment of tick infestations since these organisms may feed over periods of several days. The use of ivermectin in the treatment and control of a wide variety of arthropod parasites of livestock has been the subject of a number of reviews, which suggest that marked interspecific differences in susceptibility exist. These probably reflect differences in parasite feeding patterns, which may enhance or prevent drug contact, rather than inherent differences in sensitivity to ivermectin (Jackson, 1989). All the aforementioned, grants special attention to bioequivalence studies to respond whether the drug behaves in a similar way from a pharmacokinetic point of view and if that could be the reason for differences in efficacy studies. Lanusse et al. (2018) stated that understanding the pharmacokinetic behaviour of broad-spectrum antiparasitic drugs and the factors modulating that behaviour is highly important for maximizing their clinical efficacy and delaying the development of drug resistance.

The aim of this study was to demonstrate IVM 1% bioequivalence between two novel formulations combined with fluazuron 12.5% for subcutaneous administration on cattle.

MATERIAL AND METHODS

Experimental design

Twelve male Holstein calves (weighing 217 ± 7 kg) were randomized in parallel bioequivalence study into two groups (n = 6). The groups were aleatory identified as A and B and each group received a single dose of one or another of two novel formulations A (standard or reference) and B (test) of IVM combined with fluazuron (ivermectin 1% 0.2 mg/kg + fluazuron 12.5% 12.5 mg/kg) by subcutaneous route on the neck of the animals (both formulations have been approved as Animal Drugs Product from Ministerio de Agricultura y Pesca in Uruguay) (Table 1). Blood samples (10 mL) were collected from the jugular vein in heparinized tubes by disposable plastic syringe at 0, 0.3, 0.9, 1.3, 1.9, 2.3, 2.9, 3.3, 3.9, 4.3, 6, 9, 13, 27 and 34 days. Blood was centrifuged at 3500 rpm (10 min) and the plasma obtained was stored at -20°C

Table 1. Characteristics of the formulations A (standard or reference) and B (test) for ivermectin combined with fluazuron for use in cattle used in the study.

Formulation	Code	Ingredients	Dosage and route of administration	Pharmaceutical form	Indications
Standard or Reference	A	Ivermectin (1000 mg), fluazuron (12 500 mg) (API). Excipient q.s. (100 mL)	1.0 mL per 50 kg bodyweight, subcutaneous injection	Solution	Endectocide
Test	B	Ivermectin (1000 mg), fluazuron (12 500 mg) (API). Excipient q.s. (100 mL)	1.0 mL per 50 kg bodyweight, subcutaneous injection	Solution	Endectocide

The names of the manufacturers have been omitted for ethical reasons. API: active pharmaceutical ingredient. q.s.: quantity sufficient.

until analysis was performed. The experimental study was approved by the Comisión Honoraria de Experimentación Animal, Universidad de la República, Uruguay (CHEA, No. 506-1493294137).

In order to optimizing the well-being of the animals enrolled in the clinical trial, frequent clinical health assessments were performed to identify animals that were potentially approaching a study end point. No animal reported any local (administration site) or systemic clinical signs or symptoms due to the administration of both formulations during the study.

Analytical procedures

IVM plasma concentrations were determined by high performance liquid chromatography (HPLC-FLD) method, based on the procedure developed by Lifschitz et al. (2004) with minor modifications.

Chemicals and reagents

All substances were obtained from Sigma Chemical Company (Saint Louis, MO, USA). Standards of ivermectin (IVM, product code: I8898) and abamectin (ABM, internal standard, product code: 31732), acetonitrile (product code: 34851), methanol (product code: 34860) , n-methylimidazole (product code 336092) and trifluoroacetic acid (product code: 91707).

