ULTRASOUND FEATURES OF THE KIDNEYS, URINARY BLADDER, OVARIAN FOLLICLES AND VAGINAL SACS OF FEMALE WATER MONITOR LIZARD (*Varanus marmoratus*, Weigmann, 1834)

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ABSTRACT

Four apparently healthy female water monitor lizards (*Varanus marmoratus*), weighing 0.8-3.8 kg, were examined using an ultrasound machine equipped with a 5.0 MHz linear array scanner to determine the ultrasound appearance, dimensions and echo mean values of the urogenital organs. The kidneys of the varanids were observed caudal to the ovarian follicles, appearing as elongated strucutres with hypoechoic parenchyma and hypoechoic to hyperechoic interlobular spaces. The urinary bladder appeared as an elongated anechoic structure with hypoechoic wall. Two out of four female varanids showed ovarian follicles located laterally on both sides within the caudal half of the animal's body. Both pre-vitellogenic and vitellogenic stages were observed. Pre-vitellogenic follicles appeared round and anechoic while vitellogenic follicles appeared hypoechoic to hyperchoic in the ultrasonograms. The vaginal sacs appeared as anechoic tubular structures with hypoechoic walls. Echo mean values correlated well with the echogenicity of the different organs. The results of the study can be used for comparison with the ultrasound features of the diseased urogenital organs in the water monitor lizards.

Keywords: kidney, monitor lizard, ovarian follicle, urinary bladder, vaginal sac, Varanus

INTRODUCTION

In wildlife medicine, the application of ultrasonography has gained popularity in the assessment of health conditions. In reptiles, the use of non-invasive ultrasonographic imaging has provided vital information regarding internal morphological and functional evaluation of different organs. Of particular interest, many would recognize the valuable data derived from ultrasonographic explorations of the urogenital tract in wild animals (Kik, 2002; Valente, 2007; Enriquez *et al*., 2011).

Ultrasound, unlike radiography, can provide differentiation of various soft tissue structures and offer information on organ location and pathological changes (Schilliger, 2010). Furthermore, it is non-invasive and patients are not exposed to

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ionizing radiations. It is also beneficial during evaluation of vascular structures and may aid proper fine needle aspiration and biopsy collection (Kealy and McAllister, 2000).

In the Philippines, studies have been conducted in reticulated python, which described the ultrasonographic features of the reproductive tract (Enriquez, *et al.,* 2011), heart, liver, gall bladder and spleen (Aguisanda *et al.,* 2011). However, there are no known studies conducted in other reptiles in the Philippines, including varanids (*Varanus marmoratus*). Some of these varanids are often injured and brought to rescue centers. It is necessary, therefore, to study the structures of the this species to assist in the diagnosis and treatment of diseases.

Ultrasonography can help properly determine sex, diagnose internal abnormalities and diseases and evaluate of pregnancy. Baseline data on ultrasonographic features of the urogenital system can help improve current captive management and breeding practices, enabling the clinician to provide proper care and treatment to the animal. This study was conducted to determine the ultrasonographic features of the urogenital tract of female water monitor lizards in captivity.

MATERIALS AND METHODS

Four female water monitor lizards *(Varanus marmoratus)* of various ages, weighing 0.8-3.8 kg, with a total length of 100-110 cm and snout to vent length of 56 -66 cm, were utilized in the study. Physical examination was conducted prior to the study to ensure that the animals were apparently healthy. Animals with any signs of lethargy, presence of nasal discharges, tumors, wounds and deformities were excluded from the study. The water monitor lizards were obtained from the Protected Areas and Wildlife Bureau, Wildlife Rescue Center (PAWB-WRC), Department of Environment and Natural Resources, Diliman, Quezon City. The animal's identification, age, diet and date of acquisition of the lizards were obtained from the records of the institution. The species were identified using identification keys based on descriptions by Koch *et al.* (2010).

The animals were properly restrained manually in accordance with the techniques described by Barten (2006), and placed in dorsal recumbency. Liberal amount of ultrasonic gel was applied to the transducer and the skin to improve contact. The animal was scanned using an ultrasound Machine (Aloka Ultrasound Diagnostic Equipment, Aloka® SSD-500, Aloka Co. Ltd., Tokyo, Japan) equipped with a 5.0 MHz linear array scanner. Machine settings of overall gain: 70 dB, near gain: 15.0 dB and far gain: 2.5 dB, were maintained for all animals. The scanner was placed on the abdomen and directed cranial to caudal, and medial to lateral, to visualize the kidneys, urinary bladder, ovarian follicles and vaginal sacs. The ultrasonogram images were printed out and used to determine the size and shape of the organs. Length, width and thickness of the organs were measured using the ultrasonogram's internal scale. In addition, the echogenicity of the organs was determined through histogram analysis using Adobe Photoshop 6.0 (Adobe Systems Incorporated, 1998-2000) and were expressed as echo mean value.

