EFFECT OF ECOBIOMETRIC VARIABLES IN COELOMIC CAVITY ORGANS IN RED-TAILED BOAS

[*Efeito das variáveis ecobiométricas em órgãos da cavidade celomática em jiboias*]

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RESUMO – O emprego da ultrassonografia em modo-B em serpentes é um desafio, pois apresentam anatomia diferenciada em relação às aves e mamíferos. Assim, o presente estudo teve como objetivo descrever a topografia e características ultrassonográficas do fígado, vesícula biliar, baço e rins, além de realizar comparação de medidas e variáveis biométricas de serpentes. Cinquenta e sete jiboias (*Boa constrictor*) foram agrupadas de acordo com a massa corporal: Grupo A [30 animais (15 machos e 15 fêmeas) com massa corporal variando de 0,8 a 2,8 kg] e Grupo B [27 animais (12 machos e 15 fêmeas), massa corporal variando de 3 a 3,4 kg]. Antes dos exames de imagem, as cobras foram examinadas para avaliação de quaisquer lesões visíveis ou danos que pudessem comprometer a sua condição geral. Os exames de ultrassonografia foram realizados utilizando-se transdutores convexo (3,5 MHz), linear (7,5 MHz) e linear multi-frequencial (6-18 MHz). Os parâmetros ultrassonográficos foram realizados em modo B por meio de cortes sagitais e transversais. As medidas biométricas entre os grupos mostraram que houve variação significativa entre todos os parâmetros avaliados. O sexo não influenciou os parâmetros biométricos. No entanto, o comprimento e a massa corporal influenciaram alguns órgãos da cavidade celomática. A ultrassonografia é uma ferramenta de diagnóstico útil para a avaliação de jiboias. Além disso, os dados reportados neste estudo são importantes para dar base ao exame de ultrassom em outras espécies de serpentes.

Palavras-Chave: Biometria; ultrassom; serpentes.

ABSTRACT – B-mode ultrasound employment in snakes is a challenge because the anatomy is differentiated in relation to birds and mammals. Thus, the present study aimed to describe the topography and ultrasonographic characteristics of the liver, gallbladder, spleen, kidneys, and conduct a comparison of measurements and biometric variables in snakes. Fifty-seven red-tail boas (*Boa constrictor*) were grouped according to body mass: Group A [30 snakes (15 males and 15 females) with body mass ranging from 0.8 to 2.8 kg] and Group B [27 snakes (12 males and 15 females), body mass ranging from 3 to 3.4 Kg]. Prior to imaging, the red-tail boas received a physical examination to assess for any visible lesions or damage that could compromise their overall condition. The ultrasonographic exams were performed using an ultrasound with convex (3.5 MHz), linear (7.5 MHz), and linear multi-frequency (6-18 MHz) transducers. All ultrasound parameters were performed using sagittal and transverse B-mode. Sex did not influence the biometric parameters evaluated. However, body length and body mass influenced some organs of the coelomic cavity. Ultrasonography is a useful diagnostic tool for the assessment of the snake in red-tailed boas. In addition, data reported in this study are important as a basis for ultrasound examination for other snake species.

Keywords: Biometrics; ultrasound; snakes.

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INTRODUCTION

Snakes, lizards, and chelonian exhibit unique anatomical features and little is known about basic aspects of their morphology. This fact may be a limitation in the scientific advancement of herpetology or in clinical/surgical trials related to free-living or captive animals. In this context, ultrasonography is important noninvasive of imaging method in reptile medicine and can assist in decreasing unnecessary invasive interventions (Navarre 2006; Neto et al., 2009; Andrade et al., 2012). Additionally, ultrasonography is considered a high-sensitivity technique for the evaluation of the coelomic organs of snakes and lizards (Banzato et al., 2013, Conceição et al., 2014). However, the success of this method depends of the examiner's skill, experience, and on the knowledge of the species' morphophysiology (Schildger et al., 1994; Banzato et al., 2013). On the other hand, B-mode ultrasound employment in snakes is a challenge to the examiner, since the anatomy is differentiated in relation to birds and mammals (Canny, 1998).

The present study aimed to describe the topography and ultrasonographic characteristics of the liver, gallbladder, spleen, kidneys, and conduct a comparison of measurements and biometric variables in the red-tailed boa (*Boa constrictor constrictor*). It was hypothesized that body mass and sex may influence these parameters and feasible to sonographic evaluation of these animals.

