Evaluation of urine sucrose concentration for detection of gastric ulcers in horses

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Objective—To evaluate the use of sucrose permeability testing to detect ulcers in the gastric squamous mucosa of horses.

Animals—13 adult horses ranging from 5 to 19 years of age.

Procedure—Following induction of gastric ulcers by intermittent feed deprivation, horses underwent sucrose permeability testing (administration of sucrose by nasogastric intubation followed by collection of urine at 2 and 4 hours after intubation) and gastric endoscopy. Squamous ulcers were assigned a severity score (range, 0 to 3) by use of an established scoring system. Horses were subsequently administered omeprazole for 21 days, and sucrose testing and endoscopy were repeated. Pair-wise comparisons of urine sucrose concentration were made between horses with induced ulcers before and after omeprazole treatment. Urine sucrose concentrations also were compared on the basis of ulcer severity score.

Results—Urine sucrose concentrations and ulcer severity scores were significantly higher in horses with induced ulcers before omeprazole treatment than after treatment. Urine sucrose concentrations were significantly higher for horses with ulcer severity scores > 1. Use of a cut-point value of 0.7 mg/mL revealed that the apparent sensitivity and specificity of sucrose permeability testing to detect ulcers with severity scores > 1 was 83% and 90%, respectively. Results were similar after adjusting sucrose concentrations for urine osmolality.

Conclusions and Clinical Relevance—Urine sucrose concentration appears to be a reliable but imperfect indicator of gastric squamous ulcers in horses. Sucrose permeability testing may provide a simple, noninvasive test to detect and monitor gastric ulcers in horses. (*Am J Vet Res* 2004;65:31–39)

Gastric ulcers are common among foals and horses. Prevalence among foals ranges from 25% to 51%.[1-4] In horses ≥ 1 year old, prevalence estimates range from 58% to > 90%.[5-10] Factors that influence the prevalence of gastric ulcers include feeding practices, stall confinement, racing and race training, and illness.[11] Gastric ulcers have been associated with clinical signs in foals and horses. In foals, clinical signs of gastric ulcers can be severe; moreover, gastric ulcers can be fatal when ulceration results in gastric perforation.[12-17] Clinical signs associated with gastric ulcers in yearlings and mature horses include decreased appetite, lethargy, weight loss or poor body condition, and colic.[11,18] Signs of colic are generally mild to moderate. Although clinical signs of disease are lacking in horses with colic, gastric ulcers may diminish performance in affected horses.[18] Gastric ulcers in foals and horses develop most commonly in the squamous mucosa adjacent to the margo plicatus.[11] Excessive exposure to highly acidic gastric contents is believed to be the major cause of ulcers in these areas.[11,19,20]

Currently, the only accurate method for detecting and monitoring gastric ulcers in horses and foals is gastric endoscopy (ie, gastroscopy).[11] The high cost and limited portability of the equipment needed to perform this technique (particularly equipment needed to examine mature horses) greatly restrict the availability of gastroscopy in horses. Consequently, there is considerable need for a simple, convenient, cost-effective, and accurate method for detecting gastric ulcers in horses.

Increased gastric permeability to sucrose is a reliable indicator of gastric ulcers in rabbits, dogs, and humans.[21-23] Sucrose is rapidly hydrolyzed by the brush-border enzyme sucrase in the proximal portion of the small intestine to its monosaccharide units, glucose and fructose; this hydrolysis occurs even when there is severe damage to the small intestine.[21] The disaccharide sucrose is large enough that it is excluded from permeation by the intact gastric epithelium. Thus, permeation of sucrose across the gastric epithelium indicates a defect in the mucosa proximal to the site of hydrolysis (ie, a defect in the gastric mucosa).[21]

The purpose of the study reported here was to evaluate the accuracy of urine sucrose concentration as a measure of sucrose permeation across the gastric mucosa to indicate gastric ulcers and the severity of those ulcers in horses. The total amount of sucrose excreted in urine collected during a finite period (ie, cumulative mass) has been used to indicate the severi-

Received February 7, 2003.
Accepted July 9, 2003.
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Supported by the Southern California Equine Foundation, the Link Equine Research Endowment, and Merial Ltd.
The authors thank Justin Caldwell, Grayson Gearhardt, Elizabeth Stewart, and Kim Tran for technical assistance.
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ty of gastric damage in rabbits, dogs, and humans.[21-23] In our study, a single determination of urine sucrose concentration at a specified time point was evaluated. The rationale for this approach was that our aim was to develop a test that would be simple and could be used in field settings. Determining total sucrose excretion would be impractical in field settings because it would require collection of all urine produced during a defined period by use of a labor-intensive method such as placement of an indwelling urinary catheter.

Materials and Methods

Animals—Thirteen horses were enrolled in the study. Horses were acquired by donation to the College of Veterinary Medicine, Texas A&M University. Horses comprised 7 Thoroughbreds, 3 Quarter Horses, 2 Arabians, and 1 Peruvian Paso. Four horses were geldings, and 9 were mares. Horses ranged from 5 to 19 years of age (median, 11 years). Body weight ranged from 390 to 591 kg (median, 432 kg). Horses did not have overt signs of gastrointestinal tract disease or other diseases. Between experiments, all horses were housed at the Texas A&M College of Veterinary Medicine Research Park on concrete slabs in 12 × 12-m^2 paddocks with a covered area that provided shade. Each horse was fed 2 kg of a concentrate ration[a] twice daily, and coastal Bermuda grass hay was available ad libitum. Water was available to horses at all times. During the course of the study, horses were visually monitored for clinical signs of disease, and results of daily monitoring and feeding were recorded. The protocol for this study was approved by the Texas A&M University Laboratory Animal Care Committee.

