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Anatomic-radiographic aspects and gastrointestinal transit time in *boa constrictor amarali* stull, 1932 (Squamata, Boidae)

[*Aspectos anátomo-radiográficos e tempo de trânsito gastrointestinal em jiboia-cinzenta boa constrictor amarali stull, 1932 (Squamata, Boidae)*]

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ABSTRACT

The goal of this study was to describe the gastrointestinal transit technique in the *Boa constrictor amarali*. For that purpose, we obtained simple radiographic images of seven serpents, subsequently administering a 25mL/kg dose of barium sulfate and establishing a radiographic sequence at the following times: 5 minutes; 1, 2, 3, 6, 9, 24, 48, 72 and 96 hours, extending to 120 and 126 hours for one animal. The mean esophageal transit was 26.71 ± 19.48 hours; the mean gastric filling time was 28.57 ± 27.22 minutes and the emptying time was 60 ± 12 hours; the mean filling time of the contrast medium in the small intestine was 3 ± 2.16 hours and the emptying time was 97 ± 15.65 hours. We also obtained the mean filling time of the large intestine, which was 40 ± 11.31 hours. We found that the mean passage time of the contrast medium through the cranial gastrointestinal tract – until the complete elimination of barium sulfate from the small intestine – was 97 ± 15.65 hours. In addition to determining the gastrointestinal transit time, the technique used allowed for the morphological identification of the alimentary canal.

Keywords: reptiles,imaging, radiological contrast, gastrointestinal tract

RESUMO

O objetivo deste trabalho foi descrever a técnica de trânsito gastrointestinal em Boa constrictor amarali. Para tanto, foram obtidas radiografias simples de sete serpentes, e, subsequentemente a essas, foi administrado sulfato de bário na dose de 25mL/kg. A partir disso, foi estabelecida a sequência radiográfica nos seguintes tempos: cinco minutos; uma, duas, três, seis, nove, 24, 48, 72 e 96 horas, e em um animal estendeu-se para 120 e 126 horas. O trânsito esofágico médio foi de 26,71±19,48 horas, o tempo médio de preenchimento gástrico foi de 28,57±27,22 minutos, e o esvaziamento de 60±12 horas; a média de tempo de enchimento do meio de contraste no intestino delgado foi de 3±2,16 horas e, por fim, o esvaziamento foi de 97±15,65 horas, e ainda obteve-se o tempo médio de enchimento do intestino grosso igual a 40±11,31 horas. Desse modo, verificou-se que a média do tempo de passagem do contraste pelo trato gastrointestinal cranial – até a completa eliminação do sulfato de bário do intestino delgado – foi de 97±15,65 horas. Além da determinação do tempo de trânsito gastrointestinal, a técnica empregada permitiu a identificação morfológica do canal alimentar.

Palavras-chave: répteis, imagem, contraste radiológico, trato gastrointestinal

INTRODUCTION

Reptiles show a wide biological variety, which results in a diversity of anatomical and physiological aspects (Bernarde and Machado, 2006). There is currently an increase in the breeding of these animals in captivity, especially ophidians, for several purposes such as venom extraction, exhibition in zoos, research, and even as pets. This leads to a growing need for the professional improvement of veterinarians and, in particular, for expanding the knowledge regarding the radiographic anatomy of these patients (Vieira *et al.*, 2012).

According to Mader (2006), issues related to the gastrointestinal tract in captivity are among the major diseases that affect serpents, which may be of infectious origin or due to improper handling. The clinical signs often appear late and are nonspecific, which hinders the diagnosis and early treatment of diseases (Banzato *et al.*, 2013). Diagnostic imaging is an area of great importance for diagnosis, therapeutic orientation and disease monitoring. Compared to other imagining modalities, the radiographic examination is safe, non-invasive and widely available to clinicians, resulting in large contributions to the medical treatment of animals (Silverman, 1993).

