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Research paper

Plasma disposition, milk excretion and parasitological efficacy of mebendazole in donkeys naturally infected by Cyathostominae

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ABSTRACT

Mebendazole (MBZ) has been licensed for use in horses and donkeys, however there are no data available in the literature regarding its pharmacokinetic disposition and efficacy in donkeys. This study was designed to determine the plasma disposition, milk excretion and anthelmintic efficacy of MBZ in donkeys naturally infected by Cyathostominae. The animals were allocated to three groups, each of six donkeys. One group was untreated control (C-group) and the others were treated using a paste formulation of MBZ administered *per os* at the manufacturer's recommended horse dosage of 10 mg/kg body weight (MBZ 1) and at the double horse dosage 20 mg/kg body weight (MBZ 2). Blood and milk samples were collected at various times between 1 h and 120 h post treatment and analyzed by high performance liquid chromatography with photodiode array detector. Individual FECs (Faecal Egg Counts) were performed on each animal before the treatment (day-3) and weekly from day 7 until day 56 post treatment using a modified McMaster technique. The plasma concentrations and systemic exposure of MBZ in donkeys were relatively lower compared with the other methylcarbamate benzimidazoles. Dose-dependent plasma dispositions of MBZ were observed at the increased dosage (10 mg/kg *vs* 20 mg/kg) in donkeys. MBZ was not detected in any milk samples at a dosage of 10 mg/kg. However, the parent drug reached 0.01 μg/ml peak milk concentration at 10.66 h and AUC_{milk}/AUC_{plasma} value was 0.18 ± 0.02 at a dosage of 20 mg/kg bodyweight. This study indicated that *per os* administration of MBZ has a minimal disposition rate into the milk and may be used in lactating donkeys with zero milk-withdrawal period. The results of FECRT for both MBZ dosages were efficient (>95% efficacy) until day 28. This trial demonstrates that MBZ oral paste at horse dosage (10 mg/kg B.W.) was effective and safety for the treatment of Cyathostominae in donkeys. Therefore, similar dosage regimens of MBZ could be used for horses and donkeys.

1. Introduction

Few drugs have been licensed for use in donkeys (Lizarraga et al., 2004). Only limited information is available on the pharmacokinetics of anthelmintic drugs including some benzimidazoles (Gokbulut et al., 2006), endectocides (Gokbulut et al., 2005, 2011, 2013) and tetrahydropyrimidines (Gokbulut et al., 2014), as donkeys were often neglected species in domestic animals. Because of the lack of registered drugs for donkeys, anthelmintics licensed for horses or ruminants are used for treatment of parasitic infections in this species with same dosage. It has been reported that donkeys have a greater capacity to metabolize certain drugs compared with horses; thus, higher dosage or shorter intervals could be required for reaching and maintaining effective drug concentrations, although there are some exceptions (Matthews et al., 1997; Coakley et al., 1999; Peck et al., 2002, 1997; Lizarraga et al., 2004; Kum et al., 2009; Grosenbaugh et al., 2011; Sekkin et al., 2010).

Mebendazole (MBZ, Methyl 5-benzyl-1H-benzimidazole-2yl-carbamate) belongs to the chemical class of benzimidazole methylcarbamates and has been used for more than 40 years in veterinary and human medicine to treat a range of internal parasitic

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infections with broad-spectrum of activity. Like other methylcarbamate benzimidazoles such as albendazole and fenbendazole, the poor water solubility of MBZ reduces flexibility for drug formulation, allowing its formulation only as tablets, paste, or suspensions *per os* administration in animals and this limits its absorption and disposition in the body (McKellar and Scott, 1990). Dosage recommendations vary from 5 mg/kg to 50 mg/kg depending by host and parasite infection. The recommended dosage of MBZ is 5–10 mg/kg *per os* route in horses (McKellar and Scott, 1990). In addition, 20 mg/kg is recommended for the treatment of lungworm *Dictyocaulus arnfieldi* in donkeys and this should be administered for 5 consecutive days (Clayton and Neave, 1979).

