Received: 12 June 2017 | Accepted: 6 October 2017

DOI: 10.1111/jvp.12466

ORIGINAL ARTICLE

WILEY Journal of Veterinary Pharmacology and Therapeutics

Pharmacokinetic assessment of the monepantel plus oxfendazole combined administration in dairy cows

M. Ballent[1] | P. Viviani[1] | F. Imperiale[1] | P. Dominguez[1] | S. Halwachs[2] | H. Mahnke[2] | W. Honscha[2] | C. Lanusse[1] | G. Virkel[1] | A. Lifschitz[1]

[1]Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil (CIVETAN), CONICET-CICPBA-UNCPBA, Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Argentina

[2]Institute of Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, Universität Leipzig, Leipzig, Germany

Correspondence
Mariana Ballent and Adrián Lifschitz
Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil (CIVETAN), CONICET-CICPBA-UNCPBA, Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Argentina.
Emails: mballent@vet.unicen.edu.ar and adrianl@vet.unicen.edu.ar

Funding information
CONICET-CICPBA; Agencia Nacional de Promocion Cientifica y Tecnica (ANPCyT)

Monepantel (MNP) is a novel anthelmintic compound launched into the veterinary pharmaceutical market. MNP is not licenced for use in dairy animals due to the prolonged elimination of its metabolite monepantel sulphone ($MNPSO_2$) into milk. The goal of this study was to evaluate the presence of potential *in vivo* drug-drug interactions affecting the pattern of milk excretion after the coadministration of the anthelmintics MNP and oxfendazole (OFZ) to lactating dairy cows. The concentrations of both parent drugs and their metabolites were measured in plasma and milk samples by HPLC. $MNPSO_2$ was the main metabolite recovered from plasma and milk after oral administration of MNP. A high distribution of $MNPSO_2$ into milk was observed. The milk-to-plasma ratio (M/P ratio) for this metabolite was equal to 6.75. Conversely, the M/P ratio of OFZ was 1.26. Plasma concentration profiles of MNP and $MNPSO_2$ were not modified in the presence of OFZ. The pattern of $MNPSO_2$ excretion into milk was also unchanged in animals receiving MNP plus OFZ. The percentage of the total administered dose recovered from milk was 0.09 ± 0.04% (MNP) and 2.79 ± 1.54% ($MNPSO_2$) after the administration of MNP alone and 0.06 ± 0.04% (MNP) and 2.34 ± 1.38% ($MNPSO_2$) after the combined treatment. The presence of MNP did not alter the plasma and milk disposition kinetics of OFZ. The concentrations of the metabolite fenbendazole sulphone tended to be slightly higher in the coadministered group. Although from a pharmacodynamic point of view the coadministration of MNP and OFZ may be a useful tool, the presence of OFZ did not modify the *in vivo* pharmacokinetic behaviour of MNP and therefore did not result in reduced milk concentrations of $MNPSO_2$.

1 | INTRODUCTION

Parasitic diseases caused by gastrointestinal (GI) nematodes represent a major concern in ruminant species. The intensive use of the available drugs has led to the widespread development of anthelmintic resistance. In recent years, anthelmintic resistance in nematodes of cattle has been reported in several countries (Kaplan & Vidyashankar, 2012). The use of novel compounds and the combination of different existing anthelmintic groups seem to be the most reliable pharmacological strategies, proposed to delay the development of nematode resistance (Leathwick, 2012). Monepantel (MNP) is a novel compound launched into the veterinary pharmaceutical market in 2009 and labelled for oral administration in sheep (Hosking et al., 2010; Kaminsky et al., 2008). Its novel mechanism of action supports the high efficacy of MNP against nematodes exhibiting resistance to other anthelmintic classes (Baker et al., 2012). Unfortunately, MNP resistance was reported in sheep and goats a few years following its launch (Mederos, Ramos, & Banchero, 2014; Scott et al., 2013; Van den Brom, Moll, Kappert, & Vellema, 2015). In an attempt to preserve the efficacy of this new molecule, MNP has recently been combined with abamectin for its use in sheep. However, the coadministration of two or more drugs can lead to pharmacokinetic and/or pharmacodynamic interactions between

drug components, and the combination may consequently behave as a third drug (Alvarez et al., 2008; Chou, 2010). The introduction into the veterinary pharmaceutical market of a MNP preparation for use in cattle is desirable in the short term, as it exhibits a very high efficacy against the main GI nematodes of cattle (King et al., 2015). In lactating animals, it is assumed that the administration of MNP should be limited due to the prolonged elimination of its metabolite monepantel sulphone ($MNPSO_2$) into milk (EMA, 2013). The ATP-binding cassette (ABC) transporters are major determinants of the cellular efflux of many drugs, protecting against the toxicity of xenobiotics and influencing pharmacokinetics (Schinkel & Jonker, 2003). The breast cancer resistance protein (BCRP/ABCG2) is ubiquitously expressed at the apical membrane of important blood-tissue barriers such as the intestine and placenta (Krishnamurthy & Schuetz, 2006). BCRP is also highly expressed in the mammary gland tissues, particularly during lactation, thereby playing a central role in the active secretion of various xenobiotics into milk (Jonker et al., 2005; Lindner, Halwachs, Wassermann, & Honscha, 2013). Drug-drug interactions leading to inhibition of mammary BCRP could influence drug secretion into milk and consequently the accumulation of drug residues in milk and milk-derived products. For instance, the coadministration of triclabendazole and moxidectin to sheep results in significantly reduced levels of the BCRP substrate moxidectin in milk (Barrera et al., 2013). In this context, the main goal of this study was to evaluate the potential *in vivo* drug-drug interactions after the coadministration of MNP and oxfendazole (OFZ), which could affect the milk excretion patterns of those anthelmintics in dairy cows. The plasma and milk kinetic profiles were evaluated for both drugs and their metabolites after the administration of each compound either alone or following combined treatment.

2 | MATERIALS AND METHODS

2.1 | Experimental design, treatment and sampling procedures

The experiment was conducted in a commercial dairy farm located in Tandil, Argentina. 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). Clinically healthy lactating Jersey cows (n = 18) weighing ~600 kg were used. The animals were in the midlate lactation period. They were allocated into three (3) treatment groups of six animals each. Animals were milked twice a day using mechanical vacuum milking machines, and milk production was measured throughout the trial. The average milk production was 10 L/animal/day.

The dosages were calculated based on individual body weights, and treatments were performed as follows: MNP Group: received a single dose of monepantel (Zolvix®; Novartis Animal Health Inc., Eli Lilly and Company/Elanco, Greenfield, IN) at 2.5 mg/kg, orally; OFZ Group: received a single dose of oxfendazole (Vermox 5CO®; Over, Buenos Aires, Argentina) at 10 mg/kg, orally; and MNP + OFZ Group received monepantel and oxfendazole following the above-mentioned doses and route. Blood samples were taken from the jugular vein in heparinized blood collection prior to and at 0, 3, 8, 24, 33, 57, 81, 129, 157, 177 hr post-treatment. Plasma was separated by centrifugation at 2000 *g* for 20 min, and the recovered plasma was transferred into vials. Milk samples were collected following the usual milking scheme at 8, 20, 33, 44, 57, 81, 105, 129, 157, 177 hr post-treatment. At each sampling time, a composite milk sample (50 ml, 12 ml from each udder approximately) was collected by hand milking after discarding 30–50 ml and before the complete mechanical milking of each cow. Plasma and milk samples were stored at −20°C until HPLC analysis.