However, due to the relative scarcity of scientific information regarding the imaging and interpretation techniques, the low radiographic contrast that is inherent to soft tissues in the coelomic cavity, and the lack of radiographic parameters, radiographic examination in reptiles is commonly underused (Mader, 2006).The contrast radiographic examination of the gastrointestinal tract is of indisputable relevance; however, the description of the times and physiological parameters is limited in reptiles (Banzato *et al.*, 2012). Our literature review did not find any description of the normality standards in neotropical serpents. Therefore, the goal of this study was to describe the anatomical features and partial and total filling and emptying times of the contrast medium for each segment of the gastrointestinal tract in *Boa constrictor amarali.*

MATERIAL AND METHODS

The experiment was accepted by the Ethics Committee on the Use of Animals at the Federal University of Uberlândia (CEUA/UFU) with protocol number 037/2018 and obtained a license from the Biodiversity Authorization and Information System (SISBIO) under the number 62659.The research was divided into two steps. The first step consisted of an anatomical evaluation through the dissection of a serpent corpse belonging to the *Boa constrictor amarali* subspecies, female, young, with rostro-cloacal length of 184 centimeters, belonging to the scientific didactic collection of the Laboratory for Teaching and Research on Wild Animals (LAPAS) at the Federal University of Uberlândia (UFU), in Uberlândia, Minas Gerais, Brazil. The dissection was based on the practical dissection guide elaborated by Gomes and Puorto (1989).

The second step consisted of the radiographic evaluation of the live animals kept in captivity at the LAPAS. We used seven adult and healthy *Boa constrictor amarali* serpents, 3 male and 4 females, with an average body mass of approximately 1664±683.56g and average rostro-cloacal length (RCL) of approximately 124±15.9cm. The equipment used was the X-ray generator model Neo-Diagnomax of Medicor Mövek Röntgengyara, with a capacity of 50-500mA and 40-125kV. The values used for the radiographic examination were 55-60kV, 125mA, and 0.05s time, which were determined based on the individual body mass of the specimens.

The serpents had been fasting for at least seven days prior to the radiographic imaging. For the radiographic positioning, we used transparent containment tubes of diameters compatible with the dimensions of the serpents. The body of each animal was segmented into three or four parts, depending on each individual's rostro-cloacal length (RCL), using a blue-ink marker on the dorsal skin along the extension of the body, enabling the use of metallic number markers on the chassis in correspondence with the markings on the animal during the radiographic exposures.

First, we performed a simple radiographic examination in the dorsoventral projection of all segments of the serpents, with the radiographic cassette placed on the table and the animal positioned directly on the cassette. Then, we administered the suspension of the radiographic contrast medium based on barium sulfate (Bariogel® 100%) diluted in water at a concentration of 35%, at room temperature, via gastric probe number two. The probe was inserted until the esophagus, approximately 15cm of its length, and the contrast medium was administered at a dose of 25 milliliters per kilo (mL/kg) for each serpent (Banzato *et al.*, 2012).

Afterwards, we produced sequenced radiographic images at the following times: 5 minutes, 1 hour, 2 hours, 3 hours, 6 hours, 9 hours, 24 hours, 48 hours, 72 hours, and 96 hours. Specifically for the animal in which the contrast medium was found in the small intestine at 96 hours, we produced radiographic images at the additional times of 120 hours and 129 hours.After the radiographic exposures, the photostimulable phosphor (PSP) plate located inside the cassette was processed by a computerized radiography digitizer model CR

10-x (Agfa Health Care®).The images were analyzed for contrast filling time (first radiographic time when the contrast reaches the organ); contrast emptying time (radiographic time when the target organ is completely free of contrast), topography and filling of the esophageal mucosa until the beginning of the large intestine.

