Ultrasonographic characterization of the organs in the middle third and caudal coelomic cavity of snakes in the Boidae family

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ABSTRACT.- Martins N.B., Ferreira L.A.R., Pinto N.N.R., Mendonça C.S., Hirano L.Q.L., Santos A. & Milken V.M.F 2021. Ultrasonographic characterization of the organs in the middle third and caudal coelomic cavity of snakes in the Boidae family. Pesquisa Veterinária Brasileira 41:e06716, 2021. Laboratório de Ensino e Pesquisa em Animais Silvestres, Universidade Federal de Uberlândia, Rua Piauí s/n, Bairro Umuarama, Uberlândia, MG 38400-902, Brazil. E-mail: nathanabmartins@gmail.com

The aim of this study was the ultrasonographic evaluation of the organs in the middle third and caudal coelomic cavity of healthy snakes in the Boidae family. For such, 15 adult snakes from five species were evaluated: Boa constrictor amarali, Boa constrictor constrictor, Eunectes murinus, Epicrates assisi and Epicrates crassus. The animals were physically restrained for examination. After the application of acoustic gel over the scales the transducer was positioned in the frontal plane of the coelomic cavity. Microconvex and linear transducers at 10MHz were used. The ultrasound examination was conducted in the craniocaudal direction, with longitudinal and transversal sections to assess syntropy, echogenicity and echotexture of the organs. The liver was bilobulated, hyperechoic, homogeneous echotexture, hyperechoic capsule, with a hepatic lobe measuring 1.15±0.64 cm wide, and the presence of a central hepatic vein, measuring 0.44±0.21 cm in diameter. The gallbladder was characterized as an anechoic structure with echogenic walls and dimensions of 2.91±1.18 cm x 1.38±0.84 cm (length x width). The stomach showed a pleated and linear texture, echogenic, with hypoechoic walls measuring 0.3±0.07 cm wide. The splenopancreas was a circular and echogenic structure, homogeneous echotexture and measuring 1.18±0.62 cm in width and 1.56±0.88 cm in length. The kidneys were characterized by elongated, lobulated, hypoechoic structures, an echogenic capsule and an echogenic central line, measuring 1.05±0.7 cm in width. The testis were characterized by fusiform, hypoechoic structures, homogeneous echotexture, measuring 2.31±0.79 cm in length and 0.6±0.23 cm in width. Ovarian follicles were seen in different stages, 0.67±0.39 cm wide and 0.73±0.38 cm long. No differences were found between species of syntropy, echogenicity and echotexture of the organs of the coelomic cavity. The ultrasound examination proved to be a safe, non-invasive and efficient technique for characterizing the organs of the coelomic cavity of snakes.

INDEX TERMS: Ultrasonography, coelomic cavity, snakes, Boidae family, Boa constrictor, Epicrates spp., Eunectes murinus, diagnostic imaging, Squamata, wildlife animals.