Procedure—The study used a before-and-after design. Horses were evaluated by use of sucrose permeability testing after induction of gastric ulcers. Subsequently, omeprazole[b] (4 mg/kg, PO, q 24 h for 21 days) was administered to heal induced or naturally developing ulcers, and the same horses were evaluated by use of sucrose permeability testing a second time. The rationale for not using a crossover design with random treatment assignment was to avoid carry-over effects of omeprazole treatment prior to induction of ulcers as well as to reduce the duration and costs of the study.

Horses were maintained in 12 × 12-m^2 paddocks and fed the aforementioned diet until the evening on which ulcer induction was initiated. On that evening, horses were placed in 3 × 3-m^2 box stalls in a well-ventilated barn. Horses were fed that evening. Ulcers were induced during a period of 7 days by use of a model of intermittent feed deprivation, as described elsewhere.[21] During the ulcer-induction period, each horse was subjected to intermittent feed deprivation such that horses were alternately fed 2 kg of a concentrate ration[a] twice daily for 24 hours and then deprived of feed for 24 hours until a total of 96 hours of feed deprivation had been accumulated.[20] Water was available ad libitum throughout this period. This model reliably causes ulcers in the gastric squamous mucosa, most commonly in the region adjacent to the margo plicatus, the area most often affected in horses with naturally developing gastric ulcers.[19,20]

Horses included in the study did not routinely undergo endoscopy prior to intermittent feed deprivation. Data from preliminary studies we conducted indicated that most of the pool of 80 horses acquired for the study had some naturally developing gastric ulcers at the time of arrival, and intermittent feed deprivation sustained or exacerbated these ulcers. Thus, the intermittent feed deprivation model was used in this study to induce new ulcers or sustain-exacerbate existing ulcers. Therefore, the term ulcer induction in this report referred to induction, maintenance, or exacerbation of gastric ulcers in the horses.

Administration of sucrose and collection of specimens—The end of the food-deprivation period coincided with the beginning of data collection for sucrose permeability testing. On the morning of sucrose permeability testing, the skin overlying the left jugular vein of each horse was aseptically prepared, and a 14-gauge, 5.25-inch catheter[c] was inserted. A blood sample was collected prior to administration of sucrose, and serum was separated by centrifugation. After each horse was administered 15 mg of acepromazine,[d] the vulva or distal part of the penis, respectively, was aseptically prepared for urinary catheterization. A soft, flexible urinary catheter (ie, stallion catheter) was passed via the urethra into the bladder. Urine was emptied from the bladder and thoroughly mixed. Total urine volume was recorded, and 3 to 5 aliquots of urine (10 mL/aliquot) were placed in separate 15-mL tubes[e] that contained 10 µL of a solution of **sodium azide** (NaN_3[f]; 0.1 g/mL). The rationale for the addition of NaN_3 to the tubes was to inhibit bacteria that may have metabolized sucrose or other sugars in the urine. After collection of blood and urine samples, horses were fed 1 kg of a concentrate feed[g] prior to receiving 454 g of sucrose (10% solution in tap water) via a nasogastric tube. For each horse, blood and urine samples were collected 2 and 4 hours after sucrose administration. Total urine volume at each time point was recorded, urine was thoroughly mixed, and several (3 to 5) 10-mL aliquots were prepared, as described previously. Immediately after urine was added to each tube that contained NaN_3, urine specimens were frozen at –80°C until analysis by use of **high-performance liquid chromatography and pulsed amperometric detection (HPLC-PAD)**. Three 2-mL aliquots of serum, obtained concurrently from each horse at each sample time, were also frozen at –80°C.

Gastroscopy was performed approximately 30 to 60 minutes after collection of the 4-hour samples. After completion of sucrose permeability testing and gastroscopy, each horse was treated by administration of omeprazole (4 mg/kg, PO, q 24 h for 21 days) to heal any induced or naturally developing gastric ulcers. After the 21-day treatment period, each horse was again evaluated by use of sucrose permeability testing in accordance with the protocol described previously for horses with induced ulcers. Food was withheld from horses for 12 to 14 hours prior to collection of urine samples.

Endoscopic evaluation—Endoscopic examinations were performed by use of a 3-m equine videoendoscope.[h] In preparation for endoscopy, horses were sedated with xylazine hydrochloride[i] (0.6 to 0.8 mg/kg, IV), and a 1-m equine stomach (nasoesophageal) tube was inserted to protect the endoscope from pharyngeal retroflexion. The stomach was distended by insufflation with air through the biopsy channel of the endoscope until the squamous and glandular mucosae were visible, and the entire squamous epithelium of each horse was examined. Because horses were fed 1 kg of concentrate 4 to 5 hours before endoscopy, it was possible to see only the most dorsal portion of the glandular mucosa. Therefore, the final 6 horses were endoscopically examined the morning after sucrose administration following an additional 12 to 14 hours of feed deprivation.

To ensure proper identification of all squamous mucosal defects, gastric contents were rinsed from the mucosa with warm tap water flushed through the biopsy channel. When necessary and possible, gastric fluid was aspirated through the biopsy channel. At the conclusion of the gastric examination, the stomach was deflated by suctioning air through the biopsy channel. Videotape and still-frame images of all examinations were recorded and archived.

The videotaped and still-frame images from each of the 13 horses were used to determine gastric ulcer scores by use of an established scoring system.[24] Ulcers were scored on a

scale of 0 to 3 as follows: 0, intact mucosal epithelium that may have been red or had evidence of hyperkeratosis; 1, a small (approx < 2 cm) single ulcer or small multifocal ulcers considered to extend through the mucosa to the submucosa; 2, a large (> 2 cm) single ulcer or large multifocal ulcers considered to extend through the mucosa to the submucosa; and 3, extensive coalescing ulcers considered to extended through the mucosa; such ulcers were similar to grade-2 ulcers but would coalesce to produce areas of extensive ulceration. Images from examinations were reviewed and scored after completion of data collection from all horses; all scoring was performed by 1 of the authors (NDC), who was unaware of the treatment status of each horse.