Donkey milk is gaining popularity in some countries such as Italy, France and Belgium (Veneziano et al., 2011) since it is the closest milk to human breast milk with high lactose ratios and low fat content. It is ideal for feeding infants and in particular the rediscovery of donkey milk as a food source for children affected by cow milk protein allergy—CMPA (Iacono et al., 1992; Vincenzetti et al., 2008). Although, MBZ has been licensed for use in horses and donkeys, there are no data available in the literature concerning its pharmacokinetic disposition in any equine species. Therefore, the present study was designed to determine the plasma disposition, milk excretion, anthelmintic efficacy and egg reappearance period (ERP) of MBZ in donkeys following *per os* administrations at the dosages of 10 mg/kg and 20 mg/kg body weight (B.W.).

2. Material and methods

2.1. Study animals

Eighteen cross-breed female milking donkeys with a mean age (±standard deviation) of 10 ± 4 years, weighting in mean (±standard deviation) 294 ± 49 kg were used in the present study. The bodyweight of each animal was estimated 1 day prior to treatment (day-1) using the nomogram proposed by The Donkey Sanctuary (2003). Donkeys were milked once a day and, the milk production measured before and throughout the trial ranged from 1500 to 1800 ml/day. The study animals were tagged for identification using a numbered head collar. During the entire experimental period the donkeys were housed communally in an outdoor pen with a permanent and hilly pasture; supplementary feeding was given such as hay and concentrate (barley, wheat bran, beet pulp, oatmeal, carob). The animals had a history of grazing pasture contaminated with equine nematode parasites and have not been treated with any anthelmintics during the previous 6 months. Faecal examinations (individual Faecal Egg Counts and pooled coprocoltures) performed before the beginning of the study (days -14 and -3) showed individual counts >500 eggs per gram (EPG) and high prevalence of Cyathostominae in all studied donkeys. Water was provided *ad libitum* throughout the course of the study. This investigation was approved by the Animal Ethic Committee of University of Naples Federico II.

2.2. Experimental groups

On day-3, the experimental animals ($n = 18$) had an average of 1520 ± 522 EPG. The animals were ranked from lowest to highest epg counts. Based on increasing epg counts, replicates of 3 animals were formed. Within each replicate, animals were randomly assigned to treatment. The 18 selected donkeys were assigned consecutively to the following treatment groups of 6 animals each: mebendazole paste horse treated group (MBZ 1 group), mebendazole paste double horse dosage treated group (MBZ 2 group) and untreated control group (C-group).

The three groups were maintained together under the same conditions. In particular, they co-grazed on the same pasture throughout the study and there was not any change of diet during the lactation.

2.3. Treatment procedures

Commercially available equine formulation of mebendazole paste (Telmin^{TM}, 20%, Janssen, Italy) licensed for horses was administered to donkeys orally at the horse dosage 10 mg/kg B.W. (MBZ 1) and at the double horse dosage 20 mg/kg B.W. (MBZ 2). For each animal, the dosage was calculated on the basis of its body weight. All groups received a single treatment.

Donkeys of the C-group did not receive any treatment but were subjected to the same handling procedures as the donkeys of the treated groups, receiving *per os aqua fontis*.

All treated animals were intermittently observed for adverse reactions for 3 h (three times) during the day of treatment (day 0) and then weekly until the end of the trial.

2.4. Sampling procedures

Heparinised blood samples (10 ml) were collected by jugular venipuncture prior to drug administration and 1, 2, 4, 8, 12, 16, 24, 30, 36, 48, 56, 72, 96, and 120 h thereafter. Individual milk samples (15 ml) were also collected throughout the blood sampling period, before drug administration and thereafter atprior to drug administration and 8, 12, 24, 36, 48, 72, 96 and 120 h post treatment in order to the determination of drug milk excretion. Blood samples were centrifuged at 3000 rpm for 30 min and plasma was transferred to plastic tubes. All the plasma and milk samples were stored at −20 °C until estimation of drug concentration.

