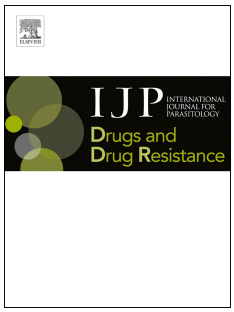

Monepantel pharmaco-therapeutic evaluation in cattle: Pattern of efficacy against multidrug resistant nematodes

Candela Canton, Lucila Canton, Adrian Lifschitz, María Paula Domínguez, Juan Torres, Carlos Lanusse, Luis Alvarez, Laura Ceballos, Ballent Mariana

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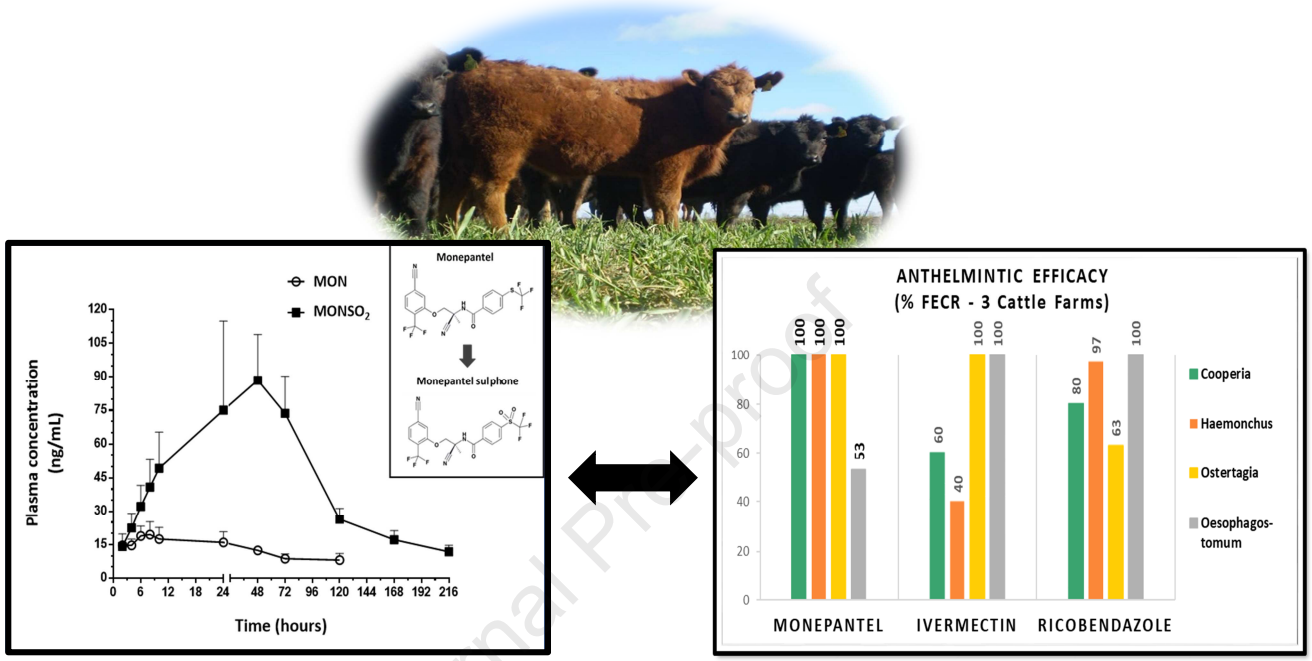

MONEPANTEL PHARMACO-THERAPEUTIC EVALUATION IN CATTLE: PATTERN OF EFFICACY AGAINST MULTIDRUG RESISTANT NEMATODES

Canton, Candela*; Canton, Lucila; Lifschitz, Adrian; Domínguez, María Paula; Torres, Juan; Lanusse, Carlos; Alvarez, Luis; Ceballos, Laura; Ballent, Mariana

Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil (CIVETAN), UNCPBA-CICPBA-CONICET, Facultad de Ciencias Veterinarias, Campus Universitario, (7000) Tandil, Argentina

*Corresponding author: Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil (CIVETAN), CONICET, Facultad de Ciencias Veterinarias, UNCPBA. Campus Universitario, 7000 Tandil, Argentina. Tel/Fax: 54- 249- 4385850. E-mail address: ccanton@vet.unicen.edu.ar

ABSTRACT

The goal of the current work was to perform an integrated evaluation of monepantel (MNP) pharmacokinetics (PK) and pharmacodynamics, measured as anthelmintic efficacy, after its oral administration to calves naturally infected with GI nematodes resistant to ivermectin (IVM) and ricobendazole (RBZ) on three commercial farms. On each farm, forty-five calves were randomly allocated into three groups (n= 15): MNP oral administration (2.5 mg/kg); IVM subcutaneous (SC) administration (0.2 mg/kg); and RBZ SC administration (3.75 mg/kg). Eight animals from the MNP treated group (Farm 1) were selected to perform the PK study. Drug concentrations were measured by HPLC. The efficacy was determined by the faecal egg count reduction test (FECRT). MNP and MNP-sulphone ($MNPSO_2$) were the main analytes recovered in plasma. $MNPSO_2$ systemic exposure was markedly higher compared to that obtained for MNP. Higher Cmax and AUC values were obtained for the active $MNPSO_2$ metabolite (96.8 ± 29.7ng/mL and 9220 ± 1720ng.h/mL) compared to MNP (21.5 ± 4.62ng/mL and 1709 ± 651ng.h/mL). The $MNPSO_2$ AUC value was 6-fold higher compared to the parent drug. Efficacies of 99% (Farm 1), 96% (Farm 2) and 98% (Farm 3) demonstrated the high activity of MNP ($P< 0.05$) against GI nematodes resistant to IVM (reductions between 27 and 68%) and RBZ (overall efficacy of 75% on Farm 3). While IVM failed to control *Haemonchus* spp. and *Cooperia* spp., and RBZ failed to control *Coooperia* spp. and *Ostertagia* spp., MNP achieved 100% efficacy against *Haemonchus* spp., *Cooperia* spp. and *Ostertagia* spp. However, a low efficacy of MNP against *Oesophagostomum* spp. (efficacies ranging from 22 to 74%) was observed. In conclusion, oral treatment with MNP should be considered for dealing with IVM and benzimidazole resistant nematode parasites in cattle.