Determination of urine sucrose concentration by use of HPLC-PAD—Prior to determination of urine sucrose concentration, one 10-mL aliquot of urine was thawed at room temperature (22°C). The sample was vortexed, and 2 mL of urine was collected into a 3-mL syringe and filtered through a syringe filter with 0.45-µm pores[j] into a 1.5-mL cryotube. Each cryotube was labeled with the identification number of the horse, date of sample collection, treatment status (ie, ulcer induction or omeprazole treated), and time of sample collection. Filtered urine (100 µL) was transferred into a polypropylene tube that contained 900 µL of 0.01% NaN_3 to create a dilution of 1 in 10. Each polypropylene tube was vortexed, and 100 µL of the mixture was transferred by pipette into a corresponding HPLC sample tube that contained 900 µL of 0.01% NaN_3 to create a dilution of 1 in 100. Tubes were then sealed and frozen at –20°C until analysis.

Analysis of urine samples to determine sucrose concentration was performed by use of HPLC-PAD.[25] Sugars were separated by use of a **sodium hydroxide (NaOH)** gradient on a metal-free HPLC system[k] at a flow rate of 1 mL/min. Three concentrations of NaOH were used to create the NaOH gradient; NaOH was stored in 2-L pressurizable plastic bottles[l] under a helium cap. An anion-exchange column[m] was used for separation of sugars. A gradient pump and controller[n] added 0.5M NaOH at a flow rate of 0.5 mL/min to narrow the NaOH gradient at the detector. Pulsed amperometric detection[o] was used to quantify sugars.[25]

Determination of urine osmolality—Urine osmolality was determined by use of an osmometer.[p] The rationale for determining urine osmolality was that horses were allowed access to water ad libitum prior to sucrose permeability testing; urine osmolality data were used to determine a ratio of urine sucrose concentration to urine osmolality to account for effects of urinary dilution on urine sucrose concentrations. Briefly, the osmometer was calibrated in accordance with the manufacturer's instructions, and the osmometer chamber was cleaned with deionized water and dried prior to use. The micropipettor furnished with the vapor-pressure osmometer and a sterile unused tip were used to dispense 10 µL of urine onto the osmometer sample disc. Osmolality was measured and results recorded. The sample chamber was cleaned with deionized water and dried between subsequent samples.

Statistical analysis—Paired urine sucrose concentrations for the 2-hour collection in horses with and without induced ulcers were analyzed by use of the Wilcoxon signrank test.[26] The association between gastric ulcer score and sucrose concentration was analyzed by use of a generalized linear model,[27] with horse as a random effect and ulcer score as a fixed effect. The Duncan multiple-range test was used for post hoc testing to compare sucrose concentrations on the basis of ulcer score. The urine sucrose concentration-to-urine osmolality ratio was determined for each sample. Statistical analyses and sensitivity and specificity determinations were repeated by use of the sucrose concentration-to-osmolality ratios. All statistical analyses were performed by use of a computer software package.[q] A value of $P \leq 0.05$ was considered significant for all analyses.

Results

Thirteen horses were subjected to intermittent feed deprivation[20] to induce ulcers. All horses tolerated the period of feed deprivation; none developed signs of abdominal discomfort or other clinically apparent effects. After accumulation of 96 hours of feed deprivation, ulcers were detected in the squamous mucosa of 11 horses; however, feed deprivation failed to induce gastric squamous ulcers in 2 horses.

Ulcer scores following feed deprivation ranged from 0 to 3 (median ulcer score, 2). Six horses had an ulcer score of 3, 4 horses had an ulcer score of 2, 1 horse had an ulcer score of 1, and the 2 aforementioned horses that failed to develop gastric squamous ulcers had ulcer scores of 0. Median ulcer score for the 11 horses with induced ulcers was 3. In 10 of these 11 horses, there was at least 1 moderate to severe area of ulceration in the gastric squamous epithelium after 96 hours of feed deprivation; only a few small ulcers were induced in 1 horse. Frequency and severity of gastric squamous ulcers were greatest at the lesser curvature adjacent to the margo plicatus and least severe or not evident at the greater curvature adjacent to the margo plicatus. We did not detect ulcers in the limited amount of the glandular portion of the stomach that could be observed in the 7 horses examined endoscopically on the day of testing, nor did we detect ulcers in the gastric glandular mucosa in any of the 6 horses that were examined the day after sucrose permeability testing.

Omeprazole treatment resulted in a significant ($P = 0.001$) decrease in ulcer severity, with ulcer scores after treatment ranging from 0 to 2 (median ulcer score, 0) for all 13 horses. After 21 days of omeprazole treatment, 2 horses had an ulcer score of 2, 4 horses had an ulcer score of 1, and 7 horses had an ulcer score of 0. In the 6 horses with ulcer scores ≥ 1, lesions were most frequently located at the lesser curvature adjacent to the margo plicatus. Excluding the 2 horses that had ulcer scores of 0 before and after treatment, the ulcer score after treatment for the remaining 11 horses ranged from 0 to 2 (median ulcer score, 1). Difference in ulcer scores (score after feed deprivation minus score after omeprazole treatment) for the 13 horses ranged from 0 to 2 (median difference, 2). Excluding the 2 horses that failed to develop ulcers after feed deprivation, the difference in ulcer scores for the remaining 11 horses ranged from 1 to 2 (median difference, 2). In each horse in which ulcers were induced, ulcer scores were lower after omeprazole treatment.

Among the 11 horses in which feed deprivation induced ulcers, sucrose concentration was significantly ($P = 0.001$) higher after ulcer induction (range, 0.24 to 7.68 mg/mL; median, 1.40 mg/mL), compared with concentrations measured after omeprazole treatment (range, 0.08 to 2.10 mg/mL; median, 0.36 mg/mL). Difference in sucrose concentrations (sucrose concentration after ulcer induction minus sucrose concentra-

tion after omeprazole treatment) ranged from 0.06 to 5.58 mg/mL (median difference, 1.24 mg/mL); in all 11 horses, the urine sucrose concentration was lower after omeprazole treatment than after ulcer induction (**Fig 1**).

