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# Evaluation of pharmacological interactions after administration of a levamisole, albendazole and ivermectin triple combination in lambs

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ABSTRACT

The goals of the current trial were (a) to characterize the plasma disposition kinetics of levamisole (LEV), albendazole (ABZ) and ivermectin (IVM), each administered either alone (single active ingredient) or as a combined formulation to lambs; (b) to compare the clinical anthelmintic efficacy of the same drugs given either separately or co-administered to lambs infected with resistant nematodes. Fifty Corriedale lambs naturally infected with multiple resistant gastrointestinal nematodes were involved in the following experimental trials: (a) "*Pharmacokinetic trial*": the animals were allocated into five groups ($n = 10$ each) and intraruminally treated with either LEV (8 mg/kg), ABZ (5 mg/kg), IVM (0.2 mg/kg), or with a LEV + ABZ + IVM combined formulation, where each active ingredient was administered at the same dose. Blood samples were collected over 15 days post-treatment and drug plasma concentrations measured by HPLC. (b) "*Efficacy trial*": the same treated groups plus an untreated control group were used to assess the comparative anthelmintic efficacy by the faecal egg count reduction test (FECRT). Although the overall LEV disposition kinetics was unaffected, significantly lower (61%) ABZ-sulphoxide and higher (71%) IVM systemic availabilities were obtained after administration of the combined formulation in comparison to those obtained after treatment with each drug alone. A multiple drug resistance situation was observed for *Haemonchus* spp. The observed efficacies were 52% (LEV), 72% (ABZ), 80% (IVM) and 87% (triple combined formulation). The results reported here contribute to the pharmaco-therapeutic knowledge on drug combinations. This type of research is crucial before further development of combined anthelmintic preparations reaches the market to deal with resistant nematode control. The co-administration of LEV + ABZ + IVM did not result in a significant advantageous anthelmintic effect compared to the treatment with IVM alone. The simultaneous/combined administration of LEV, ABZ and IVM may account for a drug–drug pharmacological interaction in infected lambs. The pharmacokinetic interaction accounted for a reduced ABZ-sulphoxide and enhanced IVM systemic exposure following the combined treatment.



## 1. Introduction

The development of resistance to the available anthelmintic drugs is a serious constraint to the control of

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gastrointestinal (GI) nematodes in sheep, goats and cattle in many regions of the world (Kaplan, 2004; Wolstenholme et al., 2004; Jabbar et al., 2006). Currently, combinations of two or more anthelmintic active ingredients are primarily being used to manage anthelmintic resistance in ruminants, and to expand the spectrum of efficacy (Geary et al., 2012). Combination of anthelmintics with a similar spectrum of nematodicidal activity and different mechanism of action/resistance has been proposed as an alternative parasite control strategy, where failure of individual drugs is documented (Anderson et al., 1988; Barnes et al., 1995; Leathwick et al., 2009). The rationale behind the use of these combined preparations is based on a lower resistance in individual worms to a formulation with multiple components (each one with different mechanism of action/resistance) compared to the treatment with a single active component. Different drug combined formulations are available in Uruguay, a country with an economically relevant sheep industry and a widespread development of parasite resistance (Nari et al., 1996; Suarez et al., 2011, 2013), as in several others countries with a similar situation.

Among many others combinations, the mixture of levamisole (LEV), an imidazothiazole compound, albendazole (ABZ), a methyl carbamate benzimidazole compound and ivermectin (IVM), a macrocyclic lactone avermectin-type compound is already available in the market. The purpose of this combined is based on the different mechanism of anthelmintic action of each active ingredient. LEV causes a spastic paralysis of susceptible nematodes by selectively gating acetylcholine receptor ion channels on nerve and muscles (Robertson and Martin, 1993); the intrinsic anthelmintic action of ABZ relies on a progressive disruption of basic cell functions as a result of their binding to parasite β-tubulin and depolymerization of microtubules (Lacey, 1990). IVM acts on ligand-gated channels, including glutamate and GABA-gated chloride channels, which are involved on nematode feeding, reproduction and locomotion (Geary et al., 1993; Feng et al., 2002; Yates et al., 2003). The different mode of action/resistance of the active ingredients included in the LEV + ABZ + IVM combination, may complementary contribute to their efficacy against resistant nematodes. However, in spite of the fact that combined preparations are already being used in parasite control, there is a need for pharmacology-based research to assess the potential pharmacokinetic (PK) and/or pharmacodynamic (PD) interactions among the active ingredients in this combined anthelmintic formulation.

A drug–drug interaction refers to the possibility that one compound may alter the intensity of the pharmacological effects of another drug given concurrently. The modified/altered effect may emerge from a change on the relationship between drug concentration and response of the organism to the drug (PD interaction) or from a change on the concentration of either one or both molecules in the organism (PK interaction). PD interactions (at site of action) would account for indifference, antagonism, additive or synergistic effects. A synergistic pharmacological effect is achieved when the combined effect of the drugs is significantly greater than the independent effect of each molecule, which could be an ideal pharmacological situation in the control of resistant parasites. PK drug–drug interactions are mainly related to enzyme induction or inhibition, competition with drug transport proteins and/or protein binding. While most of the data obtained on the pharmacological assessment of different available anthelmintic combinations would indicate that only an additive anthelmintic effect is achieved by drug combined activity, the PK interactions among anthelmintic molecules may be more common than expected. A PK interaction between ABZ and IVM in sheep has been previously reported (Alvarez et al., 2008). Additionally, the presence of triclabendazole, an halogenated BZD compound used as flukicidal in sheep and cattle, increases the plasma concentrations of IVM in sheep (Lifschitz et al., 2009). The pharmaco-parasitological evaluation of drug interactions is becoming highly relevant since drug combinations are now widely used as an alternative to control resistant helminth parasites in livestock. Therefore, the potential PK and PD drug–drug interactions occurring among LEV, ABZ and IVM should be understood before this particular drug combined formulation is recommended to be used in sheep. The main goal of the current trial was to characterize the plasma disposition kinetics of LEV, ABZ and IVM administered either alone (a single active ingredient) or as a combined formulation in parasitized lambs. Additionally, the clinical efficacy of the same drugs given either separately or co-administered to lambs infected with multiple resistant nematodes was compared.

## 2. Materials and methods

### 2.1. Animals

Fifty male and female Corriedale lambs (7–8 month old), weighing 28.8 ± 4.0 kg and naturally infected with multiple resistant GI nematodes, were involved in this trial. All animals were subjected to a veterinary examination before inclusion in the study, and shown a FAMACHA (Van Wyk and Bath, 2002) score ≤2 and a body condition score between 2 and 4. The study was conducted on a farm (*Centro de Investigación y Experimentación "Dr. Alejandro Gallinal", Florida, Uruguay*) where the failure of different anthelmintics to control GI nematodes had been previously demonstrated by the faecal egg counts reduction test (FECRT) (Castells, 2002; Bonino et al., 2010). On day −1, all lambs were individually identified and the number of nematode eggs/gram of faeces (epg) was determined (modified McMaster technique). Experimental animals had an average of 1348 epg ranging from 50 to 9850. Throughout and 60 days before starting the experiment, animals grazed on a natural pasture and had free access to water. Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy of the Faculty of Veterinary Medicine, Universidad de la República (UDELAR), Montevideo, Uruguay.

