Plasma disposition, concentration in the hair, and anthelmintic efficacy of eprinomectin after topical administration in donkeys

Cengiz Gokbulut, PhD; Antonio Di Loria, PhD; Necati Gunay, BS; Roberto Masucci, DVM; Vincenzo Veneziano, PhD

Objective—To investigate plasma disposition, concentration in the hair, and anthelmintic efficacy of eprinomectin after topical administration in donkeys.

Animals—12 donkeys naturally infected with strongyle nematodes.

Procedures—The pour-on formulation of eprinomectin approved for use in cattle was administered topically to donkeys at a dosage of 0.5 mg/kg. Heparinized blood samples and hair samples were collected at various times between 1 hour and 40 days after administration. Samples were analyzed via high-performance liquid chromatography with fluorescence detection. Fecal strongyle egg counts were performed by use of a modified McMaster technique before and at weekly intervals for 8 weeks after treatment.

Results—Plasma concentration and systemic availability of eprinomectin were relatively higher in donkeys, compared with values reported for other animal species. Concerning the anthelmintic efficacy against strongyle nematodes, eprinomectin was completely effective (100%) on days 7 and 14 and highly effective (> 99%) until the end of the study at 56 days after treatment. No abnormal clinical signs or adverse reactions were observed for any donkeys after treatment.

Conclusions and Clinical Relevance—Eprinomectin had excellent safety. The relatively high plasma concentration after topical administration could result in use of eprinomectin for the control and treatment of parasitic diseases in donkeys. (*Am J Vet Res* 2011;72:1639–1645)

Eprinomectin, a semisynthetic compound of the avermectin family, is marketed as a topical pour-on formulation for use in cattle at a dosage of 500 µg/kg.[1] Eprinomectin has been used extensively to control endoparasites and ectoparasites of dairy animals because it has a relatively high maximum residue limit, which suggests that eprinomectin can be used safely in lactating animals with no milk-withdrawal time.[2,3] Moreover, use of pour-on formulations of anthelmintics decreases the risk of injury for humans and animals, compared with that for use of injectable products, and is particularly convenient for farmers because they can apply the product easily.[4]

A paucity of data is available on the efficacy of anthelmintics used in donkeys because donkeys are often a neglected species for studies in domestic animals. Classes of drugs used in horses and ruminants are commonly extrapolated for use in donkeys without optimization of dosing regimens and determination of pharmacokinetic and pharmacodynamic properties. Because of the lack of drugs approved for use in donkeys, anthelmintics licensed for use in horses or ruminants are used at the same dosages for treatment of parasitic infections in donkeys. It has been reported that donkeys have a greater capacity to metabolize certain drugs, compared with the capacity for horses; thus, higher dosages or shorter intervals could be required to maintain effective drug concentrations in donkeys.[5–8]

Donkey milk is gaining popularity for consumption by humans in some countries, such as Italy, France, and Belgium, because it is the milk closest in composition to human milk and is ideal for feeding infants.[9]

Abbreviations

AUC	Area under the plasma concentration–time curve
Cmax	Maximum plasma concentration
FECR	Fecal egg count reduction
HPLC	High-performance liquid chromatography
MRT	Mean residence time
Tmax	Time to reach maximum plasma concentration

Received August 1, 2010.
Accepted October 26, 2010.
From the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine (Gokbulut), and ADUBILTEM Research and Development Centre (Gunay), University of Adnan Menderes, Isikli Koyu 09100 Aydin, Turkey; the Departments of Veterinary Clinical Sciences (Di Loria) and Pathology and Animal Health (Veneziano), Faculty of Veterinary Medicine, University of Naples Federico II, 80138 Naples, Italy; and Equine Practices, Clinica Nuovi Orizzonti, Via Siracusa, Castelvolturno, 81030 Caserta, Italy (Masucci).
Address correspondence to Dr. Gokbulut (cengizgokbulut@yahoo.com).

Furthermore, there is an increasing interest in raising donkeys because of the development of their use in leisure activities and onotherapy (a type of pet- or animal-assisted treatment popular in Italy that involves the use of donkeys) and, in particular, the rediscovery of donkey milk as a food source for children affected by allergies to cow milk.[10] To our knowledge, there have been no data reported regarding the pharmacokinetics and efficacy of eprinomectin against parasites in donkeys. Thus, the purpose of the study reported here was to determine the potential of topical administration as a treatment route in donkeys and to investigate the plasma disposition and anthelmintic efficacy of eprinomectin in donkeys after topical administration at a dosage used in cattle (0.5 mg/kg). Moreover, hair concentration was determined at the application sites and far from the application sites after topical administration of eprinomectin.