Because feed deprivation failed to induce ulcers in 2 horses and the 21-day treatment period did not result in ulcer scores of 0 for all horses, the relationship between urine sucrose concentration and ulcer score was examined (**Fig 2**). Median urine sucrose concentrations were calculated for each ulcer score. In 9 horses with an ulcer score of 0 after ulcer induction or omeprazole treatment, the urine sucrose concentration ranged from 0.18 to 1.63 mg/mL (median, 0.59 mg/mL). In 5 horses with an ulcer score of 1, the urine sucrose concentration ranged from 0.08 to 0.36 mg/mL (median, 0.16 mg/mL). In 6 horses with an ulcer score of 2, the urine sucrose concentration ranged from 0.15 to 2.89 mg/mL (median, 1.32 mg/mL). In the 6 horses with an ulcer score of 3, the urine sucrose concentration ranged from 0.50 to 7.68 mg/mL (median, 2.61 mg/mL). Results of the Duncan multiple-range test revealed significant differences in sucrose concentrations between horses with ulcer scores of 3 and horses with ulcer scores ≤ 2; however, significant differences in sucrose concentrations were not identified among horses with ulcer scores of 2, 1, or 0. The correlation between urine sucrose concentration and ulcer score was also analyzed by categorizing ulcer scores as ≤ 1 or > 1. Results of the generalized linear model and Duncan multiple-range test revealed that urine sucrose concentrations were significantly lower in horses with ulcer scores ≤ 1, compared with sucrose concentrations in horses with ulcer scores > 1.

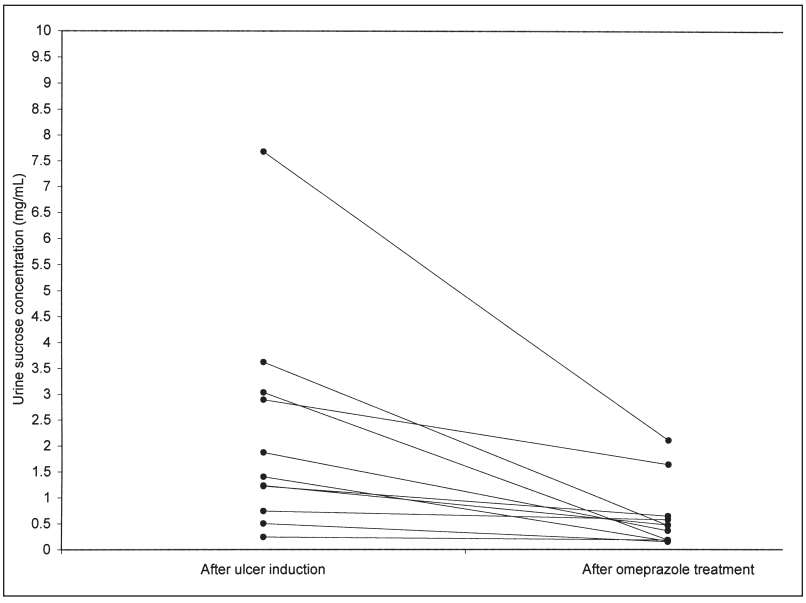

Figure 1—Urine sucrose concentration in 11 horses after induction of gastric ulcers and after 21 days of treatment with omeprazole.

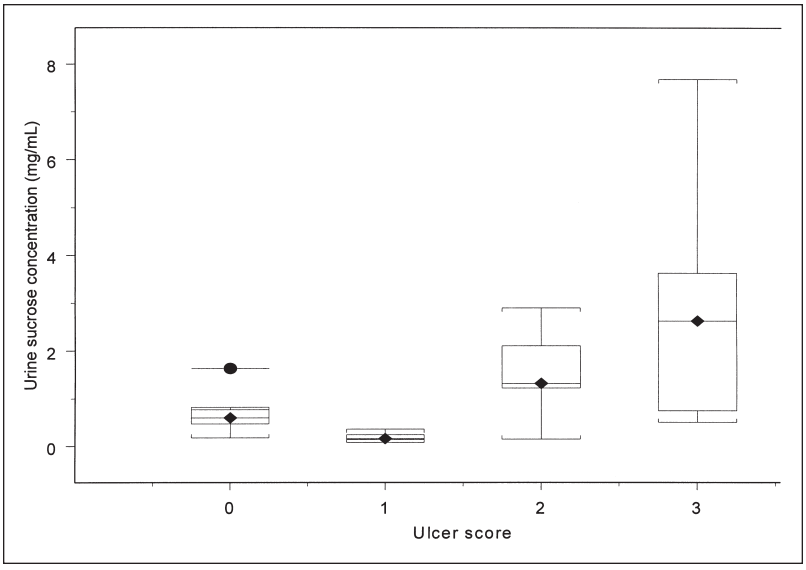

Figure 2—Box plots of urine sucrose concentrations on the basis of ulcer severity score for 13 horses evaluated after induction of gastric ulcers and after 21 days of treatment with omeprazole. Each box represents the interquartile range (25th to 75th percentiles). The horizontal line that bisects the diamond in each box represents the median value. Lines extending vertically from the box represent 95% of the data (ie, 2.5th to 97.5th percentiles). The value indicated by the circle bisected by a horizontal line represents an outlier.

The relationship between urine sucrose concentration and ulcer score was also analyzed by excluding the 2 horses with ulcer scores of 0 before and after omeprazole treatment. In this analysis, only 5 horses had an ulcer score of 0, and urine sucrose concentration for these 5 horses ranged from 0.18 to 1.63 mg/mL (median, 0.57 mg/mL). Urine sucrose concentrations for the remaining ulcer scores were as reported for all horses. Results of the generalized linear model and Duncan multiple-range test revealed a significant difference in sucrose concentrations between horses with ulcer scores of 2 or 3, compared with sucrose concentrations in horses with ulcer scores ≤ 1; however, significant differences in sucrose concentrations were not detected between horses with an ulcer score of 3, compared with sucrose concentrations in horses with an ulcer score of 2.

