SRAS Final Report: Ultrasonographic Features of the Liver, Spleen and Gallbladder of Subadult Philippine Crocodile (*Crocodylus mindorensis Schmidt, 1935)* and Indo-Pacific Crocodile *(Crocodylus porosus Schneider, 1801*)

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ABSTRACT

The study was conducted to compare the ultrasonographic features of the liver, spleen, and gallbladder of crocodiles between two species, Philippine crocodile (*Crocodylus mindorensis)* and Indo-Pacific crocodile *Crocodylus porosus* and between sex. Twelve *C. mindorensis* with six female and six male crocodiles and twelve *C. porosus* with six female and six male crocodiles were examined using an ultrasound equipped with a 6.5 MHz linear array scanner, using the ventral aspect of the abdomen as an acoustic window. The ultrasonograms were recorded and analyzed to obtain the appearance, measurements, and echogenicity of these organs. The hepatic parenchyma was a homogenous hypoechoic structure with granular texture interspersed with anechoic vessels. The gallbladder appeared as an anechoic round to slightly elongated structure with hyperechoic capsule embedded in the hepatic parenchyma. The spleen appeared as an oval-shaped structure with fine homogenous texture covered by a hyperechoic capsule. The spleen was observed to be more hypoechoic compared to the liver. Statistical analysis showed no difference in the ultrasonographic features of the liver, spleen, and gallbladder between species, and between sex. The ultrasonographic features, dimension, and echo values obtained can serve as a baseline for comparison and diagnosis of diseases in these crocodilian species.

Keywords: *gallbladder, Indo-Pacific crocodile, liver, Philippine crocodile, spleen, ultrasonography*

INTRODUCTION

Ultrasonography is a diagnostic tool that has many potential uses in reptile medicine (Stetter, 2006). It is a non-invasive procedure that provides real-time images of the internal body. It is a convenient method of assessing the anatomic size, location, and structure of an organ.

In reptiles, mainly crocodiles, ultrasonography is used to detect symptoms of illness and assess health status. Diagnostic examinations such as hematology and biochemistry provide insufficient information about the organ, thus ultrasonography is often performed. Few studies concerning the use of ultrasonography in crocodiles have been published or made.

Crocodylus mindorensis and *Crocodylus porosus* are the two crocodilian species found in the Philippines. *Crocodylus mindorensis* is an endemic species in the Philippines while *Crocodylus porosus* exists over the Indo-Pacific region (Huchzermeyer, 2003). Over time, the population of both crocodilian species has declined and is continuously facing serious threats. There is a limited

study on these crocodilian species despite their conservation and economic value. Only two veterinary textbooks are devoted to crocodiles (Martelli, 2019).

At present, data on ultrasonography in crocodiles are limited to reproductive organs. Commonly, these are about assessment of reproductive status using ultrasonography. There are no known published data on ultrasonography of the liver, gallbladder, and spleen of *Crocodylus mindorensis* and *Crocodylus porosus*.

The aim of this study was to obtain new knowledge about the use of ultrasonography in assessing the liver, spleen, and gallbladder of *Crocodylus mindorensis* and *Crocodylus porosus*. The study was conducted to provide references for information of ultrasonographic features of liver, gallbladder, and spleen that can be used for comparison and diagnosis of diseases in these crocodilian species.

MATERIALS AND METHODS

The protocols for the study have been approved by the Biodiversity Management Bureau of the Department of Environment and Natural Resources (DENR-BMB) and Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Los Baños.

Animals

Twelve subadult *Crocodylus mindorensis*, six males and six females, weighing 2.75- 20.3 kg and twelve subadult *Crocodylus porosu*s, six males and six females, weighing 4.5 – 18.23 kg were used in the study. The snout to tail length of subadult *C. mindorensis* were between 0.97 meter to 1.67 meters while the snout to tail length of subadult *C. porosus* were between 1.16 meters to 1.68 meters. The crocodiles were obtained from Palawan Wildlife Rescue and Conservation Center. As of 2009, there were 574 *C. mindorensis* in the facility (Hinlo, 2010). Only apparently healthy crocodiles were used in the study.