Materials and Methods

Animals—Twelve donkeys (8 nonpregnant females and 4 males) with a mean ± SD age of 5.0 ± 1.5 years were used in the study. Donkeys weighed between 128 and 380 kg; the body weight of each donkey was estimated 1 day before treatment by use of the nomogram proposed by a United Kingdom–based charity facility for donkeys.[11] The donkeys had a history of grazing on pasture contaminated with equine nematode parasites, but they were kept indoors and fed hay and concentrated feed formulated for horses during the study. The donkeys had not been treated with any anthelmintics during the 9 months preceding the study. Fecal examinations (fecal egg counts for each donkey and pooled coprocultures) performed before the beginning of the study revealed counts of > 150 eggs/g of feces for individual donkeys and a high prevalence of intestinal nematodes (*Cyathostomum* spp, *Poteriostomum* spp, *Triodontophorus* spp, and *Strongylus* spp) in all donkeys. The donkeys received an identification tag and were housed communally in an indoor pen until the study. Water was provided ad libitum throughout the course of the study. The study was approved by the Animal Ethics Committee of the University of Naples Federico II.

Treatments and sample collection—Extralabel administration of a pour-on formulation of eprinomectin licensed for use in cattle was used on the 12 donkeys in the present study. The dose was calculated on the basis of the body weight of each donkey. The pour-on formulation of eprinomectin[a] was administered topically at a dosage of 0.5 mg/kg along the dorsal midline of the donkeys. Pharmacokinetic assessment was performed for 8 donkeys (4 males and 4 females). Heparinized blood samples were collected via jugular venipuncture prior to drug administration (time 0) and then at 1, 2, 4, 8, 12, 16, 24, 32, 48, 72, 96, and 120 hours and 6, 8, 10, 12, 15, 20, 25, 30, 35, and 40 days after administration.

Hair samples (> 0.05 g) were also collected prior to drug administration and then at 24, 48, 72, 96, and 120 hours and 6, 8, 10, 12, 15, 20, 25, 30, 35, and 40 days after administration. Hair samples were collected with scissors from the application site and at a location far from the application site (ie, the ventral part of the thorax of each donkey) and used to determine concentrations in the hair attributable to administration of eprinomectin. To prevent cross-contamination, gloves were changed and scissors were washed with ethanol after collection of each hair sample. Blood samples were centrifuged at 2,000 × *g* for 30 minutes, and plasma was harvested and transferred to plastic tubes. All plasma and hair samples were stored at –20°C inside plastic tubes and bags, respectively, until analyzed to determine the drug concentration.

Analytic procedures—A stock solution (100 µg/mL) of a pure standard of eprinomectin[b] was prepared with acetonitrile[b] as the solvent. This stock solution was diluted to standard solutions of 5, 10, 100, 200, and 500 ng/mL and to 0.5, 1, 5, 10, and 50 µg/mL for plasma and hair samples, respectively. Standard solutions were used to provide calibration as standard curves and were added to drug-free plasma and hair samples to determine recovery.

Plasma concentration of eprinomectin was analyzed via HPLC with fluorescence detection following a solid-phase extraction procedure performed in accordance with a method described elsewhere.[12] Hair samples were analyzed via HPLC with a liquid-liquid phase extraction procedure adapted from the method described in another study.[13]

Briefly, drug-free plasma samples (1 mL) were spiked with the pure standard of eprinomectin to achieve final concentrations of 0.25, 0.5, 1, 10, 25, and 50 ng/mL Plasma samples (spiked and unknowns) were combined with 50 µL of internal standard (moxidectin[b] [250 ng/mL]) and then mixed with 1 mL of acetonitrile. The solvent-sample mixture was mixed for 5 minutes and then centrifuged at 10,000 × *g* for 10 minutes. Supernatant was transferred to a C_{18} solid-phase extraction cartridge[c] previously conditioned with 2 mL of methanol and 2 mL of deionized water. The cartridge was washed with 2 mL of a water:methanol mixture (3:1 [vol/vol]) and dried under vacuum for 1 hour. Analytes were eluted with 3 mL of methanol and concentrated to dryness at 45°C in a sample concentrator.[d] Reconstitution was performed by use of 100 mL of a solution of *N*-methylimidazole in acetonitrile (1:1 [vol/vol]). Derivatization was initiated by the addition of 150 mL of trifluoroacetic anhydride solution in acetonitrile (1:2 [vol/vol]). The samples were vortexed for 10 seconds, and 50 µL of glacial acetic acid[b] then was added. Samples were again vortexed for 10 seconds, and samples then were incubated at 65°C for 30 minutes. After incubation, samples were cooled at 4°C for 3 minutes, and an aliquot (50 µL) of this solution was injected into the chromatograph. The mobile phase consisted of acetonitrile, methanol, and ultrapure water (65:32:3 [vol/vol/vol]) and was delivered via a pump[e] at a flow rate of 1 mL/min. A nucleosil C_{18} analytic column[f] (particle size, 3 µm; dimension, 150 × 4.6 mm) with nucleosil C_{18} guard column[f] was used for analysis of the analytes. Fluorescence detection[e] was performed at an excitation wavelength of 365 nm and an emission wavelength of 475 nm.