Drug extraction and derivatization

Briefly, plasma samples (1.0 mL) were spiked with 50 µL of ABM. Acetonitrile (1 mL) and 0.25 mL of deionized water were added. After mixing for 20 min under a high speed vortexing shaker (DMT-2500 Multi-Tube Vortex Mixer, Miulab, Hangzhou Miu Instruments Co., Ltd., China), samples were centrifuged at 10.000 rpm for 10 min (Sorvall™ Legend™ Micro 17 microcentrifuges, Thermo Fisher Scientific, United States). The supernatant was transferred to a plastic tube previous to the solid extraction. The cartridges (Strata® C18-E 100 mg/1 mL) were previously activated with 2 mL of methanol and 2 mL of deionized water. The sample was passed through the cartridge using a manifold vacuum (HyperSep™ Glass Block Vacuum Manifolds, Thermo Fisher Scientific, United States) and then it was washed with 1 mL of deionized water and 1 mL of a mixture of deionized water:methanol (4:1); it was then let to dry for 5 min. A volume of 1.5 mL of methanol was used to elute the sample. The elute was evaporated to dryness under nitrogen flow (55°C/50 min). Derivatization was carried out using 100 µL of a n-methylimidazole solution in acetonitrile (1:1) and 150 µL of trifluoroacetic acid solution in acetonitrile (1:2). After completion of the reaction, 200 µL of this solution was transferred to a HPLC vial and injected into the chromatographic system (Dionex UltiMate 3000 HPLC, Thermo Fisher Scientific, United States).

Calibration curves in the range between 0.5 - 5.0 and 5.0 – 80.0 ng/mL were established using least squares linear regression analysis and correlation coefficients (r) and coefficient of variation (CV) calculated. The lower limit of quantification (LLOQ) was defined as the lowest measured concentration with a CV < 20% an accuracy of ± 20% and an absolute recovery ≥ 70%. Concentration values below the 0.5 ng/mL (LLOQ) were not considered for the kinetic analysis of experimental data.

Chromatographic conditions

A Phenomenex®Luna C18 (5 m, 100°A, 150 mm × 4.6 mm, Phenomenex, reference code: 00F-4252-E0-BV) column was used as a reversed stationary phase. The mobile phase was a mixture of acetic-acid 0.02%/methanol/acetonitrile (2/42/56) pumped at a flow rate of 1.5 mL/min. The column compartment was kept at 37°C. Fluorescence detection at an excitation wavelength of 361 nm and an emission wavelength of 463 nm.

Pharmacokinetic analysis

Noncompartmental analysis

Plasma concentration data were analysed using Pkanalyx (PKanalix version 2019R2. Antony, France: Lixoft SAS, 2020) for a noncompartmental analysis. Pharmacokinetic parameters were reported as mean ± standard deviation (SD). The statistical analysis for establishing bioequivalence was performed using pharmacokinetic parameters by a one-way ANOVA test for C_{max}, AUC and C_{max}/AUC; the Mann-Whitney U test was applied for T_{max} (R Core Team, 2020). Bioequivalence occurred whenever the 90% confidence intervals (90%CI) for a systemic exposure ratio fell entirely inside the equivalence range of 0.8 - 1.25 (Martinez et. al., 2002; FDA, 2016). In all cases, a value of $p<0.05$ was considered statistically significant.

Compartmental analysis

IVM plasma concentration data was modeled using Monolix (Monolix version 2019R2. Antony, France: Lixoft SAS, 2020). Structural and statistical models were evaluated by basic goodness of fit plots, metrics value and visual predictive checks (VPC).

RESULTS

Non-compartmental approach

Fig. 1 depicts the plasma concentration of IVM for both formulations of IVM combined with fluazuron after subcutaneous administration (mean ± SD). The most relevant pharmacokinetic parameters were calculated from a non-compartment analysis and the results are shown in Table 2.

Results show that both IVM formulations combined with fluazuron behaved in a similar way having similar C_{max} and AUC but at different times (T_{max}). Results for AUC and C_{max} were log transformed for statistical analysis (ANOVA test) to asses IVM bioequivalence between both formulations; the C_{max}/AUC coefficient was calculated for both formulation being a much more reliable indicator of absorption rate (Endrenyi et al., 1991), these data and the bioequivalence range the 90%CI are displayed in Table 3. T_{max} was depicted as a secondary bioequivalence parameter due to the fact that the sampling times were not equal and therefore the statistical analysis for T_{max} as a bioequivalence parameter described by Basson et al. (1996; 1998) could not be applied.

