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Ultrasonographic features of liver, gallbladder, spleen, kidneys, and urinary bladder of rescued long-tailed macaques, *Macaca fascicularis*

Paul CARDENIO*, Jezie ACORDA, Emilia LASTICA-TERNURA
Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of the Philippines Los Banos, Laguna, Philippines

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Abstract: This study was conducted to determine the ultrasonographic features of the liver, gallbladder, spleen, kidneys, and urinary bladder of 24 apparently healthy male and female long-tailed macaques (LTM) and the correlations of organ measurements with body weight and crown–rump length. The animals were grouped into 12 males and 12 females. Examination was performed in sedated LTM using an ultrasound machine with 6.0 MHz microconvex scanner. Ultrasound appearance, dimensions, and echotexture of the liver, gallbladder, kidneys, spleen, and urinary bladder were evaluated. The results showed that there were no sex-related differences in echo mean values, thickness, and length of the selected abdominal organs. The liver was hypoechoic to isoechoic with the right renal cortex, while spleen was isoechoic to hyperechoic to left renal cortex. Spleen was hypoechoic to isoechoic to the liver. Gallbladder and urinary bladder had hypoechoic to hyperechoic thin wall with anechoic lumen. There was a statistically significant and moderate positive correlation between the volume of the left kidney and body weight. The right kidney volume was greater than the left kidney and the total renal volume had a statistically significant and moderate positive correlation with body weight. This study established the reference values for ultrasonographic features of selected abdominal organs of rescued LTM.

Key words: Kidney, liver, long-tailed macaques, spleen, ultrasonography

1. Introduction

The long-tailed macaque, *Macaca fascicularis* (Raffles, 1821), also known as cynomolgus monkey, is a nonhuman primate species belonging to Cercopithecine group of Old World monkeys and is native to Southeast Asia. They are captive bred and raised legally and are frequently used in laboratories for biomedical researches [1,2].

Diagnostic ultrasonography is a noninvasive imaging tool commonly employed in wildlife including monkeys. Use of ultrasound to evaluate abdominal organs of monkeys has been reported in various species such as rhesus monkey (*Macaca mulatta*) [3], common marmoset (*Callithrix jacchus*) [4], owl monkey (*Aotus* sp.) [5], vervet monkey (*Chlorocebus sabaeus*) [6], and howler monkeys (*Alouatta fusca*) [7]. Abdominal ultrasonography of cynomolgus monkeys was previously studied, particularly the kidney [8], liver and gallbladder [9], and uterus [10]. Previous studies examined imported captive-bred female monkeys in the Philippines and male monkeys originated from Cambodia [8,9]. No ultrasonography study was conducted in other abdominal organs of cynomolgus monkey such as spleen and urinary bladder.

In addition, no ultrasonographic studies have yet been conducted in abdominal organs of the long-tailed macaques kept at local captivity. Hence, there may be variations in the ultrasonographic features of abdominal organs of local long-tailed macaque compared to previous ultrasonography studies in other species of laboratory and captive monkeys from other countries. Knowledge of the normal ultrasonographic anatomy is useful for diagnosis of disease and health assessment of injured captive and recently rescued long-tailed macaques. Moreover, ultrasonographic features can be used for postoperative monitoring of surgical patients, evaluation of abnormal structures, and ultrasound-guided biopsies [11,12].

It was hypothesized that the abdominal ultrasonographic evaluation and measurements of long-tailed macaques in local rescue center differ from established reference values from other laboratory-kept *M. fascicularis* from other countries. This study was conducted to determine the abdominal ultrasonographic features of liver, gallbladder, spleen, kidneys, and urinary bladder of apparently healthy male and female long-tailed macaques kept in a local rehabilitation and rescue center and the correlations of organ measurements with body weight and crown–rump length.