While dealing with the animals in order to subject them to the radiographic exposures, we assessed behavioral changes characteristic of their expression of stress, such as lunging attempts, hissing sound, excessive agitation and regurgitation. Stress was graded using three classifications marked with a "plus sign" (+), where one (+) represented mild manifestation of stress, two (++) moderate and three (+++) severe.We considered the stress manifestation to be mild when the animals allowed (physical) containment and manipulation (sounding and positioning) without difficulties or resistance; we considered stress to be moderate when the serpents were difficult to contain and manipulate, showing resistance and excessive agitation, attempting to lunge, and emitting sounds; and we considered stress to be severe when the animals also regurgitated the radiographic contrast. In addition, we recorded the variation of room temperature at the time of each radiographic examination using a digital thermometer (Incoterm®).We used simple descriptive statistics to calculate the mean and standard deviation of the emptying times of the contrast medium in the esophagus, filling and emptying times of the stomach and small intestine, and filling time of the large intestine.

RESULTS

The dissection started with an incision from the cloaca extending cranially to the mandible through the ventral midline, followed by moving away the muscles and ribs and making an incision in the coelomic membrane, allowing access to the coelomic cavity for the macroscopic analysis and photographic record of the internal organs. In a caudal position to the stomach, it was necessary to remove a considerable amount of adipose tissue enveloping the outer face of the organs to facilitate their visualization and registration. Through dissection, it was possible to perform the anatomical characterization of the esophagus, stomach, small intestine, large intestine and cloaca, as well as accessory structures and glands, such as the liver, gallbladder and pancreas (Table 1).

Table 1. Topography of the organs correlated with the percentage of the rostro-cloacal length of a young female specimen of *Boa constrictor amarali*with184cm of rostro-cloacal length

Organ	Rostro-cloacal length (cm)	Percentage(%)
Esophagus	3.8 - 77.5	2.06- 42.12
Heart	45-50	24.45 - 27.17
Right Lung	46-66	25 - 35.86
Left Lung	49-64	26.63 - 34.78
Liver	59 - 92	32.06 - 50.00
Stomach	73 - 121	39.6 - 65.76
Pylorus	112 - 121	60.87 - 65.76
Gallbladder	115-118	62.5 - 64.13
Coelomic fat	115-172.3	62.5 - 93.64
Spleen	116.5-118	63.31 - 64.13
Pancreas	117-118	63.58 - 64.13
Small intestine	121-133	65.76 - 72.28
Cecum	133.5-138.5	72.55 - 75.27
Large intestine	134-174	72.82 - 94.56

The esophagus appeared as a rectilinear canal with narrow and uniform diameter, extending up to 68cm from the serpent's RCL, increasing gradually as it neared the transition with the stomach. Topographically, it started dorsal to the trachea and assumed a position on the left antimere slightly before the end of the trachea (42cm from the RCL) (Figure 1). The mucosa showed longitudinal folds arranged parallel to each other. The total length of the esophagus was 77.5cm, corresponding to approximately 42.12% of the animal's rostro-cloacal length (RCL).

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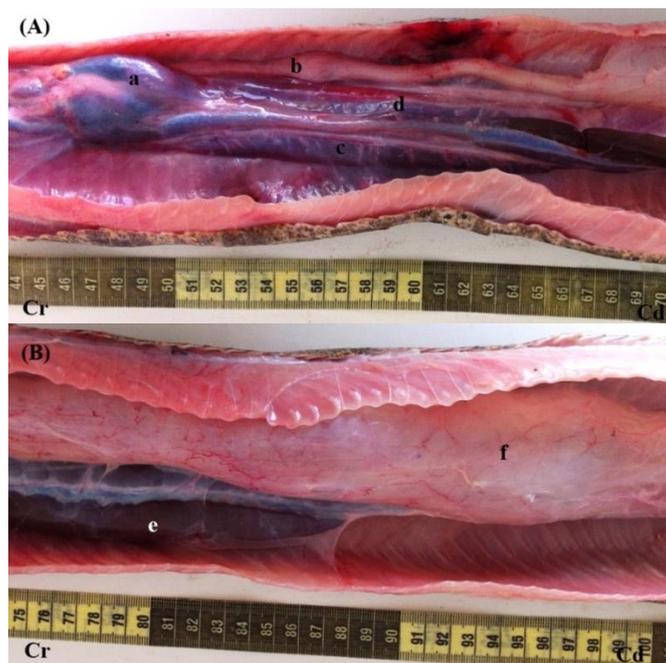
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Figure 1. Photographs of the coelomic cavity of a *Boa constrictor amarali*, proximal third. Cr,cranial; Cd, caudal. (A) heart (a); esophagus (b); right lung (c); left lung (d). (B) liver (e); stomach (f). Numeric tape - right side.

