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Ultrasonographic Evaluation of Gastro-intestinal Dysfunction in Rescued Olive Ridley Sea Turtles (*Lepidochelys olivacea*)

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ABSTRACT

Background: The olive ridley sea turtle is a common inhabitant of primarily in the Pacific and Indian Oceans. Sea turtles show poor clinical signs and due to the existence of carapace and plastron, physical examination requires complementary diagnostic methodology to provide valuable information for clinical diagnosis.

Methods: This work describes the normal ultrasonographic appearance of cervical structures and gastro-intestinal organs of seven olive ridley sea turtles that were stranded during September 2017 to April 2018 along with rescued sea turtle by Trust for Environmental and Education (TREE) foundation, Vettuvankeni, Chennai, Tamil Nadu. Ultrasound examination was performed with multi frequency Probe (3.5 MHz to 7.5 MHz) transducer. Ten soft-tissue areas of integument were used as acoustic windows.

Result: On ultrasonographic examination of the cervical-ventral acoustic window, the esophagus was imaged. The stomach was more frequently seen through the left axillary acoustic window. The liver could be imaged through both sides of the axillary window and the right axillary acoustic window was the most indicated to visualize the gallbladder. The large and small intestines could be seen in the prefemoral and post femoral acoustic window on the both the sides. The ultrasonographic measurement of various organs was done furthermore gastrointestinal dysfunctions were recorded. This study on ultrasound indices was found to be a useful diagnostic tool for evaluation of coelomic organs in olive ridley.

Key words: Chelonian, Digestive disorders, Olive ridley sea turtle, Ultrasonography.

INTRODUCTION

There are seven species of marine sea turtles worldwide and all are listed on the IUCN Red List. Marine sea turtles encompass in the world's oceans for greater than 100 million years. These sea turtles are widely distributed marine reptiles, in which many species migrate for thousands of miles and even across entire oceans between foraging and nesting grounds or breeding grounds (IUCN, 2008). The most common sea turtle species that were observed along the coast of Tamil Nadu are Green Turtle (*Chelonia mydas)*, Hawksbill (*Eretmochelys imbricate)*, Olive Ridley (*Lepidochelys olivacea*), Loggerhead Sea Turtle (*Caretta caretta)*. The Olive Ridley Turtle (*Lepidochelys olivacea*) is one of the smallest species of sea turtle in the world (80 cm). Since, injured sea turtles usually display reduced clinical signs of illness and disease diagnosis of the sea turtles is relatively poor. Advancement of various techniques is limited to the difference in anatomy, physiology and physiopathology as chelonians remain the most challenging reptiles for medical examination. A major part of the unusual anatomy and physiology of the turtles and tortoises is hardly identified and regular routine clinical examination usually applied to domestic animals provides little information about their health status (McArthur *et al.,* 2004).

Ultrasonography technique is a very supportive and easy to use diagnostic tool for exploration and identification of various disorders, particularly in the study of coelomic organs in chelonians (Martorell *et al*., 2004). The normal ultrasonographic appearance of coelomic organs and blood vessels along with Doppler wave patterns in loggerhead sea turtles were described (Valente *et al*., 2008). Ultrasonography is well established as a rapid non-invasive and non expensive method of assessing the soft structures. Therefore, it serves as a helpful tool in clinical diagnosis of rescued and rehabilitated sea turtles. Application of ultrasonographic techniques in regular practice at rehabilitation centers helps in the easy ante-

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mortem diagnosis of various pathological conditions. Utilizing ultrasonography in sea turtles warrants standardization of technique as a priority step to study normal ultrasonographic indices of gastro-intestinal system.

MATERIALS AND METHODS

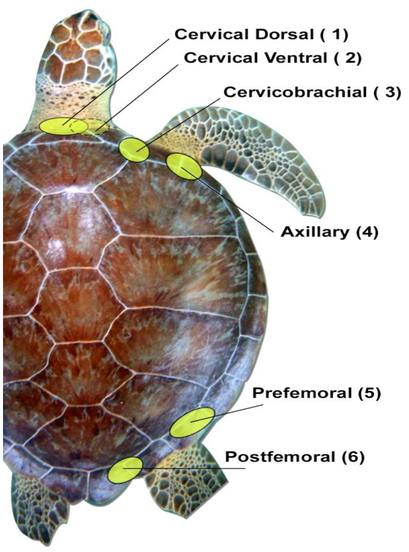
The research work was carried out on stranded Olive Ridley sea turtles (*Lepidochelys olivacea*) at various period of time which was due to ingestion of fish hook, entanglement in fishing nets as well as collision with boat propellers and was maintained in the rehabilitation facilities at the rescue center at Trust for Environmental and Education (TREE) Foundation, Vettuvankeni, Chennai, Tamil Nadu.