* Correspondence: pacardenio@up.edu.ph

2. Materials and methods

2.1. Animal and housing conditions

A total of 24 apparently healthy adult long-tailed macaques of both sexes kept at Biodiversity Management Bureau-National Wildlife Rescue and Research Center (BMB-NWRRC), Department of Environment and Natural Resources, Quezon City for at least 3 months were included in the study. Purposive sampling method was performed. Permits to conduct the study were obtained from the Institutional Animal Care and Use Committee (IACUC) - University of the Philippines Los Baños-College of Veterinary Medicine (UPLB-CVM) (Protocol number: 2018 - 0155), and Department of Environment and Natural Resources (DENR), Biodiversity Management Bureau-National Wildlife Rescue and Research Center (BMB-NWRRC) (Gratuitous Permit Number: 285).

The animals were grouped into 12 males and 12 females. The 12 males and 6 females were housed individually in a cage with squeeze-back, while the other 6 females were group-housed. The monkeys were fed once daily with fruits and vegetables, whereas water was given ad libitum. The animals were observed for 2 weeks for any behavior and physical changes. The parameters observed were body condition (weight), pelage (color, growth), face (discharge), posture (lameness, hunched), breathing, and appetite. The BMB-NWRRC Form 3 (Animal Health Record) was used during observation and physical examination. Only animals with no signs of disease based on physical examination, hematology, and blood chemistry, and observation within the last 2 weeks were considered apparently healthy.

Selected animals were fasted for 18 h. Conscious, apparently healthy monkeys were physically restrained humanely by experienced animal care technicians and 0.2 mL of zolazepam/tiletamine (Zoletil 50, Virbac, France) was administered intramuscularly for chemical restraint. Body condition, body weight, crown to rump length [13], heart and lung auscultation, rectal temperature, and dehydration were assessed after administration of a sedative.

2.2. Abdominal ultrasonography

Ultrasound examination was conducted using an ultrasound machine (Chison Digital Doppler Ultrasound System Eco 5 Vet, Chison Medical Technologies Co., Ltd., Wuxi, Jiangsu, China) with a 6.0 MHz microconvex scanner. Fixed gain setting of 100 was maintained throughout the examination. Echocardiographic evaluation was performed with the animal in right lateral recumbency, while abdominal ultrasound was performed in left, right, and dorsal recumbency. A clipper was used to shave the abdomen and lower right parasternal area of the animals. Liberal amounts of ultrasound gel were applied in the scanner and the skin. Digital images were saved in a computer for further analysis.

The protocol previously described was used with modifications [7]. The animals were examined in left lateral, right lateral, and dorsal recumbency. Ultrasound examination started with the evaluation of the liver and gall bladder, right kidney, spleen, left kidney, and urinary bladder. Optimum acoustic windows and reference borders were determined for each organ. Abdominal images of anatomical structures were evaluated for size, shape, position, echotexture, and echogenicity.

The urinary bladder was visualized in transverse and longitudinal planes. The thickness of the urinary bladder wall adjacent to the colon was measured. Kidney images were obtained in sagittal and transverse planes. The ratio between the renal cortex and medulla was determined in the sagittal plane. Kidney length and height were obtained in a longitudinal view while kidney width was measured in transverse view. The volume was measured and computed using the previously reported formula ($V = L \times W \times H \times 0.52$) [8]. Images of the spleen were visualized in longitudinal and transverse view. Spleen thickness was measured at the level of the hilus in transverse view. The liver was visualized in the sagittal and transverse planes. Thickness, echogenicity, and echotexture of liver parenchyma and vessels were measured. The shape, wall size and contents of the gallbladder were recorded.

2.3. Data management and statistical analyses

Histogram analysis and dimension measurements of images were analyzed using image analysis software (Adobe Photoshop® CC 2019). Echo mean value was obtained by evaluating a 10 × 10 mm representative section of an organ. Pearson's correlation coefficient was also used to determine the relationship between kidney sizes, body weight and crown–rump length. Pearson correlation coefficient values were interpreted as very weak (±0.00 to ±0.19), weak (±0.20 to ±0.39), moderate (±0.40 to ±0.59), strong (±0.60 to ±0.79) and very strong (±0.80 to ±1.00). Independent t-test was also used to determine differences in the means of dependent variables measured in male and female macaques. P-value < 0.05 was considered significant.