Ultrasound equipment

An ultrasound machine, Chison® Digital Color Doppler Ultrasound System ECO 5VET Echo 5 (manufactured by Chison Medical Imaging Co., Ltd) with a 6.5 MHz linear arrayscanner was used for the scanning the liver, spleen, and gallbladder. Obtained ultrasonograms were saved and recorded in a removal disk for further analysis.

The ultrasonograms obtained were analyzed using Adobe Photoshop CC 2019. The length, width, circumference, and area were measured, and echo mean values were determined using the digital analysis software.

Physical examination and selection

Only apparently healthy subadult *C. mindorensis* and *C. porosus* were selected for the study. Zookeepers assisted with the selection of subadult crocodiles. The crocodile species were identified based on morphology. Sexing was done prior to examination. Body weight, tail girth and body length (tail to snout and vent to snout) were measured and recorded. Physical examination was conducted starting from the head to the tip of the tail. Discharges of any kind were not observed on the subject. The scales covering the body, including the underside, were

examined thoroughly for external parasites or presence of any lesions and inflammation. Animals with lesions were replaced with apparently healthy ones.

Restraint and handling

Subadult crocodiles were restrained and handled properly by the skilled zookeepers. The handlers wore protective clothing such as long-sleeved shirts and boots to prevent wounds and accidents. Initially, target crocodiles were isolated from the rest of the group using a long wooden stick, after which, a rope was placed around the neck to pull the crocodile out of the water. While tugging the rope around the neck, the tail was grasped to completely immobilize the crocodile. Another handler from behind the animal secured the mouth by tying elastic rubber bands. The restrained crocodile was placed in a towel then weighed before transport to the study facility. While lying flat on the table, a co-adhesive tape (Vetwrap®) was placed over the eyes to prevent further stress to the animal. The animal was placed in dorsal recumbency while being held down by three handlers.

Ultrasonography

The gain of the ultrasound was adjusted and fixed to 130 reaching a midlevel echogenicity. A liberal amounts of ultrasound gel was applied on the scales to improve penetration of sound waves and image quality. The organs of concern were the liver, spleen, and gallbladder. There were three acoustic windows for viewing these organs in ultrasonography: left flank, right flank, and abdomen. The abdomen was used as the acoustic window for viewing these three organs. A 6.5 MHz linear array scanner was applied on the scales of the ventral abdomen, starting from cranial to caudal then medial to lateral sides of the body to view the liver, spleen, and gallbladder. The bilobed liver envelopes the heart of crocodiles. The heart may serve as landmark for visualizing the liver. The morphology, thickness and echogenicity of the liver was obtained. The scanner was moved sideways to further observe the liver. The scanner was moved midway to locate the gallbladder and the spleen. The spleen lies close to the gallbladder and can be viewed with same acoustic windows as the gallbladder. All the organs were described, and their dimensions and echogenicities were obtained and recorded.

Statistical analysis

All values obtained from the different parameters were tested for normality using Shapiro- Wilk test. Normal values were subjected to student T-Test to test for presence of significant variation between sex and species at $\alpha=0.05$, otherwise non-normal values were subjected to Mann-Whitney test.

RESULTS AND DISCUSSION

The thick scales and osteoderms of the crocodiles interfere with obtaining good quality sonogram of the organs. To solve the problem, a liberal amount of gel was applied on the scales before scanning. This improved the image quality of the scanned organs. The thick scales and osteoderms of reptiles can affect the penetration of sound waves thus resulting to poor image quality (Stetter, 2006). Ultrasound gel absorbed by scales for 30 minutes may improve the image quality according to Stetter (2006). Using generous amount of ultrasound gel can help in obtaining the best images in reptiles with thick, calcified scales (Peers, 2011).

Uniform setting of the ultrasound machine is imperative for comparative measurement of echogenicity of the organs among the study population. The gain was fixed at 130 reaching a midlevel echogenicity. A homogenous image quality, neither hypoechoic nor hyperechoic, matters to prevent misdiagnosis (Silkowski, 2010). Low frequency linear transducers, 5 MHz and 3 MHz are recommended for large reptiles such as crocodiles (Peers, 2011). Low frequency allows an adequate depth of wave penetration through the reptilian skin (Hochleithner and Sharma, 2019). In this study, a 6.5 MHz linear array scanner was used to scan the liver, spleen, and gallbladder. It provides good quality images of the organs of subadult *Crocodylus mindorensis* and *Crocodylus porosus*. The three main organs assessed in the study were liver, gallbladder, and spleen.

