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NORMAL ULTRASONOGRAPHIC IMAGING OF THE LOGGERHEAD SEA TURTLE (*Caretta caretta*)

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Summary

This work describes the normal ultrasonographic appearance of cervical structures and coelomic organs of loggerhead sea turtles. Twenty live, and five dead juvenile and subadult loggerhead sea turtles were studied. Ten soft-tissue areas of integument were used as acoustic windows: cervical-dorsal and cervical-ventral, left and right cervicobrachial, left and right axillary, left and right prefemoral and left and right postfemoral acoustic windows. Anatomical cross-sections were performed on the dead turtles to provide reference data. The fourth and fifth cervical vertebrae, the spinal cord, and the venous sinuses of the external jugular vein are clearly seen on the cervical-dorsal acoustic window. On the cervical-ventral acoustic window, the esophagus and the heart were imaged. The stomach was more frequently seen through the left axillary acoustic window. Although the liver could be imaged through both sides, the right axillary acoustic window was the most indicated to see the gallbladder. The large and small intestines and the kidneys could be seen in the right and left prefemoral acoustic windows; the latter were easily identified due to the intense renal vasculature. This study shows that ultrasonography is a useful tool for diagnosis in juvenile and subadult loggerhead sea turtles.

Keywords: ultrasound, Loggerhead Sea Turtle, *Caretta caretta*, echoanatomy, anatomy

Introduction

The loggerhead sea turtle (*Caretta caretta*) is the most common species accidentally captured by fisheries in the western Mediterranean Sea. Juveniles and subadult animals are those most affected by the captures. According to (IUCN, 2004) the species is listed as endangered (EN A1abd) and the estimated annual number of subadult loggerhead sea turtles accidentally captured every year on the Spanish Mediterranean coast is more than 20,000 (Aguilar and Pastor 1995). Captured turtles are usually released back to the sea with a fish-hook still lodged, and it is estimated that between 20% and 30% of these individuals might die due to the lesions caused by them (Aguilar and Pastor 1995). The main cause of admission of sea turtles in the rescue centres is the ingestion of fishhooks; other causes include lesions by entanglement in fishing nets, collision with boat propellers and ingestion of garbage. As in other chelonian species, the shell severely limits examination of internal organs, and the diagnosis based only on a clinical examination is usually very poor.

Ultrasonography is well-established as a rapid, non-invasive and non-expensive method of assessing soft structures; therefore, it serves as a helpful tool for clinical diagnosis in turtle rehabilitation. Its main disadvantage is the lack of well-described ultrasonographic standards for sea turtles. Although general considerations on ultrasound in reptiles could be found in some chapters of wildlife medicine books (Jackson and Sainsbury 1992, Silverman and Janssen 1996, Whitaker and Krum 1999, Wilkinson and others 2004) and papers (Sainsbury and Gili 1991, Gaudron and others 2001, Schumacher and Toal 2001), only few works systematically describe the normal appearance and the best way to visualize the organs (Pennick and others 1991, Martorell and others 2004). Furthermore, anatomical differences among chelonian species have a reflective effect upon ease of scanning. The reproductive tract of adult female Olive Ridley (*Lepidochelys olivacea*) and Kemp's Ridley (*Lepidochelys kempii*) sea turtles has been studied by Rostal and others (1989, 1990 and 1994). These authors recommend ultrasound as a good technique to evaluate the gonad structure, egg development and presence of atretic follicles. To the authors' knowledge, published data about normal ultrasonography of the loggerhead sea turtle are not available.

The aim of this work is to describe the normal ultrasonographic appearance of cervical structures and coelomic organs of the loggerhead sea turtle and to provide images of frozen cross-sections for anatomical reference.

Materials and Methods

A total of 25 loggerhead sea turtles (*Caretta caretta*), with a minimum straight carapace length (SCLmin) of 26.0 cm to 58.5 cm and weights of 3.5 kg to 26.8 kg were used in this study. Twenty live turtles, eight juveniles and 12 subadults, were used for the ultrasound proposal, and five dead juvenile turtles were used to provide the anatomical information. Turtle measurements were based on Bolten (1999). According to Pont and Alegre (2000), turtles with a SCLmin of 21-40 cm, and those with 41-65 cm were considered juvenile and subadults, respectively. Sex could not be identified because they were sexually immature specimens. All animals were accidentally caught in pelagic long-line sets and fishing nets along the northwestern Mediterranean coast (40°31'- 42°26' N and 0°32'-3°10' E), Spain, and were temporally housed in the rehabilitation facilities of the Rescue Centre for Marine Animals (CRAM), Premiá de Mar, Barcelona, Spain. Only turtles in good condition, based on physical, radiographic and haematological parameters, were used in this study. From the turtles captured in longlines, we included only those in which the hook was superficially attached in the buccal cavity.