RESUMO.- [Caracterização ultrassonográfica dos órgãos do terço médio e caudal da cavidade celomática de serpentes da família Boidae.] O objetivo do estudo foi realizar a avaliação ultrassonográfica de órgãos do terço médio e caudal da cavidade celomática de serpentes híbridas da família Boidae. Para isto, foram avaliadas 15 serpentes adultas de cinco espécies: Boa constrictor amarali, Boa constrictor constrictor, Eunectes murinus, Epicrates assisi e Epicrates crassus. Os animais foram contidos fisicamente para a realização do exame. Após...
aplicação de gel acústico sobre as escamas, o transdutor foi posicionado no plano frontal da cavidade celômática. Foram utilizados transdutores microconvexo e linear em 10MHz. O exame ultrassonográfico foi conduzido no sentido craniocaudal, com cortes longitudinais e transversais para avaliar sintopia, ecogenicidade e ecotextura dos órgãos. O fígado se apresentou bilobulado, hiperecoico, ecotextura homogênea, cápsula hiperecoica, com lobo hepático medindo 1,15±0,64 cm de largura, e presença de uma veia central hepática, medindo 0,44±0,21 cm de diâmetro. A vesícula biliar foi caracterizada como uma estrutura anechoica de paredes ecogênicas e dimensões de 2,91±1,18 cm x 1,38±0,84 cm (comprimento x largura). O estômago apresentou-se com o aspecto pregueado e linear, ecogênico, com paredes hiperecoicas medindo 0,3±0,07 cm de largura. O esplenopâncreas se apresentou como uma estrutura circular e ecogênica, ecotextura homogênea e medindo de 1,18±0,62 cm de largura e 1,56±0,88 cm de comprimento. Os rins se caracterizaram por estruturas alongadas, lobuladas, hiperecoicas, cápsula ecogênica e uma linha central ecogênica, medindo 1,05±0,7 cm de largura. Os testículos foram caracterizados por estruturas fusiformes, hiperecoicas, ecotextura homogênea, medindo 2,31±0,79 cm de comprimento e 0,6±0,23 cm de largura. Foram visualizados folículos ovarianos em diferentes estágios, de 0,67±0,39 cm de largura e 0,73±0,38 cm de comprimento. Não foram encontradas diferenças entre espécies de sintonia, ecogenicidade e ecotextura dos órgãos da cavidade celômática. O exame ultrassonográfico demonstrou-se uma técnica segura, não invasiva e eficiente para caracterização dos órgãos da cavidade celômática de serpentes.

TERMOS DE INDEXAÇÃO: Ultrassonografia, cavidade celômática, serpentes, Boidae, bódeos, celoma, diagnóstico por imagem, Óvidos, animais selvagens.

INTRODUCTION

With the growing demand to assist wild and exotic animals in veterinary medicine, the anatomical knowledge is extremely important to elaborate the diagnosis and for the success in choosing a therapeutic protocol (Bortolini et al. 2013). Ultrasound, as well as radiography, are noninvasive diagnostic imaging techniques largely used in the veterinary practice (Muzzi et al. 2001). The success of the examination will depend on variables such as anatomical knowledge of the species and the ability and experience of the operator (Oliveira et al. 2013).

The diagnostic imaging, although largely used, is still a challenge in reptile medicine due to the scarcity of normality parameters, mainly when dealing with animals of the Brazilian fauna (Bortolini et al. 2012, Augusto & Hildebrandt 2014). Thus, anatomical studies are necessary in healthy animals to characterize and standardize the normality references of the organs (Bortolini et al. 2012).

Several snake species in the Boidae family are raised in captivity by zoos, breeders, collectors and tutors (Banzato et al. 2012). However, there is little literature in studies that evaluate the coelomic cavity of snakes in this family through ultrasound. Researches were reported on *Boa constrictor* to evaluate the liver, spleen and urogenital tract (Andrade et al. 2012), on *Eunectes murinus*, *Boa constrictor constrictor*, *Corallus hortulanus* and *Epicrates cenchrus* evaluating the reproductive organ (Garcia et al. 2015) and on *Boa constrictor imperator* evaluating the organs in the coelomic cavity (Banzato et al. 2012). No studies were found in the species *Boa constrictor amarali*, *Epicrates assisi* and *Epicrates crassus*, making this work the first of its kind.

Therefore, the aim of the present study was to characterize the organs in the middle third and caudal coelomic cavity of healthy snakes in the Boidae Family through ultrasound, such as *B. constrictor amarali*, *B. constrictor constrictor*, *E. murinus*, *E. assisi* and *E. crassus*.

MATERIALS AND METHODS

The research was authorized under protocol 037/2018 from the Committee for the Ethics in Animal Use (CEUA) at the “Universidade Federal de Uberlândia” (UFU), and Biodiversity Authorization and Information System (SISBIO) under protocol 62659. The study was done in the months of August and September 2019.

