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Development of a technique for contrast radiographic examination of the gastrointestinal tract in ball pythons (*Python regius*)

Tommaso Banzato, DVM; Elisa Russo, DVM; Luca Finotti, DVM; Alessandro Zotti, DVM, PhD

Objective—To develop a technique for radiographic evaluation of the gastrointestinal tract in ball pythons (*Python regius*).

Samples—10 ball python cadavers (5 males and 5 females) and 18 healthy adult ball pythons (10 males and 8 females).

Procedures—Live snakes were allocated to 3 groups (A, B, and C). A dose (25 mL/kg) of barium sulfate suspension at 3 concentrations (25%, 35%, and 45% [wt/vol]) was administered through an esophageal probe to snakes in groups A, B, and C, respectively. Each evaluation ended when all the contrast medium had reached the large intestine. Transit times through the esophagus, stomach, and small intestine were recorded. Imaging quality was evaluated by 3 investigators who assigned a grading score on the basis of predetermined criteria. Statistical analysis was conducted to evaluate differences in quality among the study groups.

Results—The esophagus and stomach had a consistent distribution pattern of contrast medium, whereas 3 distribution patterns of contrast medium were identified in the small intestine, regardless of barium concentration. Significant differences in imaging quality were detected among the 3 groups.

Conclusions and Clinical Relevance—Radiographic procedures were tolerated well by all snakes. The 35% concentration of contrast medium yielded the best imaging quality. Use of contrast medium for evaluation of the cranial portion of the gastrointestinal tract could be a reliable technique for the diagnosis of gastrointestinal diseases in ball pythons. However, results of this study may not translate to other snake species because of variables identified in this group of snakes. (*Am J Vet Res* 2012;73:996–1001)

Snakes, similar to other animals that feed at irregular prolonged intervals, have a complex digestive physiologic process. However, in contrast to other animals, they have pronounced physiologic and morphological gastrointestinal responses to feeding. Comprehensive studies^{1–3} have been conducted on the digestive physiology of snakes. However, to our knowledge, no detailed studies have been performed to evaluate the use of contrast radiography to examine gastrointestinal transit in snakes.

Many gastrointestinal diseases can affect snakes. Most common pathological conditions are the consequence of husbandry-related problems (temperature too high or too low, unsuitable humidity, or inappropriate cage substrate); stress (loud noises or a paucity of hiding places in a cage); gastrointestinal parasitism; gastroenteritis from viral, fungal, or bacterial infec-

ABBREVIATIONS

ETT	Esophageal transit time
GET	Gastric emptying time
OGF	Onset of gastric filling
OLIT	Onset of large intestine transit
OSIT	Onset of small intestine transit
SITT	Small intestine transit time

tions; or intestinal occlusions caused by foreign bodies (pieces of cage substrate [eg, bark, wood chips, gravel, or towels], impaction, or tumors^{4,5}). Ileocolic intussusception has also been reported.⁶ Similar to these conditions in other nonreptilian species, the signs of gastrointestinal tract disease are nonspecific and include anorexia, emesis, regurgitation, diarrhea, and abdominal distention.⁴

Contrast radiography is a standard procedure for the evaluation of gastrointestinal transit in dogs and cats with vomiting, palpable abdominal masses, signs of acute abdominal pain, or suspected intestinal foreign body.⁷ Furthermore, analysis of the literature^{8–10} suggests that contrast radiography could also be a useful tool for the diagnosis of disorders of the gastrointestinal tract in reptiles.

The purpose of the study reported here was to develop a technique for radiographic evaluation of the cra-

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nial portion of the gastrointestinal tract in ball pythons by the use of contrast medium. In particular, our objectives were to determine the concentration of contrast medium for optimum results and time at which radiographic images should be obtained after administration of contrast medium and to describe radiographic features in this species.

Materials and Methods

Samples—Two evaluations were conducted on ball pythons (*Python regius*) during the study. The first evaluation involved 10 cadavers of ball pythons, and the second involved 18 live adult ball pythons. Informed consent was obtained from all clients for use of their snakes in the study. The study was conducted under the approval of the Padua University Ethical Committee.