The stomach had a fusiform aspect and was located in the left antimere, extending from 73 to 121cm of the RCL (39.67% to 65.76% of the RCL), continuing from the liver (Figure 1). The pylorus was easily identified at 112 to 121cm of the RCL (60.87% to 65.76%), which was the terminal part of the organ, with muscular appearance and curvilinear disposition, slightly similar to a horizontal "s" figure. We found the gallbladder and pancreas medially to the pylorus. Together with the spleen, they assumed a triad form arrangement. Analyzing the lumen of the organ did not allow us to determine the exact transition between esophagus and stomach. The mucosa showed a large amount of folds arranged longitudinally (Figure 2).

The liver was elongated and located in the right antimere at 59 to 92cm of the RCL, positioned approximately in the middle (50%) of the animal's body (Figure 2).The small and large intestines were surrounded by coelomic fat, which was removed to allow the visualization of the organ. The loops of the small intestine were arranged transversely and showed few flexures. It was possible to delimit two parts of the organ: the duodenum and the jejunum-ileum (Figure 2). The duodenum forms the initial part of the intestine,

and the jejunum-ileum portion is connected to the mesentery. The total length of the organ, measured after removal from the coelomic cavity and fully stretched, was 193.5cm. The mucosa showed villi.

The large intestine was composed of the cecum and the colorectum segment. The cecum is seen as a small tubiform extension (measuring 6cm in length), located just after the end of the small intestine. The colorectum segment started immediately after the end of the jejunum-ileum portion, appearing as a canal with diameter larger than the small intestine and showing a rectilinear disposition, ending in the cloaca (Figure 2).Seven serpents underwent the radiographic procedure and it was possible to observe the esophageal emptying and gastric filling in all of them. However, one animal was excluded from the experiment in the second radiographic period (one hour), as it regurgitated a large amount of contrast and showed signs of severe stress. Consequently, the number of animals in the study was reduced to six. From these six animals, we obtained data on the emptying times of the stomach and small intestine and the beginning of the filling of the large intestine.

Figure 2.Photographs of the coelomic cavity of a *Boa constrictor amarali*. (A) A segment of the gastrointestinal tract showing the gastric lumen. Cr, cranial; Cd, caudal; stomach (a); pylorus (b). (B) Stomach (a); gastric mucosa folds (b). (C) Pylorus (b), gallbladder (d); duodenum (e); jejunum-ileum (f); left ovary (g); right ovary (h); cecum (i); colorectum (j).

The radiographic differentiation of the organs of the alimentary canal was achieved by means of radiopaque contrast. It was possible to visualize the esophagus, stomach and intestines based on the RCL of each animal in the study. Details of the esophageal mucosa were observed in all animals, with the esophageal folds seen as thin and parallel. Regarding the distribution of the contrast medium in the esophagus, we observed the segmentation of barium sulfate through the canal (Figure 4). The filling of the entire esophagus was observed in the first time (time zero) of the radiographic sequence, 5 minutes after the administration of the radiological contrast medium. The estimated emptying time varied between 9 and 48 hours, with a mean of 31±19.41 hours (Table 2).