Fifteen adult animals from the Boidae family were used, coming from the “Laboratório de Ensino e Pesquisa em Animais Silvestres” (LAPAS-UFU), of these, one *Boa constrictor constrictor*, one *Eunectes murinus*, three *Epicrates assisi*, three *Epicrates crassus* and seven *Boa constrictor amarali*. Upon physical examination all animals were healthy.

The animals were identified, weighed and the cloacal rostrum length was measured with a 1.5m measuring tape. The identification was done with numbered tags in plastic individual organization boxes, where the animals stayed during the study. The weight was measured with a digital scale.

Sexing was done through the introduction of a lubricated edge probe in the cloaca, in the caudal direction, and confirmed through ultrasound examination. Animals in which the probe passed 8 to 12 sub-caudal scales were considered male, and when the probe passed only two to four sub-caudal scales the specimen was considered female. There was a total of six females and nine males. The corporeal mass and rostrum-cloacial length of each species are described in Table 1.

The snakes were kept under fasting for seven days previous to the ultrasound examination. The animals were physically restrained with a herpetological hook and then manually contained for approximately ten minutes for ultrasonographic evaluation. They stayed in an anatomical position and a layer of water-soluble gel was applied on the windows to allow the transmission of ultrasonic waves in the transducer contact with the scales. The acoustic window was obtained through the frontal plane and the scan was done in the craniocaudal direction through a GE Logiq F6® device, a real time bidimensional equipment. The frequency choice (5-10 MHz) and shape of the transducer (microconvex or linear) varied according to the size of the animals.

The coelomic cavity was divided in three thirds and the scan was done in the craniocaudal direction of the middle and caudal third. The beginning of the evaluation was marked by a cardiac and large vessel image, which serves as anatomical reference for the cranial limit of the liver. All structures were evaluated in sagittal and transversal planes.

Morphology, syntropy, echogenicity and ecotexture of the liver, gallbladder, stomach, intestinal loops, kidneys, testis and ovarian follicles were evaluated and measured.

The ultrasonographic exam and measurements of the organs were done by a single operator. The images obtained through ultrasound were evaluated for reference description and comparison among species in the study.

A simple descriptive statistical analysis, with average and standard deviation of organ dimensions was done in Excel.
RESULTS

There were no differences among species regarding syntropy, morphology, echogenicity and echotexture of the evaluated organs. However, for measurement of the organs in the coelomic cavity, the animals were separated in species due to variations in corporal mass. The dimension of the organs is represented in Table 2.

The liver was located and evaluated in the sagittal plane, right after the end of the cranial third and beginning of the middle third of the animal, in a caudal position to the heart apex. The organ was bilobulated, with echogenicity varying from hypoechoic in relation to the splenopancreas and slightly hyperechoic in relation to the kidney, with homogeneous echotexture, surrounded by a hyperechoic capsule and with the presence of a tubular structure, with hyperechoic walls, and anechogenic content, characterizing the hepatic vein (Fig.1).

The gallbladder (GB) was evaluated in the longitudinal and transverse planes, was not directly connected to the liver and was located on the right side, caudal to the stomach and cranial to the spleen and pancreas, at the end of the animal’s middle third. It presented itself as a structure of anechogenic content surrounded by a hyperechoic wall (Fig.1). It was not possible to see the bile duct.

The stomach was located dorsal to the liver and cranial to the GB, halfway through middle third of the animal. It was elongated, pleated, linear, echogenic with the hyperechoic walls and heterogeneous echotexture, in the longitudinal and transverse planes (Fig.1). In some animals, the organ was found without content and in others with food content and gases.

The intestine was located caudal to the stomach, in the middle and caudal third of the coelomic cavity. The organ was evaluated in the longitudinal and transversal planes, but it was not possible to delimit the organ contours in most animals, due to the presence of gases, faeces or food content in its lumen, therefore, the organ was not measured. It was seen only in one specimen of Epicrates crassus, in the specimen of Eunectes murinus and in a Boa constrictor amarali snake.

