Pharmacokinetics of intrarectal omeprazole in alpacas

T. MARMULAK*

S. STANLEY[†]

P. H. KASS[‡]

V. WIEBE[§]

D. McKEMIE[¶] &

N. PUSTERLA**

*Veterinary Medical Teaching Hospital, University of California, Davis, CA, USA; [†]California Animal Health and Food Safety Laboratory, University of California, Davis, CA, USA; [‡]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, USA; [§]Department of Pharmacy, Veterinary Medical Teaching Hospital and Associate Clinical Professor of Medicine and Epidemiology, University of California, Davis, CA, USA; [¶]California Animal Health and Food Safety Laboratory, University of California, Davis, CA, USA; **Department of Medicine and Epidemiology, Veterinary Medical Teaching Hospital, University of California, Davis, CA, USA

Marmulak, T., Stanley, S., Kass, P.H., Wiebe, V., McKemie, D., Pusterla, N. Pharmacokinetics of intrarectal omeprazole in alpacas. *J. vet. Pharmacol. Therap.* doi: 10.1111/j.1365-2885.2009.00149.x.

The purpose of this study was to evaluate the pharmacokinetics of omeprazole in three different vehicles when administered rectally to six alpacas. Alpacas were given single doses of omeprazole (4 mg/kg) in a double-blinded, randomized cross-over design with a 1 week washout period. Omeprazole formulations consisted of (1) Treatment A: omeprazole paste mixed in surgical lubricant (2) Treatment B: omeprazole capsule contents in 8.4% sodium bicarbonate and (3) Treatment C: omeprazole capsule contents in surgical lubricant and 8.4% sodium bicarbonate solution. Plasma samples were drawn at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 300 and 480 min. Omeprazole plasma concentrations were determined by high-pressure liquid chromatography-mass spectrometry. Pharmacokinetic results demonstrated median peak plasma concentrations (C_{max}) of 7.35 (3.2–15.2), 7.30 (1.7–10.9) and 8.65 (1.8–19.3) ng/mL and median area under the concentration curve ($AUC_{(0-180)}$) of 747 (237–1681) min·ng/mL, 552.9 (39–1063) min·ng/mL, and 972 (107–1841) min·ng/mL for treatments A, B and C, respectively. The median half-lives were similar between groups: 38, 50, and 53 min. As a result of the low measured omeprazole plasma concentrations, it is assumed that rectal absorption of omeprazole is poor in alpacas and not an effective route of administration.

INTRODUCTION

Third compartment ulcers are a commonly reported and serious problem in llamas and alpacas. Camelids of all ages appear to be affected (Smith *et al.*, 1994). Despite the high incidence of ulcers in these species, to date, few treatments have shown efficacy. For treatment of acute active ulcers, proton pump inhibitors have demonstrated the most efficacies across species lines (Gisbert *et al.*, 2001).

Several studies have looked at the pharmacokinetics of the proton pump inhibitor, omeprazole, in camelids. In a study of intravenous omeprazole in llamas, Christensen *et al.* (2001) demonstrated that doses of 0.4 mg/kg and 0.8 mg/kg produced plasma area under the concentration curve (*AUC*) values of 67 740 min·ng/mL (1.29 h μg/mL) and 202 200 min·ng/mL (3.37 h·μg/mL). This significantly suppressed acid production of the third compartment suggesting that proton pump inhibitors are effective even at relatively low doses if adequate plasma levels can be obtained.

Following the use of high dose (12 mg/kg) omeprazole given orally, peak plasma concentrations of 0.12 μg/mL and an *AUC* of 22 800 min·ng/mL (0.38 h·μg/mL) were reported in llamas, suggesting that omeprazole is poorly absorbed via oral administration (Poulsen *et al.*, 2005). It is postulated that the acid labile drug is mostly destroyed before absorption can occur in the small intestine (Christensen *et al.*, 2001). In addition, omeprazole undergoes hepatic first pass metabolism in the liver, further reducing plasma drug concentrations (Andersson *et al.*, 1991). Given the difficulties in attaining therapeutic concentrations orally in the camelid and the expense of intravenous therapy, other routes of drug administration are currently being evaluated.