Table 2. Filling and emptying times, mean and standard deviation of the transit of the barium sulfate-based contrast medium through the gastrointestinal tract of six specimens of *Boa constrictor amarali*

Animal	Filling				Emptying		
	Esophagus (min)	Stomach (min)	Small intestine (h)	Large intestine (h)	Esophagus (h)	Stomach (h)	Small intestine (h)
1	0	5	1	48	48	72	96
2	0	5	6	24	48	48	96
3	0	60	2	48	24	72	126
4	0	5	1	24	48	48	96
5	0	5	6	48	9	72	96
6	0	60	2	48	9	48	72
Mean	0	23.33	3	40	31	60	97
St. Dev.	0	28.40	2.37	12.39	19.41	13.14	17.15

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Folds in the gastric mucosa were observed in all animals in the experiment with longitudinal and wavy dispositions and seen as more prominent from the middle third of the organ. As in the esophagus, the contrast medium presented a segmented distribution through the stomach. We did not visualize any reflux of the contrast medium from the stomach into the esophageal lumen. It was possible to recognize the pylorus in all the serpents; however, morphological variations were detected, as it was more stretched in four serpents and had more flexures in two. A peristaltic wave was visualized in the stomach of three of the six serpents studied (Figure 3).
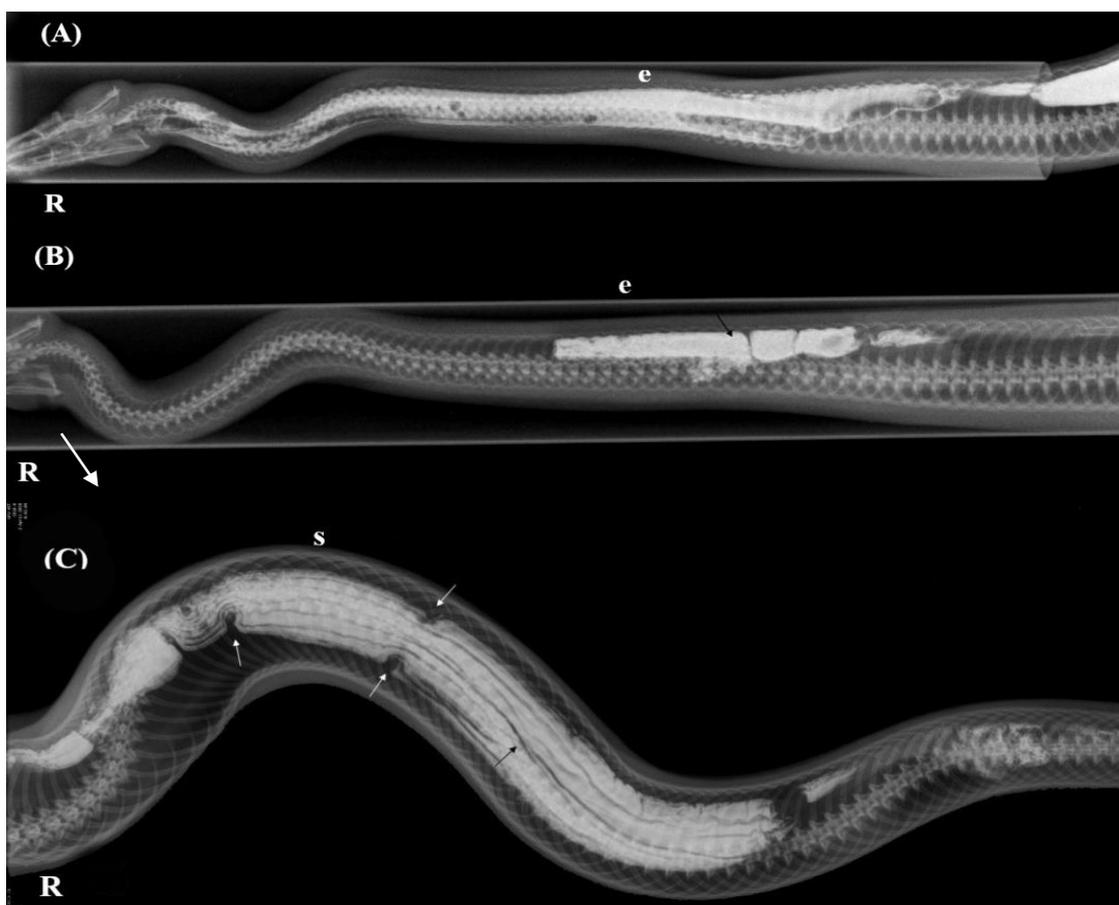

Figure 3.(A) Radiographic image of the cranial third of a *Boa constrictor amarali* serpent obtained 5 minutes after administration of the contrast medium, contained in an acrylic tube, notice the esophagus (e).R, right side. (B) Radiographic image obtained 1 hour after administration of the contrast medium; notice the segmentation (black arrow) of the contrast medium in a portion of the esophagus(e) (C) In the middle third, 6 hours after administration of the contrast medium, notice the stomach (s), gastric fold (black arrow); stomach peristalsis (white arrows).

