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Radiographic anatomy and barium sulfate contrast study of the gastrointestinal tract of eastern box turtles (*Terrapene carolina carolina*)

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Abstract

Gastrointestinal disorders are an important cause of morbidity in box turtles (*Terrapene carolina Carolina*), however published information is currently lacking on the normal radiographic anatomy, transit, and emptying times of the gastrointestinal tract. A total of 15 healthy box turtles were recruited for this prospective, anatomic, reference interval study. Three-view radiographic series (vertical beam dorsoventral, horizontal beam latero-lateral, and horizontal beam rostrocaudal views) were acquired prior to contrast administration, and following contrast administration at 0, 20, 40, 60, and 90 min, 2, 4, 8, 12, and 24 h post administration, and every 24 h thereafter until all contrast was eliminated (15 mL/kg barium sulfate diluted to 30% weight per volume was administered via orogastric gavage). Vertical beam dorsoventral and horizontal beam latero-lateral views were of excellent quality to identify gastrointestinal structures. The horizontal beam rostrocaudal view immediately postcontrast administration provided gastric and pyloric identification but had lesser diagnostic use at later time points due to anatomical superimposition. The gastrointestinal tract was composed of a tubular stomach, a pyloric sphincter near midline, a duodenum with a cranial flexure in the right cranial coelomic cavity, small intestines within the right coelom, a small cecal bulb, and a transverse and descending colon. Contrast media entered the large intestine by 24 h in all turtles, and a pyloro-colic indentation was noted at the proximal descending colon. The large intestinal emptying was highly variable due to the interindividual variability of contrast sequestration within the cecal bulb. Findings from the current study serve as a reference on the gastrointestinal anatomy, transit, and emptying times in healthy eastern box turtles; and introduce a novel, horizontal beam, rostrocaudal view for gastrointestinal contrast studies in chelonians.

KEYWORDS
cecum, radiology, *Testudines*

1 | INTRODUCTION

Anorexia is a common presenting complaint of both captive and free-ranging chelonians. Primary gastrointestinal illness, such as trauma, foreign material, torsion, and neoplasia, must be ruled out prior to pursuing secondary causes of anorexia. Positive contrast radiography of the chelonian gastrointestinal tract was described by Jackson and Faid to be useful in identifying gastrointestinal structures, outlining the gastrointestinal lumens, and assessing motility.[1] Subsequently, gastrointestinal positive contrast studies in chelonians have been reported in clinical cases in the diagnosis of organ integrity,[2] gastrointestinal strictures,[3] intraluminal foreign bodies,[4] gastrointestinal obstruction,[5] and esophagostomy tube placement.[6]

Contrast radiography with barium sulfate, iodinated contrast, and a combination of these agents has been used to describe the normal gastrointestinal anatomy of a wide variety of chelonian species, including red-eared sliders (*Trachemys scripta elegans*),[7] leopard tortoises (*Stigmochelys pardalis*),[8] Greek tortoises (*Testudo hermanni*),[9,10] red-footed tortoises (*Geochelone carbonaria*),[11] yellow-spotted river turtles (*Podocnemis unifilis troschel*),[12] West African mud turtles (*Pelusios castaneus*),[13] Geoffroy's side-necked turtles (*Phrynops geoffroanus*),[14] D'Orbigny's sliders (*Trachemys dorbigni*),[15] Arrau turtles

(*Podocnemis expansa*),[16] Zarudni's spur-thighed tortoises (*Testudo graeca zarudnyi*),[17] and loggerhead sea turtles (*Caretta caretta*).[5,18] Only one of the aforementioned studies used two orthogonal views to evaluate contrast transit time,[8] and none used a horizontal beam rostrocaudal view.

Mirroring the diversity of life histories and feeding strategies, the gastrointestinal anatomy, transit times, and emptying times within Testudines is diverse. For example, two closely related box turtles (*Terrapene carolina* and *Terrapene ornata*) occupying the same geographic region were found to have significantly different gastrointestinal transit times and digestive efficiencies.[19] Establishing the normal radiographic appearance of the gastrointestinal tract as well as the normal gastrointestinal transit and emptying times for each species is important to accurately identify abnormalities presented in a clinical setting.

Turtles and tortoises are commonly held as privately owned pets.[20] The common box turtle (*Terrapene carolina*) is a primarily terrestrial chelonian found free-ranging in North America. This species is classified as "Vulnerable" by the International Union for Conservation of Nature due to a decline from habitat destruction, pollution and pesticide effects, vehicle strikes, increased predation, and collection as personal pets.[21] The only published report of gastrointestinal contrast radiography in the box turtle investigated orally administered iohexol in two turtles with traumatic gastric eviscerations in eastern box turtles (*Terrapene carolina carolina*), a subspecies of box turtle that inhabits the eastern United States.[2] However, no studies have been previously performed describing contrast radiography in healthy eastern box turtles. This study's objective is to describe the normal radiographic gastrointestinal anatomy, transit, and emptying times in healthy adult eastern box turtles following orogastric administration of barium sulfate with three-view whole body radiographs.

2 | MATERIALS AND METHODS

This prospective, anatomic, reference interval study was performed in accordance with the regulations of the Institutional Animal Care and Use Committee of North Carolina State University (protocol #18-054-O) and included rehabilitated eastern box turtles housed at the North Carolina State University College of Veterinary Medicine. All turtles had been admitted for an illness or injury, were successfully treated, and were being housed during winter months prior to their planned release in the late spring. All turtles enrolled in the full study were deemed healthy on the basis of a physical examination, packed cell volume, total solids, and a plasma biochemistry panel (VetScan 2 Avian Reptilian Profile Plus, Abaxis, Inc., Union City, CA 94587). Decisions for subject inclusion and exclusion were made by consensus of two authors, one of whom was an ACZM-certified specialist in zoological medicine (E.L.H. and O.A.P.).