Although rectal administration of proton pump inhibitors has been successfully used in other species, it has not been evaluated to date in camelids. In this study, we sought to evaluate the rectal absorption of omeprazole in three different formulations: (1) Treatment A: omeprazole paste mixed in surgical lubricant (2) Treatment B: omeprazole capsule contents in 8.4% sodium

bicarbonate and (3) Treatment C: omeprazole capsule contents in surgical lubricant and 8.4% sodium bicarbonate solution.

MATERIALS AND METHODS

Study design

This was a double blind, prospective, pharmacokinetic study that evaluated three different vehicle formulations of omeprazole given rectally to alpacas. Animals ($N = 6$) were randomized into three groups of two animals each. Each group received a single dose each of one of the three formulations. A 1 week washout period was assigned between crossing over to the subsequent formulations. Therefore, each animal received a single dose of all three formulations over the 3-week-study period and each animal served as its own positive control for comparisons between formulations.

Animals

Six healthy castrated males, ages ranging from 2 to 3 years old, from privately owned herds were used for this study. Six days before the study was to begin, the animals were brought to UC Davis and allowed to acclimate in a pen with outdoor access for the duration of the study. They had free access to grass hay and water. The animals were determined to be healthy on the basis of a complete physical examination prior to enrollment in the study. Animals were not on any other medications during the study period. Each animal was weighed with weights ranging from 54 kg to 87 kg. At the completion of the study, the animals were returned to their herd of origin. The study was conducted in compliance with the University of California, Davis protocol for animal care and use.

Drugs

Omeprazole formulations were prepared just prior to dosing to eliminate problems caused by inadequate stability. Omeprazole solutions are known to be inactivated rapidly over time if not maintained at an alkaline pH (Pilbrant & Cederberg, 1985; Mathew *et al.*, 1995). Oral solutions of omeprazole are stable in 8.4% sodium bicarbonate (Quercia *et al.*, 1997). Vehicles were chosen for the three formulations based on stability, solubility and viscosity. Clinically available vehicles were evaluated for uniform drug distribution within the mixture. The pH of each formula was measured just prior to drug administration using pH strips. Each animal was randomized to receive a 4 mg/kg omeprazole rectal dose of one of the following three formulations: Formulations A, B and C.

Formulation A consisted of the commercial omeprazole paste (Gastrogard® 37% paste, Merial Ltd, Duluth, GA, USA) dissolved in surgical lubricant (Surgilube®, Fougera & Co., Melville, NY, USA). The surgical lubricant primarily consisted of: water, propylene glycol and chlorhexidine gluconate. The thick omeprazole paste readily mixed into a uniform aqueous formulation with the surgical lubricant. It was felt by the authors that the resulting formulation had sufficient viscosity for prolonged rectal retention times but was aqueous enough to allow dispersion of drug within the rectum. A 4 mg/kg dose of the paste was calculated for each animal and the dose amount weighed out using an analytical scale adjusting for the fact that only 37% of the weight was active drug. This dose was then mixed with surgical lubricant and brought up to a final volume of 25 mL before being placed into a 60 mL catheter tip syringe (Kendall monoject™, Tyco Healthcare Group, Mansfield, MA, USA).

A sodium bicarbonate solution was chosen as a second vehicle as a result of the known stability of this compounded product for oral use in humans (Quercia *et al.*, 1997). Formulation B was made as follows; thirty omeprazole 20 mg capsules (omeprazole delayed-release capsules, Kremers Urban, Mequon, WI, USA) were opened and their contents placed in a mortar and dissolved in 10 mL sodium bicarbonate 8.4% solution (8.4% Sodium Bicarbonate injectable solution, Hospira, Inc., Lake Forest, IL, USA). The beads were allowed to soften in the sodium bicarbonate solution for 5 min before being ground by mortar and pestle to a fine powder. Another 30 mL of sodium bicarbonate solution was then added to the mixture to make 40 mL of a 15 mg/mL solution. The 4 mg/kg dose for each alpaca was drawn up and sodium bicarbonate added to a total volume of 25 mL.

Formulation C consisted of omeprazole capsule contents mixed in a mortar with 8.4% sodium bicarbonate solution and surgical lubricant. It was made by dissolving the contents of thirty 20 mg omeprazole capsules in 10 mL of sodium bicarbonate 8.4% solution in a mortar until softened and then grinding with a pestle until a homogenous solution was formed. Thirty mL of surgical lubricant was then added to increase the viscosity of the formulation and to produce a final concentration of 15 mg/mL. The dose of 4 mg/kg omeprazole for each alpaca was drawn up and surgical lubricant added to a final volume of 25 mL.

