COMPARISON OF GASTROGRAFIN TO BARIUM SULFATE AS A GASTROINTESTINAL CONTRAST AGENT IN RED-EARED SLIDER TURTLES (*TRACHEMYS SCRIPTA ELEGANS*)

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Red-eared slider turtles (*Trachemys scripta elegans*) commonly develop intestinal obstruction. The gastrointestinal transit time in turtles tends to be longer than in other animals, making a rapid diagnosis of obstruction difficult. Fifteen red-eared sliders were given either Gastrografin® or 30% w/v barium sulfate orally to compare ease of administration, transit time, and image quality. Each contrast medium was easy to administer but barium sulfate had to be administered more slowly (mean = 40 s) than Gastrografin® (mean = 20 s) to prevent regurgitation. The mean transit and emptying time of Gastrografin® was at least 9 h faster than barium sulfate at all time points except gastric transit. Both contrast media had a smooth, uniform appearance that outlined the mucosa with well-defined margins within the stomach and proximal small intestine. Dilution of Gastrografin® occurred as it progressed through the intestines, resulting in decreased opacity in the distal small intestine and colon. Pre-administration packed cell volume and total serum protein levels of four turtles receiving Gastrografin® were compared with levels at 24-, 96-, and 168-hours postadministration as well as to four control turtles not receiving contrast medium. Packed cell volume and total serum protein levels did not significantly differ among the Gastrografin® and control group. From a clinical perspective, administration of Gastrografin® allows for quicker results with only minor hematologic changes in red-eared sliders, but visualization of this contrast medium in the lower gastrointestinal tract may be insufficient for an accurate diagnosis. *Veterinary Radiology & Ultrasound, Vol. 51, No. 1, 2010, pp 42–47.*

Key words: barium sulfate, Gastrografin®, gastrointestinal contrast study, red-eared sliders, *Trachemys scripta elegans*.

Introduction

RED-EARED SLIDERS (*Trachemys scripta elegans*) are semi-aquatic, omnivorous turtles native to the southern United States. They are one of the most frequently encountered chelonians in clinical practice. They are also used extensively in research, in areas such as ophthalmology,[1] otology,[2] reproductive physiology,[3] and species conservation.[4] Gastrointestinal obstruction is common in captive and free-ranging chelonians. Tortoises and semi-aquatic turtles, such as red-eared sliders, develop bowel obstruction by ingestion of bedding substrate and other indigestible materials.[5] Fishing line, plastic bags, and other nonradiopaque foreign bodies can lead to complete gastrointestinal obstruction in fresh- and saltwater turtles.[5,6] As in other species, intestinal obstruction can also be caused by neoplasia and functional problems such as volvulus and intussusception.[5] Although the alimentary tract of turtles is short, the transit time is longer compared with other vertebrate animals; this can make a rapid diagnosis of gastrointestinal obstruction difficult when relying on the transit time of an intestinal contrast medium.

Radiographic contrast media administered via an orogastric tube are often used to aid in the diagnosis of intestinal obstruction and foreign bodies. Barium sulfate has been used as a gastrointestinal contrast medium in chelonians.[6–10] Gastrografin®* is a water-soluble, hyperosmolar, iodinated (66% diatrizoate meglumine and 10% diatrizoate sodium), radiopaque contrast medium that has a rapid transit time in the Greek tortoise,[11] cat,[12] and dog.[13] Aside from passing more quickly than barium, iodinated contrast medium retains its radiographic opacity in the gastrointestinal tract of turtles for a longer period of time than in other species.[6] These factors, along with greater safety in animals with suspected intestinal perforation or subsequent surgery, make Gastrografin® an appropriate choice for contrast radiography of the gastrointestinal tract of red-eared slider turtles. However, a hyperosmolar contrast medium may lead to hypovolemia and dehydration

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*E.R. Squibb and Sons, Princeton, NJ.

due to fluid loss from the bowel.[6,11,12,14,15] This is especially true of small animals, neonates, and critically ill patients.[6,12,14,15]

General recommendations for gastrointestinal contrast studies in turtles have focused on larger sea turtles[6] and herbivorous terrestrial chelonians[8–11] but a detailed comparison of gastrointestinal contrast radiography in smaller semi-aquatic turtles is lacking. Our primary objective was to compare Gastrografin® with barium sulfate in regard to ease of administration, transit time, and image quality. A second objective was to determine if administration of a hypertonic contrast medium caused significant hematologic changes in healthy, adult red-eared slider turtles.