Turtles were housed individually in black plastic tubs that measured 50 × 30 × 15 cm and that were lined with newspaper substrate. The enclosure also included a shallow water dish that was changed daily and a plastic shelter. Ambient temperature within the permanent housing room ranged from 22.2 to 24.4°C, and ultraviolet B lighting was provided (Zoo Med Reptisun 10.0 UVB, 48," 1219 mm, 32 watts; Zoo Med Laboratories, Inc., San Luis Obispo, CA) placed approximately 30-60 cm above the open enclosure. Turtles were soaked in a shallow bath of room temperature water daily for approximately 30 min, as part of their normal husbandry routine while in captivity. Each turtle was offered a daily diet of 3 g blackberries, 3 g blueberries, and 4 g raw sweet potato except for the experimental fasting period 48 h prior to and 72 h following contrast administration. All turtles were allowed at least 2 weeks to acclimate to the environment, husbandry routine, and diet prior to the contrast study. In addition, all turtles included in this study were observed eating and defecating regularly prior to contrast administration.

On the day of contrast administration, turtles were moved in their plastic tubs to the study room (ambient temperature range: 20-25.6°C) at least 30 min prior to contrast administration and held there until radiographs of the first 12 h were complete. For the following days of data collection, turtles were moved to the study room for brief periods of time needed for radiograph acquisition.

A pilot study was conducted on four adult eastern-box turtles (two males, two females) from the rehabilitation population to determine optimal contrast dosage. All procedures were performed by two authors (E.L.H. and O.A.P.) with the assistance of the turtles' caretakers. Prior to contrast administration, a three-view survey radiographic series was acquired via digital radiography (CPI CMP 200 DR, Communications & Power Industries LLC., Palo Alto, CA; kVp 85, mAs 2.5; Eklin EDR6, Eklin Medical Systems, Santa Clara, CA; and Canon CXDI-50G, Canon U.S.A., Inc., Melville, NY). The turtles were placed in sternal recumbency, and a vertical beam dorsoventral view, a horizontal beam latero-lateral view, and a horizontal beam rostrocaudal view were obtained. Survey radiographs were performed within 2 h prior to contrast administration. The four pilot study turtles were divided into two groups and administered either 10 or 20 mL/kg barium sulfate suspension (105% weight per volume, E-Z-EM Canada Inc., Lake Success, NY 11042) diluted to 30% weight per volume with water via an orogastric tube. These dosages were based on previously published recommendations for reptiles.[22,23] Gastric gavage was performed with the turtles unsedated using manual restraint. A 12 French red rubber catheter lightly coated with sterile lubricant was inserted through the oral cavity and esophagus and advanced into the stomach just cranial to the level of the plastron hinge. Turtles were held in a vertical position to aide tube placement, during contrast administration, and for approximately 30 s following contrast administration to prevent regurgitation. Following contrast administration, a three-view radiographic series was obtained as previously described. Radiographs were obtained immediately after contrast administration at time 0, 20, 40, 60, and 90 min, 2, 4, 6, 8, 12, and 24 h, and then every 24 h until contrast was cleared from the colon or until 792 h (33 days), whichever occurred first. No further radiographs were taken after 792 h due to predetermined seasonal release back into their native habitat.

Based on the data acquired from the pilot study, a volume of 15 mL/kg 30% weight per volume barium sulfate suspension was

chosen for the remainder of the study population (n = 11, seven males, four females). This dose was chosen because turtles administered 10 mL/kg 30% weight per volume of barium sulfate in the pilot study exhibited filling of the proximal duodenum similar to the 20 mL/kg group but had incomplete filling and lack of distension of the stomach. The turtles receiving 20 mL/kg 30% weight per volume of barium sulfate in the pilot study exhibited appropriate distension of the stomach but increased filling of the proximal duodenum. Contrast administration and radiographic views were obtained by the same two authors (E.L.H. and O.A.P.) as described for the pilot study. Radiographs were assessed based on a consensus of two authors, one of whom was an ACVR certified veterinary radiologist (E.B.C. and E.L.H.). Evaluation criteria included diagnostic quality (diagnostic, nondiagnostic) and radiographic features (Horos2K, Ver. 2.2.0, Bernex, Switzerland). Radiographic features assessed included technique, exposure, positioning, gastric filling following contrast administration, gastric transit time, gastric emptying time, small intestinal transit time, small intestinal emptying time, large intestinal transit time, and large intestinal emptying time. Images were also assessed for lesions such as mucosal irregularities, luminal filling defects, luminal stenosis, or delayed emptying compared to gastrointestinal studies in other chelonian species.[7,8]

Computed tomography was performed on a deceased free-range, adult male eastern box turtle using a 64-slice helical scanner (Siemens Syngo CT 2006A). This turtle was not part of the study population and died secondary to suspected respiratory viral disease. Procedures were performed by two authors, one of whom was an ACVR certified veterinary radiologist (E.B.C. and E.L.H.). The plastron was removed, and the gross appearance of the gastrointestinal tract was normal. The turtle was placed in dorsal recumbency, and two image acquisitions were acquired. First, the pylorus was ligated using nylon suture, and 30% weight per volume barium sulfate was administered into the stomach via a 12 French red rubber catheter to moderate distension to opacify the stomach through the pylorus. The second series was obtained after ligation of the ileum, with a barium enema performed with 30% barium sulfate administered via a red rubber catheter to moderately distend and opacify the colon. Image acquisition parameters consisted of slice thickness 1.0 mm, pitch 0.8, tube rotation time 1.0 s, 236 mAs, 210 kV, with a 512 × 512 matrix. A transverse multi-slice dataset was acquired in soft tissue and bone algorithms and reconstructed into 3.0 and 1.0 mm transverse sequences, with sagittal and dorsal plane reconstructions. Three-dimensional volume rendered images were generated using image analysis software (Mimics, Ver. 21.0 and 3-matic Research, Ver. 13.0, Materialize NV, Belgium; Horos2K, Ver. 2.2.0, Bernex, Switzerland).

Data were analyzed, descriptive statistics were performed, and graphs were generated (Excel 2016, Microsoft, Redmond, WA 98052) by a single author (E.L.H.). Contrast transit and emptying times were determined for the stomach, small intestines, and large intestines. The transit time was defined as the time postcontrast administration that contrast media was first noted in the gastrointestinal segment (stomach, small intestine, large intestine). The emptying time was defined as the time post contrast administration that all contrast media had moved aboral and the gastrointestinal segment was clear of all contrast material.

3 | RESULTS

3.1 | Study population

Two male and two female, adult eastern box turtles (276-358 g) comprised the pilot study population. Seven male and four female, adult eastern box turtles (260 - 459 g) comprised the study population. One of the pilot study turtles were enrolled in the full study after exhibiting a normal appetite and confirming the complete clearance of gastrointestinal contrast material. All turtles were determined to be adult based on carapace size and body weight.