The analytic methods used for eprinomectin in plasma and hair samples were validated prior to the start of the study. The analyte was identified with the retention times of the pure reference standard. Recov-

eries of the analytes were measured by comparison of the peak areas from 6 spiked plasma and hair samples with the areas resulting from injection of standards prepared in acetonitrile. The limit of detection and limit of quantification were determined for the HPLC method. The limits were determined on the basis of the SD and slope of the curve at the lowest concentrations. Calibration curves were fitted by use of 6 concentrations that ranged from 0.25 to 50 ng/mL for plasma and 0.5 to 50 µg/mL for hair.

Parasitologic analysis (fecal examinations and anthelmintic efficacy)—Fecal samples were obtained from the rectum of each of the 12 donkeys 3 days before the start of the study, on day 0, and on days 7, 14, 21, 28, 35, 42, 49, and 56 after treatment. Fecal samples were stored in a refrigerator at 4°C as proposed in another study.[14] Fecal egg counts were performed within 4 hours after sample collection. Fecal egg counts for individual donkeys were determined via a modified McMaster technique with a sensitivity of 10 eggs/g of feces by use of a sucrose flotation solution (specific gravity, 1.250).[15]

Individual fecal samples obtained on each day of sample collection were incubated at 27°C for 7 to 10 days for larval identification. Only fecal samples collected 3 days before the start of the study were pooled for analysis. Third-stage larvae were identified by use of the morphological keys proposed by the Ministry of Agriculture, Fisheries and Food.[15] When a pooled coproculture had ≤ 100 third-stage larvae, all were identified; however, when a pooled coproculture had > 100 third-stage larvae, only 100 were identified.

To determine the efficacy of eprinomectin against intestinal strongyles at each day of sample collection, the mean number of eggs per gram of feces was calculated in accordance with guidelines established by the World Association for the Advancement of Veterinary Parasitology.[16,17] For each donkey, the percentage efficacy was calculated in terms of FECR at each day of sample collection by use of the following equation:

$$\text{FECR} = (\text{[mean EPG before treatment} - \text{mean EPG after treatment]/mean EPG before treatment}) \times 100$$

where EPG is the number of eggs per gram of feces.

Pharmacokinetic and statistical analysis of data—The plasma concentration versus time curves obtained after each treatment in individual donkeys were fitted with a software program.[g] Pharmacokinetic parameters for each donkey were analyzed via noncompartmental model analysis for topical administration. The Cmax and Tmax were obtained from the plotted plasma concentration–time curve in each donkey. The trapezoidal rule was used to calculate the AUC, and MRT from 0 to infinity was calculated by use of the following equation:

$$MRT_{0-\infty} = AUMC_{0-\infty}/AUC_{0-\infty}$$

where $AUMC_{0-\infty}$ is the area under the moment curve from 0 to infinity and $AUC_{0-\infty}$ is the AUC from 0 to infinity. Terminal $t_{1/2}$ was calculated as $t_{1/2\lambda z} = -\ln(2)/\lambda_z$, where λz is the first-order rate constant associated with the terminal (log-linear) portion of the curve.

Table 1—Mean ± SD pharmacokinetic parameters for eprinomectin in 8 donkeys after topical administration (0.5 mg/kg) of a pour-on formulation licensed for use in cattle.

Parameter	Mean ± SD
$t_{1/2\lambda z}$ (d)	6.33 ± 0.90
Cmax (ng/mL)	14.17 ± 5.98
Tmax (d)	3.38 ± 0.74
AUC (ng•d/mL)	129.44 ± 36.69
AUMC (ng•d²/mL)	1,121.27 ± 250.20
MRT (d)	8.95 ± 1.78

$t_{1/2\lambda z}$ = Terminal $t_{1/2}$. AUMC = Area under the moment curve.

