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Silva Meireles, Yara; Serra Shinike, Flávia; Matte, Douglas Rodrigo; Oliveira Morgado, Thaís; Vincoletto Kempe, Guilherme; Ramiro Corrêa, Sandra Helena; Lopes de Souza, Roberto; Brandini Néspoli, Pedro

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Ultrasound characterization of the coelomic cavity organs of the red-footed tortoise (*Chelonoidis carbonaria*)

Caracterização ultrassonográfica dos órgãos da cavidade celomática do jabutipiranga (*Chelonoidis carbonaria*)

Yara Silva Meireles[I] Flávia Serra Shinike[I] Douglas Rodrigo Matte[I] Thaís Oliveira Morgado[II] Guilherme Vincoletto Kempe[II] Sandra Helena Ramiro Corrêa[II] Roberto Lopes de Souza[III] Pedro Brandini Néspoli[I]

ABSTRACT

Herein it was describe sonographic morphology and sintopy of the bowels of the coelomic cavity in the red-footed tortoise. Coelomic cavity of 19 males and 19 females were scanned through cervical and prefemoral access with a multifrequency sector transducer. Morphology, syntopy and echogenicity of the heart, thyroid, liver, gallbladder, reproductive organs, stomach, small intestine, large intestine, urinary bladder and kidneys were evaluated. The heart showed two atria and one ventricle with a thick, trabecular wall. The thyroid was oval and hyperecoic, visualized in the cardiac portion of the ultrasound. The liver, gallbladder and digestive system were similar to those seen in mammals and turtles. However, the tortoise liver was relatively more hyperechoic than mammals. The kidneys appeared as triangular structures, which were hypoechoic, homogeneous and vascularized; the bladder was observed mostly as being elongated with anechoic content, and its wall appeared as a thin hyperechoic line when free fluid was present. The testes were observed to be elongated, homogeneous and more hyperechoic than kidneys. The ovarian follicles were seen as hyperechoic, echogenic balls of variable size and quantity, the oviduct as a sigmoid tubular structure and the eggs as thin hyperechoic lines with posterior acoustic shadowing. In some animals, there were variable amounts of fluid around the heart and in the coelomic cavity.

Key words: *chelonians, ultrasound, tortoise, Testudines,* ***Chelonoidis carbonaria***.

RESUMO

Neste trabalho é descrita a morfologia ecográfica e sintopia das vísceras da cavidade celomática do jabutipiranga. Foram examinados 19 machos e 19 fêmeas por meio de acesso cervical e pré-femoral com um transdutor setorial multifrequencial. Foram avaliadas morfologia, sintopia e ecogenicidade do coração, tiróide, fígado, vesícula biliar, órgãos reprodutivos, estômago, intestino delgado, intestino grosso, bexiga urinária e rins. O coração mostrou dois átrios e um ventrículo com uma parede trabecular e espessa. Na base cardíaca pode-se visibilizar a tireóide oval e ecogênica. O fígado, vesícula biliar e sistema digestivo foram semelhantes ao observado em mamíferos e testudinos. No entanto, o fígado dos quelônios mostrou-se mais hiperecóico em relação ao dos mamíferos. Os rins foram vistos como estruturas triangulares, hipoecóicas, homogêneas e vascularizadas; a bexiga foi observada na maior parte como alongada com conteúdo anecóico, e sua parede foi vista como uma fina camada hiperecóica. Os testículos foram observados como estruturas alongadas, homogêneas e hiperecóicas em relação aos rins. Os folículos ovarianos foram vistos como esferas ecogênicas a hiperecogênicas de tamanho e quantidade variável, o oviduto como uma estrutura tubular sigmóide e os ovos como finas linhas hiperecogênicas com sombra acústica posterior. Em alguns animais, havia quantidades variáveis de líquido em volta do coração e na cavidade celomática.

Palavras-chave: *quelônios, ultrassom, Testudinos,* ***Chelonoidis carbonaria***.