The rescued Olive Ridley sea turtles were manually restrained on pneumatic automobile tubes with high enough to avoid injury to the handler in addition to that protective gloves were used by handler to avoid injuries from flipper blows and bites. The sedation was not essential and head, neck, limbs were extended as necessary. While, both in-water examination and out-water examination were done by placing a clean moist cloth on the carapace of turtle. The soft-tissue areas of integument which was used as acoustic windows were cervical- dorsal acoustic window, cervical-ventral acoustic windows, right and left cervicobrachial, right and left axillary, right and left prefemoral, right and left postfemoral acoustic windows (Fig 1). Ultrasonographic examinations were performed with a real time, B-mode scanner (Esoate Mylab-20) using sector electronic transducers and frequencies which were used are 3.5, 5.0 and 7.0MHz. The coupling gel was placed on the surface of the transducer and was oriented mainly on the horizontal plane as oriented parallel to the plastron and carapace (Valente *et al*., 2007, Wyneken and Witherington, 2001 and Stetter, 2006).

RESULTS AND DISCUSSION

Ultrasonographic studies of seven rescued Olive Ridley Sea Turtle revealed the following information on the gastrointestinal system: The esophagus was visualized at the ventral-cervical and left-cervicobrachial acoustic windows (Fig 2). The echogenecitiy of lumen was coarser echogenic folds (keratinized papillae) were visualized on cross sections (Fig 3). Stratification of layers of wall was appreciated. The mean±S.E measurement of the wall thickness leaving the esophageal folds was about 0.30±0.02 cm. The contraction and relaxation of

ACOUSTIC WINDOWS



ECHOES ACCESS

1. The echoes pass obliquely cranial to the edge of the carapace (nuchal scute)
2. The echoes pass in parallel dorsally to the plastron, between the cranial edge (epiplastron bone), medial ends of coracoid bones and ventral face of the cervical vertebra.
3. The echoes pass between the coracoid bone and the acromion process of the scapula.
4. The echoes pass between the shoulder joint and the cranial border of the bridge joining the carapace and plastron.
5. The echoes pass between the caudal border of the bridge joining the carapace and the plastron and cranial face of the femur.
6. The echoes are limited dorsally by the carapace and the proximal end of the femur.

Acoustic-window and echo accesses used for the ultra-sound scan of sea turtles. The diagram shows only the right side.

Fig 1: Acoustic windows.

esophagus was captured which revealed normal esophageal motility

The visualization of stomach was made in one out of seven cases through the left axillary acoustic window (Fig 4). The image obtained on ultrasonography was a partial cross section image of stomach with irregular inner wall and fluid within the lumen.

The intestines both large and small intestine was visualized in the prefemoral acoustic window (Fig 5). The small intestine was stratified with five layers as serosa, muscularis, submucosa, mucosa and lumen. The mean±S.E thickness of the layers was 1.40±0.06 cm. The large intestine was visualized with thick wall and stratification was not appreciated as that of small intestine. The mean±S.E thickness of wall measured was 1.52±0.08 cm.

Normal intestinal loops (Fig 6) in four cases (4/7), thickened intestinal loops in one (1/7) and fluid filled loops in one (1/7) gas filled intestinal loops in one (1/7) was identified on ultrasonographic examination.

Coelomic fluid and (free fluid) was identified in two cases (Fig 7) and an inflammatory change was recorded in one of the recently rescued turtle in the cervico-brachial and in coelomic space adjacent to kidneys.

Liver was identified from the prefemoral acoustic windows (Fig 8) as longitudinal section with echogenic and granular parenchyma measuring 6.98±0.08 cm when measured from cranial to caudal. Hepatic vessels were visualized as anechoic tubular structures; gall bladder was visualized at the prefemoral acoustic windows as an anechoic spherical to oval in structure.

The morphology of sea turtles is adapted to its feeding habits which were evident even in esophagus which is marked by cervical papillae and was evicted towards stomach (Porter 1972; Wyneken and Witherington, 2001; Pressler *et al.,* 2003; Wilkinson, 2004; Magalhaes *et al.,* 2012).