The work described here reports for the first time an integrated assessment of MNP pharmaco-therapeutic features and highlights the need to be considered as a highly valuable tool to manage nematode resistant to other chemical families.

Keywords: Monepantel - Cattle - Resistant nematodes - Pharmaco-parasitological assessment

1.INTRODUCTION

Considering the increasing prevalence and worldwide dissemination of gastrointestinal (GI) nematodes resistant to most of the available anthelmintic families, drug resistance is considered one of the main sanitary problems in extensive cattle production systems today (Kaplan, 2020). During the last decades, chemical control has been mainly based on the use of only three anthelmintic chemical families: macrocyclic lactones (ML), benzimidazoles (BZD) and imidazothiazoles. Furthermore, since GI parasitism has a high impact on animal production, these anthelmintic drugs have been intensively used at short intervals in different cattle production grazing systems worldwide. This heavy reliance on anthelmintics to control parasitism and the limited implementation of refugia-based sustainable control programmes have led to the development of resistance to all the available chemical groups. Unfortunately, resistance is becoming a worldwide serious problem, particularly in countries such as New Zealand (Waghorn et al., 2006), Brazil (Ramos et al., 2016), Australia (Rendell, 2010), Uruguay (Mederos et al., 2019), United States (Kaplan, 2020) and Argentina (Cristel et al., 2017) among many others. Despite the complex current situation regarding the widespread development of anthelmintic resistance, dependence on chemically-based control continues to be high since it is still the most practical option for parasite control on commercial beef cattle farms.

The increasing levels of resistance to all traditional drug classes and the still high dependence on anthelmintics for controlling parasitic nematodes, have encouraged the introduction of new molecules with different modes of action into the veterinary

pharmaceutical market. The compound monepantel (MNP) is a compound of a new family of anthelmintics, the amino-acetonitrile derivatives, developed to treat ruminants infected with GI nematodes (Kaminsky et al., 2008). Its mode of action is different from the other available anthelmintic families since it acts as a positive allosteric modulator of the nematode specific acetylcholine receptor MPTL-1 (Rufener et al., 2009, 2010). MNP binding to this receptor results in a constant uncontrolled flux of ions and finally in a depolarization of muscle cells leading to nematode paralysis (Epe and Kaminsky, 2013). The cellular target of MNP, the MPTL-1 receptor, is so far only present in nematodes, which might explain the excellent tolerability of MNP in mammals and its high efficacy against multidrug-resistant parasites to other anthelmintic classes in sheep and cattle (Baker et al., 2012; King et al., 2015). The first formulation of MNP, launched in 2009, was licensed for exclusive use in sheep, and some years later was also introduced in a limited number of countries as an oral formulation for use in cattle (King et al., 2015). The disposition kinetics and distribution to target tissues of MNP have been previously described in sheep (Lifschitz et al., 2014), and some data on plasma profiles in dairy cows have been also reported (Ballent et al., 2017). However, until now there have been no published reports regarding the relationship between MNP pharmacokinetics and its efficacy against resistant GI nematodes in beef cattle.

The goal of the work described here was to perform an integrated evaluation of MNP pharmacokinetics (PK) and pharmacodynamics (PD), assessed as anthelmintic efficacy, after its oral administration to calves naturally infected with GI nematodes resistant to ivermectin (IVM) and ricobendazole (RBZ) on three commercial farms.

2. MATERIAL AND METHODS

2.1. Field Trial

This study was conducted on three cattle commercial farms located in the Humid Pampean Region, Argentina. All farms (Farms 1, 2 and 3) had a grazing system of meat production representative of Argentina bovine production. The resistance status of the nematode population characteristic of each farm was previously determined by the faecal egg count reduction test (FECRT) (Canton et al., 2019). In this way, the study included two farms with a predominance of IVM and RBZ-resistant nematode population (Farms 1 and 3) and one farm with only an IVM-resistant nematode population (Farm 2).

2.2. Animals

All the farms involved in the trial raise calves acquired from other producers. The herd on each farm from which the animals were selected were treated with levamisole prior to the study to remove their worm infections. It is important to point out that resistance to levamisole has not been reported in this region of Argentina (Cristel et al., 2017). They had then grazed on the study farms for at least two months prior to the study, which ensured that their parasite burden was native from each Farm. All the animals had free access to water.

