

# Pharmacokinetics of ponazuril after oral administration to healthy llamas (*Lama glama*)

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**Objective**—To determine the pharmacokinetics after oral administration of a single dose of ponazuril to healthy llamas.

**Animals**—6 healthy adult llamas.

**Procedures**—Ponazuril (20 mg/kg) was administered once orally to 6 llamas (day 0). Blood samples were obtained on days 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 9, 11, 14, 21, 28, 35, 42, and 49. Serum ponazuril concentrations were determined by use of a validated reverse-phase high-performance liquid chromatography assay with UV absorbance detection. Pharmacokinetic parameters were derived by use of a standard noncompartmental pharmacokinetic analysis.

**Results**—Mean  $\pm$  SD area under the serum concentration–time curve was  $7,516 \pm 2,750$  h•mg/L, maximum serum ponazuril concentration was  $23.6 \pm 6.0$  mg/L, and the elimination half-life was  $135.5 \pm 16.7$  hours. Serum concentration of ponazuril peaked at 84 hours (range, 48 to 120 hours) after administration and gradually decreased but remained detectable for up to 35 days after administration. No adverse effects were observed during the study period.

**Conclusions and Clinical Relevance**—The rate and extent of absorption following oral administration of a single dose of ponazuril were sufficient to result in potentially effective concentrations, and the drug was tolerated well by llamas. At this dose, ponazuril resulted in serum concentrations that were high enough to be effective against various Apicomplexans on the basis of data for other species. The effective ponazuril concentration that will induce 50% inhibition of parasite growth for *Eimeria macusaniensis* in camelids is currently unknown. (*Am J Vet Res* 2011;72:1386–1389)

*Eimeria macusaniensis* is a large coccidian parasite of the phylum Apicomplexa that causes fatal enteritis in camelids throughout the world.<sup>1–3</sup> Camelids may be affected at any age, and they often have nonspecific clinical signs, such as weight loss, lethargy, and decreased appetite.<sup>3</sup> However, some clinically affected animals have gastrointestinal signs that range from mild nonhemorrhagic diarrhea to severe enteritis associated with substantial protein loss, and it may even result in death.<sup>1,3,4</sup> Currently, there are no drugs approved for the treatment of camelids infected with *E macusaniensis*, and the authors are not aware of any controlled studies performed to evaluate the efficacy of available anticoccidial products. However, some of the anticoccidial

## ABBREVIATIONS

AUC <sub>0–∞</sub>	Area under the serum concentration–time curve from time 0 to infinity
C <sub>max</sub>	Maximum serum concentration
MRT	Mean residence time
t <sub>1/2</sub>	Terminal half-life
T <sub>max</sub>	Time to maximum serum concentration

drugs that have been used include sulfonamides, amprolium, decoquinate, and ponazuril.<sup>3,5,6</sup> Response of clinically affected camelids to these compounds appears to be controversial. Although some clinicians maintain that standard anticoccidial drugs, such as amprolium and decoquinate, are ineffective or marginally effective against *E macusaniensis*, their administration may have been associated with substantial clinical improvement in 1 report.<sup>3</sup> Thus, current treatment practices are empirical and primarily extrapolated from protocols used to treat coccidia infections in other species.

Of concern is the potential for interspecies differences in drug metabolism. Phylogenetic divergence, such as digestive functions (ruminant vs nonruminant or carnivore vs herbivore), between species affects drug absorption.<sup>7</sup> For example, in 1 study,<sup>8</sup> investigators detected major differences in the metabolism of ivermectin between cattle and camels. Camelids are considered

Received June 23, 2010.

Accepted August 11, 2010.

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Presented in abstract form at the American College of Veterinary Internal Medicine Forum, Anaheim, Calif, June 2010.

The authors thank Dr. Steven A. Ripp for technical assistance.

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pseudoruminants because of the remarkable anatomic and histologic differences of their forestomachs, compared with those of true ruminants.<sup>9</sup> Thus, extrapolation of treatment protocols from other species, even ruminants, may provide an inappropriate treatment regimen for camelids (ie, dosage is too low, frequency of administration is insufficient, or dosage is too high and leads to toxicosis).