During the ultrasonographic examinations, the animals were manually restrained in ventral recumbency on a bucket, high enough to avoid the limbs contacting the table. No sedation was necessary. The head, neck or limbs were extended as needed. Eyes were kept blind through a mask and the body surface was kept wet using a sopping towel. Ten soft-tissue areas of integument were used as acoustic windows: cervical- dorsal and cervical-ventral, left and right cervicobrachial, left and right axillary, left and right prefemoral and left and right postfemoral acoustic windows (Figure 1). Colour and pulsed doppler were applied in the large vessels. Ultrasonographic examinations were performed with a real-time, B-mode scanner (Computed Sonography Siemens 128XP/10, Acuson) using sector electronic transducers and frequencies of 4.0, 5.0 and 7.0 MHz. Coupling gel (Polaris II, GE Medical Systems, Leonhard Lang GmbH, Archenweg 56, A-6020; Innsbruck, Austria) was placed on the surface of the transducer, which was oriented mainly on the horizontal plane (the one oriented parallel to the plastron and carapace). Other planes were not used due to size limitations of the acoustic windows and transducer. Images were recorded using videotapes. Records of the ultrasound images were made with a thermal printer.

Anatomical sections were performed on three frozen turtles, each one in an oriented plane: sagital plane, frontal plane and transverse plane. Two other dead turtles were dissected to improve the morphological data. Anatomical terminology followed Wyneken (2001).

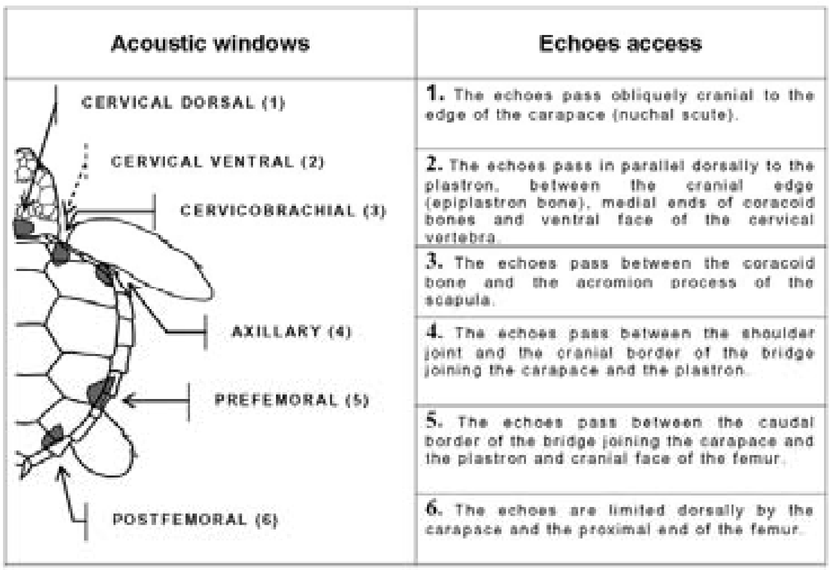

Figure 1. Acoustic-window and echo accesses used for the ultrasound scan of loggerhead sea turtles. The diagram shows only the right side

Results and Discussion

The fourth and fifth cervical vertebrae, the spinal cord, and the venous sinuses of the external jugular vein (also called cervical-dorsal sinus) were clearly seen in a transverse and transverse-oblique scan on the cervical-dorsal acoustic window (Figure 2, A-C). The ultrasonographic contour of the vertebrae was visible with echogenic borders followed by acoustic shadowing due to the strong reflection from the osseous tissue. The vertebral arch and the spinal cord were clearly recognized and could be used as landmarks to identify the right and left cervical-dorsal sinuses, which lay dorso-lateral to the vertebrae. The sinuses are broad dilatations of the external jugular vein (Figure 2, B-F) and were ultrasonographically identified as superficial, rounded, distensible hypo-echogenic structures connected to the venous vessels. The connection between left and right cervical sinuses was seen through the anastomosis with the vertebral vein, which lay centrally on the vertebral arch (Figure 2: B, D). The echoes of the circular blood flow inside the sinuses were sometimes visible. The dimensions of the sinuses were variable but, in general, relatively greater than those cited by Whitaker and Krum (1999). These authors stated that the dorsal-cervical sinus in 25-kg turtle was about 1 x 2 cm in a transverse section, while we found measurements of about 2.5 x 3.5 cm and 1.8 x 4.2 cm in a 26.8-kg and 58.5-cm SCLmin turtle, and in an 8.6-kg and 39- cm SCLmin turtle, respectively. The disparity could be related to different factors. Because reptiles are ectothermal animals, and the sinuses are vascular structures related to thermo-regulation, changes in their flow and dimensions are expected due to external temperature variation, as occurs in the front flippers (Hochscheid and others 2001). The turtles used in this study were scanned in the summer, and consequently underwent relatively high temperatures (25°C to 35°C) during the transportation and the procedure. Therefore, an increase in sinus size could be due to a vasodilatation response. Additionally, the inclination angle of the transverse oblique scan could also be related to the larger size found because of the longitudinally slanted section of the vessels, which could justify the greater length. On the other hand, compared to the anatomical sections, it was observed that the sinuses were not only a single dilation of the jugular vein, but also a complex of small cavities separated by fine membranous walls (Figure 2: D, F), which could not be completely visualized ultrasonographically.