Area under the curve from time 0 extrapolated to infinity (AUC_{0-inf}) was not statistically different between formulations after ANOVA test on the log transformed data to assure normality ($p>0.05$); no statistical difference was observed on any of the rest of the pharmacokinetic parameters obtained from the non-compartmental analysis of IVM.

Compartmental approach

IVM plasma concentration vs time data was modeled using Monolix (Monolix version 2019R2. Antony, France: Lixoft SAS, 2020. http://lixoft.com/products/monolix/). Parameter estimates, residual standard error (%) and visual predictive check graphs (VPC) were the applied criteria for the selection of the best fit. The PK model has a central compartment (volume V1), a peripheral compartment (volume V2, intercompartmental clearance Q), and a linear elimination (clearance Cl) with a first order absorption (rate constant ka). Results for this model are presented in Table 4. Bioavailability (F) was fixed to 1 for the registered formulation (Formulation A). Model acceptance was validated using the VPC graphics comparing simulated data against the data obtained from the experiment (Fig. 2).

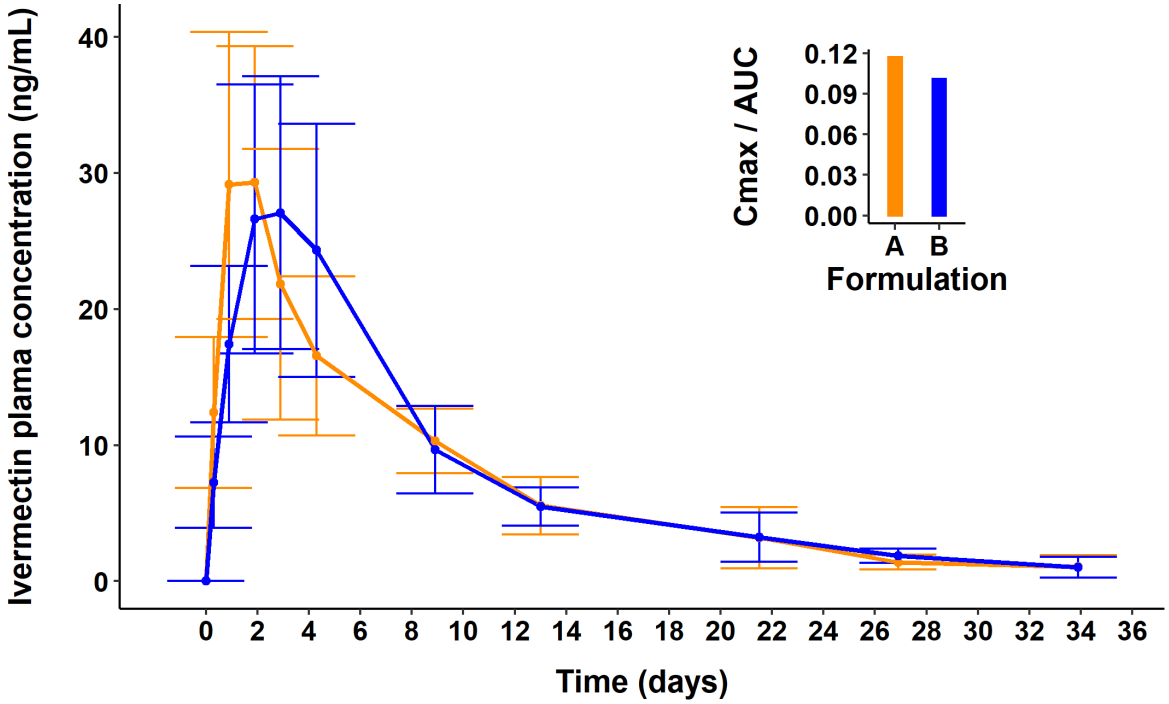

Figure 1. Mean plasma concentration of ivermectin (IVM) (ng/mL) for both IVM formulations (A and B) combined with fluazuron, after subcutaneous administration (0.2 mg/kg IVM and 12.5 mg/kg fluazuron, single dose) to 12 calves.