Anatomic evaluation—The cadavers of 10 ball pythons (5 males and 5 females) with a mean \pm SD body weight of 0.9 ± 0.5 kg and mean body length (snout to vent) of 89.4 ± 17.6 cm were used in this evaluation. Snakes had died as a result of various causes. Cadavers were stored at -20°C for a mean of 1 month and were dissected prior to the beginning of the experiment. The objective of these dissections was to provide an unambiguous description of the gastrointestinal tract of ball pythons. The gastrointestinal tract of each of the 10 cadavers was carefully analyzed to characterize the anatomic structures, topography, and individual variations.

Radiographic evaluation—Eighteen client-owned healthy ball pythons (10 males and 8 females) were included in the evaluation. Mean \pm SD body weight was 1.3 ± 0.4 kg, and mean body length (snout to vent) was 97.2 ± 27.4 cm. Snakes were considered healthy on the basis of medical history and results of a complete physical examination. The study period lasted from June to September 2010.

Radiographic procedures began within 4 hours after each snake was admitted at the Department of Veterinary Clinical Sciences of the University of Padua, Italy. During the radiographic procedures, snakes were housed in plastic cages at a steady environmental temperature of 28°C , which was maintained via air conditioning. All snakes were not fed for at least 7 days (range, 7 to 14 days) before examination. Chemical restraint for the snakes was not needed.

The snakes were allocated into 3 groups (groups A, B, and C; 6 snakes/group). A barium sulfate^a suspension was orally administered (25 mL/kg) as a contrast medium. The suspension was administered at a concentration of 25%, 35%, and 45% (wt/vol) for groups A, B, and C, respectively. Contrast medium was administered through an esophageal probe by manually restraining each snake and gently opening its mouth. A gauze pad was placed over the operator's hand to prevent injury to both the snake and the operator. To obtain comparable

results among snakes and to obtain images in which the body of each snake was as straight as possible, each snake was placed in a 6.3-cm-diameter radiolucent pipe; the pipe had been sectioned along its longitudinal axis to enable it to be rapidly opened for insertion of each snake and subsequent removal of each snake immediately after the radiographic procedure.

Radiographic images were all obtained with the same computed radiography device.^b Dorsoventral radiographic images were obtained by use of the following settings: 52 to 58 kV (depending on the size of the snake), 300 mA, and 0.02 seconds. No grid was used.

An initial snake (included in group A) was used to verify the feasibility of the procedures and to establish a proposed time course for the procedures. We considered the radiographic procedures concluded when all of the contrast medium had reached the large intestine. The initial snake was radiographed 5, 10, 20, and 30 minutes and 1, 2, 3, 6, 9, 12, 24, 48, and 72 hours after administration of contrast medium. On the basis of results for this initial snake, it was decided that images of all remaining snakes should be obtained 5 minutes and 1, 2, 3, 6, 9, 12, 24, 48, and 72 hours after administration of contrast medium.

Imaging data analysis—To assess transit time, the radiographic variables ETT, OGF, GET, OSIT, OLIT, and SITT were evaluated. The ETT was defined as the first radiographic time point at which no residual contrast medium was seen in the esophagus. The OGF was defined as the first radiographic time point at which contrast medium could be seen inside the stomach. The GET was defined as the first radiographic time point at which contrast medium was detected leaving the stomach; GET was considered complete when the contrast medium in the stomach consisted of only contrast medium that remained adhered to the gastric folds. The OSIT was defined as the first radiographic time point at which contrast medium could be seen in the small intestine. The OLIT was defined as the first radiographic time point at which contrast medium could be seen in