The ultrasonogram of the hepatic parenchyma appears as homogenous hypoechoic structure with granular texture interspersed with anechoic vessels surrounded with hyperechoic outline (Figure 1). A hyperechoic subcutaneous fat body may also be observed. The ultrasonogram of the liver of *Crocodylus mindorensis* (Figure 1A and B) and *Crocodylus porosus* (Figure 1C and D) are similar in appearance. Longitudinal and transverse scans of the liver were obtained to measure the thickness (maximum vertical length) and echogenicity. No significant differences were observed in the mean thickness and mean echogenicity of the liver between the two species and between sex (Table 1).

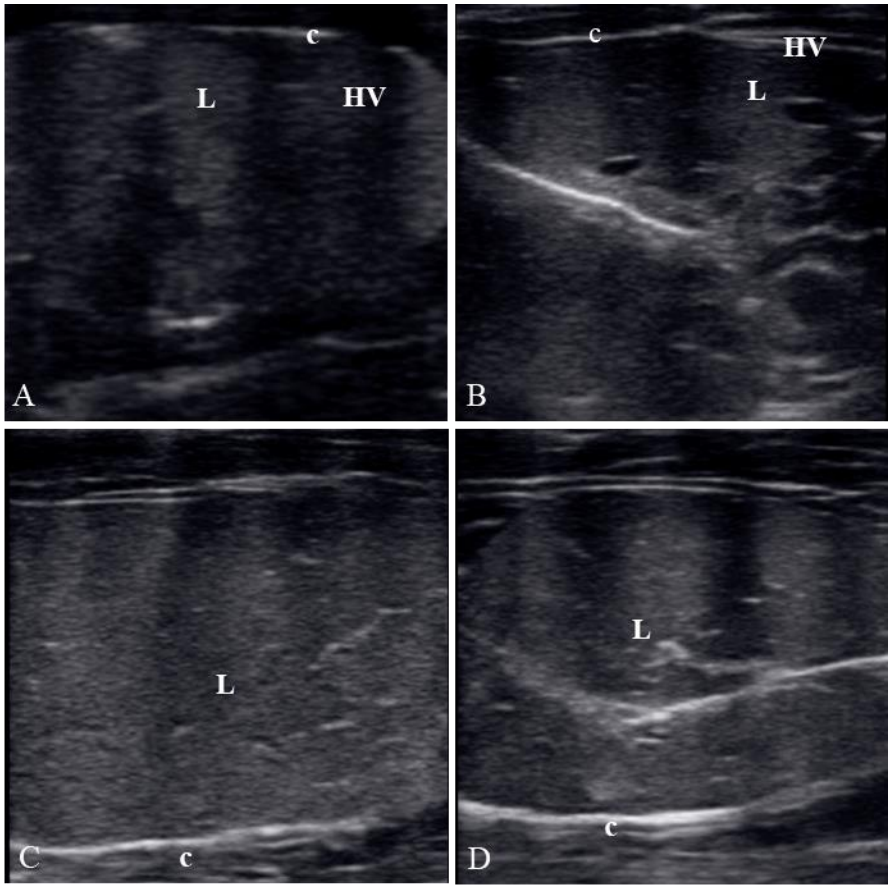


Figure 1. Ultrasonographic features of the liver of (**A**) female *Crocodylus mindorensis* (**B**) male *Crocodylus mindorensis* **(C)** female *Crocodylus porosus* and (**D**) *male Crocodylus porosus* (L= liver parenchyma; c=capsule; HV=hepatic blood vessels).

Table 1. Mean ± SD thickness, width and echo mean values of the hepatic parenchyma of *Crocodylus mindorensis* and *Crocodylus porosus*. No significant differences were observed between groups by sex and species ($p > 0.05$).

