Endoscopy via a gastric cannula to monitor the development of ulcers in the pars esophagea in pigs after consumption of a finely ground feed combined with a period of withholding of feed

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Objective—To develop an endoscopic technique for use in monitoring development of gastric ulcers via a gastric cannula during withholding of feed and administration of a finely ground diet to pigs. Animals—6 pigs weighing between 60 and 70 kg. Procedure—A gastric cannula was surgically inserted adjacent to the pars esophagea in each pig. Pigs were fed a finely ground diet for two 7-day periods that were separated by a 48-hour period during which feed was withheld. Endoscopic examination via the gastric cannula was used to monitor development of ulcers in the pars esophageal region of the pigs during the 48-hour period of feed withheld and subsequent 7-day feeding period. An ulcer score was assigned during each endoscopic examination. A final examination was performed during necropsy and compared with results for the final endoscopic examination. Results—Consumption of a finely ground diet for 7 days resulted in progressive erosive damage to the pars esophageal region of the stomach. Further significant increases in ulcerative damage were detected after 24 and 48 hours of withholding of feed. Final examination during necropsy did not reveal significant differences from results obtained during the final endoscopic examination. Conclusions and Clinical Relevance—Endoscopic examination via a gastric cannula was an effective means of monitoring ulcer development in the pars esophagea of pigs. Feeding a finely ground diet and withholding of feed induced endoscopically observable ulcers in the stratified squamous epithelial region of the stomach. Direct visual examination during necropsy confirmed the accuracy of endoscopic examination. (Am J Vet Res 2002;63:1076–1082)
gastric flange. A central opening (diameter, 2 cm) was created in the oval section of a silicon sheet, and the barrel of the cannula was inserted through this opening. The barrel of the cannula and the silicon sheet were then affixed to each other by use of medical-grade silicon adhesive. Similarly, an oval-shaped section of polyester fiber mesh (length, 5 cm; width, 6 cm) was attached to the barrel of the cannula at a location approximately 2.5 cm from the section of the silicon sheet; the fiber mesh served as an extragastric flange.

Surgical procedure—Feed was withheld from pigs for 24 hours prior to surgery. Anesthesia was induced by administration of a combination product containing tiletamine hydrochloride-zolazepam hydrochloride (3 mg/kg, IM). An orotracheal tube was then inserted, and anesthesia was maintained by administration of isoflurane vaporized in 100% O2. Throughout anesthesia, pigs were administered saline (0.9% NaCl) solution at a rate of 15 ml/kg/h, IV, via a lateral ear vein.

Pigs were positioned in dorsal recumbency, and the ventral surgical site was prepared by use of a commercial iodine-based solution. A ventral midline incision (length, approx 10 cm) was made, beginning at the xiphoid process and extending caudally to expose the abdominal cavity. In the stomach of pigs, a small outpouching can be seen adjacent to the pars esophagea (Fig 1). This outpouching was identified and clamped with Doyen forceps to prevent gastric fluid from entering the surgical site. An inchion approximately 2.5 cm in length was made in the outpouching. The end of the cannula with the affixed silicon sheet was inserted, leaving the polyester mesh outside the stomach. A purse-string suture pattern, using 2-0 polyglactin 910, and the skin was closed by use of a simple interrupted sutures of 2-0 polyglactin 910. A cannula has been inserted into the stomach and secured by use of a purse-string suture. Polyester fiber mesh affixed to the cannula and the silicon sheet were then affixed to each other by use of medical-grade silicon adhesive. Similarly, an oval-shaped section of polyester fiber mesh (length, 5 cm; width, 6 cm) was attached to the barrel of the cannula at a location approximately 2.5 cm from the section of the silicon sheet; the fiber mesh served as an extragastric flange.

Figure 1—Photographs of the surgical procedure used to insert a cannula into the area immediately adjacent to the pars esophageal region of the stomach of pigs. Panel A—A 10-cm incision is made on the ventral midline, beginning at the xiphoid process and extending caudally, and the stomach is exposed. The cranial extent of the incision is toward the bottom of the photograph. Notice the outpouching of the stomach adjacent to the pars esophagea (arrow). Panel B—The outpouching has been isolated with Doyen intestinal forceps (arrows). Panel C—A cannula has been inserted into the stomach and secured by use of a purse-string suture. Polyester fiber mesh affixed to the cannula is secured to the seromuscular layer of the stomach with simple interrupted sutures. Panel D—The cannula is exteriorized on the left side of the body wall caudal to the last rib.