2.2 | Analytical procedures

2.2.1 | Reagents

Pure reference standards of monepantel and monepantel sulphone were gently donated by Novartis Animal Health (Basel, Switzerland). Stock solutions (1 mg/ml) were prepared in acetonitrile. Standards of oxfendazole, fenbendazole, fenbendazole sulphone and oxibendazole were purchased from Sigma-Aldrich (Carlsbad, CA, USA). Stock solutions (1.5 mg/ml) were prepared in methanol at pH 3. Acetonitrile and methanol solvents used during the extraction and drug analysis were HPLC grade and purchased from J.T. Baker® (Center Valley, PA, USA). Water was double distilled and deionized using a water purification system (Simplicity®, Millipore, Sao Paulo, Brazil).

2.2.2 | Drug extraction and chromatographic analysis of monepantel and metabolites

The chemical extraction of MNP from plasma (0.5 ml) and milk (1.0 ml) spiked and experimental samples was performed following the technique described by Karadzovska et al. (2009) and adapted by Lifschitz et al. (2014). The parent drug and the sulphone metabolite were extracted from plasma and milk by the addition of 0.5 ml water and 2.0 ml acetonitrile followed by shaking in a high-speed vortexing shaker (Multi-tube Vortexer, VWR Scientific Products, West Chester, PA, USA) for 15 min. After centrifugation (BR 4i Centrifuge, Jouan®, Saint Herblain, France) at 2000 *g* for 15 min at 10°C to allow phase separation to take place, the clear supernatant was transferred to a 10 ml plastic tube and mixed with 6 ml of pure water. The mixture was vortexed for 5 s and transferred to a polymeric sorbent solid-phase extraction cartridge (Strata-X 33 Im Polymeric sorbent 60 mg, Phenomenex Torrance, CA, USA) previously conditioned with 1.0 ml acetonitrile and 1.0 ml water. All samples were added, and the cartridges were then washed with 2.0 ml of acetonitrile:water (30:70, v/v), dried with air for 1 min and eluted with 1.5 ml of acetonitrile. The eluted volume was evaporated (40°C) to dryness in a vacuum concentrator (Speed-Vac®, Savant, Los Angeles, CA, USA) and then reconstituted with 250 µl of mobile phase. Experimental and fortified plasma and milk samples were analysed for MNP and OFZ and their metabolites by HPLC. Fifty microlitres of each sample were injected in a Shimadzu Chromatography system (Shimadzu Corporation,

Kyoto, Japan), with two LC-10AS solvent pumps, an automatic sample injector (SIL-10A), an ultraviolet-visible spectrophotometric detector (UV) (SPD-10A), a column oven (Eppendorf TC-45, Eppendorf, Madison, WI, USA) set at 30°C, and a CBM-10A data integrator. Data and chromatograms were collected and analysed using the Class LC10 software (SPD-10A, Shimadzu Corporation, Kyoto, Japan). A C_{18} reversed-phase column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 μm, 4.6 × 250 mm) was used for separation. Elution of MNP and $MNPSO_2$ from the stationary phase was carried out at a flow rate of 0.8 ml/min (MNP) using acetonitrile:methanol:water (60:8:32, v/v/v). The drug or metabolite was detected at 230 nm. Under the described chromatographic conditions, the retention times (min) were established at 8.7 ($MNPSO_2$) and 10.8 (MNP).

2.2.3 | Drug extraction and chromatographic analysis of oxfendazole and metabolites

The chemical extraction of OFZ from plasma and milk of experimental samples was performed following the analytical techniques described by Virkel, Lifschitz, Pis, and Lanusse (2002) (plasma) and Moreno, Imperiale, Mottier, Alvarez, and Lanusse (2005) (milk), with minor modifications. Briefly, aliquots of plasma (0.5 ml) and milk (1 ml) were placed into a 5 ml plastic tube and spiked with the internal standard oxibendazole (1.0 μg/ml, final concentration). Drug molecules were extracted from both biological matrixes by the addition of 0.5 ml water and 2.0 ml acetonitrile and vortexing for 15 min. After centrifugation at 2000 *g* for 15 min at 10°C, the supernatant was transferred to a 10 ml plastic tube and mixed with 6 ml of pure water. The mixture was vortexed for 5 s and transferred to C_{18} cartridges (Strata®, Phenomenex, CA, USA) connected to a vacuum manifold. The cartridges had been previously conditioned with 0.5 ml of methanol, followed by 0.5 ml of water. All samples were added, and the cartridges were then washed with 0.5 ml of water, dried with air for 1 min and eluted with 2 ml of methanol. The eluted volume was evaporated (40°C) to dryness in a vacuum concentrator and then reconstituted with 250 μl of mobile phase. Identification of OFZ and its metabolites was performed by comparison with retention times of pure reference standards. A C_{18} reversed-phase column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 μm, 4.6 × 250 mm) was used for separation. The elution of the analytes from the stationary phase was carried out at a flow rate of 1.2 ml/min using acetonitrile and ammonium acetate buffer (0.025 M, pH 6.6) as the mobile phase. The gradient was changed linearly from 39:61 (acetonitrile:ammonium acetate buffer) to 50:50 in 7 min and then maintained for 8 min and modified to 39:61 in 1 min, where it was maintained for over 2 min. The detection of drug/metabolites was carried out at a wavelength of 292 nm. The retention times (min), under the described chromatographic conditions, were established at 3.5 (OFZ); 5.7 ($FBZSO_2$); 7.1 (OBZ) and 12.7 (FBZ).

2.2.4 | Validation procedures

A complete validation of the analytical procedures for the extraction and quantification of MNP, OFZ and their derivatives in plasma and milk of dairy cattle was performed before the analysis of the experimental samples. The linearity of the method was tested after analytical calibration curves for each analyte in plasma and milk were obtained. For MNP and $MNPSO_2$, blank samples were fortified with each compound in a range of 0.004 to 0.5 μg/ml (plasma) and 0.025 to 4 μg/ml (milk). For OFZ and its derivatives fenbendazole (FBZ) and fenbendazole sulphone ($FBZSO_2$), calibration curves were built in a range between 0.02 to 0.63 μg/ml (plasma) and 0.05 to 0.31 μg/ml (milk). Calibration curves were constructed at the beginning of the validation process using eight (8) points (four replicates per point). The least squares linear regression analysis was used, and correlation coefficients were calculated. Linearity was established to determine the analytes concentration/detector response relationships. The precision of the extraction and chromatography procedures (intraday variability) was estimated by calculating the coefficients of variation (CV) of plasma and milk samples containing known analyte concentrations. The accuracy of the method was defined as the closeness of the measured value to the true value. The limits of drug detection and quantification were established. Concentration values below the quantification limit were not considered for the kinetic analysis. The linear regression lines for all analytes showed correlation coefficients ≥0.99. Intraday variability in plasma and milk showed CV <7% (MNP); <6% ($MNPSO_2$); <6% (OFZ); <4% ($FBZSO_2$) and <7% (FBZ). The accuracy of the method in plasma and milk showed a relative error <13% (MNP); <11% ($MNPSO_2$); <5% (OFZ and $FBZSO_2$) and <8% (FBZ). The limits of quantification (LOQ) for both MNP and $MNPSO_2$ were 0.004 μg/ml (plasma) and 0.01 μg/ml (milk). For benzimidazoles, the LOQ was established at 0.01 μg/ml (OFZ); 0.025 μg/ml (FBZ and $FBZSO_2$) in both matrices. The limits of detection (LOD) were 0.002 μg/ml (plasma) and 0.005 μg/ml (milk) for MNP and $MNPSO_2$. The LOD for OFZ, FBZ and $FBZSO_2$ was 0.005 μg/ml in both biological fluids. Validation and analytical procedures were performed within the period where the stability of analytical standards was demonstrated (Karadzovska et al., 2009; Moreno et al., 2012). No interferences among analytes were observed under current chromatographic conditions.