	Crocodylus mindorensis			*Crocodylus porosus*		
	Male	Female	Mean	Male	Female	Mean
Thickness (cm)	3.81 ± 0.57	3.47 ± 0.60	3.64 ± 0.58	3.77 ± 0.99	3.86 ± 0.14	3.77 ± 0.81
Echo mean	35.96 ± 6.80	42.92 ± 20.79	39.44 ± 15.19	46.36 ± 12.55	49.66 ± 18.31	48.01 ± 15.06

The liver of the crocodiles is composed of two lobes with the right lobe larger than the left lobe. Bilobed livers are also present in lizards and chelonians (Hochleithner and Sharma, 2019). The liver of the crocodile can be visualized on the ventral abdomen. This follows the window for imaging liver in crocodiles as described by Martelli (2019). The heart served as a landmark for visualizing the liver. The liver can be scanned caudal to the heart.

The hyperechoic outline surrounding the hepatic parenchyma is the Glisson's capsule. Similar with the encapsulated liver of pythons and lizards, the liver of the crocodiles is covered by Glisson's capsule (Van Wilpe, 2012). According to Hochleithner and Sharma (2019), the hepatic parenchyma of the lizards and chelonians is homgenous relatively hypoecehoic with anechoic blood vessels. This is comparable to the hepatic parenchyma of *Crocodylus mindorensis* and *Crocodylus porosus*. In a study by Martelli and Kot (2012), the ultrasonogram of hepatic parenchyma of *Crocodylus siamensis* appears as homogenous hypoechoic structure with granular appearance. It can be implied that the appearance of the hepatic parenhcyma is similar among the crocodilian species. In all reptiles, the liver has a uniformly hypechogenic structure with anechogenic blood vessels (Schilliger, 2010) .

Increased echogenicity and enlargement of the liver is associated with fatty liver degeneration (Hochleithner and Sharma, 2019). Diseases such as Herpes virus, West Nile virus, chlamydiosis and mycoplasmosis cause severe diffuse hepatitis (Martelli, 2019). Hepatitis is best diagnosed n ultrasound and may appear as heterogenous pattern with anechoic areas in the parenchyma.

The ultrasonogram of the gallbladder appears as a round to slightly oval anechoic structure with a hyperechoic wall embedded in the hepatic parenchyma (Figure 2). There is no difference in the appearance of the gallbladder between *Crocodylus mindorensis* (Figure 2A and B) and *Crocodylus porosus* (Figure 2C and D). Comparison of the mean dimensions of the gallbladder showed no significant differences between the two species and sexes as shown in Table 2. However, statistical analysis of the mean echogenicity of the male *Crocodylus porosus* is significantly larger than the male *Crocodylus mindorensis.*