Material and Methods

Fifteen, male, wild-caught red-eared slider turtles were studied. Turtles weighed 437–696 g (mean = 566 g; median = 562.5 g) with a straight carapace length of 15–17 cm (mean = 16.3 cm; median = 16.36 cm). The turtles were group-housed in a 315 l capacity self-flushing aquatic tank in an AALAC-International accredited research facility in accordance with the established guidelines.[16] The ambient temperature in the room was 23–25°C with tank temperature maintained at 26.1–28.3°C (mean = 26.6°C) by an inline water heater† and two 300 W submersible aquatic heaters.‡ Light cycle in the room was 12 h on, 12 h off. Two basking areas were provided for the turtles with the use of 160 W ultraviolet flood lamps§ situated 45.7 cm (18 in) above each area to maintain basking temperatures at 32.2–33.8°C. Fourteen days before and during the contrast studies, turtles were fed a complete pelleted freshwater turtle diet¶ fortified with calcium and reptile vitamins.¶ Food was withheld 48 h before contrast administration. Turtles were fed 55–58 g of pellets placed into the aquatic tank at 24-h postadministration of contrast medium and at 72-h intervals thereafter until the contrast studies were complete.

To administer the contrast media, turtles were restrained manually without sedation and their neck fully extended. The turtles were positioned 90° vertically to facilitate passage of an orogastric tube and to prevent regurgitation they remained in this position until swallowing was confirmed. A plastic oral speculum was constructed by transecting the proximal one-third of a 1.0 ml tuberculin syringe‖ and filing the cut end smooth. An 8 French (2.7 cm × 56 cm) red rubber feeding tube** was lubricated at the distal end,†† inserted through the lumen of the speculum, and manually advanced to the stomach. The appropriate tube length was ascertained for each turtle by measuring the distance from midplastron to the proximal end of the speculum when inserted into the oral cavity with the neck fully extended.

Gastrografin® (660 mg diatrizoate meglumine and 100 mg diatrizoate sodium/ml) was administered at a dosage of 1 ml/200 g body weight to one group of seven red-eared sliders while 30% w/v barium sulfate‡‡ was administered to a group of eight red-eared sliders at a dosage of 1.6 ml/200 g. The time from initial contrast medium administration to final swallowing was recorded for each turtle. If the tube had to be repositioned, timing was stopped and restarted when contrast medium administration recommenced. Immediately after administration, each turtle was positioned in ventral recumbency and dorsoventral projections were made using a direct flat panel digital radiography system.§§ Images were acquired immediately after contrast medium administration as well as at 20-, 40-, 60-, and 90-mins postadministration; 2-, 4-, 6-, 8-, 12-, 24-, 32-, 48-, 56-h postadministration; and subsequently at 24-h intervals until all contrast medium had cleared the large intestine. Gastric, small intestinal, and large intestinal emptying time was defined as the time from initial contrast administration to when there was no longer any radiographically detectable contrast medium in the corresponding compartment of the gastrointestinal tract. This was quantified in each turtle, as well as when contrast medium first appeared in the small and large intestine. Gastric transit was determined by recording the time from initial contrast administration to its first appearance in the duodenum, and small intestinal transit was defined as the time from first appearance of contrast medium in the duodenum to its first appearance in the large intestine. All procedures were approved by the Institutional Animal Care and Use Committee of Stanford University.