The protocol for this study has been approved by the Protected Areas and Wildlife Bureau of the Department of Environment and Natural Resources and the Institutional Animal Care and Use Committee of the University of the Philippines Los Baños College of Veterinary Medicine.

RESULTS AND DISCUSSION

Upon ultrasound examination, the kidneys, urinary bladder, ovarian follicles and vaginal sacs were successfully visualized in all animals, except for the ovarian follicles in two animals. Few limitations were encountered in the course of this study. First was the slight difficulty of obtaining clear sonogram images due to the presence of highly keratinized scales of the varanids. This was resolved by placing plenty of ultrasound gel through the scales of the animals before scanning with the transducer. Another technique used to obtain better image quality would be soaking the animal in warm water and imaging the organs with the scanner submerged in water (Stahl, 2007; Stetter, 2006). Secondly, as with the study of Enriquez *et al*. (2011), slight difficulty in obtaining ultrasonograms in juveniles or smaller animals was also noted in this particular study. It is recommended to use higher frequency transducers with good standoff pad if it is inevitable to utilize juvenile lizards, as pointed out by Stahl (2007). However, the use of 5.0 MHz frequency scanner in this study proved to be sufficient to provide good quality images for the corresponding size of animals.

Physical restraint in lizards is important for the safety of both the animal and the examiner. Small lizards can be restrained by a single hand over the thorax and neck; middle-sized animals should be restrained with one hand on the thorax, neck and the front legs, while the other hand holds the hind quarter and tail. Other aggressive and big lizards can just be examined in the enclosure (Coke, 2008). Furthermore, they may be enveloped in a towel to protect the handler from claws, teeth and tail. One hand may grasp the forelimbs around the shoulders and neck. The thumb and index fingers should be placed just caudal to the head to prevent bites. The second hand on the hindquarters should be over the dorsal pelvis. The tail may be tucked under one arm. The use of towel or gauze over the eyes will help in calming the animal (Barten, 2006). Animal restraint is generally based on the patient's size and mobility; general anesthesia is rarely required during ultrasound examination (Hochleitner and Hochleitner, 2004; Isaza *et al*., 1991; Sainsbury and Gilic, 1991; Schildger *et al.*, 1994).

The kidneys in all animals were visualized in the lateral sides of the pelvic cavity, caudal to the ovaries. They were located intra-pelvic beneath the abdominal fat. The transducer can be placed against the lateral base of the tail and in a craniodorsal orientation towards the pelvic inlet to visualize the kidneys. The kidneys were not divided into medulla, cortex and pelvis as is seen in mammals, particularly in dogs and cats (Kealy and McAllister, 2000; Nyland and Mattoon, 1995; Mannion, 2006), in agreement with Stahl (2007). Grossly, the kidneys are seen as paired structures with the presence of lobes and brown in color. Morphological changes altering the shape and size of the kidneys should be noted to determine the presence of diseases such as neoplasia and cysts (Hernandez-Divers and Hernandez-Divers, 2001).

The renal ultrasonograms showed an elongated organ with no distinction between medulla and cortex. The kidneys appeared homogenous with hyperechoic and hypoechoic transverse partitions, which represent the interlobular spaces (Figure 1). Morphologically, reptile kidneys lack a renal pelvis and loop of Henle, and have few nephrons resulting to failure in urine concentration (Barten, 2006). Similar to what Stahl (2007) observed in his study, the kidneys appeared more hyperechoic than the fat bodies. Hernandez-Divers and Hernandez-Divers (2001) also stated that the kidneys of lizards are homogenous and more hyperechoic than the fat bodies and have a distinct granular and speckled image but the capsule may not be seen. The posterior segments of the kidneys, called sexual segments, are sexually dimorphic; these become swollen and contribute seminal fluid during the breeding season (Barten, 2006).