Approximately 13 cm of the cannula barrel was exteriorized and situated in a ventrodorsal orientation. The linea alba and subcutaneous tissues were closed by use of a simple continuous pattern of 2-0 polyglactin 910, and the skin was closed by use of a simple continuous pattern of 2-0 nylon. A rubber washer (diameter, 4 cm) was affixed to the exposed barrel of the cannula by use of silicon adhesive; the washer prevented the cannula from re-entering the abdominal cavity. An adapter made of polyvinyl chloride tubing was used as a plug and inserted into the open end of the cannula. A metal hose clamp was used to secure the adapter in position and to prevent accidental loss of the plug during the endoscopic phase of the study when the plug was repeatedly removed and reinserted. In our experience, repeated manipulation of the plug causes a slight loss in resiliency of the silicon tubing, which enables the plug to come out of the cannula unless it is secured in position.

Buprenorphine (0.03 mg/kg, IM) was administered to each pig prior to the end of surgery to provide analgesia during recovery from anesthesia. After recovery from anesthesia, each pig was returned to its pen and resumed typical activities for 10 days before the start of the experiment.

Feeding protocol—Prior to the study, pigs had ad libitum access to the coarsely ground (particle size, 600 µm) diet. On day 1 of the study, pigs were randomly assigned to 2 groups (3 pigs/group). The second group of pigs began the study after the first group, so that only 3 pigs were examined per day. On days 1 through 7, pigs were provided ad libitum access to a finely ground (particle size, < 400 µm) diet. On day 8 of the study, pigs were fasted for 24 hours to allow emptying of the stomach. On day 9, pigs were fasted for 12 hours. On day 10, pigs were fasted for 8 hours. On day 11, pigs were fasted for 6 hours. On day 12, pigs were fasted for 4 hours. On day 13, pigs were fasted for 2 hours. On day 14, pigs were fasted for 1 hour. On day 15, pigs were fasted for 30 minutes. On day 16, pigs were fasted for 15 minutes. On day 17, pigs were fasted for 10 minutes. On day 18, pigs were fasted for 5 minutes. On day 19, pigs were fasted for 3 minutes. On day 20, pigs were fasted for 2 minutes. On day 21, pigs were fasted for 1 minute.
days 8 and 9, feed was withheld from the pigs, and pigs again had ad libitum access to the finely ground diet on days 10 through 16.

**Endoscopic examinations**—Pigs were sedated and restrained in right lateral recumbency. Endoscopic examination was performed to determine the status of the pars esophagea on day 1 at the beginning of the study and on day 7 prior to the onset of the period of feed withholding. Additional endoscopic examinations were conducted on days 8 and 9 to monitor the course of ulcer development during the 48-hour period of feed withholding. Endoscopic examinations also were conducted daily from days 10 to 16 to document the health status of the pars esophagea following resolution of feed withholding.

Three of the pigs permitted the cannula plug to be removed prior to sedation and recumbency, which facilitated gravity drainage of the stomach contents. In the remaining pigs, plugs were removed after the pigs were sedated. The gastric lumen was lavaged with a minimum of 420 ml of water. The flexibility of the cannula as it exited through the body wall enabled the lavage water and gastric contents to be drained by tilting the cannula and pointing it downward. In some pigs that had greater than average filling of the stomach (ie, immediately after consumption of a meal), it was necessary to position the pig in left lateral recumbency to enable adequate drainage of gastric contents. For all endoscopic examinations, drainage was sufficient to prevent chyme or lavage fluid from obstructing subsequent examination of the pars esophagea.

Examination of the pars esophagea was performed by use of a flexible fiberoptic endoscope. A laparoscopic 1-seal reducer cap was fitted to the barrel of the cannula, creating an airtight seal for insufflation of the stomach. Following insertion of the endoscope, the stomach was insufflated to provide an unobstructed view of the stratified squamous epithelium of the pars esophagea. A camera was fitted to the eyepiece of the endoscope to record images.

An ulcer scoring system described by Eisemann and Argenzio(2) was used to monitor the progress of ulcerative damage. During each endoscopic examination, the epithelial surface of the pars esophagea was scored by use of a 7-point scale (1, smooth healthy tissue; 2, cornification evident; 3, more extensive cornification with elongated papillae; 4, completely cornified with extensive development of papillae; 5, tissue erosions evident; 6, more extensive erosions with small ulcers; and 7, completely ulcerated).

On day 16, pigs were euthanatized by administration of an overdose of sodium pentobarbital (0.2 ml/kg, IV). After the pigs were euthanatized, a final endoscopic examination was performed, and the stomachs were then removed to enable direct visual examination. Comparison of results for the final endoscopic examination and direct visual observation during necropsy allowed us to assess accuracy of the endoscopic procedure.

