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Clinical ultrasonography in loggerhead sea turtles (*Caretta caretta*): imaging of pathological features

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ABSTRACT: Ultrasound scans were used to image pathological features in sea turtles. Scans were carried out in 19 loggerhead sea turtles, weighing from 2 to 21 kg, during the course of clinical examinations using 3.5 and 7.5 MHz sector transducers. The examination was performed after placing turtles in dorsal recumbency. Turtles were manually restrained and, in order to find the most suitable acoustic windows, were held down either by their heads (left and right cervicobrachial windows), front flippers (left and right axillary windows) or back flippers (left and right prefemoral and postfemoral windows). The right and left cervicobrachial windows allowed visualisation of the liver and gallbladder; the stomach was localised through the left prefemoral acoustic window; the intestinal loops were observed through the left and right prefemoral acoustic windows; the bladder was identified through both prefemoral acoustic windows. The pathological findings were as follows: idiopathic *ileus*, an intestinal linear foreign body and smooth muscle hypertrophy, presence of calculi and sediment in the gallbladder. These results highlight the importance of ultrasound examination along with clinical examination in sea turtles for the evaluation of coelomic pathologies.

Keywords: ultrasound; loggerhead sea turtle; *Caretta caretta*

The loggerhead sea turtle (*Caretta caretta*) is a common inhabitant of the Mediterranean Sea. This species nests particularly on the Sicilian and Calabrian coasts. As an endangered species, its conservation involves also the protection of nesting sites, where juveniles and adults are often captured by fisheries (De Florio et al. 2005).

Sea turtles show poor clinical signs and, due to the presence of carapace and plastron, physical examination requires complementary diagnostic procedures to provide useful information for clinical diagnosis (Penninck et al. 1991; Lieberman and Rosskopf 1984).

Radiological examination is the best method in order to detect the presence of a swallowed fishhook, but it has limited value to identify the internal organs of sea turtles, due to the shell and the lack of visceral fat (Silverman 1989; Penninck et al. 1991; Rubel et al. 1991; Deshaw et al. 1996; Silverman and Janssen 1996; Stetter 2000). Laparoscopic examinations can be performed, even if there could be problems in using this technique (Blanvillain et al. 2008). In our experience such risks are inherent to post-surgical recovery in wild aquatic animals (i.e. infections or dehiscence of the wound).

In aquatic animal medicine, ultrasound is a very helpful and easy-to-use tool for investigation and diagnosis, particularly in the study of the coelomic organs in chelonians (Penninck et al. 1991; Martorell et al. 2004; Valente et al. 2007; Margheri et al. 2011; Kot et al. 2012; Macri et al. 2013a; Macri et al.2013b; Macri et al. 2015). Regarding chelonian reproduction, ultrasound examinations have proven to be an efficient non-invasive method for monitoring ovarian development and activity in sea turtles and reproductive activity in adult males (Rostal et al. 1990; Valente et al. 2007; Manire et al. 2008).

Valente et al. (2007, 2008) described the normal sonographic appearance of coelomic organs in the loggerhead sea turtle and identified ultrasound-accessible blood vessels in *Caretta caretta* and their Doppler waveform patterns. Since the information regarding pathological patterns are still unsatisfactory or too dissimilar from the ones now used for

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diagnostic purposes (Valente et al. 2007; Valente et al. 2008), in the present study we hypothesised that ultrasonographic imaging would help practitioners in the diagnosis of the various pathologies affecting sea turtles. Therefore, the aim of this study was to perform ultrasound examination on sea turtles (*Caretta caretta*) rescued near the Sicilian coast.

Ultrasound techniques, as well as different pathological and normal patterns, are reported and discussed.

MATERIAL AND METHODS

Ultrasound examinations were performed on 19 loggerhead sea turtles (*Caretta caretta*) of different sizes and weights belonging to the Rescue Centres of the Sicilian Nature Fund of Catania (Fondo Siciliano per la Natura, Catania, Italy) and the World Wildlife Fund for Nature of Messina (Italy). The scans were carried out using an ultrasound machine (Sigma IRIS 440, Kontron Instruments, Italy), with ultrasound multifrequency probes (3.5–7.5 MHz). The following acoustic windows were used: left and right cervicobrachial, left and right axillary, left and right prefemoral and postfemoral. The examination was done after placing the animals in dorsal recumbency; the turtles were manually restrained according to Valente et al. (2007). In brief, turtles were held down by their heads and their front or back flippers, depending on the most suitable acoustic window. Their eyes and body surface were covered by disposable wet wipes.