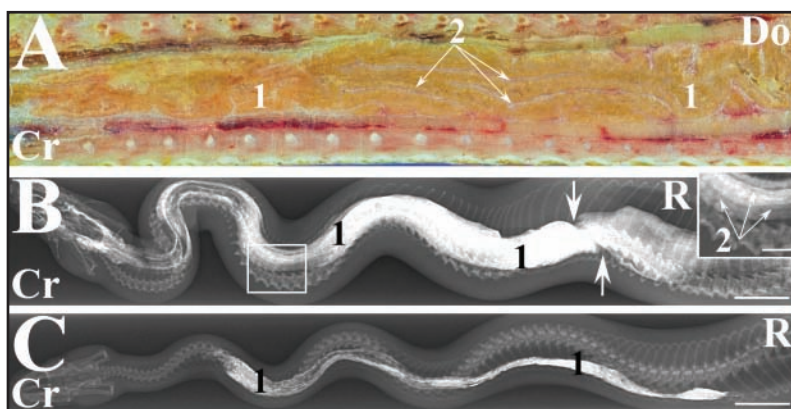


Figure 1—Photograph of a gross longitudinal section of the esophagus in a ball python (*Python regius*) cadaver (A) and dorsoventral radiographic views of the esophagus of a live ball python 5 minutes (B) and 1 hour (C) after oral administration of contrast medium (25 mL of barium sulfate suspension/kg at 1 of 3 concentrations [25%, 35%, and 45% {wt/vol}], which was administered through an esophageal probe to snakes in each of 3 groups. Notice the appearance of the esophagus (1) and the esophageal folds (2). At 5 minutes after administration of the contrast medium, there is normal dilation of the esophagus (large white arrows). Inset—Larger view of the normal-appearing esophageal folds. Cr = Cranial. Do = Dorsal. R = Right. In panels B and C, bar = 1 cm.

the large intestine. The SITT was defined as the first radiographic time point at which no residual contrast medium was visible in the small intestine; the SITT corresponded to the end of the evaluation.

To determine the concentration of contrast medium that provided the best radiographic imaging quality, images were evaluated by 3 investigators (TB, ER, and AZ). Images were assessed on the basis of general distribution of contrast medium (flocculation and segmentation in the small intestine), which were each graded on a scale from 1 to 4 (grade 1 = extremely evident and grade 4 = not detected). Images were also as-

sessed for visibility of esophageal folds, gastric folds, intestinal villi, the pylorus, and superimposed loops of small intestine (when present); these were graded on a scale from 1 to 4 (1 = poor, 2 = fair, 3 = good; and 4 = excellent). When the interpretations of the 3 investigators were not unanimous, a consensus was reached, and that value was used as the final grade.

Regurgitation of contrast medium—The number of snakes that regurgitated immediately after administration of the contrast medium was recorded as part of the assessment.

Statistical analysis—Because of the small quantity of data, statistical analysis could not be performed to determine whether the transit time was related to barium concentration. Mean \pm SD values of transit time for each radiographic variable for each group as well as the entire sample population were reported. Variations in quality of images among the groups were tested with both the Kruskal-Wallis test for the 3 groups and Dunn multiple comparison test to compare the groups in pairs. Mean \pm SD for each group and the entire sample population were reported. All analyses were performed with commercially available software.^c Values of $P < 0.05$ were considered significant.

Results

Anatomic evaluation—Gross anatomic findings related to the esophagus and stomach were consistent among all the cadavers. However, both the gross appearance and topography of the small intestine differed among the cadavers.

The esophagus appeared as a long structure starting at the base of the head and ending at the caudal lobe of the left lung (Figure 1). The stomach appeared as a saccular structure starting at the caudal lobe of the left lung and extending beyond the caudal lobe of the right lung (Figure 2). Gross anatomic variability was detected in the small intestine (Figure 3). Aspects of intestinal loops and villi visible in some cadavers differed from an almost straight small intestine with less evident villi detected in other cadavers.

Radiographic evaluation—The esophagus, stomach, and small and large intestines were easily identified, regardless of the concentration of contrast medium. Distribution patterns of contrast medium in the esophagus and stomach were consistent among all snakes, whereas the radiographic patterns differed in the small intestine, regardless of the concentration of contrast medium.