MATERIALS AND METHODS

This study followed the guidelines for the care and use of laboratory animals and was approved by the Ethics in Research with Animals Committee of the Evandro Chagas Institute (CEPAN/IEC protocol nº 002/2012) and System for Authorization and Information on Biodiversity from Chico Mendes Institute for Biodiversity (SISBIO/ICMBIO protocol nº 34280-1).

All red-tailed boas had been confiscated by Brazilian environmental agencies and were incorporated into a rescue colony (Sítio Xerimbabo, Santo Antônio Tauá, Pará, Brazil).

They were each presumed as adults but exact age was unknown and housed in brick enclosures covered with tiles and wire netting. Females were housed in terrariums with up to 5 individuals (2m x 1m x 1.5m) and males in individual terrariums (0.3m x 1m x 0.5m). These enclosures were positioned in a north–south orientation to receive 12 hours of natural light each day. The average temperature was 33°C, and humidity was 85%. Snakes were fed rats (*Rattus norvegicus*) every 15 days and had been last fed 7-10 days prior to ultrasound examination.

Fifty-seven red-tail boas (*Boa constrictor*) were grouped according to body mass: Group A [30 snakes (15 males and 15 females) with body mass ranging from 0.8 to 2.8 kg] and Group B [27 snakes (12 males and 15 females), body mass ranging from 3 to 3.4 Kg]. Prior to imaging, the snakes received a physical examination to assess for any visible lesions or damage that could compromise their overall condition. Each snake was identified by a microchip (Animalltag® São Carlos, São Paulo, 13563-306, Brazil) placed horizontally to the vertebral axis, in the last third of the animal's body.

Only red-tail boas with good or regular body conditions were included in this study. Body condition was assessed subjectively, and scored as: good - those snakes with well-developed constrictor muscle and ribs unapparent and not palpable; regular - those snakes that had developed constrictor muscle, but with little apparent and palpable ribs. Body mass and total length of body, and snout-vent length were recorded. Body mass was determined using a scale with 200 gram increments (Pesola Scale®, Baar, CH-6340, Switzerland). Based on those characteristics, the animals were considered of normal size for this species.

Under manual restraint, the snakes were positioned in ventral recumbency on a square table with an opening (20cm x 10cm) to enable application of a transducer with acoustic transmission gel (Carbogel®, São Paulo, São Paulo, Brazil). The ultrasonographic exams were performed using an ultrasound with convex (3.5 MHz), linear (7.5 MHz) (Logiq α 100®, GE Healthcare Bio-Sciences Corp. Piscataway, USA), and a linear multi-frequency (6 to 18MHz) transducers (MyLab30 VET Gold®, Esaote, Genoa, Liguria, Italy). All ultrasound parameters were performed using sagittal and transverse B-mode. The images were obtained and analyzed similarly to those previously described and outlined below (Isaza et al., 1993[ab]; Neto et al., 2009; Stahlschmidt et al., 2011; Banzato et al., 2012[ab]).

Liver images were evaluated positioning the transducer on the right ventrolateral area, caudal to heart and cranial to stomach. This organ was examined in the sagittal and transverse planes in order to evaluate their localization, shape, echotexture (homogeneous or heterogeneous), echogenicity (anechoic, hypoechoic, hyperechoic or mixed), and size. The height (H) of the left/right hepatic lobes and thickness of the hepatic vein were measured (cm) from sagittal scans (Figure 1A). The width (W) at the liver parenchyma was measured from transverse scans (Figure 1B).

Gallbladder was visualized because their anechoic appearance, which was evaluated according to localization, wall, repletion, contour (defined or non-defined) and appearance of the content (anechoic, hypoechoic, hyperechoic or mixed). The length (L) and height (H) were obtained from sagittal plane, and the width (W), transverse plane (Figure 1C and D, respectively).