The filling of the stomach began at five minutes in four animals and at one hour in two animals. The complete emptying of the contrast medium from the organ ranged from 48 to 72 hours, with a mean of 60±13.14 hours (Table 1). Small intestine flexures were seen in all the animals evaluated. The filling time of the small intestine ranged from one to six hours, with a mean of 3±2.37 hours. Regarding the distribution pattern of the radiographic contrast medium, two animals presented segmentation and two other animals presented flocculation (Figure 4). The emptying time of the small intestine ranged from 72 to 129 hours, with a mean of 97±17.15 hours (Table 1).

The differentiation between the large and small intestines was based on the dimensions of the organ, the most rectilinear disposition of the large

intestine and the topography, in addition to the location of the cecum, seen as an appendix filled with radiographic contrast. The filling time of the large intestine ranged from 24 to 48 hours, with a mean of 40±12.39 hours (Table 1). The mean cranial gastrointestinal transit time observed until the complete absence of contrast medium in the small intestine of the *Boa constrictor amarali* specimens studied using this technique was 97±17.15 hours. Although the thermal conditions of the environment were not controlled, the variations during the experiment were considered small (23.8 and 27°C).
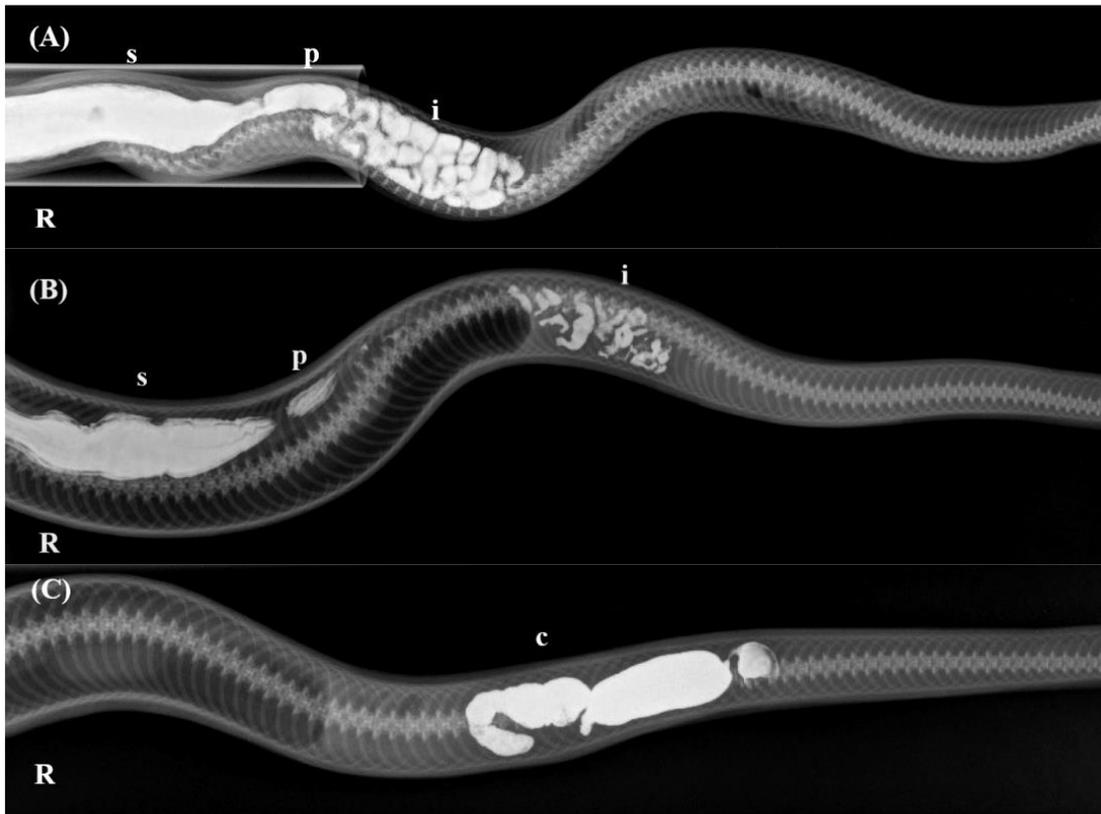