INTRODUCTION

Red-footed tortoises are chelonians with terrestrial habits, belonging to the Testudinidae family, being found in tropical areas of South America (LEVINE & SCHAFER, 1992). In Brazil the red-footed tortoise (***Chelonoidis carbonaria***) is likely the most numerous chelonian species kept in captivity as a pet (PINHEIRO & MATIAS, 2004) and currently

[I]Setor de Diagnóstico por Imagem, Hospital Veterinário (HOVET), Universidade Federal de Mato Grosso (UFMT), Cuiabá, MT, Brasil.
[II]Setor de Animais Silvestres, Hospital Veterinário (HOVET), Universidade Federal de Mato Grosso (UFMT), Cuiabá, MT, Brasil.
[III]Setor de Clínica Cirúrgica, Hospital Veterinário (HOVET), Universidade Federal de Mato Grosso (UFMT), Av. Fernando Corrêa da Costa, n 2367, 78060-900, Bairro Boa Esperança, Cuiabá, MT, Brasil. E-mail: lopesdesouza.roberto@gmail.com. Corresponding author.
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represents a large proportion of the wild animals routinely attended by veterinarians. (Supress text) Regarding the ultrasound examination, previously, only directed ultrasonography of the reproductive system has been performed for the red-footed tortoise (TEIXEIRA, 2009) and for other species of turtles (KUCHLING, 1989; ROBECK et al., 1990; ROSTAL et al., 1990; MANIRE et al., 2008; PEASE et al., 2010; BLANCO et al., 2012), as well as general descriptions of turtle anatomy (PENNINCK et al., 1991; VALENTE et al., 2007; REDROBE, 2010). (Supress text) As with its use on other wild and domestic species, ultrasound can be a very useful tool for the diagnosis of many diseases that affect the red-footed tortoise. Among these diseases, we are familiar with hepatic steatosis, which presents increased diffuse echogenicity (DIVERS & COOPER, 2000; NYLAND et al., 2005; DUTRA, 2007; REDROBE, 2010), the deposition of uric acid in the kidneys and heart, which can be visualized as hyperechoic deposits (REDROBE, 2010), reproductive disorders, such as retention of eggs, which can be caused by cancer or granulomatous lesions in the reproductive system (FRYE, 1991), and the intake of foreign bodies, which may also occur in these animals and can be detected by ultrasound (PENNINCK, 2005).

Considering the need to establish a morphological basis for the sonographic interpretation of this species, the present study aims to describe techniques for scanning and identifying the morphology, patterns of echogenicity and syntopy of the organs in the coelomic cavity of the red-footed tortoise (***Chelonoidis carbonaria***).

MATERIALS AND METHODS

In this study, 38 tortoises were used, 19 males and 19 females, weighing between 4 and 10kg, with a carapace length between 30 and 50cm, all without any apparent clinical symptoms. The animals were kept at the Zoological Park. The study was approved by the Ethics Committee on Animal Research (CEPA) 23108010550/13-8. Animals were fasted from solid 12 hours and chemical restraint was performed with ketamine (40mg kg^{-1}) and midazolam (2mg kg^{-1}) by intramuscular injection.

For the sonographic procedures, the animals were kept in the plastral recumbency with limbs free. The coelomic cavity was evaluated from the ventral, right and left cervical acoustic windows and from the right and the left prefemoral windows in longitudinal and transverse sections to the axis of the animal. A layer of water-soluble gel was applied and the test was performed with real-time equipment in the B mode (MyLab Five® /Esaote Pie MedicalTM, Genova, Italy) with a multifrequency transducer electronics sector arrangement in phase and at 4-7.5MHz.

The morphology, syntopy and echogenicity of the bowels were evaluated. The images obtained by ultrasonography were evaluated, and comparisons were made between the individual tortoise's own organs and the organs of similar species and mammals. The heart rate was determined by measuring the number of ventricular contractions during a one-minute period. The measurements for the digestive tract wall and for thyroid and testicular thickness were obtained through the program R 2.15.1.