According to Bleakney (1965) and Wyneken and Witherington, (2001) papillae in esophagus helps in transport/ facilitate the food to stomach by removing excess water and avoid regurgitation during diving. Microscopically these esophageal mucosa are made of keratinized squamous epithelium which protect them against damages caused by friction generated by food passage and also absence of glands suggest it acts only as organ food transport.

Ultrasonographic studies conducted by Valente *et al.* (2008) and Majo *et al.* (2016) described esophagus (Fig 2) as a coarse echogenic structure identified as the keratinized papillae when visualized through the distal end of the esophagus at the ventral-cervical and left cervicobrachial acoustic window. The echogenecitiy was coarse with visualization of the echogenic folds with the cross sections of the esophageal lumen was in accordance with studies of the previous authors who worked in sea turtles.

There was no pathological change recorded ultrasonographically in the present study in all the seven rescued Sea Turtle. The thickness of wall by Chen *et al.* (2015) described various measurements of esophagus in

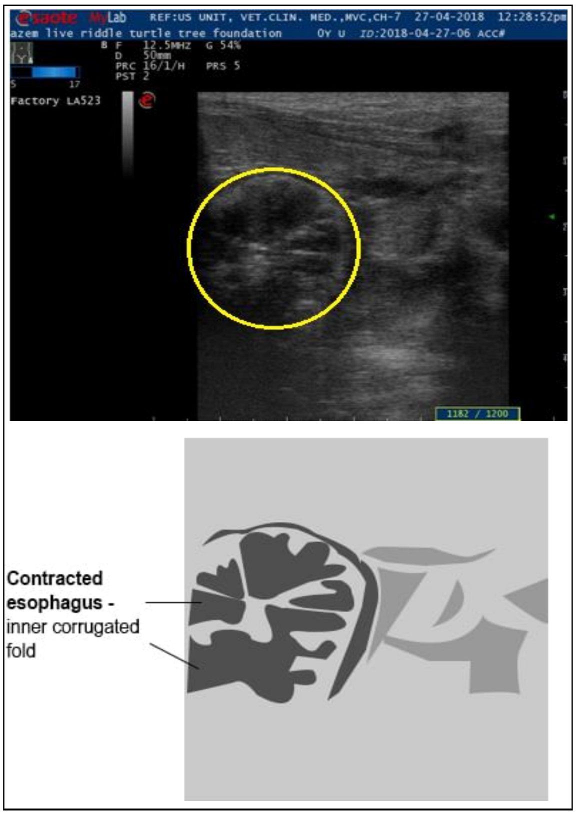


Fig 2: Contracted oesophagus - inner corrugated fold.

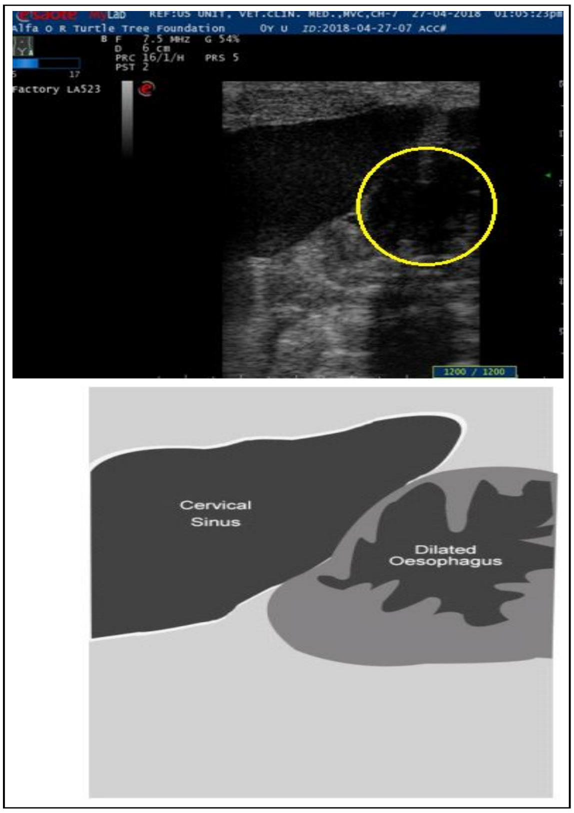


Fig 3: Dilated oesophagus with mild corrugation - keratinized papillae (marked area).

green turtle hatchlings as 0.1856 cm (1856 mm) and rest of the all layers are 0.05 cm (514 mm).The measurement of esophageal wall leaving the esophageal fold in the present study of Olive Ridley Sea Turtles revealed 0.30 cm (30 mm). The measurement of the esophageal wall however needs further studies in correlation with anatomical and histological section using the dead specimens whichever obtained for postmortem studies. However, the ultrasonographic measurements of the wall thickness as 0.05 cm which was done in the green turtle could not be correlated with the present findings as the study was conducted in the hatchlings and they missed few layers in measurements due to their very small size.