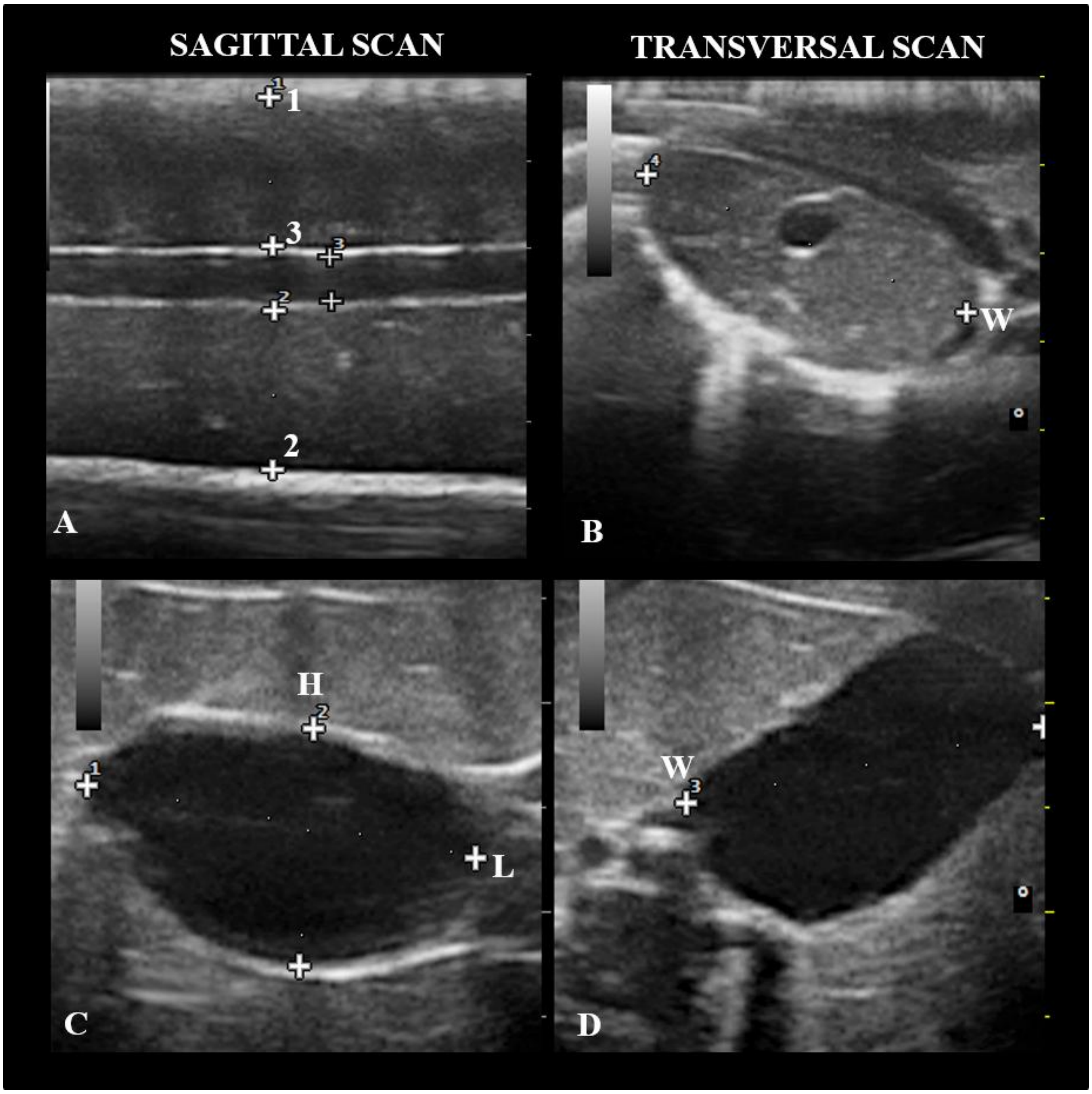

Figure 1. Hepatic measurements in red-tailed boas (*Boa constrictor constrictor*). (A) Height of the right and left parenchyma lobes, and thickness of the hepatic vein measured on sagittal view (1, 2, and 3; respectively). (B) Transversal scan, conducted at the largest diameter of the liver, where the liver width (W) was measured. (C and D) Measurement of the gallbladder. (C) Sagittal plane was measured height (H) and length (L) of the gallbladder. (D) Transversal plane was measured the width (W).

Localization, echotexture (homogeneous or heterogeneous), echogenicity (anechoic, hypoechoic, hyperechoic or mixed) and biometry (length -L; and height - H) of the spleen were obtained from the sagittal scan (Figure 2A), and the width spleen in the transverse scan (Figure 2B). Gallbladder and splenic volumes (cm^3) were estimated using the formula for volume of spheroid bodies: [$V = \pi (L \times H \times W) / 6$].