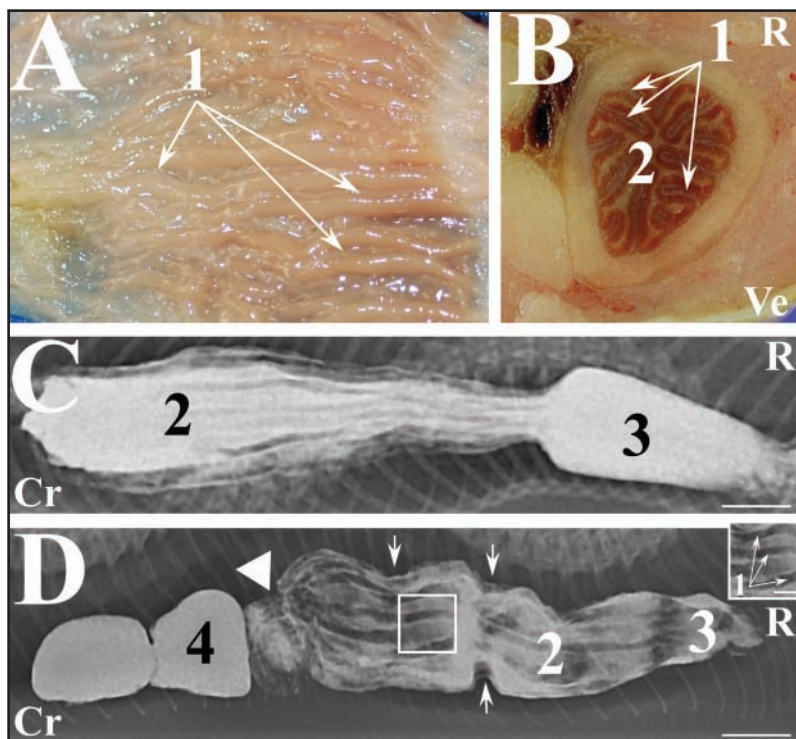


Figure 2—Photographs of the gross appearance of the internal surface of the stomach (A) and a transverse cross section of the stomach (B) in the cadaver of a ball python and dorsoventral radiographic views of the stomach of a live python 1 hour after oral administration of contrast medium (C and D). Notice the gastric folds (1), stomach (2), pylorus (3), and esophagus (4). In panel C, the pylorus appears oval. In panel D, the pylorus appears elongated and there is evidence of peristaltic waves (large white arrows) and an unusual pattern of gastric folds in the proximal portion of the stomach (white arrowhead). Inset—Enlarged view of the normal-appearing gastric folds. The pylorus appears elongated. Ve = Ventral. See Figure 1 for remainder of key.

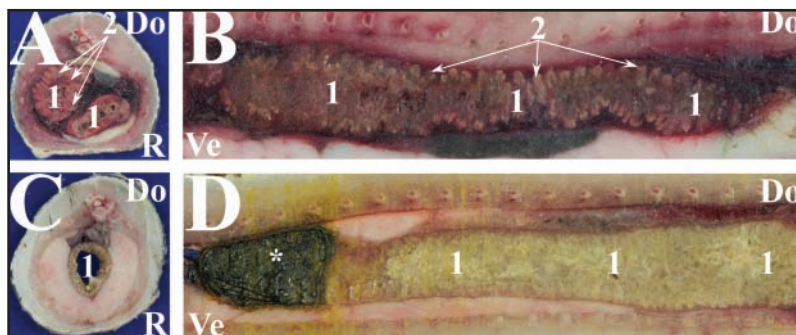


Figure 3—Photographs of a transverse section (A and C) and longitudinal section (B and D) of the small intestine (1) of the cadaver of a ball python. In panels A and B, notice the intestinal loops and evident villi (2). In panels C and D, notice that the villi are less evident and the small intestine has a rectilinear appearance. The gallbladder is indicated (asterisk). See Figure 1 for remainder of key.

Long longitudinal esophageal folds were often visible as thin, parallel, undulated filling defects within the organ. Dilation of the esophagus was visible in all snakes at the first radiographic time point (5 minutes after administration of contrast medium). Segmentation in the esophagus was a consistent finding and likely was a result of peristaltic activity associated with a long ETT (Figure 1). However, flocculation was not evident in the esophagus, despite a long ETT.

Peristaltic activity of the stomach was evident in 15 of 18 snakes. The gastric folds were visible as thick longitudinal filling defects within the stomach (Figure 2). Reflux from the stomach to the esophagus was not observed; moreover, the gastric folds appeared to be extremely tight in the cranial portion of the stomach. The pylorus was identified in all snakes as the terminal part of the stomach. The pylorus appeared oval shaped in some snakes, whereas it was more elongated in other snakes.