Results

The donkeys were observed throughout the study. No adverse reactions were observed in any of the donkeys treated with the eprinomectin pour-on formulation, and no licking behavior was observed. The analytic procedures and HPLC analysis of eprinomectin

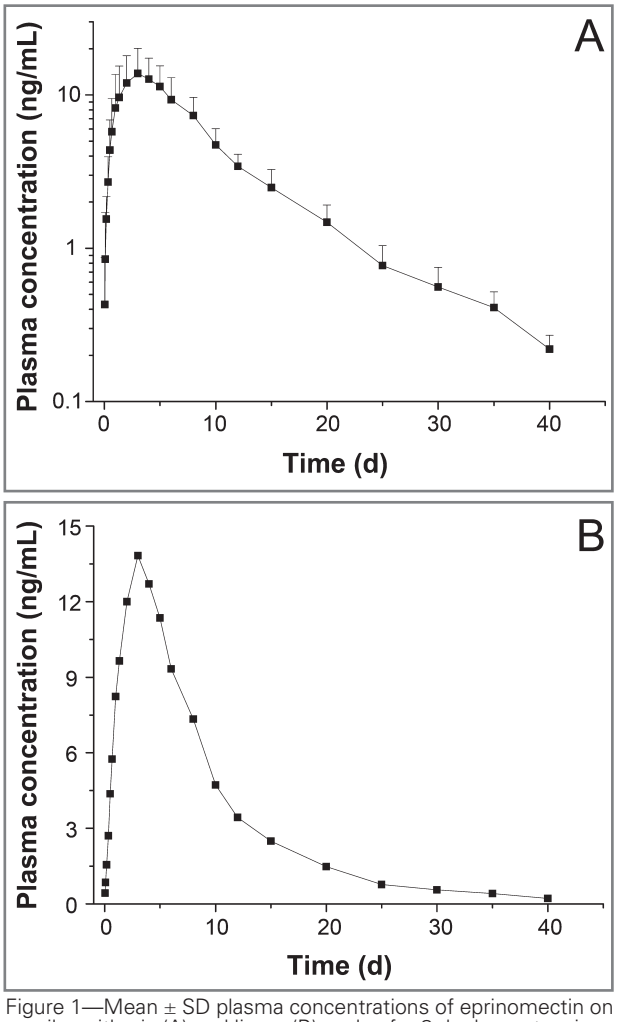

Figure 1—Mean ± SD plasma concentrations of eprinomectin on semilogarithmic (A) and linear (B) scales for 8 donkeys at various times after topical administration (0.5 mg/kg) of a pour-on formulation licensed for use in cattle. Day of eprinomectin administration was designated as day 0.

were validated. Mean recovery of eprinomectin from plasma was 88.91% with a relative SD < 10%. The limit of detection and limit of quantification for eprinomectin were 0.019 and 0.21 ng/mL, respectively. The interassay and intra-assay precision of the extraction and chromatography procedures were evaluated by processing on different days 6 replicate aliquots of drug-free donkey plasma samples that contained known amounts of eprinomectin. The precision determined at each concentration was < 15% of the coefficient of variation, and accuracy ranged from 95% to 102%.

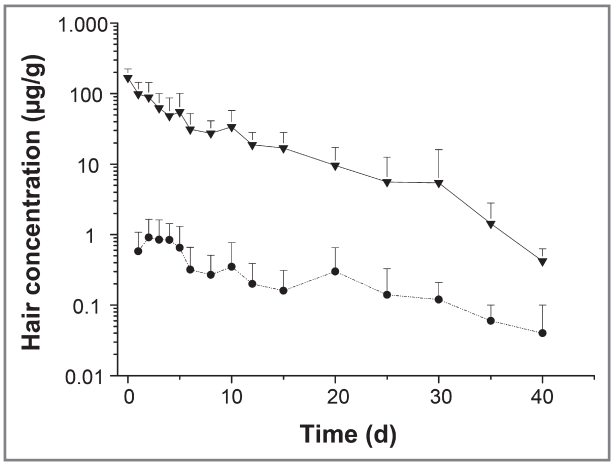

Figure 2—Mean ± SD eprinomectin concentration in hair samples collected from the application site (inverted triangles) and far from the application site (circles) in 8 donkeys at various times after topical administration (0.5 mg/kg) of a pour-on formulation licensed for use in cattle. Day of eprinomectin administration was designated as day 0.

Pharmacokinetic parameters of eprinomectin after topical administration (0.5 mg/kg) were summarized (**Table 1**). Mean plasma concentration versus time curves were plotted on semilogarithmic and linear scales (**Figure 1**). In addition, concentrations of eprinomectin in hair samples collected from the application site and far from the application site were plotted (**Figure 2**). The Cmax was 14.17 ng/mL, and Tmax was 3.38 days. The AUC and MRT of eprinomectin were 129 ng•d/mL and 8.95 days, respectively.

All donkeys shed strongyle eggs in their feces on the day of treatment (day 0). The individual and arithmetic mean of strongyle egg counts and FECRs at the various times of sample collection were summarized (**Table 2**). The mean strongyle egg count on day 0 was 1,361 eggs/g of feces (range, 200 to 3,190 eggs/g of feces). After treatment, 1 donkey was shedding 10 eggs/g of feces by day 21. By day 35, 3 donkeys were shedding eggs, and by day 49, 4 donkeys were shedding strongyle eggs. However, FECR remained high throughout the study period. The FECR was 100% on days 7 and 14 and > 99% from day 21 until the end of the study 56 days after treatment.