### 2.2. Chemicals

Standards of ABZ, ABZ-sulphoxide (ABZSO), ABZ-sulphone ($ABZSO_2$), oxibendazole (OBZ, internal standard),

LEV, IVM and abamectin (ABM, internal standard) were obtained from Sigma Chemical Company (Saint Louis, MO, USA). Cibeles, Uruguay, provided the combined LEV + ABZ + IVM commercial suspension (RaiderPlus®, Cibeles, Uruguay), as well as the experimental formulations containing each of the single active (LEV, ABZ or IVM) ingredients. The final drug concentration in each formulation (both single and combined) was confirmed by the Uruguayan Regulatory Agency (DILAVE, Uruguay).

### 2.3. Experimental design, treatments and sampling

All parasitized lambs were ranked according to epg counts and then divided into five groups of 10 animals each based on epg values, to perform the PK and efficacy trials. Each group received on day 0 by the intraruminal (i.r.) route the following treatments: LEV (8%) at the dose of 8 mg/kg; ABZ (5%) at the dose of 5 mg/kg; IVM (0.2%) at the dose of 0.2 mg/kg; LEV + ABZ + IVM (combined treatment) (8, 5 and 0.2%, respectively) at the same individual dose for each active ingredient. For the efficacy trial, an untreated group was kept as a control.

### 2.4. PK trial

Six animals randomly selected from the LEV, ABZ, IVM or LEV + ABZ + IVM treated groups were used in the PK trial. Blood samples (6 mL) were collected by venipuncture into 10 mL heparinised Vacutainers® tubes (Becton Dickinson, NJ, USA), prior to drug administration and at 0.5, 1, 3, 6, 9, 12, 18 and 24 h (LEV treatment); 1, 3, 6, 9, 12, 18, 24, 36 and 48 h (ABZ treatment); 1, 3, 6, 9, 12 and 24 h, and 2, 3, 6, 9, 12 and 15 days (IVM treatment); 0.5, 1, 3, 6, 9, 12, 18, 24 and 36 h, and 2,3, 6, 9, 12 and 15 days (combined treatment) post-treatment. The plasma samples were immediately centrifuged at $3000 \times g$ for 15 min and stored at $-20\,^{\circ}C$ until analysis by high performance liquid chromatography (HPLC).

### 2.5. Efficacy trial: egg count reduction

Faecal samples were individually collected from the rectum of each animal pre-treatment (day −1) and at 7 (LEV and control groups) or 14 (all groups) days post-treatment. Epg counts were performed by the McMaster technique modified by Roberts and O'sullivan (1950). Additionally, the genus and species of the nematodes recovered from parasitized lambs were determined by the identification of the third stage larvae ($L_3$) recovered from pooled faecal cultures obtained from each experimental group (Maff, 1986). Since the egg counts observed in the current trial were highly variable (including no egg counts in some animals of the control group at day 14), the anthelmintic efficacy and the 95% confidence limits (CL) were calculated by the Jeffreys interval, as described by Dobson et al. (2012), where the low confidence limits (LCL) for a binomial proportion is calculated using the method described by Brown et al. (2001). Here the name 'Jeffreys interval' is used to describe a confidence interval (CI) derived from Bayesian procedures assuming non-informative priors (Dobson et al., 2012). In terms of FECRT the Jeffreys interval define $n$ as the total number of eggs counted pre-treatment, $x$ the total number of eggs counted post-treatment, $p$ the proportion of resistant eggs ($p = x/n$) and

$$\text{Efficacy}\quad (\%) = 100 \times (1 - p).$$

### 2.6. Analytical procedures

*LEV analysis*: After a liquid–liquid chemical extraction, plasma LEV concentrations were quantified from plasma samples by HPLC, following a method previously developed (De Baere et al., 2003). Briefly, plasma samples (1 mL) were mixed with 2 mL of sodium hydroxide and 4 mL of extraction solvent (hexane/isoamyl alcohol, 95:5, v/v), and shaken (multi-tube vortexer, VWR Scientific Products, West Chester, PA, USA) over 10 min and then centrifuged (Jouan®, BR 4i Centrifuge, Saint Herblain, France, $2000 \times g$, 10 min, 10 °C) to allow phase separation. Thereafter, the organic phase was collected and concentrated to dryness in a vacuum concentrator (Speed-Vac®, Savant, Los Angeles, CA, USA), and then reconstituted with 150 μL of mobile phase. Fifty microliters of each solution were injected into the chromatographic system. The HPLC equipment was a Shimadzu 10 A-HPLC System (Kyoto, Japan) with a UV detector set at 225 nm. The limit of quantification (LOQ) of LEV was 0.10 μg/mL, and mean absolute recovery percentages for concentrations ranging between 0.10 and 1 μg/mL ($n = 6$) were 90.6 with coefficient of variation (CV) of 7.2%.

*ABZ/metabolites analysis*: Plasma samples (1 mL) were spiked with 10 μL of OBZ (100 μg/mL), as internal standard. ABZ and its metabolites were extracted from plasma as previously described (Alvarez et al., 2008), using disposable C18 columns (RP-18, 100 mg, Strata®, Phenomenex, CA, USA), which were previously conditioned with 0.5 mL of methanol (HPLC grade), followed by 0.5 mL of water. All samples were applied to the cartridge and then sequentially washed with 2 mL of water and eluted with 2 mL of HPLC grade methanol, concentrated to dryness in a vacuum concentrator (Speed-Vac®, Savant, Los Angeles, CA, USA) and then reconstituted with 150 μL of mobile phase. Fifty microliters of each solution were injected into the chromatographic system. ABZ, ABZSO and $ABZSO_2$ plasma concentrations were determined by HPLC (Shimadzu 10 A-HPLC System, Kyoto, Japan) with a UV detector set at 292 nm following a method previously developed (Alvarez et al., 1999). The limit of quantification for ABZ/metabolites was 0.05 μg/mL. Mean absolute recovery percentages for concentrations ranging between 0.1 and 1 μg/mL ($n = 6$) were 91.4 (ABZSO), 89.9 ($ABZSO_2$) and 86.3% (ABZ) with CV of 6.7, 6.9 and 6.3%, respectively.