**Statistical analysis**—Each pig was considered as an experimental unit. Because 1 pig was not able to complete the entire study, data from only 5 pigs was included in statistical analysis, which was accomplished by use of a statistical software program. Data were analyzed by use of a 1-way ANOVA, and post hoc comparisons were performed by use of a Tukey test. Significance was declared at values of \( P < 0.05 \).

**Results**

**Sedation and restraint**—Five of 6 pigs completed the study. During surgical insertion of the cannula, 1 pig developed respiratory distress that caused complications during recovery from anesthesia and subsequent episodes of sedation. That pig died during the fourth endoscopic examination.

Initial attempts at sedation involved IM administration of a combination of xylazine hydrochloride (0.02 mg/kg) and ketamine hydrochloride (0.03 mg/kg). However, of the 5 pigs that completed the study, 1 was not fully responsive to this combination, causing difficulty during endoscopic examination because of paddling movements of the pig's limbs. A second pig became hyperexcitable after administration of the xylazine-ketamine combination. Several additional dosages of the xylazine-ketamine combination were tried on these pigs, but sedation was inadequate. Therefore, pigs were sedated by administration of a combination product that contained tiletamine-zolazepam (0.02 mg/kg, IM), which was effective in 4 pigs. However, 1 of these pigs became progressively less sedate with the tiletamine-zolazepam product over a period of 3 days during the study and was thereafter sedated by administration of medetomidine hydrochloride (0.02 mg/kg, IM), which was reversed by administration of atipamezole (0.02 mg/kg, IM).

**Endoscopic examination**—Insertion of the endoscope via the gastric cannula allowed observation of the stomach of all pigs (Fig 3). On day 1, pigs had minimal evidence of ulcers (Fig 4). Four of the 5 pigs had a normal appearing pars esophagea, which was characterized by an elevated, glistening, white epithelium that was demarcated from the surrounding gastric mucosa. The fifth pig had evidence of minor damage that was characterized by loss of apical epithelial tissue, although none of the erosions appeared to reach the basement layers. After being provided the finely ground diet for 7 days, all 5 pigs had substantial evidence of injury to the pars esophagea (Fig 5). The injury was characterized by extensive loss of epithelium with keratinization of any remaining epithelium.
Pigs were not allowed access to feed on days 8 and 9. By 24 hours after the onset of withholding of feed, the pars esophagea had a significant increase in the extent and depth of loss of the epithelium (Fig 6). After 48 hours of feed withholding, the pars esophageal region contained from 2 to 5 ulcers. Without exception, ulcers originated along the crest of the gastric folds within the pars esophagea as they emerged from the cardia. Additional ulcers then progressed throughout the pars esophageal region.

From days 10 to 16, pigs were again provided the finely ground diet. This resulted in a gradual improvement in the health of the stratified squamous epithelium from days 11 to 15 (data not shown), and the epithelium was significantly improved by day 16.

The study was terminated 7 days after resumption of feeding, and pigs were euthanatized. There was not a significant difference between scores assigned during the final endoscopic examination and scores assigned on the basis of direct visual examination during necropsy (Fig 6). Results for the final endoscopic examination varied by a score of 0.33 from the mean score assigned during direct visual examination during necropsy, indicating close agreement between endoscopic and direct visual examinations. This degree of agreement was achieved despite the fact that the score for direct visual examination during necropsy in 1 pig did not correlate with score for the final endoscopic examination. In that pig, the cannula placement induced a fold in the stomach wall that partially obscured the pars esophageal region. Insertion of the endoscope into the lumen of the stomach, accompanied by full retroflexion, was inadequate to enable us to completely observe the pars esophagea, which accounted for the discrepancy.

One detail that could not be fully appreciated endoscopically was the 3-dimensional aspect of the pars esophagea. This region is normally elevated above the surrounding mucosa in healthy pigs, and this was clearly evident in the pigs reported here. However, after loss of the epithelial layer, the pars esophagea was level with or lower than the surround-
ing mucosa. This subtle difference was difficult to detect endoscopically.

Discussion

The IM administration of tiletamine-zolazepam at a dosage of 0.02 mg/kg was consistently effective in inducing sedation in 4 of 5 pigs in which it was used. It induced a mild state of sedation that lasted for approximately 30 minutes. This allowed investigators to easily handle the pigs yet enabled them to recover rapidly (ie, within 30 minutes after injection) and completely. Future studies should include a determination of the effect of various sedatives on the pigs prior to initiation of a feeding regimen.