The spleen and pancreas were visualized as a single structure, called splenopancreas in all species, located caudally to the GB, in the end of the middle third of the animal. The spleen was characterized by a small, circular structure, hyperechoic with respect to the liver, homogeneous echotexture and surrounded by a hyperechoic capsule. The pancreas was slightly hypoechogenic in relation to the spleen with homogeneous echotexture (Fig.2). It was not possible to see it in all animals.

The kidneys were seen in the caudal third of the coelomic cavity in the longitudinal plane, with the right kidney located more cranial than the left. They were characterized by an elongated, lobulated structure, hypoechoic in relation to the liver and splenopancreas, heterogeneous echotexture, surrounded by a hyperechoic capsule, with the central region of minor echogenicity and an echogenic central line. The cortical

Table 1. Body mass, in kilograms (kg), and rostro-cloacal length (CRC) in centimeters (cm), of snakes of the Boidae family from the “Laboratório de Ensino e Pesquisa em Animais Silvestres” of the “Universidade Federal de Uberlândia”

| Species                  | Weight (kg) | CRC (cm) |
|-------------------------|-------------|----------|
| Boa constrictor constrictor | 1.922       | 140      |
| Boa constrictor amarali | 1.058 2.046 | 102 153  |
| Epicrates assisi        | 1.900       | 150      |
| Epicrates crassus       | 0.600 0.650 | 102 104  |
| Eunectes murinus        | 12          | 245      |

Table 2. Measurement, in centimeters, of the organs of the middle and caudal thirds of the coelomic cavity of snakes of the Boidae family. The minimum (Min) and maximum (Max) measurements were obtained for each species, and the mean (M) and standard deviation (SD) were obtained for all species in general

| Species                  | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Mean | SD     |
|-------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|------|
| Epicrates assisi (n=3)  | 0.57| 1.61| 0.39| 0.84| 0.64| 1.81| 1.23| 2.16|     |     | 1.15±0.64|      |
| Epicrates crassus (n=3) | 0.39| 0.83| 0.26| 0.30| 0.26| 0.71| 0.42| 0.36|     |     | 0.44±0.21|      |
| Boa constrictor amarali (n=7) | 2.6 | 4.53| 1.58| 2.17| 1.36| 3.9 | 4.11| 3.06| 2.91±1.18|      |
| Boa constrictor constrictor (n=1) | 0.92| 1.22| 0.5 | 0.74| 0.95| 2.98| 1.4 | 2.41| 1.38±0.84|      |
| Eunectes murinus (n=1) | 0.88| 1.27|     |     | 0.62| 0.92| 2.21|     | 1.18±0.62|      |

(-) not shown, GBW = gallbladder (width), GBL = gallbladder (length). HL = hepatic lobe (width), HV = hepatic vein (width), KW = kidney (width), OL = ovarian follicles (length), OW = ovarian follicles (width), SW = stomach wall (width), SPL = splenopancreas (length), SPW = splenopancreas (width), TL = testicle (length), TW = testicle (width).
region is hyperechogenic with respect to the medulla (Fig. 2). Adrenal glands and ureters were not seen in any animal.

The genital organs were located caudally to the GB and cranially to the kidneys, in the caudal third of the coelomic cavity. In males, the testis were viewed in the longitudinal and transverse plane and characterized as fusiform structures, with regular contours, homogeneous and hyperechoic parenchyma in relation to all structures (Fig. 3), however it was not possible to identify them in all animals in the study. No epididymis was observed in any of the animals.

Ovarian follicles were visualized in different stages, in the longitudinal and transversal planes. They were characterized as ovoid structures, with anechoic and hypoechogenic content, with homogeneous echotexture, thin, regular and hyperechoic walls (Fig. 3). The pre-vitellogenic follicles were smaller, spherical and anechoic and were seen only in the species *E. crassus*. Vitellogenic follicles, however, were larger and hyperechoic and were seen in the species *B. constrictor constrictor* and *B. constrictor amarali*. However, it was not possible to visualize the follicles in all females in the study.