*IVM analysis*: The extraction of IVM from spiked and experimental plasma samples was carried out following the well established technique (Alvinerie et al., 1993, slightly modified by Lifschitz et al., 1999). Plasma samples (1 mL) were placed into a 5 mL plastic tube and spiked with 50 μL of ABA (internal standard, 2 ng/10 μL). Drug molecules were extracted by addition of 0.5 mL acetonitrile for 10 min under a high speed vortexing shaker (Multi-tube Vortexer, VWR Scientific Products, West Chester, PA, US). After mixing, the sample was sonicated (Ultrasound Bath, Lab-Line Instrument, Inc., Melrose Park, OL, US)

and centrifuged (BR 4i Centrifuge, Jouan®, Saint Herblain, France) at 2000 × *g* for 10 min at 5 °C. The clear supernatant was transferred to a tube, and the procedure repeated. The total supernatant was transferred to $C_{18}$ columns (RP-18, 100 mg, Strata®, Phenomenex, CA, USA) using a manifold vacuum (Baker spe-24G, Phillipsburg, USA). The cartridges were previously conditioned with 2 mL of methanol, followed by 2 mL of water. All samples were applied and then sequentially washed with 1 mL of water, 1 mL methanol:water (1:4), dried with air for 5 min and eluted with 1.5 mL of methanol. The eluted volume was evaporated (60 °C) to dryness in a vacuum concentrator (Speed-Vac®, Savant, Los Angeles, CA, USA), derivatised and an aliquot of 100 μL was injected in the chromatographic system. Plasma IVM concentrations were determined by HPLC (Shimadzu 10 A-HPLC System, Kyoto, Japan) with a fluorescence detector. The limit of quantification was 0.1 ng/mL and mean absolute recovery percentages for concentrations ranging between 0.1 and 30 ng/mL ($n = 6$) were 87% with CV of 6.4%.

In all cases, values below LOQ were not included in the PK analysis.

### 2.7. PK analysis of the data

Non-compartmental PK analysis for the plasma concentration versus time curves for LEV, ABZ metabolites and IVM for each individual animal after the different treatments were conducted using the R software (version 2.14.0) (Team, 2008). The peak concentration ($C_{max}$) and time to peak concentration ($T_{max}$) were recorded directly from the measured concentration data. The elimination half-life ($T_{1/2el}$) was calculated as $\ln 2/\lambda_{el}$, where the terminal elimination rate constant ($\lambda_{el}$), was calculated by performing regression analysis using data points belonging of the terminal phase concentration–time plot. The area under the plasma concentration–time curve from zero up to $T_{max}$ ($AUC_{0-T_{max}}$) or the limit of quantification ($AUC_{0-LOQ}$) was calculated by means of the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to infinity ($AUC_{0-\infty}$) by dividing the last experimental concentration by the terminal elimination rate constant ($\lambda_{el}$).

### 2.8. Statistical analysis of the data

The PK parameters and concentration data are reported as arithmetic mean ± SD. The PK parameters $AUC_{0-LOQ}$ and $C_{max}$ obtained for each drug after its administration alone or as a combined treatment were compared by Student *t*-test. $T_{max}$ were compared using nonparametric Wilcoxon two-sample test. In all cases, a value of $P < 0.05$ was considered statistically significant. The PK parameters $C_{max}$ and $AUC_{0-LOQ}$ were used to determine potential drug–drug interactions. Geometric mean ratios (GMR) of the $C_{max}$ and $AUC_{0-LOQ}$ for the drug used in a combination/alone and the 90% confidence interval (90%CI) of the GMR were determined. It was concluded that a significant interaction had occurred whenever the 90%CI for a systemic exposure ratio fell entirely outside the equivalence range of 0.8–1.25 (FDA, 2012).

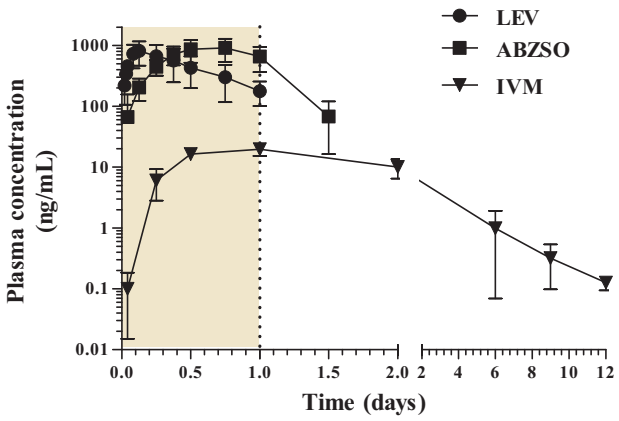


**Fig. 1.** Comparative mean (±SD) plasma concentration profiles ($n = 6$) for levamisole (LEV), albenzadole sulphoxide (ABZSO) and ivermectin (IVM), obtained after the intraruminal administration of LEV (8 mg/kg), albendazole (5 mg/kg) and IVM (0.2 mg/kg) as a triple combination in nematode infected lambs. Shaded area indicates the LEV, ABZSO and IVM simultaneous plasma concentration.

The total number of nematode eggs (raw data) counted in each group (pre- and post-treatment) were compared by a negative binomial generalized lineal model (Dobson et al., 2012). To perform the assessment of the comparative efficacy of the single and combined treatments, the egg count reduction was used as a "clinical end-point", following the criteria suggested by the bioequivalence guidelines of the Food and Drug Administration (FDA, 2006). The analysis compares the test product (LEV + ABZ + IVM), and the different "reference" products (LEV, ABZ or IVM used alone). Additionally, both the test and the reference products were compared separately to a placebo (untreated control group). It is important to note that the group, not the individual animal, was the experimental unit. The 90%CI was used as the best method for evaluating clinical end-point studies. Assuming that the test and reference products have been shown to be superior to the untreated control, the determination of equivalent efficacy was based upon the 90%CI of the ratio between the two products. The statistical analysis was performed using the R software, version 2.15.0 (Team, 2008).

## 3. Results

No adverse events were observed in treated animals, showing a good tolerability to all the assessed formulations. The concentration of each active compound was within the expected range for all the experimentally prepared formulations (combined formulation, LEV, ABZ and IVM single formulations). ABZ parent drug was not detected in plasma at any time post-treatment. ABZSO (active metabolite) and $ABZSO_2$ (inactive metabolite) were the main analytes recovered in plasma after ABZ treatment. LEV, ABZSO and IVM were simultaneously measured in the plasma obtained from lambs treated with the combined formulation (Fig. 1). The $AUC_{0-LOQ}$ for LEV, ABZSO and IVM represent ≥80% of the $AUC_{0-\infty}$ for each compound, showing that the sampling time design was adequate. The plasma disposition kinetics for LEV, ABZSO and IVM showed marked differences. While IVM was detected up to

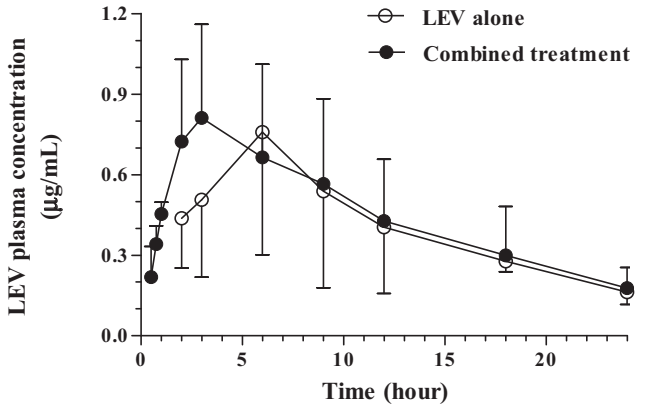


**Fig. 2.** Comparative mean (±SD) plasma concentration profiles ($n = 6$) for levamisole (LEV), obtained after its intraruminal administration (8 mg/kg) alone or as a triple combination (combined treatment) with albendazole and ivermectin in nematode infected lambs.