Similar to the gross anatomic findings, the distribution pattern for contrast medium in the small intestine was rather inconsistent among the live snakes, regardless of the concentration of contrast medium administered. Nevertheless, image analysis allowed the identification and description of 3 distribution patterns for the contrast medium, with each pattern characterized by common radiographic features.

Distribution pattern 1 was observed in 4 snakes (1 snake in group A, 2 snakes in group B, and 1 snake in group C; Figure 4). The OSIT started during the first radiographic time point (5 minutes after administration of contrast medium). The intestinal loops and the villi were evident during the entire transit through the small intestine. There was a clear distinction between the small and large intestines.

Distribution pattern 2 was observed in 6 snakes (3 snakes in group A, 1 snake in group B, and 2 snakes in group C; Figure 5). The OSIT started during the first radiographic time point. The contrast medium outlined less evident intestinal loops in addition to an overall more rectilinear appearance of the small intestine. Villi were clearly evident until 24 hours after administration of contrast medium. There was a clear distinction between the small and large intestines.

Distribution pattern 3 was observed in 8 snakes (2 snakes in group A, 3 snakes in group B, and 3 snakes in group C; Figure 6). The OSIT started during later radiographic time points (3

to 6 hours after administration of contrast medium). Furthermore, the contrast medium did not outline

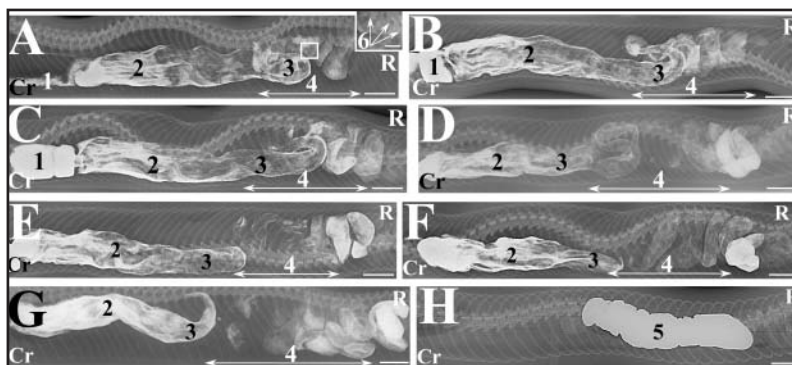


Figure 4—Dorsoventral radiographic views of the small intestine of a python in group A 5 minutes and 3, 6, 9, 12, 24, 48, and 72 hours (A through H, respectively) after oral administration of contrast medium (barium sulfate suspension) at a concentration of 25% (wt/vol). Distribution of the contrast medium was defined as distribution pattern 1 (evident villi and intestinal loops). The inset provides an enlarged view of the normal-appearing intestinal villi. Notice the esophagus (1), stomach (2), pylorus (3), small intestine (4), large intestine (5), and intestinal villi (6). Bar = 1 cm. See Figure 1 for remainder of key.

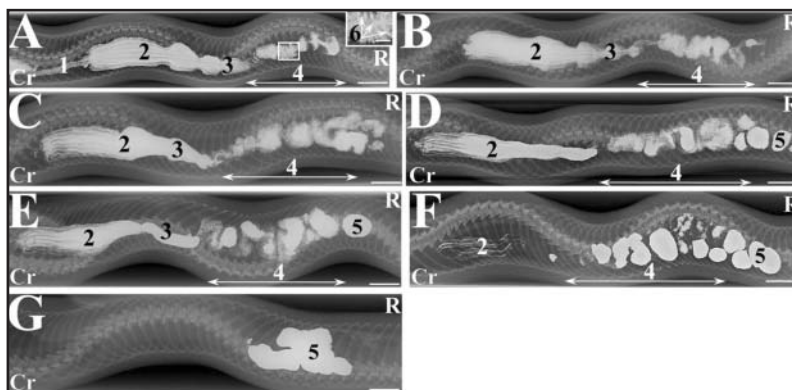


Figure 5—Dorsoventral radiographic views of the small intestine of a python in group B 5 minutes and 3, 6, 9, 12, 24, and 48 hours (A through G, respectively) after oral administration of a dose (25 mL) of contrast medium at a concentration of 35% (wt/vol). Distribution of the contrast medium was defined as distribution pattern 2 (evident villi and less-evident intestinal loops with an overall rectilinear appearance of the small intestine). Bar = 1 cm. See Figures 1 and 4 for remainder of key.