2.2.5 | Pharmacokinetic analysis

The plasma and milk concentration *vs.* time curves obtained after treatment of each individual animal were fitted with the PK Solutions 2.0 (Ashland, OH, USA) computer software. Pharmacokinetic parameters were determined by the noncompartmental analysis. The peak plasma concentration (C_{max}) was obtained from the plotted plasma concentration–time curve of each individual animal. The area under the concentration-versus-time curves (AUC) was calculated by the trapezoidal rule (Gibaldi & Perrier, 1982) and further extrapolated to infinity. The terminal (elimination) half-life ($t_{1/2el}$) was calculated as $\ln 2/\lambda z$, where ln2 is the natural logarithm of 2 and λz, the slope of the terminal phase. The λ_z was determined performing regression analysis using at least four points of the terminal phase of the concentration–time plot. The percentage of elimination of drug into milk was calculated using the following equation:

$$\%\ \text{dose excreted into milk} = (V_{milk} \times c_{milk}/D) \times 100$$

where V_{milk} is the individual milk production (ml); c_{milk} is the drug concentration in milk (μg/ml), and D is the total administered dose (mg).

The withdrawal time for $MNPSO_2$ in milk was calculated using the following formula:

Withdrawal time: 1.44 ln (C^0/tolerance) × ($t_{1/2\,el}$) (Riviere & Sundlof, 2009), where C^0 is the initial concentration in the body, tolerance is the maximum residue limits in milk, and $t_{1/2\,el}$ is the terminal half-life.

2.2.6 | Statistical analysis

Plasma and milk concentrations of the analysed drugs/metabolites and all the estimated pharmacokinetic parameters are reported as mean ± SD. Mean pharmacokinetic parameters for the analysed compounds were statistically compared using Student's *t* test. The assumption that the data obtained after treatments have the same variance was assessed. A nonparametric Mann–Whitney test was used where significant differences among standard deviations were observed. A similar procedure was used to compare drug concentrations measured in plasma and milk. The statistical analysis was performed using the Instat 3.0 software (GraphPad Software, CA, USA). A value of $p < .05$ was considered statistically significant.

3 | RESULTS

MNP and its sulphone metabolite were detected in plasma up to 33 and 177 hr postadministration of a single oral dose of MNP, respectively. Pharmacokinetic data obtained from MNP Group have been reported by Mahnke et al. (2016). MNP concentrations were only detected during a short period. The sulphone metabolite was the main analyte recovered in plasma. $MNPSO_2$ exhibited significantly higher systemic exposure compared to the parent drug ($MNPSO_2$/MNP AUC ratio = 23.1 ± 10.5) ($p < .05$). In milk samples, MNP was detected up to 33 hr (not shown) and $MNPSO_2$ derivative up to 177-hr postoral administration of MNP. Milk concentrations of $MNPSO_2$ were significantly higher compared to those measured in plasma. The calculated AUC of $MNPSO_2$ in milk was 6.75 ± 3.54-fold higher than that observed in plasma. The percentage of the total administered dose recovered from milk was 0.09 ± 0.04% (MNP) and 2.79 ± 1.54% ($MNPSO_2$).

Plasma concentration profiles of MNP and $MNPSO_2$ were not significantly modified in the presence of OFZ. MNP was detected up to 33-h post-treatment, and its mean plasma C_{max} was 0.092 μg/ml. Similar to the control group, the main metabolite $MNPSO_2$ was also detected up to 177-hr post-treatment, and its mean plasma C_{max} was 0.43 μg/ml. The pattern of MNP and $MNPSO_2$ milk excretion was also unchanged in animals receiving MNP plus OFZ. MNP was detected in milk only in the first two sample points (8 and 33 hr post-treatment). The mean concentrations of MNP in milk ranged between 0.29 μg/ml and 0.05 μg/ml. The percentage of the total dose excreted in milk was 0.06 ± 0.04% (MNP) and 2.34 ± 1.38% ($MNPSO_2$) after the combined treatment of MNP and OFZ. The concentration profiles of $MNPSO_2$ obtained after MNP administration either alone or combined

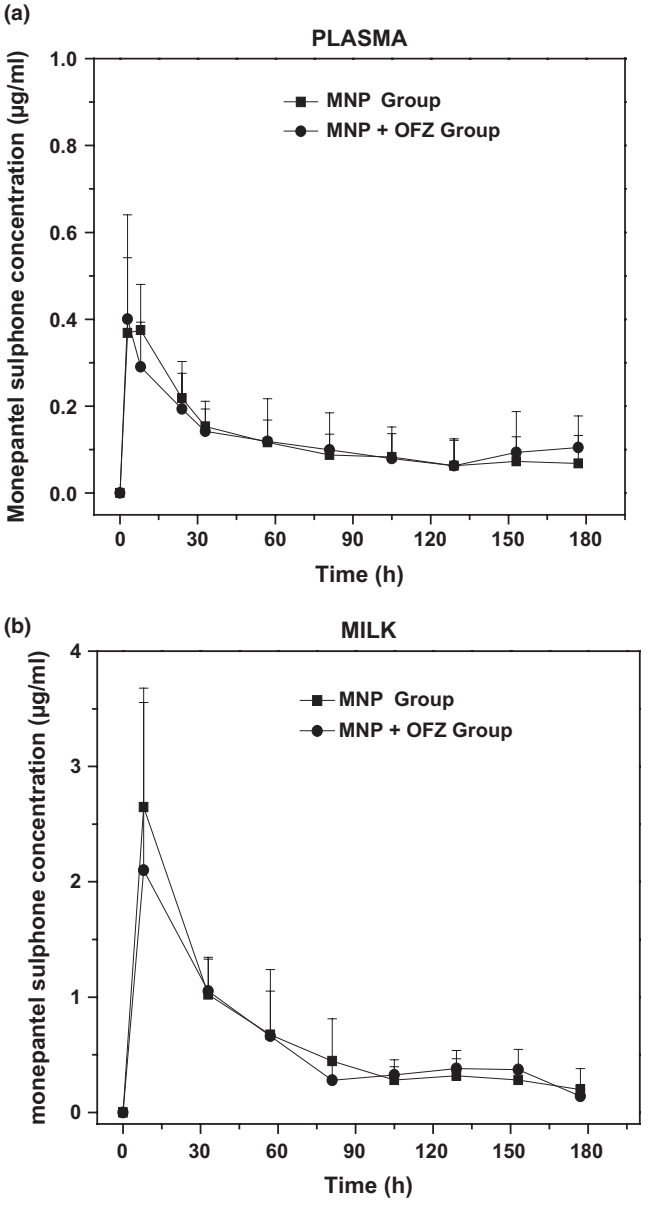

FIGURE 1 Comparative (mean ± SD, $n = 6$ per group) monepantel sulphone ($MNPSO_2$) plasma (a) and milk (b) concentration profiles (μg/ml) obtained after the oral administration of the parent drug monepantel (MNP) at 2.5 mg/kg, either alone or coadministered with oxfendazole (OFZ) (10 mg/kg, oral) to dairy cows

treatment with OFZ are shown in Figure 1. The pharmacokinetic parameters calculated for $MNPSO_2$ in plasma and milk are summarized in Table 1.