Drug administration

Animals were randomized via a simple random sampling method into pairs to receive one of the three formulations listed above. The alpaca pairs were placed in separate pens during drug administration and each animal was marked with a separate tag number. The veterinarian delivering the dose was blinded to the formulation being administered. A lubricated French 14 urethral catheter (Kendall Sovereign™ feeding tube and urethral catheter, Tyco Healthcare) was inserted into the rectum of each alpaca. The catheter was inserted by the same veterinarian in a consistent manner to assure that formulas were placed in the same area of the rectum in each animal. Using a catheter tip syringe, a 4 mg/kg dose of one of the omeprazole formulations (A, B, or C) was administered via bolus fashion. Following administration, each animal was observed for the remainder of the day and time points of defecation were noted, as this may have impacted drug retention time. Animals were allowed free access to food and water at all times. Following a 1 week washout period, animals were crossed over to the subsequent randomly assigned treatment group for two more cycles.

Sample collection and processing

Blood samples (10 mL) for omeprazole plasma concentrations were collected via a pre-placed intravenous jugular vein catheter (16 GA, 3¼ inch angiocatheter) and then placed into heparinized tubes (BD Vacutainer®, BD, Franklin Lakes, NJ, USA) immediately before (time 0), and 5, 10, 15, 30, 45, 60, 90, 120, 180, 300, and 480 min postomeprazole rectal administration. After collection, each catheter was flushed with 3 mL of 10 units/mL heparin in saline flush. Before subsequent blood samples, approximately 2 mL of blood was withdrawn and disposed of from the catheter to prevent dilution of samples with the flush. Samples were placed directly on ice and processed within 2 h. The blood samples were removed from ice and then centrifuged at $1509 \times g$ for 10 min. Plasma was aliquoted into 4 mL cryotubes in duplicates. The samples were then stored at -80 °C until analysis at the K.L. Maddy Equine Analytical Chemistry Laboratory.

Sample processing

Plasma omeprazole concentrations were determined by high-pressure liquid chromatography (HPLC) – linear ion trap mass spectrometry (LTQ) (Thermo Fisher Scientific Inc., Waltham, MA, USA). Briefly, extraction of samples was performed using a protein precipitation procedure: 0.5 mL of a 9:1 acetonitrile (ACN):water with 25 ng of d3-omeprazole internal standard were added to 0.5 mL of plasma. The samples were agitated using a vortex for 1 min, then allowed to settle while refrigerated at 4 °C for 20 min. Samples were then agitated for another 20 sec and centrifuged at $3830 \times g$ at 4 °C.

Standard curve preparation

The omeprazole (USP cat. No. 1478505, C/D/N Isotopes Inc., Pointe-Claire, Quebec) and d3-omeprazole (USP cat. No. D-6264, C/D/N Isotopes Inc.) used to make the standard curve were 1 mg/mL stock solutions prepared in methanol. Control plasma samples were prepared from healthy untreated alpacas to check for any interfering peaks on the chromatograph. A standard curve was prepared with alpaca plasma over a concentration range of 0.1 ng/mL to 100 ng/mL. Standard software (Xcalibur, Thermo Fisher Scientific Inc.) was used for data acquisition and processing. The data points were linear with a coefficient of determination (r^2) of >0.99. The lower limit of quantification was 0.1 ng/mL.

Omeprazole analysis

Separations were accomplished on a reverse-phase, 2.0×100-mm, 3.0-μm particle size column maintained at ambient temperature (ACE®, Advanced Chromatography Technologies, C18, Aberdeen, Scotland), preceded by a 4×4-mm, 5-μm particle guard column (Hypersil BDS C18, Agilent Technologies, Palo Alto, CA, USA). Flow rate was 0.4 mL/min and an injection volume of 10 μL of the supernatant was used. The initial mobile phase composition was 5% Acetonitrile (ACN) with 0.2% formic acid and 95% formic acid (0.2%) in water. The gradient changed to 90% ACN + 0.2% formic acid over 4.6 min, and at 5 min 51 sec the solution was changed back to the initial conditions for the remainder of the total run time of 11 min. MS/MS/MS analysis was performed with full-scan mass spectra collected in positive electrospray ionization mode (ESI). For omeprazole m/z 346 was isolated, fragmented with a collision energy of 26 and isolation width of 2.7 followed by fragmentation of the daughter ion m/z 198 at a collision energy of 28 and isolation of width of 1.5. d3-Omeprazole used m/z 349 with the same scan event settings. M/Z 179.9 was used for quantization of both omeprazole and the internal standard at a retention time of 5.1 min for each. The plasma concentration for omeprazole was calculated via linear regression analysis.