On day -1, 60 (Farms 1 and 3) or 80 (Farm 2) male Aberdeen Angus calves, aged 9–11 months old, naturally infected with GI nematodes resistant to IVM and RBZ (Farms 1 and 3)

or resistant to IVM (Farm 2), were checked for worm egg per gram (EPG) counts, ear-tagged, and the individual body weights were recorded. The animals for inclusion in the trial were then selected based on the EPG counts. Forty-five (45) animals on each farm, with at least 100 EPG on day -1, were selected for inclusion in the study. Experimental animals had an average of 508 EPG counts ranging from 100 to 2440 on Farm 1, 274 EPG counts ranging from 100 to 660 on Farm 2, and 450 EPG counts ranging from 140 to 1440 on Farm 3.

Animal procedures and management protocols were approved by the Ethics Committee (act 11/2020) of the Facultad de Cs. Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina.

2.3. Treatments

On each farm (1, 2 and 3), all parasitized animals (n= 45) were ranked according to EPG counts, and then randomly assigned into three groups of 15 animals each: MNP: animals were treated with MNP (Zolvix®, 2.5% solution, Elanco, Argentina) by the oral route at a dose of 2.5 mg/kg; IVM: animals were treated with IVM (Ivomec®, 1% solution, Boehringer Ingelheim, Argentina) by the subcutaneous (SC) route at 0.2 mg/kg and RBZ: animals were treated with RBZ (Bayverm PI®, 15% solution, Bayer, Argentina) by the SC route at 3.75 mg/kg. The mean EPG were similar ($P > 0.05$) across all groups on each farm at the beginning of the trial.

2.4. Monepantel PK trial

The PK trial was carried out on Farm 1. Eight randomly selected animals from the MNP treated group were used in the PK trial. Blood samples (10 mL) were taken from the jugular vein in heparinised Vacutainer® tubes (Becton Dickinson, NJ, USA) before treatment and at 2,4, 6, 8 and 10 h and 1, 2, 3, 5, 7 and 9 days post-treatment. Plasma was separated by centrifugation at 3000 g for 15 min, placed into plastic tubes and frozen at -20°C until analysis by High Performance Liquid Chromatography (HPLC).

2.5. Anthelmintic efficacy trial: faecal egg count reduction test and coprocultures

Faecal samples were individually collected directly from the rectum of each calf during pre-treatment (day -1) and again on day15 post-treatment. A modified McMaster technique with a sensitivity of 10 EPG (Roberts and O'sullivan, 1950) was used to analyse the faecal samples and estimate EPG counts. Additionally, 10 g of faeces (obtained from an individual animal and/or from a pool of each experimental group) was used to prepare coprocultures on each sampling day. The nematode genera and species were identified through the third-stage larvae recovered from these coprocultures (MAFF, 1986). Third stage larvae (L_3) were collected by the Baermann technique and approximately 100 L_3 were differentiated from each sample. Thus, the relative participation of each genus per experimental group was determined.

The anthelmintic efficacy of the different treatments was assessed by the faecal egg count reduction test (FECRT), calculated according to the following formula (McKenna, 1990):

$$FECRT\ (\%) = 100\ (1 - [T2/T1])$$

where T2 is the arithmetic mean EPG count in each treated group at 15 days post-treatment, and T1 is the arithmetic mean EPG count in each treated group on day -1. The 95% confidence intervals were calculated as reported by Coles et al. (1992). Besides, efficacy against different genera was calculated by dividing the mean faecal egg count of each treatment group at day -1 and 15 post-treatment, by the proportion of L_3 of each genus in the associated coproculture (McKenna, 1990).

2.6. Analytical procedures

MNP and its metabolite, MNP-sulphone ($MNPSO_2$), concentrations were determined in plasma by HPLC with UV detection. Briefly, MNP/$MNPSO_2$ were extracted from plasma (0.5 mL) by the addition of 1 mL of acetonitrile. The preparation was mixed with a high-speed shaker (Multi Tube Vortexer, VWR Scientific Products, West Chester, PA) for 15 minutes at room temperature to allow phase separation. The solvent-sample mixture was centrifuged at 2000 *g* for 10 min at 4 °C and the supernatant was manually transferred into a clean tube. This volume was evaporated to dryness under a gentle stream of dry nitrogen at 56 °C in a water bath. Finally, the dried residue was reconstituted with 250 µL of mobile phase (acetonitrile:methanol:water 60:8:32, v/v/v) and 200 µL of this solution was injected directly into the chromatography system.

MNP plasma concentration was determined by HPLC (Shimadzu 10 A-HPLC System, Kyoto, Japan) with a UV detector set at 230 nm following a method previously developed (Ballent et al., 2017; Lifschitz et al., 2014). A C_{18} reversed-phase column (Kromasil, Eka Chemicals,