Ponazuril is a metabolite of toltrazuril and is approved by the FDA for the treatment of infections attributable to *Sarcocystis neurona* in horses.<sup>10</sup> In addition, ponazuril is an effective anticoccidial drug in several host species (sheep,<sup>11</sup> goats,<sup>12</sup> chickens,<sup>13</sup> mice,<sup>14</sup> cattle,<sup>15,16</sup> dogs,<sup>17</sup> pigs,<sup>18</sup> and rabbits<sup>19</sup>) and against other Apicomplexans (*Toxoplasma gondii*,<sup>20</sup> *Neospora caninum*,<sup>21,22</sup> and *Sarcocystis neurona*<sup>10,21</sup>). In contrast to results for other anticoccidial drugs, ponazuril has coccidiocidal action against all intracellular forms or developmental stages and does not interfere with the development of natural immunity.<sup>23</sup> Ponazuril currently is used to treat *E macusaniensis* infections in camelids despite a lack of information regarding its efficacy. Therefore, the purpose of the study reported here was to describe the pharmacokinetics of ponazuril in healthy llamas following oral administration of a single dose.

## Materials and Methods

**Animals**—Six healthy adult (4 client-owned<sup>a</sup> and 2 university-owned) llamas (*Lama glama*) were used in the study. The llamas (4 males and 2 females) ranged from 3 to 13 years of age (mean  $\pm$  SD, 6.3  $\pm$  4.0 years). Body weight of the llamas ranged from 153 to 173 kg (mean, 158  $\pm$  16 kg). Llamas were housed as a group in a pasture. Llamas had ad libitum access to hay and water and were fed a pelleted ration. The University of Tennessee Institutional Animal Care and Use Committee approved the experimental protocol.

**Preparation for the experiment**—Two days before the beginning of the study, the llamas were placed in indoor stalls in pairs in a well-ventilated, climate-controlled barn. Attitude and appetite of llamas were assessed twice daily, and rectal temperature, heart rate, respiratory rate, and motility of the first compartment were assessed once daily. The day before the onset of the experiment, a 14-gauge, 20-cm, long-term, over-the-wire catheter<sup>b</sup> was aseptically inserted in the right jugular vein of each llama for use in collection of blood samples. Food was removed 10 hours before onset of the experiment.

**Experimental design**—All llamas received a single dose of ponazuril<sup>c</sup> (20 mg/kg, PO). The drug was administered via a syringe, and care was exercised to ensure that animals ingested the entire dose. Blood samples were collected from the jugular catheter or via venipuncture into sterile red-top tubes without additives immediately before (time 0) and 0.5, 1, 2, 3, 4, 5, 6, 7, 9, 11, 14, 21, 28, 35, 42, and 49 days after administration. Blood samples were allowed to clot and then were centrifuged (3,220  $\times$  g for 10 minutes); serum was harvested and stored at  $-80^{\circ}\text{C}$  until analyzed.

**Drug analysis**—Serum samples were analyzed by use of a validated reverse-phase high-performance liq-

uid chromatography assay with UV-absorbance detection to determine ponazuril serum concentrations in each llama. Briefly, serum samples (50  $\mu\text{L}$ ) were fortified with 100  $\mu\text{L}$  of an internal standard (toltrazuril; 250  $\mu\text{g}/\text{mL}$ ). Acetonitrile (450  $\mu\text{L}$ ) was added to precipitate serum proteins. Samples were then centrifuged at 8,500  $\times$  g for 5 minutes. Following centrifugation, the supernatant was transferred to another vial and diluted with 800  $\mu\text{L}$  of acetonitrile–0.1% formic acid (dilution of 1:3 [vol/vol]). Diluted samples were then analyzed by use of reverse-phase high-performance liquid chromatography.

Chromatographic separation of the compounds was performed on a 5- $\mu\text{m}$  4.6  $\times$  150-mm column<sup>d</sup> with an isocratic flow of 0.5 mL/min. The mobile phase consisted of a mixture of acetonitrile (54%) and 0.1% formic acid solution (46%). Ponazuril and the internal standard toltrazuril were detected at a UV absorbance of 254 nm.

Standard curves for analysis were prepared by fortifying untreated llama serum with ponazuril to yield a linear portion of the curve ( $R^2 = 0.993$ ) for concentrations that ranged from 0.3 to 100  $\mu\text{g}/\text{mL}$ . Accuracy and precision of the assay were assessed by repeated measures of quality-control samples at concentrations throughout the calibration curve.<sup>24</sup> Accuracy was determined as  $\pm 6.9\%$  and  $\pm 9.0\%$  for intraday and interday measurements, respectively. Precision as a measure of reproducibility of the assay was 7.2% and 7.3% for intraday and interday measurements, respectively.

**Pharmacokinetic analysis**—The pharmacokinetic parameters  $t_{1/2}$ ,  $T_{\text{max}}$ ,  $C_{\text{max}}$ ,  $\text{AUC}_{0-\infty}$ , oral clearance, and MRT were calculated for ponazuril. Pharmacokinetic parameters were determined via noncompartmental analysis of the obtained serum concentration-time curves by use of a software program.<sup>e</sup>

## Results

Analysis of serum samples revealed substantial absorption of ponazuril after oral administration of a single dose of ponazuril to healthy llamas. Samples obtained be-

Table 1—Measured serum concentrations of ponazuril in samples obtained from 6 healthy llamas (*Lama glama*) following oral administration of a single dose of ponazuril (20 mg/kg).