Plasma and milk concentrations of OFZ were also assessed in the current trial. Plasma concentrations of OFZ were measured up to 57 hr following oral drug administration. The mean C_{max} of OFZ was 0.36 ± 0.18 μg/ml. The sulphone metabolite ($FBZSO_2$) was the most important metabolite detected after the OFZ oral treatment. The mean C_{max} value of $FBZSO_2$ was 0.14 ± 0.06 μg/ml, and the plasma AUC_{FBZSO2}/AUC_{OFZ} ratio was 0.53 ± 0.07. Trace amounts of fenbendazole (FBZ), the thioether compound produced by ruminal sulphoreduction of OFZ, were detected in plasma with a mean C_{max} of

TABLE 1 Mean ± SD (n = 6 per group) pharmacokinetic parameters of monepantel sulphone ($MNPSO_2$) obtained in plasma and milk after the oral administration of monepantel (MNP) at 2.5 mg/kg either alone (MNP Group) or with oxfendazole (OFZ) (10 mg/kg, oral) (MNP + OFZ Group) to dairy cows

	Plasma		Milk	
Pharmacokinetic parameters	MNP Group	MNP + OFZ Group	MNP Group	MNP + OFZ Group
C_{max}	0.40 ± 0.13	0.43 ± 0.21	2.65 ± 0.91	2.23 ± 1.46
T_{max}	5.50 ± 2.74	4.67 ± 2.58	8.00 ± 0.00	20.3 ± 20.6
AUC	21.2 ± 9.21	20.7 ± 13.9	123 ± 43.5	111 ± 36.8
$T_{1/2}$el	73.1 ± 51.5	88.4 ± 62.6	60.3 ± 15.2	71.7 ± 21.0

C_{max} (μg/ml): peak plasma concentration. T_{max} (hr): time to peak plasma concentration. AUC_{total} (μg-h/ml): area under the concentration versus time curve extrapolated to infinity. T½ el (hr): elimination half-life.

0.04 ± 0.03 μg/ml. In milk, OFZ concentrations were also measured up to 57 hr postadministration. There were no significant differences between OFZ concentrations detected in plasma or milk samples. For OFZ, the calculated AUC milk-to-plasma ratio was 1.26 ± 0.12. The amount of OFZ recovered from milk was 0.05 ± 0.03% of the total administered dose. The coadministration of OFZ with MNP did not alter the plasma and milk disposition kinetics of this compound. The C_{max} of OFZ in the coadministered group was 0.45 ± 0.12 μg/ml. The AUC milk-to-plasma ratio obtained after the coadministration with MNP was 1.14 ± 0.42 and the amount of OFZ recovered from milk was 0.05 ± 0.03% of the total administered dose, the same percentage found in OFZ Group. The concentrations of $FBZSO_2$ tended to be slightly higher in the coadministered group. The plasma AUC_{FBZSO2}/AUC_{OFZ} ratio in the coadministered group was 0.65 ± 0.14. The plasma and milk concentration profiles of OFZ and $FBZSO_2$ obtained after the OFZ administration either alone or combined with MNP are shown in Figures 2 and 3, respectively. Tables 2 and 3 show the main plasma and milk pharmacokinetic parameters of OFZ and $FBZSO_2$, respectively, obtained after the OFZ administration either alone or coadministered with MNP.

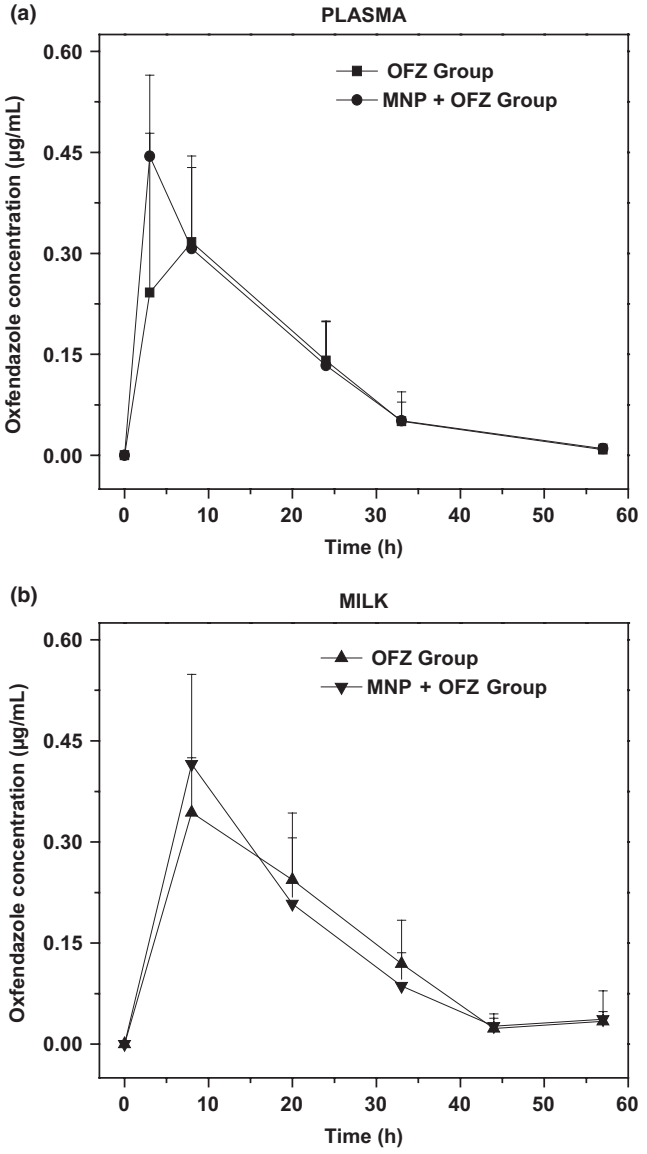

FIGURE 2 Comparative (mean ± SD, n = 6 per group) oxfendazole (OFZ) plasma (a) and milk (b) concentrations (μg/ml) obtained after its oral administration at 10 mg/kg, either alone or coadministered with monepantel (MNP) (2.5 mg/kg, oral) to dairy cows