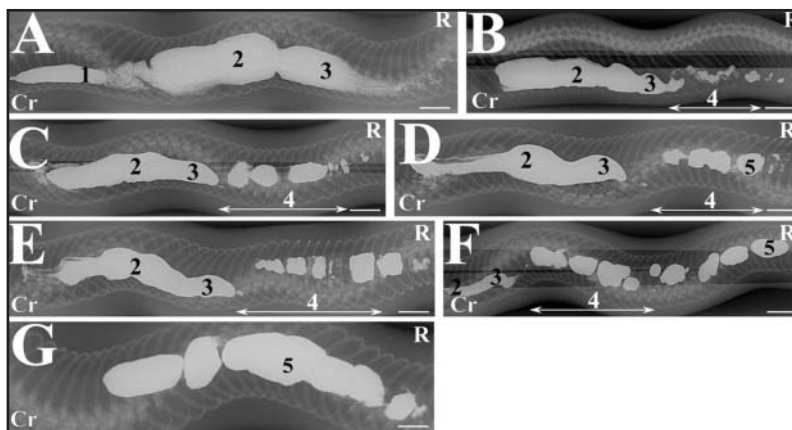


Figure 6—Dorsoventral radiographic views of the small intestine of a python in group B 5 minutes and 3, 6, 9, 12, 24, and 48 hours (A through G, respectively) after oral administration of a dose (25 mL) of contrast medium at a concentration of 35% (wt/vol). Distribution of the contrast medium was defined as distribution pattern 3 (no recognizable structures evident). Radiolucent bands visible in panels B and F are attributable to the presence of free air within a 6.3-cm-diameter radiolucent pipe that was used to help immobilize the snake during the radiographic procedures. Bar = 1 cm. See Figures 1 and 4 for remainder of key.

any recognizable intestinal structures (ie, intestinal loops or villi). The contrast medium was completely segmented and there was diffuse flocculation. The distinction between the small and large intestines could only be detected on the basis of thickness and amount of contrast medium.

Table 1—Mean \pm SD times for each radiographic variable in 3 groups of ball pythons (*Python regius*) orally administered contrast medium.*

Variable	Group A	Group B	Group C	All snakes
ETT	7.16 \pm 8.44	8.50 \pm 8.36	5.00 \pm 2.44	6.89 \pm 6.74
OGF	1.00 \pm 0.89	1.00 \pm 0	0.83 \pm 0.40	0.94 \pm 0.54
GET	38.00 \pm 15.95	44.00 \pm 18.07	40.00 \pm 12.40	40.67 \pm 14.92
OSIT	1.66 \pm 1.33	2.16 \pm 1.94	2.33 \pm 1.97	2.05 \pm 1.70
OLIT	44.33 \pm 22.85	22.00 \pm 20.31	20.50 \pm 15.46	28.94 \pm 21.70
SITT	56.00 \pm 12.40	48.00 \pm 15.17	52.00 \pm 9.80	50.82 \pm 12.34

Values are reported in hours.
 *A dose (25 mL/kg) of barium sulfate suspension at 3 concentrations (25%, 35%, and 45% [wt/vol]) was administered through an esophageal probe to groups A, B, and C, respectively; there were 6 snakes/group.

Mean \pm SD values of the transit time were determined for each radiographic variable (Table 1). To provide a more graphic representation of transit of contrast medium in relation to time, the number of snakes in which contrast medium was detected in the esophagus, stomach, small intestine, and large intestine at each radiographic time point were plotted (Figure 7).

The mean \pm SD values for the assessments performed by the investigators and differences among the 3 groups were summarized (Table 2). Flocculation was detected in all 3 groups; however, values did not differ significantly among groups. Segmentation appeared to decrease as the concentration of barium increased. Group A had a segmentation pattern with a significantly higher score than did the other 2 groups. Esophageal and gastric folds were similarly visible in group A and group B but became less evident in group C. The pylorus was clearly visible in all 3 groups. Visibility of small intestinal villi did not differ significantly among the 3 groups. Visibility of the superimposed intestinal loops was good and did not differ significantly among the 3 groups.