3. Results and discussion

A total of 24 adult long-tailed macaques were examined in this study. The 12 males had a mean weight of 4.3 ± 0.96 kg and mean crown–rump length of 39.37 ± 4.95 cm. The 12 females had a mean weight of 3.54 ± 0.53 kg and mean crown–rump length of 39.88 ± 3.12 cm. The reported mean weights in this study were comparable with the previous report wherein adult males aged 3.5–6 years old weighed 4.4 ± 0.83 kg, while females aged 5–6 years old weighed 3.6 ± 0.78 kg [14]. In contrast, some studies reported higher body weights in adult males (6.4 ± 1.74 kg) and females (4.5 ± 1.05 kg) aged 4–16 years old [13]. Males were significantly heavier when compared to females (P = 0.013) but did not differ significantly in crown–rump length in this study (P = 0.767). On the contrary, it was reported that males had higher crown–rump length in males when compared to females [13]. The monkeys had an average rectal temperature of 37.9 ± 0.83 °C (35.6–39.50), respiratory rate of 53.5 ± 8.57 (36–66) breaths per minute and heart rate of 193.9 ± 14.47 (156–225) beats per minute.

3.1. Liver

The liver generally had homogenous hypoechoic echotexture located within the rib cages. The intercostal view of the liver did not provide a good acoustic window to examine the liver; hence, the probe was placed in the subxiphoid region and directed cranially. Figure 1 shows the sagittal and transverse views of the liver and gallbladder. The liver displayed a mirror image artifact due to the lung-diaphragm interface appearing as a hyperechoic band. Distinct liver lobes were difficult to distinguish.

The portal vessels have hyperechoic wall when compared to the hypoechoic wall of hepatic veins (Figure 1). The mean diameter of the main portal vein was 5.2 ± 0.59 mm. This is higher when compared to the mean diameter of 4.4 mm reported earlier [9]. The liver thickness between males (43.3 ± 7.29 mm) and females (44.5 ± 4.71 mm) were not significantly different (P = 0.637). In addition, the echo mean of the liver parenchyma of males (29.9 ± 17.65) was not significantly different from those of females (22.8 ± 9.44) (P = 0.235).

The liver was isoechoic with the right renal cortex at the level of the caudate lobe. To support this observation, the echo mean value (26.4 ± 14.3) of the liver parenchyma at the subxiphoid region was not significantly different from the echo mean value of the right renal cortex (20.5 ± 14.86, P = 0.344). In contrast, it was reported that the liver parenchyma was more hypoechoic than the kidney parenchyma [9].

There was a very weak positive correlation between the thickness of liver and the body weight (r = 0.181, P = 0.389) and height (r = 0.096, P = 0.654) of long-tailed macaques; however, it was not statistically significant.

3.2. Gallbladder

The gallbladder appeared as a round structure within the liver parenchyma with homogenous anechoic fluid with no luminal debris and thin hypoechoic to hyperechoic wall (Figure 1). It was expected to be located within a fossa on the inferior surface of the large median lobe [11]. Gallbladder appeared bilobed in two males and one female. The mean thickness of the gallbladder wall was 0.8 ± 0.17 mm which is thinner when compared to gallbladder wall of howler monkeys [7]. The mean lumen diameter of males (10.8 ± 2.71 mm) was not significantly different from those of females (12.1 ± 2.85 mm) (P = 0.294). The echo mean value of the gallbladder lumen was 1.7 ± 2.09. Proper visualization of the sagittal and transverse section of the gallbladder is essential for evaluating the presence of sludge, cystolith and also for performing an ultrasound-guided cholecystocentesis in these monkeys [15].