Image quality was determined for both contrast agents by having at least two veterinarians, including one board-certified radiologist, scrutinize the radiographs. Areas of interest that were specifically reviewed included: gastric and intestinal filling, contrast medium transit time, mucosal pattern; and where applicable, absorption of contrast medium.

Approximately 0.2 ml of blood was collected from the subcarapacial venous sinus,[17] from four turtles receiving iodinated contrast medium and four control turtles that did not receive contrast medium. Packed-cell volume (PCV) was determined by collecting blood in heparinized glass capillary tubes, centrifugation in a hematocrit centrifuge at 11,700 rpm for 3 min ,¶¶ and subsequent assessment with a

†Edstrom Industries, Waterford, WI.
‡Aqueon, Franklin, WI.
§T-Rex Products Inc., Chula Vista, CA.
¶Fluker's, Port Allen, LA.
‖Monoject, Tyco Healthcare, Mansfield, MA.
**Sovereign, Tyco Healthcare, Mansfield, MA.
††Surgilube, E. Fougera & Co., Melville, NY.
‡‡Liquid E-Z-Paque, E-Z-EM Inc., Lake Success, NY.
§§TruDR™, Sound Technologies, Carlsbad, CA.
¶¶Autocrit™ Ultra 3, BD Diagnostics, Sparks, MD.

hematocrit chart. A clinical refractometer||| was used to determine total serum protein level. Four hematocrit tubes per turtle were collected at each time point; the average values from the four samples were determined for each turtle and used for later comparison.

Independent *t*-tests were used to compare PCV and total protein values between the control turtles and turtles receiving Gastrografin® preadministration (0-h) and 24-, 96-, and 168-h postadministration. Analysis of variance (ANOVA) was used for repeated measures analysis and to test for three time trends: linear, quadratic, and cubic to determine differences in PCV and total protein levels over time within the group receiving contrast medium. Statistical significance was defined as $P<0.05$. All statistical analyses were performed with the use of SPSS Version 16 software.***

Results

Each contrast medium was relatively easy to administer, except in one turtle that experienced esophageal perforation during administration. The neck of this turtle escaped the handler's grasp and retracted into the shell causing the distal end of the orogastric tube to perforate the esophagus. Although this turtle was exempted from the study after barium sulfate was noted in the coelomic cavity it remained stable over the following 14 days, with a normal appetite and no signs of illness. Regardless, the turtle was euthanized to document any abnormalities associated with barium sulfate leakage. Grossly, clumps of pale white, moist, brittle foreign material was found in the adventitial tissues of the dorsal esophageal wall, and circumferentially around the coelomic trachea and the greater vessels of the heart. Histologically, multifocal areas of hemorrhage and heterogranulomatous coelomitis were noted around the esophagus, trachea, and greater vessels of the heart in association with large amounts of intra- and extrahistiocytic dull gray-brown, finely granular, refractile foreign body material that was consistent with barium. Acid-Fast and Gram staining of histologic sections ruled out the presence of secondary bacterial infections, including mycobacterial infection.

Three of the seven turtles (43%) receiving barium sulfate regurgitated barium into the cranial aspect of the esophagus, which was visible within the caudal aspect of the oral cavity during administration. When barium sulfate was administered at a constant rate over 30–60 s (mean = 40 s), no turtle experienced regurgitation into the oral cavity. No reflux of Gastrografin® was noted even when administered over 15–25 s (mean = 20 s) intervals. No evidence of contrast medium aspiration was observed.