3.2 | Contrast administration and radiograph acquisition

During contrast administration, two turtles regurgitated a small volume into their oral cavity estimated at less than 5% of the contrast administered. In both instances, contrast administration was halted temporarily, any pressure against the ventral surface of the neck from manual restraint was relieved, and contrast administration was resumed shortly after with no further complications. The remaining turtles had no complications during contrast administration. The dose of 15 mL/kg provided mild gastric distension and filling of the proximal duodenum. All three radiographic views were successfully obtained at all time points.

3.3 | Radiographic anatomy and contrast study assessment

All radiographic studies were assessed to be of diagnostic quality. One turtle was missing its right front foot, distal radius, and distal ulna (turtle 1), one turtle was missing its right pelvic limb (turtle 8), and one turtle had a right femoral intramedullary pin from prior surgery for a fracture (turtle 9); these abnormalities were healed prior to initiation of the study. No other pathology was identified, and no studies needed to be discontinued. In all turtles prior to contrast administration, it was difficult to identify and differentiate gastrointestinal structures, and no ingesta were present within the region of the upper gastrointestinal tract. Barium sulfate administration via the described method immediately provided positive contrast images of the entire stomach, pyloric sphincter, and duodenum at time 0 (Figure 1A-C), with subsequent passage through the small intestine, cecal bulb, transverse colon, and descending colon (Figure 1D-F).

A small volume of esophageal contrast was present in some turtles at time zero, and in one turtle (turtle 10), a small volume of esophageal contrast was present at 10 min that was not present at time zero, indicating gastroesophageal reflux. All gastrointestinal structures were in the ventral half of the coelom, ventral to the lungs. In all turtles, the stomach was tubular in shape with more bulbous dilation at the level

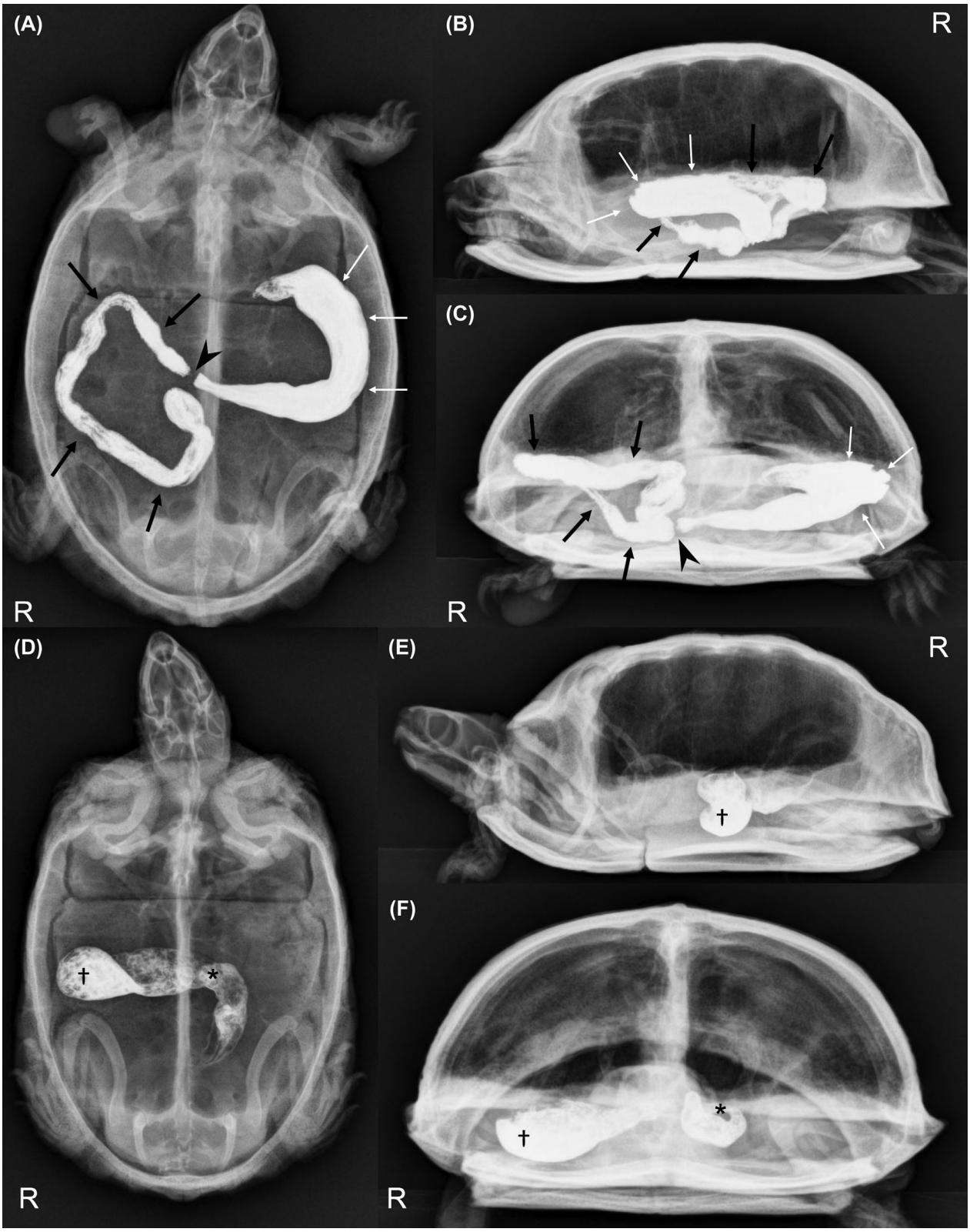

FIGURE 1 A and D, Dorsoventral; B and E, latero-lateral; and C and F, rostroocaudal radiographic views at 20 min (A-C) and 84 h (D-F) following orogastric gavage with 15 mL/kg barium sulfate 30% weight per volume in two healthy, adult eastern box turtles (*Terrapene carolina carolina*) (kVp 85, mAs 2.5). In parts (A)-(C), the stomach (white arrows) and duodenum (black arrows) are contrast enhanced. The pyloric sphincter (A, C; black arrowhead) is seen as a circumferential band-like filling defect just right of vertebral column within the mid-ventral coelom. The turtle depicted in (A)-(C) also has a healed, right, mid-antebrachial amputation from previous trauma. In parts (D)-(F), the cecal bulb (black dagger), transverse colon, and descending colon are contrast enhanced. The pyloro-colic indentation (D, F; black asterisk) is seen as focal narrowing of the contrast column within the proximal descending colon. The heterogeneous appearance of the contrast material in the turtle depicted in parts (D)-(F) is likely secondary to ingesta present within the large intestines