12 days post-treatment, LEV and ABZSO were only recovered in the bloodstream up to 24 and 36 h post-treatment, respectively. The plasma concentrations vs. time profiles for LEV, ABZSO and IVM after their administration alone or as a triple combination to parasitized lambs, are shown in Figs. 2–4, respectively. Table 1 summarizes the plasma PK parameters for LEV, ABZSO and IVM obtained after the i.r. administration of each drug either alone or as a combined formulation.

The presence of ABZSO and IVM did not affect the plasma disposition kinetics of LEV after its i.r. administration to lambs, with the exception of the partial AUC ($AUC_{0-T_{max}}$), where a higher value ($P < 0.05$) was observed after the administration of the combined formulation (Fig. 2, Table 1). The $AUC_{0-LOQ}$ value of LEV obtained after LEV administration ($8.63 \pm 5.22$ µg h/mL) was similar to that obtained after the combined treatment ($10.5 \pm 5.73$ µg h/mL). Furthermore, no statistical differences among other LEV PK parameters were observed in the LEV alone treated group compared to the animals treated with the combined formulation (Table 1). The ABZSO plasma disposition kinetics was altered in the presence of

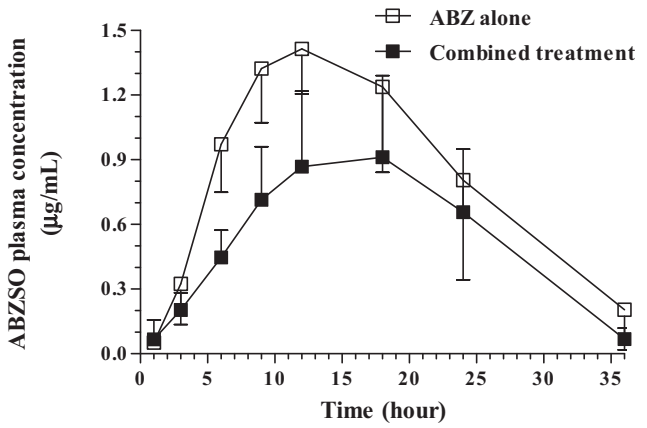


**Fig. 3.** Comparative mean (±SD) plasma concentration profiles ($n = 6$) for albendazole sulphoxide (ABZSO), obtained after the intraruminal administration of albendazole (ABZ) (5 mg/kg) alone or as a triple combination (combined treatment) with levamisole and ivermectin in nematode infected lambs.

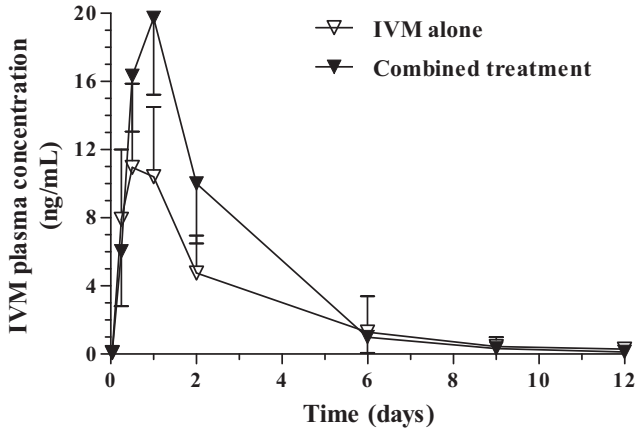


**Fig. 4.** Comparative mean (±SD) plasma concentration profiles ($n = 6$) for ivermectin (IVM), obtained after its intraruminal administration (0.2 mg/kg) alone or as a triple combination (combined treatment) with levamisole and albendazole to nematode infected lambs.

LEV and IVM (Fig. 3, Table 1). Significantly ($P < 0.05$) lower $AUC_{0-LOQ}$ and $C_{max}$ values were obtained for the combined treatment ($AUC_{0-LOQ} = 19.4 \pm 7.90$ µg h/mL; $C_{max} = 0.96 \pm 0.35$ µg/mL), compared to those observed in the ABZ alone treated group ($AUC_{0-LOQ} = 30.7 \pm 9.01$ µg h/mL; $C_{max} = 1.50 \pm 0.21$ µg/mL). A similar trend was observed in other PK parameter such as the $AUC_{0-T_{max}}$. The

**Table 1**
Plasma pharmacokinetic parameters (mean ± SD) for levamisole (LEV), albendazole sulphoxide (ABZSO) and ivermectin (IVM), obtained after the intraruminal administration of LEV (8 mg/kg), albendazole (5 mg/kg) or IVM (0.2 mg/kg), by separated (alone) or as a triple combination (combined treatment) in nematode infected lambs.

| Pharmacokinetic parameters | Experimental groups | |
|---|---|---|
| | Drug alone | Combined treatment |
| LEV | | |
| $T_{max}$ (h) | 5.33 ± 1.63 | 3.33 ± 1.37 |
| $C_{max}$ (µg/mL) | 0.78 ± 0.43 | 0.87 ± 0.34 |
| $AUC_{0-LOQ}$ (µg h/mL) | 8.63 ± 5.22 | 10.5 ± 5.73 |
| $AUC_{0-\infty}$ (µg h/mL) | 10.5 ± 4.52 | 11.4 ± 5.82 |
| $AUC_{0-T_{max}}$ (µg h/mL) | 0.54 ± 0.38 | 1.37 ± 0.50* |
| $T_{1/2el}$ (h) | 5.97 ± 1.74 | 5.62 ± 1.52 |
| ABZSO | | |
| $T_{max}$ (h) | 12.0 ± 5.02 | 13.0 ± 4.10 |
| $C_{max}$ (µg/mL) | 1.50 ± 0.21 | 0.96 ± 0.35* |
| $AUC_{0-LOQ}$ (µg h/mL) | 30.7 ± 9.01 | 19.4 ± 7.90* |
| $AUC_{0-\infty}$ (µg h/mL) | 31.0 ± 9.09 | 20.8 ± 6.72* |
| $AUC_{0-T_{max}}$ (µg h/mL) | 9.89 ± 1.57 | 5.38 ± 1.68* |
| $T_{1/2el}$ (h) | 3.94 ± 1.10 | 5.89 ± 4.05 |
| $ABZSO_2$/ABZSO $AUC_{0-LOQ}$ | 0.15 ± 0.03 | 0.32 ± 0.09* |
| IVM | | |
| $T_{max}$ (d) | 0.92 ± 0.58 | 1.00 ± 0.00 |
| $C_{max}$ (ng/mL) | 12.5 ± 2.92 | 19.7 ± 4.49* |
| $AUC_{0-LOQ}$ (ng d/mL) | 30.9 ± 11.6 | 51.6 ± 16.2* |
| $AUC_{0-\infty}$ (ng d/mL) | 31.2 ± 11.7 | 51.9 ± 16.2* |
| $AUC_{0-T_{max}}$ (ng d/mL) | 6.50 ± 3.33 | 12.5 ± 2.96* |
| $T_{1/2el}$ (d) | 1.48 ± 0.05 | 1.47 ± 0.05 |