4 | DISCUSSION

Drug combinations have been successfully used as a strategy to expand the efficacy spectrum of antiparasitic drugs. Additionally, in an attempt to manage anthelmintic resistance in ruminants, combinations of two or more compounds are primarily being used (Geary et al., 2012). However, potential pharmacokinetic and/or pharmacodynamic interactions between drugs may occur. The modified effect may result from a change in the concentration of either one or both drugs in the organism (pharmacokinetic interaction) or from a change in the relation between drug concentration and response of the organism to the drug (pharmacodynamic interaction) (Lanusse, Lifschitz, & Alvarez, 2015). In this context, the efflux activity of ATP-binding cassette (ABC) transporters represents an important molecular mechanism of clinically relevant drug-drug interactions (Marchetti, Mazzanti, Beijnen, & Schellens, 2007). The interaction of OFZ with BCRP was corroborated *in vitro* using MDCKII cells transduced with human BCRP (Merino et al., 2005). An interesting experimental approach was recently carried out by cloning the BCRP from lactating mammary gland tissues of dairy cows into MDCKII-bABCG2 cells

(Wassermann et al., 2013). The interaction of MNP and $MNPSO_2$ with ruminant BCRP was shown (Halwachs, Wassermann, & Honscha, 2014). Moreover, BCRP-mediated transport of the main active MNP metabolite $MNPSO_2$ was demonstrated by flux studies using polarized MDCKII-bABCG2 monolayers (Mahnke et al., 2016). As the concomitant drug administration may induce drug-drug interactions affecting both pharmacokinetics and drug accumulation into milk, the present study aimed to investigate the effect of combined treatment of the novel anthelmintic drug MNP with the benzimidazole anthelmintic OFZ on both plasma and milk concentrations in lactating dairy cows.

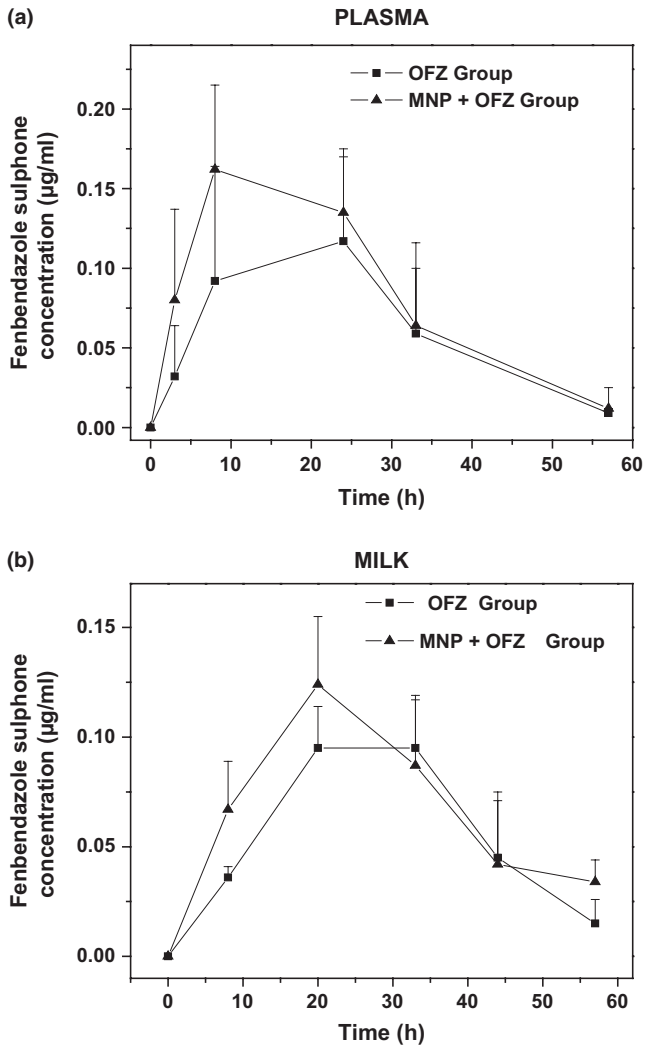

FIGURE 3 Comparative (mean ± SD, n = 6 per group) fenbendazole sulphone ($FBZSO_2$) plasma (a) and milk (b) concentrations (µg/ml) obtained after the oral administration of oxfendazole (OFZ) at 10 mg/kg, either alone or coadministered with monepantel (MNP) (2.5 mg/kg, oral) to dairy cows

In line with previous pharmacokinetic studies in sheep (Karadzovska et al., 2009; Lifschitz et al., 2014), bovine plasma concentration profiles of MNP showed a rapid decline in the parent drug. Moreover, $MNPSO_2$ represents the main metabolite detected in biological samples of dairy cattle similar to sheep (Lifschitz et al., 2014). Interspecies differences in $MNPSO_2$ disposition in plasma were observed between cattle and sheep. The C_{max} and AUC values of $MNPSO_2$ were 6.5- and 4-fold higher compared to those reported in sheep, respectively (Lifschitz et al., 2014). Similarly, a higher systemic exposure of other sulphone metabolites of anthelmintics including albendazole and fenbendazole has been observed in cattle compared to sheep (Lanusse & Prichard, 1993). The higher *in vivo* production of these sulphone metabolites could be related to the higher expression levels (Girolami et al., 2016) and metabolic activities (unpublished observations) of cytochrome P-450 (CYP) 1A isoenzymes in cattle compared to sheep liver. Moreover, a different pattern of liver metabolism of MNP in sheep and cattle may explain the observed pharmacokinetic differences between both species, whereas in sheep, the formation of the sulphone metabolite is based on the enzymatic activity of both flavin-monooxygenase (FMO) and CYP, in cattle MNP is converted into $MNPSO_2$ only in a CYP-mediated metabolism reaction (Ballent et al., 2016).

In the present work, the plasma concentration profiles of OFZ were in accordance with those reported in previous studies in dairy cattle (Moreno et al., 2005). Similarly, concentrations of the metabolite $FBZSO_2$ and the parent drug FBZ were also quantified. The presence of FBZ in the bloodstream indicates a presystemic sulphoreduction of OFZ after its oral administration. In fact, it has been shown that OFZ is reduced back to its parent thioether (FBZ) by the ruminal and intestinal microflora and may be as a source of FBZ in the digestive tract (Virkel et al., 2002). In the liver, FBZ is re-oxidized to OFZ, a process which was shown to be mediated mainly by the FMO enzymatic system in cattle (Virkel, Lifschitz, Sallovitz,

TABLE 2 Mean ± SD (n = 6 per group) pharmacokinetic parameters of oxfendazole (OFZ) obtained in plasma and milk after its oral administration at 10 mg/kg either alone (OFZ Group) or with monepantel (MNP) (2.5 mg/kg, oral) (MNP + OFZ Group) to dairy cows

	Plasma		Milk	
Pharmacokinetic parameters	OFZ Group	MNP + OFZ Group	OFZ Group	MNP + OFZ Group
C_{max}	0.36 ± 0.18	0.45 ± 0.12	0.34 ± 0.08	0.39 ± 0.14
T_{max}	7.17 ± 2.00	3.83 ± 2.00	8.00 ± 0.00	8.00 ± 0.00
AUC	7.11 ± 2.24	7.88 ± 2.30	8.99 ± 3.06	8.50 ± 2.64
$T_{1/2}$el	10.7 ± 4.24	9.12 ± 1.05	11.5 ± 2.21	10.2 ± 2.33

C_{max} (µg/ml): peak plasma concentration. T_{max} (hr): time to peak plasma concentration. AUC_{total} (µg-h/ml): area under the concentration vs. time curve extrapolated to infinity. T½ el (hr): elimination half-life.