In the present study the stomach could not be visualized through the left prefemoral acoustic windows in six out of seven cases as suggested by Majo *et al.* (2016) the visualization was made only in one (1/7) of the case through left axillary windows which was similar to the findings of Valente *et al.* (2007) who reported that stomach was frequently imaged through the left axillary windows. In the present study stomach (Fig 4) was visualized partially as more echogenic content. The difficulty in restraining the animal during the procedure might be the reason for difficulty in visualization. However, the findings of more echogenic content in one of the case correlated with the similar findings of previous studies reported by Valente *et al.* (2007) and Majo *et al.* (2016).

The more echogenic visualization of stomach in one case might be due to presence of intra-luminal gas and food contents which was in concurrence with Valente *et al.* (2007). A true data could be obtained about pathology of stomach only when further extensive research is carried out in more number of immediately rescued, stranded or during egg laying season. Further, a detailed study on the ultrasonography in correlation to anatomy and pathology of stomach in animals could yield better results.

The large and small intestine was visualized in the prefemoral acoustic windows as described by Valente *et al.* (2007). The wall of the small intestine had stratification of five layers like those in that of mammals (Mattoon *et al.*, 2002 and Valente *et al.*, 2007).The serosa and submucosal was identified as echogenic lines. The muscular layer was hypoechoic to anechoic, among which mucosa was thickest and hypoechoic.

In the present study stratification of the layers of small intestine was identified (Fig 5) and was measured and the mean thickness was 1.40cm. Majo *et al.* (2016) described the wall thickness. Large intestine was visualized with no separate layers and this lack of layering might be due to excessive goblet cells in large intestine.

According to Majo *et al.* (2016) out of 19 cases, five had ileus with accumulation of fluid and dilated by thickened food material and bowel thickness as 1.30 to 2.25 cm and with mechanical ileus 1.70 to 3.75 cm.

In the present study there was regular peristalsis in all the cases. While two cases had intestinal thickening however the cause could not be identified, it might be due

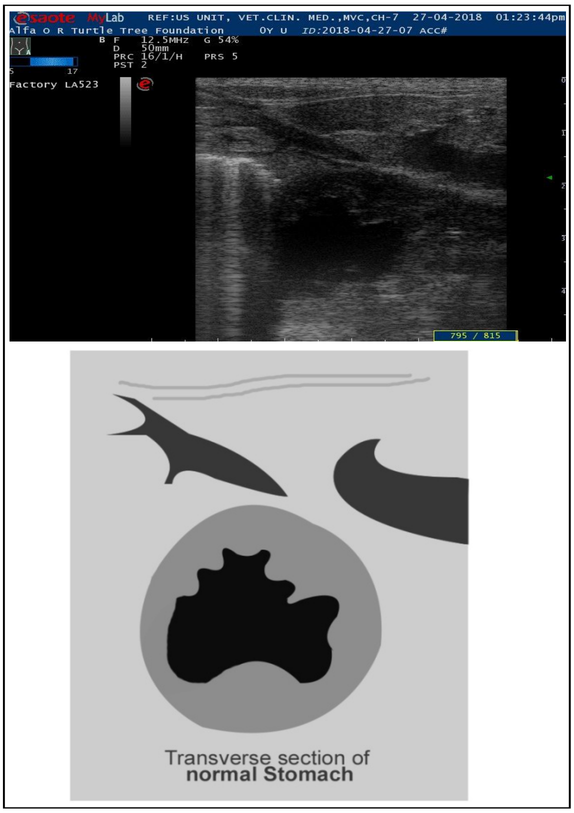


Fig 4: Stomach transverse section.