$T_{max}$: time to peak plasma concentration; $C_{max}$: peak plasma concentration; $AUC_{0-LOQ}$: area under the concentration vs. time curve from 0 up to the limit of quantification; $AUC_{0-\infty}$: area under the concentration vs. time curve extrapolated to infinity; $AUC_{0-T_{max}}$ : area under the concentration vs. time curve form 0 up to $T_{max}$; $T_{1/2el}$: elimination half-life. $ABZSO_2$: albendazole sulphone.

* Differences between columns are statistically different at $P < 0.05$.

$ABZSO_2$ plasma AUC value obtained after ABZ administration alone ($4.49 \pm 1.67$ μg h/mL) was similar to that obtained after the LEV + ABZ + IVM combined treatment ($5.72 \pm 1.02$ μg h/mL). Additionally, a higher ($P < 0.05$) $ABZSO_2$/ABZSO $AUC_{0-LOQ}$ ratio was observed following the triple combination treatment (Table 1).

The IVM plasma disposition was modified by its co-administration with LEV and ABZ (Fig. 4, Table 1). Significantly ($P < 0.05$) higher IVM $AUC_{0-LOQ}$, $AUC_{0-\infty}$, $C_{max}$, and $AUC_{0-T_{max}}$ values were obtained after the combined treatment, compared to those observed in the group treated with IVM alone (Table 1). No statistical differences were observed in other IVM PK parameters such as $T_{max}$ and $T_{1/2el}$. In all cases, $AUC_{0-LOQ}$ represent ≥80% of $AUC_{0-\infty}$ showing that the time-sampling design was adequate.

The faecal egg counts obtained for all the experimental groups before treatment (trial day −1) and at 14 days post-treatment, including the total egg counted (raw data) by group and the efficacies, are shown in Table 2. At trial day −1, similar mean epg counts were observed among the different experimental groups (ranging between 1085 and 1820 epg). At trial day 14 in all treated groups at least one animal had zero epg counts. Unexpectedly, one animal in the control group also had zero egg counts, which suggests the high aggregation in the epg counts observed in the current experiment (negative binomial distribution aggregation parameter $k = 0.3806$, Table 2). The overall efficacy levels observed at 14 days post-treatment (Table 2) indicate the presence of GI nematodes resistant to either LEV, ABZ and IVM. The anthelmintic efficacy was 87 (combined treatment), 80 (IVM treatment), 72 (ABZ treatment) and 52% (LEV treatment). Egg counts in LEV-treated animals and the efficacy observed after LEV single administration did not differ ($P > 0.05$) when estimated at either 7 or 14 days post-treatment. The $L_3$ composition (%) observed after faecal culture of pooled samples collected from untreated control animals and from the different treated groups at 14 days post treatment, showed the predominance of both, *Haemonchus* spp. and *Trichostrongylus* spp. which are surviving the LEV, ABZ and IVM individual treatments. However, based on the $L_3$ composition, only *Haemonchus* spp. survived the combined treatment (Table 2).

Table 3 summarizes the magnitude of exposure ratios obtained from the PK and efficacy trials. The degree of drug-to-drug interaction was demonstrated by the GMR combined/alone treatments (90%CI) for both ABZSO and for IVM (see Table 3). A significantly ($P < 0.05$) reduction on total epg counts was observed after the IVM alone or following the LEV + ABZ + IVM combined treatment, compared to the untreated control group (Table 3). The total egg counts did not show differences ($P > 0.05$) in comparison to the untreated control following the administration of both LEV and ABZ given as single active ingredient.

## 4. Discussion

Drug–drug interactions may arise from PK changes caused by absorption, distribution, metabolism and/or excretion interactions, which may alter PK/PD relationship. The pattern of tissue distribution, and hence the active drug concentrations achieved at the site of action may be either increased or decreased when an interaction occur. The aim of the study described here was to determine a potential drug–drug interaction among LEV, ABZ and IVM used as a combination in lambs parasitized with multidrug resistant nematodes.

A PK interaction between LEV, ABZ and/or IVM was shown. No significant PK changes were observed for LEV after its co-administration with ABZ and IVM in lambs (Table 1). However, the absorption rate of LEV was increased (increased $AUC_{0-T_{max}}$) due to the presence of ABZ (ABZSO) and/or IVM. On the other hand, the ABZSO systemic exposure was substantially modified by the presence of LEV and/or IVM. ABZSO plasma concentration profiles were significantly lower after the combined treatment, resulting in a significantly lower (39%) $AUC_{0-LOQ}$ and $C_{max}$ values (Table 1). Conversely, an increase on IVM plasma concentrations was observed after the combined treatment, leading to a 71 and 58% of increment on IVM $AUC_{0-LOQ}$ and $C_{max}$ values, respectively, compared to the IVM alone treatment.

Overall, the main plasma changes observed in the systemic exposure of the three drugs after their simultaneous administration in lambs were on $AUC_{0-LOQ}$ and $C_{max}$ (ABZSO and IVM). Since the 90%CI of the GMR for the $AUC_{0-LOQ}$ and $C_{max}$ (ABZSO and IVM) were both outside the "no effect" interval (0.8–1.25) (FDA, 2006), the observed PK changes could be considered as clinically relevant. However, the real clinical impact of the decreased ABZSO and/or increased IVM concentrations on their efficacy against resistant nematodes should be cautiously considered. No differences were observed in other plasma PK parameters such as $T_{max}$ and $T_{1/2el}$, between the single or combined treatments. Since the concentration of each active compound was within the expected range for all the experimentally used preparations (combined formulation, LEV, ABZ and IVM formulations), differences in plasma availability of ABZSO and IVM observed after either the combined or the individual treatments, could not be related to accidental excess/reduction on drug concentration within the used experimental formulation.