Through the cervical-ventral acoustic window, the heart was dorso-ventrally flattened and located between the two hepatic lobes. The atria, during diastole, could be identified as oval anechogenic-paired structures with a fine echogenic wall (Figure 3, A). The ventricle had a homogenously echogenic appearance, with distinction of a thick wall with trabecular lining (Figure 3: A, B, D). Due to the slow heart rate, the echoes corresponding to blood flow and atrial diastole were easily recognized during ventricular systole. Only an atrioventricular valve was clearly seen as a short horizontal echogenic line centrally placed between the atria and ventricle. In juvenile specimens the best way to see the heart was the cervical-ventral acoustic window. Through this point, all three chambers and the large vessels on the cardiac base could be identified on a horizontal plane. By this access the scan usually passes along the dorsal aspect of the heart, where the pulmonary and subclavian arteries could be seen together with the aortic arches (Figure 3, A-D).

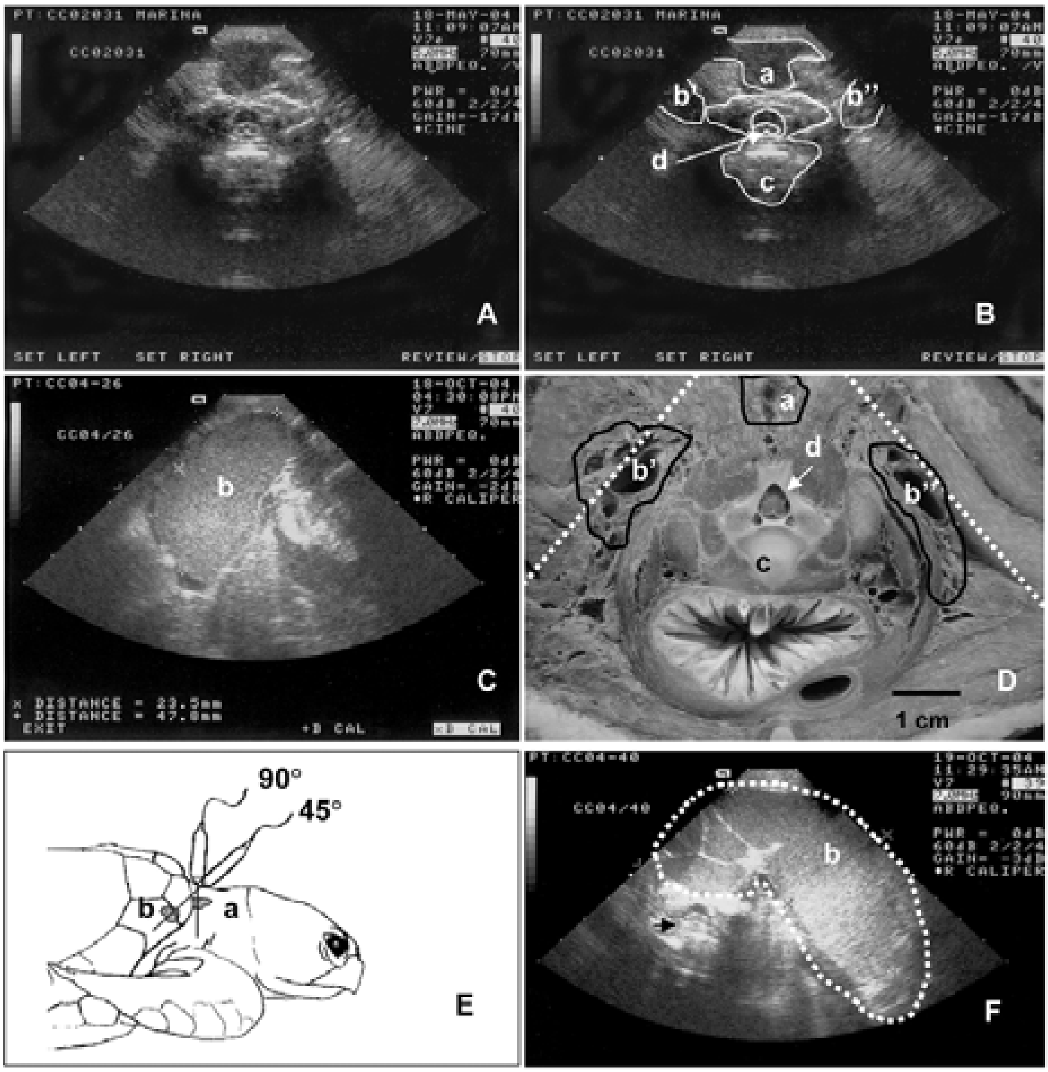

Figure 2: Ultrasound evaluation of the cervical-dorsal structures of the loggerhead sea turtle: a, vertebral vein, anastomosis point to transversal vein; b'-b'', sinuses of external jugular vein, c, vertebral body; d, spinal cord. A and B: Ultrasound images of a transversal scan. C: Ultrasound image of oblique scan of the venous cervical sinus (b) of the jugular vein. D: anatomical transversal cross-section corresponding to transversal scan. E: illustration showing the transversal and transversal oblique planes preferred for imaging of the cervical venous sinuses (a and b, sinues of the jugular vein). F: Ultrasound image of the complex of jugular sinuses seen in a 45° oblique scan, the black arrow indicates the cervical vertebra.

In subadult turtles, the right and left cervicobrachial acoustic windows allowed partial access to the heart and the complete visualisation of the right and left aortic arches and pulmonary arteries, respectively.

The distal end of the oesophagus could be seen through the ventral-cervical and left cervicobrachial acoustic windows as a coarse echogenic structure, identified as the keratinised papillae (Figure 4, A). Although recognised in the necropsies as a lobular structure tissue associated with the fat around the large cardiac vessels, the thymus was not clearly identified in this study due to the similar granular appearance of the esophagus.

The stomach was placed on the left side of the coelomic cavity. Although it could be partially seen in the left cervicobrachial, axillary and prefemoral acoustic windows, it was more frequently imaged through the left axillary acoustic window (Figure 4: B-C). A good landmark to locate this organ was the subscapular muscle seen in a horizontal scan as a hypoechogenic rounded structure followed by a fine echogenic line, the coelomic membrane (Figure 4: B - D). The stomach was usually seen dorsal to this muscle in an oblique dorsal scan (30°-35°).