TABLE 3 Mean ± SD (n = 6 per group) pharmacokinetic parameters of fenbendazole sulphone ($FBZSO_2$) obtained in plasma and milk after the oral administration of oxfendazole (OFZ) at 10 mg/kg either alone (OFZ Group) or with monepantel (MNP) (2.5 mg/kg, oral) (MNP + OFZ Group) to dairy cows

	Plasma		Milk	
Pharmacokinetic parameters	**OFZ Group**	**MNP + OFZ Group**	**OFZ Group**	**MNP + OFZ Group**
C_{max}	0.14 ± 0.06	0.17 ± 0.05	0.10 ± 0.02	0.13 ± 0.04
T_{max}	22.8 ± 8.11	22.8 ± 8.11	22.8 ± 8.11	20.2 ± 10.0
AUC	3.76 ± 1.33	4.83 ± 0.63	3.57 ± 1.12	4.45 ± 1.30
$T_{1/2}$el	9.30 ± 1.90	6.78 ± 2.84	8.59 ± 3.75	5.60 ± 2.29
AUC_{FBZSO2}/AUC_{OFZ}	0.53 ± 0.07	0.65 ± 0.14	0.40 ± 0.05	0.58 ± 0.13

C_{max} (μg/ml): peak plasma concentration. T_{max} (hr): time to peak plasma concentration. AUC_{total} (μg-h/ml): area under the concentration versus time curve extrapolated to infinity. T½ el (hr): elimination half-life.

Pis, & Lanusse, 2004) and also OFZ is converted to $FBZSO_2$ by the CYP450 system (Short, Flory, Hsieh, & Barker, 1988). The absence of *in vivo* pharmacokinetic interactions between MNP and OFZ may indicate, among other ADME-related mechanisms, that different enzymatic pathways are involved in the biotransformation of these anthelmintic drugs. However, the $FBZSO_2$ formation tended to be higher in cows that also received MNP (Figure 3). The AUC_{FBZSO2}/AUC_{OFZ} ratio was 1.23-fold (plasma) and 1.45-fold (milk) in the co-administered group compared to the administration of OFZ alone. Recently, both the CYP450-related specific activities and the mRNA expression of CYP3A24 were increased in the liver of sheep treated with a therapeutic dose of MNP (Stuchlíková et al., 2015). The effect of MNP on CYP induction evidenced in other animal species may explain the tendency of the increased FBZSO2 formation in the dairy cows that received OFZ plus MNP.

It is well recognized that the extent of xenobiotic partitioning between the bloodstream and milk is a complex process based on different factors. The physicochemical properties of the drug, dosing rate and route of administration, as well as the animal species and milk composition, are relevant factors that determine the percentage of the drug dose excreted into milk (Alvinerie, Sutra, Galtier, & Mage, 1999). Compared to plasma, $MNPSO_2$ displayed a high distribution and accumulation in milk (milk-to-plasma ratio 6.75). In contrast, milk-to-plasma concentrations ratio of OFZ was approximately 1 for the whole sampling period. The milk-to-plasma ratio may allow the prediction of potential active BCRP-mediated drug secretion and, in consequence, residual accumulation of BCRP substrates into milk (Alvarez et al., 2006). Therefore, the current data corroborate results recently obtained by *in vitro* BCRP flux studies (Mahnke et al., 2016) showing active BCRP-mediated secretion of $MNPSO_2$.

Although OFZ may be used in dairy cattle, MNP is not licensed to be used in lactating ruminants. The relevance of the presence of drug milk residues is depicted by the maximum residue limits (MRL) established by regulatory authorities. Maximum residue limits (MRL) for MNP have been established only for edible ovine and caprine tissues. Recently, the European Commission has established a MRL for $MNPSO_2$ (the marker residue of MNP) for ovine and caprine milk at 170 μg/kg. Using this MRL and based on the elimination half-life of $MNPSO_2$ in milk of dairy cows, the calculated withdrawal time for MNP was 168 hr. The use of the elimination half-life in milk is useful to determine the discard times when regulatory withdrawal times are not available (Riviere & Sundlof, 2009). Hence, the current results indicate a long persistence and relevant drug residue levels of $MNPSO_2$ in milk of dairy cows. Therefore, the use of MNP is less to be practical in the different dairy production systems, and any strategy reducing milk elimination of its active metabolite $MNPSO_2$ may be beneficial.

In this context, concomitant administration of multiple drugs could lead to modifications in the secretion pattern of BCRP substrates with potential effects on milk residues. Benzimidazole methylcarbamates, such as albendazole sulphoxide (ABZSO) and OFZ, have been reported to interact with BCRP *in vitro* (Merino et al., 2005). Moreover, OFZ showed an inhibitory effect on efflux activity in MDCKII-BCRP cell monolayers, with an apparent higher potency compared with their respective thioethers, albendazole and flubendazole (Mahnke et al., 2016). In fact, these authors also corroborated that OFZ reduces the *in vitro* $MNPSO_2$ net efflux mediated by BCRP in MDCKII-bABCG2 cells (Mahnke et al., 2016). Thus, for drugs exhibiting a high secretion rate into milk as it was observed for $MNPSO_2$, the administration of MNP parent drug in combination with other BCRP substrates such as OFZ may modify the drug excretion pattern into milk. Previous reports showed some success in achieving this purpose. The secretion rate of the antimicrobial danofloxacin into milk was reduced by the presence of the anthelmintic ivermectin (Real et al., 2011) or the natural isoflavones genistein and daidzein (Perez et al., 2013). The coadministration of the halogenated BZD triclabendazole with either moxidectin or danofloxacin to sheep reduced moxidectin but not danofloxacin levels in milk (Barrera et al., 2013).

Altogether, the current results showed the absence of drug-drug interactions between both BCRP substrates, $MNPSO_2$ and OFZ. Although the presence of OFZ modified the secretion of $MNPSO_2$ in the *in vitro* model (Mahnke et al., 2016), in the current work, the plasma concentrations of both anthelmintic drugs were unchanged as compared to the administration of each compound alone. The lack of *in vivo* drug-drug interactions may be due to the relative drug concentrations of substrates reached at the biophase, the involvement of other transporter proteins and factors related to drug metabolism.

However, it was also noticed a high variability in the kinetic parameters of $MNPSO_2$ and OFZ in the coadministered group compared to animals receiving a single administration of anthelmintics. This variability may reflect potential drug-drug interactions produced after the concomitant treatment with both drugs. The reduced number of animals used to perform the current PK trial may mask the potential differences between experimental groups.

In milk, the $MNPSO_2$ concentration profile was similar after the coadministration with OFZ without significant modification in the percentage of the total dose excreted into milk. The withdrawal time calculated for MNP in dairy cattle after its coadministration with OFZ was 184 hr, reflecting the fact that $MNPSO_2$ excretion in milk was not modified by the combined treatment. These results highlight the importance of performing *in vivo* studies in field conditions to confirm the clinical relevance of potential pharmacokinetic drug-drug interactions including BCRP-mediated drug cellular efflux. In the work reported here, concurrent concentrations of OFZ and $MNPSO_2$ in the plasma and mammary epithelial cells of dairy cattle seem to have been insufficient to modify the $MNPSO_2$ excretion into milk. Moreover, as summarized in Table 2, the coadministration with MNP did not alter the main pharmacokinetic parameters of OFZ compared to its administration alone. It was noticed that combination of both drugs.