The association between urine sucrose concentration and ulcer score for these 11 horses was also examined by categorizing ulcer scores as ≤ 1 or > 1. Results of the generalized linear model and the Duncan multiple-range test revealed that urine sucrose concentration was significantly less in horses with ulcer scores ≤ 1 than in those with ulcer scores > 1.

In urine samples collected 4 hours after sucrose administration for the 11 horses in which feed deprivation induced gastric ulcers, we detected significant (P = 0.01) differences in urine

sucrose concentrations before and after omeprazole treatment. The association between urine sucrose concentration and ulcer score was analyzed for the data at the 4-hour collection. Results of the generalized linear model and Duncan multiple-range test revealed a significant difference between sucrose concentrations for horses with ulcer scores of 3, compared with sucrose concentrations in horses with ulcer scores ≤ 2. The association between urine sucrose concentration and ulcer score was also considered by categorizing ulcer scores as ≤ 1 or > 1. Results of the generalized linear model and Duncan multiple-range test revealed that urine sucrose concentration was significantly less in horses with ulcer scores ≤ 1, compared with concentrations in horses with ulcer scores > 1.

After examining the distribution of urine sucrose concentrations in samples collected 2 hours after sucrose administration, we determined that a cut-point value of 0.7 mg/mL would be appropriate to evaluate the apparent sensitivity and specificity of the urine sucrose concentration test to detect ulcer scores ≤ 1 or > 1 in these 11 horses. The rationale for categorizing ulcerations as ≤ 1 or > 1 was the observed distribution of the data (values of sucrose concentrations on the basis of ulcer score) and the fact that the authors considered ulcers with a score > 1 most likely to be of clinical importance to a horse and perceived by owners and veterinarians as clinically or physically important. Use of the cut-point value of 0.7 mg/mL for sucrose concentration and dichotomization of ulcer scores resulted in an apparent sensitivity of 83% (10/12) and apparent specificity of 90% (9/10).

Urine osmolality for the samples collected 2 hours after sucrose administration for all 13 horses after ulcer induction ranged from 508 to 1,445 mOsm (median, 1,034 mOsm); the urine osmolality for the 2-hour sample of these horses after omeprazole treatment ranged from 608 to 1,638 mOsm (median, 1,063 mOsm). Difference in osmolality after induction of ulcers and after omeprazole treatment ranged from –637 to 359 mOsm (median, –74 mOsm); the difference between values after ulcer induction and omeprazole treatment was not significant.

Value of the urine sucrose concentration-to-urine osmolality ratio in samples collected 2 hours after sucrose administration for the 13 horses after ulcer induction ranged from 0.0003 to 0.0066 mg/mL/mOsm (median, 0.0013 mg/mL/mOsm); the ratio in samples collected 2 hours after sucrose administration for the 13 horses after omeprazole treatment ranged from 0.0001 to 0.0015 mg/mL/mOsm (median, 0.0004 mg/mL/mOsm). This ratio for samples collected 2 hours after sucrose administration was significantly (P = 0.003) higher after ulcer induction, compared with the ratio after treatment with omeprazole (ie, after adjusting for urine osmolality, sucrose concentrations were significantly higher after ulcer induction).

Considering only the 11 horses in which feed deprivation induced ulcers, the urine osmolality values for samples collected 2 hours following sucrose administration after ulcer induction ranged from 508 to 1,445 mOsm (median, 913 mOsm). After omeprazole treatment, urine osmolality 2 hours after sucrose administration ranged from 608 to 1,638 mOsm (median, 1,063 mOsm). The median difference in urine osmolality between values after ulcer induction and after omeprazole treatment ranged from –637 to 359 mOsm (median, –84 mOsm); the difference between values after ulcer induction and omeprazole treatment was not significant.

The ratio of urine sucrose concentration to urine osmolality in samples collected 2 hours after sucrose administration for the 11 horses after ulcer induction ranged from 0.0004 to 0.0066 mg/mL/mOsm (median, 0.0002 mg/mL/mOsm); the ratio in samples collected 2 hours after sucrose administration for the 11 horses after omeprazole treatment ranged from 0.0001 to 0.0015 mg/mL/mOsm (median, 0.0003 mg/mL/mOsm). This ratio in samples collected 2 hours after sucrose administration was significantly (P = 0.002) higher after ulcer induction than after treatment with omeprazole (ie, after adjusting for urine osmolality, sucrose concentrations were significantly higher after ulcer induction). The difference in ratios after ulcer induction and after omeprazole treatment ranged from –0.0001 to 0.0053 mg/mL/mOsm (median, 0.0018 mg/mL/mOsm). Only 1 horse had a negative value (–0.001) for this difference in ratios.

Because feed deprivation failed to induce ulcers in 2 horses and the 21-day treatment period did not result in ulcer scores of 0 for all horses, the relationship between the urine sucrose concentration-to-urine osmolality ratio and ulcer score was examined. Urine sucrose concentration-to-urine osmolality ratios were calculated for each ulcer score. In 9 horses with an ulcer score of 0, the urine sucrose concentration-to-urine osmolality ratio ranged from 0.0002 to 0.0015 mg/mL/mOsm (median, 0.0005 mg/mL/mOsm). In 5 horses with an ulcer score of 1, the urine sucrose concentration-to-urine osmolality ratio ranged from 0.0001 to 0.0004 mg/mL/mOsm (median, 0.0002 mg/mL/mOsm). In 6 horses with an ulcer score of 2, the urine sucrose concentration-to-urine osmolality ratio ranged from 0.0002 to 0.0038 mg/mL/mOsm (median, 0.0013 mg/mL/mOsm). In the 6 horses with an ulcer score of 3, the urine sucrose concentration-to-urine osmolality ratio ranged from 0.0007 to 0.0066 mg/mL/mOsm (median, 0.0026 mg/mL/mOsm). Results of the Duncan multiple-range test revealed significant differences in ratios between horses with ulcer scores of 3 and horses with ulcer scores ≤ 2. Ratios differed, but not significantly, between horses with ulcer scores of 3 and horses with ulcer scores of 2 (P = 0.092) and between horses with ulcer scores of 2 and horses with ulcer scores of 1 (P = 0.076); no significant differences in ratios were detected between horses with ulcer scores of 2 through 0. The correlation between urine sucrose concentration-to-urine osmolality ratio and ulcer score was also evaluated by categorizing ulcer scores as ≤ 1 or > 1. Results of the generalized linear model and Duncan multiple-range test revealed that ratios were significantly (P = 0.046) lower in horses with ulcer scores ≤ 1 than in horses with ulcer scores > 1. The ratio for horses with ulcer scores ≤ 1 ranged from 0.0001 to 0.0015 mg/mL/mOsm (median, 0.0004 mg/mL/mOsm), and the ratio for horses with ulcer scores > 1 ranged from