RESULTS

In the cervical scan windows, the heart was seen in the midline between the liver lobes and presented the ventricle in a "V" shape with a homogeneous, echogenic, thick wall with thin and elongated trabeculae and with two rounded atria during diastole with echogenic walls, thin-walled when compared to the ventricle, with projections from the inner surface of varying sizes. The content of the atria and ventricles can be seen as hypoechoic in turbulence, and there was posterior acoustic enhancement. A hyperechoic thin wall separating the two atria was visualized, and two atrioventricular valves could be seen as being thin, hyperechoic and monocuspid at the end of the same lines where the opening formed the aspect of an inverted "Y" (Figure 1A, B and C). The mean heart rate was approximately 35 ± 6.26 beats per minute (bpm). In the cardiac basis scan, the thyroid ($1.50x1.05\pm0.24x0.22$cm) was visualized in 27 animals and showed an oval form, which was homogeneous and hyperechoic, with well-defined limits (Figure 1D).

On the lateral edge to the midline of the cervical windows, the liver was visualized and presented a granular texture with homogeneous and increased echogenic parenchyma relative to the cardiac content, intestine and kidneys (Figure 1E). The margins of this pattern were seen only when there was fluid content between the lobes and were smooth (Figure 1F). Hepatic vessels were seen as linear, and sometimes as tortuous tubular structures with thin walls that were isoechoic and hyperechoic to the liver; these vessels also showed hypoechoic content with echogenic particles in a unidirectional flow, which were positive to Doppler. Concomitant fluid between lobes of the liver was observed in only 4 animals; these animals also had a higher hepatic echogenicity. At the lateral edge of the right cervical acoustic window, the gallbladder was observed between the hepatic lobes with thin walls that were hyperechoic to isoechoic, with anechoic content and posterior acoustic enhancement. The amount of content was considered to be moderate in most animals. The format was variable according to the filling and cutting. The gallbladder was usually rounded in cross section and slightly elliptical in longitudinal section (Figure 1D).

The evaluation of the gastrointestinal tract was performed by visualization of the walls and the luminal contents, which presented as hyperechoic and sometimes prevented the visualization of deeper structures. The stomach was seen by the left cervical window and exhibited a hypoechoic wall with folds of varying sizes, and sometimes, the identification of an even less echoic layer in the outer wall was possible (Figure 2A). Distance between the folds was 0.23$\pm$0.09cm. The small intestine was observed in all windows tested and showed parietal stratification in four layers. The mucosal layer was thicker and hypoechoic, the muscular presented itself as thin and hypoechoic to anecoic, and the submucosa and serosa were thin and hyperechoic (0.35$\pm$0.08cm) (Figure 2B). In most cases, this stratification was difficult to see. The large intestine was observed through the prefemoral acoustic windows with thin walls, hypoechoic and without evident parietal stratification (0.1$\pm$0.05cm) (Figure 2C).

In scans of the prefemoral acoustic windows, the kidneys were observed in the dorso-caudal direction as compact hypoechoic structures relative to the testicles and liver, with homogeneous parenchyma, medium texture, smooth margins and intense vascularization. The capsule and the pelvis were not visible (Figure 3A). Longitudinal section in relation to the axis of the animal showed triangular and linear tubular structures that contained hypoechoic and echogenic particles in a unidirectional flow that could be seen projecting caudally into the hilus region in some animals. In cross-section, kidneys could be visualized to a larger extent.

During scanning of the prefemoral windows, the bladder was visualized in some animals, and sometimes it was difficult to identify because its format varied according to the degree of distension, and because its wall took the form of some adjacent structures, the bladder could be confused with free anechoic content in the cavity. In animals with mild to moderate repletion, the bladder was seen in the cranioventral projection as piriform and when filled, some had elongated lobes, one in the cranioventral direction and another in the craniodorsal direction. The bladder wall was better visualized when there was free anechoic content, which appeared as a thin hyperechoic line filled with anechoic content (Figure 3B).