Cultures of pooled fecal samples performed at day 0 contained *Cyathostomum* spp, *Poteriostomum* spp, *Triodontophorus* spp, and *Strongylus vulgaris*. Cultures of fecal samples from 1 donkey at days 21 and 28 and from 3 donkeys on day 35 until the end of the study and

Table 2—Strongyle egg counts and FECR after topical administration of eprinomectin (0.5 mg/kg) to 12 donkeys.

	Day of study*									
Variable	**–3**	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**
Egg counts for individual donkeys (No. of eggs/g of feces)										
Mean	1,204	1,361	0	0	0.8	2.5	5.8	3.3	3.3	5.0
Range†	300–3,300 (0)	200–3,190 (0)	— (12)	— (12)	0–10 (11)	0–30 (11)	0–40 (9)	0–20 (9)	0–10 (8)	0–30 (8)
FECR (%)	—	—	100	100	99.9	99.8	99.6	99.7	99.7	99.6
Upper CL	—	—	—	—	100	100	100	100	100	100
Lower CL	—	—	—	—	99	98	98	99	99	99

*Day of topical administration of eprinomectin was designated as day 0. †Values in parentheses are the number of donkeys with an egg count of 0.
— = Not applicable. CL = Confidence limit.

Table 3—Plasma pharmacokinetic parameters for eprinomectin after topical administration in several animal species.

Species	**Dosage (µg/kg)**	**Cmax (ng/mL)**	**Tmax (d)**	**AUC (ng•d/mL)**	**$t_{1/2}$ (d)**	**MRT (d)**	**Reference**
Donkey	500	14.17	3.38	129.44	6.33	8.95	—
Buffalo	500	2.7	1.44	11.43	NR	4.49	2
Cattle							
Bos taurus	500	43.8	2.02	241.21	2.03	4.16	18
Bos indicus	500	8.8	1.30	30.63	1.95	3.38	19
Sheep (Istrian Pramenka)	500	2.2	2.20	13.60	5.40	7.70	20
	1,000	5.3	1.50	33.70	12.20	9.00	20
Goat (Saanen)	500	2.2	0.75	8.24	2.44	2.67	21
	1,000	2.98	0.99	15.68	3.04	3.69	21
	500	5.6	2.55	72.31	7.46	9.42	22
Camel	500	1.8	1.50	6.26	NR	5.30	23

— = Not applicable; results determined in the present study. NR = Not reported.

from 4 donkeys on day 49 until the end of the study had few *Cyathostomum* spp larvae.

Plasma pharmacokinetic parameters of eprinomectin for the donkeys of the present study were compared with those of other animal species following topical administration[2,18–23] (**Table 3**).

Discussion

The pharmacokinetic behavior of endectocides is considerably affected by the route of administration, formulation of the drug, and interspecies and interindividual variation.[24] Although many studies[25–31] have been performed to determine the pharmacokinetic behavior of endectocides in horses, there is little information on the plasma pharmacokinetics of endectocides in donkeys.[32,33]

Large variations in pharmacokinetics are evident among animal species after topical administration of eprinomectin as a result of anatomic and physiologic variation among animal species and irregular absorption of drug from the site of application associated with differences in coat length and hair density. In the present study, the plasma concentrations and systemic exposure of eprinomectin in donkeys were lower, whereas $t_{1/2}$ and MRT were longer, compared with results for cattle[18] administered eprinomectin at the same dosage. The origin of this difference is probably self-licking or licking other cattle that make the systemic availability and plasma disposition variable and unpredictable, considering that no licking behavior was observed in the donkeys of the present study. In contrast to cattle, donkeys typically do not have self-licking behavior. Large differences between animals that lick and those that do not lick have been observed after topical administration of ivermectin or doramectin.[34–36] Prevention of licking results in an extended terminal plasma $t_{1/2}$ and in a lower systemic availability and fecal excretion of ivermectin in cattle.[34]

The pharmacokinetic behavior of eprinomectin in the present study after topical administration in donkeys differed substantially from that in buffalo, sheep, goats, and camels (Table 3). Plasma concentration and systemic exposure of eprinomectin are much higher in donkeys, compared with those in the aforementioned animals, after topical administration. Although the reason for the higher systemic exposure of eprinomectin in donkeys, compared with systemic exposure in other animal species, is unclear, the relatively high AUC in donkeys can be explained by a low plasma clearance or a relatively high bioavailability. Differences among animal species may relate to histologic and physiologic variations in structure of the skin and coat because it has been reported that the comparative number of cell layers, epidermal and corneum thickness, and blood flow to various parts of the skin differ among animal species.[37] Moreover, hair follicle density and length of coat and the secretions from sweat and sebaceous glands are important variables for differences in drug absorption from the skin among animal species.[38]