3.3. Spleen

The spleen was visualized with the probe placed parallel to the left caudal rib cage, and it was sometimes directed cranially. It appeared as tongue-like structure adjacent to the stomach. The echogenicity of the spleen in long-tailed macaques varied from hypoechoic to dense hyperechoic parenchyma with hyperechoic capsule (Figure 2). The mean thickness of the spleen in males (16.3 ± 4.96 mm)

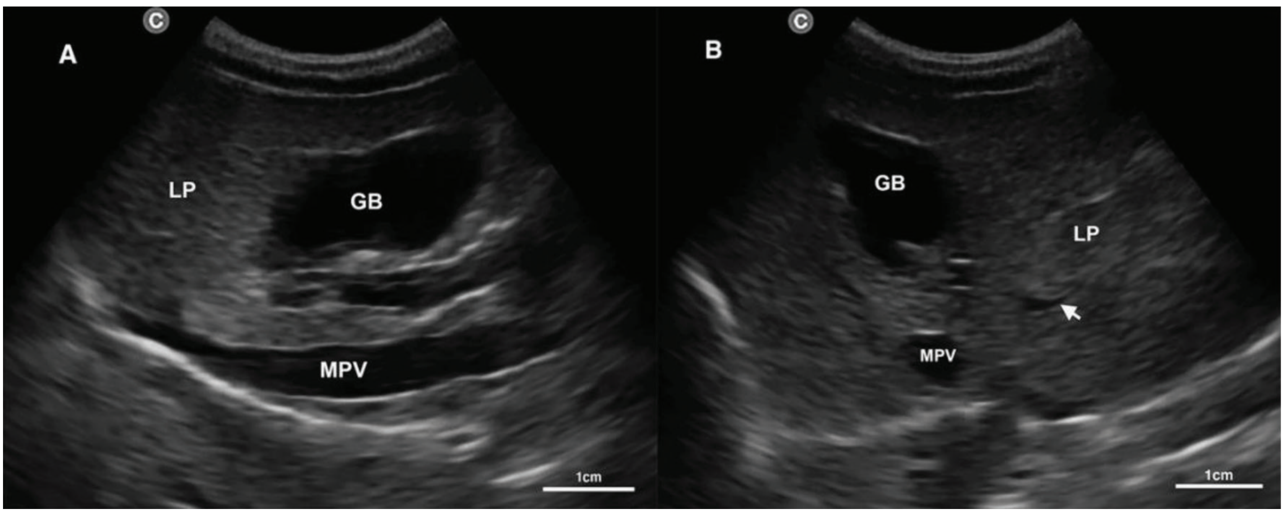

Figure 1. Sagittal (A) and transverse (B) views of the liver parenchyma (LP) and gall bladder (GB) of a male long-tailed macaque. Main portail vein (MPV) has an echogenic wall compared to the wall of hepatic vein (arrow).