Variability in transit time existed in both groups of turtles. Figure 1 summarizes the gastrointestinal transit times for Gastrografin® and barium sulfate with data expressed as mean ± standard error of the mean. Contrast medium was noted in the small intestine immediately after administration in two of the seven (29%) turtles receiving iodinated contrast medium and in one of the turtles (14%) receiving barium sulfate. However, barium was on average 1 h faster than Gastrografin® at reaching the duodenum postadministration. Gastrografin® was approximately 10 h faster at reaching the large intestine and emptying of the stomach, and 9 h faster at small intestinal emptying. The mean large intestinal emptying time was 36 h faster with iodinated contrast medium compared with barium sulfate. Multiple radiopaque granular opacities were noted within the pylorus of one turtle that received Gastrografin® (Fig. 2). This same turtle also had evidence of ingesta within the stomach on survey images despite the 48-h fast before contrast medium administration. In this instance, gastric emptying was considered complete when all contrast medium had left the stomach, except for the granular radiopaque particles.

Within the stomach, each contrast medium had a smooth, uniform texture and coated the mucosa with well-defined margins (Figs. 3A and 4A). The pyloric sphincter was easily detected with both agents as it created a filling defect between the aboral portion of the stomach and the small intestine (Figs. 3B and 4B). Barium sulfate progressed slowly through the small intestine, still having a smooth uniform texture with well-defined mucosal margins (Fig. 3C). Gastrografin® progressed more rapidly through the small intestine, resulting in progressive distension and decreased contrast medium opacity (Fig. 4C). As expected, barium sulfate maintained its opacity as

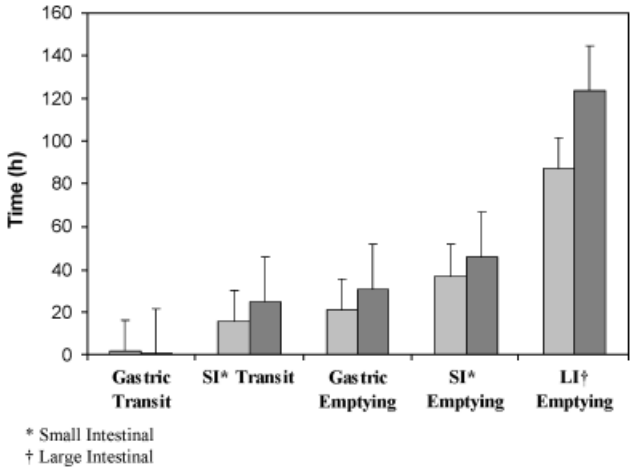

FIG. 1. Gastrointestinal transit times of Gastrografin® (solid bar) and barium sulfate (cross-hatched bar) in hours. Data are expressed as mean ± standard error of the mean ($n=7$ per group). *Small Intestinal; †Large Intestinal.

||| Erma Inc., Tokyo, Japan.
***SPSS Inc, Chicago, IL.

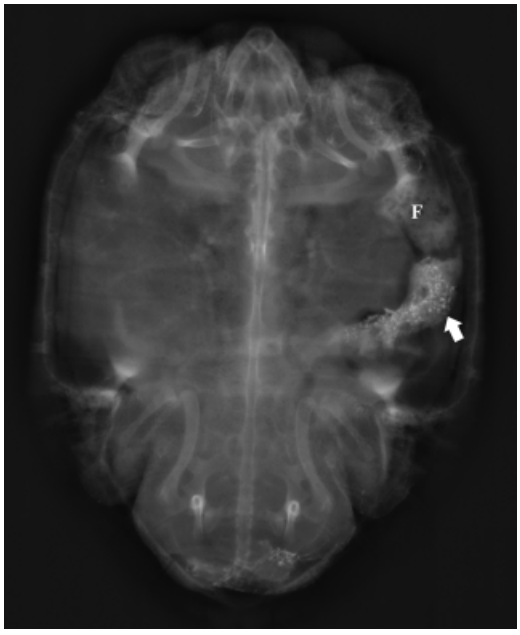

FIG. 2. Dorsoventral radiograph 8 h after administration of Gastrografin®. Note the multiple granular opacities in the pylorus (white arrow) and the ingesta in the fundus (F).

it progressed through the gastrointestinal tract (Fig. 3D). The persistently opaque nature of barium made determination of its entry into the proximal aspect of the colon easier than with Gastrografin®, which was visible within the lumen of the proximal aspect of the colon but was significantly less opaque when compared with its appearance in the stomach (Fig. 4D).