FIGURE 2 Dorsoventral radiographic views of two healthy, adult eastern box turtles (*Terrapene carolina carolina*) (kVp 85, mAs 2.5). Images demonstrate the two morphologies of the lesser curvature of the stomach, as a tight and folded "U" shape (A) or a more subtle arc of the lesser curvature (B). Images were acquired at 20 min following orogastric gavage of 15 mL/kg of 30% weight per volume barium sulfate

of the fundus. The gastric cardia was within the left cranial quadrant of the coelom, varying from cranial to caudal to the hinge of the plastron. The body of the stomach partially superimposed the left marginal scutes on dorsoventral views, then coursed medially toward midline as the pyloric antrum. The lesser curvature of the stomach varied in appearance with some appearing as a tight "U" shape while others had only a subtle arc (Figure 2). In some turtles, longitudinal rugal folds were visible (Figure 3A). This was especially common as contrast material exited the stomach lessening the volume contrast material within the stomach and decreasing the distension of the stomach (Figure 3B). The pyloric sphincter was visible in all turtles on the dorsoventral view and some turtles on the rostrocaudal view, as a circumferential band-like filling defect, centered over the vertebral column on midline or just left of midline within the center of the coelom (Figures 1A and 1C). From the pylorus, a wide cranial flexure of the duodenum coursed cranioventrolaterally into the right cranial coelomic quadrant, just caudal to the hinge of the plastron. From the cranial duodenal flexure the duodenum deviated from its cranioventrolateral orientation and coursed caudodorsally as the descending duodenum along the right lateral margin of the coelom (Figure 1A-C). The duodenum then coursed medially at the cranial margin of the pelvic inlet to connect to the remainder of the small intestine. Similar to the stomach, longitudinal folds/filling defects were visible within the duodenum, with a few turtles also having visible transverse folds/filling defects.

The remainder of the small intestine was coiled within the right coelom caudal to the pectoral scute and extending between approximately 40-75% of the lateral width of the coelom, oftentimes crossing to the left of the vertebral column on the dorsoventral view (Figure 4). A small cecal bulb was positioned ventrally in the right mid-coelom. Notably, many turtles retained contrast within the cecal bulb subsequent to passage of contrast from the remainder of the colon (Figure 5). In the live turtles, the transverse and descending colon were positioned dorsal to the small intestines. The rostrocaudal course of the descending colon is relatively parallel to the rostrocaudal course of the duodenum. Focal narrowing of the contrast column, termed the pyloro-colic indentation, was present at the proximal descending colon, secondary to compression from the adjacent gastric body coursing toward the pyloric antrum (Figure 1D-E). In turtles whose

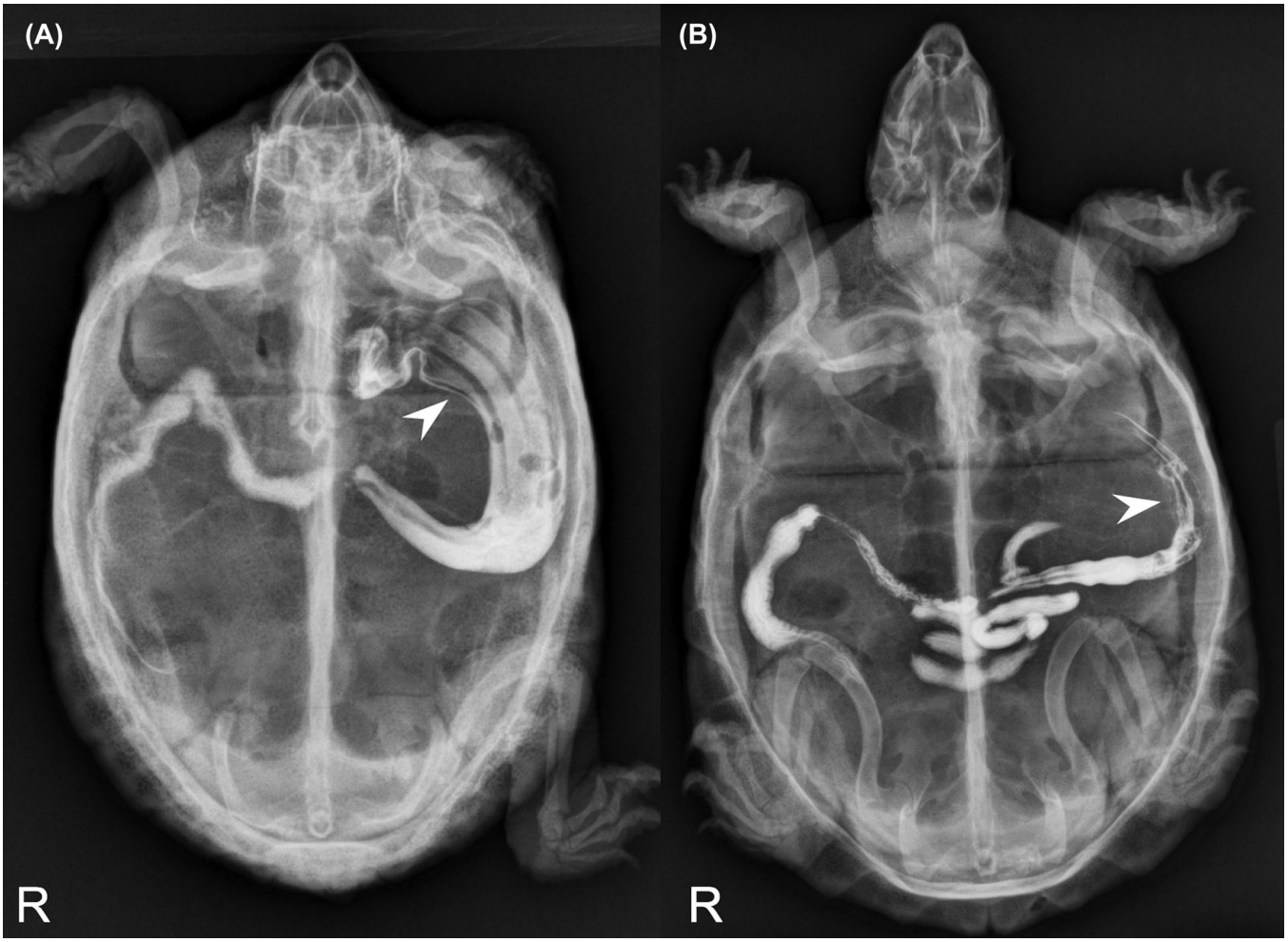

FIGURE 3 Dorsoventral radiographic views of two healthy, adult eastern box turtles (*Terrapene carolina carolina*) (kVp 85, mAs 2.5). Images demonstrate visible rugal folds within the stomach (white arrowheads) following orogastric gavage of 15 mL/kg of 30% weight per volume barium sulfate immediately after contrast administration (A) and 6 h following contrast administration (B)