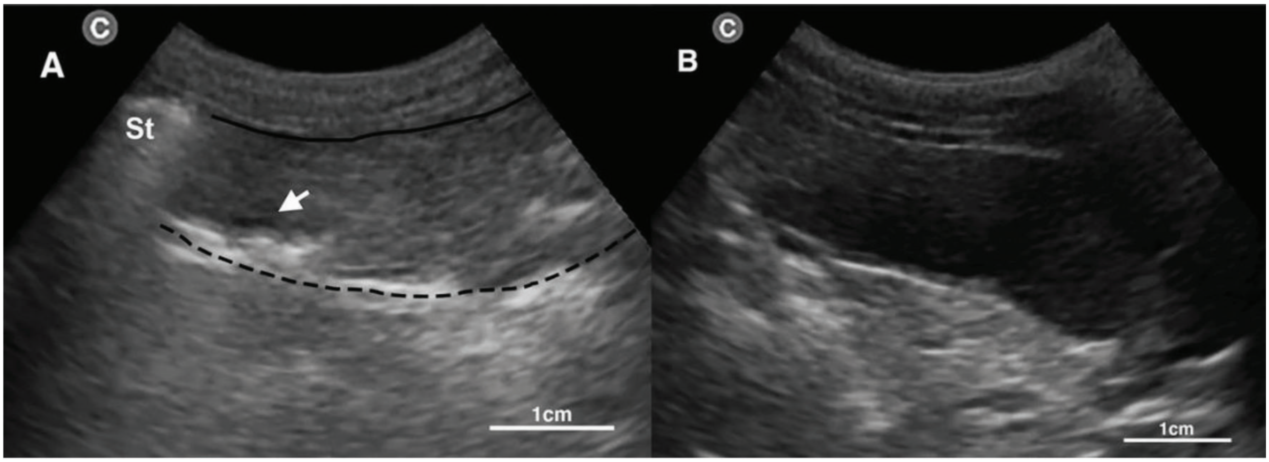

Figure 2. Sagittal view of the spleen (outlined) of male (A) and female (B) long-tailed macaque. Splenic parenchyma appears as a dense hyperechoic (A) or hypoechoic (B) structure adjacent to the stomach (ST) with hyperechoic capsule and anechoic splenic vessel (arrow).

did not differ noticeably from those of the females (17.6 ± 1.89 mm) (P = 0.389). Furthermore, spleen thickness had very weak positive correlation with the body weight in males (r = 0.094, P = 0.772) and females (r = 0.118, P = 0.716), but it was not statistically significant. This result was similar to previous study results in common marmoset [4]. The echo mean value for both sexes was 28.6 ± 17.90 and it was not statistically different in males (28.7 ± 17.25) and females (28.4 ± 19.29) (P = 0.976). In addition, the echo mean value of splenic parenchyma (28.6 ± 17.90) did not differ statistically with the echo mean value of liver parenchyma (26.4 ± 14.30) (P = 0.744); therefore, spleen can be observed to be isoechoic to the liver which agrees with previous report [8].

3.4. Kidneys

The kidney was viewed as just caudal and dorsal to the rib cage in both sexes. In a few instances, the probe was directed cranially to view the right kidney. The kidneys had a hyperechoic capsule and hyperechoic renal pelvis. The renal cortex appeared hypoechoic when compared to hypoechoic to anechoic medulla. The kidneys generally were bean-shaped, but triangular shape was seen in the left and right kidneys in some monkeys. This is in agreement with previous reports that cynomolgus monkeys may have nonidentical kidneys [8]. The right kidney was seen always caudal to the caudate lobe of the liver, while the left kidneys varied in location but can be visualized caudal to the spleen and dorsal to the colon (Figure 3). The cranial pole of the left kidney was seen in contact with the spleen and cranial pole appeared tapering as previously reported [8].

The mean length of left kidneys and right kidneys were not significantly different (P = 0.131). In contrast, the width of left kidneys and right kidneys had a significant difference (P = 0.009). In addition, right kidney had higher height values when compared to left kidney (P < 0.001). The reported values in this study were comparable to the previous report [8]. Since the height and width of right kidneys were higher as compared to the left kidneys, the right kidney volume was significantly greater as compared to the left kidney volume (P < 0.001). The greater volume estimation in the right kidneys also agrees with the previous study [8]. Measurements of male kidneys were also presented in the present study in contrast to the previous study wherein only kidneys of female long-tailed macaques were measured [8]. The total renal volume for both kidneys was 11.47 ± 2.39 cm^3, and was moderately correlated with body weight (r = 0.432, P = 0.0395), which agrees with the earlier observation [8]. However, there was no statistical significance between the length, width, height, and volume of the left and right kidneys of long-tailed macaques based on sex (Table).