175 Bohus, Sweden, 5 µm, 4.6 × 250 mm) was used for separation. Elution of MNP and $MNPSO_2$
176 from the stationary phase was carried out at a flow rate of 0.8 mL/min (MNP) using
177 acetonitrile/methanol/water (60:8:32, v/v/v). Under the described chromatographic
178 conditions, the retention times (min) were established at 9.3 ($MNPSO_2$) and 12.5 (MNP).
179 There was no interference of endogenous compounds in any of the chromatographic
180 determinations. A calibration curve in the range between 4-400 ng⁄mL was prepared for
181 both molecules. The plasma calibration curve had a correlation coefficient ≥ 0.998. Mean
182 absolute recovery percentages for concentrations ranging between 4 and 400 ng⁄mL (n= 6)
183 were 74.9% (MNP) and 74.1% ($MNPSO_2$) with coefficients of variation (CV) of 14.1% and
184 15.7, respectively. Accuracy (expressed as the relative error) and precision (expressed as the
185 coefficient of variation) were 10% and 5.2%, respectively. The limit of quantification (LOQ)
186 was established at 4 ng⁄mL for MNP and $MNPSO_2$, which is the lowest concentration
187 measured with a recovery higher than 70% and a CV < 20%. In all cases, concentration values
188 below the LOQ were not considered for the kinetic analysis of experimental data.
189
190 *2.7. Pharmacokinetic analysis of the data*
191 The concentration vs. time curves for MNP and $MNPSO_2$ in plasma for each animal after the
192 different treatments was fitted with the PK Solution 2.0 software (Summit Research Service,
193 CO, USA). The peak concentration (Cmax) and time to peak concentration (Tmax) were
194 recorded directly from the measured concentration data. The elimination half-life ($T_{½el}$) and
195 absorption half-life ($T_{½abs}$) were calculated as $\ln 2/\lambda_{el}$ and $\ln 2/k_{abs,}$ respectively, where λ_{el} is
196 the elimination rate constant and k_{abs} represents the first-order absorption rate constant.

The rates were calculated by performing regression analysis using data points belonging to the terminal or absorption phase concentration-time plot. The area under the plasma concentration-time curve from zero up to the quantificationlimit (AUC_{0-LOQ}) was calculated using 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 (λ_{el}). Statistical moment theory was applied to calculate the mean residence time (MRT) according to Perrier and Mayersohn (1982). PK analysis of the experimental data was performed using a non-compartmental model method.

2.8. Statistical analysis of the data

The PK parameters and concentration data are reported as arithmetic mean ± Standard Deviation (SD). PK parameters for MNP and $MNPSO_2$ were statistically compared using Student t-test. Faecal egg counts (reported as arithmetic mean ± SD) were compared by non-parametric Kruskal–Wallis test. A value of $P<0.05$ was considered statistically significant. The statistical analysis was performed using the Instat 3.0 software (Graph Pad Software, CA, USA).

3. RESULTS

MNP and $MNPSO_2$ were the main analytes recovered in plasma after oral administration of MNP to cattle. The mean (± SD) plasma concentrations profiles of MNP and its $MNPSO_2$ metabolite are shown in Fig. 1. $MNPSO_2$ systemic exposure was markedly higher compared to that obtained for MNP. It accounted for >80 % of the total amount of the analytes recovered in plasma. While low concentrations of MNP were measured in plasma only up to

219 120 h (5 days) post-administration, the persistence of the sulphone metabolite was longer in
220 the bloodstream, being recovered up to 216 h (9 days). These differences were reflected in
221 the values estimated for the main PK parameters. Table 1 summarizes the plasma PK
222 parameters for MNP and $MNPSO_2$ obtained after the oral administration of MNP to cattle.
223 Higher Cmax and greater AUC values were obtained in plasma for $MNPSO_2$ compared to
224 MNP. In fact, the AUC value for $MNPSO_2$ were 6-fold higher compared to those reported for
225 the parent drug ($MNPSO_2$/MNP AUC ratio= 5.99 ± 2.08).
226
227 Table 2 shows the overall faecal egg counts (arithmetic mean) and reduction percentages of
228 faecal egg counts (FECR) (undifferentiated) with its 95% lower and upper confidence
229 intervals obtained for all experimental groups on Farms 1, 2 and 3. The results of the FECRT
230 with 99%, 96% and 98% of reduction for MNP on Farms 1, 2, and 3, respectively,
231 demonstrated the high efficacy of this amino-acetonitrile derivative against GI nematodes
232 resistant to IVM and RBZ in cattle. In fact, the low efficacies obtained for IVM (43%, 68% and
233 27% of reduction) confirm the presence of resistant parasites to this anthelmintic. On the
234 other hand, the overall efficacy for RBZ on Farm 2 was98%, demonstrating that this farm was
235 the only one included in the study with a predominance of a RBZ-susceptible nematode
236 population. Although the total efficacy for RBZ on Farm 1 was 94%, the 95% lower
237 confidence interval for this anthelmintic was less than 90%, indicating an initial level of
238 resistance. Finally, a higher level of resistance for RBZ was reported on Farm 3, where an
239 overall reduction of 75% confirms the presence of resistant GI nematodes. In this context,
240 whilst on Farms 1 and 2 significant ($P< 0.05$) differences were only observed between EPG

241 counts post-IVMand MNP treatments, on Farm 3, the EPG counts after MNP were
242 significantly (P< 0.05) lower than the egg counts after both IVM and RBZ.
243
244 The anthelmintic efficacies against *Cooperia* spp., *Haemonchus* spp., *Ostertagia* spp. and
245 *Oesophagostomum* spp. for the different treatments on Farms 1, 2 and 3, are shown in Table
246 3. On Farms 1 and 3 IVM failed to control *Haemonchus* spp. and *Cooperia* spp., showing
247 efficacies ranging from 0% to 80%. In the case of Farm 2, only IVM-resistant *Cooperia* spp.
248 was present, being the others GI nematode genera susceptible to RBZ. The BZD treatment
249 failed to control *Cooperia* spp. and *Ostertagia* spp. on Farms 1 and 3 (FECR below 90% for
250 both nematode genera). In contrast, MNP was the only treatment that achieved 100%
251 efficacy against *Cooperia* spp., *Haemonchus* spp. and *Ostertagia* spp., including against
252 resistant parasites (99% against *Ostertagia* spp. on Farm 3). However, MNP failed to control
253 *Oesophagostomum* spp., showing low efficacies of 74%, 22% and 64% against this genus on
254 Farms 1, 2, and 3, respectively.
255 Finally, no adverse events were observed in any of the cattle treated with MNP.
256