On the basis on clinic examination, ultrasound was performed in nine subjects with pathological signs (Group A).Ten additional animals (Group B) underwent ultrasound examination to exclude asymptomatic lesions. The percentage of visualisation of anatomic structures from different acoustic windows was noted.

RESULTS

The turtles had a straight-line carapace length between 32 and 63 cm and a weight ranging from

Table 1. Clinical, radiological and ultrasound findings

	Clinical and/or X-ray exam	Ultrasound
1	skin lesions at head and nape regions	nothing to report
2	fishing line leaking from cloaca	absence of peristalsis, intestinal stasis, coelomic fluid
3	ocular changes	nothing to report
4	hook in oesophagus; discoloured and soften areas of the carapax	echogenic dots were visible floating within the bladder
5	fishing line leaking from cloaca	absence of peristalsis, coelomic fluid, hyperechoic bladder content
6	hook in oesophagus	dilated loops, coelomic fluid
7	dehydration, softened plastron, partial absence of rhamphotheca	nothing to report
8	dehydration, softened plastron, fishing line leaking from cloaca	the ultrasound scan was not performed through the inguinal window due to intestinal gas
9	nothing to report	nothing to report
10	nothing to report	intestinal idiopathic muscular hypertrophy
11	hook in oesophagus surgically removed 5 days previously	absence of peristalsis, liquid in the bowel, coelomic fluid
12	hook in oesophagus surgically removed 5 days previously	normal bowel peristalsis, echogenic gallbladder content, echogenic dots were visible floating within the bladder
13	nothing to report	nothing to report
14	hook in oesophagus	nothing to report
15	fishing line leaking from cloaca	adynamic ileus, coelomic fluid, fishing line
16	nothing to report	nothing to report
17	nothing to report	nothing to report
18	nothing to report	nothing to report
19	anorexia	calculi in the gallbladder.

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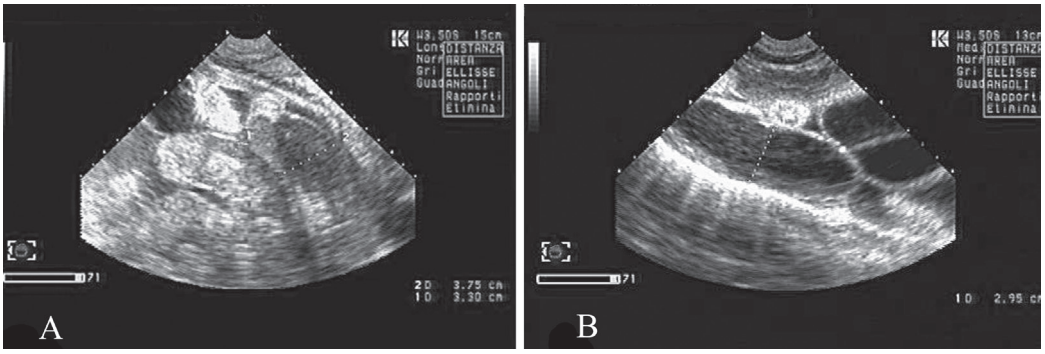

Figure 1. Intestine – **A** and **B**; adynamic ileus and dilated loops

2 to 21 kg; according to Pont and Alegre (2000), the subjects examined were juvenile and sub-adult ones (Pont and Alegre 2000).

Clinical examination

One turtle presented cutaneous lesions in the head region; another presented ocular lesions; three turtles showed the presence of fishing-line protruding from the oral cavity and, as later confirmed by an X-ray examination, a hook lodged in the oesophagus. Two animals underwent surgery to remove the hook five days before the ultrasound scan. Four turtles had a fishing line attached to the cloaca. One animal showed signs of dehydration, softening of the carapace and a fracture of the rhamphotheca. Seven subjects showed no clinical symptoms. All the above data, along with ultrasound reports are summarised in Table 1.