The pharmacokinetic disposition of eprinomectin after topical administration in donkeys in the present study differed substantially from that of ivermectin reported in horses[31] at the same dosage and via the same route of administration. In the study reported here, a shorter Tmax (3.38 days), higher Cmax (14.17 ng/mL), and larger AUC (129.4 ng•d/mL) but shorter $t_{1/2}$ (6.33 days) and MRT (8.95 days) of eprinomectin were detected in donkeys, compared with those detected after topical administration of ivermectin in horses (Tmax, 4.33 days; Cmax, 4.29 ng/mL; AUC, 59.93 ng•d/mL; $t_{1/2}$, 7.18 days; and MRT, 11.8 days).[31] The origin of this difference is unclear. Differences in the physical-chemical structure between eprinomectin and ivermectin are the most likely reason because the endectocides have structural features that affect their pharmacokinetics. Substitution of the C-4 hydroxyl group with acyl, amino, or thio groups improves the solubility and tissue distribution of avermectins, and this feature has been used for the epiacetyl amino substitution in eprinomectin that retains and enhances the potency and spectrum of activity of this avermectin, which is effectively absorbed percutaneously and possesses a very low plasma-to-milk partitioning ratio (0.17) that permits use in lactating dairy cattle[39] (plasma-to-milk partitioning ratio for ivermectin in dairy cattle, 0.76).[40] The low plasma-to-milk partition coefficient of eprinomectin suggests that structural conformation independent of absolute lipid solubility may be important for transfer between the mammary gland capillary vasculature and alveolar epithelium. Moreover, differences in the physiologic and histologic structure of the skin or coat between donkeys and horses probably play important roles for transfer of the drug from the surface of the skin into the systemic circulation.

Despite these differences in pharmacological assessment, eprinomectin has a broad spectrum of activity against endoparasites and ectoparasites in several large animal species. Regarding the intestinal nematodes, eprinomectin has high efficacy in cattle,[39,41] sheep,[42] and goats.[43] There are scarce data on the use of avermectins in donkeys. Furthermore, to our knowledge, no data regarding the pharmacokinetic behavior and parasitological efficacy of eprinomectin against intestinal strongyles in donkeys have been reported.

Relatively high concentrations and slow degradation of eprinomectin in hair samples collected from the application site were observed in the present study. In addition, detectable concentrations in hair samples collected far from the application site were likely attributable to the excretion of eprinomectin by sebaceous glands (after absorption of a fraction of eprinomectin). It has been found that moxidectin is excreted by sebaceous glands.[44] These results suggest that eprinomectin was available at the skin surface for a long period after topical administration and thus probably provided long persistence of efficacy against ectoparasites in donkeys. In addition, the $t_{1/2}$ for concentrations in the hair at the application site was much longer (27.06 days), compared with the $t_{1/2}$ for plasma (6.33 days). This lack of parallelism between the 2 curves could suggest that the $t_{1/2}$ of absorption is not the limiting rate for eprinomectin disposition in donkeys.

Relatively high drug concentrations detected in the coat of donkeys may result in potential contamination to humans, especially if the donkeys are used for milking. The people who milk the donkeys should be aware of this concern and take precautions

to prevent cross-contamination after topical drug administration.

Analysis of results of the present study indicated that topical application of eprinomectin at the dosage recommended for cattle (0.5 mg/kg) did not cause safety issues in donkeys. Eprinomectin was tolerated well by all the donkeys, with no adverse reactions following treatment.

The present study indicated that the plasma concentration of eprinomectin after topical administration is relatively greater in donkeys, compared with that in other animal species (Table 3). Eprinomectin was highly effective against intestinal strongyles in all the donkeys, and higher FECRs were observed after topical application. The persistence of eprinomectin on the coat at the application site and far from the application site after topical administration may prolong the efficacy against ectoparasites, especially for nonbloodsucking parasites such as biting lice. Considering the pharmacokinetic disposition and anthelmintic efficacy, topical administration of eprinomectin could be used for the control and treatment of parasitic diseases in donkeys. Nevertheless, further studies are required to determine the milk excretion and milk-withdrawal time for eprinomectin after topical administration in milking donkeys.

a. Eprinex pour-on formulation, 0.5% wt/vol, Merial, Assago, Italy.
b. Sigma Chemical Co, St Louis, Mo.
c. AccuBOND, 200 mg/3 mL, Agilent, Waldron, Germany.
d. Maxi-dry Plus, Heto Lab Equipment, Allerød, Denmark.
e. 1100 Series, Agilent, Waldron, Germany.
f. Luna, Phenomenex, Macclesfield, Cheshire, England.
g. WinNonlin, version 5.2, Pharsight Corp, Mountain View, Calif.