In conclusion, potential drug-drug interactions at the mammary gland level should be considered when BCRP substrates are coadministered in dairy animals. The involvement of efflux proteins in the milk secretion of antiparasitic compounds may play a relevant role in their clinical use in lactating animals due to the presence of residues in milk and its derived products. $MNPSO_2$ was extensively excreted in milk following oral administration of MNP to dairy cattle. Although, from a pharmacodynamic point of view, the coadministration of MNP and OFZ may be a useful tool, the presence of OFZ did not modify the *in vivo* pharmacokinetic behaviour of MNP and therefore did not result in reduced milk concentrations of $MNPSO_2$.

ACKNOWLEDGMENTS

The research at the Laboratorio de Farmacologia, CIVETAN, Facultad de Cs. Veterinarias, UNCPBA is supported by CONICET-CICPBA and Agencia Nacional de Promocion Cientifica y Tecnica (ANPCyT) (PICT 1432), Argentina. We thank Novartis Animal Health for providing MNP and $MNPSO_2$ pure analytical standards.

ORCID

M. Ballent http://orcid.org/0000-0002-1811-6832

REFERENCES

Alvarez, L., Lifschitz, A., Entrocasso, C., Manazza, J., Mottier, L., Borda, B., … Lanusse, C. (2008). Evaluation of the interaction between ivermectin and albendazole following their combined use in lambs. *Journal of Veterinary Pharmacology and Therapeutics*, *31*, 230–239. https://doi.org/10.1111/j.1365-2885.2008.00953.x

Alvarez, A. I., Merino, G., Molina, A. J., Pulido, M. M., McKellar, Q. A., & Prieto, J.G. (2006). Role of ABC transporters in veterinary drug research and parasite resistance. *Current Drug Delivery*, *3*, 199–206. https://doi.org/10.2174/156720106776359195

Alvinerie, M., Sutra, J. F., Galtier, P., & Mage, C. (1999). Pharmacokinetics of eprinomectin in plasma and milk following topical administration to lactating dairy cattle. *Research in Veterinary Science*, *67*, 229–232. https://doi.org/10.1053/rvsc.1999.0312

Baker, K. E., George, S. D., Stein, P. A., Seewald, W., Rolfe, P. F., & Hosking, B. C. (2012). Efficacy of monepantel and anthelmintic combinations against multiple-resistant Haemonchus contortus in sheep, including characterisation of the nematode isolate. *Veterinary Parasitology*, *186*, 513–517. https://doi.org/10.1016/j.vetpar.2011.11.060

Ballent, M., Virkel, G., Maté, L., Viviani, P., Lanusse, C., & Lifschitz, A. (2016). Hepatic biotransformation pathways and ruminal metabolic stability of the novel anthelmintic monepantel in sheep and cattle. *Journal of Veterinary Pharmacology and Therapeutics*, *39*, 488–496. https://doi.org/10.1111/jvp.2016.39.issue-5

Barrera, B., González-Lobato, L., Otero, J. A., Real, R., Prieto, J. G., Álvarez, A. I., & Merino, G. (2013). Effects of triclabendazole on secretion of danofloxacin and moxidectin into the milk of sheep: Role of triclabendazole metabolites as inhibitors of the ruminant ABCG2 transporter. *The Veterinary Journal*, *198*, 429–436. https://doi.org/10.1016/j.tvjl.2013.07.033

Chou, T. C. (2010). Drug combination studies and their synergy quantification using the Chou-Talalay method. *Cancer Research*, *70*, 440–446. https://doi.org/10.1158/0008-5472.CAN-09-1947

EMA (2013). The European Medicines Agency. Committee for Medicinal Products for Veterinary Use, CVMP European public MRL assessment report (EPMAR) Monepantel (caprine and ovine milk). MA/CVMP/741250/2011.

Geary, T. G., Hosking, B. C., Skuce, P. J., von Samson-Himmelstjerna, G., Maeder, S., Holdsworth, P., … Vercruysse, J. (2012). World Association for the Advancement of Veterinary Parasitology (WAAVP) Guideline: Anthelmintic combination products targeting nematode infections of ruminants and horses. *Veterinary Parasitology*, *190*, 306–316. https://doi.org/10.1016/j.vetpar.2012.09.004

Gibaldi, M., & Perrier, D. (1982). *Pharmacokinetics*, 2nd edn. Revised and Expanded. New York, USA: Marcel Dekker Inc.

Girolami, F., Spalenza, V., Benedetto, A., Manzini, L., Badino, P., Abete, M. C., & Nebbia, C. (2016). Comparative liver accumulation of dioxin-like compounds in sheep and cattle: Possible role of AhR-mediated xenobiotic metabolizing enzymes. *Science of the Total Environment*, *571*, 1222–1229. https://doi.org/10.1016/j.scitotenv.2016.07.150

Halwachs, S., Wassermann, L., & Honscha, W. (2014). A novel MDCKII in vitro model for assessing ABCG2-drug interactions and regulation of ABCG2 transport activity in the caprine mammary gland by environmental pollutants and pesticides. *Toxicology In Vitro*, *28*, 432–441. https://doi.org/10.1016/j.tiv.2013.12.015

Hosking, B. C., Stein, P. A., Karadzovska, D., House, J. K., Seewald, W., & Giraudel, J. M. (2010). Effect of route of administration on the efficacy and pharmacokinetics of an experimental formulation of the amino-acetonitrile derivative monepantel in sheep. *Veterinary Record*, *166*, 490–494. https://doi.org/10.1136/vr.b4830

Jonker, W., Merino, G., Musters, S., van Herwaarden, E. A., Bolscher, E., Wagenaar, E., … Schinkel, A. H. (2005). The breast cancer resistance protein (BCRP/ABCG2) concentrates drugs and carcinogenic xenotoxins into milk. *Nature Medicine*, *11*, 127–129. https://doi.org/10.1038/nm1186

Kaminsky, R., Ducray, P., Jung, M., Clover, R., Rufener, L., Bouvier, J., … Mäser, P. (2008). A new class of anthelmintics effective against drug-resistant nematodes. *Nature*, *452*, 176–180. https://doi.org/10.1038/nature06722

Kaplan, R. M., & Vidyashankar, A. N. (2012). An inconvenient truth: Global Worming and anthelmintic resistance. *Veterinary Parasitology*, *186*, 70–78. https://doi.org/10.1016/j.vetpar.2011.11.048

Karadzovska, D., Seewald, W., Browning, A., Smal, M., Bouvier, J., & Giraudel, J. M. (2009). Pharmacokinetics of monepantel and its sulfone metabolite, monepantel sulfone, after intravenous and oral administration in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, *32*, 359–367. https://doi.org/10.1111/jvp.2009.32.issue-4

King, J., George, S., García, R., Baker, K., Stein, P., Forster, S., & Hosking, B.C. (2015). Zolvix® Plus Cattle – Efficacy against gastro-intestinal nematodes infecting cattle. Proceedings of 25th International Conference of the World Association for the Advancement of Veterinary Parasitology, pp 263.

