ULTRASOUND EXAMINATION IN REPTILES: RESTRAINT, POSITIONNING, ARTIFACTS AND PATIENT EXAMINATION


INTRODUCTION
Two dimensional (2-D) ultrasonography is a particularly well-adapted tool for exploring soft tissue in reptiles (liver, kidney, bladder, genital system, heart and large blood vessels at the base of the heart, as well as the digestive tract). Unlike radiography, it can differentiate various soft tissue structures and provide information regarding organ location and pathologic changes. It is non-invasive, painless, without any reported adverse side effect, and does not expose the patient or the staff to ionizing radiations. It provides both a static and a dynamic cross-sectional image of the organ under exploration and can be an excellent adjunct to radiography in these animals. Moreover, ultrasonography allows carrying out ultrasound-guided biopsies, without the need for surgical laparotomy, and it can also be used for sex determination in species in which there is no sexual dimorphism.

In reptiles, no distinction is made between “thoracic ultrasonography” and "abdominal ultrasonography" as these animals have only one single general cavity, called coelomic cavity or thoraco-abdominal cavity. Like other medical imaging modalities, ultrasonography requires a good knowledge of the internal organ topographical anatomy of the animals concerned (Figures 1,2,3).

EQUIPMENT
A great range of ultrasound equipment is currently available to the veterinary practitioner. Most of these units are suitable to reptiles. Selection of the appropriate transducer is a trade-off between spatial resolution of the image and imaging depth: lower frequencies produce less resolution but image deeper into the body. That’s why the size of the patient dictates the transducer probes that are needed: smaller animals (snakes, most lizards and small chelonians) require the use of a 10 or 15 MHz transducer as a 5 to 8 MHz transducer is used for medium to large size patients. A 3.5 MHz probe may be warranted for very large reptiles such as big crocodiles, giant tortoises, large monitors or sea turtles. We routinely use two probes: one microconvex probe with a frequency switchable from 5 to 6.6 to 8 MHz and a linear array transducer with a frequency switchable from 10 to 15 MHz. The linear array is usually used in snakes and lizards. In chelonians, we prefer the use of the biconvex sector scanner because of the narrow space of the acoustic windows in these reptiles: the linear transducer is too large to fit in the cervical, axillary or prefemoral areas.

RESTRAINT
Animal restraint is based on the patient’s size and its mobility. General anaesthesia is rarely required. However, often several helpers may be necessary to restrain the animal. Many small lizards, snakes, crocodilians, turtles and tortoises can be imaged with manual restraint. In non cooperative species (aggressive and dangerous animals or tortoises which are strongly retracted into their shell), a sedation may be necessary. Care should be taken to the movements of the turtles’ or lizards’ legs as their claws can damage the probe.
POSITIONING
Except in chelonians of course (because of the plastron), the best window for visceral organ imaging in reptiles is created by placement of the transducer on the ventral surface (ventral approach) \(^{4,5,9,13,15}\). Thus, the lizard, snake or crocodile patient can be restrained either in dorsal or in ventral recumbency. In dorsal recumbency, the patient is either manually restrained or anesthetized. In ventral recumbency, two methods can be used to facilitate the transducer placement: the patient can be simply elevated off the table and the transducer is then placed on the animal’s ventrum, or the animal can be placed on a fenestrated table (with access from below) allowing for transducer placement \(^{5,15}\).

In snakes, a lateral approach in ventral recumbency is excellent for assessing the ovarian activity in females. It’s also much more comfortable for the snake itself, which is easier to restrain in this manner without any anesthesia.

Turtles and tortoises are probably the reptiles in which ultrasound examination is the most challenging: even more than in radiographic examinations, the shell considerably limits the access of ultrasonic waves to the content of the coelomic cavity. Therefore, few approaches are available. The transducer can be positioned only in two or three places \(^{5,6,8,9,10,13,15,17}\):

- in the cervicobrachial fossa, between the cranial side of the front leg and the base of the neck. The chelonian can be held head up or horizontally,
- in the axillary fossa, between the caudal side of the anterior limb and the "plastron-carapace" junction,
- in the pre-femoral fossa, just cranial to the rear leg (between the "plastron-carapace" junction and the cranial side of the posterior limb).

In our experience, this last approach is the most useful one. The axillary window is usually too confined in most terrestrial chelonians and freshwater turtles to be of use. This approach has been prescribed in marine turtles to scan the liver, heart and pectoral muscles \(^{15,17}\).

In certain turtles whose shells are not very ossified (Trionyx sp., Malacochersus tornieri), the probe may be placed directly on the shell \(^{17}\).