**DISCUSSION**

For the ultrasonographic exam a layer of water-soluble gel was applied to the scales of all animals, as well as to the transducer in order to minimize the occurrence of artifacts. Artifacts in the study occurred due to gases in the gastrointestinal tract, making visualization and characterization difficult. The main artifacts in the ultrasonographic exam of snakes are the acoustic barrier in the keratin on the scales, the presence of ribs in almost the entire corporal extension, air in the lungs and air sacs, gases

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Fig. 1. Ultrasonographic images of organs in the coelomic cavity of *Eunectes murinus*. (A) Bilobulated liver, slightly hyperechoic, homogeneous echotexture, with hyperechoic capsule and hepatic vein with hyperechoic walls, with anechogenic content (blue arrow). (B) Gallbladder (GB) with anechoic content and thin echogenic wall (green arrow). (C) Stomach represented by the yellow arrow, located dorsal to the liver and hepatic vein (blue arrow) and cranial to GB (green arrow). (D) Stomach with pleated and linear aspect, echogenic, heterogeneous echotexture, with hypoechogenic walls (yellow arrow). Source: Diagnostic Imaging Sector at the Veterinary Hospital of the “Universidade Federal de Uberlândia”.
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Fig. 2. Ultrasonographic image of coelomic organs from *Boa constrictor amarali*. (A) Spleen is characterized by a circular, small, echogenic structure with homogeneous echotexture (red arrow), while the pancreas is slightly hypoechoic in relation to the spleen with homogeneous echotexture (yellow arrow). (B) Kidney presenting itself as an elongated, lobulated, hypoechoic structure, heterogeneous echotexture, echogenic capsule and an echogenic central line (purple arrow). Source: Diagnostic Imaging Sector at the Veterinary Hospital of the “Universidade Federal de Uberlândia”.

Fig. 3. Ultrasound images of genital organs of snakes of the Boidae family. (A) Testicle of *Epicrates assisi*, with fusiform shape, hypoechoic aspect and homogeneous echotexture (red arrow). (B) Pre-vitellogenic ovarian follicles (anechogenic aspect) of the species *Epicrates crassus* (yellow arrow). (C) Vitellogenic ovarian follicles (hypoechoogenic aspect) of the *Boa constrictor constrictor* species (green arrow). (D) Vitellogenic follicles (green arrow) and pre-vitellogenic follicle (yellow arrow) in a specimen of the species *B. constrictor constrictor*. Source: Diagnostic Imaging Sector at the Veterinary Hospital of the "Universidade Federal de Uberlândia".
The gallbladder was visible in all animals and proved to be of 5.74±1.34cm, values similar to those found in the present study (Aguisanda et al. 2011). The stomach was identified in all animals in the present study, empty or filled with food or gas content, in the region dorsal to the liver and cranial to GB. The gastric wall was 0.3±0.07cm wide. The stomach can be easily identified in snakes, however, the presence of luminal or adjacent gas makes it difficult to characterize the organ (Isaza et al. 1993). The organ was partially visualized in some snakes, starting from the final third of the liver, with hypoechoic walls (Matayoshi et al. 2012).

In the present study, it was not possible to evaluate the stratification of the gastrointestinal tract. The five layers of the mucosa of the digestive tract, classically described in mammals, are visible only in large reptiles (Wilkinson et al. 2004, Silverman 2013, Stetter 2013). In one study, the gastric and pyloric walls had two layers, a thin outer echo layer and an inner anechoic layer. Gastric folds were evidenced in longitudinal and transverse scans, as well as the absence of cardia (Banzato et al. 2012).