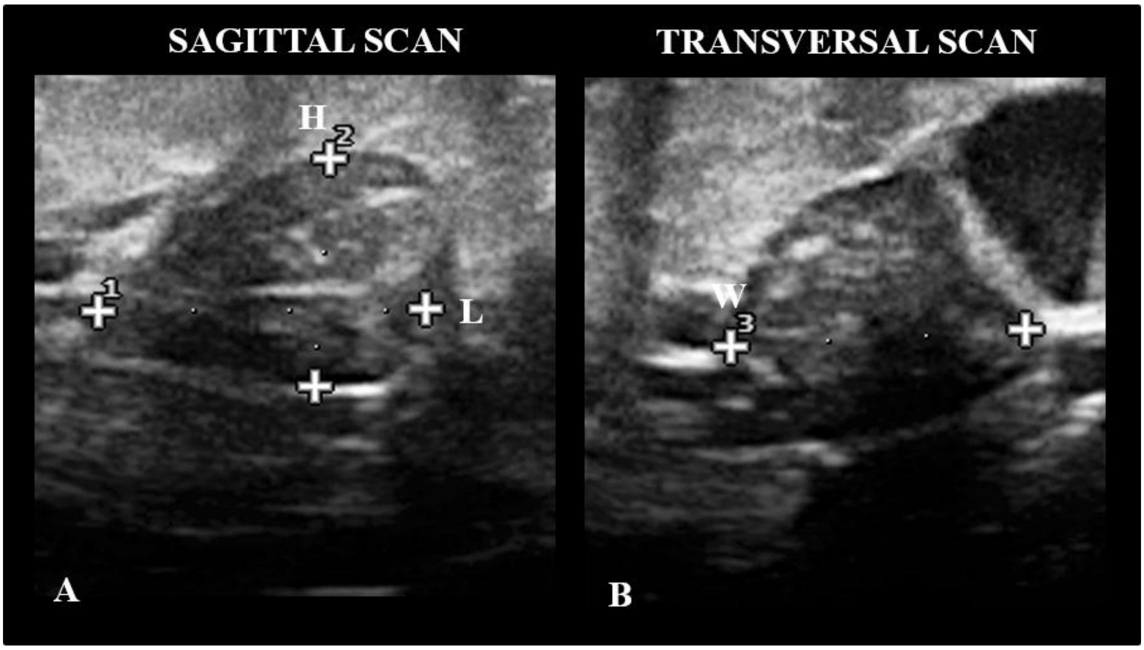

Figure 2. Spleen measurements in red-tailed boas (*Boa constrictor constrictor*). (A) Sagittal plane was measured height (H) and length (L) of spleen. (B) Transversal plane was measured the width (W).

Each kidney was examined in the sagittal and transverse planes in order to evaluate their outline, shape, localization, echotexture (homogeneous or heterogeneous), echogenicity (anechoic, hypoechoic, hyperechoic or mixed), and size. The kidneys were measured in the medial portion, obtaining height (H) in the sagittal plane and width (W) in the transverse plane (Figure 3).