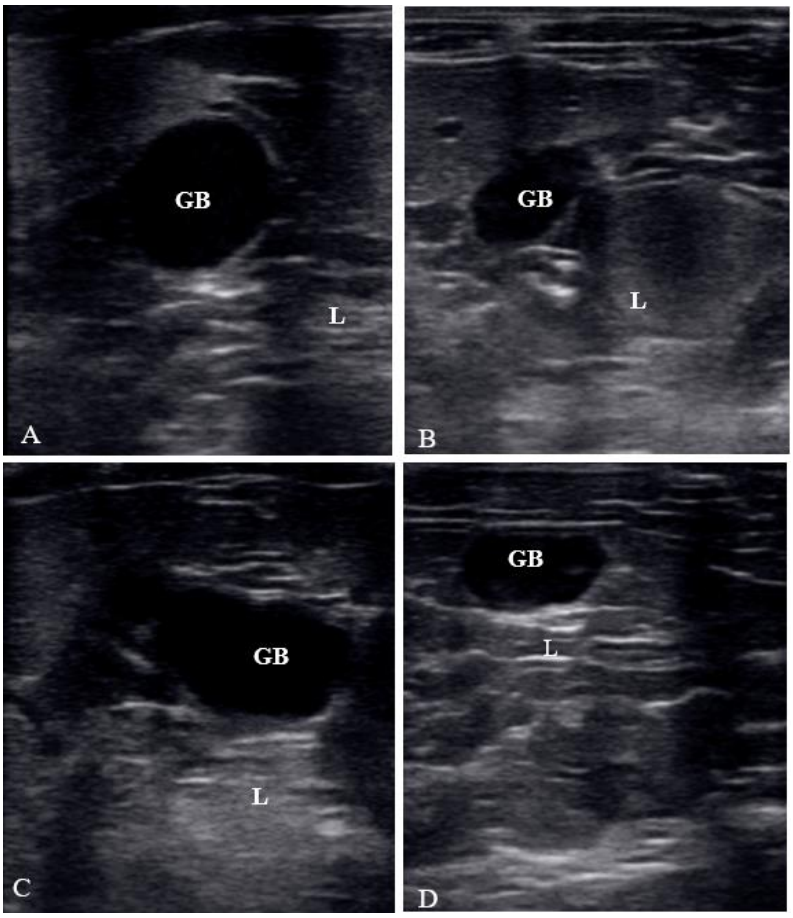


Figure 2. Ultrasonographic features of the gallbladder of (**A**) female *Crocodylus mindorensis* (**B**) male *Crocodylus mindorensis* **(C)** female *Crocodylus porosus* and (**D**) *male Crocodylus porosus.* (GB=gallbladder; L= liver parenchyma)

The gallbladder is attached to the right lobe of the liver in crocodiles (Nevarez, 2019). Similar with lizards and chelonians, the gallbladder is present in the right lobe of the liver (Hochleithner and Sharma, 2019). The gallbladder of the crocodile is associated with the hepatic parenchyma unlike in snakes, where the gallbladder is separated. The ventral abdomen is an ultrasonographic window for visualizing the gallbladder (Martelli, 2019). The transducer was placed on the ventral aspect of crocodile to view the gallbladder. Locating the hepatic parenchyma is a convenient way of finding the gallbladder.

The anechoic sonogram appearance of the gallbladder is similar with the lizards, chelonians, and snakes (Hochleithner and Sharma, 2019). Presence of echogenic materials in the gallbladder may indicate thickening of the bile (Hochleithner and Sharma, 2019). Choleliths or gallbladder stones may be an incidental finding when conducting ultrasound. These can result from a mild infection in crocodiles (Huchzermeyer, 2003). The stones appear as granular or focal area with or without acoustic shadow.

Table 2. Mean ± SD length, width, area, circumference, and echo mean values of the gallbladder of *Crocodylus mindorensis* and *Crocodylus porosus.* Means in the same row in the same group with superscripts are different ($p < 0.05$).

	Crocodylus mindorensis			*Crocodylus porosus*		
	Male	Female	Mean	Male	Female	Mean
Length (cm)	1.69 ± 0.47	1.22 ± 0.60	1.46 ± 0.57	1.25 ± 0.40	1.39 ± 0.35	1.32 ± 0.37
Width (cm)	1.72 ± 0.52	1.18 ± 0.51	1.45 ± 0.57	1.98 ± 0.85	1.28 ± 0.41	1.63 ± 0.73
Area (cm^2)	2.40 ± 1.10	1. 43 ± 0.92	1.92 ± 1.09	3.39 ± 2.15	1.81 ± 0.83	2.60 ± 1.76
Circumference (cm)	6. 32 ± 2.21	4.46 ± 1.86	5.39 ± 2.18	8.38 ± 3.05	5.57 ± 1.90	6.97 ± 2.83
Echo mean	0.20 ± 0.29[a]	0.91 ± 0.70	0.55 ± 0.63	1.93 ± 1.54[a]	1.54 ± 1.29	1.74 ± 1.37

The spleen appears as a fine homogenous echogenic oval-shaped organ surrounded with a hyperechoic capsule (Figure 3). The appearance of the spleen is similar between *Crocodylus mindorensis* (Figure 3A and B) and *Crocodylus porosus* (Figure 3C and D). The area of the spleen of female *Crocodylus porosus* is significantly larger than the female *Crocodylus mindorensis* (Table 3). In the study, the spleen of the crocodiles was observed to be more hypoechoic compared with the liver. The echo mean value of the spleen of *Crocodylus mindorensis* is 20.60 ± 11.02 and *Crocodylus porosus* is 28.20 ± 14.36 while the echo mean value of the liver of *Crocodylus mindorensis* is 39.44 ± 15.19 and *Crocodylus porosus* is 48.01 ± 15.06, thus the spleen is more hypoechoic.