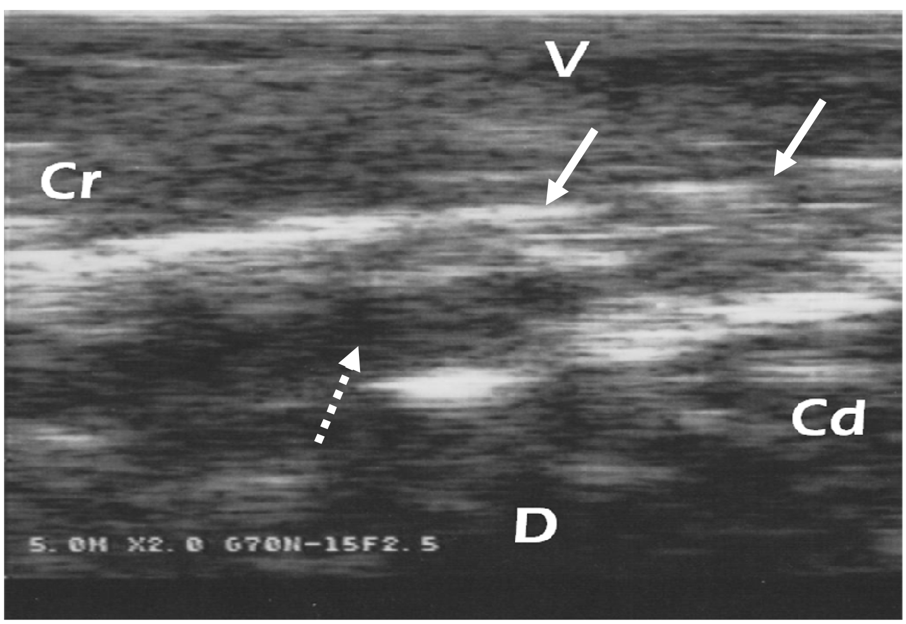

Figure 1. Longitudinal scan of the kidney in female water monitor lizard (*Varanus marmoratus*) showing hyperechoic to hypoechoic walls (solid arrows) and homogeneous parenchyma (dotted arrow). (Cr= cranial; Cd= Caudal; V= ventral; D= dorsal).

The dimensions of the left and right kidneys are shown in Table 1. The results show no significant differences in the mean dimensions and echo mean values of left and right kidneys. This means that the left and right kidneys have similar sizes and echogenicities.

Table 1. Mean±S.D. dimensions and echo mean values of kidneys, urinary bladder and vaginal sacs in female *Varanus marmoratus* (Weigmann, 1834).

Organ	Length (cm)	Width (cm)	Echo mean value
Left kidney	8.30±0.1	2.75 ±0.5	86.42±6.5
Right kidney	8.27±0.2	3.09±0.4	84.73±3.9
Urinary bladder	3.60±1.0	1.14 ±0.4	52.66±4.6
Left vaginal sac	4.21±0.8	0.73±0.4	59.83±3.6
Right vaginal sac	4.08±0.7	0.75±0.5	64.48±5.1

The urinary bladder was visualized in the caudal part of the abdomen and appeared as an irregular elongated structure with anechoic lumen and hypoechoic walls (Figure 2). Sometimes, hypoechoic areas or corpuscular echogenicities could be observed in some parts of the urinary bladder. Mader (2006) described the urinary bladder as a thin-walled anechoic structure that occupies the caudal coelom. Some lizards have a urinary bladder and all produce hypoosmotic urine containing nitrogenous waste as uric acid, which readily precipitate. The urinary bladder

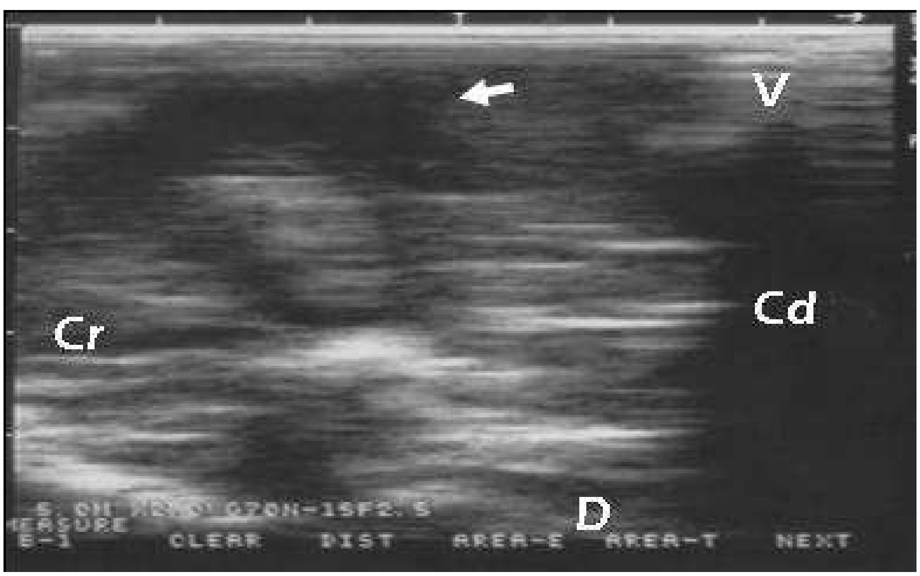

Figure 2. Longitudinal scan of the urinary bladder (arrow) in female water monitor lizard (Varanus marmoratus) showing anechoic lumen with hypoechoic walls. (Cr= Cranial; Cd= Caudal; V= ventral; D= dorsal).