Data analysis/statistics

Data were analyzed using a noncompartmental method. Plasma concentration time points were graphed by hand on semi-logarithmic paper. C_{max} and T_{max} were derived from the concentration vs. time curve. AUC was calculated using the trapezoidal rule. Half-life was calculated using the slope of the elimination phase of the curve according to standard equations.

Following HPLC/LTQ analysis, omeprazole plasma concentration data were noted not to be normally distributed. Given that each animal served as its own control, a Friedman test was used to assess differences among pharmacokinetic parameters between treatment arms. Values of $P < 0.05$ were considered significant. If a significant difference between treatment arms was found for any of the pharmacokinetic variables, then a Wilcoxon signed rank test was used to determine where that significant difference lay. Standard statistical software was used (StatXact-8, Cytel Studio Software Corp., Cambridge, MA, USA).

RESULTS

Plasma pharmacokinetic omeprazole parameters are summarized in Table 1. Overall, plasma omeprazole concentrations were extremely low being measured in ng/mL rather than ug/mL. Omeprazole was detectable in the plasma in all but one alpaca 5 min postdrug administration. The mean plasma concentration vs. time curves for each treatment arm is shown in Fig. 1.

No significant differences were found among AUC, half life, and C_{max} between formulations. When comparing T_{max} values, there was a significant difference between groups ($P = 0.028$). A Wilcoxon signed rank test ($P = 0.031$) determined that the significant difference was between the T_{max} values of formulation A (median: 90 min) and B (median: 30 min).

No adverse effects were observed in the alpacas. The mean time to defecation from drug administration in this study was 106 min. Average time to defecation for treatment A, B and C were 135, 90 and 92 min, respectively. This indicated that rectal exposure time was relatively consistent between groups. The pH of each formulation was measured at the time of administration with average pHs for formulas A, B and C being 7.0, 9.0 and 8.7,

Table 1. Pharmacokinetic parameters of six alpacas following a single intrarectal dose (4 mg/kg) of one of three omeprazole formulations

Alpaca	Formulation A				Formulation B				Formulation C			
	C_{max} (ng/mL)	$AUC_{(0-180)}$ (min·ng/mL)	Half-life (min)	T_{max} (min)	C_{max} (ng/mL)	$AUC_{(0-180)}$ (min·ng/mL)	Half-life (min)	T_{max} (min)	C_{max} (ng/mL)	$AUC_{(0-180)}$ (min·ng/mL)	Half-life (min)	T_{max} (min)
1	3.9	237.5	40	60	1.7	39.7	25	15	1.8	144.7	50	45
2	3.2	342.5	45	120	6.3	158.0	35	15	2.7	107.5	30	10
3	11.7	1234.1	35	180	8.3	913.5	50	60	11.2	994.5	55	90
4	7.8	647.0	35	90	3.6	192.2	80	60	9.6	1088.1	90	60
5	15.2	1680.6	35	45	10.9	1063.4	60	30	19.3	1841.4	30	90
6	6.9	847.3	95	90	10.2	946.0	50	30	7.7	950.3	55	15
Median (range)	7.35 (3.2–15.2)	747 (237.5–1680.6)	38 (35–95)	90 (45–180)	7.3 (1.7–10.9)	552.9 (39.7–1063.4)	50 (25–80)	30 (15–60)	8.65 (1.8–19.3)	972 (107.5–1841.4)	53 (30–90)	53 (10–90)