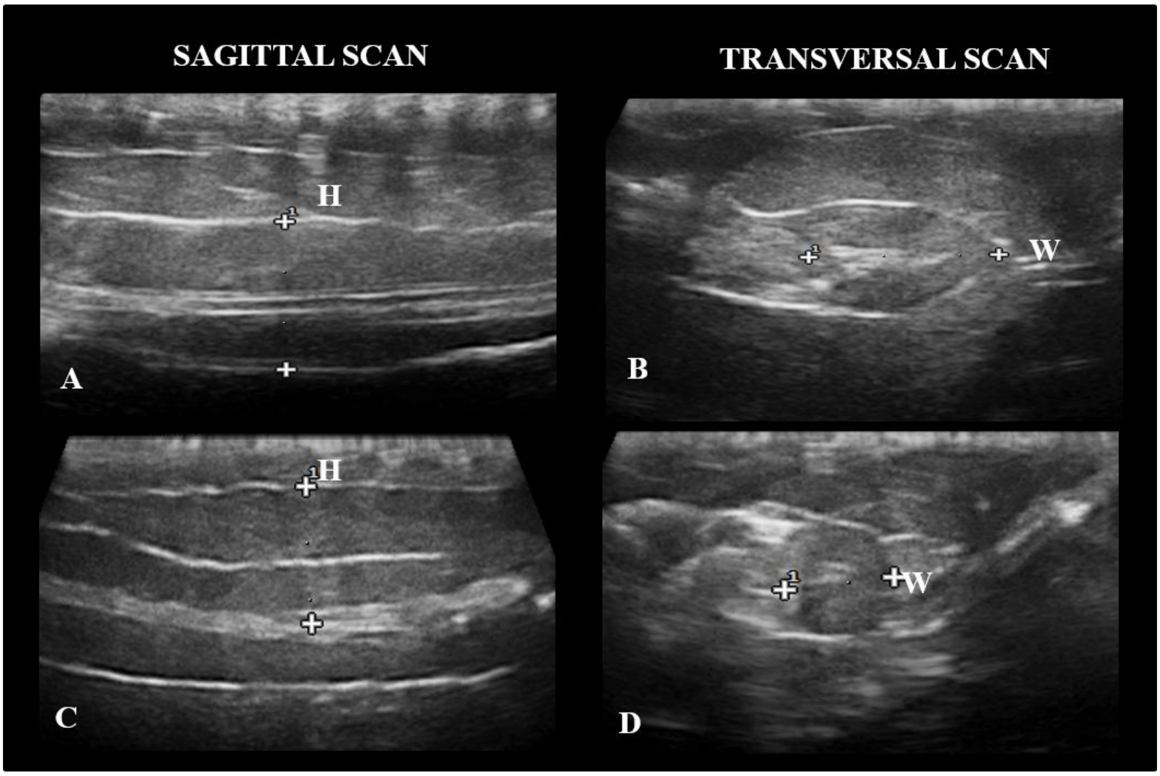

Figure 3. (A and B) Measurements of the right kidney in red-tailed boas (*Boa constrictor constrictor*). (A) Height of the right kidney (H) was measured in sagittal scan. (B) Transversal scan, conducted at the largest diameter of the right kidney, where the kidney width (W) was measured. The same measurements were performed for the left kidney(C and D).

A factorial ANOVA (3 groups x 2 sexes) with Tukey's post-hoc was used to evaluate differences between groups for all biometric and organ parameters. Linear regression equations were created between body length/body mass with all organ parameters. However, were considered only the equations that showed determination coefficients (R^2) greater than 0.50 and $P < 0.05$, which was calculated with the aid of the BioEstat software (version 5.3; Instituto de Desenvolvimento Sustentável Mamirauá, Tefé, Amazonas, Brazil).

RESULTS

Biometric measurements between groups showed that there was significant variation between all parameters. However, sex did not influence in these variables (Table 1).

Table 1. Biometric data (mean ± standard deviation [SD]) of red-tailed boa (*Boa constrictor*) grouped according to body mass, body length, and snout-vent length.

Parameters*	Sex	N	Group A (0.8 - 2.8 kg)	N	Group B (3 - 5.4 kg)
Body mass (Kg)	♂	15	1.93 ± 0.61	12	4.12 ± 0.93
	♀	15	1.71 ± 0.60	15	3.89 ± 0.85
	♂♀	30	1.82^B ± 0.60	27	3.99^A ± 0.88
Body length (cm)	♂	15	154.07 ± 21.70	12	192.25. ± 17.28
	♀	15	140.40 ± 28.08	15	185.07 ± 21.79
	♂♀	30	147.23^B ± 25.21	27	188.26^A ± 19.88
Snout-vent length (cm)	♂	12	137.17 ± 20.38	7	167.14 ± 13.78
	♀	13	127.31 ± 26.42	8	173.00 ± 18.57
	♂♀	25	127.05^B ± 35.35	15	170.27^A ± 16.21

♂ (male); ♀ (female). *Measurements were made 7–10 days postprandial. Different uppercase, superscripted letters in the same row indicate ($P < 0.05$).