Faecal samples were taken from the rectum, or from freshly voided, from each study animal, were stored in a refrigerator (4 °C) and within 12 h individual faecal egg counts (FEC) were performed, before the start of the trial (day-3), at days 7, 14, 21, 28, 35, 42 and 56 after treatment (Nielsen et al., 2010).

2.5. Analytical procedures

A stock solution (100 μg/ml) of pure standard of MBZ (Sigma, St. Louis, MO, USA) was prepared using acetonitrile (Sigma, St. Louis, MO, USA) as the solvent. This was diluted to give 0.01, 0.05, 0.1, 0.5, 1, 5 μg/ml standard solutions for plasma and milk samples for calibration as standard curves and to add to the drug-free plasma and milk samples to determine the recovery.

The plasma and milk concentrations of MBZ were analysed by high performance liquid chromatography (HPLC) with photodiode array detector following liquid–liquid extraction procedure according to a previously described method (Dawson et al., 1982). Briefly, drug-free plasma or milk samples (1 ml) were spiked with standards to reach the following final concentrations: 0.005, 0.01, 0.05, 0.1, and 0.5 μg/mL. Ammonium hydroxide (100 L, 0.1 N, pH 10) was added to 10 ml-ground glass tubes containing 1 ml spiked or experimental plasma or milk samples. After mixing for 15 s, 6 mldiethyl etherwas added. The tubes were shaken on a slow rotary mixer for 10 min. After centrifugation at 3000 × *g* for 10 min, the diethyl ether layer (5 ml) was transferred to a thin-walled 10 ml-conical glass tube and and concentrated to dryness at 40 °C in a sample concentrator (Maxi-dry plus, Heto Lab. Equipment, Denmark). The dry residue was re-suspended with 250 μL dimethyl sulphoxide (DMSO). Then the tubes were placed in an ultrasonic bath and finally, 100 μL of this solution were injected into the chromatographic system.The mobile phase consisted of acetonitrile and 0.05 M aqueous ammonium phosphate buffer (50:50, v/v) and was delivered (1100 Series QuatPump, Agilent, Waldron, Germany) at a

Table 1
Mean (±SD) plasma and milk pharmacokinetic parameters of mebendazole (MBZ) following *per os* administration to donkeys at 10 mg/kg body weight ($n = 6$) and 20 mg/kg bodyweight ($n = 6$).

Parameters	Plasma (10 mg/kg)	Plasma (20 mg/kg)	Milk (10 mg/kg)	Milk (20 mg/kg)
$t_{1/2\lambda z}$ (h)	11.97 ± 4.38	13.13 ± 3.85	–	8.40 ± 3.54
t_{max} (h)	7.33 ± 3.93	8.00 ± 0.00	–	10.66 ± 3.29
C_{max} (μg/ml)	0.04 ± 0.01	0.07 ± 0.01	–	0.01 ± 0.00
$AUC_{0\to\infty}$: (μg h/ml)	0.78 ± 0.35	1.42 ± 0.20	–	0.29 ± 0.04
$AUMC_{0\to\infty}$: (μg.h^2/ml)	19.49 ± 7.86	33.78 ± 10.38	–	7.08 ± 0.90
$MRT_{0\to\infty}$: (h)	20.34 ± 7.59	23.43 ± 5.55	–	17.63 ± 8.74
AUC_{milk}/AUC_{plasma}	–	–	–	0.18 ± 0.02
$C_{max\text{-}milk}/C_{max\text{-}plasma}$	–	–	–	0.20 ± 0.03