ARTIFACTS
The acoustic barrier of the keratin on the reptile scales, the presence of bones (ribs, girdles and dermal bones) and air (lung air and intestinal gas because of the lack of a diaphragm) and the interference of the unshed dermis and the underlying air during ecysis in snakes and lizards are the main artifacts encountered in these animals when dealing with ultrasound. All these limitations can actually prevent penetration of ultrasonic waves through the patient’s body. To minimize these artifacts, copious amounts of acoustic coupling gel can be liberally applied on the skin, at least 5 minutes before application of the transducer. This is necessary to reduce trapped air on the irregular skin and under the scales but in spite of this precaution, ultrasonic examination can be very difficult in some species whose scales are ossified (Tiliqua, Tachydosaurus, Heloderma). The use of a latex glove filled with gel placed between the skin and the transducer allows for better image quality. Finally, the patient can be partially submerged in a warm water bath and then transducer-skin contact is not necessary. The probe can even be pressed with gel against the plastic container itself \(^{5,6,8,9,10,13,15}\).

PATIENT EXAMINATION
As in other animals, ultrasonographic examination of reptiles must respect a rigorous protocol: in snakes whose body shape is long and slender (Fig 1), the examination begins at the level of the heart and ends at the cloaca. In lizards (Fig 2), it begins, as with the dog and the cat, by the liver and the gallbladder and continues with the other organs in a
predetermined order (for example: digestive tract, gonads, kidneys, urinary bladder, heart). In turtles (Fig 3), positioning the probe in the axillo-cervical fossa allows, in a first step, visualising the heart, stomach and liver. Placed next in the inguino-femoral cul-de-sac, it allows visualising the echostructure of the liver, digestive tract, the bladder and the gonads.

In both human and animal medicine, “short-axis” cross-sections are obtained by taking sections perpendicular to the principal axis of the concerned organ, that is to say perpendicular to the body of the animal. In contrast, “long-axis” cross-sections are obtained by taking a cross-section parallel to the principal axis of the organ, in other words parallel to the body of the animal.

The heart of Serpentes, Lacertilia and Testudines has a single ventricular cavity, two atria, a tubular sinus venosus ending in the right atrium and three efferent arterial trunks: right aortic arch, left aortic arch and pulmonary arterial trunk. The heart of crocodilians is a four-cavity heart, fairly close to that of mammals and birds. It is located almost in the middle of the body, opposite the 11th and 12th ventral scale from the junction between the two anterior limbs. The heart of Serpentes is elongated, located caudal (15 to 25%) to the head and close to the thyroid, at the cranial pole of the lung(s), just cranial to the liver, and facing the most caudal tracheal rings. It is mobile inside the coelomic cavity. This implies that during the ultrasonography examination, the operator must at times move the probe cranially or caudally a few centimetres in relation to its initial position. Three approaches to define the ultrasound windows are used in succession. The majority of the ultrasound examination can be carried out by placing the probe ventrally in relation to the heart. This ventral approach makes it possible to view the organ from the caudal ventricular apex to the cranial atria, and examine the sinus venosus, the atrioventricular junctions and the three arterial trunks. Two other approaches, known as right and left intercostal approaches, are obtained by laterally positioning the probe. These approaches may be used to complete the ventral cranial examination and obtain a lateral visualization of the three arterial trunks and both atria that proceed into the single ventricle.

The heart of certain Lacertilia (scincidae, iguanidae, helodermatidae, agamidae and gekkonidae) is located at the level of the base of the neck, under the bony pectoral girdle which constitutes a real barrier to ultrasound. In these lizards, the ultrasonography approach route is cranio-caudal, the probe being positioned in front of and under this solid plastron. In varanidae and teiidae, the heart is located in front of the liver in a mesenchymatous sheet called the descendens cordis and can be accessed via a mid-cœlom ventral approach.

The echocardiographic examination of reptiles allows diagnosing pericardial effusion, endocarditis, myocarditis and pericarditis lesions, valvulopathy, parasite infestations and tumours. It also allows visualising the presence of uric acid crystals on the myocard, indicator of visceral gout.