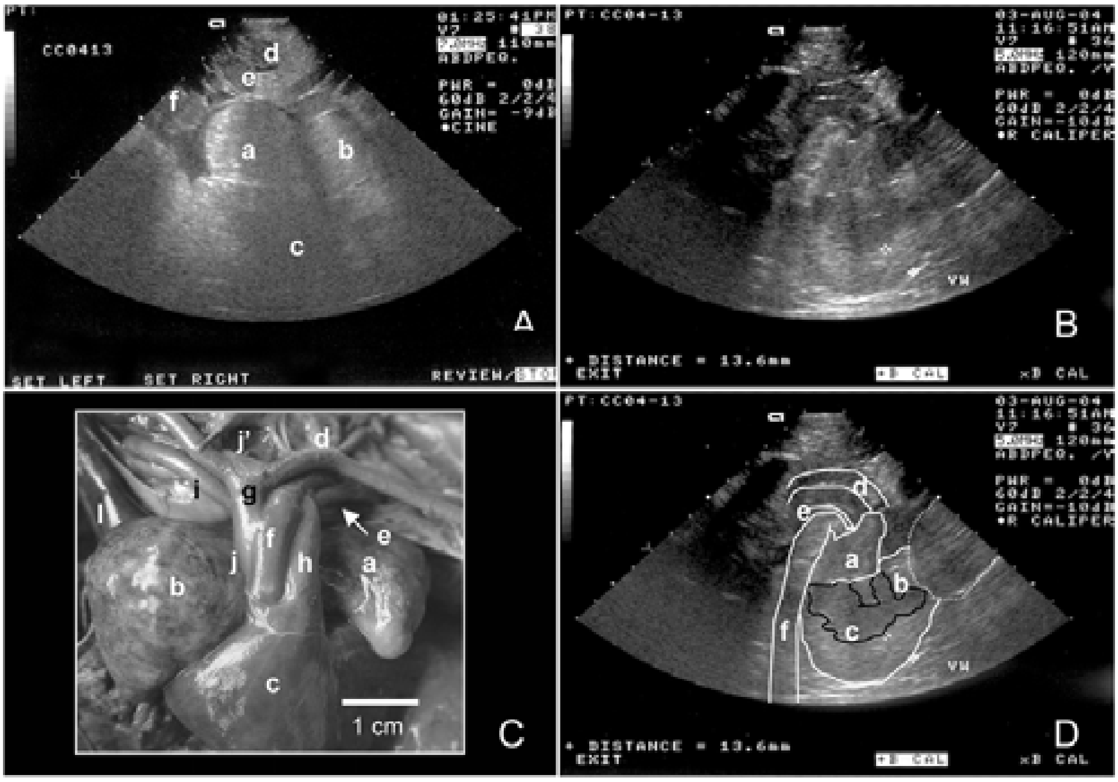

Figure 3: Ultrasonographic images of heart of the loggerhead sea turtle taken from cervical-ventral acoustic window: a, left atrium; b, right atrium; c, ventricle; d, left subclavian artery; e, left pulmonary artery; f, left aorta. A, B and D: Ultrasonographic image taken from cervical-ventral acoustic window. C: Ventral view of anatomical dissection of the heart of juvenile loggerhead sea turtle injected with latex, the right atrium is slighty dilated by the latex. A, left atrium; b, right atrium; c, ventricle; d, left subclavian artery; e, left pulmonary artery; f, left aorta; g, brachicephalic