Figure 4.(A) In the middle third radiographic image, *Boa constrictor amarali serpent,* 48 hours after administration of the contrast medium. R, right side; showing the stomach (s); pylorus (p); small intestine (i); (B) In another animal, in the middle third radiographic image, 72 hours after administration of the contrast medium. Notice the flocculation of the contrast medium in the small intestine (i). Pylorus (p) with segmentation of the contrast; (C) Distal third radiographic image,96 hours after administration of the contrast medium. Notice the filled large intestine, notice the colorectum segment (c).

Two of the seven serpents used in the experiment regurgitated. One of them regurgitated a small amount of contrast medium shortly after its administration (prior to the first radiographic exposure) and prior to the ninth radiographic shot, at 72 hours. We noticed the presence of a small amount of regurgitated radiographic contrast medium in the animal's plastic box. We carried on with the radiographic sequence of this animal until the complete emptying of the contrast medium from the small intestine. The other serpent regurgitated shortly after the second radiographic time, one hour after its administration, and was excluded from the experiment. Regarding the stress level manifested by the animals during the course of the experiment resulting from their manipulation, three animals showed mild stress (+), one moderate (++), and two severe (+++). However, it was possible to finish the examination even with the stressed animals, and none of them died, which demonstrates that the technique is safe for the species.

DISCUSSION

The dissection of a specimen is important for the macroscopic recognition and description of the topographic anatomy of the structures that compose the gastrointestinal tract and its accessory organs (liver and pancreas). In addition to allowing the determination of the position of organs in relation to neighboring organs and direct identification of their morphology, this knowledge contributes to radiographic interpretation. On simple radiographic images, the organs of the coelomic cavity showed little radiographic definition, as described by Silverman (1993), which makes it difficult to assess the structures. However, the administration of the radiographic contrast medium enabled a clear visualization of the organs filled by it.

The radiographic examination described in this study was tolerated well by five of the seven animals used, as they did not regurgitate the contrast medium and their stress levels were mild. It is important to note that serpents are animals susceptible to postprandial manipulation, and regurgitation is an escape mechanism for these animals in stressful situations (Campagner, 2011). The placement of the esophageal probe and the administration of the barium sulfate suspension were procedures performed with ease and considered relatively safe as long as the serpent is properly contained. We did not use any chemical containment of the animals under study, considering the influence of drugs on gastrointestinal transit time mentioned by Pinto *et al.* (2014).

Pinto *et al.* (2014) mention that one possible but rare complication in this examination is the formation of enteroliths due to the slow progression of the contrast medium through the gastrointestinal tract of reptiles. Pizzutto *et al.* (2000) advocated for the concomitant administration of barium sulfate with mineral oil in the assessment of the gastrointestinal transit in testudines in order to prevent the contrast medium from becoming dry in the colon. However, in this study, we administered only the barium solution diluted in water, as the only study found on a contrasted examination of the gastrointestinal tract in serpents, prepared by Banzato *et al.* (2012), did not use mineral oil. One of the serpents used in the experiment excreted a large amount of the contrast on the fifth day of the evaluation, after the last radiographic evaluation.