257 **4. DISCUSSION**

258 Since GI parasitism negatively affects weight gain in grazing animals (Charlier et al., 2014a),
259 parasite control is necessary to ensure adequate production levels on beef cattle farms.
260 Alternative nematode control strategies, such as grazing management, host genetic
261 resistance and helminth vaccines, are now being developed for further reduce reliance on
262 chemically-based parasite control (Charlier et al., 2014b). However, dependence on

263 anthelmintics continues to be high, since it is still being the most practical tool for parasite
264 control on large scale commercial beef cattle farms. Due to the enormous difficulties
265 involved in the development of novel anthelmintic molecules, such as the lastly introduced
266 amino-acetonitrile derivative MNP, it is essential to understand its pharmacological
267 behaviour to optimize its use in cattle under natural field conditions. The work described
268 here reports for the first time an integrated assessment of MNP pharmacokinetics and
269 pharmacodynamics (measured as anthelmintic efficacy), in cattle naturally infected with GI
270 nematodes resistant to IVM and RBZ on a field trial performed on three different commercial
271 farms.
272
273 The MNP plasma disposition kinetics has not been described in beef cattle. However, in line
274 with previous PK studies in sheep (Karadzovska et al., 2009; Lifschitz et al., 2014) and dairy
275 cows (Ballent et al., 2017), a rapid decline in the plasma profiles of the parent drug and the
276 recovery of the $MNPSO_2$ metabolite as the main analyte detected in the bloodstream, were
277 observed in beef calves in the current trial. The metabolic conversion of MNP into $MNPSO_2$
278 also involves the production of an intermediate sulphoxide derivative (Karadzovska et al.,
279 2009), which is rapidly and almost completely converted into $MNPSO_2$, being undetectable
280 in plasma of MNP treated animals. In fact, the Cmax of the sulphone metabolite was four
281 times higher than the corresponding parent concentration (21.5 *vs* 96.8 ng/mL for MNP and
282 $MNPSO_2$, respectively). Moreover, when MNP reached the Cmax (at 8 h post-oral
283 treatment), the $MNPSO_2$ metabolite was already about twice as high. Since $MNPSO_2$ is an
284 active metabolite against nematodes (Karadzovska et al., 2009), its high plasma and GI

285 exposure greatly contribute to the overall MNP nematodicidal efficacy. In fact, the ratio of
286 the total plasma AUC of $MNPSO_2$ over the total AUC of MNP in both species, exhibited higher
287 systemic exposure for $MNPSO_2$ compared to the parent drug after the oral administration of
288 MNP. However, interspecies differences in $MNPSO_2$ systemic availability were observed
289 between cattle and sheep. While Lifschitz et al. (2014) reported a $MNPSO_2$/MNP AUC ratio
290 of about 12 in sheep, a 50% lower value is described for that ratio after oral administration
291 of MNP in cattle (Table 1). This finding may be explained by the different patterns of MNP
292 liver metabolism (S-oxidation) between sheep and cattle. The rate of MNP conversion into
293 $MNPSO_2$ was five-fold higher in sheep compared to cattle (Ballent et al., 2016). While in
294 sheep, the formation of the sulphone metabolite is based on the enzymatic activity of both
295 flavin-monooxygenase (FMO) and cytochrome P- 450 (CYP), in cattle MNP is converted into
296 $MNPSO_2$ only in a CYP- mediated metabolic reaction (Ballent et al., 2016). These interspecies
297 differences do not necessarily imply lower exposure of worms to the active drug. Moreover,
298 considering MNP anthelmintic activity may be mainly based on a considerable
299 drug/metabolite accumulation in the GI tissues and fluid contents during the first 2 to 3 days
300 post-treatment, the different patterns of MNP liver metabolism between sheep and cattle
301 should not affect its efficacy against GI nematodes (Lifschitz et al. 2014).
302
303 The results of the current PK assessment in cattle and those reported in sheep by Lifschitz et
304 al. (2014) on the characterization of MNP accumulation in target tissues, give strong
305 pharmacological support to the anthelmintic efficacy findings. The increasing worldwide
306 prevalence of GI nematodes resistant to most of the traditional anthelmintic groups such as

ML and BZD, therapeutic failures associated with anthelmintic resistance has enormous economic importance of global significance, particularly in countries where weather and production conditions contribute to a high incidence of parasitism. For instance, resistance to IVM was diagnosed in 93% of the farms tested in Argentina, while resistance to RBZ was diagnosed in 28% of the farms included in a nation-wide survey (Cristel et al.,2017). The main resistant genera were *Cooperia* spp. and *Haemonchus* spp. to IVM, and *Ostertagia* spp. and *Cooperia* spp.to RBZ (Cristel et al., 2017). Therefore, the efficacy of MNP was evaluated in scenarios where the nematode population was representative of the real situation on most commercial cattle farms. In this context, the efficacy results showed 99%, 96% and 98% of reduction for MNP on Farms 1, 2 and 3, respectively. These results demonstrated the high efficacy of MNP against resistant GI nematodes in cattle. Only limited information is available on MNP efficacy against GI nematodes in cattle (King et al. 2015). In that particular trial, MNP was administered in a combined formulation with abamectin. However, the reported efficacy results are consistent with those observed in our current trial with efficacies measured by FECR ranging from 98.3 to 99.9%. Similarly, the efficacy results observed in the present work are consistent with several studies in sheep (Bustamante et al., 2009; Hosking et al., 2009; Kaminsky et al., 2009; Sager et al., 2009). Bustamante et al. (2009) also evaluated MNP efficacy against IVM resistant nematode parasites. The low IVM efficacies obtained in the current work (43%, 68% and 27% of reduction on Farms 1, 2 and 3), confirm the presence of resistant nematode populations to this ML anthelmintic. Additionally, MNP was the only treatment that achieved >95% both in the overall efficacy and in the 95% lower confidence interval.