0.0002 to 0.0066 mg/mL/mOsm (median, 0.0017 mg/mL/mOsm).

After examining the distribution of urine sucrose concentration-to-urine osmolality ratios for samples collected 2 hours after sucrose administration, a cut-point value of 0.0005 mg/mL/mOsm was used to evaluate the apparent sensitivity and specificity of the use of the ratio to detect ulcer scores ≤ 1 or > 1 in these 11 horses. Use of the cut-point value of 0.005 mg/mL/mOsm for the ratio and dichotomization of ulcer scores resulted in an apparent sensitivity of 92% (11/12 horses) and apparent specificity of 80% (8/10). Thus, adjusting for urine osmolality improved sensitivity of the test but reduced specificity.

Discussion

Prevalence of gastric ulcers can be as high as 90% among Thoroughbreds actively engaged in training or racing.[9,10] The availability of a simple, noninvasive test to detect and monitor gastric ulcers in horses at risk for developing this condition or with clinical signs consistent with gastric ulcers is greatly needed. The primary objective of the study reported here was to determine whether sucrose permeability testing could be used to accurately predict gastric ulcers and the severity of gastric ulcers in horses.

When there is healthy gastric mucosa, sucrose is transported to the small intestine where it is enzymatically hydrolyzed by the brush-border enzyme sucrase to its monosaccharide units, glucose and fructose. Sucrose is a relatively large molecule that is unable to cross the intact gastric mucosa to any detectable extent. Therefore, only trace amounts of sucrose appear in the urine when there are no defects in the gastric mucosa. However, when there is damage to the gastric mucosa, sucrose is able to penetrate the gastric wall and enter the circulatory system.[22-24] Once in the circulatory system, sucrose is filtered from the blood by the kidneys and is concentrated and excreted in the urine.[21-23]

Analysis of results of the study reported here indicates that the urine sucrose concentration is useful for identifying horses with endoscopically visible gastric ulcers. Urine sucrose concentrations were higher in horses with induced ulcers before omeprazole treatment than in these same horses after omeprazole treatment. Moreover, urine sucrose concentrations appeared to increase with increasing gastric ulcer score. Sucrose concentrations for horses with an ulcer score > 1 were significantly higher than those for horses with an ulcer score ≤ 1. This may be of clinical use because, in the authors' experiences, gastric ulcers with a score ≤ 1 are rarely deemed clinically important.

When considering results for all 13 horses, significant differences were observed between those with ulcer severity scores of 3 and ulcer severity scores ≤ 2. These differences remained significant even when data from the 2 horses in which feed deprivation did not induce ulcers were excluded from analysis. Values of urine sucrose concentration appeared to increase with increasing ulcer score, particularly for ulcer scores ≤ 1. Given that even an experienced endoscopist may be inaccurate with regard to detection of gastric ulcers[28] and the inherent problems of repeatability and validity with scoring gastric ulcers, it is possible that urine sucrose concentration may more accurately indicate the extent of gastric mucosal defects than results of endoscopic examination.

Horses in the study were allowed ad libitum access to water prior to sucrose permeability testing. Because we used urine sucrose concentration as the outcome, differences among horses in urine concentration may have resulted from differences in water consumption, renal blood flow, glomerular filtration, or fluid resorption in the renal tubules. To assess the extent to which urine concentration influenced our results, we assessed the urine sucrose concentration-to-urine osmolality ratios. Results were essentially unchanged: the urine sucrose concentration-to-urine osmolality ratios were significantly higher after ulcer induction than after omeprazole treatment, and these ratios were significantly higher for horses with ulcer scores > 1 than for horses with ulcer scores ≤ 1. The same pattern of increasing values with increasing ulcer score was observed for the urine sucrose concentration-to-urine osmolality ratios as was observed for the urine sucrose concentrations.

In other studies[21-23,29] of sucrose permeability, the total mass of sucrose appearing in the urine during a 5-hour period was used as the outcome of interest. For the study reported here, urine sucrose concentration 2 hours after sucrose administration was selected as the outcome of interest. We used a single concentration at a specified time point for several reasons. First, determining the total mass of sucrose in urine requires collecting all urine produced; without the use of an endoscope, it is impossible to confirm that the entire volume of urine in the bladder has been evacuated. Second, it would be impractical in a field setting to insert an indwelling urinary catheter in a horse to collect urine for several hours. Third, our goal was to establish a simple and cost-effective test whereby the bladder could be emptied as much as possible via catheterization, sucrose could be administered intragastrically, and a urine sample could be collected at a predetermined time for determination of urine sucrose concentration. A potential limitation of a single-sample urine sucrose concentration is that the concentration would be dependent on the hydration status of the horse and other factors that influence the capacity to concentrate urine. The concentration of sucrose in urine would be influenced by the extent to which the urine had been concentrated. For example, a horse that was dehydrated might concentrate its urine to conserve body water, resulting in a higher urine sucrose concentration than if the horse were not dehydrated. All horses included in this study were allowed ad libitum access to water, except for the 4 hours during which we were collecting urine samples. Presumably, the hydration status of each horse was relatively constant. Therefore, urine concentration would be relevant only when there was considerable intra- and interindividual variability among the horses with respect to hydration status and capacity to concentrate urine. As described previously, results were similar when we examined urine sucrose concentration or the urine sucrose concentration-to-urine osmolality ratio

as the outcome of interest. Therefore, we believe that differences in urine concentration among the horses had little, if any, effect on the results of this study. Although the apparent sensitivity and specificity differed for the 2 outcomes, these differences were small and not important. The sensitivity and specificity of the test may differ among horses with compromised renal blood flow or renal dysfunction.

