ORIGINAL ARTICLE

ULTRASONOGRAPHIC FINDINGS IN THE LIVER, GALLBLADDER AND KIDNEYS OF CAPTIVE RETICULATED PYTHONS (*Python reticulatus,* SCHNEIDER*,* 1801) (REPTILIA: PYTHONIDAE) WITH PNEUMONIA

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ABSTRACT

Ultrasonography was performed using a 6.5 MHz linear array scanner in order to determine the ultrasonographic features of the liver, gallbladder and kidneys of 12 captive reticulated pythons with pneumonia in comparison with macroscopic findings. The organs were examined using an ultrasound machine equipped with a 6.5 MHz scanner and echo mean values were calculated. The liver appeared elongated with a hypoechoic parenchyma and a large anechoic central hepatic vein with a hyperechoic wall. The gallbladder was observed as a large elliptical structure with a hypoechoic wall and an anechoic lumen. The kidneys appeared as hypoechoic granulated, elongated structures with the right kidney located more cranially than the left. No differences were observed between ultrasonographic and gross measurements of the different organs. Histogram analysis showed that there was a distinct increase in the Emean values of all structures, except the kidneys which showed decrease in Emean values. The results suggest that pneumonia causes changes in the echogenicity of the liver, gallbladder and kidneys in reticulated pythons.

Keywords: echo mean, gallbladder, kidneys, liver, pneumonia, python, ultrasound

INTRODUCTION

In wildlife medicine, where many of the animals handled are either protected, important for conservation and/or are highly valuable, the need for an accurate, painless and safe way of health monitoring as well as disease diagnosis without having a negative impact during the process of providing veterinary care is important. Ultrasonography meets all these criteria and has been found to be beneficial to wildlife such as reptiles wherein the diagnoses of diseases are difficult since pathognomonic signs are very few and manifestations of clinical signs are usually observed in the latter stages of the disease progression (Hernandez-Divers, 2008). In the Philippines, the use of ultrasound in the field of wildlife and exotic animals are yet to be explored thoroughly. Normal ultrasonographic features of the heart, liver, gall bladder, spleen (Aguisanda *et al.,* 2011), kidneys (Enriquez *et al.,* 2011) and the reproductive tract (Enriquez *et al.,* 2011) of apparently healthy reticulated pythons have been studied in the Philippines, but studies with diseased animals are still lacking. One of the most common diseases in snakes is pneumonia or lower respiratory tract disease and it has a predilection to pythons and boas especially captive snakes (Mayer *et al.,* 2009). Diagnosing this disease requires systemic evaluation due

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to high possibility of a secondary infections involving adjacent organs such as the heart, liver, gallbladder, spleen, pancreas and kidneys (Murray, 2006). Even with an aggressive therapeutic regimen, prognosis for pneumonia is still dependent on the state of the animal upon presentation (Murray, 2006; Mayer *et al*., 2013).

This makes early and accurate disease diagnosis essential for the management and treatment of pneumonia especially for wildlife species such as the snakes that seldom show signs of illness. Effective disease monitoring is crucial in order to execute an early response to possible infection and prevent disease progression. This study, therefore, was conducted to determine the ultrasonographic changes in the liver, gall bladder and kidneys in reticulated pypthons with pneumonia. The results of this study will, hopefully, assist the clinician in arriving at a more accurate diagnosis and prognosis of the disease condition.

MATERIALS AND METHODS

Animals

Twelve reticulated pythons from the Biodiversity Management Bureau-Wildlife Rescue Center (BMB-WRC), Department of Environment and Natural Resources, Quezon City were used as subjects in the study. Pythons with clinical signs of pneumonia were selected from a list of animals due for euthanasia that was provided by the BMB-WRC.

An initial screening was done in the selection of the animals with pneumonia. An ocular examination was performed and the animals were inspected for the occurrence of anorexia, dehydration, poor body condition and presence of mouth lesions, respiratory sounds, foamy and or ropey saliva, nasal exudates and caseous lesions in the mouth. Animals with at least three of the above clinical signs were included in the study. The animals were restrained chemically using Tiletamine-Zolazepam (Zoletil®, Virbac, Carros, France) at the dose of 5 mg/kg prior to ultrasound examination.

Ultrasonography

Ultrasound examination was conducted on the ventral surface of the snake using an ultrasound machine (WED – 3100V, Shenzhen Well.D Medical Electronics Co., Ltd., Shenzhen, China) equipped with a 6.5 MHz micro-convex scanner with the animal placed in dorsal recumbency. The intercostal approach was also done in scanning the length of the animal to locate and identify the organs. Each organ was scanned in both sagittal (long-axis) and transverse (short-axis) views. The liver, gall bladder and kidneys were examined and ultrasonograms of the different organs were obtained. Measurements of the liver, gall bladder and left and right kidneys were also determined.