trunk; h, pulmonary trunk; i, right pulmonary artery; j, j', right aorta; l, right precava vein.

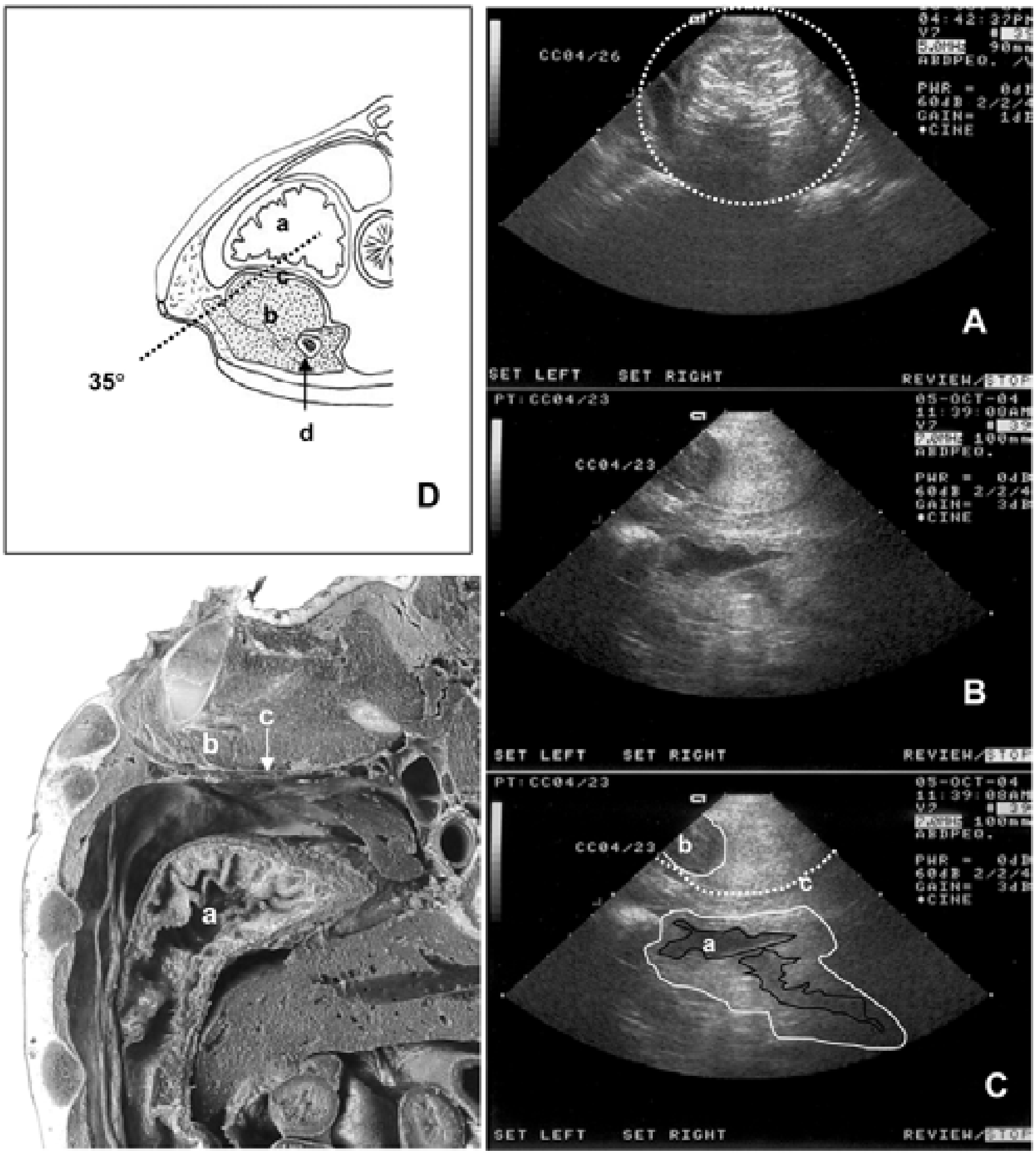

Figure 4: Ultrasound evaluation of the esophagus and stomach of the loggerhead sea turtle. A: Ultrasound image of esophagus, cross-section scan. B-C: Ultrasound image of stomach taken from left axillary acoustic window: a, stomach lumen; b, subscapular muscle; c, coelomic membrane. D: Illustration showing the oblique image plane preferred for imaging the stomach: a, stomach lumen; b, subscapular muscle; c, coelomic membrane; d, coracoid. E: Corresponding frontal gross anatomical seccion.