C_{max} = peak plasma concentration, $AUC_{(0-180)}$ = area under the concentration curve from time 0 to 180 min, T_{max} = time to peak plasma concentration, Formulation A = a commercial omeprazole paste dissolved in surgical lubricant, Formulation B = omeprazole capsule contents dissolved in 8.4% sodium bicarbonate, Formulation C = omeprazole capsule contents mixed in surgical lubricant and 8.4% sodium bicarbonate solution.
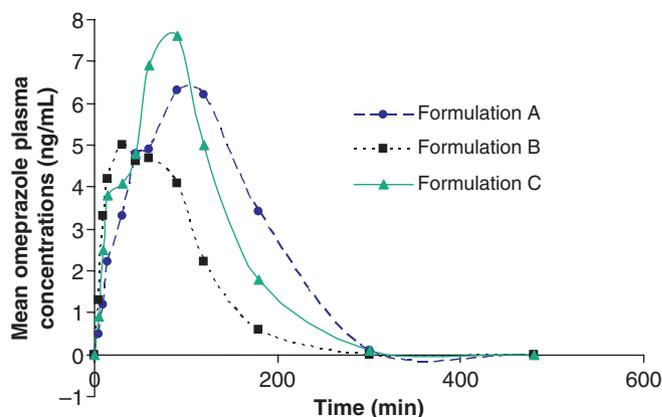

Fig. 1. Average omeprazole plasma concentration vs. time curves for three rectally administered omeprazole formulations in alpacas ($N = 6$). Standard deviation bars are not depicted as a result of high variability in the data. Formulation A = a commercial omeprazole paste dissolved in surgical lubricant, Formulation B = omeprazole capsule contents dissolved in 8.4% sodium bicarbonate, Formulation C = omeprazole capsule contents mixed in surgical lubricant and 8.4% sodium bicarbonate solution.

respectively. The neutral to alkaline pHs of these formulations suggest that the stability of these formulations was adequate for the short time between drug preparation and administration (Pilbrant & Cederberg, 1985; Mathew *et al.*, 1995).

DISCUSSION

Rectal administration of omeprazole has been studied in rats, rabbits and humans (Sastry *et al.*, 1993; Eun *et al.*, 1995; Choi *et al.*, 1996). In humans, no significant differences in the *AUC* of omeprazole were noted following oral and rectal administration suggesting that the bioavailability is similar between oral and rectal routes (Choi *et al.*, 1996). Choi *et al.* (1996) further express that the equivalent bioavailabilities between routes may be related to the fact that following rectal administration to humans, omeprazole significantly avoids hepatic first pass metabolism. It is known that rectal administration can partially circumvent hepatic first pass metabolism and result in higher plasma concentration values for drugs, such as omeprazole, which are significantly metabolized via this route.

As a result of the very poor oral absorption of omeprazole in camelid species, we hypothesized that rectal administration might circumvent hepatic first pass metabolism and result in therapeutic plasma drug concentrations. With omeprazole, area under the plasma concentration curve (*AUC*) is considered a better predictor of the clinical efficacy than peak plasma concentrations because it gives a better approximation of the exposure of the animal to the drug over time (Lind *et al.*, 1983). In llamas, intravenous doses of omeprazole (0.4 and 0.8 mg/kg) resulted in 8 h plasma *AUC*s of 67 770 and 202 200 min·ng/mL, respectively, which significantly suppressed third compartment acid production (Christensen *et al.*, 2001).

In our study, the median AUC_{0-180} for treatments A, B and C were 747.1, 552.9 and 972.4 min·ng/mL, all well below concentrations reported to be therapeutic in camelids and other species. The extremely poor absorption via this route of administration was surprising and remains poorly understood. Several factors may have contributed to the extremely limited absorption via this route.

In this study, we did not evacuate the rectums of the alpacas prior to drug administration. Feces may potentially bind drug and interfere with absorption. This factor may have contributed to some degree to poor rectal absorption, but would not be considered a major contribution to the extremely poor absorption noted here.

In a previous study in equines at UC Davis, the commercial omeprazole paste (Gastrogard®) was given rectally to horses following evacuation of feces. The resulting plasma concentrations were also noted to be low and had a direct correlation to the time of first defecation. The thick omeprazole paste was noted to be defecated undissolved when administered rectally (Rand *et al.*, 2008). It was speculated from this study, that a more aqueous formulation might improve drug dispersion and rectal mucosal contact time.

Retention times prior to voiding of feces in our study should have allowed for ample time for drug absorption. Average retention times of the three treatment arms A, B and C were 135, 90 and 92 min, respectively. There were two animals (alpacas 1 and 2) in Formulation B that defecated early and resulted in low *AUC*s. However, the majority of the animals in this study did retain the drug for a moderately long period of time, it is not felt by the authors to have played a major role in the low plasma concentrations of omeprazole noted.