References

1. Shoop WL, Egerton JR, Eary CH, et al. Eprinomectin: a novel avermectin for use as a topical endectocide for cattle. *Int J Parasitol* 1996;26:1237–1242.
2. Dupuy J, Sutra JF, Alvinerie M, et al. Plasma and milk kinetic of eprinomectin and moxidectin in lactating water buffaloes (*Bubalus bubalis*). *Vet Parasitol* 2008;157:284–290.
3. Baoliang P, Yuwan W, Zhende P, et al. Pharmacokinetics of eprinomectin in plasma and milk following subcutaneous administration to lactating dairy cattle. *Vet Res Commun* 2006;30:263–270.
4. Hennessy DR. Modifying the formulation or delivery mechanism to increase the activity of anthelmintic compounds. *Vet Parasitol* 1997;72:367–382.
5. Welfare R, Mealey KL, Matthews N, et al. Pharmacokinetics of gentamicin in donkeys. *J Vet Pharmacol Ther* 1996;19:167–169.
6. Mealey KL, Matthews NS, Peck KE, et al. Comparative pharmacokinetics of phenylbutazone and its metabolite oxyphenbutazone in clinically normal horses and donkeys. *Am J Vet Res* 1997;58:53–55.
7. Coakley M, Peck KE, Taylor TS, et al. Pharmacokinetics of flunixin meglumine in donkeys, mules, and horses. *Am J Vet Res* 1999;60:1441–1444.
8. Peck KE, Matthews NS, Taylor TS, et al. Pharmacokinetics of sulfamethoxazole and trimethoprim in donkeys, mules, and horses. *Am J Vet Res* 2002;63:349–353.
9. Monti G, Bertino E, Muratore MC, et al. Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: an in vivo and in vitro study. *Pediatr Allergy Immunol* 2007;18:258–264.
10. Carroccio A, Cavataio F, Montalto G, et al. Intolerance to hydrolysed cow's milk proteins in infants: clinical characteristics and dietary treatment. *Clin Exp Allergy* 2000;30:1597–1603.
11. The Donkey Sanctuary. Donkey nomogram. Available at: drupal.thedonkeysanctuary.org.uk/files/donkeys/Nomogram.pdf. Accessed Sep 18, 2010.
12. Danaher M, O'Keeffe M, Glennon JD, et al. Development and optimisation of an improved derivatisation procedure for the determination of avermectins and milbemycins in bovine liver. *Analyst (Lond)* 2001;126:576–580.
13. Scott EW, McKellar QA. The distribution and some pharmacokinetic parameters of ivermectin in pigs. *Vet Res Commun* 1992;16:139–146.
14. Nielsen MK, Vidyashankar AN, Andersen UV, et al. Effects of fecal collection and storage factors on strongylid egg counts in horses. *Vet Parasitol* 2010;167:55–61.
15. Ministry of Agriculture Fisheries and Food. *Manual of veterinary parasitological laboratory techniques*. London: Her Majesty's Stationery Office, 1986.
16. Duncan JL, Abbott EM, Arundal JH, et al. World Association for the Advancement of Veterinary Parasitology (WAAVP): second edition of guidelines for evaluating the efficacy of equine anthelmintics. *Vet Parasitol* 2002;103:1–18.
17. Coles GC, Jackson F, Pomroy W, et al. The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol* 2006;136:167–185.
18. Alvinerie M, Sutra JF, Galtier P, et al. Pharmacokinetics of eprinomectin in plasma and milk following topical administration to lactating dairy cattle. *Res Vet Sci* 1999;67:229–232.
19. Bengone-Ndong T, Ba MA, Kane Y, et al. Eprinomectin in dairy zebu Gobra cattle (*Bos indicus*): plasma kinetics and excretion in milk. *Parasitol Res* 2006;98:501–506.
20. Hodoscek L, Grabnar I, Milcinski L, et al. Linearity of eprinomectin pharmacokinetics in lactating dairy sheep following pour-on administration: excretion in milk and exposure of suckling lambs. *Vet Parasitol* 2008;154:129–136.
21. Dupuy J, Chartier C, Sutra JF, et al. Eprinomectin in dairy goats: dose influence on plasma levels and excretion in milk. *Parasitol Res* 2001;87:294–298.
22. Alvinerie M, Lacoste E, Sutra JF, et al. Some pharmacokinetic parameters of eprinomectin in goats following pour-on administration. *Vet Res Commun* 1999;23:449–455.
23. Bengoumi M, Hidane K, Bengone N, et al. Pharmacokinetics of eprinomectin in plasma and milk in lactating camels (*Camelus dromedarius*). *Vet Res Commun* 2007;31:317–322.
24. McKellar QA, Benchaoui HA. Avermectins and milbemycins. *J Vet Pharmacol Ther* 1996;19:331–351.
25. Marriner SE, McKinnon I, Bogan JA. The pharmacokinetics of ivermectin after oral and subcutaneous administration to sheep and horses. *J Vet Pharmacol Ther* 1987;10:175–179.
26. Perez R, Godoy C, Palma C, et al. Plasma profiles of ivermectin in horses following oral or intramuscular administration. *J Vet Med A Physiol Pathol Clin Med* 2003;50:297–302.
27. Perez R, Cabezas I, Godoy C, et al. Pharmacokinetics of doramectin and ivermectin after oral administration in horses. *Vet J* 2002;163:161–167.
28. Perez R, Cabezas I, Garcia M, et al. Comparison of the pharmacokinetics of moxidectin (Equest) and ivermectin (Eqvalan) in horses. *J Vet Pharmacol Ther* 1999;22:174–180.
29. Perez R, Godoy C, Palma C, et al. Plasma disposition and fecal elimination of doramectin after oral or intramuscular administration in horses. *Vet Parasitol* 2010;170:112–119.
30. Gokbulut C, Nolan AM, McKellar QA. Pharmacokinetic disposition and faecal excretion of pyrantel embonate following oral administration in horses. *J Vet Pharmacol Ther* 2001;24:77–79.
31. Gokbulut C, Cirak VY, Senlik B, et al. Comparative plasma dispositions and bioavailability of ivermectin following oral and pour-on administration in horses. *Vet Parasitol* 2010;170:120–126.
32. Scott EW. Pharmacokinetics of ivermectin in donkeys and ponies, in *Proceedings*. 15th Meet Assoc Vet Clin Pharmacol Ther 1997;20–21.
33. Gokbulut C, Boyacioglu M, Karademir U. Plasma pharmacokinetics and faecal excretion of ivermectin (Eqvalan paste) and doramectin (Dectomax, 1%) following oral administration in donkeys. *Res Vet Sci* 2005;79:233–238.
34. Laffont CM, Alvinerie M, Bousquet-Melou A, et al. Licking be-