The one likely explanation for the observed PK changes could be based on a drug–drug interaction at the efflux transport level. As part of the ABC superfamily, P-glycoprotein (Pgp) is a transmembrane protein located in the apical side of intestinal cells that participate in the ATP-dependant efflux of a broad range of structurally and functionally unrelated compounds out of the cell (Gerlach et al., 1986). Pgp plays a key role in the biliar, urinary and intestinal elimination of different unrelated compounds. IVM is largely excreted in bile and faeces as the parent drug, with less than 2% excreted in the urine (Chiu et al., 1990). IVM has been described as a specific Pgp substrate (Didier and Loor, 1996; Pouliot et al., 1997) which is actively secreted from the rat intestine (Laffont et al., 2002). IVM has been also proposed as a multidrug resistance protein 1 and 2 (MRP 1 and 2) substrate (Lespine et al., 2006). Additionally, it is known that ABZSO is actively secreted into the intestinal lumen (Redondo et al., 1999), likely due to a combination of passive diffusion and active transport. Pgp, MRP2 and the breast cancer resistance protein (BCRP) have been proposed as the main carriers involved in ABZSO

**Table 2**
Nematode egg counts[a] (range) and total number of eggs counted in each experimental group pre (day −1) and post treatment (day 14), obtained in nematode infected lambs intraruminally treated with levamisole (LEV, 8 mg/kg), albendazole (ABZ, 5 mg/kg) or ivermectin (IVM, 0.2 mg/kg), each alone or in combination (combined treatment). The efficacy of the treatments, 95% confidence limits (95% CL) and third stage ($L_3$) larvae composition (%) are also shown.

| Experimental groups | Mean epg (range) | | Total number of eggs[b] | | Efficacy %[c] (95% CL) | $L_3$ composition (%) | |
|---|---|---|---|---|---|---|---|
| | Pre treatment | Post treatment | Pre treatment | Post treatment | | *Haem.* | *Trich.* |
| LEV | 1380 (100–5300) | 670 (0–2500) | 277 | 102 | 52 (45:57) | 20 | 80 |
| ABZ | 1820 (50–9850) | 510 (0–1800) | 364 | 134 | 72 (67:76) | 54 | 46 |
| IVM | 1285 (100–4550) | 260 (0–1400) | 257 | 52 | 80 (74:84) | 85 | 15 |
| LEV + ABZ + IVM | 1085 (50–2650) | 140 (0–500) | 217 | 28 | 87 (82:91) | 100 | 0 |
| Untreated control | 1170 (50–3450) | 1490 (0–11100) | 234 | 298 | – | 55 | 45 |

[a] Arithmetic mean.
[b] Total number of eggs (raw data) counted in each group.
[c] Estimated according Dobson et al. (2012). *Haem*: *Haemonchus* spp.; *Trich.*: *Trichostrongylus* spp.

intestinal efflux transport (Merino et al., 2003). In contrast, ABZ parent drug does not seem to interact with Pgp (Merino et al., 2002, 2005; Dupuy et al., 2010), MRP2 or BCRP1 (Merino et al., 2005). ABZSO is mainly eliminated in the liver through the formation of $ABZSO_2$, although biliar excretion (free and conjugated) has been described in sheep (Hennessy et al., 1989; Alvarez et al., 1999). The relative involvement of the biliary and intestinal excretion mechanisms for ABZ metabolites and IVM in sheep, and their potential competition needs to be elucidated. However, since both ABZSO and IVM have been indicated as Pgp substrates, an interaction at this level should be considered. ABZSO competition on IVM efflux (at either intestinal or biliar level) may help to explain the significantly higher IVM AUC and $C_{max}$ values observed in lambs after treatment with the triple combination.

Oppositely, after the combined treatment, ABZSO systemic exposure (estimated as $C_{max}$ and $AUC_{0-LOQ}$) was significantly reduced compared to that achieved after the ABZ alone administration. An IVM inhibition/competition in ABZ/ABZSO transport mediated-absorption could account for the altered absorption pattern; however, the real contribution of such a mechanism is unknown. Interestingly, it has been previously reported that ABZSO plasma AUC was significantly higher after the i.r. administration of ABZ combined with IVM, compared to ABZ alone (Alvarez et al., 2008), which was attributed to a combined effect on metabolism and drug efflux transporter interactions between ABZ/ABZSO and IVM. However, Alvarez et al. (2008) administered IVM by the subcutaneous route. Consequently, the lower IVM concentrations achieved at the GI level after the subcutaneous route compared to the oral/i.r.

**Table 3**
Changes on the pharmacological exposure and parasitological effects on nematode infected lambs intraruminally treated with levamisole (LEV, 8 mg/kg), albendazole (ABZ, 5 mg/kg) or ivermectin (IVM, 0.2 mg/kg), each alone or as a triple combination (combined treatment).

| Experimental groups | Pharmacokinetic trial % change on exposure | | Efficacy trial % change on exposure | |
|---|---|---|---|---|
| | Pharmacokinetic parameter | *Combined/alone treatment* GMR[a] (90% CI) | *Treated/untreated* (95% CI)[b] | *Combined/alone treatment* (90% CI)[c] |
| LEV | $AUC_{0-LOQ}$ | 1.30↔ (0.54:3.14) | 0.44 (0.10:2.00) | 0.22* (0.05:0.80) |
| | $C_{max}$ | 1.22↔ (0.68:2.20) | | |
| ABZ | $AUC_{0-LOQ}$ | 0.61↓ (0.46:0.81) | 0.34 (0.07:1.52) | 0.27* (0.09:0.79) |
| | $C_{max}$ | 0.61↓ (0.40:0.92) | | |
| IVM | $AUC_{0-LOQ}$ | 1.71↑ (1.18:2.46) | 0.17* (0.03:0.78) | 0.53 (0.12:2.37) |
| | $C_{max}$ | 1.58↑ (1.22:2.04) | | |
| LEV + ABZ + IVM | – | – | 0.09* (0.02:0.43) | – |

*Symbols*: ↑, significantly increase; ↓, significantly decrease; ↔, not determined interaction (the 90% CI surrounding the GMR was within 0.80–1.25%).
[a] GMR = geometric mean ratio; CI = confidence interval.
[b] Negative binomial distribution means ratio and 95% confidence interval.
[c] Negative binomial distribution means ratio and 90% confidence interval. When the confidence interval includes a value of 1, the means between treatments are not statistically significant different. $C_{max}$: peak plasma concentration; $AUC_{0-LOQ}$: area under the concentration vs. time curve from 0 up to the limit of quantification.
* Statistical significant differences.

route (Lloberas et al., 2012), would determine a different "magnitude" on the drug–drug interaction. It seems clear that the extension of the PK interaction depends on the route of administration of the drugs under assessment. On the other hand, the contribution of LEV (current trial) on the observed PK changes should not be underestimated. In fact, LEV modified the PK behaviour of both ABZ and IVM in humans orally treated (Awadzi et al., 2004). Such PK-based drug–drug interaction was similar to that described in the current work in lambs, resulting in a lower (ABZSO) and higher (IVM) plasma drug exposure induced by the presence of LEV, while the plasma systemic exposure of LEV was slightly altered in the first few hours post treatment by the presence of either ABZ or IVM.