Concentrations are expressed as mean ± SD with fluazuron (12.5 mg/kg; 2.5%), after subcutaneous administration to 12 calves.

Table 2. Non-compartment parameters for ivermectin (0.2 mg/kg; 1%) in two different formulations (A and B) combined.

Pharmacokinetic parameter	A	B	p
AUC_{0-t} (ng/d)	246.8 ± 41.0	264.8 ± 52.8	0.526
AUC_{t-inf} (ng/d)	14.9 ± 9.3	16.8 ± 14.0	0.794
AUC_{0-inf} (ng/d)	261.7 ± 41.4	281.5 ± 44.4	0.442
Beta	0.1 ± 0.02	0.09 ± 0.03	0.734
$t_{1/2}$ (d)	7.3 ± 2.0	8.2 ± 2.7	0.532
Vd (L)	1.74 ± 0.34	1.9 ± 0.83	0.682
Cl (L/d)	0.17 ± 0.03	0.16 ± 0.02	0.794
T_{max} (d)	1.4 ± 0.5*	2.9 ± 1.2	0.027
C_{max} (ng/mL)	30.9 ± 10.5	29.0 ± 9.8	0.749
MRT (d)	9.5 ± 3.0	10.7 ± 3.7	0.554

AUC_{0-t}: area under the concentration vs. time curve from 0 up to the limit of quantification; AUC_{t-inf}: area under the concentration vs. time curve form the limit of quantification up to infinity; AUC_{0-inf}: area under the concentration vs. time curve extrapolated to infinity; beta: slow elimination rate constant ; $T_{1/2el}$: elimination half-life; Vd: volume of distribution; CL: clearance ; T_{max}: time to peak plasma concentration; C_{max}: peak plasma concentration; MRT: medium retention time. *Differences between columns are statistically different at $p<0.05$, Mann-Whitney U-test.

Table 3. Bioequivalence for ivermectin between two formulations (formulation A and B) combined with fluazuron, after subcutaneous administration (0.2 mg/kg IVM and 12.5 mg/kg fluazuron, single dose) to 12 calves.

Pharmacokinetic parameter	Log transformed values		Ratio* (µB/µA)[90%CI]	p	Bioequivalence criteria
	Formulation A	Formulation B			
AUC_{0-t} (ng/d)	5.49	5.56	1.01 [0.974 - 1.05]	0.587	Pass
AUC_{0-inf} (ng/d)	5.55	5.63	1.01 [0.982 - 1.05]	0.451	Pass
C_{max} (ng/mL)	3.36	3.31	0.98 [0.861 - 1.12]	0.814	Pass
C_{max}/AUC_{0-inf}	0.61	0.59	0.96 [0.866 - 1.08]	0.620	Pass
T_{max} (d)	1.40	2.90		0.0274**	Fail

AUC_{0-t}: area under the concentration vs. time curve from 0 up to the limit of quantification; C_{max}: peak plasma concentration. Two pharmaceutical formulations are considered bioequivalent when the ratio µB/µA confidence intervals (90%CI) for both AUC and C_{max} between formulation A and B result between 0.8 and 1.25 (Martinez et. al., 2002). **Compared using Mann-Whitney U-test. Significant statistical difference when $p<0.05$.