C_{max}: peak plasma concentration; t_{max}: time to reach peak plasma concentration; $AUC_{0\to\infty}$: area under the (zero moment) curve from time 0 to infinity, $AUMC_{0\to\infty}$: area under the moment curve from time 0 to infinity; $MRT_{0\to\infty}$: mean residence time; $t_{1/2\lambda z}$: terminal half-life.

flow rate of 1 ml/min. A nucleosil C_{18} analytical column (Luna, 3 μm, 150 mm x 4.6 mm, Phenomenex, Macclesfield, Cheshire, UK) with nucleosil C_{18} guard column(Phenomenex, Macclesfield, Cheshire, UK) was used for analysis of the molecules. Photodiode array detection (1100 Series, Agilent, Waldron, Germany) was at a wavelength of 254 nm.

2.6. Analytical method validation

The analytical methods used for MBZ in plasma and milk samples were validated prior to the start of the studies. Calibration graph for MBZ in the range 0.005–0.5 μg/mL plasma and milk were prepared using drug-free plasma and milk samples from donkeys.

The analyte was identified with the retention times of pure reference standard. Recoveries of the molecule under study were measured by comparison of the peak areas from spiked plasma and milk samples with the areas resulting from direct injections of standards prepared in acetonitrile. The inter and intra-assay precision of the extraction and chromatography procedures were evaluated by processing replicate aliquots of drug-free donkey plasma and milk samples containing known amounts of the drug on different days.

2.7. Coprological examinations: anthelmintic efficacy and egg reappearance period (ERP)

Individual faecal egg counts were determined using a modified McMaster technique with a detection limit of 10 EPG, using a Sheather's saturated sugar solution with a specific gravity of 1.250 (Reinemeyer and Nielsen 2013; Lester and Matthews, 2014).

On each sampling day, individual faecal samples were incubated at 27 °C for 7–10 days for larval identification. Only before the start of the trial (days -14 and -3) were performed pooled coprocoltures. Third stage larvae were identified using the morphological keys proposed by M.A.F.F. (1986). When a coprocolture had 100 or less third stage larvae, all were identified; when a coprocolture had more than 100 larvae, only 100 were identified.

To determine the efficacy of MBZ against intestinal strongyles at each faecal sampling time, arithmetic mean of EPG was calculated following the WAAVP guidelines (Coles et al., 1992, 2006). For each groups (MBZ 1–MBZ 2) percent efficacy (%) was calculated in terms of Faecal Egg Count Reduction (FECR) at the different days according to the formula:

$$\text{Efficacy (\%)} = \frac{\text{mean EPG control group} - \text{mean EPG treated group}}{\text{mean EPG control group}} \times 100$$

The cutoff value chosen for establishing efficacy was FECR > 90% as reported by Kaplan and Nielsen (2010) for benzimidazole drugs. In addition, 95% confidence interval (CI) were included to give a more accurate indication of the data (Vidyashankar et al., 2007): Lower CI of 80% was selected for classify resistance for MBZ treatments.

The ERP was evaluated as suggested by the American Association of Equine Practitioners (AAEP) Parasite Control Guidelines (Nielsen et al., 2013) and it was defined for benzimidazoles as the interval time (expressed in weeks post-treatment) when the percent reduction in FEC decreases below a cutoff value of 80% based on the group arithmetic mean FEC. The expected cyathostomin egg reappearance period is 28–35 days (4–5 weeks) as indicated for benzimidazoles by Nielsen et al. (2013).

2.8. Pharmacokinetics and statistical analysis of data

The plasma concentration *vs* time curves obtained after each treatment in individual animals, were fitted with the WinNonlin software program (Version 5.2, Pharsight Corporation, Mountain View, CA, USA). The pharmacokinetic parameters for each animal were analysed using non-compartmental model analysis for *per os* administration. The maximum plasma concentration (C_{max}) and time to reach maximum concentration (t_{max}) were obtained from the plotted concentration-time curve of each drug in each animal. The trapezoidal rule was used to calculate the area under the plasma concentration time curve (AUC). The mean residence time (MRT) was calculated by the linear trapezoidal rule with extrapolation to infinity. Similar equations were used to determine the pharmacokinetics of MBZ in milk.