Removal of the cannula plug prior to sedation of the pigs and use of an accompanying lavage, when necessary, allowed adequate drainage of stomach digesta. In those instances in which a pig was sedated prior to plug removal, lavage and draining required shifting the pig into a lateroventral position in left lateral recumbency. Adequate drainage of the stomach was critical to the success of this technique, because it enabled the pigs to be continuously fed prior to endoscopic examination. In other studies, feed was withheld from pigs for 12 to 72 hours prior to transoral endoscopic examination. Because withholding of feed is 1 method for inducing ulcers in pigs, it would be desirable to separate this potentially confounding ulcerogenic effect. Conversely, the length and flexibility of the gastric cannula used in the study reported here allowed drainage of the stomach contents, permitting continuous feeding of the pigs and eliminating the confounding effect of feed withdrawal.

Additionally, insertion of an endoscope via the esophagus requires general anesthesia, prolonging the recovery period and delaying the return to feed consumption. The sedative techniques used in the study reported here allowed the pigs to be handled easily and examined with greater frequency with less risk and distress to the pigs. The pigs completely recovered within 30 minutes after the endoscopic examinations and, in most cases, began to eat again.

Insertion of an endoscope via the esophagus has several other drawbacks. Insertion of an endoscope through the pars esophagea increases the chance of generating iatrogenic damage that may be misinterpreted as naturally occurring ulcerative damage. Also, examination of the pars esophagea would require complete retroflexion of the endoscope, partially obscuring the region being examined.

The endoscopic examination reported here allowed the pars esophageal region of the stomach to be clearly observed and examined, except that the elevation of the pars esophagea relative to the surrounding mucosa could not be fully appreciated. Monitoring the pars esophagea over time provides this technique with a major advantage over other types of methods used in studies designed to evaluate treatment or prevention of gastric ulcers. Such studies typically require a comparative slaughter design in which a representative group of pigs is euthanatized at the beginning of the study to establish a baseline for comparison. Additional pigs would need to be euthanatized throughout the study to monitor changes in the pars esophagea, requiring a much larger number of animals. In addition, the health of the pars esophagea can change rapidly in response to withholding of feed and administration of treatments; thus, frequent examinations would be required to detect and monitor changes, increasing the number of pigs required in a comparative slaughter design. Studies such as this have been conducted by our laboratory group to determine the effects of animal crowding and size of feed particles; those studies required 80 pigs. Use of endoscopic examination via a gastric cannula would have greatly reduced the number of pigs needed for completion of those studies.

The study reported here was terminated 7 days after the resumption of feeding for 2 reasons. First, efficacy of the endoscopic examination for monitoring day-to-day changes in the health status of the pars esophageal region of the stomach was confirmed during the period of feed withholding. Severity of the damage induced by feed withdrawal, mimicking the anorectic condition seen secondary to other illnesses, was a key finding of this study, because it confirms the potential for use of feed withholding to induce ulcers for study. Second, the study was deliberately ended prior to complete restoration of the health status of the pars esophagea to allow comparison of results for the endoscopic examination with results for direct visual examination during necropsy. Similarity of results between the final endoscopic and direct visual examinations confirms the efficacy of the endoscopic technique for monitoring gastric health in swine.

The pars esophageal region in all pigs except 1 was healthy prior to the start of the feeding protocol, suggesting that any subsequent health changes were probably caused by the finely ground diet. However, a control group of pigs fed a nonulcerogenic coarsely ground diet would have been beneficial for comparison of dietary effects on ulcer formation (ie, ulcers in pigs on a nonulcerogenic diet would have implicated the techniques used rather than the diet). Before the technique described here is adapted as a replacement for large-scale, comparative slaughter studies, this type of comparative study should be performed.

We detected some pitfalls with the technique used in this study. For example, repeated contact with the endoscope generated a hemorrhagic ulcer in the proper gastric mucosa of 1 pig. Placement of the cannula in the correct position was critical. Gastric cannulas typically are inserted in the greater curvature of the stomach. However, in the study reported here, the cannula was placed adjacent to the pars esophagea. This provided 2 advantages. First, when entering the stomach via the cannula, the endoscope was immediately adjacent to the pars esophagea. This allowed for a more rapid identification of the pars esophagea and decreased the amount of time required for an examination. Second, by limiting the distance the endoscope must be inserted to locate the pars esophagea, the chance of iatrogenic injury was reduced, and the amount of gastric contents that accumulated on the tip of the endoscope that may have obscured visibility was reduced. In 4 of 5 pigs, this more proximal cannula
position allowed unobstructed observation of the entire pars esophageal region. However, in 1 pig, the surgical procedure induced a fold in the stomach wall that partially obscured the pars esophagea.