In general, the stomach showed a thin wall with folds seen as thin hypoechogenic lines, and produced poor images due to the presence of intraluminal gas and contents (Figure 4: B-C).

The liver was visualised as an echogenic structure with granular parenchyma (Figure 5, A). Hepatic vessels were seen as anechogenic tubular structures either in transverse or longitudinal sections (Figure 5, A). The best accesses to this organ were through the left and right axillary acoustic windows (Figure 5, B). The right axillary acoustic window was the one most indicated to see the gallbladder, which had an anechogenic spherical/oval appearance within the right hepatic lobe (Figure 5: A, C). The postcava (also called right hepatic vein) could be identified on entering the right atrium via the sinus venous valve (Figure 5, D) by this acoustic window.

Figure 5: Ultrasound evaluation of liver of the loggerhead sea turtle: A: Ultrasonographic image taken from right axillary acoustic windows: a, branches of right hepatic vein (Postcava); b, hepatic parenchyma; c, subscapular muscle, d, gallbladder. B: Corresponding frontal gross anatomical seccion: a, right hepatic vein; b, hepatic parenchyma; c, subscapular muscle; d, gallbladder; e, area of the right atrium; f, sinuses of jugular vein. C: Ultrasound image of the hepatic parenchyma: d, cross-section of the gallbladder. D: Ultrasound image of the liver and heart: a, right hepatic vein (Postcava); b, hepatic parenchyma; c, subscapular muscle; d, right atrium.

The large and small intestines could be seen in the prefemoral acoustic window from both sides. Loops of small intestine were more frequently imaged on the right side. The ultrasonographic image of the intestine was similar to that of mammals (Mattoon and others 2002), and stratification in five layers could be recognised. The wall of the small intestine was thicker than that of the large intestine (Figure 6, A-B). The serose and submucose layers were easily identified as echogenic lines. The muscular layer, between them, had a hypoechoic to anechoic appearance. The mucosa was the thickest hypoechoic layer (Figure 6, C). On the contrary, the wall of the large intestine did not have clearly distinguishable layers.