The pharmacokinetic parameters are reported as mean ± SD (standard deviation). Pharmacokinetic parameters lognormally distributed were statistically compared with a one-way analysis of variance (ANOVA). All statistical analyses were performed by using MINITAB for Windows (release 12.1, Minitab Inc., State College, PA, USA). Mean values were considered significantly different at $P < 0.05$.

For comparison of the anthelmintic efficacy of both MBZ dosages, statistical analysis of data were performed on arithmetic mean (AM) EPG counts using the parametric t-test to compare differences between treatment groups forsignificance at the $P < 0.01$ level.

3. Results

Clinically, no adverse reaction was observed in any of the donkeys treated with MBZ during the study. The analytical procedures and HPLC analysis of MBZ were validated. The linear regression lines for MBZ in the range between 0.005–0.5 μg/ml showed correlation coefficients of 0.9987. The mean recoveries of MBZ from plasma and milk were 88.87% ± 4.37and 75.33% ± 5.32. The detection limit of the analytical technique was 0.001 μg/ml; the quantification limit was 0.005 μg/ml for plasma and milk samples. The inter- and intra-assay precisions (mean ± SD) of the analytical

Table 2
Strongyle egg counts in eggs per gram (EPG) and percentage reductions in faecal egg counts (FECR) for donkeys treated with MBZ paste at a horse dosage −10 mg/kg B.W. (MBZ 1—group) and for donkeys treated with mebendazole paste at a double horse dosage—20 mg/kg B.W. (MBZ 2-group), compared with control untreated group (C-group) at each time study points.

Day	C group		MBZ 1 group		P value	FECR (%)	Upper–lower 95% CI	MBZ 2 group		*P* value	FECR (%)	Upper–lower 95% CI
	EPG AM	EPG Range	EPG AM	EPG Range				EPG AM	EPG Range			
−3	1347	1000–1800	1680	900–2240	0.1492	–	–	1533	730–2680	0.5858	–	
7	857	450–1170	3	0–20	0.0000	**99.6**	100–98.4	10	0–40	0.0000	**98.8**	99.9–97.1
14	942	500–1340	3	0–20	0.0000	**99.7**	100–98.4	7	0–30	0.0000	**99.3**	99.9–97.8
21	1185	890–1450	12	0–30	0.0000	**99.0**	99.7–98.3	12	0–30	0.0000	**99.0**	99.7–98.0
28	1528	1110–2030	23	10–50	0.0000	**98.5**	99.2–97.5	32	10–80	0.0000	**97.9**	99.1–95.2
35	1157	610–2000	175	10–340	0.0007	84.9	94.3–80.8	128	10–230	0.0004	88.9	95.9–82.9
42	982	520–1370	378	190–610	0.0015	61.5	90.7–65.8	305	40–450	0.0008	68.9	89.4–62.7
49	945	600–1680	570	80–970	0.1040	36.5	80.9–41.4	472	50–1100	0.0549	50.1	86.1–32.1
56	738	530–1130	648	180–1170	0.6065	12.2	78.6–37.5	683	70–1000	0.7509	7.5	75.9–17.5

*Parametric *t*-test (MBZ-groups *vs* C-group) values are significantly different $P < 0.001$ – arithmetic means (AM) – Confidence Intervals (CI).

technique presented a coefficient of variation of 6.11% ± 2.22 and 4.91% ± 3.12 for plasma and 5.34% ± 2.88 and 5.84% ± 3.65 for milk analysis, respectively.