Necropsy

The animals were euthanized according to the protocol of the BMB-WRC. Magnesium sulphate (50%) was injected into the skull through the foramen magnum after each ultrasound procedure. Necropsy was performed in accordance with the procedures by Terrell *et al.,* (2007). The organs were examined *in situ* before *in toto* evisceration of the organs. The length and width of the liver, gallbladder, spleen and kidneys were measured using a caliper while the volume of each organ was acquired using the displacement method.

Analysis

Analysis of the ultrasonograms was done using Adobe Photoshop® CS3 Extended software. The echo mean (Emean) values of the histogram of the liver (hepatic parenchyma, central hepatic vein wall, and central hepatic vein lumen), gallbladder and the left and right kidneys were calculated.

Echo mean values and organ measurements during necropsy and using the ultrasonograms were tested using Wilk-Shapiro test for normality. Significant variation was tested using Student's t-test for normality and Mann-Whitney test for samples that were not normally distributed. Pearson's Correlation test and Spearman's test for correlation were used to determine the presence of correlation between the independent and dependent variables at $\alpha = 0.05$.

RESULTS

Liver

Using ultrasonography, the liver was found caudal to both the heart and the lungs. The liver was identified as an elongated, hypoechoic structure (Figure 1) that was easily recognizable due to the presence of a large anechoic streak with a hyperechoic wall which was identified as the central hepatic vein (Figures 1 and 2). No differences in the length and width of the liver were observed between ultrasonographic and gross measurements (Table 1). Histogram analysis of the liver revealed Emean values of 63.91 ± 10.67 for the hepatic parenchyma and 154.83 ± 8.46 and 57.46 ± 11.39 for the central hepatic vein wall and lumen, respectively (Table 2). Gross examination of the liver showed that 25% of the animals showed no signs of visible lesions while 17% had adhesions, 25% had milliary, white-raised pinpoint nodules on the surface of the organ, 25% had both adhesions and milliary white nodules while 8% of the samples had adhesions and presence of diffused raised brownish lesions that had a mottled appearance.

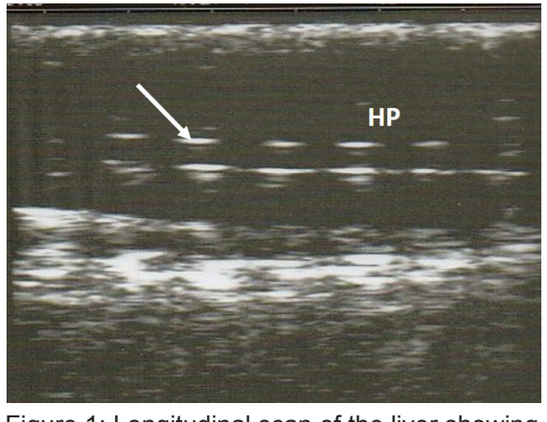

Figure 1: Longitudinal scan of the liver showing hypoechoic parenchyma (HP) and a large central hepatic vein (arrow) with a hyperechoic wall and an anechoic lumen.

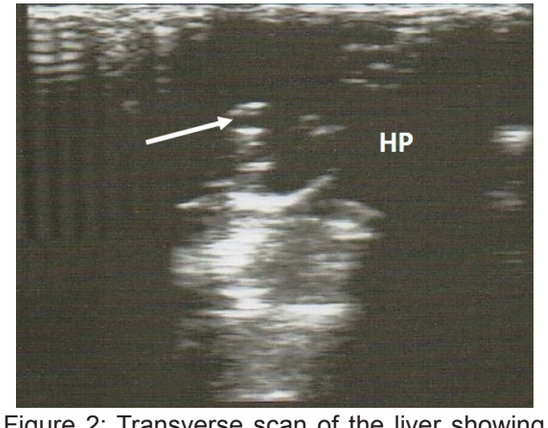

Figure 2: Transverse scan of the liver showing hypoechoic parenchyma (HP) and a large central hepatic vein (arrow) with a hyperechoic wall and an anechoic lumen.

Table 1. Mean ± SD of the dimensions of the liver, gallbladder and kidneys acquired grossly and through ultrasonography of reticulated pythons with pneumonia.