Ultrasound examination of Group A

Five subjects showed findings of idiopathic *ileus* (atonic intestinal loops with accumulation of fluid or dilated by the accumulation of food material); the bowel diameter appeared larger than the control group (Figure 1 A and B). The bowel diameters

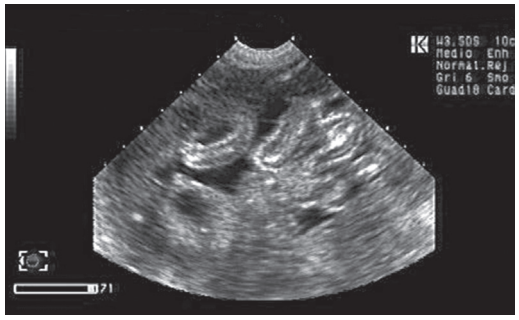

Figure 2. Intestine; normal intestinal pattern, free of coelomic fluid

of turtles with and without signs of ileus were compared using Student's *t*- test at $P < 0.05$.

In six subjects with regular peristalsis, the diameter of the loops ranged from 1.30 to 2.25 cm (mean 1.67 cm), while in the subjects with findings indicative of mechanical ileus, the bowel diameter ranged from 1.70 to 3.75 cm (mean 2.79 cm).

The two-tailed P value was 0.02, indicating significance.

Five subjects showed coelomic fluid between the intestinal loops (Figure 2) or in the bladder area (Figure 3).

In the gallbladder of two subjects, one of which was anorexic and the other which had undergone surgery five days previously, an echogenic/hyperechoic content, which caused distal acoustic shadowing, was found.

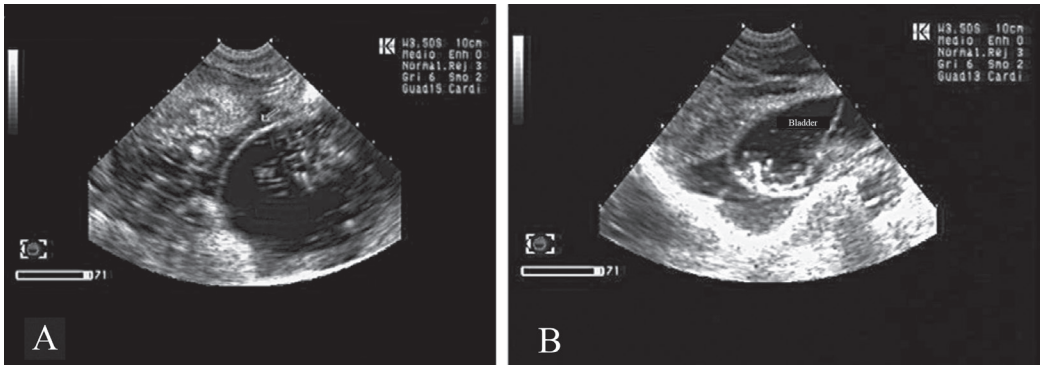

Figure 3. Bladder – **A** and **B**; presence of free-floating hyperechoic material within bladder

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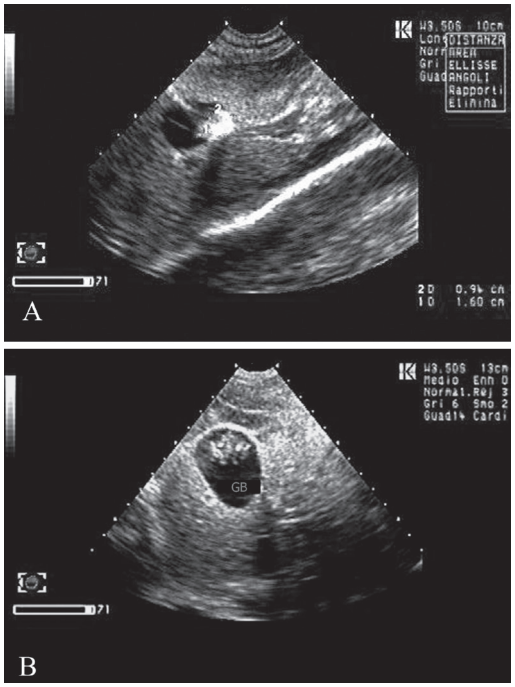