Pharmacokinetic parameters of MBZ in plasma and milk following *per os* administration at dosages of 10 and 20 mg/kg are given in Table 1. The mean plasma and milk concentration *vs* time curves are shown in Fig. 1. Dose-dependent plasma dispositions of MBZ were observed at the increased dosage (10 mg/kg *vs* 20 mg/kg) in donkeys. MBZ reached the peak plasma concentrations (C_{max}: 0.04 and 0.07 μg/ml) at 7.33 and 8.00 h after at dosages of 10 and 20 mg/kg bodyweight, respectively. MBZ was not detected in any milk samples after *per os* administration at a dosage of 10 mg/kg. However, the parent drug reached 0.01 μg/ml peak concentration at 10.66 h and AUC_{milk}/AUC_{plasma} value was 0.18 at a dosage of 20 mg/kg bodyweight.

Regarding the parasitological examinations, pre-treatment EPG of 1347, 1680 and 1533 were observed for the control, MBZ 1 and MBZ 2 groups, respectively. At the start of the study the pre-treatment EPG were not significantly different ($P > 0.1$) between groups. Total EPG counts and efficacy values for both treated groups (MBZ 1 and MBZ 2) compared with the control untreated group (C-group) at each time study points are shown in Table 2. The results of the post-treatment EPG for both MBZ treatment groups were significantly different ($P < 0.001$) from the control group until day 28 following treatments. The MBZ formulations were efficient (>95% efficacy) on days 7, 14, 21 until day 28. The percentage reductions in faecal egg counts for the MBZ 1 group, compared to the C-group, were 99.6% on day 7; 99.7% on day 14; 99.0% on day 21 and 98.5% on day 28. The percentage reductions in faecal egg counts for the MBZ 2 group, compared to the C-group, were 98.8% on day 7; 99.3% on day 14; 99.0% on day 21 and 97.9% on day 28.There was no significant difference in anthelmintic efficacy between the two MBZ dosages. The ERP value were 35 days (5 weeks) for both treated MBZ groups. In all studied donkeys, coprocultures performed before the treatments revealed the presence of Cyathostominae. Faecal coltures performed on different days from C-group confirmed the presence of Cyathostominae. Coprocultures post-treatments revealed the presence of larvae of Cyathostominae in both MBZ groups.

4. Discussion

The pharmacokinetics and activity of benzimidazoles are particularly influenced by the physicochemical properties and metabolic pathways of the active molecules. These compounds are only sparingly soluble in water and their absorption and pharmacokinetics are affected by their aqueous solubility (Ngumuo et al., 1984). The rapid dissolution and consequent absorption and elimination of some benzimidazoles explain their relatively short residence in the body. Thus more soluble compounds have shorter residence in comparison to less soluble benzimidazoles, which are absorbed over prolonged periods (McKellar and Scott, 1990). The differences in efficacy of the benzimidazoles against parasites *in vivo* have been attributed largely to different pharmacokinetic profile of each compound within the host (Bogan, 1983).

MBZ is a broad spectrum anthelmintic which is effective against the major intestinal parasites of horses such as large and small strongyles, *Parascaris* spp. and *Oxyuris equi*.