pelvic limbs were flexed within the shell, the descending colon and caudal duodenal flexure were displaced medially.

The current protocol provided excellent quality imaging of the stomach, pylorus, and duodenum. However, there was inconsistent opacification of the mid to distal small intestine and colon at the time points acquired. These portions of the bowel were identified as focal segmental opacification by the contrast material.

3.4 | Contrast transit and emptying times

Descriptive statistics and graphical depiction of the contrast transit and emptying times are located in Table 1 and Figure 6. Contrast media was present in the stomach and duodenum in all turtles immediately post contrast administration (gastric and small intestinal transit time of 0 h). Gastric and small intestinal emptying occurred in all turtles by 48 h post contrast administration (range: 3-48 and 12-48 h, respectively; Figure 6A). The mean emptying time for the stomach and small intestines was 20.2 and 32.7 h, respectively. Contrast media reached the colon in all turtles by 24 h (mean large intestinal transit time: 15.6 h; range 8-24 h). The large intestinal emptying time was highly variable (Figure 6B), ranging from 48 to over 792 h (mean 357.8 h). Most commonly, the prolonged total contrast emptying was due to contrast sequestration within the cecal bulb or dilution of contrast material adherent to fecal material.

3.5 | CT modeling

The three-dimensional CT volume rendered images (Figure 7) of the deceased adult male box turtle further clarified the radiographic gastrointestinal anatomy characteristics. In particular, these models improved characterization of the pyloric sphincter and its relation to the pyloro-colic indentation.

4 | DISCUSSION

Based on our review of the literature, this is the first published study evaluating normal contrast radiography and gastrointestinal contrast

FIGURE 4 Dorsoventral radiographic view of a healthy, adult eastern box turtles (*Terrapene carolina carolina*) (kVp 85, mAs 2.5). Image demonstrates contrast material within the small intestines at 6 h following orogastric gavage of 15 mL/kg of 30% weight per volume barium sulfate

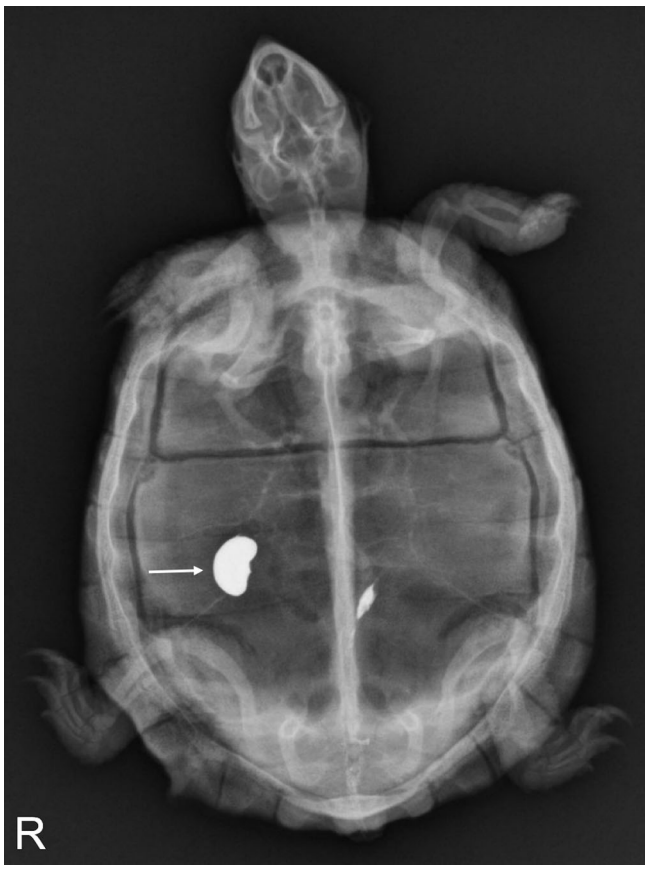

FIGURE 5 Dorsoventral radiographic view of a healthy, adult eastern box turtles (*Terrapene carolina carolina*) (kVp 85, mAs 2.5). Sequestration of contrast material is present within the cecal bulb (white arrow) at 60 h following orogastric gavage of 15 mL/kg of 30% weight per volume barium sulfate. A small volume of residual contrast material is also present within the proximal descending colon

transit times in healthy eastern box turtles and the first study evaluating three-view contrast radiography in any chelonian species. This study described the normal gastrointestinal anatomy, transit, and emptying times of healthy eastern box turtles based on orally administered barium sulfate. Orogastric gavage of barium sulfate diluted to 30% weight per volume at a dose of 15 mL/kg resulted in minimal side effects. The advantages of barium sulfate include cost effectiveness, widespread availability to practitioners, and the decreased visibility of iodinated contrast materials in similarly sized turtles due to dilution as it passes through the gastrointestinal tract.[7] Barium sulfate is contraindicated in cases where gastrointestinal perforation or compromise is suspected, a gastrointestinal tract obstruction is known, and when the patient has a hypersensitivity to barium sulfate.[24–26] Administration of gastrointestinal material is also not recommended in dogs and cats if endoscopy is planned within 24 h.[27] In chelonian species with total contrast transit times much longer than dogs and cats, the time to endoscopy may need to be prolonged even further.

The barium protocol applied in our study provided excellent image quality of the stomach and proximal small intestine and moderate image quality of the mid to distal small intestine and large intestine.