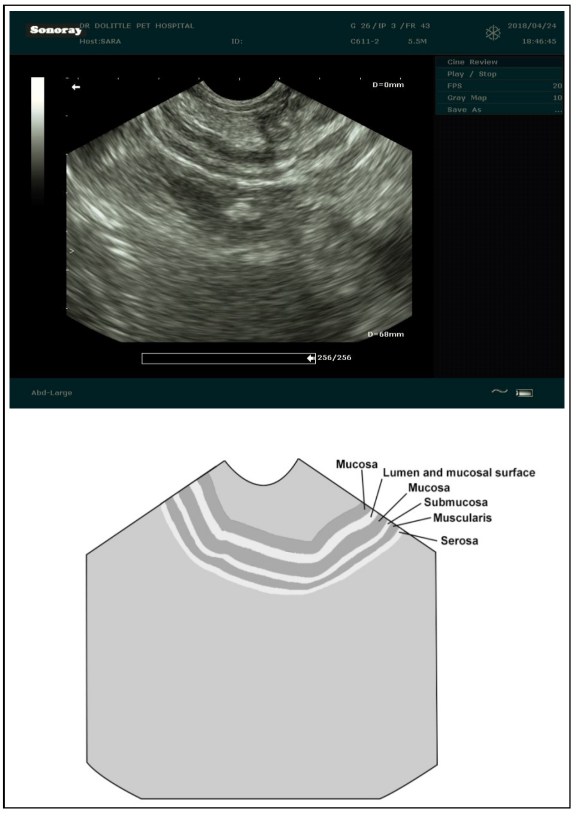


Fig 5: Intestines-longitudinal section.

to chronic inflammatory changes as discussed by Valente *et al.* (2007). In domestic mammals, cat and horses with similar pattern was observed by Diana *et al.* (2003), where there will be thickened intestinal walls due to chronic inflammatory changes.

According to Stander *et al.* (2010) ultrasonographic appearance of the gastrointestinal tract of puppies suffering from parvoviral enteritis was characterized by fluid-filled small intestine in 92.5 per cent of subjects and stomach and colon in 80.0 and 62.5 per cent of subjects respectively and observed a mild amount of anechoic free peritoneal fluid in 26 subjects. He also reported that significant increase in the mean sum of thickness of the submucosa, muscularis, muscularis and serosa was noted and in humans with Crohn's Disease has similar findings. In accordance with Maffe *et al.* (2015) bowel is considered to be thickened when the measurement of the wall is more than 3mm and opined that ultrasonography helps in identification of extra intestinal features associated with active Crohn's Diseases, such as mesenteric fat hypertrophy, enlarged regional lymph nodes and free fluid accumulation in intraperitoneal free fluid. He also suggested that stenoic intestine has a thickened wall, narrow lumen along with reduced or on peristalsis and associated with pre-stenoic dilatation greater than 25 mm in diameter with liquid and air in Lumen. So in our study the thickened intestinal loops strongly suggest chronic inflammatory changes or changes might be due to adaptations in rescue centre.

Fluid filled loops are indicative of ileus as described by Valente *et al.* (2007) and Majo *et al* (2016). Gas filled loop was noticed in one case which was in colon. Gas might be accidental entry through the cloacae as described by Majo *et al.* (2016). Echogenic content might be food material as formed in other mammals. The presence of coelomic fluid adjacent to intestines might be due to the chronic inflammation. In the present study fluid filled loops, gas filled and thickened loops recorded were in comparison with the studies of described conclusions indicating inflammatory changes of intestines and chronicity.

The liver was visualized in the prefemoral acoustic windows in all the cases. Valente *et al.* (2007) used the left and right axillary window to visualize the liver. Majo *et al.* (2016) described various per cent of visualization of liver as 52 to 63 per cent in the right and left cervico-brachial acoustic windows and 52 to 73 per cent in the right and left prefemoral acoustic window, whereas partial images of the hepatic parenchyma with transverse and oblique scan planes was also obtained through axillary windows.

CONCLUSION

Normal acoustic windows were identified and present study was hypothesized in understanding ultrasonographic imaging which would facilitate clinicians in the diagnosis of the various pathologies affecting sea turtles. This study will be enlightening to report the observed patterns as they have not been described earlier in the literature. The inability to carry out histopathological or cytopathological examinations