The liver was located in the longitudinal plane at the beginning of the second third of the animal, caudal to the cardiac apex. The liver parenchyma showed well defined, echogenicity ranging from hypoechoic in relation at spleen, keeping echotexture homogeneous throughout its length. The vase liver was visualized coursing throughout the medial aspect of the liver in the longitudinal plane. This showed definite shape increase the echogenicity of the walls. In the transverse plane was possible to visualize the portal veins and hepatic located in the dorsal and ventral planes, respectively. Measurements of liver parameters showed significant variations ($P < 0.05$) between groups A and B. However, there was no significant change ($P > 0.05$) between the sexes (Table 2).

The gallbladder was located caudal to liver, at the end of the second third. It was visualized with anechoic bladder inside it, and hyperechoic wall and defined contour. The dimensions of gallbladder varied according to the state food of snakes. There were no significant differences when variables were compared between sexes. For comparisons between groups, there were significant differences between all parameters (Table 2).

The spleen showed variable location, caudal or ventral in relation to the gallbladder, the cranial and female gonads. The positioning varied with gallbladder fullness and with follicles development in females. Furthermore, the organ shape defined hyperechoic echogenicity capsule, triangular shape, isoechoic in relation to the renal parenchyma and homogeneous echotexture. Variables spleen showed significant differences ($P < 0.05$) between groups A and B. However, no significant differences were found between sexes (Table 3).

The kidneys were located caudal to female gonads and cranial to vent at the end of the middle third of the coelomic cavity. It was difficult to visualize male gonads. The location of the kidneys in males was due to the identification of the ultrasound shape, performing scans from the caudal to the gallbladder until the region cranial to the cloaca. Approximately in 60% (34/57) of snakes it was difficult to visualize the shape, homogeneous echotexture, the echogenicity was isoechoic in relation to the spleen, and more echogenic and granulated than intra-abdominal adipose bodies. In 40% (23/57) the right kidney showed better visualization compared to the left. Distinction among renal pelvis, medulla and cortex of the red-tailed boas could not be observed.

Table 2. Biometric data (mean ± standard deviation [SD]) of liver parameters of red-tailed boa (*Boa constrictor*) grouped according to body mass.

Parameters*	Sex	N	Group A (0.8 – 2.8 kg)	N	Group B (3 - 5.4 kg)
Height of right lobe (cm)	♂	15	0.78 ± 0.13	12	0.90 ± 0.22
	♀	15	0.80 ± 0.21	15	1.07 ± 0.21
	♂♀	30	0.79^{B} ± 0.17	27	1.00^{A} ± 0.23
Height of left lobe (cm)	♂	15	0.78 ± 0.12	12	0.96 ± 0.23
	♀	15	0.84 ± 0.23	15	1.23 ± 0.32
	♂♀	30	0.81^{B} ± 0.18	27	1.11^{A} ± 0.31
Thickness of hepatic vein (cm)	♂	15	0.31 ± 0.07	12	0.37 ± 0.07
	♀	15	0.31 ± 0.08	15	0.38 ± 0.09
	♂♀	30	0.31^{B} ± 0.07	27	0.38^{A} ± 0.08
Hepatic transversal diameter (cm)	♂	15	1.46 ± 0.34	12	1.90 ± 0.56
	♀	15	1.45 ± 0.42	15	2.05 ± 0.56
	♂♀	30	1.46^{B} ± 0.37	27	1.99^{A} ± 0.55
Gallbladder length (cm)	♂	15	3.77 ± 1.23	12	4.42 ± 1.05
	♀	15	2.37 ± 0.99	15	4.40 ± 0.88
	♂♀	30	3.07^{B} ± 1.31	27	4.41^{A} ± 0.94
Gallbladder height (cm)	♂	15	1.37 ± 0.33	12	1.71 ± 0.30
	♀	15	1.10 ± 0.36	15	1.59 ± 0.49
	♂♀	30	1.23^{B} ± 0.37	27	1.64^{A} ± 0.42
Gallbladder width (cm)	♂	15	1.60 ± 0.40	12	1.96 ± 0.31
	♀	15	1.43 ± 0.47	15	1.94 ± 0.29
	♂♀	30	1.51^{B} ± 0.44	27	1.95^{A} ± 0.29
Gallbladder volume (cm^3)	♂	15	4.94 ± 3.18	12	7.98 ± 3.32
	♀	15	2.25 ± 1.49	15	7.49 ± 3.60
	♂♀	30	3.60^{B} ± 2.80	27	7.70^{A} ± 3.42

♂ (male); ♀ (female). * Measurements were made 7–10 days postprandial. Different uppercase, superscripted letters in the same row indicate ($P < 0.05$).