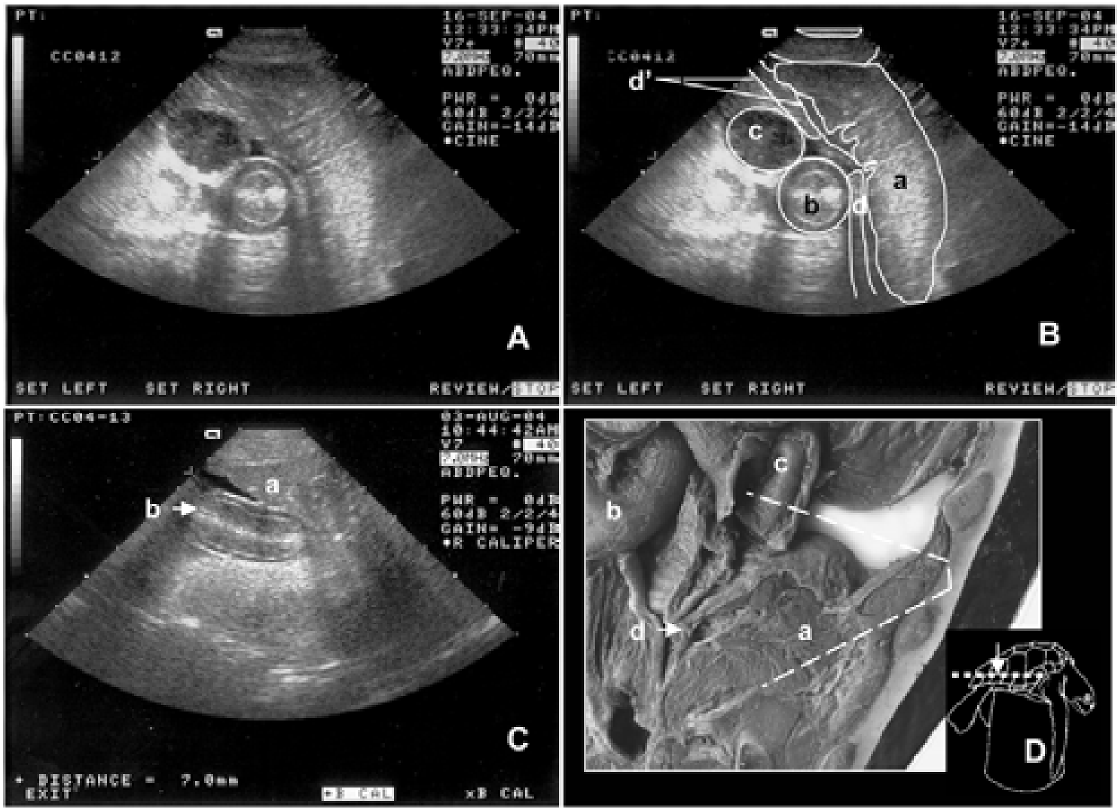

Figure 6: Ultrasound evaluation of the kidney and intestines of the loggerhead sea turtle. A-B: Ultrasonographic images taken from right prefemoral acoustic window: a, right kidney; b, cross-section of small intestine; c, cross-section of large intestine; d and d', blood supply of the kidney. C: Ultrasound image of the kidney (a) and longitudinal section of small intestine (b). D: Illustration showing dorsal view of the imaged area in a frontal anatomical section: a, kidney; b, loop of small intestine; c, section of large intestine; d, renal vein.

The kidneys were easily identified due to the intense renal vasculature, which could be used as a landmark. They could be imaged superficially from the prefemoral acoustic window (Figure 6, D) from the respective side, and showed a uniformly echogenic area, comma like, without a distinct renal pelvis and capsule. The great renal vein was easily seen, cranial to the kidney (Figure 6, A-B).

The urinary bladder could be seen, only when it was completely filled, as an anechoeic globular structure (Figure 7: A, C). It was better identified from the left prefemoral acoustic window (Figure 7, B). Echogenic dots were seen floating within and were considered normal. They could be related not only to the presence of urate crystals but also to parasites or fecal material, which could opportunistically enter as a result of its closed connection with the cloaca (Wyneken 2001). Vesical parasites were found in one of the three dissected turtles (unpublished data).

According to Wyneken (2001), sea turtles have two small, accessory urinary bladders connected to the urinary bladder, each located laterally to the neck of the urinary bladder and dorsal to the pubis. In this study, the neck of the urinary bladder could not be imaged due to the acoustic shadow of the pubic bone. These structures were not found in the gross-anatomy detailed dissections of our work (unpublished data).

The reproductive tract, the spleen, pancreas and adrenal glands could not be identified in this study. The pancreas extends as an irregular strip along the duodenum just past the stomach. The spleen has an oval form located below the pancreas. These organs were not ultrasonographically accessible due to their small size, midline location and the artifacts caused by intestinal loops surrounding them. They were also not documented in a previous study in California desert tortoises (*Xerobates agassizi*) (Penninck and others 1991).

The post-femoral acoustic windows allowed access to the caudal end of the kidney only in a few larger subadult turtles. In juvenile turtles just the femur and muscular mass could be imaged through this point, it was deemed inadequate for the ultrasonographic evaluation of any coelomic structures.

In recent decades ultrasound imaging has been frequently used to evaluate the reproductive status in many reptilian and mammalian species (Küchling 1989, Owens and Kraemer 1990, Casares and others 1997, Rostral and others 1998, Brook and others 2000, Brook and others 2004). Some species of reptiles are not sexually dimorphic, and in these cases the ultrasonographic imaging of the gonads is a reliable and inexpensive method to identify the sex of the animals and select them for reproductive programmes (Morris and Allison 1996). In general, the loggerhead sea turtle do not exhibit differentiated external sexual characteristics until they reach the adult size (SCL>65 cm), which occurs at more than 20 years old (Bjorndal and others 2001). This work reveals that sex identification in subadult turtles can not be performed using ultrasonographic imaging. The small non-developed gonads are mistaken for renal parenchyma, and the tubular structures, such as oviduct and deferent canals, are not visible. The same limitation was found in Kemp's Ridley sea turtles, where only ovarian follicles with sizes greater than 5 mm in diameter, and oviducts with eggs, were ultrasonographically recognised (Rostal and others 1990).