Regurgitation of contrast medium—

One snake in group A, 2 snakes in group B, and 1 snake in group C regurgitated a small amount of contrast medium immediately after administration. None of the snakes regurgitated contrast medium later during the study.

Discussion

The radiographic procedure described in the study reported here was tolerated well by all snakes. Administration of contrast medium was easy to perform and a relatively safe procedure, although it must be mentioned that all the snakes in the study were client-owned animals and were used to manipulation. Furthermore, the use of a radiolucent pipe allowed comparable images to be obtained without the need for chemical restraint of the snakes.

All snakes had not been fed for at least 7 days, which has been reported as the time necessary for the gastrointestinal tract of pythons to return to a resting phase after feeding.¹ The minimum

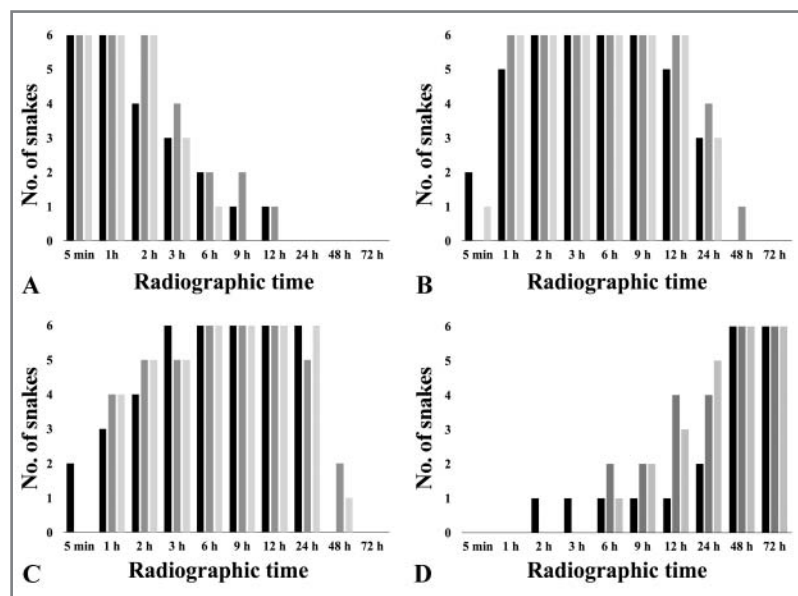


Figure 7—Number of snakes in each of 3 groups (A [black bars], B [dark gray bars], and C [light gray bars]) that had contrast medium in the esophagus (A), stomach (B), small intestine (C), and large intestine (D) at various time points after oral administration of contrast medium. A dose (25 mL/kg) of barium sulfate suspension at 3 concentrations (25%, 35%, and 45% [wt/vol]) was administered through an esophageal probe to groups A, B, and C, respectively (6 snakes/group).

Table 2—Mean \pm SD values for image quality of contrast radiographs of the cranial portion of the gastrointestinal tract of 3 groups of ball pythons.*

Variable	Group A	Group B	Group C	All snakes
Flocculation	2.15 \pm 1.20	2.49 \pm 1.08	2.19 \pm 1.32	2.28 \pm 1.21
Segmentation	1.57 \pm 1.04†	2.21 \pm 1.24	2.23 \pm 1.34	2.00 \pm 1.24
Visibility of esophageal folds	3.35 \pm 0.84	3.08 \pm 1.03	2.19 \pm 0.99†	2.96 \pm 1.05
Visibility of gastric folds	2.92 \pm 1.15	3.06 \pm 0.98	1.93 \pm 0.86†	2.62 \pm 1.12
Visibility of pylorus	2.92 \pm 0.99	3.24 \pm 0.84	3.05 \pm 0.79	3.07 \pm 0.89
Visibility of small intestine villi	3.11 \pm 0.95	3.36 \pm 0.73	2.87 \pm 1.08	3.10 \pm 0.95
Visibility of superimposed loops of small intestine	2.27 \pm 1.03	2.75 \pm 1.08	2.33 \pm 0.92	2.47 \pm 1.04

Flocculation and segmentation in the small intestine were each graded on a scale from 1 to 4 (grade 1 = extremely evident and grade 4 = not detected). Visibility of esophageal folds, gastric folds, intestinal villi, the pylorus, and superimposed loops of small intestine were graded on a scale from 1 to 4 (1 = poor, 2 = fair, 3 = good, and 4 = excellent).
 †Within a row, value differs significantly ($P < 0.05$) from the mean value of the other 2 groups.