The concentration of 35% barium sulfate was determined based on the contrast technique of the gastrointestinal tract in a *Python regius* serpent developed by Banzato *et al.* (2012), in which this concentration enabled the identification of the esophageal and stomach folds, which were also visualized in our experiment. The 25mL/kg dose was also determined based on that same study, as it took into consideration the great extension capacity of the gastrointestinal tract in serpents. However, other gastrointestinal transit studies on reptiles found in the literature used lower doses of barium sulfate, in animals such as Testudines (Lopes, 2006; Moraes, 2007) and in *Caiman crocodiles crocodiles* (Pereira *et al.*, 2014). Those studies used a 10mL/kg dose of barium sulfate associated with mineral oil.

The radiographic sequence established after the administration of the barium sulfate suspension enabled the visualization and imaging record of the filling and emptying times of the contrast medium in the structures studied. The esophageal transit time was estimated taking into account that the contrast medium was administered directly in the canal.

Regarding the distribution pattern of the contrast medium, Banzato *et al.* (2012) used two terms to characterize the organization of barium sulfate in the gastrointestinal tract, namely segmentation and flocculation. Segmentation was defined in radiographic terms by the non-homogeneous distribution of the contrast medium in the gastrointestinal tract. Flocculation, which was seen in the small intestine of some animals, was defined as the distribution of contrast in small clusters with no uniform distribution across the organ, which can impair the outline of the mucosa by the contrast medium.

According to Banzato *et al.* (2012), the probable cause of flocculation is related to the prolonged transit from the stomach to the small intestine in some serpents. In this study, the two animals that presented flocculation also presented longer stomach emptying times, which is consistent with the author's hypothesis. In the species analyzed in this study, *Boa constrictor amarali*, the mean gastrointestinal transit time (recorded until the complete emptying of the small intestine) was

longer (97±15.65 hours) when compared to the *Python regius* (48±15.17 hours) studied by Banzato *et al*. (2012). In addition, it was longer than in the *Caiman crocodiles crocodilus*, mean 74.8±22.8 hours (Pereira *et al.*, 2014), in the *Iguana iguana*, mean 16 hours (Smith; Dobson; Speen, 2001), and in the *Trachemys dorbignyi* and *Trachemys scripta elegans*, mean 3.47 days (Moraes, 2007).These descriptions demonstrate the importance of studies on different species, as there are interspecific peculiarities.

In the species studied in this research, *Boa constrictor amarali*, the mean gastrointestinal transit time (until the complete emptying of the small intestine), at ambient temperature ranging between 23.8 and 27°C, was higher (97±15.65 hours) when compared to the *Python regius* at ambient temperature of 28°C (48±15.17 hours) studied by Banzato *et al*. (2012). It was also higher than in the *Caiman crocodiles crocodilus*, (74.8±22.8 hours) at ambient temperature of 27°C (Pereira *et al*., 2014), in the *Iguana iguana* (16 hours) at ambient temperature between 27 and 29°C (Smith *et al*, 2001), and in the *Trachemys dorbignyi* and *Trachemys scripta elegans* (3.47 days) at ambient temperature around 27°C (Moraes, 2007). These descriptions demonstrate the importance of studies in different species since there are interspecific peculiarities.

In addition, it is important to consider the ambient temperature in studies with reptiles using contrast media, since these animals are ectothermic and require adequate external climatic conditions, with a minimum temperature of 19ºC to perform their organic functions. Many aspects of the physiology of these animals are closely linked to temperature, including the rate of passage of food through the gastrointestinal tract, which can vary according to the amount of mass ingested, temperature, conformation and length of the serpents' gastrointestinal tract (Mader, 2006).

CONCLUSION

The cranial gastrointestinal transit time in *Boa constrictor amarali* was on average 97±15.65 hours, considering room temperature between 23.8 and 27°C, as well as the filling times of the stomach, small intestine and large intestine, and the emptying times of the esophagus, stomach and small intestine. In addition, in the radiographic image it was possible to identify morphological characteristics of each anatomical segment of the digestive system of the subspecies *Boa constrictor amarali*, outlined by the contrast medium.

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