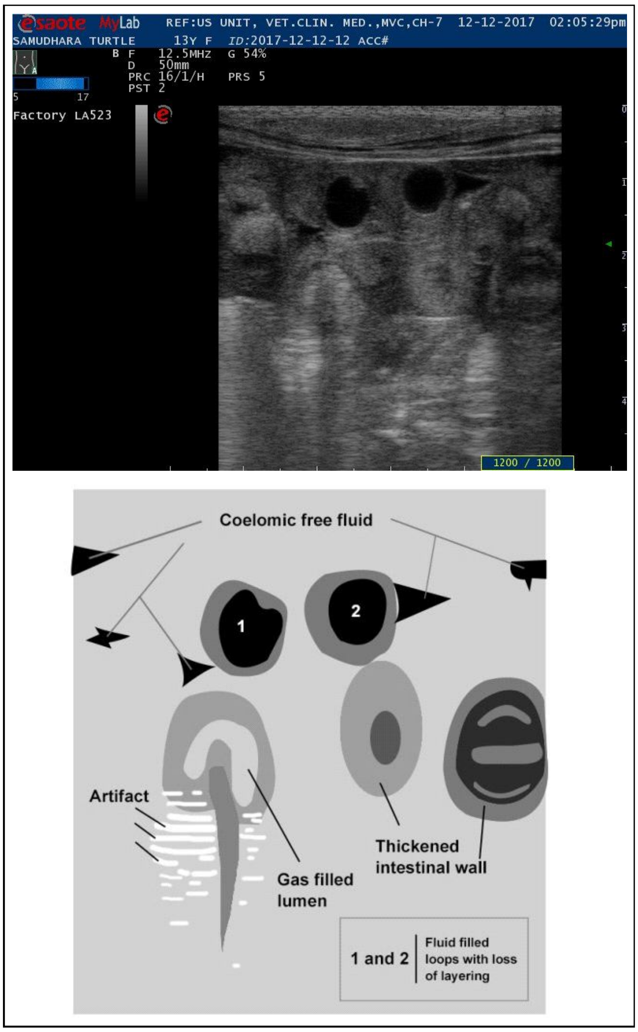


Fig 6: Normal Intestinal loops.

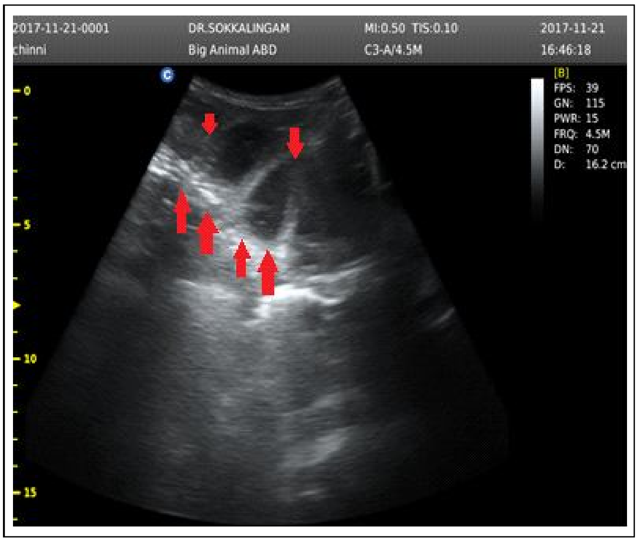


Fig 7: Intestines distended and fluid filled (arrows).

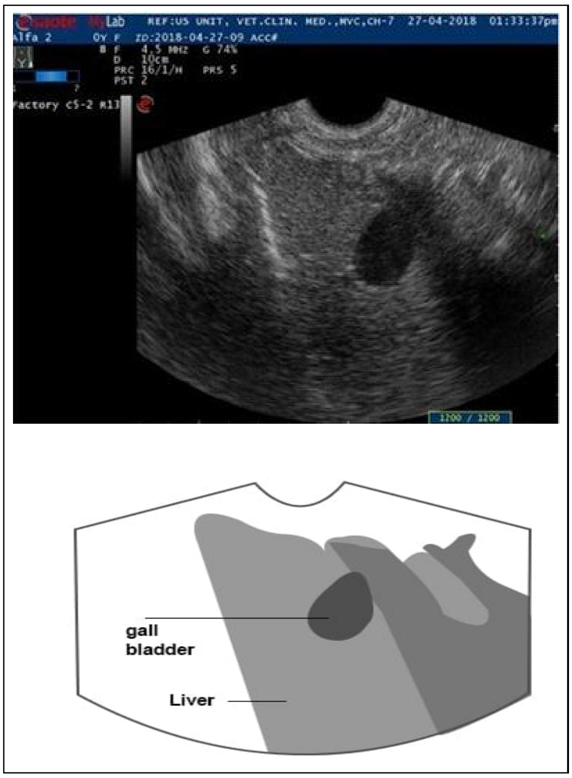


Fig 8: Liver and gall bladder.

in order to get hold of a diagnosis of some suspected ultrasound patterns may be a limitation of this work. Our study represents a novel contribution on evaluation of gastro-intestinal disorders in sea turtles.

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Author contribution

Dr.D.S- assessment of ultrasound indices and interpretation of results of ultrasound images.

Dr. M.PVR- assessment of Olive ridley invasive as well as non-invasive procedures and interpretation of results.

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