The PCV and total protein levels did not vary significantly as a function of contrast medium administration across turtles at specific time points (0-, 24-, 96-, 168-h) or across time; $P \geq 0.69$ and $P \geq 0.44$, respectively. When comparing PCV and total protein levels within the turtles receiving contrast medium, PCV levels did not significantly vary across time ($P \geq 0.136$) nor did total protein levels ($P \geq .086$). Using repeated measures ANOVA, the total protein levels of the group receiving contrast medium had quadratic and cubic time trends that were statistically significant; $P = 0.030$ and 0.028, respectively. For the quadratic trend, total protein levels were low at baseline (mean = 3.10 g/dl), increased in between (mean = 3.53 g/dl), and then decreased at the end (mean = 3.20 g/dl). When analyzing the cubic trend, total protein levels were low at baseline (mean = 3.10 g/dl), increased after 24 h (mean-3.70 g/dl), decreased at 96 h (mean = 3.35 g/dl), and then decreased slightly more at 168 h (mean = 3.20 g/dl).

Discussion

In both groups of turtles, the contrast media were easily administered without radiographically observable signs of aspiration. However, at a dosage of 1.6 ml/200 g (8 ml/kg) the 30% w/v barium sulfate solution had to be administered at a rate that was on average twice as long (40 vs. 20 s) as that of Gastrografin® to prevent regurgitation. Based on the dosage of each contrast medium, the turtles that received barium sulfate received 60% more volume than the Gastrografin® group. However, when a slower rate of administration was used for barium sulfate, regurgitation did not occur in any turtle. As manually restraining a red-eared slider turtle without the use of anesthesia can be technically demanding, the faster administration rate of Gastrografin® could be a distinct advantage over barium sulfate.

The esophageal perforation occurred in a turtle receiving barium sulfate due to a handler error in physical restraint, causing the neck of the turtle to retract caudally into the shell resulting in a sigmoid or "S-shape" formation to the esophagus; and continual advancement of the orogastric tube despite proper extension of the turtle's head and neck.