TABLE 1 Descriptive statistics of the contrast transit for the stomach, small intestine, and large intestine in 11 healthy, adult eastern box turtles (*Terrapene carolina carolina*) after orogastric gavage of 15 mL/kg of 30% weight per volume barium sulfate. The transit time of the stomach and small intestines was 0 h in all turtles

	Gastric Emptying (h)	Small Intestinal Emptying (h)	Large Intestinal Transit (h)	Large Intestinal Emptying (h)
Mean	20.2	32.7	15.6	357.8
Median	24	36	12	240
Mode	24	24	12	552
Standard Deviation	12.1	8.7	6.5	199.3

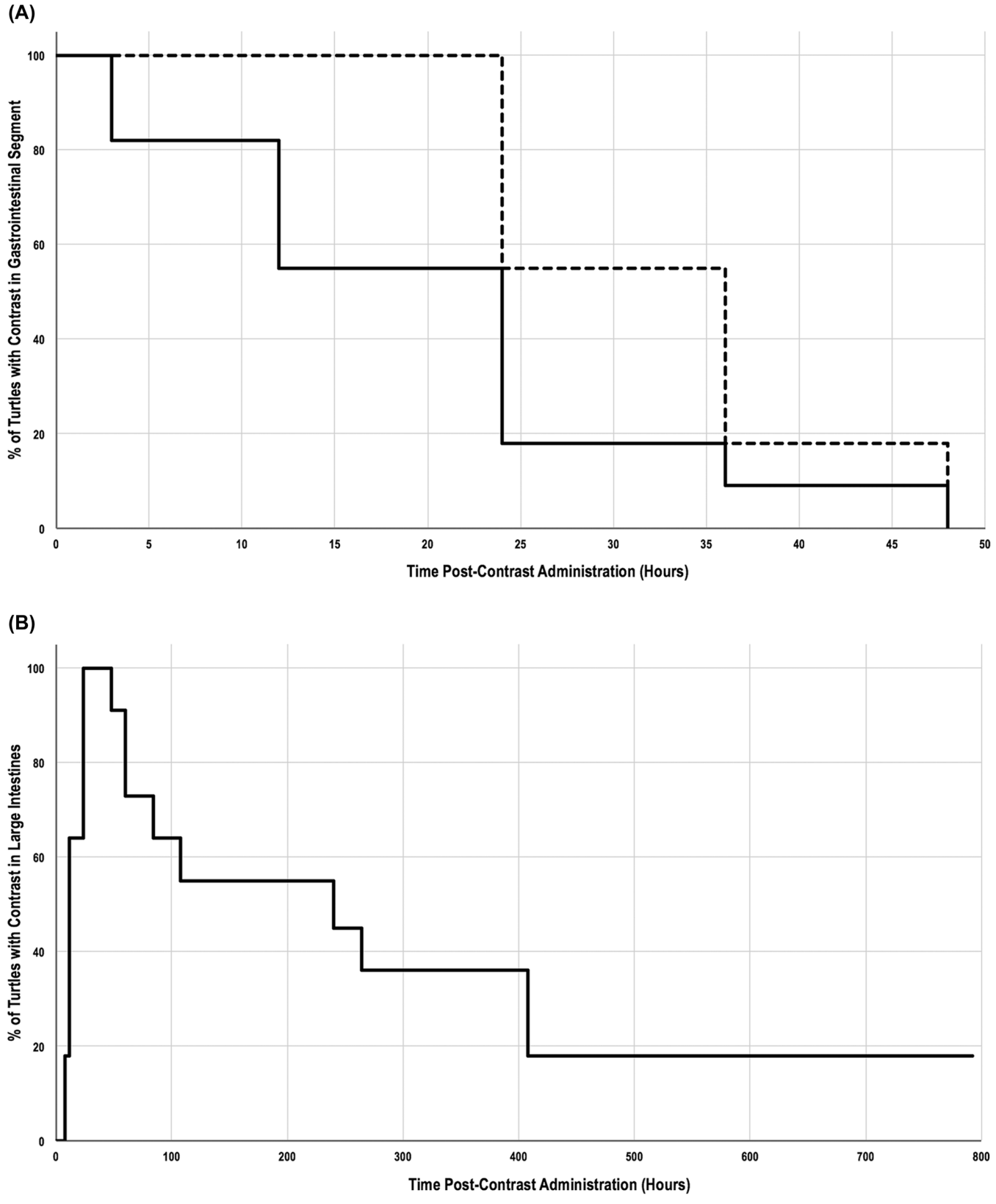

FIGURE 6 The percentage of healthy, adult eastern box turtles (*Terrapene carolina carolina*) with contrast visible in the gastrointestinal tract following orogastric gavage of 15 mL/kg of 30% weight per volume barium sulfate. A, The percentage of turtles with contrast visible in the stomach (A; solid line) and small intestines (A; dashed line). B, The percentage of turtles with contrast visible in the large intestines (B; solid line)