Knowledge of normal anatomy is essential for the interpretation of diagnostic techniques. Chelonians have well-protected internal viscera, and the few body parts not covered by the carapace or plastron are internally limited by the bone of the proximal end of the limbs (scapular and pelvic girdles), which usually limit scanning to one plane. Poor image quality could also be a consequence of the great size of the turtle in relation to the transducer frequency, the amount of fat tissue and the degree of emptiness of the gastrointestinal tract. High-frequency transducers used in this study (7 MHz) were suitable to assess in detail superficial structures like the cervical venous sinuses, stomach, intestines and kidneys. In juvenile turtles, this probe displayed good images of the kidneys and the heart when the latter was seen through the cervical- ventral acoustic window. In subadult animals the transducer of 5MHz was more frequently used, and it allowed a good balance between penetration and resolution. The transducer of 4MHz was used infrequently because of its low resolution. Due to the direct access of the kidneys in the prefemoral acoustic windows, as in other chelonian species, this point could be used to perform ultrasound-guided biopsies of this organ. The hepatic and renal parenchymas were easily imaged and could be evaluated for morphologic alteration in shape and size, as well as for the presence of neoplasia, cysts, abscesses and papillomas.

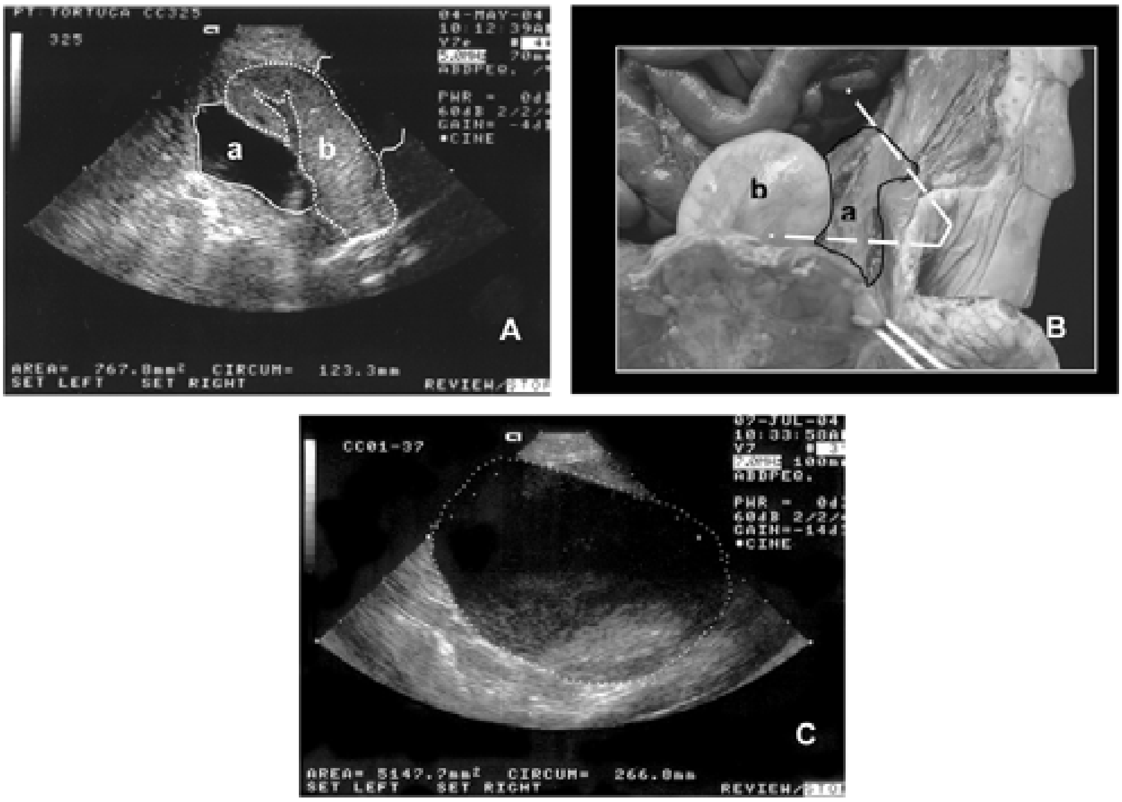

Figure 7: Ultrasound evaluation of the kidney and urinary bladder of the loggerhead sea turtle. A: Ultrasonographic image of urinary bladder (a) and kidney (b). B: Corresponding gross anatomy of the scanned area. C: Full urinary bladder with echogenic dots.

Due to the slow digestive transit time, the presence of food in the gastrointestinal tract in turtles with a fasting period of 24 to 48h produced multiple artifacts and poor images, mainly of the stomach. Concerning the digestive tract, the diagnosis of obstructive processes due to garbage ingestion or severe mucosal damage caused by fishing hooks (accordion effect) could also be possible.

Although it is known in mammals that low-level echoes are returned from fat in certain areas of the body (Nyland and others 2002), low-resolution images are also obtained from some fat turtles, mainly using the cervicobrachial acoustic windows.

This study shows that ultrasonography can be a useful tool for the diagnosis of different internal lesions in juvenile and subadult loggerhead sea turtles. It is inexpensive, safe and easy to use and with practice, most of the internal organs can be located and examined.

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