Figure 4. Gallbladder; **A** – gallstones; **B** – biliary sludge

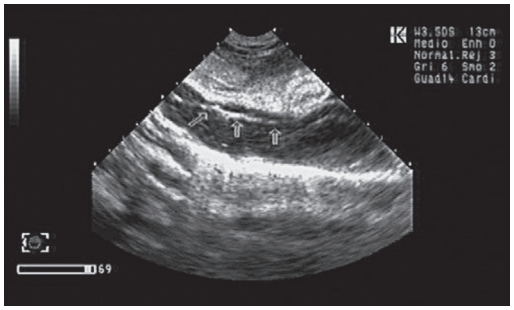

Figure 5. Intestine; linear foreign bodies

Figure 6. Intestine; hypertrophy of the muscle wall of the intestine

One case revealed a hyperechoic structure due to the presence of gallstones (Figure 4 A and B).

One subject, with a fishing-line protruding from the cloaca and an ultrasound image of *ileus*, showed an intestinal loop inside with fluid content and a hyperechoic line connected to the linear foreign body (Figure 5); in these cases, the increased bowel transversal dimension was significant, recalling a similar case described in a cat by Tidwell and Penninck (1992).

Ultrasound examination of Group B

One subject without any anamnestic indication of pathology in the gastrointestinal tract, showed a thicker intestinal loop at the level of *muscularis propria* (Figure 6).

In the other nine subjects findings were considered normal according to literature data, as described below.

Liver and gallbladder. The liver was located laterally and slightly dorsally to the heart, with the gallbladder housed on the ventral surface of its right lobe. The right and left cervicobrachial windows allowed visualisation of the organ in a percentage of the subjects varying from 52% to 63%, respectively; through the right and left prefemoral windows, visualisation was obtained in 52% to 73% of the subjects. Partial images of the hepatic parenchyma with transverse and oblique scan planes were also obtained through the axillary windows.

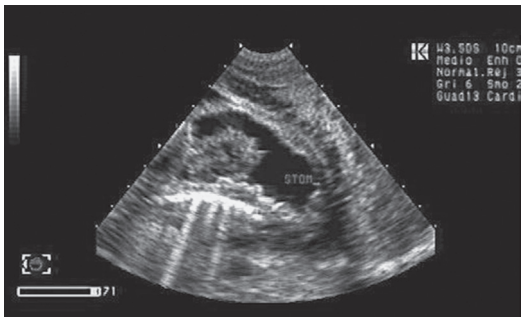

Figure 7. Stomach; gastric material

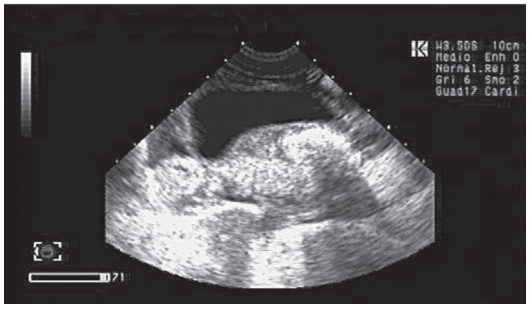

Figure 8. Colon; presence of gas in the colon with hyperechoic content with acoustic shadowing

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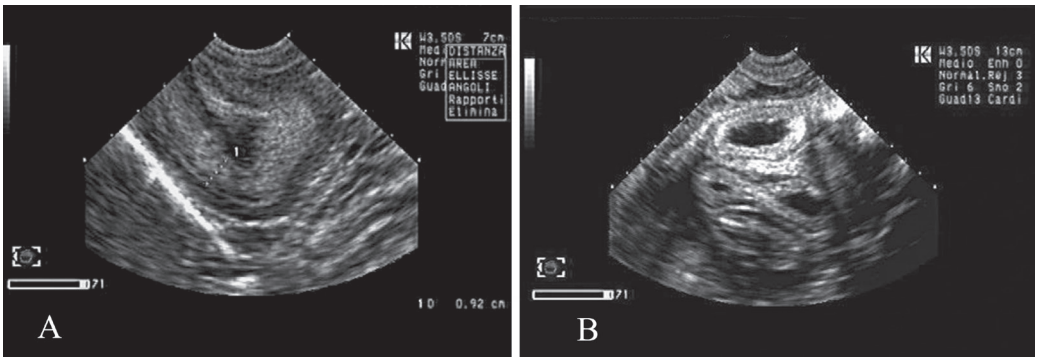

Figure 9. Intestine; **A** – echogenic material without acoustic shadowing; **B** – fluid pattern

Stomach. The stomach, curved and situated to the left of the coelomic cavity, was displayed through the left prefemoral acoustic window only in five subjects (26%). It was identified by the presence of its fluid content. The presence of echogenic material was found in one subject (Figure 7).