To clarify these apparently contradictory results it is necessary to introduce the impact that LEV may have in the triple anthelmintic combination. As it was suggested in a previous work (Awadzi et al., 2004) carried out in humans, LEV might have decreased ABZSO and increased IVM exposures in relation with their respective single administrations due to its interaction at the efflux transporter level. In the case of IVM a decreased in both intestinal and hepatic excretion, because of the inhibition of efflux transporter, could be envisaged. Contrarily, the inhibition of efflux carriers at the hepatobiliary membrane may allow ABZSO to stay longer in the hepatocyte and then to be metabolized faster for yielding $ABZSO_2$. It should be taken into consideration that the molar dose of LEV is much higher than that of IVM, and then its influence on drug transport interaction may become predominant. So, both IVM and ABZSO exposure changes may have been induced by LEV.

Assuming this hypothesis is true, let us analyze the findings reported by Alvarez et al. (2008). Considering that systemic IVM molar concentration could have not been enough for inhibiting ABZSO efflux transportation towards the bile, IVM interaction at the enterocyte should be now taken into account. As it is known, IVM has a very long half-life, and its elimination is carried out exclusively by secretion, and mainly through the intestine. So, it is highly likely that local concentration at the enterocyte would be enough to antagonize ABZSO efflux protein binding, and then to avoid the substance recycling at the apical membrane of intestinal cell. The final result could be a lower intestinal clearance of ABZSO, which even lower in relation with the hepatic clearance, leads to a reduction of its total clearance. In summary, LEV could have overridden the effect produced by IVM on the ABZSO PK. The result is a lower exposure of ABZSO instead of its probable plasma level increase when IVM + ABZ administration is compared to ABZ given alone. Cytochrome P-450 enzymes and efflux transporters, expressed in the intestine and/or in the liver, play important roles in drug clearance and/or bioavailability (Fagiolino et al., 2011), and depending on which membrane the interaction are taking place different PK consequences will be achieved. Inhibiting hepatic apical efflux transporters causes drug to remain trapped within hepatocytes and if the compound is a substrate for metabolizing enzymes its metabolism will increase. If the drug is not subject to metabolic transformation but rather is eliminated by biliary excretion, its clearance hepatic may decrease due to decreased excretion into bile (Shugarts and Benet, 2009).

On the other hand, both ABZSO and IVM together could be able to antagonize LEV binding to efflux transporter during the absorption of the three substances, and hence to allow LEV enter the body in a faster rate. Once the permeation from the gut lumen to the systemic blood circulation finishes, body dilution may reduce the impact that ABZSO and IVM could have on LEV PK, and then LEV becomes the predominant interacting agent. For this reason the exposure changes were relevant for IVM and ABZSO, while they were just reduced to the first part of the LEV plasma concentration–time curve.

From the results reported here, it is evident that multiple resistance against LEV, ABZ and IVM was present, since for all the experimental groups the percentage of reduction in egg faecal counts was less than 95% (with 95% confidence levels <90%) (Table 2). The faecal cultures showed *Haemonchus* spp. and *Trichostrongylus* spp. as the main nematodes resistant to LEV, ABZ and IVM. However, only *Haemonchus* spp. was able to survive to the triple combination treatment, indicating that multiple drug resistance may occur. After LEV and ABZ treatments given separately, the faecal nematode egg counts resulted similar to those observed in the untreated control group. Only after the IVM alone or combined LEV + ABZ + IVM treatment, the total egg counts significantly differ from the control. Thus, in farms where multiple-resistant nematode populations are present, the use of drug combinations may be an alternative to improve the chemical control. In fact, the use of drug combinations for managing existing resistance has been proposed as a potential useful tool (Geary et al., 2012). However, the use of either a triple combined treatment or IVM alone resulted in a similar nematode control, which appears to indicate that at least in some cases and based on previous diagnosis of resistance, the use of drug combinations should be avoided.

## 5. Conclusions

The finding reported here indicates that after the simultaneous i.r. administration of LEV, ABZ and IVM to lambs, drug to drug interactions occur. While further work may be required to evaluate the impact of this pharmacological interaction, a reduced ABZSO and enhanced IVM systemic exposure was observed. The co-administration of LEV + ABZ + IVM did not result in a clinically significant enhanced anthelmintic effect compared to IVM alone, with the disadvantage of an increased resistance selection pressure over parasite populations. The findings described here clearly point out the need for further and deeper pharmacological-based research to identify the advantage/disadvantage of use of combined drug preparations for anthelmintic control in livestock.

## Conflict of interest statement

The authors declare that they have no competing interests.

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## References

Anderson, N., Martin, P.J., Jarret, R.G., 1988. Mixtures of anthelmintics: a strategy against resistance. Aust. Vet. J. 65, 62–64.

Alvarez, L., Sanchez, S., Lanusse, C., 1999. In vivo and *ex vivo* uptake of albendazole and its sulphoxide metabolite by cestode parasites: relationship with their kinetic behaviour in sheep. J. Vet. Pharmacol. Ther. 22, 77–86.

Alvarez, L., Lifschitz, A., Entrocasso, C., Manazza, J., Mottier, L., Borda, B., Virkel, G., Lanusse, C., 2008. Evaluation of the interaction between ivermectin and albendazole following their combined use in lambs. J. Vet. Pharmacol. Ther. 31, 230–239.

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

Awadzi, K., Edwards, G., Opoku, N., Ardrey, A., Favager, S., Addy, E., Attah, S., Yamuah, L., Quartey, B., 2004. The safety, tolerability and pharmacokinetics of levamisole alone, levamisole plus ivermectin, and levamisole plus albendazole, and their efficacy against *Onchocerca volvulus*. Ann. Trop. Med. Parasitol. 98, 595–614.

Barnes, E., Dobson, R., Barger, I., 1995. Worm control and anthelmintic resistance: adventures with a model. Parasitol. Today 11, 56–63.

Bonino, J., Bonino, L., Pereira Neto, O., 2010. Efficacy of monepantel (Zolvix®) on internal parasite control on sheep production systems from Uruguay. Congreso Mundial de Buiatria, Chile.

Brown, L.D., Cai, T.T., Das Gupta, A., 2001. Interval estimation for a binomial proportion. Stat. Sci., 101–117.

Castells, D., 2002. Nuevo enfoque en el control parasitario de ovinos. FAO Animal Production and Health Paper.

Chiu, S.H.L., Green, M.L., Baylis, F.P., Eline, D., Rosegay, A., Meriwether, H., Jacob, T.A., 1990. Absorption, tissue distribution, and excretion of tritium-labeled ivermectin in cattle, sheep, and rat. J. Agric. Food Chem. 38, 2072–2078.

De Baere, S., Cherlet, M., Croubels, S., Baert, K., De Backer, P., 2003. Liquid chromatographic determination of levamisole in animal plasma: ultraviolet versus tandem mass spectrometric detection. Anal. Chim. Acta 483, 215–224.