Located cranioventrally to the kidneys, the testes were elongated structures that were homogeneous and hyperechoic relative to the renal parenchyma, with well-defined boundaries and sometimes with a thin hyperechoic line on their borders. Most of the time, the testes were elongated on a transverse scan (Figure 3C and D). Only the thickness can be measured and the average was 1.79$\pm$0.44cm for the left and 1.95$\pm$0.4cm to the right. Sometimes the right testicle cannot be visualized due to the intense fullness of the colon. In one animal, the epididymis could be seen between the testis and kidney; this structure was poorly defined and reticulated, with numerous rounded structures with anechoic centers and hyperechoic walls.

The female reproductive tract could be observed through the cervical and prefemoral windows. Visualization of the ovaries was possible by cervical access and through the prefemoral window, where they were located adjacent to the oviduct. The ovarian follicles were observed as spherical structures, hyperechoic relative to the oviduct, homogeneous, with clear limits, in quantity and of different sizes. Echogenicity of the ovarian follicles increased proportional to size, with the largest having acoustic shadowing and increased echogenicity in a gradient. The oviduct, which was located between the follicles and cranial to the kidneys, was presented as a tubular structure with a sigmoid aspect, showing hypoechoic walls with poorly defined boundaries. The content was visualized as echogenic in variable amounts (Figure 3E). Eggs were seen as thin hyperechoic lines with acoustic shadowing. The ones with little calcium deposition were seen with anechoic content on the outer part and hyperechoic content in the innermost part.

There was a variable amount of free anechoic content on the coelomic cavity in 34 animals around the heart (Figure 1C); in 12 animals between the liver lobes (Figure 1F); and in 22 animals around the intestines, testis, oviduct, ovaries and bladder (Figure 3B). In the heart, no hyperechoic line could demonstrate that the source of liquid was inside or outside the pericardium, even when it was observed concomitantly with fluid between the liver lobes. In most animals in which liquid was observed between the lobes, liquid was also observed around the caudal organs.