The kidneys of long-tailed macaques have distinct corticomedullar junctions, but they appeared poorly distinct in some examined monkeys. The left cortex: medulla ratio did not differ significantly between two kidneys (P = 0.788). This is in contrary to the gross morphologic study of cynomolgus macaques which reported that cortex was shorter as compared to medulla with an average of 1 : 1.25 or 0.80 ratio.[16] The echo mean of the right renal cortex was significantly higher when compared to echo mean or right medulla (P = 0.0053). However, in some monkeys, the renal medulla had high echo mean value ranging from 0.67 to 29.41. The echo mean value of the left renal cortex was not significantly different from the echo mean values of the right renal cortex (P = 0.803). Similarly, the echo mean value of left renal medulla was not different from the echo mean values of the right renal medulla (P = 0.674).

The left renal cortex generally appeared hypoechoic when compared to spleen, but it appeared isoechoic to the

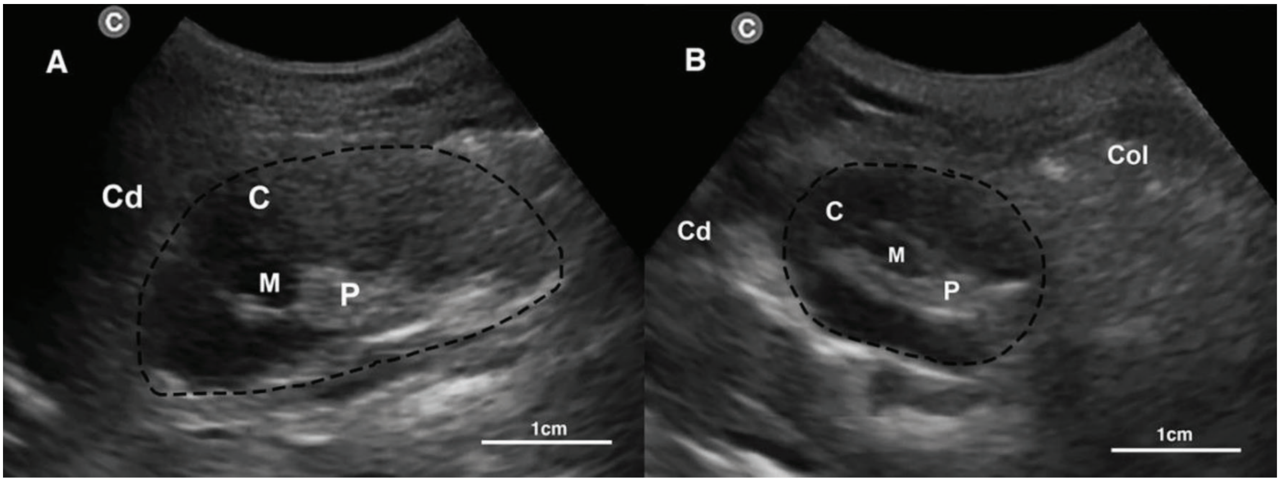

Figure 3. Sagittal (A) and transverse (B) views of the right kidney (outlined) of a male long-tailed macaque. Renal pelvis (P), cortex (C), medulla (M), caudate liver lobe (Cd), Colon (Col).

Table. Comparison of mean ± SD values of ultrasound measurements and calculated parameters of left and right kidneys of male (n = 12) and female (n = 12) rescued long-tailed macaques (n = 24).

Parameters	Male		Female		Overall	
	Left	Right	Left	Right	Left	Right
Length (mm)	36.3 ± 5.43	37.8 ± 5.47	36.5 ± 5.15	39.4 ± 3.98	36.4 ± 5.17	38.6 ± 4.75
Width (mm)	15.6 ± 2.21	17.8 ± 2.54	15.1 ± 2.29	16.4 ± 2.04	15.2 ± 2.22*	17.1 ± 2.36*
Height (mm)	16.8 ± 2.40	19.3 ± 2.59	16.4 ± 1.65	19.1 ± 3.32	16.6 ± 2.02*	19.2 ± 2.91*
Cortex echo mean	26.4 ± 16.15	20.8 ± 15.58	17.9 ± 14.34	20.1 ± 14.79	22.2 ± 15.54	20.5 ± 14.86
Medulla echo mean	15.2 ± 9.04	9.6 ± 8.63	7.3 ± 8.20	10.6 ± 9.73	11.2 ± 9.35	10.1 ± 9.01
Cortex: medulla ratio	1.6 ± 0.45	1.3 ± 0.31	1.2 ± 0.09	1.3 ± 0.47	1.4 ± 0.37	1.3 ± 0.39
Volume (cm^3)	2.9 ± 1.21	6.8 ± 1.91	4.8 ± 1.45	6.4 ± 1.41	4.9 ± 1.31*	6.6 ± 1.65*