Krishnamurthy, P., & Schuetz, J. D. (2006). Role of ABCG2/BCRP in biology and medicine. *Annual Review of Pharmacology and Toxicology*, *46*, 381–410. https://doi.org/10.1146/annurev.pharmtox.46.120604.141238

Lanusse, C., Lifschitz, A., & Alvarez, L. (2015). Basic and clinical pharmacology contribution to extend anthelmintic molecules lifespan. *Veterinary Parasitology*, *212*, 35–46. https://doi.org/10.1016/j.vetpar.2015.07.015

Lanusse, C., & Prichard, R. (1993). Clinical pharmacokinetics and metabolism of benzimidazole anthelmintics in ruminants. *Drug Metabolism Review*, *25*, 235–279. https://doi.org/10.3109/03602539308993977

Leathwick, D. M. (2012). Modelling the benefits of a new class of anthelmintic in combination. *Veterinary Parasitology*, *186*, 93–100. https://doi.org/10.1016/j.vetpar.2011.11.050

Lifschitz, A., Ballent, M., Virkel, G., Sallovitz, J., Viviani, P., & Lanusse, C. (2014). Accumulation of monepantel and its sulphone derivative in tissues of nematode location in sheep: Pharmacokinetic support to its excellent nematodicidal activity. *Veterinary Parasitology*, *203*, 120–126. https://doi.org/10.1016/j.vetpar.2014.02.049

Lindner, S., Halwachs, S., Wassermann, L., & Honscha, W. (2013). Expression and subcellular localization of efflux transporter ABCG2/BCRP in important tissue barriers of lactating dairy cows, sheep and goats. *Journal of Veterinary Pharmacology and Therapeutics*, *36*, 562–570. https://doi.org/10.1111/jvp.2013.36.issue-6

Mahnke, H., Ballent, M., Baumann, S., Imperiale, F., von Bergen, M., Lanusse, C., … Halwachs, S. (2016). The ABCG2 efflux transporter in the mammary gland mediates veterinary drug secretion across the blood-milk barrier into milk of dairy cows. *Drug Metababolism and Disposition*, *44*, 700–708. https://doi.org/10.1124/dmd.115.068940

Marchetti, S., Mazzanti, R., Beijnen, J. H., & Schellens, J. H. (2007). Clinical relevance of drug and herb drug interactions mediated by the ABC transporter ABCB1 (MDR1, P-glycoprotein). *Oncologist*, *12*, 927–941. https://doi.org/10.1634/theoncologist.12-8-927

Mederos, A. E., Ramos, Z., & Banchero, G. E. (2014). First report of monepantel Haemonchus contortus resistance on sheep farms in Uruguay. *Parasitology Vectors*, *7*, 598. https://doi.org/10.1186/s13071-014-0598-z

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 Metabolism and Disposition*, *33*, 614–618. https://doi.org/10.1124/dmd.104.003319

Moreno, L., Imperiale, F., Mottier, L., Alvarez, L., & Lanusse, C. (2005). Comparison of milk residue profiles after oral and subcutaneous administration of benzimidazole anthelmintics to dairy cows. *Analytica Chimica Acta*, *536*, 91–99. https://doi.org/10.1016/j.aca.2004.12.069

Moreno, L., Lopez-Urbina, M. T., Farias, C., Domingue, G., Donadeu, M., Dungu, B., … González, A. E. (2012). A high oxfendazole dose to control porcine cysticercosis: Pharmacokinetics and tissue residue profiles. *Food and Chemical Toxicology*, *50*, 3819–3825. https://doi.org/10.1016/j.fct.2012.07.023

Perez, M., Otero, J. A., Barrera, B., Prieto, J. G., Merino, G., & Álvarez, A. I. (2013). Inhibition of ABCG2/BCRP transporter by soy isoflavones genistein and daidzein: Effect on plasma and milk levels of danofloxacin in sheep. *Veterinary Journal*, *196*, 203–208. https://doi.org/10.1016/j.tvjl.2012.09.012

Real, R., Egido, E., Perez, M., Gonzalez-Lobato, L., Barrera, B., Prieto, J. G., … Merino, G. (2011). Involvement of breast cancer resistance protein (BCRP/ABCG2) in the secretion of danofloxacin into milk: interaction with ivermectin. *Journal of Veterinary Pharmacology and Therapeutics*, *34*, 313–321. https://doi.org/10.1111/jvp.2011.34.issue-4

Riviere, J.E., & Sundlof, S.F. (2009). Chemical residues in tissues of food animals. In J. E. Riviere & M. G. Papich (Eds.), *Veterinary pharmacology and therapeutics*, 9th edn. (pp 1453–1462). Ames, IA: Wiley-Blackwell.

Schinkel, A. H., & Jonker, J. W. (2003). Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: An overview. *Advanced Drug Delivery Reviews*, *55*, 3–29. https://doi.org/10.1016/S0169-409X(02)00169-2

Scott, I., Pomroy, W. E., Kenyon, P. R., Smith, G., Adlington, B., & Moss, A. (2013). Lack of efficacy of monepantel against *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. *Veterinary Parasitology*, *198*, 166–171. https://doi.org/10.1016/j.vetpar.2013.07.037

Short, C. R., Flory, W., Hsieh, L. C., & Barker, S. A. (1988). The oxidative metabolism of fenbendazole: A comparative study. *Journal of Veterinary Pharmacology and Therapeutics*, *11*, 50–55. https://doi.org/10.1111/j.1365-2885.1988.tb00120.x

Stuchlíková, L., Matoušková, P., Bártíková, H., Vokřál, I., Lamka, J., Štolcová, T., … Skálová, L. (2015). Monepantel induces hepatic cytochromes p450 in sheep in vitro and in vivo. *Chemicol-Biological Interactions*, *227*, 63–68. https://doi.org/10.1016/j.cbi.2014.12.025

Van den Brom, R., Moll, L., Kappert, C., & Vellema, P. (2015). *Haemonchus contortus* resistance to monepantel in sheep. *Veterinary Parasitology*, *209*, 278–280. https://doi.org/10.1016/j.vetpar.2015.02.026

Virkel, G., Lifschitz, A., Pis, A., & Lanusse, C. (2002). In vitro ruminal biotransformation of benzimidazole sulphoxide anthelmintics: Enantioselective sulphoreduction in sheep and cattle. *Journal of Veterinary Pharmacology and Therapeutics*, *25*, 15–23. https://doi.org/10.1046/j.1365-2885.2002.00373.x

Virkel, G., Lifschitz, A., Sallovitz, J., Pis, A., & Lanusse, C. (2004). Comparative hepatic and extrahepatic enantioselective sulfoxidation of albendazole and fenbendazole in sheep and cattle. *Drug Metabolism and Disposition*, *32*, 536–544. https://doi.org/10.1124/dmd.32.5.536

Wassermann, L., Halwachs, S., Baumann, D., Schaefer, I., Seibel, P., & Honscha, W. (2013). Assessment of ABCG2-mediated transport of xenobiotics across the blood-milk barrier of dairy animals using a new MDCKII in vitro model. *Archives of Toxicology*, *87*, 1671–1682. https://doi.org/10.1007/s00204-013-1066-9

How to cite this article: Ballent M, Viviani P, Imperiale F, et al. Pharmacokinetic assessment of the monepantel plus oxfendazole combined administration in dairy cows. *J vet Pharmacol Therap*. 2017;00:1–9. https://doi.org/10.1111/jvp.12466