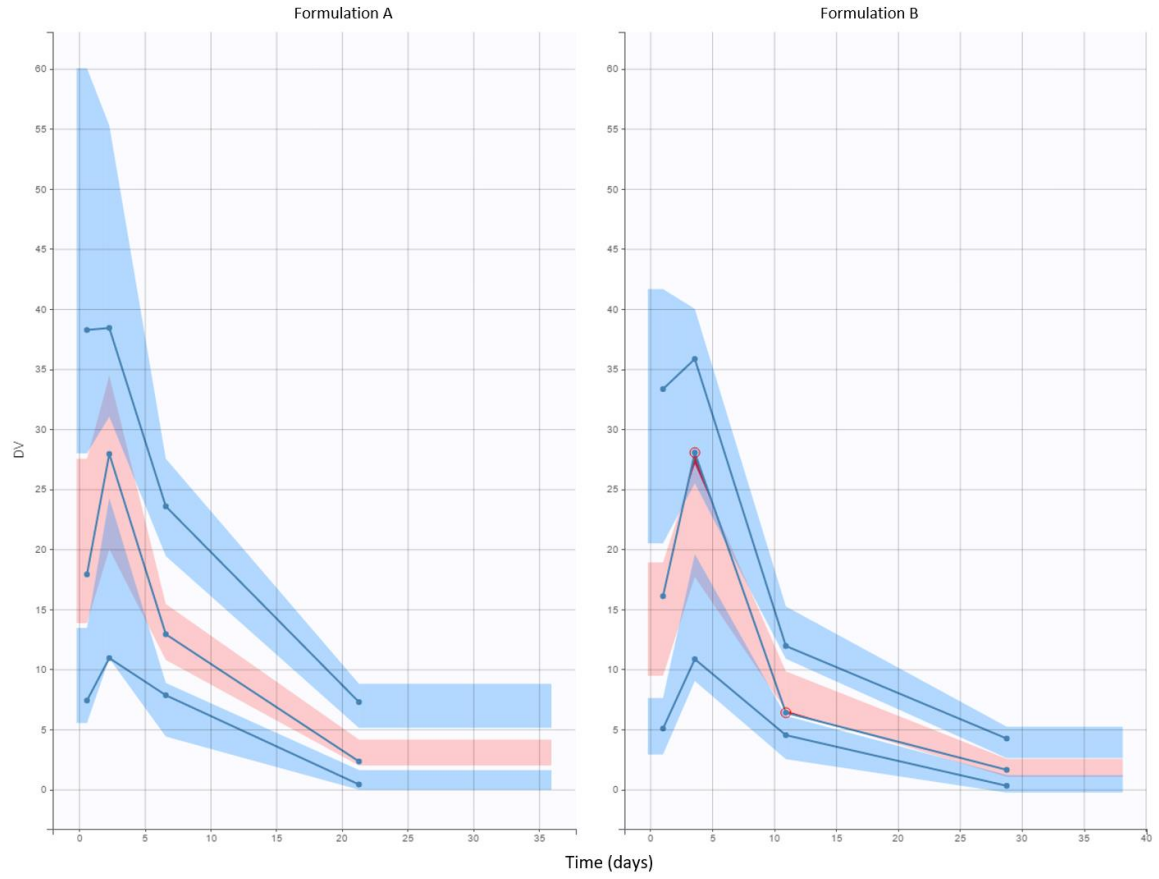

Figure 2. Visual Predictive Check (VPC) for ivermectin 1% in combination with fluazuron 2.5%, after subcutaneous administration (0.2 mg/kg IVM and 12.5 mg/kg fluazuron, single dose) to 12 calves, stratified by formulation (A and B).

Table 4. Pharmacokinetic parameters estimate and residual standard error (%) for ivermectin (0.2 mg/kg; 1%) combined with fluazuron (12.5 mg/kg; 2.5%), after subcutaneous administration to 12 calves.

Pharmacokinetic parameter	Estimate	R.S.E.(%)
ka (d)	1.55	16.0
beta_ka_B	-0.932	22.8
Cl (L/d)	158	5.5
V1 (L)	1110	17.2
Q (L/d)	48.9	34.2
V2 (L)	566	49.3
F_pop	1	
Standard deviation of the random effects		
omega_V1 (L)	0.485	21.5
Error model parameters		
A	0.639	24.7
B	0.16	14.4

ka: absorption rate constant; beta_Ka_B: covariate of formulation B on absorption rate constant; Cl: clearance; V1: volume of distribution for the central compartment; Q: intercompartmental clearance; V2: volume of distribution for the peripheral compartment; F_pop: population biodisponibility (fixed to 1); omega_V1: random effect for V1.