Parameter	Gross	Ultrasound	*P* value
Liver			
Length (cm)	38.38 ± 6.04	40.17±17.13	0.72893
Width (cm)	2.28 ± 0.51	2.55±0.53	0.90712
Volume (cm³)	67.83 ± 40.02	-	-
Weight (g)	85.67 ± 42.85	-	-
Circumference of CHV (cm)	-	1.2±0.39	-
Gall bladder			
Length (cm)	6.28±2.01	5.96±1.43	0.59712
Width (cm)	3.57±0.99	3.06±0.33	0.13212
Volume (cm³)	37.42±32.65	21.03±8.56	0.24663
Weight (g)	39.42±35.66	-	-
Circumference (cm)	15.71±4.75	15.18±0.67	0.37381
Right kidney			
Length (cm)	17.05±4.26	16.5±3.34	0.75079
Width(cm)	1.6 ± 0.38	1.61±0.37	0.94696
Volume (cm³)	12.08±4.34	21.61±12.55	0.094244
Weight (g)	12.83±4.55	-	-
Left kidney			
Length (cm)	15.34±3.98	13.75±3.85	0.38496
Width(cm)	1.7±0.42	1.74±0.44	0.81927
Volume (cm³)	13.25±4.94	19.57±10.15	0.19742
Weight (g)	13 ± 4.79	-	-

CHV: central hepatic vein.

Gall bladder

The gallbladder was observed to be located caudal to but not attached to the liver at the third quadrant of the animal's body. Visualization of the gallbladder varied in distance but was found a few centimeters caudal to the caudal border of the liver. It appeared as an anechoic, large elliptical structure (Figure 3) with a hypoechoic wall. No differences in the length, width, volume and circumference of the gallbladder were observed between gross and ultrasonographic measurements (Table 1). Histogram analysis showed that the lumen of the gallbladder had an Emean of 55.12 ± 9.58 (Table 2). No gross lesions were apparent upon examination of the gallbladder aside from its varying sizes in different snakes.

Table 2. Mean ± SD of the echo mean values of the liver and its associated structures, gallbladder and kidneys of reticulated pythons with pneumonia.

Parameter	Emean values
Liver parenchyma	63.91 ± 10.67
Central hepatic vein wall	154.83 ± 8.46
Central hepatic vein lumen	57.46 ±11.39
Gallbladder	55.12 ± 9.58
Right Kidney	54.01 ± 11.41
Left Kidney	53.84 ± 12.09

Kidneys

The kidneys were located in the fourth quadrant of the body with the cloaca as the reference point. From the cloaca, the transducer was moved cranially in order to locate the kidneys. Upon identification, the left and right kidneys were found to be hypoechoic granulated, elongated structures with the right kidney located more cranially than the left. An area of overlap was observed during ultrasonography wherein the caudal right kidney was visualized with the cranial left kidney (Figure 4). No differences in the length, width and volume of the left and right kidneys between gross and ultrasonographic measurements were observed (Table 1). Histogram analysis showed that the right and left kidneys had Emean values of 54.01 ± 11.41 and 53.84 ± 12.09, respectively (Table 2). Gross examination of the kidneys revealed that 92% of the animals had no apparent gross lesions on both the left and right kidneys while the other 8% were congested with pale areas on both left and right kidneys as well as thickened capsule and web-like adhesions.

DISCUSSION

Ultrasound findings in reticulated pythons with pneumonia in this study showed that the appearance of the liver, gallbladder and kidneys of all samples were in agreement with the findings of Aguisanda *et al.* (2011) and as described by Stetter (2006) and Schilliger (2010) in apparently healthy reticulated pythons. This implies that changes in the gross appearance of the different organs observed in the diseased pythons in this study cannot be detected by ultrasonography.

The circumference of the central hepatic vein obtained through ultrasonography in the present study was found to be smaller than that observed in apparently healthy animals by Aguisanda *et al.* (2011). A decrease was also found between the mean length of the liver acquired through ultrasonography in this study compared to that of the findings of Aguisanda *et al.* (2011). The mean length of the animals used in this study (264.67 ± 43.15 cm) was similar to that used by Aguisanda *et al.* (2011) *(*264.46 40.57 cm). These findings suggest that both mean length of the liver and circumference of the central hepatic vein decrease in reticulated pythons with pneumonia.

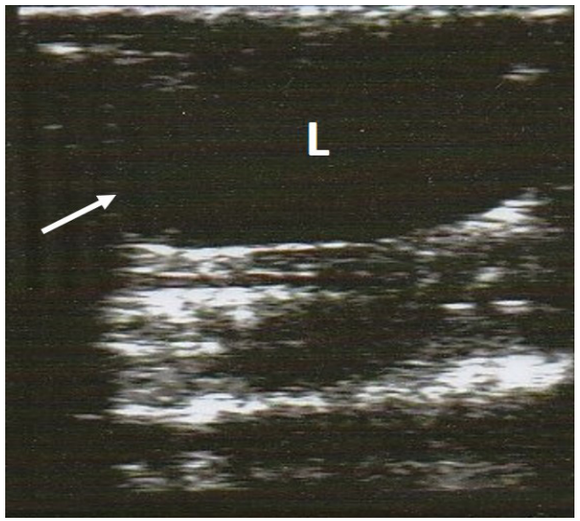

Figure 3: Longitudinal scan of the gallbladder showing anechoic lumen (L) and hypoechoic wall (arrow).