Didier, A., Loor, F., 1996. The abamectin derivative ivermectin is a potent P-glycoprotein inhibitor. Anticancer Drugs 7, 745–751.

Dobson, R., Hosking, B., Jacobson, C., Cotter, J., Besier, R., Stein, P., Reid, S., 2012. Preserving new anthelmintics: a simple method for estimating faecal egg count reduction test (FECRT) confidence limits when efficacy and/or nematode aggregation is high. Vet. Parasitol. 186, 79–92.

Dupuy, J., Alvinerie, M., Ménez, C., Lespine, A., 2010. Interaction of anthelmintic drugs with P-glycoprotein in recombinant LLC-PK1-mdr1a cells. Chem. Biol. Interact. 186, 280–286.

Fagiolino, P., Vázquez, M., Eiraldi, R., Maldonado, C., Scaramelli, A., 2011. Influence of efflux transporters on drug metabolism: theoretical approach for bioavailability and clearance prediction. Clin. Pharmacokinet. 50, 75–80.

FDA/CVM guidance document #35 of 1996, and released in November, 2006 http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052363.pdf

FDA draft guidance for Industry, Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling, 2012 http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf

Feng, X.P., Hayashi, J., Beech, R.N., Prichard, R., 2002. Study of the nematode putative GABA type-A receptor subunits: evidence for modulation by ivermectin. J. Neurochem. 83, 870–878.

Geary, T., Sims, S., Rhomas, E., Vanover, L., Davis, J., Winterrowd, C., Klein, R., Ho, N., Thompson, D., 1993. *Haemonchus contortus*: ivermectin-induced paralysis of the pharynx. Exp. Parasitol. 77, 88–96.

Geary, T.G., Hosking, B.C., Skuce, P.J., von Samson-Himmelstjerna, G., Maeder, S., Holdsworth, P., Pomroy, W., Vercruysse, J., 2012. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) Guideline: anthelmintic combination products targeting nematode infections of ruminants and horses. Vet. Parasitol. 190, 306–316.

Gerlach, J.H., Endicott, J.A., Juranka, P.F., Henderson, G., Sarangi, F., Deuchars, K.L., Ling, V., 1986. Homology between P-glycoprotein and a bacterial haemolysin transport protein suggests a model for multidrug resistance. Nature 324, 485–489.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics, second ed. Marcel Dekker Inc., New York, pp. 45–109.

Hennessy, D., Steel, J., Lacey, E., Eagleson, G., Prichard, R., 1989. The disposition of albendazole in sheep. J. Vet. Pharmacol. Ther. 12, 421–429.

Jabbar, A., Iqbal, Z., Kerboeuf, D., Muhammad, G., Khan, M.N., Afaq, M., 2006. Anthelmintic resistance: the state of play revisited. Life Sci. 79, 2413–2431.

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

Lacey, E., 1990. Mode of action of benzimidazoles. Parasitol. Today 6, 112–115.

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

Leathwick, D., Hosking, B., Bisset, S., McKay, C., 2009. Managing anthelmintic resistance: is it feasible in New Zealand to delay the emergence of resistance to a new anthelmintic class? N. Z. Vet. J. 57, 181–192.

Lespine, A., Dupuy, J., Orlowski, S., Nagy, T., Glavinas, H., Krajcsi, P., Alvinerie, M., 2006. Interaction of ivermectin with multidrug resistance proteins (MRP1, 2 and 3). Chem. Biol. Interact. 159, 169–179.

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

Lifschitz, A., Virkel, G., Ballent, M., Sallovitz, J., Lanusse, C., 2009. Combined use of ivermectin and triclabendazole in sheep: *in vitro* and *in vivo* characterisation of their pharmacological interaction. Vet. J. 182, 261–268.

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

Maff, H., 1986. Manual of Veterinary Parasitological Laboratory Techniques. Her Majesty's Stationery Office, London.

Merino, G., Alvarez, A.I., Prieto, J.G., Kim, R.B., 2002. The anthelminthic agent albendazole does not interact with p-glycoprotein. Drug Metab. Dispos. 30, 365–369.

Merino, G., Molina, A., García, J., Pulido, M., Prieto, J., Alvarez, A., 2003. Intestinal elimination of albendazole sulfoxide: pharmacokinetic effects of inhibitors. Int. J. Pharm. 263, 123–132.

Merino, G., Jonker, J.W., Wagenaar, E., Pulido, M.M., Molina, A.J., Alvarez, A.I., Schinkel, A.H., 2005. Transport of anthelmintic benzimidazole drugs by breast cancer resistance protein (BCRP/ABCG2). Drug Metab. Dispos. 33, 614–618.

Nari, A., Salles, J., Gil, A., Waller, P.J., Hansen, J.W., 1996. The prevalence of anthelmintic resistance in nematode parasites of sheep in Southern Latin America: Uruguay. Vet. Parasitol. 62, 213–222.

Pouliot, J.F., L'Heureux, F., Liu, Z., Prichard, R., Georges, E., 1997. Reversal of P-glycoprotein-associated multidrug resistance by ivermectin. Biochem. Pharmacol. 53, 17–25.

Redondo, P., Alvarez, A., Garcia, J., Larrode, O., Merino, G., Prieto, J., 1999. Presystemic metabolism of albendazole: experimental evidence of an efflux process of albendazole sulfoxide to intestinal lumen. Drug Metab. Dispos. 27, 736–740.

Roberts, F., O'sullivan, P., 1950. Methods for egg counts and larval cultures for strongyles infesting the gastro-intestinal tract of cattle. Crop Pasture Sci. 1, 99–102.

Robertson, S., Martin, R., 1993. Levamisole-activated single-channel currents from muscle of the nematode parasite *Ascaris suum*. Br. J. Pharmacol. 108, 170–178.

Shugarts, S., Benet, L.Z., 2009. The role of transporters in pharmacokinetics of orally administered drugs. Pharm. Res. 26, 2039–2054.

Suarez, G., Alvarez, L., Castells, D., Correa, O., Fagiolino, P., Lanusse, C., 2011. Comparative drug systemic exposure and clinical efficacy against resistant nematodes in lambs treated with different albendazole formulations. J. Vet. Pharmacol. Ther. 34, 557–564.

Suarez, G., Alvarez, L., Castells, D., Correa, O., Fagiolino, P., Lanusse, C., 2013. Relative bioavailability and comparative clinical efficacy of different ivermectin oral formulations in lambs. BMC Vet. Res. 9, 27.

Team, R.D.C., 2008. R: A language and environment for statistical computing. R Foundation Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0 http://www.R-project.org

Van Wyk, J.A., Bath, G.F., 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet. Res. 33, 509–529.

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

Yates, D.M., Portillo, V., Wolstenholme, A.J., 2003. The avermectin receptors of *Haemonchus contortus* and *Caenorhabditis elegans*. Int. J. Parasitol. 33, 1183–1193.