*The difference between means P-value ≤ 0.05

spleen in some monkeys. In support to this observation, the echo mean value of the left renal cortex (22.2 ± 15.54) did not differ statistically with that of spleen (28.6 ± 17.90) (P = 0.195). The similar echogenicity of the spleen and left renal cortex had been reported in laboratory-kept cynomolgus macaques [8]. In addition, the similarity in echo mean values between the two organs may be due to hypoechoic spleen and hyperechoic left renal cortex in some monkeys. More echogenic renal cortex as compared to spleen and liver was observed in other species of monkeys such as common marmoset [4], vervet monkeys [6], and squirrel monkeys [17].

The left kidney height (r = 595, P = 0.040) and volume (r = 0.810, P = 0.001) in males had significant positive moderate and very strong correlation with body weight, respectively. The width of the right kidney of males had a significant and moderate positive correlation with the crown-rump length (r = 0.792, P = 0.002). In contrast, no significant correlation was observed between body weights, crown–rump length and ultrasound measurements in both left and right kidneys of female. There was a statistically significant moderate positive correlation between the volume of the left kidney (r = 0.404, P = 0.050) and body weight. On the contrary, the volume of the right kidney was not statistically significant despite having a weak positive correlation with body weight (r = 0.291, P = 0.166). In addition, the crown–rump length or height of the long-tailed macaques had very weak moderate positive correlation to right kidney (r= 0.138, P = 0.519) and left kidney (r = 0.329, P = 0.116), respectively, but it was not statistically significant.

3.5. Urinary bladder

The urinary bladder was a round or oval structure cranial to the pubis, with homogenous anechoic content and a hypoechoic to hyperechoic thin wall. The thickness of the bladder wall (0.9 ± 0.10 mm) was lower as compared to the reported thickness in howler monkeys [7]. The colon cast irregular shadows, while the urinary bladder produced distant acoustic enhancement. In males, urination prior to sedation resulted in difficulty in the visualization of the lumen and wall of the urinary bladder. No cystoliths or thick echogenic debris were observed in any of the monkeys.

Physical and chemical restraints are standard practices in zoo and laboratory animal immobilization. These are necessary to ensure the welfare of the animal and the handlers during a physical examination, treatment of wounds, tattooing, and in minor surgeries [18]. The long-tailed macaques in this study were not trained prior to the blood collection and ultrasound evaluation; therefore, sedating the monkeys was favorable in order to perform complete physical examination and faster conduct of ultrasound evaluation. In addition, these rescued monkeys are usually sedated during the testing for tuberculosis and all physical examination prior to quarantine and placement to monkey area of the center. Hence, ultrasound evaluation of abdominal organs can be performed concurrently during these examinations.

The obtained measurements, references, and descriptions reported in this study can be used by the rescue centers in the country during ultrasound evaluations of long-tailed macaques. Moreover, ultrasound evaluation of other abdominal organs such as adrenal glands, pancreas, ovaries, and mesenteric lymph nodes can provide valuable information and warrant further study.

This study established the optimal acoustic windows, reference measurements and description of ultrasonogram of the liver, gallbladder, spleen, kidneys, and urinary bladder of long-tailed macaques in a rescue and rehabilitation center.

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Conflict of interest
The authors declare that there are no conflicts of interest.

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