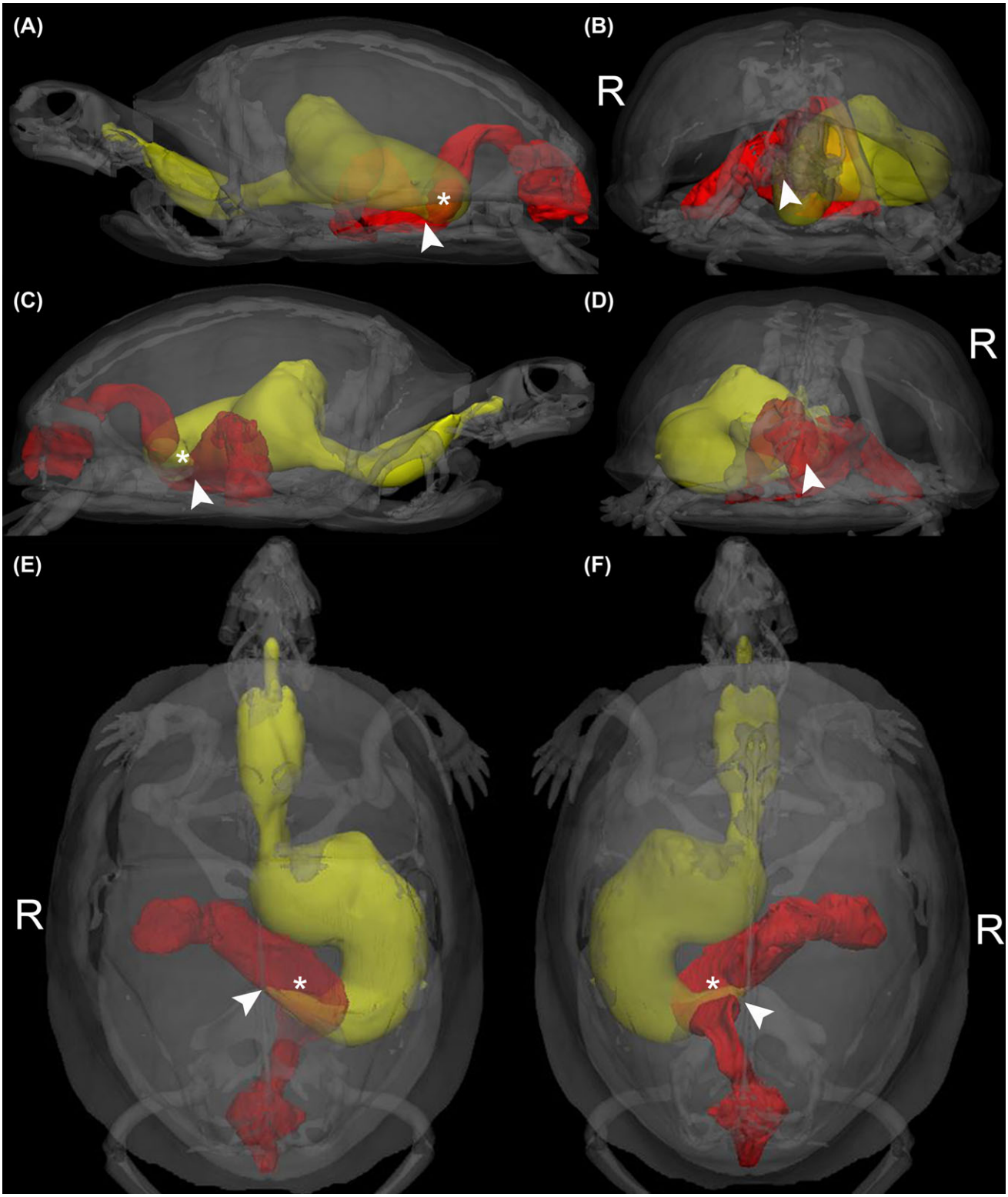

FIGURE 7 Three-dimensional CT volume reconstructions of a deceased, adult, male Eastern Box turtle (*Terrapene carolina carolina*) following filling of the stomach (yellow shading) and large intestine (red shading) with barium (slice thickness 1.0 mm, pitch 0.8, tube rotation time 1.0 s, 236 mAs, 210 kV, with a 512 × 512 matrix). The skeleton is shaded in gray. Images are reconstructions as viewed from left lateral (A), rostrocaudal (B), right lateral (C), caudorostral (D), ventrodorsal (E), and dorsoventral (F). Images have been oriented as they would be viewed anatomically as opposed to radiographic hanging convention in order to demonstrate the relationship of the gastrointestinal anatomy. The pyloric sphincter (white arrowhead) that was ligated with suture in this turtle is visible overlying the vertebral column in the ventrodorsal (E) and dorsoventral (F) views, just dorsodistal to the crossing of the pyloric antrum and descending colon, termed the pyloro-colic indentation (white asterisk). The pyloric sphincter is not visible on the rostrocaudal (B) and caudorostral (D) views, but its location is marked for reference [Color figure can be viewed at wileyonlinelibrary.com]

Barium sulfate 30% weight per volume dilution was selected based on the findings of a study in red-eared sliders, a similarly sized chelonian species.[7] The volume of barium sulfate administered was selected based on recommendations for reptiles[22,23] and data from the pilot study in these individuals. It is notable that this volume dosage is almost double the volume dosage used in a similar study in red-eared sliders[7] and only two turtles in this study experienced mild gastroesophageal reflux and minimal oral regurgitation. A slow rate of administration of barium sulfate was likely a contributing factor to the low complication rate compared to the red-eared slider study.[7]

A standard three-view radiographic technique is recommended in chelonians to evaluate gastrointestinal anatomy and pathology.[1] The vertical beam dorsoventral view provided the most valuable information regarding location of contrast material, while the horizontal beam lateral view aided differentiation of the dosrsoventral orientation of structures. The rostrocaudal view was the least useful due to the degree of superimposition, but this view obtained immediately after contrast administration did allow good discrimination of the stomach, pyloric antrum, and pyloric sphincter.

All turtles in this study had contrast material in the stomach and duodenum immediately after contrast administration. Because the proximal small intestinal fill was noted in the pilot studies with smaller volumes that resulted in incomplete stomach distension, it is suspected that the pyloric sphincter but not cardiac sphincter of this species may easily allow transit of liquid contents under minimal pressure, such as during slow orogastric gavage of contrast media.

There was no radiographic evidence of food in any turtles' stomach after 48 h of withholding food, indicating that this length of time is sufficient to allow complete emptying of the stomach with the diet fed in this experiment. This is supported by the contrast findings in this study, as all turtles had gastric contrast emptying within 48 h. Fasting for 48 h may be sufficient for gastric emptying depending on the particular diet and medical condition in in the individual. In the fasted turtles, the gastrointestinal structures were poorly visualized but contrast administration greatly enhanced the identification of the stomach and proximal small intestines and with a lesser degree of opacification of the mid to distal small intestines and colon. Contrast media was present in the colon in all animals by 24 h. In a clinical setting where the evaluation of the stomach and small intestines is indicated, a reasonable approach to a contrast radiography study in eastern box turtles would include three-view series (vertical beam dorsoventral, horizontal beam lateral, and horizontal beam rostrocaudal) prior to contrast administration and immediately after contrast administration, with subsequent two-view series (vertical beam dorsoventral and horizontal beam lateral) at multiple time points through the first 8 to 12 h. To best evaluate contrast transit through the small intestines, the contrast study may need to be extended up to 24 h depending on the individual turtle's transit time. Contrast emptying from the cecal bulb and colon was highly variable and dependent on contrast sequestration in the cecal bulb and frequency of defecation. Due to this high variability, large intestinal barium sulfate transit time may be difficult to use to differentiate between normal and pathologic large intestinal motility in eastern box turtles. If large intestinal disease is of concern, an enema administration of positive contrast media may be more appropriate to better visualize these structures.[5]

Compared to the eastern box turtles in this study, leopard tortoises and Greek tortoises had a longer mean large intestinal transit time and prolonged gastric and small intestinal emptying times, and leopard tortoises, West African mud turtles, D'Orbigny's sliders, and Geoffrey's side neck turtles had shorter large intestinal emptying times.[8,9,13–15] However, compared to the eastern box turtles in this study, longer large intestinal emptying times were noted in yellow-spotted river turtles, Arrua turtles, and red-footed tortoises.[11,12,16] It is important to note that each of these contrast radiography studies were completed under differing parameters, including type and formulation of contrast media, so results may not be comparable.