The intestine was only visualized in three animals, located caudal to the stomach, in the middle and caudal third of the coelomic cavity and it was not possible to define the contours of the organ in most animals. It corroborates the literature, which mentions that the ultrasound visualization of the intestinal loops is extremely difficult due to the presence of gases, feces or food content in its lumen (Redrobe & Wilkinson 2002, Raiti 2004). The small intestine can be mistaken for large vessels. It is necessary to use Doppler for differentiation, however, in mode B it was possible to visualize intestinal motility (Purduoi et al. 2016).

In the present study, it was not possible to view the splenopancreas in all animals. According to the literature, the spleen and pancreas are difficult to locate and visible on ultrasound examination, in most species of reptiles (Schildger et al. 1994). The identification and positioning of the splenopancreas depend on variables such as the degree of gallbladder repletion and the development of ovarian follicles (Andrade et al. 2012). The python spleen had an average diameter of 3.33±0.39cm, higher than those found in the present study, possibly due to the difference between the species studied, size and body mass (Aguisanda et al. 2011).

Stetter (2013) reported difficulty in identifying reptile kidneys by ultrasound examination. In the present study, the organ was easily seen in the larger species, however, it was more difficult to identify in the smaller species (Epicrates crassus), and it was not possible to view it in all animals. In snakes, the kidneys are usually located in the caudal third of coelomic cavity, the right being more cranial than the left. The kidneys can be seen in association with fatty bodies of snakes, which was also observed in larger species (Andrade et al. 2012).

The results obtained in the present study regarding ovarian follicles corroborate the literature. The pre-vitellogenic follicles were smaller, spherical and anechoic, measuring 0.4x0.36cm and 0.37x0.35cm in diameter. Vitellogenic follicles, on the other hand, were larger and hyperechoic, with 1.18x1.11cm and 0.37x0.35cm in diameter. Vitellogenic follicles, on the other hand, were larger and hyperechoic, with 1.18x1.11cm and 0.37x0.35cm in diameter. In females, regardless of the season, ovarian follicles are easily identified, which did not occur in the present study, performed outside the breeding season (Stetter 2013).
According to Garcia et al. (2015), the pre-vitellogenic follicles can be recognized as small grouped spheres, in the form of a ‘grape bunch’, anechogenic or hypoechogenic, and homogeneous, measuring 0.40x2.50cm, values above that found in the present study. The authors described the vitellogenic follicles as larger and hyperechogenic following each other, in the form of a ‘pearl necklace’, with a dimension of 1.60x5.70cm, being greater values than those found in this study.

The present study was not carried out in breeding season, which may have made it difficult to visualize the testis and resulted in greater variations in the dimensions of the organs. The mating season of boidae is different between species, due to the difference in mass and body size between them. However, in general, females of boidae show vitellogenesis concentrated in autumn-winter (Prado, 2006).

According to Garcia et al. (2015), in the reproductive season, the testis are symmetrical, have regular and defined contours, homogeneous parenchyma, measuring 6.20cm in length in E. murinus, 8.70cm in Boa constrictor constrictor and 6.10cm in Epicrates cenchria, values above those found in the males in the study. In the non-reproductive period, the organs had smaller dimensions or were not seen. According to Stetter (2013), during the non-reproductive season, the testis usually regress. At the beginning of the reproductive season, the gonads increase in size, becoming more apparent. Some authors have described the presence of an epididymis in snakes (Langlada et al. 1994), but in the study by DeNardo (2006) this structure was not observed, which corroborates the data obtained.

CONCLUSIONS

The ultrasound evaluation of the celomic cavity of snakes can be easily performed and used as a complementary exam in the diagnosis of diseases. The evaluation of healthy snakes of the Boidae family allowed the characterization of organs and obtaining measures to establish normality parameters. The syntopy, echogenicity and ecotexture of the organs did not vary according to the different species, only the measurement values of the organs, due to the difference in mass and body size between them.

Conflict of interest statement.- The authors have no competing interests.

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