Drug binding to the vehicles may also have affected our results. In our study, three water soluble vehicles were chosen. A more lipophilic vehicle was considered as a base for a formulation in our study, but it was found that omeprazole did not mix well into these vehicles when compounded. In addition, vehicles such as mineral oil, may also act as laxatives, which could negatively impact retention time and thus our results. We speculated that dissolving the paste in a vehicle such as surgical lubricant would decrease the viscosity of the paste and permit greater surface area exposure of the drug to the rectal mucosa, potentially increasing overall rectal absorption. It may be that the drug preferentially bound to the aqueous vehicle and was not available for transmucosal absorption.

Differences in vehicle viscosity may also account for the statistical difference in T_{max} between Formulations A and B. Formulation A was the most viscous, and Formulation B, the least viscous formulation. Increased viscosity can result in a decreased rate of drug absorption as a result of a slower rate of movement of drug molecules to the absorbing membrane resulting in a longer T_{max} for Formulation A when compared with Formulation B.

Other factors including physiologic differences in the alpacas rectal mucosal tissue or blood supply may also contribute to the extremely low drug concentrations achieved following rectal administration of omeprazole in our study. Further evaluation of other drugs known to be highly absorbed via rectal administration (diazepam) may provide further clues as to the poor absorption noted here. In conclusion, rectal omeprazole administration in alpacas failed to achieve levels known to be therapeutic in other camelids. It does not appear to be a viable route of drug administration in this species.

REFERENCES

Andersson, T., Bergstrand, R. & Cederberg, C. (1991) Influence of acid secretory status on absorption of omeprazole from enteric coated granules. *British Journal of Clinical Pharmacology*, **31**, 275–278.

Choi, M.S., Chung, S.J. & Shim, C.K. (1996) Rectal Absorption of Omeprazole from Suppository in Humans. *Journal of Pharmaceutical Sciences*, **85**, 893–894.

Christensen, J.M., Limsakun, T., Smith, B.B., Hollingshead, N. & Huber, M. (2001) Pharmacokinetics and pharmacodynamics of antiulcer agents in llama. *Journal of Veterinary Pharmacology & Therapeutics*, **24**, 23–33.

Eun, K.H., Lee, Y.H. & Shim, C.K. (1995) Rectal absorption of omeprazole from suppositories in rabbits. *Archives of Pharmacal Research*, **18**, 219–223.

Gisbert, J.P., Gonzalez, L., Calvet, X., Roqué, M., Gabriel, R. & Pajares, J.M. (2001) Proton pump inhibitors versus H2-antagonists: a meta-analysis of their efficacy in treating bleeding peptic ulcer. *Alimentary Pharmacology & Therapeutics*, **15**, 917–926.

Lind, T., Cederber, C., Ekenved, G., Haglund, U. & Olbe, L. (1983) Effect of omeprazole-a gastric proton pump inhibitor- on pentagastrin stimulated acid secretion in man. *Gut*, **24**, 270–276.

Mathew, M., Gupta, V.D. & Bailey, R.E. (1995) Stability of omeprazole solutions at various ph values as determined by high-performance liquid chromatography. *Drug Development and Industrial Pharmacy*, **21**, 965–971.

Pilbrant, A. & Cederberg, C. (1985) Development of an oral formulation of omeprazole. *Scandinavian Journal of Gastroenterology*, **20**, 113–120.

Poulsen, K.P., Smith, G.W., Davis, J.L. & Papich, M.G. (2005) Pharmacokinetics of oral omeprazole in llamas. *Journal of Veterinary Pharmacology & Therapeutics*, **28**, 539–543.

Quercia, R.A., Fan, C., Liu, X. & Chow, M.S. (1997) Stability of omeprazole in an extemporaneously prepared oral liquid. *American Journal of Health-System Pharmacy*, **54**, 1833–1836.

Rand, C.L., Stanley, S. & Pusterla, N. (2008) Effects of intrarectally administered omeprazole paste on gastric juice ph in adult healthy horses. *Journal of Veterinary Internal Medicine*, **22**, 709.

Sastry, M.S.P., Diwan, P.V. & Krishna, D.R. (1993) Comparative evaluation of ulcer prevention efficacy of orally, rectally and sublingually administered omeprazole in three acute gastric ulcer models in rats. *Fundamental and Clinical Pharmacology*, **7**, 77–80.

Smith, B.B., Pearson, E.G. & Timm, K.I. (1994) Third compartment ulcers in the llama. *Veterinary Clinics of North America: Food Animal Practice*, **10**, 319–330.