DISCUSSION

In Uruguay, according to official data, the number of available veterinary formulations containing IVM for the control of ticks in cattle rises to nine different IVM 1% and twenty-seven IVM 3.15% formulations for use in veterinary medicine (MGAP, 2020). The lack of information on the pharmacokinetic profile for IVM between formulations, and of the differences on manufacturing and quality of ingredients, becomes problematic when it comes to interchangeability of pharmaceutical products for the control of cattle ticks.

The pharmacokinetic profiles of IVM in cattle (Lo et al., 1985) and ewes (Suarez et. al., 2013) have been shown to be substantially affected by the composition of the administered formulation. The comparative plasma disposition applying non-compartmental analysis of IVM reported in these studies were evaluated after subcutaneous or oral administration on cattle and sheep, respectively. The best fitted pharmacokinetic model was adjusted and the final decision was based on parameters estimates and residual standard error (%). McKellar and Gokbulut (2012) expresses that differences in body condition, breed, gender, feeding, and parasitism substantially affect the plasma disposition kinetics of macrocyclic lactones, therefore the current study was conducted using cattle with similar characteristics, uniformly distributed among experimental groups and free of tick infestation. This becomes of great importance when conducting parallel designs, since the experimental design has a lower power than cross-over design for bioavailability (Toutain and Koritz, 1997).

Avermectins and milbemycins are highly lipophilic substances that are extensively distributed from the bloodstream to different tissues. The extensive tissue distribution of the endectocide compounds in cattle agrees with the Vd values obtained in the current study (1800 - 1914 L for A and B formulations, respectively). Distribution into adipose tissue, particularly in liver and fat may account for the large Vd obtained for these com-

pounds compared to other antiparasitic drugs. Lanusse et al. (1997) stated that the persistence of the broad-spectrum antiparasitic activity of endectocide compounds relies on their disposition kinetics and pattern of plasma/tissues exchange in the host, where slight modifications to their plasma/tissue exchange pattern and/or disposition kinetics may result in substantial changes in their concentration and residence time at the site of parasite location which, in turn, would alter the potency and persistence of their antiparasitic activity. Taking this into account, bioequivalence study becomes more and more important as to understand any major changes in the pharmacokinetic behavior of any drug when administered in different formulations (Martinez et al., 2002). There were no statistically significant differences in AUC and C_{max} between both formulations under study, and only T_{max} showed statistical difference ($p<0.05$). T_{max} for formulation B was 103% longer compared with that for formulation A; these results imply a slower absorption rate constant for formulation B after s.c. administration on cattle. The difference in T_{max} between both products may be due to differences in the manufacturing process.

The difference observed may not affect the efficacy/persistence of the antiparasitic activity against most susceptible strains of target endo- and ectoparasites, but differences in the activity against the dose-limiting parasites are likely to occur (Lifschitz et al., 2004). Being that there was no significant statistical difference between medium retention time (MRT, $p<0.05$) between formulation A and B, it is correct to assume a similar persistence for ivermectin and hence achieving drug concentrations in the different target tissues, where the sustained attainment of drug levels toxic to the parasites is critical for the resultant efficacy (Lifschitz et al., 2004). Suarez et al. (2013) conducted relative bioavailability studies for IVM under three different formulations for oral administration on lambs, and the effect on nematodes control. The pharmacokinetic profile for IVM showed no statistical difference between the three formulations, with differences on the impact on endoparasites.

Among the parameters considered for bioequivalence studies, Endrenyi et al. (1991) stated that C_{max}/AUC is the most reliable for the study of the absorption rate between different formulations. This is due to the fact that the C_{max}/AUC ratio is independent of the amount of absorbed drug and is also independent of the intrasubject variability. In this scenario, both formulations showed no statistical difference ($p<0.05$) indicating that there was no difference between the absorption rate for ivermectin between the formulations under study; thus we are thrown to believe that both formulations would behave in a similar way in a scenario of animals with different burdens of parasites (both internal and external). This last statement would need to be verified by field trials, using both formulations under similar conditions.