Intestinal loops were observed through the left and right prefemoral acoustic windows, respectively, in 73% and 78% of the subjects examined, as a region that originates from the pyloric sphincter and then curves upward from left to right, becoming an entangled organ located medially in the coelomic cavity up to the cloaca. Aspects of normal mucosa (Figure 2), the presence of gas in the colon with hyperechoic content and acoustic shadowing (Figure 8), as well as echogenic material without acoustic shadowing (Figure 9A) were observed. In some cases, the presence of fluid in the loops enabled excellent visualisation of the layers of the walls (Figure 9B).

Bladder. This was easily identifiable through the prefemoral acoustic windows in the turtles of both groups, just above the large intestine and under the pelvis. The presence of hyperechoic material was revealed in two subjects due to calculosis (Figure 10). The presence of hyperechoic material with and without comet-tail artefacts was found inside the bladder of another subject (Figure 3 A and B).

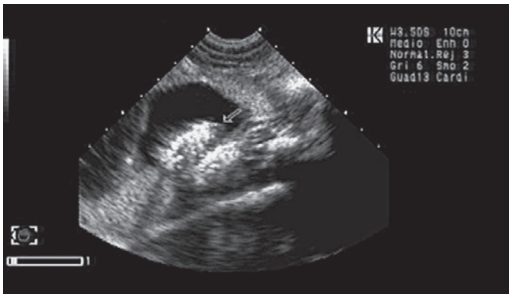

Figure 10. Bladder; prefemoral windows; calculus with acoustic shadowing

DISCUSSION

Loggerhead sea turtles are often recovered along the Ionian Sea, the southern Adriatic and the Sicilian coasts, generally because of ingestion of fishing hooks and other foreign bodies (Tomas et al. 2002; Casale et al. 2004; Oros et al. 2004; De Florio et al. 2005).

The poor radiopacity of foreign bodies (nylon fishing-lines, plastic bags, etc.), along with the frequent absence of clinical signs, can delay the diagnosis of a gastrointestinal obstruction and intestinal intussusception (De Vico et al. 2003; Di Bello et al. 2006). In our study, the ultrasonography allowed the diagnosis of *ileus* likely caused by ingestion of fishing-lines and/or fishing hooks in subjects with only indicative signs of gastroenteric pathology, sometimes accompanied by the presence of coelomic fluid. In one case it was also possible to recognise the presence of the fishing line. This finding, unlike what reported by Penninck et al. (1991) in *Xerobates agassizii*, the presence of coelomic fluid is not a frequent occurrence in *C. caretta*, and its clinical significance is unknown (Penninck et al. 1991). The thickening of the intestinal muscular layer reported in some mammals, such as cats and horses, has been related to chronic stenosis or chronic inflammatory enteropathies (Diana et al. 2003; Penninck 2008). In our case it was not possible to detect the cause of intestinal wall thickening.

In the literature these ultrasound intestinal patterns have never been described in sea turtles. For this reason we consider it interesting to report our evaluations.

The presence of gallstones in the anorexic subject is likely related to malnutrition. Echogenic dots

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were visible floating within the bladder and have been linked to the presence of urate crystals, parasites or faecal material (Wyneken 2001).

The evidence of comet-tail artefacts was indicative of gas, which probably entered accidentally via the cloaca.

In this study, we chose to place the examined turtles in dorsal recumbency as suggested by Pease et al. (2010).

In the present study superior visualisation of coelomic organs, such as stomach intestinal loops and the urinary bladder, was obtained using the same acoustic windows reported by Valente et al. (2007), with the exception of the liver that was more visible through the prefemoral acoustic window.

The inability to perform histopathological or cytopathological examinations in order to obtain a diagnosis of some suspect ultrasound patterns may be a limitation of this study, although we still think that it is instructive to report the observed patterns as they have not been described before in the literature. Our study represents a novel contribution to the evaluation of coelomic disorders in sea turtles. Some subjects with pathological findings were sent to rehabilitation centres or were set free into the sea, so we did not have the possibility to perform necroscopies on subjects after death.

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