MBZ, fenbendazole and oxfendazole were registered benzimidazole anthelmintics for use in donkeys in some countries including UK and Australia (McKellar and Scott, 1990). The pharmacokinetic profile of oxfendazole and albendazole in donkeys has been reported before (Gokbulut et al., 2006). However, the pharmacokinetic disposition of MBZ is firstly reported in the present study. Our results indicate that the plasma disposition of MBZ was different compared to those of oxfendazole and albendazole studied in donkeys at the same dosages (Gokbulut et al., 2006). It has been reported that the peak plasma concentrations and areas under the curve of oxfendazole (C_{max}: 0.49 μg/ml, AUC: 5.17 μg.h^2/ml) and albendazole (C_{max}: 0.08 μg/ml, AUC: 0.84 μg.h^2/ml) were higher than those of MBZ (C_{max}: 0.04 μg/ml, AUC: 0.78 μg.h^2/ml), respectively. Nevertheless, in the present study the $t_{1/2}$ (11.97 h) and MRT (20.34 h) values of MBZ are much longer compared with those of oxfendazole ($t_{1/2}$: 4.49 h, MRT: 10.95 h) and albendazole ($t_{1/2}$: 6.65 h, MRT: 9.15 h) at the same dosage (10 mg/kg). These differences probably reflect lower water solubility and subsequent lower absorption and/or more extensive the first-pass metabolism of MBZ compared with oxfendazole and albendazole. Similarly, low systemic availability has been observed with MBZ in human and it has been suggested that it is a result of the very low level of absorption of MBZ from the gastrointestinal tract (Munst et al., 1980). High intestinal concentrations could be effective against intestinal nematodes that inhabit the gut lumen, but very low plasma concentrations of MBZ such as demonstrated in horses, may not be effective against migrating larval stages of large and small strongyles, and encysted cyathostomin larvae. It has been indicated that the benzimidazoles including MBZ (at a dosage of 8.8 mg/kg bodyweight) were highly effective against adult large and small strongyles, adult *Oxyuris equi* and the 4th stage larvae in horses (Colglazier et al., 1977).

The results obtained in the present study plainly indicate that an increase in MBZ dosage is associated with elevation in the plasma level of MBZ in donkeys. Significantly higher AUC and C_{max} values for MBZ were observed after *per os* administration at 20 mg/kg compared to the treatment at 10 mg/kg (Table 1).

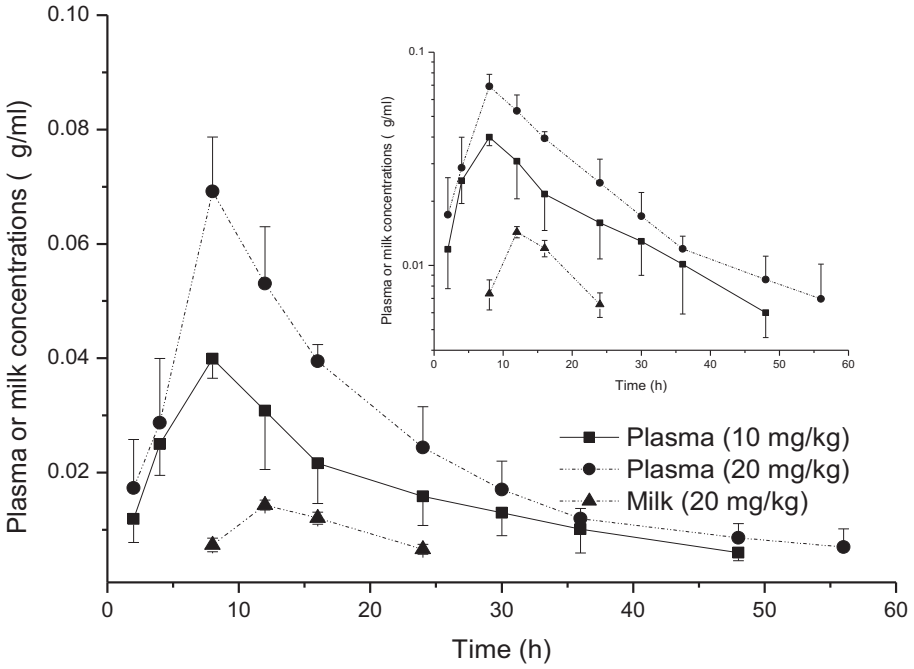

Fig. 1. Mean (±SD) plasma and milk concentrations (μg/g) *vs* time curves of mebendazole (MBZ) following *per os* administration to donkeys at 10 mg/kg body weight ($n = 6$) and 20 mg/kg bodyweight ($n = 6$). (Smaller graph: semi-logarithmic plasma and milk concentration *vs* time curves of MBZ).