haviour and environmental contamination arising from pour-on ivermectin for cattle. *Int J Parasitol* 2001;31:1687–1692.
35. Laffont CM, Bousquet-Melou A, Bralet D, et al. A pharmacokinetic model to document the actual disposition of topical ivermectin in cattle. *Vet Res* 2003;34:445–460.
36. Sallovitz JM, Lifschitz A, Imperiale F, et al. Doramectin concentration profiles in the gastrointestinal tract of topically-treated calves: influence of animal licking restriction. *Vet Parasitol* 2005;133:61–70.
37. Riviere JE, Papich MG. Potential and problems of developing transdermal patches for veterinary applications. *Adv Drug Deliv Rev* 2001;50:175–203.
38. Monteiro-Riviere NA, Baynes RE, Riviere JE. Animal skin morphology and dermal absorption. In: Roberts MS, Walters KA, eds. *Dermal absorption and toxicity assessment*. 2nd ed. New York: Informa Healthcare USA Inc, 2008;17–36.
39. Shoop WL, Demontigny P, Fink DW, et al. Efficacy in sheep and pharmacokinetics in cattle that led to the selection of Eprinomectin as a topical endectocide for cattle. *Int J Parasitol* 1996;26:1227–1235.
40. Toutain PL, Campan M, Galtier P, et al. Kinetic and insecticidal properties of ivermectin residues in the milk of dairy cows. *J Vet Pharmacol Ther* 1988;11:288–291.
41. Dorny P, Demeulenaere D, Smets K, et al. Persistent efficacy of topical doramectin and eprinomectin against *Ostertagia ostertagi* and *Cooperia oncophora* infections in cattle. *Vet Rec* 2000;147:139–140.
42. Cringoli G, Rinaldi L, Veneziano V, et al. Efficacy of eprinomectin pour-on against gastrointestinal nematode infections in sheep. *Vet Parasitol* 2003;112:203–209.
43. Cringoli G, Rinaldi L, Veneziano V, et al. Effectiveness of eprinomectin pour-on against gastrointestinal nematodes of naturally infected goats. *Small Rumin Res* 2004;55:209–213.
44. Dupuy J, Sutra JF, Alvinerie M. Pharmacokinetics assessment of moxidectin long-acting formulation in cattle. *Vet Parasitol* 2007;147:252–257.