appears as a thin-walled organ located intra-pelvic and caudal to the kidneys. Beuchat as cited by Davis and DeNardo (2007), concluded that there is no phylogenic and habitat generalization that could be made on the occurrence of urinary bladder in lizards. Water may be reabsorbed from the bladder resulting in post-renal concentration of urine (Barten, 2006). Thus, the anechoic urine may have hyperechoic particles of urates floating within it (Redrobe, 2006). This serves to explain the hypoechoic areas observed in some parts of the urinary bladder.

The dimensions and echo mean values of the urinary bladder are shown in Table 1. The echo mean value of the urinary bladder lumen was lower than the parenchyma of the left and right kidneys. This confirms the fluid nature of the urine present in the urinary bladder compared to the renal parenchyma.

The ovarian follicles were located caudal and dorsal to the liver in a paramedian position, and cranial to the kidneys, in agreement with the observation of Stahl (2007). Out of four female varanid samples, only two showed the presence of ovarian follicles. The appearance and position of the ovarian follicles varied depending on their developmental stage. Stahl (2007) stated that only gravid animals are usually readily imaged. The ovarian follicles were classified into two stages: pre-vitellogenic and vitellogenic. Pre-vitellogenic follicles had rounded anechoic appearance while vitellogenic follicles, exhibited round, hypoechoic to hyperechoic patterns (Figure 3). Mader (2006) stated that pre-vitellogenic follicles appeared round and anechoic and arranged in clusters, whereas post-ovulatory

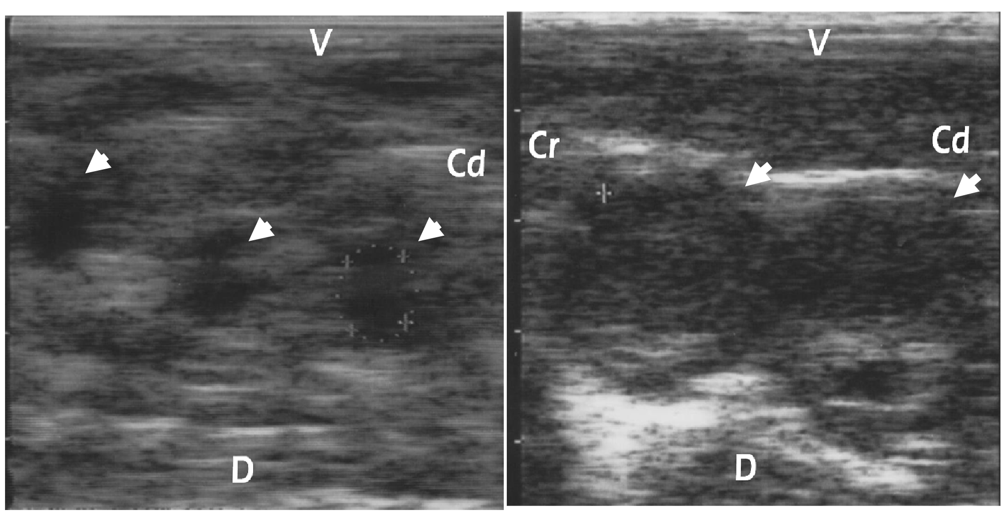

Figure 3. Ultrasonograms of pre-vitellogenic (arrowheads, left) and vitellogenic follicles (arrowheads, right) in female water monitor lizard (Varanus marmoratus). Pre-vitellogenic follicles appear as small round anechoic structures while vitellogenic follicles appear as large round hypoechoic structures. (Cd= Caudal; V= ventral; D= dorsal).

follicles or vitellogenic ones are more oblong and appear in a line (Mader, 2006). They have variable echogenicity with hypoechoic centers and hyperechoic outer lines or the shell. Redrobe (2006), on the other hand, stated that the ovaries of female lizards under the sonogram appear round and hypoechoic. They are detected in the mid-abdomen and dorsally positioned. Pre-vitellogenic second stage follicles are more easily detectable; they can be up to 2.5 cm, round and appear hyperechoic. When the follicles become vitellogenic, it becomes ovoid. There is hyperechoic albumen in the caudal half of the egg and hyperechoic yolk in the ventral half.