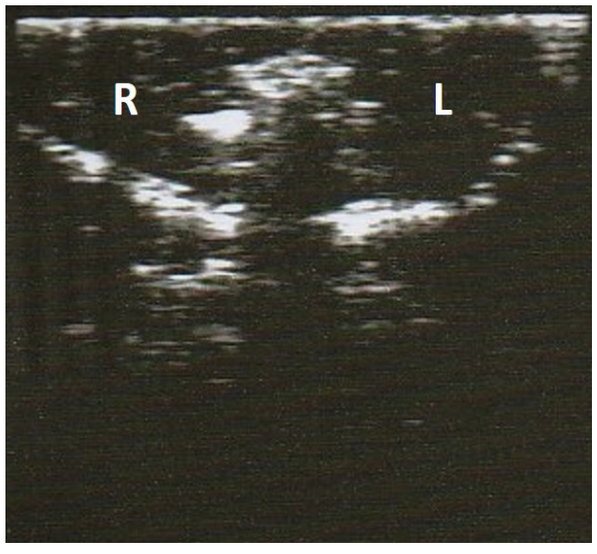

Figure 4: Transverse scan of the right (R) and left (L) kidneys showing an echoic, granulated parenchyma.

Histogram analysis was performed to measure the Emean values of the liver, gallbladder and kidneys. For the liver, a distinct increase in the Emean values of the liver parenchyma and cental hepatic vein wall and lumen compared to the findings of Aguisanda *et al.* (2011) was obtained. This may suggest that the presence of hepatic parenchymal degeneration in diseased animals compared to healthy animals. There was a distinct increase in echogenicity of the gall bladder lumen as compared to the Emean value obtained by Aguisanda *et al.* (2011). This suggests the possibility of occurrence of biliary stasis in reticulated pythons with pneumonia. There was also a distinct increase in the mean circumference and length of the gall bladder acquired through ultrasound compared to the values stated by Aguisanda *et al.* (2011). This finding suggests that the gallbladder of the animals used in this study that were diagnosed with pneumonia increased in size compared to those that were apparently healthy. A weak positive relationship was found between Emean of the lumen of the gallbladder and the circumference of the gallbladder acquired grossly measurement (r = 0.231) and through ultrasound (r = 0.22263). This implies that an increase in the circumference of the gallbladder weakly affects the echogenicity of the gallbladder lumen.

Histogram analysis of the Emean values of the left and right kidneys were all within normal distribution. Variations between the Emean values of the left and right kidneys were found to be insignificant (p = 0.9733) while their Emean values were found to have a very strong positive relationship (r = 0.9318). This indicates that as the echogenicity of the right kidney increases so does the left. There was deviation in the echogenicity of the kidneys from normal where in a decrease in the echogenicity of both the left and right kidneys was apparent when compared to the findings of Enriquez *et al.* (2011). This implies that there is a distinct decrease in the Emean value of the kidneys of reticulated pythons with pneumonia.

Statistical analysis showed that the Emean values of the liver, gallbladder and kidneys were correlated and it was found that relationship between the liver and gallbladder had a very strong positive relationship (r = 8182). A very strong positive relationship was also found between the Emean of the gallbladder and the right (r= 0.8341) and left kidneys (r = 0.97404) while the relationship between the liver and the right and left kidneys was found to be negligible (r = 0.0013) and weakly positive (r = 0.259), respectively. This suggests that as the echogenicity of the liver increases, so does the gallbladder's and as the echogenicity of the gallbladder lumen increases, so does the echogenicity of the left and right kidneys. Albeit the relationship between the kidneys and the liver was found to be negligible to weak, it could be attributed to the distance between the organs. This suggests that relationship between the organs may be attributed to their anatomical location and proximity with one another.

Ultrasonography may not be able to detect and visualize the gross lesions found on the liver, gall bladder and kidneys of reticulated pythons diagnosed with pneumonia, but it can be used to monitor changes in organ dimensions such as length, width and weight. With the aid of histogram analysis, changes in the echo mean value of different organs can be used to indirectly monitor and assess the health conditions of reticulated pythons, especially in cases where the history and general health condition of the animal are unknown, which is a dilemma often observed in animal rescue and rehabilitation centers. The findings in this study can be helpful in the diagnosis and prognosis of suspected cases of pneumonia in reticulated pythons.

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