1-week period of not feeding was required to obtain comparable results and to reduce the stress of the snakes; snakes are particularly vulnerable to stress during the immediate postfeeding period.

The dose of contrast medium administered to the snakes in the present study was the dose used in green iguanas in another study⁸; other authors¹⁰ reported a lower dose for gastrointestinal tract evaluation in reptiles. It is the authors' opinion that the dose used in the study reported here is the most suitable because of the unique capacity for distention of the gastrointestinal tract in snakes.¹⁻³

The anatomic outline of some gastrointestinal tract organs within the snakes of the present study was extremely good and enabled investigators to view structures that typically are not visible in survey radiographs. The consistent appearance of the esophagus and stomach should help veterinary practitioners in detecting anomalies in these organs. Despite the mean \pm SD values of the various radiographic variables (Table 1), it is important to notice that the last radiographic time point at which contrast medium was visible inside the stomach of all snakes, except for 1, was 24 hours after administration of contrast medium (Figure 7). Furthermore, the last radiographic time point at which contrast medium was detected within the small intestine was 48 hours after administration of contrast medium.

Snakes are described as not having a cardia portion of the stomach.¹¹ However, despite the lack of a recognizable specific anatomic structure, the lack of reflux of contrast medium from the stomach to the esophagus suggested the existence of an effective physiologic mechanism that separated the stomach and esophagus.

Gross anatomic differences in the small intestine were detected. Three distribution patterns of contrast medium in the cranial portion of the gastrointestinal tract were identified within the snakes of the present study. The 3 radiographic patterns detected in the study may have been related to the gross anatomic findings observed in snakes (snakes have high intraspecific anatomic differences, some of which even involve the lungs¹²). Another possible explanation is that they may have been the expression of various gastrointestinal digestive phases or morphological changes attributable to each snake's captivity conditions, regardless of the similar period of no feeding.

Snakes are poikilothermic animals, and gastrointestinal transit time and morphological adaptation depend on the species of snake, environmental temperature, seasonal changes, diet, and interval since the last meal.^{1-3,10} The present study was conducted under standardized conditions; however, the environmental and nutritional conditions of each snake before entrance into the study could not be controlled and standardized.

It was beyond the scope of the present study to determine the nature of the gastrointestinal variability. Nevertheless, we believe that the radiographic findings reported may be considered comprehensive of the multiple aspects of clinically normal snakes that would be examined in clinical practice.

The imaging data analysis did not reveal significant differences between groups A and B with regard to visibility of the internal topography of the gastrointestinal tract, whereas the use of a high concentration of con-

trast medium, as in group C (45% [wt/vol]), reduced visibility of both esophageal and gastric folds. Group A (concentration of contrast medium, 25% [wt/vol]) had a significantly higher value for segmentation than did the other 2 groups. Flocculation was detected in all 3 groups, and this most likely was related to the slow transit from the stomach to the small intestine identified in some snakes, rather than to the concentration of contrast medium administered. On the basis of analysis of the data, the best imaging quality was obtained for group B (concentration of contrast medium, 35% [wt/vol]).

Analysis of results of the present study suggested that contrast radiography could be a reliable tool for use in assessing the anatomic and functional aspects of the cranial portion of the gastrointestinal tract in ball pythons. However, the great variability among snakes observed in the present study and the differences among snake species^{13,14} do not permit the results to be used as a reference for species other than ball pythons, unless the anatomic and physiologic differences are known.

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- a. Barium Sulfate 400 g, Caesar & Loretz GmbH, Hilden, Germany.
 - b. Kodak point-of-care CR-360 system, Carestream Health Inc, Rochester, NY.
 - c. GraphPad Prism, version 4.00 for Windows, GraphPad Software Inc, San Diego, Calif.
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