329

330 It should be considered that GI parasitism in cattle always involves different parasite genera.
331 In this sense, while on Farms 1 and 3 IVM failed to control *Cooperia* spp. and *Haemonchus*
332 spp., on Farm 2 *Cooperia* spp. was the only genus resistant to IVM. *Cooperia* spp. is
333 commonly present in the cases of IVM resistance in cattle. In fact, resistant *Cooperia* spp.
334 was recovered in 100% of the farms where resistance to IVM were present in a survey carried
335 out in Argentina in 2017 (Cristel et al. 2017). *Cooperia* spp. is one of the genera in which
336 resistance to IVM is more frequent not only because it is a "dose-limiting" parasite for IVM
337 (Benz et al., 1989), but also because routine IVM treatments are administered in the absence
338 of any significant larval population in refugia (Sauermann and Leathwick 2018). However,
339 similarly to our findings, some studies have also reported both *Cooperia* spp. and
340 *Haemonchus* spp. resistant to IVM (Anziani et al., 2004; Ramos et al., 2016; Canton et al.,
341 2018). Although RBZ achieved higher overall efficacies than IVM, the BZD treatment did not
342 show effective control against all the GI nematodes present on Farms 1 and 3. Indeed, on
343 these farms, RBZ failed to control *Cooperia* spp. and *Ostertagia* spp. (FECR below 90% for
344 both nematode genera). In contrast, MNP was the only treatment that achieved 100%
345 efficacy against *Cooperia* spp., *Haemonchus* spp. and *Ostertagia* spp. Similar results were
346 found in different studies in sheep against resistant GI nematodes. Hosking et al. (2008) and
347 Sager et al. (2009) demonstrated high (>95%) efficacy of MNP administered orally to sheep
348 against GI nematodes resistant to either BZ or levamisole. Furthermore, Steffan et al. (2011)
349 and Baker et al. (2012) showed almost 100% efficacy of MNP against GI nematodes multiple

350 resistant to BZ, levamisole and ML. Although those studies were performed in sheep, their
351 results and resistance scenarios were comparable with the current trial of MNP in cattle.
352
353 Efficacy of MNP against *Oesophagostomum* spp. is a particularly relevant issue due to
354 efficacy results failed to meet an adequate reduction. The findings of the present study in
355 cattle demonstrated that MNP failed only to control *Oesophagostomum* spp., with efficacies
356 ranging from 22% to 74%. Similarly, it has been reported in sheep that *Oesophagostomum*
357 was only reduced by 88% (Sager et al., 2009) and 61.9% (Bustamante et al., 2009).
358 Furthermore, Hosking et al. (2009) also found efficacies below 90% against this nematode in
359 sheep. In fact, the dose of 2.5 mg/kg was established as a suitable minimum dose rate
360 (Kaminsky et al., 2009), because lower doses failed to control *Oesophagostomum* spp.,
361 which was established as the dose-limiting nematode for MNP (Hosking et al., 2010).
362 Although a reduced sensitivity of this genus to MNP may explain its low efficacy, Lifschitz et
363 al. (2014) suggested that a PK-related issue should contribute to this limited therapeutic
364 response in sheep. The lower concentration of MNP achieved in the large intestine mucosa
365 (225 ng/g) compared to that measured in the small intestine mucosa (562 ng/g in the ileum
366 and 762 ng/g in the duodenum) may explain the efficacy levels obtained against
367 *Oesophagostomum* spp. (Lifschitz et al., 2014), situation that could also occur in cattle. The
368 PK/PD of MNP against GI nematodes may suggest that the high concentrations of MNP
369 parental drug achieved in the GI contents and mucosa during 48-72 h after its oral
370 administration are relevant to the effectiveness of this compound (Lifschitz et al., 2014).
371

372 The activity of MNP against multidrug-resistant parasites, which is based on its novel mode
373 of action, is a highly favorable element. However, resistance to MNP has occurred on the
374 field within less than 2 years of the product first being used in sheep and goats in New
375 Zealand. In this first report of resistance in goats excessively treated with the amino-
376 acetonitrile derivative, MNP was ineffective against at least two GI nematode species,
377 *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Scott et al., 2013). Moreover,
378 Mederos et al. (2014) found *Haemonchus contortus* resistant to MNP on sheep farms in
379 Uruguay. Lack of efficacy of MNP was also reported on sheep farms in the Netherlands (van
380 den Brom et al., 2015), Brazil (Cintra et al., 2016), Australia (Sales and Love, 2016), Argentina
381 (Illanes et al., 2018) and the United Kingdom (Hamer et al., 2018; Bartley et al., 2019).
382 Considering that resistance to MNP has already been reported in sheep in different
383 countries, it is essential to understand the mechanisms of resistance to this compound. In
384 this way, the presence of multiple separate mutations in theMPTL-1gene in field-derived *H.*
385 *contortus* and *T. circumcincta* isolates may at least partly explain MNP resistance (Bagnall et
386 al., 2017; Turnbull et al., 2019). The reports of resistance highlight the need to learn from
387 the use of this anthelmintic on sheep farms. It is essential to maintain the awareness on the
388 possibility of development of resistance to MNP in cattle nematode parasites, which includes
389 the need to follow appropriate guidelines of parasite control (Bartley et al., 2019).
390
391 Overall, there is no published reports on the simultaneous assessment of the relationship
392 between the PK performance and the anthelmintic therapeutic response to MNP in cattle.
393 The results of the current work determined that the oral route is a very efficient