Time (d)*	Mean $\pm$ SD	Range
0	NA	BLQ
0.5	7.23 $\pm$ 1.93	1.50–15.49
1	16.20 $\pm$ 1.00	12.97–19.76
2	19.79 $\pm$ 2.18	12.58–27.25
3	20.94 $\pm$ 1.68	16.10–26.08
4	21.76 $\pm$ 2.80	13.96–30.60
5	20.12 $\pm$ 2.32	14.30–27.43
6	19.98 $\pm$ 2.45	13.16–26.05
7	16.21 $\pm$ 2.46	7.25–22.84
9	15.39 $\pm$ 2.13	8.41–22.89
11	13.84 $\pm$ 2.21	7.13–19.81
14	10.62 $\pm$ 2.65	4.69–18.30
21	4.34 $\pm$ 1.01	1.88–8.26
28	1.85 $\pm$ 0.51	0.64–3.43
35	0.75 $\pm$ 0.16	0.33–1.23
42	NA	BLQ
49	NA	BLQ

Values reported are mg/L.  
\*Time of ponazuril administration was designated as day 0.  
BLQ = Below limit of quantification. NA = Not applicable.

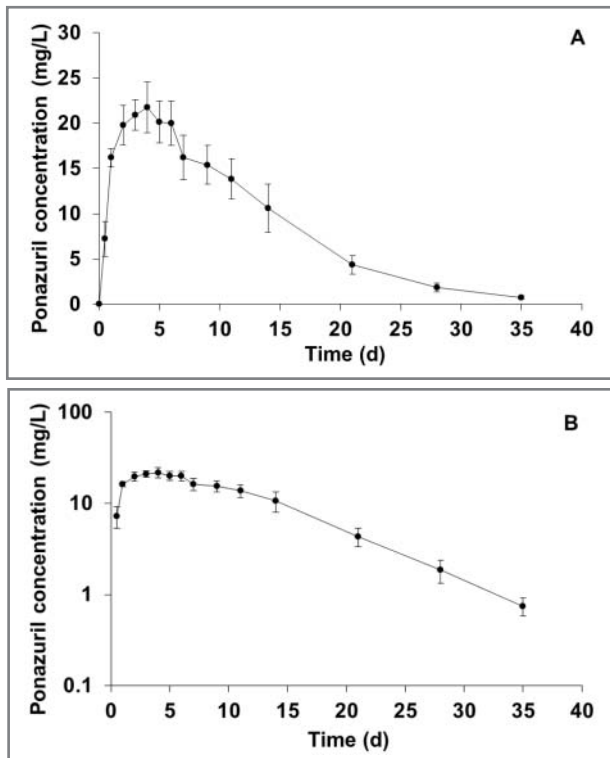


Figure 1—Serum concentration–time curve for ponazuril following oral administration of a single dose (20 mg/kg) to 6 healthy llamas (*Lama glama*; A) and a semilogarithmic graph of the serum concentration–time curve for ponazuril (B). Values reported are mean  $\pm$  SD. Time of ponazuril administration was designated as time 0.

fore drug administration had no detectable concentrations of ponazuril. Mean  $\pm$  SD serum ponazuril concentration was  $7.23 \pm 4.73$  mg/L at the first sample collection after administration (ie, 0.5 days). The serum concentration of ponazuril was below the limit of quantification in samples obtained  $\geq 42$  days after drug administration (Table 1). The serum concentration–time profile for ponazuril was determined (Figure 1).

Mean  $\pm$  SD  $T_{max}$  was  $84 \pm 25$  hours, and mean  $C_{max}$  was  $23.6 \pm 6.0$  mg/L (range, 16.6 to 30.6 mg/L; Table 2). The elimination of ponazuril was slow, with a mean serum  $t_{1/2}$  of  $135.5 \pm 16.7$  hours. Llamas varied in their ability to eliminate ponazuril, with  $t_{1/2}$  ranging from 115.5 to 160.0 hours. Mean  $AUC_{0-\infty}$  was  $7,516 \pm 2,750$  hours $\cdot$ mg/L. Mean oral clearance was  $3.0 \pm 1.12$  mL/h/kg, and mean MRT was  $251.3 \pm 32.6$  hours. Adverse effects were not observed in any of the llamas during the study period.