Horses in this study were administered a uniform amount of sucrose (454 g), regardless of their body weight. Consequently, the dosage of sucrose (0.8 to 1.3 g/kg) varied among horses. This variability in dosage could have explained some of the variability among horses in urinary concentrations of sucrose; however, the correlation between urine sucrose concentration in samples obtained 2 hours after sucrose administration and body weight was not significant. Nevertheless, administration of a fixed dosage (1 g/kg) of sucrose rather than a fixed amount (454 g) may improve performance of the test. Because our long-term goal was to develop a test that will be clinically useful in field settings, our rationale for the administration of 454 g to each horse was that accurate body weights often are not available to veterinarians in field settings and that it is easy to purchase or measure 454 g of table sugar.

Variability in urine sucrose concentration among horses was apparent (Fig 1). One horse had the highest urine sucrose concentration after ulcer induction and after omeprazole treatment. The reason for this apparent outlier is unclear. Concentrated urine could have contributed to this finding because the horse had the second-highest urine osmolality after ulcer induction and the highest urine osmolality after treatment with omeprazole; however, this was an unlikely explanation because the urine sucrose concentration-to-urine osmolality ratio after ulcer induction in this horse remained an outlier. The horse did have ulcers categorized as severe after ulcer induction that improved and were categorized as moderate after omeprazole treatment. The extent of microscopic and macroscopic mucosal lesions in this horse may have been considerably worse than those of other horses. Alternatively, other unmeasured factors that could influence sucrose permeability testing (eg, rate of gastric emptying) could have caused this finding. Such outliers may yield false-positive results (ie, urine sucrose concentrations indicating ulcers when they truly do not have ulcers) for a dichotomous outcome based on a specific cut-point value. The extent and impact of such outliers remains to be determined in studies involving a larger population of horses with naturally developing ulcers.

Urine sucrose concentrations above the cut-point value of 0.7 mg/mL in horses with ulcer scores of 0 or 1 may have resulted from microscopic but not grossly evident defects of the gastric mucosa at the time of endoscopy. Evidence exists that the findings of endoscopy, gross pathologic examination, and microscopic examination of gastric ulcers in horses may be discordant or divergent.[23,28] Investigators concluded in 1 study[23] that increased sucrose permeability in dogs was correlated more closely with generalized mucosal damage than with discretely visible ulcers. In another study,[28] the correlation between endoscopic, necropsy, and microscopic pathologic findings of gastric ulcers in horses was evaluated. Analysis of results of that study indicates that endoscopy does not accurately predict the number of gastric ulcers because small glandular ulcers (approx 5 mm in diameter) may be missed when the endoscopist is inexperienced and gastric contents or improper insufflation may mask ulcers. Furthermore, those investigators did detect a weak correlation between severity scores determined during endoscopy and microscopic determination of ulcer depth. However, the endoscopist in that study misclassified 57% of the nonglandular gastric ulcers as superficial, whereas microscopic examination revealed that those ulcers were deep and extended to the submucosa and tunica muscularis. Sucrose is absorbed across damaged gastric mucosa; thus, it is possible that the lack of grossly evident ulcers despite microscopic evidence of damage to the mucosa at the time of endoscopy may result in increased absorption of sucrose.

It is also possible that urine sucrose concentrations > 0.7 mg/mL in those horses with ulcer scores of 0 or 1 for the squamous mucosa may have been the result of ulcers in the gastric glandular mucosa that were not detected during endoscopy. Because the first 7 horses consumed a meal approximately 4 hours prior to endoscopy, examination of the gastric glandular mucosa was limited. As a result, we elected to endoscopically examine the final 6 horses on the morning after sucrose permeability testing following 12 to 14 hours of feed deprivation. This method allowed complete examination of the gastric squamous mucosa and most of the glandular mucosa. Glandular ulcers were not detected in the 6 horses in which the glandular mucosa was examined, and results for those 6 horses did not appear to differ from results for horses with similar ulcer scores that did not have endoscopic examination of the glandular mucosa, indicating that the horses with ulcer scores of 0 or 1 for the squamous mucosa were less likely to have glandular ulcers. However, endoscopic examination can fail to detect glandular mucosal ulcers.[28] Potential problems with this modification of our study design were that ulcer status could have changed during the 24-hour period between collection of the urine sample and endoscopy and that our method of evaluation differed for the first 7 and the last 6 horses; however, results for the last 6 horses did not differ qualitatively or quantitatively from results for the first 7 horses that were endoscopically examined immediately after sucrose testing. Thus, we do not believe that this difference in timing of endoscopic examinations biased our results.

In 1 study,[21] investigators reported that gastric damage induced by nonsteroidal anti-inflammatory agents or ethanol could be detected by an increase in sucrose permeability. Those same investigators used sucrose permeability testing to detect gastric ulcers in 189 human patients; they reported a sensitivity of 84% and specificity of 97%.[22] Sucrose permeability testing has been used to detect gastric ulcers and gastric carcinoma in humans.[29] In that study, a sensitivity of 79% and specificity of 94% were reported for detecting gastric ulcers.