Table 3. Biometric data (mean ± standard deviation [SD]) of spleen parameters of red-tailed boa (*Boa constrictor*) grouped according to body mass.

Parameters*	Sex	N	Group A (0.8 - 2.8 kg)	N	Group B (3 - 5.4 kg)
Length (cm)	♂	15	1.49 ± 0.33	12	1.82 ± 0.39
	♀	15	1.45 ± 0.35	15	1.93 ± 0.30
	♂♀	30	1.47^{B} ± 0.34	27	1.89^{A} ± 0.34
Height (cm)	♂	15	0.94 ± 0.20	12	1.17 ± 0.23
	♀	15	0.92 ± 0.21	15	1.25 ± 0.13
	♂♀	30	0.93^{B} ± 0.20	27	1.22^{A} ± 0.18
Width (cm)	♂	15	1.28 ± 0.26	12	1.39 ± 0.26
	♀	15	1.18 ± 0.28	15	1.40 ± 0.20
	♂♀	30	1.23^{B} ± 0.27	27	1.40^{A} ± 0.22
Volume (cm^3)	♂	15	0.98 ± 0.46	12	1.61 ± 0.77
	♀	15	0.89 ± 0.52	15	1.81 ± 0.51
	♂♀	30	0.94^{B} ± 0.48	27	1.72^{A} ± 0.63

♂ (male); ♀ (female). * Measurements were made 7–10 days postprandial. Different uppercase, superscripted letters in the same row indicate ($P < 0.05$).

Renal parameters were similar in significant differences ($P < 0.05$) between groups A and B. However, significant differences ($P < 0.05$) between the right and left kidneys in renal height parameter, while the width was not significant between the left and right kidney. No significant difference between sex and side (right and left) in all parameters (Table 4). Significant differences were observed when compared renal variables and groups.

Table 4. Biometric data (mean ± standard deviation [SD]) of kidneys parameters of red-tailed boa (*Boa constrictor*) grouped according to body mass.

	Right kidney - height (cm)				Right kidney - width (cm)			
	♂	♀	♂♀	Total	♂	♀	♂♀	Total
Group A	1.11 ± 0.21 (n = 15)	1.02 ± 0.15 (n = 15)	1.06^{B} ± 0.18 (n = 30)	1.14^{a} ± 0.21 (N = 57)	0.90 ± 0.21 (n = 15)	0.84 ± 0.27 (n = 15)	$O.87^{B}$ ± 0.24 (n = 30)	0.97^{a} ± 0.29 (N = 57)
Group B	1.17 ± 0.15 (n = 12)	1.26 ± 0.24 (n = 15)	1.22^{A} ± 0.21 (n = 27)		1.05 ± 0.33 (n = 12)	1.09 ± 0.29 (n = 15)	1.08^{A} ± 0.30 (n = 27)	
	Left kidney - height (cm)				Left kidney - width (cm)			
	♂	♀	♂♀	Total	♂	♀	♂♀	Total
Group A	1.01 ± 0.16 (n = 15)	0.94 ± 0.12 (n = 15)	0.98^{B} ± 0.14 (n = 30)	1.04^{b} ± 0.19 (N = 57)	0.92 ± 0.25 (n = 15)	0.77 ± 0.23 (n = 15)	0.84^{B} ± 0.25 (n = 30)	0.91^{a} ± 0.30 (N = 57)
Group B	1.12 ± 0.29 (n = 12)	1.09 ± 0.16 (n = 15)	1.11^{A} ± 0.22 (n = 27)		0.90 ± 0.35 (n = 12)	1.04 ± 0.31 (n = 15)	0.98^{A} ± 0.33 (n = 27)	

♂ (male); ♀ (female). Means with different letters represent $p < 0.05$. Capital letters indicate comparison across groups. Lowercase letters indicate comparison across right and left kidneys.

Table 5 shows the linear regression equations between body length-body mass with all significant parameters of red-tailed boa.

Table 5. Linear regression equations between body length-body mass with all significant parameters of red-tailed boa (*Boa constrictor*).