The C_{max} and AUC values of MBZ increased from 0.04 μg/ml and 0.78 μg.h/mL at a dosage of 10 mg/kg to 0.07 μg/ml and 1.42 μg.h/mL at the higher dosage (20 mg/kg), respectively. Consequently, the increased dosage regime could be a strategy to provide higher plasma concentration and thus to improve the efficacy against the target parasites such as migrating larval or tissue stages of strongyles and lungworms in donkey, although the efficacy of such strategies should be confirmed before they can be recommended.

There are significant differences in the pharmacokinetics of benzimidazole anthelmintics between monogastrics and ruminant species (Marriner and Bogan, 1981; McKellar et al., 1990). The plasma disposition of MBZ in the present study differs substantially from those observed in sheep and goats previously reported (Galtier et al., 1994; Pandey and Roy, 1998). The present study indicates that the plasma concentration of MBZ after *per os* administration are relatively lower in donkeys compared with those observed in sheep and goats. In sheep, C_{max} (0.107 μg/ml) and AUC values (3.27 μg.h/ml) of MBZ after *per os* administration at a dosage of 25 mg/kg were greater than those observed for donkeys in this study after a dosage of 20 mg/kg. Moreover, Pandey and Roy (1998) reported that the plasma concentration of MBZ was maintained for 120 h and the peak concentration of MBZ was 5.8 μg/ml after *per os* administration at a dosage of 40 mg/kg. The reason for the lower systemic exposure in donkeys compared with that observed in ruminant species is probably related with differences in the physiological or anatomic properties between the animal species. Anatomic features influence the passage of digesta, and the bioavailability and pharmacokinetics of anthelmintics may be affected by different gut transit time in animal species (McKellar and Scott, 1990). The benzimidazole anthelmintic drugs have lower bioavailability in monogastric animals (*e.g.* dog and horse), which have relatively faster gut transit time than ruminants, since rapid passage of food in the gut causes a decrease in the bioavailability of the drugs.

Because of the relatively low systemic availability of MBZ, the low level of excretion of MBZ in milk (milk to plasma ratio = 0.18 at doble dosage) in association with a low MRL value led to advocate the use of MBZ in lactating donkeys at a dosage of 10 mg/kg bodyweight.

Therapeutic equivalence was demonstrated for MBZ 1 (horse dosage) and MBZ 2 (double horse dosage) based on FECR test. In this study, both MBZ dosages were highly effective (>95%) against cyathosmoninae in donkeys until 28 days post treatment. The cut-off values (mean percent reduction in FEC) for interpreting results of strongyle FECRT suggested in the guidelines of the AAEP for benzimidazoles, reported a range of 95–99% efficacy expected in the absence of resistance in horses. The cyathostomins parasitizing the studied donkeys were apparently susceptible to mebendazole, as confirmed by FECR values. The Egg Reappearance Period (ERP) of benzimidazole drugs in the horses is not expected to exceed 4–5 weeks (Nielsen et al., 2013); the ERPs found in our study for both MBZ treated Groups (horse and double horse dosages) following *per os* administration in donkeys are fairly similar (5 weeks) to that noted in horses parasitized by benzimidazoles susceptible small strongyle populations. The two dosages studied of MBZ did not seem to modify the anthelmintic efficacy in donkeys.

5. Conclusion

The donkey is not a small horse and requires species specific pharmacological and parasitological trials.The present study indicated that *per os* administration of MBZ has a minimal disposition rate into the milk and may be used in lactating donkeys with zero milk-withdrawal period at horse dosage of 10 mg/kg B.W. The results of FECRT for both MBZ dosages were efficient (>95% efficacy) until day 28, demonstrating that MBZ oral paste at horse dosage was effective and safety for the treatment of Cyathostominae in donkeys.

In conclusion, similar dosage regimens of MBZ could be used for horses and donkeys and, this member of benzimidazole could be used as anthelmintic for strongyles control program in donkey farms for the production of milk for human consumption.

Conflict of interest

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