The spleen lies on the dorsal mesentery close to the duodenal loop (Huchzermeyer, 2003). The shape of the spleen resembles a pear with a broad cranial end. The spleen may be round to elongated, depending on the reptile species. Visualizing the spleen on ultrasonography can be difficult because the organ is positioned deep to the intestine (Hochleithner and Sharma, 2019). One method of visualizing the spleen is finding the gallbladder, then locating the spleen below it (Martelli and Kot, 2012). This method was effective in locating the spleen in the study.

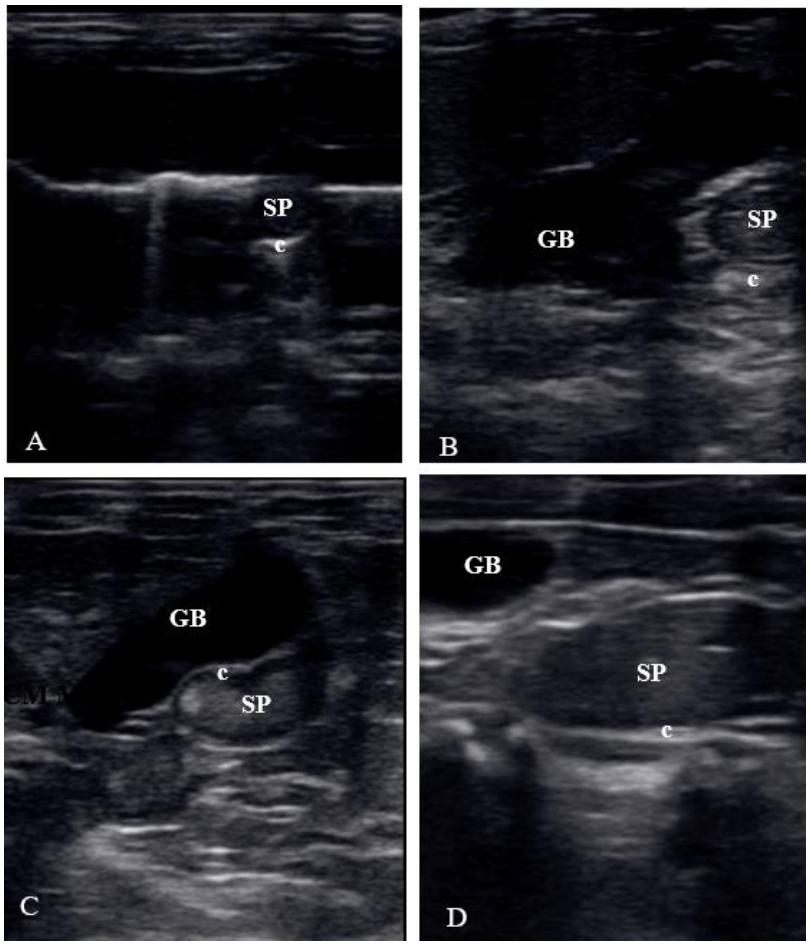


Figure 3. Ultrasonographic features of the spleen of (**A**) female *Crocodylus mindorensis* (**B**) male *Crocodylus mindorensis* **(C)** female *Crocodylus porosus* and (**D**) *male Crocodylus porosus.* (SP=spleen; c=capsule; GB=gallbladder).

The spleen is covered by a strong capsule (Huchzermeyer, 2003). The splenic capsule is the hyperechoic outline surrounding the splenic parenchyma. According to Hochleithner and Sharma (2019), the texture of the spleen is similar with the testes. The ultrasonogram of the spleen in *Crocodylus siamensis* appears as homogenous hypoechoic structure with hyperechoic outline (Martelli and Kot, 2012). This is similar with the hypoechoic spleen of *Crocodylus mindorensis* and *Crocodylus porosus*. The spleen of lizards and snakes is observed to be markedly echogenic than the liver (Martelli, 2019).

Increased size of the spleen, splenomegaly, indicates infection or septicemia (Huchzermeyer, 2003). Changes in the echogenicity of the splenic parenchyma may indicate a pathological disease. Diffuse splenic change can occur from lymphoma or mast cell neoplasia while focal splenic change can be associated with benign or malignant neoplasia (Mannion, 2006b).

Ultrasonography is a non-invasive diagnostic imaging tool useful for assessment of the liver, spleen, and gallbladder of *Crocodylus mindorensis* and *Crocodylus porosus*. It is effective in obtaining size, shape, and margination of the organs in crocodiles.