394 administration route for MNP in beef cattle. This is particularly relevant when the described
395 high systemic exposure of the anthelmintically active MNP and $MNPSO_2$ exposure is
396 considered. MNP achieved effective control of GI nematodes with multiple anthelmintic
397 resistance to ML and BZD. The widespread appearance of resistant parasites highlights the
398 need for novel anthelmintics acting at novel target sites to be used in cattle, such as MNP.
399 However, it is now crucial to accomplish adequate management of this novel compound to
400 prolong its lifespan and optimize parasite control based on diagnosis and treatment
401 strategies implemented on an individual cattle farm basis. The findings described here
402 contribute to the knowledge on MNP pharmacology and efficacy against resistant GI
403 nematodes in beef cattle.
404

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409

410 **CONFLICT OF INTEREST STATEMENT**

411 There are no potential conflicts of interest associated with this study.
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Figure Legends

Figure 1

Plasma concentration profiles of monepantel (MNP) and monepantel sulphone ($MNPSO_2$) obtained after the oral administration of monepantel (2.5 mg/kg) to parasitized calves (n=8). The insert shows the chemical structures of MNP and an its anthelmintically active metabolite $MNPSO_2$.

Table 1

Plasma pharmacokinetic parameters (mean ± SD) for monepantel (MNP) and monepantel sulphone ($MNPSO_2$) obtained after the oral administration of MNP (2.5 mg/kg) to naturally parasitized calves.

MONEPANTEL		
Pharmacokinetic parameters	**MNP**	**$MNPSO_2$**
T_{max} (h)	8.00 ± 1.51[a]	41.3 ± 17.9[b]
C_{max} (ng/mL)	21.5 ± 4.62[a]	96.8 ± 29.7[b]
$AUC_{0\text{-}LOQ}$ (ng.h/mL)	1709 ± 651[a]	9220 ± 1720[b]
$AUC_{0\text{-}\infty}$ (ng.h/mL)	2174 ± 783[a]	10242 ± 1405[b]
MRT (h)	112 ± 40.8[a]	99.3 ± 21.0[a]
$T_{½el}$ (h)	81.0 ± 31.0[a]	57.6 ± 13.9[a]
$T_{½abs}$ (h)	1.74 ± 0.66[a]	9.79 ± 4.06[b]
Ratio of the AUC $MNPSO_2$/MNP	-	5.99 ± 2.08

T_{max}: time to peak plasma concentration; C_{max}: peak plasma concentration; $AUC_{0\text{-}LOQ}$: area under the plasma concentration vs. time curve from 0 to the quantification limit; $AUC_{0\text{-}\infty}$**:** area under the concentration-time curve extrapolated to infinity; MRT: mean residence time; $T_{½el}$: elimination half-life; $T_{½abs}$: absorption half-life (the value express the metabolite formation half-life for $MNPSO_2$).

Pharmacokinetic parameters with different superscript letters are statistically different ($P<0.05$).

Table 2

Nematode egg per gram counts (EPG, arithmetic mean, range) and reduction percentages of faecal egg counts (FECR) (undifferentiated) with its 95% lower and upper confidence intervals, after the oral administration of monepantel (MNP, 2.5 mg/kg), and the subcutaneous administration of ivermectin (IVM, 0.2 mg/kg) and ricobendazole (RBZ, 3.75 mg/kg) to naturally parasitized calves.

	FARM 1			**FARM 2**			**FARM 3**		
Experimental Group	**EPG Counts (range)**		**FECR[1] (CI)**	**EPG Counts (range)**		**FECR[1] (CI)**	**EPG Counts (range)**		**FECR[1] (CI)**
	Day -1	**Day 15**		**Day -1**	**Day 15**		**Day -1**	**Day 15**	
MNP (oral)	547[a] (100-2440)	5.6[a] (0-20)	**99%** (97-99)	188[a] (100-400)	8[a] (0-20)	**96%** (90-98)	374[a] (140-740)	7[a] (0-20)	**98%** (95-99)
IVM (sc)	469[a] (100-1460)	269[b] (0-1060)	**43%** (0-73)	351[a] (100-660)	111[b] (0-320)	**68%** (42-83)	498[a] (140-1360)	362[b] (20-1520)	**27%** (0-69)
RBZ (sc)	508[a] (140-1380)	31[a] (0-120)	**94%** (85-97)	283[a] (120-580)	3[a] (0-20)	**98%** (94-99)	480[a] (140-1140)	115[b] (0-320)	**75%** (45-89)

[1]FECR estimated according to McKenna, (1990). CI: lower and upper confidence intervals.
EPG counts on each column with different superscript letters are statistically different (P<0.05).