A finely ground diet was used in this study to maximize the potential ulcerative damage for use in determination of the efficacy of repeated endoscopic examinations. Consumption of a finely ground diet induced ulcerative damage in as little as 7 days, with a further increase in damage evident within 24 hours after withholding of feed. Such ulcers do not heal when the finely ground diet is maintained.\textsuperscript{4,6} Although returning to the finely ground diet after withholding of feed appeared to improve the health status of the pars esophagea in the pigs reported here (Fig 6), this should not be misconstrued as a beneficial effect of the diet. Indeed, between days 1 and 7, the finely ground diet induced an increase of 2 units in the ulcer score. Most likely, the finely ground diet ameliorated the ulcerogenic effect of feed withholding by diluting the amount of bile and acid in the gastric lumen. This would have been insufficient to allow for a minor improvement in the health of the pars esophageal region.

Other studies\textsuperscript{4—7} indicate that a finely ground diet is 1 of the primary causes of gastric ulcers in swine, although current management practices dictate the use of finely ground diets to increase animal performance. In comparison, ample evidence exists that a coarsely ground diet limits the induction of ulcers and, if given after ulcerative damage has occurred, can actually enhance repair.\textsuperscript{7} A finely ground diet increases the fluidity of gastric contents and the rate of gastric emptying, which interferes with the normal segregation of bile and acid to the more protected distal portion of the stomach.\textsuperscript{15,16} In comparison, Lang et al\textsuperscript{17} and Regina et al\textsuperscript{17} documented that a coarsely ground diet results in the partitioning of bile and acid away from the susceptible pars esophageal region of the stomach. Regardless of the diet, gastric contents have some buffering effect on acid secreted during digestion. However, bile and acid can more readily reflux into the proximal portion of the stomach because of the lack of any buffering digesta during a period of feed withholding, exacerbating injury to the pars esophagea.\textsuperscript{18}

The study reported here confirms that a finely ground diet induces moderate damage to the stratified squamous epithelial tissue, which may predispose pigs to more severe hemorrhagic ulcers should there be an anorectic period associated with other diseases or changes in management. For example, a commercial operation that transports pigs from 1 location to another for various stages of growth or development, such as just prior to the grow-finish period, could induce stress-related anorexia in addition to feed deprivation during transit. Lawrence et al\textsuperscript{19} monitored the effect of transportation and the subsequent 24-hour period on the health of the gastric stratified squamous epithelium and reported an extensive increase in the amount of damage seen in this region of the stomach of pigs. Additionally, feed intake during the first 24 hours after being relocated was greatly reduced.\textsuperscript{19} Those authors suggest that feeding a coarsely ground diet, which can have salutary effects on the stomach of pigs, for several days immediately following arrival would allow for repair of damaged stratified squamous epithelium induced by transportation and stress-related anorexia and limit ulcerogenesis occurring during subsequent consumption of a finely ground finishing diet.

Feeding a finely ground diet and withholding of feed induced endoscopically observable ulcers in the stratified squamous epithelial region of the stomach. Direct visual examination during necropsy confirmed the accuracy of endoscopic examination. Endoscopic examination via a gastric cannula was an effective means of monitoring ulcer development in the pars esophagea of pigs, and this technique can be used in future studies to limit the numbers of animals required to obtain meaningful results. Furthermore, this technique could be used to evaluate the effects of potential treatments for gastric ulcers.

\textsuperscript{5} Tenderfoot, Tandem Products Inc, Minneapolis, Minn.
\textsuperscript{6} Sileo Medical Grade Tubing, Technical Products, Decatur, Ga.
\textsuperscript{7} Sileo Medical Grade Sheeting, Technical Products, Decatur, Ga.
\textsuperscript{8} RTV Silicone Adhesive, Applied Silicone Corp, Ventura, Calif.
\textsuperscript{9} Mersilene Mesh, Ethicon, Somerville, NJ.
\textsuperscript{10} Telazol, Fort Dodge Animal Health, Overland Park, Kan.
\textsuperscript{11} Povidone Iodine Cleansing Solution, Allegiance Healthcare Corp, McGaw Park, Ill.
\textsuperscript{12} Tubing adapter, Genova Products Inc, Davison, Mich.
\textsuperscript{13} Olympus fiberoptic endoscope, Olympus Optical Co Ltd, Tokyo, Japan.
\textsuperscript{14} SigmaStat, SPSS Science, Chicago, Ill.

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