## Discussion

Ponazuril is a triazine derivative with therapeutic properties against protozoan parasites of the phylum Apicomplexa.<sup>25</sup> Its efficacy against coccidia has been established in numerous species including sheep,<sup>11</sup> goats,<sup>12</sup> chickens,<sup>13</sup> mice,<sup>14</sup> cattle,<sup>15,16</sup> dogs,<sup>17</sup> pigs,<sup>18</sup> and rabbits.<sup>19</sup> Although ponazuril is currently used to treat *E macusaniensis* infections in camelids, there are no data that support its efficacy for this indication. The pharmacokinetics of orally administered ponazuril in healthy llamas were characterized in the present study.

Ponazuril was absorbed following a single dose (20 mg/kg, PO) and was detected in the serum of all 6 llamas, with drug concentrations detected at 12 hours after administration. Mean  $\pm$  SD  $C_{max}$  in llamas ( $23.6 \pm 6.0$  mg/L) was higher than that observed in cattle<sup>26</sup> (4.56 mg/L) receiving ponazuril at a dosage of 5 mg/kg and horses<sup>27</sup> (11.17 mg/L) receiving ponazuril at a dosage of 10 mg/kg, but this appears to be a dose-dependent effect. The dose-adjusted  $C_{max}$  is extremely similar in all 3 species, with values of 1.18, 0.91, and 1.12 mg/L for a 1 mg/kg dose in llamas, cattle, and horses, respectively. The mean  $C_{max}$  varied among the 6 llamas tested, with the peak concentration ranging from 16.6 to 30.6 mg/L. Similarly, the serum ponazuril  $t_{1/2}$  in each llama varied from 115.5 to 160 hours (mean, 135.5 hours). The mean plasma  $t_{1/2}$  of ponazuril has been reported as 58 hours for cattle<sup>26,28</sup> and 61.4 hours for horses.<sup>27</sup> Analysis of our data suggests that ponazuril is eliminated much slower (almost a 2-fold difference) in llamas than in cattle and horses. Alternatively, the intersubject and interspecies variability observed in the  $t_{1/2}$  of ponazuril may be influenced by the rate of oral absorption, which can be affected by potential binding of ponazuril to food particles within the gastrointestinal tract. Thus, the increased  $t_{1/2}$  in llamas may in fact be a result of flip-flop pharmacokinetics and therefore a reflection of the ponazuril absorption rate rather than its elimination rate constant. Although ponazuril was detected in the serum at the first sample obtained 12 hours after drug administration, the absorption rate appeared slow and variable, with  $T_{max}$  ranging from 48 to 120 hours (mean  $\pm$  SD,  $84 \pm 25$  hours).

We were unable to determine the oral bioavailability of ponazuril in llamas because we did not have access to a formulation that would allow for IV administration. Similarly, we ignored whether there was a difference in  $t_{1/2}$  between IV and oral administration. However, on the basis of data our research group has obtained during our experiments, we believe that ponazuril is absorbed after oral administration to llamas and that it has a relatively long serum half-life (135.5 hours).

To our knowledge, the in vitro susceptibility of *E macusaniensis* to ponazuril and other anticoccidial drugs has not been investigated in camelids. However, studies<sup>21,29</sup> have revealed that ponazuril administered at a dosage of 5  $\mu$ g/mL inhibits the development of several tissue cyst-forming coccidia (*T gondii*, *N caninum*, and *S neurona*) in tissue cultures. Investigators in another study<sup>30</sup> found that toltrazuril concentrations of 1  $\mu$ g/mL caused 50% inhibition of parasite growth in *Eimeria tenella*.

For the study reported here, we concluded that ponazuril is tolerated well by llamas and that the rate and extent of absorption following oral administration are sufficient to result in potentially effective serum concentrations. In addition, ponazuril has a long  $t_{1/2}$  that results in sustained exposure after oral administration of a single dose. The serum concentration of ponazuril after a single orally administered dose (20 mg/kg) in llamas reaches therapeutic concentrations for the control of coccidiosis for at least 21 days. Further studies are needed to evaluate the in vitro effects and clinical efficacy of ponazuril against *E macusaniensis*,

the bioavailability of ponazuril, and the establishment of an appropriate administration regimen in camelids.

- a. Provided by Dr. Susan Gawarecki, Pathfinder Farm, Andersonville, Tenn.
- b. Milacath No. 1410, Mila International, Erlanger, Ky.
- c. Marquis 15% paste, generously provided by Bayer Pharmaceutical Co, Kansas City, Mo.
- d. SymmetryShield RP<sub>18</sub>, Waters, Milford, Mass.
- e. WinNonlin, version 5.2, Pharsight, Cary, NC.

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