Table 3. Mean ± SD length, width, area, circumference, and echo mean values of the spleen of *Crocodylus mindorensis* and *Crocodylus porosus.* Means in the same row in the same group with superscripts are different ($p < 0.05$).

	Crocodylus mindorensis			*Crocodylus porosus*		
	Male	Female	Mean	Male	Female	Mean
Length (cm)	1.64 ± 0.48	1.27 ± 0.29	1.46 ± 0.42	1.30 ± 0.33	1.07 ± 0.17	1.19 ± 0.28
Width (cm)	1.45 ± 0.33	1.34 ± 0.33	1.39 ± 0.32	1.33 ± 0.27	1.54 ± 0.20	1.43 ± 0.25
Area (cm^2)	2.66 ± 0.97	1.79 ± 0.59^a	2.22 ± 0.89	2.27 ± 0.78	2.54 ± 0.47^a	2.41 ± 0.63
Circumference (cm)	6.57 ± 1.62	5.22 ± 0.96	5.90 ± 1.45	5.87 ± 1.23	6.30 ± 0.71	6.08 ± 0.98
Echo mean	24.39 ± 13.32	16.81 ± 7.43	20.60 ± 11.02	22.29 ± 9.80	34.12 ± 16.54	28.20 ± 14.36

CONCLUSION

Ultrasonography is a non-invasive diagnostic tool that is valuable in reptilian medicine. However, few and limited studies provide information concerning use of ultrasonography in crocodiles. This is the first study comparing the ultrasonographic features of the liver, spleen, and gallbladder of crocodiles between species, *Crocodylus mindorensis* and *Crocodylus porosus*, and between sex.

Twelve apparently healthy subadult *Crocodylus mindorensis* with six male and six female crocodiles and 12 apparently healthy subadult *Crocodylus porosus* with six male and six female crocodiles were selected for the study. An ultrasound machine equipped with 6.5 MHz linear array scanner was used to examine the liver, spleen, and gallbladder of crocodiles positioned in dorsal recumbency. The ventral aspect of the abdomen served as the acoustic window for the examination of all crocodiles. The appearance, dimensions and echogenicity of the liver, spleen, and gallbladder in the ultrasonograms were obtained and analyzed.

The hepatic parenchyma appeared as homogenous hypoechoic structure with granular texture interspersed with anechoic vessels. The parenchyma is surrounded by a hyperechoic capsule. The heart served as a landmark for locating the liver. The gallbladder is embedded in the hepatic parenchyma. It is an anechoic round to slightly elongated structure with hyperechoic capsule. The spleen is located caudal to the gallbladder. It appears as oval-shaped structure with fine

homogenous texture covered by a hyperechoic capsule. The spleen was observed to be more hypoechoic compared to the liver.

No significant differences were observed in the mean thickness and mean echogenicity of the liver between the two species and between sex. Comparison of the mean dimensions and mean echogenicity values of the gallbladder showed no significant differences between the species. However, the mean echogenicity of the male *Crocodylus porosus* is significantly larger than the male *Crocodylus mindorensis.* Statistical analysis of the mean length, width, circumference, and echogenicity values of the spleen showed no significant differences across the two species and across sex. However, the area of the spleen of female *Crocodylus porosus* is significantly larger than the female *Crocodylus mindorensis*

The results of the study suggest no difference in the ultrasonographic features of the liver, spleen, and gallbladder between species, *Crocodylus mindorensis* and *Crocodylus porosus*, and between sex. The ultrasonographic features, dimension and echo values obtained from apparently healthy subadult crocodiles can serve as a baseline for comparison and diagnosis of diseases in these crocodilian species using ultrasonography.

For further studies, comparison of the ultrasonographic features of the liver, spleen, and gallbladder in adult *Crocodylus mindorensis* and *Crocodylus porosus* can be performed. The data obtained can be used to compare and assess changes with the ultrasonographic features of the subadult and adult crocodiles. One of the limitations encountered in the study was poor image quality due to thick scales of the crocodiles. The effect of increased ventral thickness in obscuring the image quality can also be assessed by comparing the echogenicity and sonograms of the subadult and adult crocodiles.

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