In the study reported here in which we used a urine

sucrose concentration cut-point value of 0.7 mg/mL in the urine sample collected 2 hours after sucrose administration, the apparent sensitivity of urine sucrose testing for detecting horses with gastric ulcer scores ≤ 1 or > 1 was 83% and apparent specificity was 90%. Use of the urine sucrose concentration-to-urine osmolality ratio and a cut-point value of 0.0005 mg/mL/mOsm resulted in sensitivity of 92% and specificity of 80%. These findings closely agree with results of sucrose permeability tests conducted in other species.[22,29] Sensitivity and specificity determined for the study reported here should be interpreted carefully because of the crossover design (observations among horses were not independent, and we did not study 2 distinct groups of horses that did or did not have gastric ulcers). Moreover, we cannot recommend a cut-point value for diagnosis (eg, 0.7 mg/mL) because additional research is needed to evaluate sucrose permeability testing in a population of horses with and without naturally developing gastric ulcers to evaluate the clinical accuracy and establish a cut-point value for this test. However, analysis of our results indicates that sucrose permeability testing could be clinically useful.

Urine sucrose concentrations 2 hours after sucrose administration were consistently higher than concentrations 4 hours after sucrose administration (data not shown). Analysis of the data on urine sucrose concentrations in samples collected 4 hours after sucrose administration also revealed significant ($P = 0.01$) differences in urine sucrose concentrations between horses with and without induced gastric ulcers. Analysis of these findings indicates that there may be a range of time for collection of urine samples after sucrose administration (2 to 4 hours) for which useful results may be obtained, thus making the test more flexible for clinicians with respect to the timing of sample collection.

In the design of this study, omeprazole was used to treat and prevent ulcers for a duration less than that recommended by the manufacturer (ie, 21 days rather than the recommended 28 days). This may have contributed to the finding that 6 horses had ulcer scores > 1 at the conclusion of the 21-day treatment period. The rationale for the use of a 21-day treatment period was that preliminary data collected in 5 horses indicated that a 21-day treatment period was sufficient to heal induced gastric ulcers. Nevertheless, ulcer scores were significantly ($P = 0.001$) reduced after treatment with omeprazole in the horses reported here. This significant difference in ulcer score was observed even when data from the 2 horses that failed to develop gastric ulcers after feed deprivation were included (ie, ulcer scores of 0 in these horses before and after omeprazole treatment).

The feed-deprivation method of ulcer induction was selected because it does not cause clinical signs of discomfort and results in ulcers in the squamous mucosa adjacent to the margo plicatus, the area most commonly affected in horses.[20] Feed deprivation was unsuccessful in inducing ulcers of the squamous mucosa in 2 horses. The extent to which induced gastric ulcers resemble naturally developing ulcers is unknown. Thus, sucrose permeability testing to detect gastric ulcers needs to be evaluated in horses with and without naturally developing gastric ulcers. As described, horses were not examined endoscopically prior to intermittent feed deprivation. Consequently, ulcers in some horses after feed deprivation may have reflected ulcers that were already there prior to feed deprivation and that were unchanged or made more severe by intermittent feed deprivation.

One possible explanation for our finding that urine sucrose concentrations were lower among horses after treatment with omeprazole than among horses after induction of ulcers was that there was an effect of omeprazole separate from that of mucosal healing. We believe this is highly unlikely for the following reasons. First, horses were not administered omeprazole on the day of sucrose administration. Because the permeation of sucrose is in the stomach, the effects of omeprazole that interfere with absorption would have to be local and there would not have been omeprazole in the stomach of the horses at the time of sucrose permeability testing. Second, although omeprazole delays gastric emptying in other species, we believe the net effect of delayed gastric emptying would be to increase the opportunity for sucrose permeation by increasing the contact time of sucrose with the gastric mucosa. Third, urine sucrose concentration correlated with ulcer severity score regardless of treatment status, indicating that the observed association was more likely attributable to gastric ulcers than to an undetermined effect of omeprazole. Fourth, our results are consistent with studies[21-23,25,29] in other species, indicating that sucrose permeability testing is a reliable indicator of gastric ulcers. Although we cannot exclude the possibility that the acid-neutralizing effect of omeprazole was a factor, we are not aware of data from other species indicating that increased intragastric pH is associated with reduced sucrose permeation.

Development of sucrose permeability testing in which sucrose concentration in blood samples is determined at a specified time after administration of sucrose would be more practical for clinical use. To our knowledge, a method for reliably detecting the low concentration of sucrose in blood samples has not been developed. However, blood samples collected from horses in this study would be available for testing if such a method were developed.

[a]Horsechow 100, Purina Mills, St Louis, Mo.
[b]Gastrogard, Merial Inc, Atlanta, Ga.
[c]Angiocath, Becton-Dickinson Infusion Therapy Systems Inc, Sandy, Utah.
[d]Acepromazine, Vedco Inc, St Joseph, Mo.
[e]Falcon polypropylene conical tubes, Becton, Dickinson & Co, Franklin Lakes, NJ.
[f]Sodium azide, Sigma Chemical Co, St Louis, Mo.
[g]Omolene 200, Purina Mills, St Louis, Mo.
[h]Olympus GIF-100, Olympus America Inc, Melville, NY.
[i]Xylazine, Vedco Inc, St Joseph, Mo.
[j]0.45-µm syringe filter, VWR Scientific Products, West Chester, Pa.
[k]Autosampler 717 plus, Waters Corp, Milford, Mass.
[l]2-L plastic bottle (part No. 44129), Dionex Corp, Sunnyvale, Calif.
[m]Carbopac PA10 analytical and guard columns, Dionex Corp, Sunnyvale, Calif.
[n]GS50 gradient pump, Dionex Corp, Sunnyvale, Calif.
[o]ED40 electrochemical detector, Dionex Corp, Sunnyvale, Calif.
[p]5500 vapor pressure osmometer, Wescor Inc, Logan, Utah.
[q]SAS statistical software, version 8.2, SAS Institute Inc, Cary, NC.

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