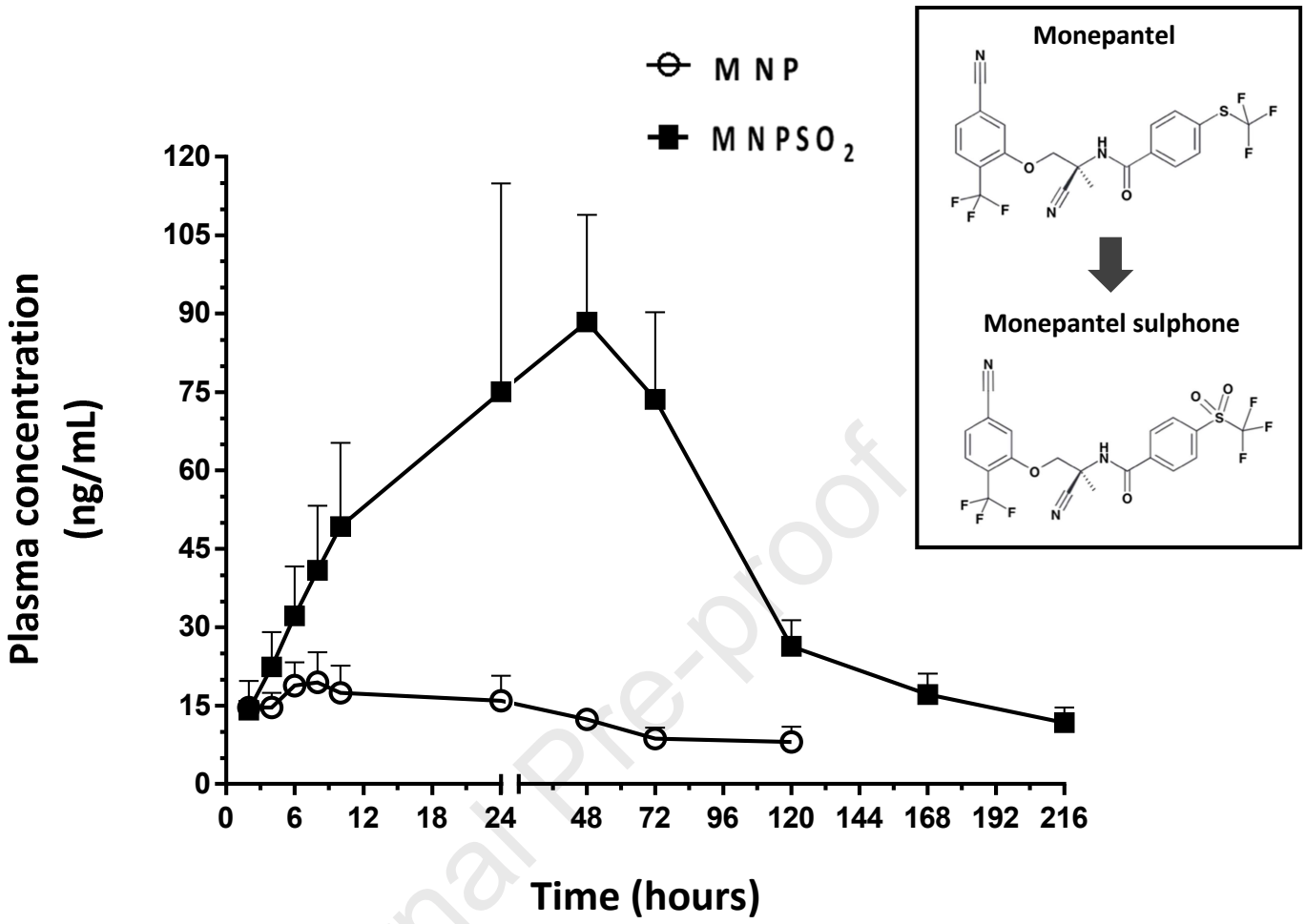
Table 3

Reduction percentages of faecal egg counts (FECR) for *Cooperia*, *Haemonchus, Ostertagia* and *Oesophagostomum* spp. after the oral administration of monepantel (MNP, 2.5 mg/kg), and the subcutaneous administration of ivermectin (IVM, 0.2 mg/kg) and ricobendazole (RBZ, 3.75 mg/kg) to naturally parasitized calves.

Genus - Treatment	**FECR[1] Day 15**		
	FARM 1	**FARM 2**	**FARM 3**
***Cooperia* spp.**			
MNPoral	100%	100%	100%
IVMsc	80%	56%	43%
RBZsc	86%	99%	54%
***Haemonchus* spp.**			
MNPoral	100%	100%	100%
IVMsc	19%	100%	0%
RBZsc	99%	95%	98%
***Ostertagia* spp.**			
MNPoral	100%	100%	99%
IVMsc	100%	100%	100%
RBZsc	89%	100%	0%
***Oesophagostomum* spp.**			
MNPoral	74%	22%	64%
IVMsc	100%	100%	100%
RBZsc	100%	100%	100%

[1]FECR estimated according to McKenna, (1990).

Figure 1



HIGHLIGHTS

- MNP and its anthelmintically active metabolite $MNPSO_2$ were the main analytes recovered in plasma
- The $MNPSO_2$ AUC value was 6-fold higher compared to the parent drug
- MNP obtained overall efficacies of 96-99% against IVM and BZD resistant nematode parasites in cattle
- MNP failed to control *Oesophagostomum* spp.
- The work described here reports for the first time an integrated assessment of MNP pharmaco-therapy features

CONFLICT OF INTEREST STATEMENT

There are no potential conflicts of interest associated with this study.