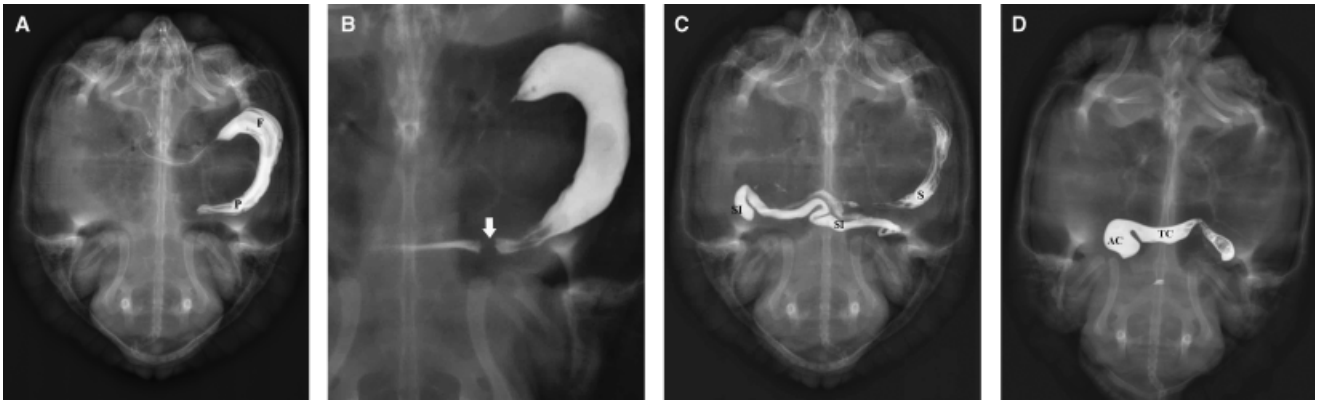

FIG. 3. (A) Dorsoventral radiograph immediately after administration of barium sulfate. Gastric folds are visible in the fundic (F) and pyloric (P) regions of the stomach. (B) Dorsoventral radiograph one hour after administration of barium sulfate. The pyloric sphincter (white arrow) creates a filling defect between the aboral portion of the stomach and the small intestine. (C) Dorsoventral radiograph 24 h after administration of barium-sulfate. Barium has moved slowly through the small intestine (SI), outlining the mucosa with well-defined margins (S, stomach). (D) Dorsoventral radiograph 48 h after administration of barium sulfate. Barium in the ascending (AC) and transverse (TC) colon has retained its opacity making it easy to distinguish entry into the colon.

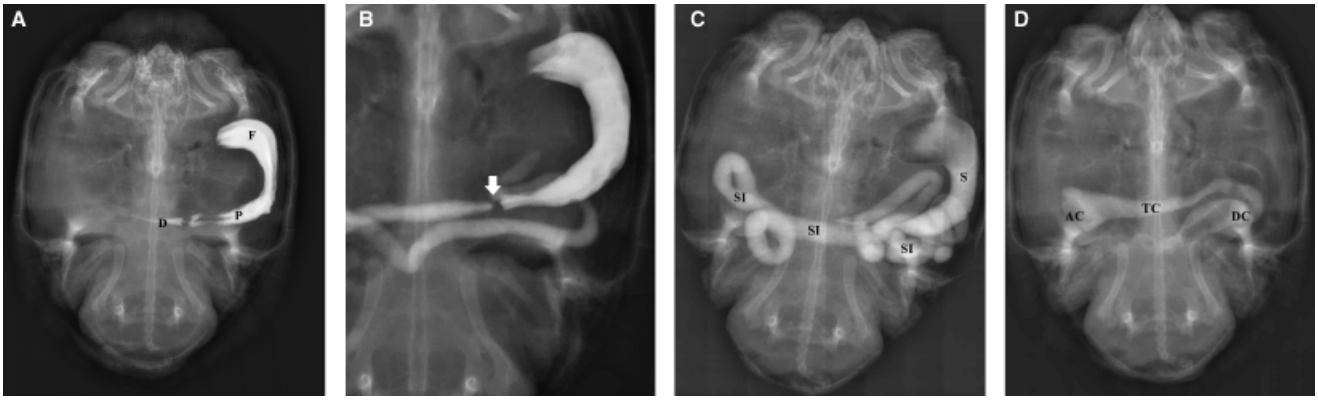

FIG. 4. (A) Dorsoventral radiograph immediately after administration of Gastrografin®. Gastric folds are visible in the fundic (F) and pyloric (P) regions of the stomach. There is also a small amount of Gastrografin® in the proximal duodenum (D). (B) Dorsoventral radiograph one hour after administration of Gastrografin®. The pyloric sphincter (white arrow) creates a filling defect between the aboral portion of the stomach and the small intestine. (C) Dorsoventral radiograph 8 h after administration of Gastrografin®. Contrast medium has moved rapidly through the small intestine (SI) resulting in progressive distension and decreased contrast medium opacity (S, stomach). (D) Dorsoventral radiograph 32 h after administration of Gastrografin®. Contrast medium is still visible in the ascending (AC), transverse (TC), and descending (DC) colon, but it is less opaque than when it was in the stomach and small intestine.