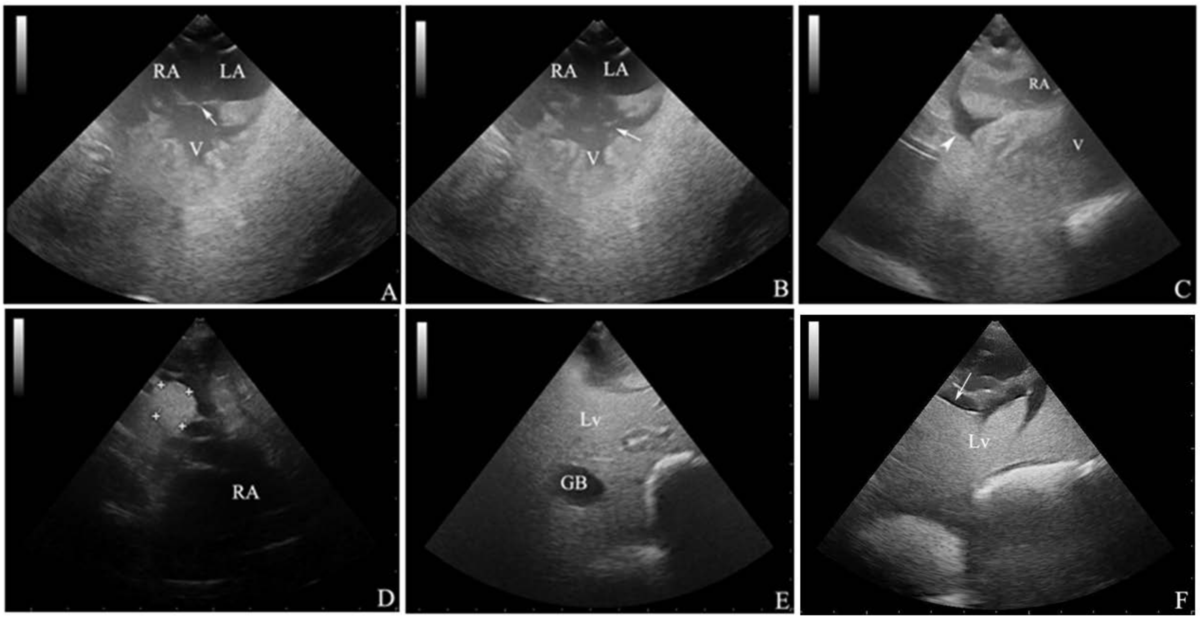

Figure 1 - Ultrasonographic image of the heart, thyroid and liver tortoise viewed through the right and left cervical windows. A) Atrium and ventricles filled with hypoechoic content. The ventricle shows trabecular wall and the atrioventricular valves are closed (arrow). B) Atrioventricular valves open (arrow). C) The atrium shows projections of the inner surface. It is possible visualize fluid around the heart (arrow head). D) Thyroid hyperechoic oval in shape with well-defined limits. E) Liver with hyperechoic granular texture and rounded gallbladder with anechoic content and posterior acoustic enhancement. F) Free fluid anechoic (arrow) among hepatic lobes showing tapered edges and regular margins. V, Ventricle, RA, Right atrium; LA, left atrium; Lv, Liver, GB, Gallbladder.

DISCUSSION

Despite the limitations related to the carapace, scan of the coelomic cavity was satisfactory in all animals examined, with an evaluation of the various viscera possible through the cervical and prefemoral windows. Unlike other descriptions from the literature (PENNINCK et al., 1991; SILVERMAN, 2006; VALENTE et al., 2007; REDROBE, 2010), the pre-axillary and femoral windows were not used here because a low-resolution image was produced, most likely due to the thick skin in these regions. Anesthesia was required for full extension of the limbs (SCHUMACHER & YELEN, 2006; REDROBE, 2010), resulted in better image quality and reduced the animal stress.

The sector transducer settings allowed a large area of the cavity to be scanned from small acoustic windows and restricted access to the transducer. Frequencies used were appropriate for the exam, but the high and medium (7.5 and 6.6 MHz) were more appropriate for assessing most of the structures. Due to poor image resolution, the lower frequency of 4 MHz was used less often, especially for the visualization of deeper structures.

The cardiac ventricle showed a thick, trabecular wall, as observed in California desert tortoises (***Xerobates agassizi***), in sea turtles (***Caretta caretta***) and in red-eared sliders (***Trachemys scripta elegans***) (PENNINCK, 1991; VALENTE et al., 2007; POSER et al, 2011). The location of the ventricle between the hepatic lobes and the thin-walled atrium was also seen in other studies (PENNINCK et al., 1991; VALENTE et al., 2008). However, in this study, two atria, with projections on the inner surface, were observed. In some studies, atrioventricular valves were also observed as mobile hyperechoic lines (PENNINCK et al., 1991; POSER et al., 2011). Nevertheless, another study reported the observation of only one atrioventricular valve, which appeared as a short, horizontal, echogenic line located in the center between the atrium and ventricle (VALENTE et al., 2007). The average heart rate was similar to that described in other studies (FONTENELLE et al., 2000; VALENTE et al., 2008). Despite the reptile heart have no union with pericardium and be anchored by gubernaculum cords ligament in