Ultrasonography is generally used in lizards as an obstetrical tool, specifically, to determine follicular development or in sex determination in juveniles of monomorphic species (Coke, 2008). Moreover, diagnosic ultrasound helps in knowing the reproductive status of female reptiles by determining ovarian activity and pregnancy (Stahl, 2007). The inactive ovaries are usually anechoic and small in snakes and lizards; the eggs present in the oviducts appear to have two distinct layers: the more superficial anechoic albumen which forms a black ring at the periphery and the deeper echogenic vitellus (Hernandez-Divers and Hernadez-Divers, 2001; Hochleitner and Hochleitner, 2004; Isaza *et al.*, 1993; Sainsbury and Gilic, 1991; Schildger *et al.,* 1994).

The developmental stages of the ovarian follicles were determined based on the differences in the ultrasonographic images and sizes (Table 2). The vitellogenic follicles were bigger, longer and wider than pre-vitellogenic follicles. In addition, the

Table 2. Dimensions and echo mean value of the ovarian follicles in female *Varanus marmoratus* (Weigmann, 1834).

Monitor lizard	Developmental stage	Circumference (cm)	Length (cm)	Width (cm)	Echo mean value
1	Pre-vitellogenic	2.63	0.85	0.9	43.93
2	-	-	-	-	-
3	Vitellogenic	9.73	3.1	2.1	96.22
4	-	-	-	-	-

- : Not observed.

echo mean value of vitellogenic follicles was higher than that of pre-vitellogenic follicles, suggesting that the vitellogenic follicles were more echogenic than pre-vitellogenic follicles. The results showed that as the follicles mature, the size and echo mean values of the follicles increase.

The vaginal sacs of the water monitor lizards appeared as anechoic to hypoechoic tubular structures with hypoechoic walls, tapering from cranial to caudal (Figure 4). The dimensions and echo mean values of the left and right vaginal sacs

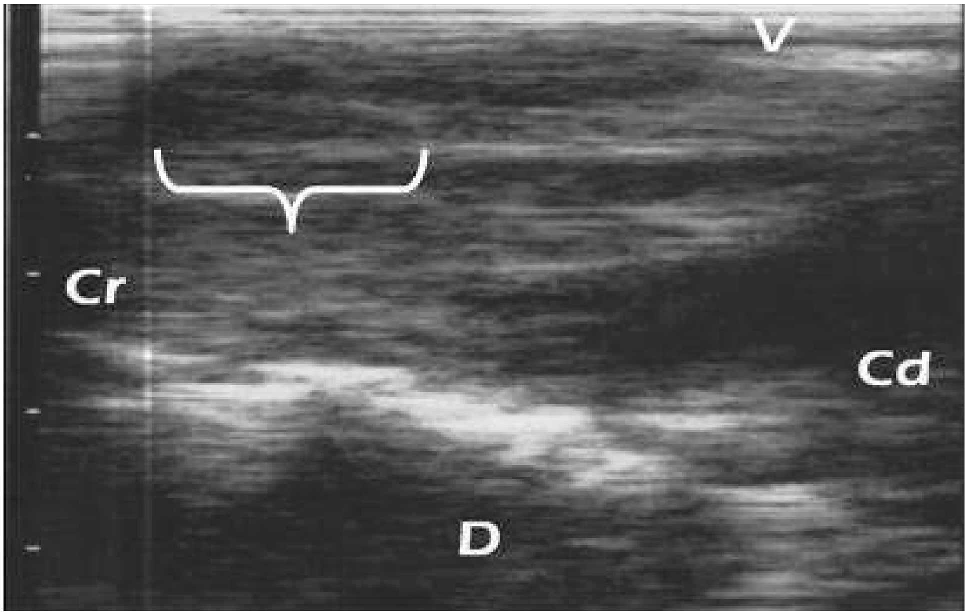

Figure 4. Longitudinal scan of the vaginal sac (bracket) in female water monitor lizard (*Varanus marmoratus*) showing a tapering structure with hypoechoic walls and less echogenic middle. (Cr= cranial; Cd= Caudal; V= ventral; D= dorsal).

are listed in Table 1. No differences in the dimensions and echo mean values between left and right vaginal sacs were observed.

The results of the study showed that the kidneys, urinary bladder, ovarian follicles and vaginal sacs of water monitor lizards could be visualized ultrasonographically using a 5.0 MHz frequency scanner. The dimensions and echo mean values of the different organs can be utilized in the diagnosis of diseases and disorders of the urinary and reproductive organs of female water monitor lizards.

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