In retrospect, it would have been preferable to remove the tube completely from the oral cavity and restrain the turtle again with the head and neck extended as much as possible. Proper restraint for orogastric tube placement may require chemical sedation in some patients although anesthesia was not used in this research project in order to avoid its reported effects on gastrointestinal motility.[18,19]

The gastrointestinal transit time of barium sulfate in the red-eared slider turtles in our study were longer than those of mammals[20,21] and green iguanas[22] but shorter than recorded in loggerhead sea turtles (*Caretta caretta*),[6] Greek (*Testudo graeca*)[8] and Galapagos (*Chelonoidis nigra*)[23,24] tortoises. This supports previous findings that gastrointestinal transit time is shorter in omnivorous turtles compared with herbivorous turtles.[23] In herbivores, the longest gut transit is the cecum and colon.[23] In this study the large intestines had the longest transit time in the Gastrografin® and barium sulfate groups, with a mean of 72 and 98 h, respectively. Ecologically this makes sense given that as red-eared sliders mature into adults they rely on a more vegetative diet,[25,26] requiring longer transit within the cecum and colon to allow for hindgut fermentation of plant material.

Gastrografin® was more rapid at passing through the gastrointestinal tract at all time points, except for entry into the small intestine. One explanation, is that barium sulfate, a thicker contrast medium, was given in a larger volume, which could have resulted in over-filling of the stomach lumen, stimulating gastric contractions and subsequent propulsion into the proximal duodenum.

Gastrografin® causes mucosal inflammation in the colon of rats and secondary hyperperistalsis has been used to explain the rapid intestinal transit in humans and dogs,[12,13,27] yet this has not been specifically identified in the gastric mucosa. The rapid small intestinal transit of Gastrografin® that resulted in progressive distension and decreased contrast medium opacity is likely a result of the hyperosmolar properties of this contrast medium. The decreased opacification and reduced mucosal adherence of Gatrografin® within the large intestines made its transit through the ascending, transverse, and descending colon harder to determine when compared with barium sulfate. These characteristics of Gastrografin® make it less ideal for contrast radiography of the lower gastrointestinal tract when orally administered to turtles. A contrast medium enema would be an alternative route of administration to better visualize the lower intestines. One study used a nonionic iodinated contrast medium diluted 1:1 with sterile saline to perform contrast medium enemas in seven loggerhead sea turtles (*Caretta careta*).[6] The contrast medium enemas provided visualization of the small and large intestinal tracts allowing identification of fishing lines and evaluation of the length of intestines obstructed by the nylon lines. However, the authors note that placement of a rectal probe into the distal colon in order to administer the contrast medium is not easily performed in smaller turtles.[6]

The multiple granules noted in the pylorus of the turtle in Fig. 2 are likely due to precipitation of the diatrizoate salts in Gastrografin® that formed when the salts were exposed to the more acidic gastric environment of the turtle, which had ingesta in the stomach on survey radiographs. This has been reported with Gastrografin® in humans and cats,[12] as well as the Greek tortoise.[11]

As mentioned previously, the high osmolality of Gastrografin® (1900 mOsm/L) can cause a decrease in plasma volume by drawing interstitial fluid into the lumen of the gastrointestinal tract. When administered as an enema to puppies less than 7 days old, Gastrografin® produced an increase in hematocrit and serum osmolality within 1-h.[15] Our results show that red-eared slider turtles that were

normovolemic before Gastrografin® administration had no significant changes in PCV and total protein levels compared with control turtles. Additionally, there were no significant changes in PCV levels. Turtles receiving Gastrografin® had only minor increases in total protein levels after 24 h but total protein then decreased again at 96–168 h, indicating that the turtles were able to compensate for the loss of fluid into the gastrointestinal tract.

In summary, barium sulfate and Gastrografin® are relatively safe to administer to nonanesthetized, adult red-eared slider turtles as long as the turtles are properly restrained, and the contrast medium is administered at an appropriate volume and rate. Gastrografin® did not result in an increase in PCV or total protein levels of normovolemic, adult red-eared sliders. Gastrografin® had more rapid gastrointestinal transit than barium sulfate, which allows for more rapid study completion, but the decreased opacity and mucosal adherence noted within the lower gastrointestinal tract may be insufficient to reach an accurate diagnosis of intestinal obstruction. A contrast enema may be a better diagnostic approach to visualize the luminal contents and mucosal margins of the distal small intestine, cecum, and colon in a timely manner.

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