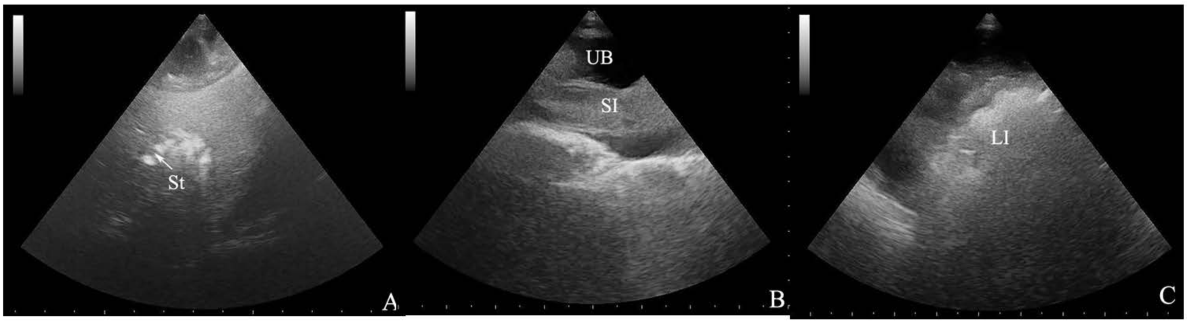

Figure 2 - Ultrasonographic image of gastrointestinal system of the tortoise, seen through the left cervical window (stomach) and prefemoral windows (small and large intestine). A) Stomach with hypoechoic pleated wall (arrow) with gaseous content. B) Small intestine shows parietal stratification in four layers. C) Larg intestine with gaseous content and thin hypoechoic wall. St, Stomach; UB, Urinary bladder; SI, Small Intestine; LI, Large Intestine.

some groups (JACOBSON, 2007), the exact origin of extracardiac fluid can not be determined since no hyperechoic interface was observed concurrently (MARTORELL et al., 2004), even when there was liquid between liver lobes. Although the heart was seen in all animals, the thyroid was not observed in all, perhaps as a result of the different conformations of the carapace among individuals, which limited the acoustic window.

The liver and gallbladder showed similar standards to those described in mammals and turtles (MARTORELL et al., 2004; NYLAND et al., 2005; STETTER, 2006), but all animals showed high echogenicity in the liver relative to adjacent organs. It is inferred that increased hepatic echogenicity be natural in this species, however, we can not rule out the occurrence of liver disease that leads to increased echogenicity as hepatocellular lipidosis (FRYE, 1991; DIVERS & COOPER, 2000; NYLAND et al., 2005; DUTRA, 2007; REDROBE, 2010).

The gastrointestinal system was similar to that seen in mammals and turtles (MARTORELL et al., 2004). However, the luminal contents made impossible better images to be obtained, especially in the stomach and large intestine, due to the production of artifacts in the sluggish digestive tract in this species (PIZZUTTO et al., 2001). We suggest a longer period of fasting, such as 24 hours, for good visibility of the stomach. The parietal stratification of the small intestine was also difficult to see in some animals, which is possible with high frequency (7.5MHz) and close contact with the transducer.

In this species, the kidneys were described morphologically as pyramidal, with convolutions looking like brain, and when devoid of the pelvis and the urinary bladder, as elongated and bilobed (FARIA, 2003). This phenomenon explains the renal triangular shape observed in this study, the appearance of the renal parenchyma and the variable form of the bladder according to the degree of repletion. However, the observed renal margins were smooth without macroscopic convolutions. One study also reported difficulty in differentiating the bladder from free fluid (PENNINCK et al., 1991). Identifying the bladder was easier when the wall was visualized with free fluid, as observed in another study (MARTORELL et al., 2004). The tubular hypoechoic structures in the renal hilar region were possibly the renal arteries. Additionally, the format and kidney echogenicity observed herein also differed from those of other turtle species. In red-eared sliders (***Trachemys scripta elegans***), the kidneys have a lobed appearance and are isoechogenic with surrounding tissues and, in sea turtles (***Caretta caretta***), present as oval or comma-shaped structures and are hyperechoic with the surrounding soft tissues in appearance, in addition to containing a small hyperechoic focus that does not project acoustic shadows (MARTORELL et al., 2004; VALENTE et al., 2007; PEASE et al, 2010). However, in the desert tortoise (***Xerobates agassizi***), the kidneys showed as triangular shapes, although they have also been described as loosely lobulate, which was not seen in this study (PENNINCK et al, 1991) In this study, no distinction was observed between the kidney and the pelvis, medulla and cortex or the ureters (PENNINCK et al., 1991; MARTORELL et al., 2004). The echogenicity of the testes was higher compared to the kidneys in this study, unlike other studies in turtles, which describe slightly hypoechoic testes as the kidneys (REDROBE, 2010). The ultrasonographic appearance of the female reproductive tract was similar to that