Parameters (x) in relation to body length (y)	N	Equations	R^2	P
Body length (cm)	57	$y = 0.0314^{**}x-2.6096^{**}$	0.7547	< 0.01
Snout-vent length (cm)	40	$y = 0.0353^{**}x-2.6778^{**}$	0.7485	< 0.01
Gallbladder volume (cm^3)	57	$y = 0.2251^{**}x+1.3405^{**}$	0.5376	< 0.01
Spleen volume (cm^3)	57	$y = 1.5388^{**}x+0.8344^{**}$	0.5353	< 0.01
Parameters (x) in relation to body mass (y)	**N**	**Equations**	**R^2**	**P**
Body mass (Kg)	57	$y = 24.0689^{**}x+102.6786^{**}$	0.7547	< 0.01
Gallbladder length (cm)	57	$y = 16.8858^{**}x+105.1972^{**}$	0.5317	< 0.01
Gallbladder height (cm)	57	$y = 46.4535^{**}x+96.7602^{**}$	0.5051	< 0.01
Gallbladder volume (cm^3)	57	$y = 6.4210^{**}x+129.8384^{**}$	0.5697	< 0.01

These equations have been based on linear model ($y = ax + b$), where ''y'' represents the dependent variable and ''x'' is the independent variable. The letters ''a'' (regression coefficient) and ''b'' (intercept) represent the coefficients estimated by the least squares method. R^2 (determination coefficient); **, $P < 0.01$; ns, not significant.

DISCUSSION

In spite of the existence of some literature on ultrasound in reptiles, there is little information about of coelomic organs evaluation in snakes. Given the clinical relevance related to the use of this technique in wild animals, this study was important to provide data for evaluation of the coelomic organs in red-tailed boa by ultrasonography. In addition, previous studies attempted to standardize echocardiograph techniques in snakes and to describe the localization of cardiac structures in the anatomic planes (Conceição et al., 2014). However, there are few information on the measures, evaluation, and imaging findings of the other organs of the coelomic cavity in snakes. Thus, the present study provides information and unreleased from that point of view.

Although male and female snakes are born with the same size, growth rates and age of sexual maturation may be different, determining sexual dimorphism in the later stages of life (Matias et al., 2011). However, in our study only the snout-vent length variable was different between sexes, this can be explained by males accommodate, in this space, the hemipenes and muscles retractors

(Pizzatto et al., 2007). Similar results were found in studies performed with biometrics of *Bothrops alternatus* (Mesquita & Brites, 2003). The significant differences found between groups and the body parameters occurred due to the increase of the mass between the groups.

The results related liver ultrasound evaluation were similar to those found by other authors (Isaza et al., 1993[ab]; Neto et al., 2009; Banzato et al, 2012[ab]). Some authors reported that for better visualization of the liver, the dorsolateral positioning the transducer would be more appropriate (Banzato et al., 2012[a]), contradicting the results of this study, which positioned the transducer in the ventrolateral plan. The sonographic features of gallbladder in animals in this study were similar to those previously described (Isaza et al., 1993[ab]; Stetter, 2006; Banzato et al, 2012[ab]). However, some authors reported that was not possible visualize the gallbladder in the ventrolateral scan (Neto et al., 2009).

The spleen may be connected with the pancreas, with a defined shape and slightly hyperechoic echogenicity relative to adjacent structures (O'Malley, 2005; Jacobson, 2007). Some authors reported that spleen visualization was difficult by stomach gases or small size of the organ, and reported greater ease of visualization using cross sections (Banzato et al. 2012a). Similar results were found in the present study.

According to Schildger et al. (1994), there is no distinction among renal pelvis, medulla, and cortex of snakes as observed in mammals. Similar description was observed at the present study. Right kidney was significantly larger than the left in *Crotalus durissus, Bothrops neuwiedi,* and *B. moojeni* (Silva, 2008). These results may explain the differences found for the variables height, analyzed for each kidney. The topographical location of the kidneys was similar to that previously described (Augusto, 2001; Stetter, 2006; Jacobson, 2007; Neto et al., 2009; Banzato et al., 2012[a]).

CONCLUSION

In conclusion, sex did not influence the biometric parameters evaluated. However, body length and body mass influenced some organs of the coelomic cavity. Ultrasonography is a useful diagnostic tool for the assessment of the snake in red-tailed boas. In addition, data reported in this study are important as a basis for ultrasound examination for other snake species.

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