IVM (200 µg/kg, 1%) pharmacokinetic behavior on cattle after subcutaneous administration in combination with Fluazuron (12.5 mg/kg, 2.5%) was modeled using Monolix (2019R2), adjusting a two-compartmental model with first order elimination (Cl), an absorption constant (ka) and an inter-compartment transfer constant (Q). The result for this model showed that the only covariate needed to be applied was the formulation effect on ka. As reported by Lo et al. (1985), the pharmacokinetic profile of IVM is greatly affected by the differences in formulations. The absorption rate (ka) for formulation A is 2.5 times faster compared to that of formulation B (4.7/d and 1.8/d, respectively) indicating that there is a great difference between the speed in which IVM ingress to the central compartment between both formulations, with the possible differences on the efficacy for the treatment of different parasites (both internal and external). The formulation was only relevant for the absorption rate constant while there was no effect on the rest of the pharmacokinetic parameters. The clinical impact of this difference is yet to be assessed by field trials on cattle infected by different parasite populations.

Lifschitz et al. (2004) compared pharmacokinetic parameters for doramectin and IVM in cattle using 42 animals divided into 6 groups (n = 7) for testing six endectocide formulations (doramectin, abamectin and four IVM formulations available on

the market), measuring plasma concentrations up until 35 days (840 hours) after the start of the experiment. The results for the bioequivalence between the four IVM formulations were similar to those obtained in our experiment, with AUC, C_{max} and T_{max} showing differences among the four IVM formulations. The AUC value found in our experiment does not differ from the ones reported by Lifshitz et al. (2004), where they reported an AUC varying from 242 ± 40.1 up to 308 ± 41.8 (ng day/mL) for the four different IVM formulations, while we obtained AUC for both formulations to be 246.8 ± 41.0 and 264.8 ± 52.8 ng/d (A and B respectively); T_{max} and C_{max} follow the same pattern, showing differences not only between the formulations tested by Lifschitz et al. (2004), but also when compared to the ones we obtained in our experiment. The cited authors reported a C_{max} ranging from 22.0 ± 6.86 up to 32.7 ± 4.35 ng/mL while we obtained a C_{max} of 30.9 ± 10.5 and 29.0 ± 9.8 ng/mL (A and B formulations respectively); for T_{max}, the reported values by the mentioned authors ranges from 1.14 ± 0.38 up to 4.29 ± 2.06 days; we obtained a T_{max} of 1.4 ± 0.5 and 2.9 ± 1.2 days (A and B formulations respectively). The similarity in the differences between the four formulations tested by Lifschitz et al. (2004), and the two formulations tested in our experiment serves only to enforce the importance of conducting bioequivalence studies to characterize the different formulations available on the market for the treatment of cattle parasites.

Study limitations

To improve the pharmacokinetic model construction, samples at closer times intervals are needed to better understand the absorption phase.

CONCLUSIONS

We conclude that the two novel formulations combined of IVM 1% with fluazuron 12.5%, are bioequivalent for administration of IVM in cattle. This shows that carrying out bioequivalence studies are of great importance for understanding the potential interchangeability between formulations available in the pharmaceutical market. Further studies would be needed to evaluate the complementarity between both compounds (IVM and fluazuron) as well as the clinical impact of the pharmacokinetic profiles in cattle infected with parasites.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The selection of the approved formulations did not respond to any particular interest to compare the quality among them.

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AUTHOR CONTRIBUTION:

Contribution	Robaina D	Alvariza S	Suárez G
Concepts or ideas	x		x
Design	x	x	x
Definition of intellectual content	x	x	x
Literature search	x		
Experimental studies	x		
Clinical trial	x		x
Data acquisition	x		
Data analysis	x	x	x
Statistical analysis	x	x	x
Manuscript preparation	x		
Manuscript editing	x		x
Manuscript review	x	x	x

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