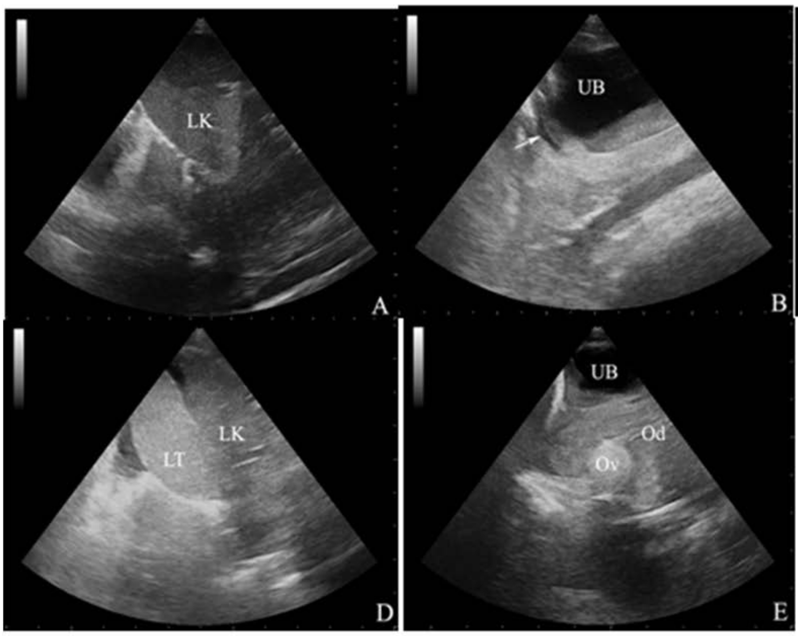

Figure 3 - Ultrasound image of the genitourinary system, seen from the left and right prefemoral windows. A) Kidney hypoechoic and vascularized with triangular shape in longitudinal view. B) Bladder with piriform shape, thin walled hyperechoic and anechoic content. Presence of free anecoic content cranial to the bladder (arrow). C) Testis hyperechoic and elongated shape. D) Cranial location and increased echogenicity of the testis in relation to the kidney. E) Oviduct with sigmoid appearance and ovary with echogenic rounded shape. LK, Left kidney, UB, Urinary bladder, LT, Left testis, Od, Oviduct, Ov, ovary.

described in other studies (MARTORELL et al., 2004; MANIRE et al., 2008). However, in our study, echogenic luminal contents were also observed in the oviducts of most animals. The ovarian follicles could be quantified and measured at different stages of development; however, only a small portion of the follicles can be seen (TEIXEIRA, 2009).

The presence of free anechoic content in the coelomic cavity was variable, but in most animals, this free anecoic content was observed between hepatic lobes and also in other areas through the prefemoral window. When a lesser quantity is primarily seen in this region, the observation of free content is inferred. Lizards and turtles usually have a small amount of free coelomic fluid with no evidence of disease (STETTER, 2006). However, more studies should be conducted in order to characterize a normal pattern in this species. In this study, no spleen, pancreas or adrenal glands were seen, most likely due to their small size or to the presence of artifacts caused by surrounding bowel loops (PENNINCK et al., 1991; MARTORELL et al., 2004; VALENTE et al., 2007). The esophagus and lungs were also not identified.

Ultrasound provided the detection and evaluation of red-footed tortoise coelomic organs through the cervical and prefemoral acoustic windows. The heart and reproductive tract resemble that seen in other turtles, while the liver, gall bladder and gastrointestinal tract, in addition, are also similar to that seen in mammals, despite the liver has increased echogenicity. The urinary tract differed from other species of testudines with major variation observed in the organs format. Free fluid was seen in more than half of the animals, which suggests that small amount is normal in this species, although further studies are necessary for this assertion.

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