Eastern box turtles have a unique combination of gastrointestinal anatomical features (Figure 8), although similarities exist compared to many other chelonians. Red-eared sliders and Zarudni's spur-thighed tortoises have a clear pyloric sphincter, very similar to those noted in this study.[7,17] The small intestines of the Greek tortoises, red-eared sliders, yellow spotted-river turtles, red-footed tortoises, and Zarudni's spur-thighed tortoises coursed laterally through the abdomen and extended to the lateral margins of both the right and left coelom, whereas the eastern box turtles' small intestines were mostly confined to the right coelom.[7,9,11,12,17] The colon of leopard tortoises, Greek tortoises, and loggerhead sea turtles are longer and more convoluted compared to the colon of the red-eared slider and the eastern box turtle.[7,8,18] These anatomical differences highlight the importance of species-specific anatomical references when using diagnostic radiography.

In conclusion, findings from the current study indicated that barium sulfate (30% weight per volume, 15 mL/kg) could be slowly administered by orogastric gavage with minimal consequence and provided excellent quality positive contrast radiographic images describing the gastrointestinal anatomy of healthy, adult eastern box turtles. Findings also supported fasting box turtles for 48 h prior to contrast administration to allow gastric emptying. Authors recommend obtaining vertical beam dorsoventral, horizontal beam lateral, and horizontal beam rostrocaudal radiographic views prior to contrast administration and immediately following contrast administration, with subsequent vertical beam dorsoventral and horizontal beam lateral radiographic views at 1, 2, 4, 8, and 12 h following contrast administration to evaluate the transit of barium through the stomach and small intestines. Contrast studies may need to be extended depending on individual emptying time and the upper gastrointestinal parameters of interest. Large intestinal transit time had high variability in this population due to cecal bulb sequestration of contrast media and may be difficult to interpret in clinical settings. Future studies are needed to characterize the effects of illness, environmental temperature, and food consumption on the gastrointestinal transit times of turtles.

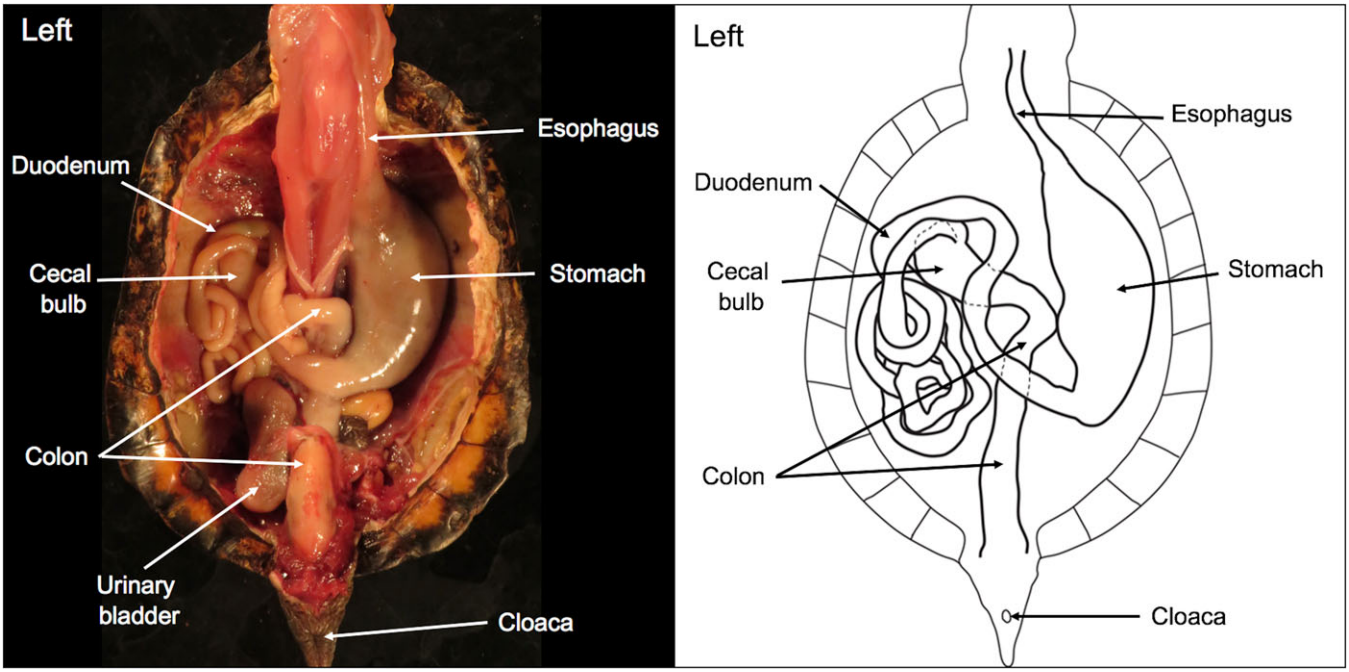

FIGURE 8 A, Photograph of a dissection of an adult male box turtle (*Terrapene carolina carolina*) in dorsal recumbency. The plastron and pectoral girdles have been removed, and the urinary bladder is reflected from midline to the left of the distal colon for better visualization. B, Line drawing of the gastrointestinal anatomy of an adult male box turtle in dorsal recumbency [Color figure can be viewed at wileyonlinelibrary.com]

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Category 1

(a) Conception and Design: Houck, Cohen, Lewbart, Petritz

(b) Acquisition of Data: Houck, Cohen, Womble, Petritz

(c) Analysis and Interpretation of Data: Houck, Cohen, Petritz

Category 2

(a) Drafting the Article: Houck, Cohen, Womble, Petritz

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Category 3

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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