The single lobed cylindrical liver of Serpentes is tubular in form. It begins just behind the heart and extends to the middle of the stomach. Snakes have a typical large hepatic vein, visualized as an anechoic stripe that helps differentiate the liver from fat bodies. Their large round anechoic gallbladder is located far behind the caudal extremity of the liver, at the mid body. In turtles and lizards, the liver is two-lobed (with the right lobe larger than the left) and partially covers the gallbladder. It is always close to the heart and can be easily visualized. It has a uniformly hypoechoic structure in all reptiles and contains anechogenic blood vessels. Compared to abdominal fatty bodies, it is generally less echogenic than adipose tissue in lizards.
The **stomach** can be found close to the caudal end of the liver in snakes. It can be visualized if it’s filled with prey, air or fluid. In chelonians and lizards, it is dorsal and caudal to the left hepatic lobe.

The five layers of the **digestive tract** mucosa, described classically in mammals, are visible only in large reptiles (large boidae, monitor lizards). The relative size of the different segments of the digestive tract varies depending on the diet: in carnivorous species, the stomach is well developed, often containing air or gastroliths, while the intestine is rather short. In herbivorous species, the stomach is rather reduced in size and the caecum and the colon (sacculated in the green iguana) are particularly voluminous and filled with faecal matter.

The **spleen** in large snakes may be identified thanks to its circular form and to its more marked echogenicity than the liver. It is found behind the liver, close to the gallbladder and the pancreas, in a cranial position in relation to the gonads. In monitor lizards, however, it is quite difficult to locate because of its small size.

The **pancreas** is always less echogenic than the liver. It is particularly difficult to visualise, as is the spleen in Testudines. In monitor lizards, on the other hand, it is quite visible.

The inactive **ovaries** are anechogenic and small in snakes and lizards, whereas they are hyperechogenic in Testudines. In lizards and snakes, the eggs present in the oviducts seem to have two distinct layers: the more superficial layer, formed of anechogenic albumen (forming a black ring at the periphery) and the deeper layer, constituted of more echogenic vitellus. Around the circumference of the eggs, is found the shell, more or less echogenic depending on the degree of calcification. On the contrary, the ovarian follicles, constituted of vitellus, are very echogenic and do not show an image of concentric circles like calcified eggs. In ovoviviparous species, skeletons and movements of embryos are easily visualised.

Ultrasonography is probably the most important tool for the assessment of the ovaries in chelonians, and particularly in the diagnosis of follicular stasis, which is a common reproductive disorder in most of reptiles. Vitellogenic follicles are spherical, homogenous, echodense structures of up to 25 mm in mediteranean tortoises. They may be clustered like grapes and fill a significant part of the coelomic cavity.

**Testes**, which are slender and fusiform in shape, are difficult to image in snakes.

The **kidneys** are located caudally with respect to the gonads. In lizards, they are intra-pelvic, but in the case of nephromegaly (frequent in case of renal failure) they are visible both in a cranial and a caudal position with respect to the pelvic girdle and can be palpated through the coelomic cavity and easily visualized during ultrasound examination of the coelomic cavity. The transducer can then be placed against the sublombar fossa and directed towards the pelvic inlet. It can also be placed against the lateral base of the tail and the beam directed cranially toward the pelvis outlet. In snakes, the right kidney is cranial in relation to the left kidney. There is no distinction between renal pelvis, medulla and cortex as is the case with mammals. The kidneys present a homogeneouse echostructure, more echogenic and granulated than intra-abdominal adipose bodies.

Chelonian kidneys are in a retrocoelomic position. They are generally crescent shaped, fairly voluminous, only slightly lobulated. They are located close to the carapace, caudal to the gonads and the bladder, under the most caudal portions of the lungs. The center of the kidney is often hypoechogenic and the remainder more or less hyperechoic. These organs are difficult to examine by ultrasound: the probe must be
directed dorsally to the maximum extent 17. In snakes, they are typically plurilobulated and the right one is cranial to the left one.
The urinary bladder (and the accessory bladders, when present) is (are) located in a caudo-ventral position, cranial to the pelvis. It does not exist in snakes. Its wall is thin and its content anechogenic (black). It is only easily visible when filled with urine. Uric acid particles in suspension are often visible in it2,3,4,5,6,8,9,10.

CONCLUSION
Ultrasound examination is a very useful diagnostic tool in reptile medicine. It is an excellent compliment to radiography to provide information regarding organ location and pathologic changes. The ventral approach is the main ultrasound display window that provides an almost complete exploration of the coelomic cavity in snakes, lizards and small sized or juvenile crocodilians. In snakes, despite artifacts induced by the ribs, lungs and air sacs, the lateral approach is also useful to explore the liver, the gall bladder and particularly the ovaries in females. In turtles and tortoises, the prefemoral approach is the main ultrasound window to explore the enigmatic interior of these particular reptiles. Examination of the heart is performed by a cervico-brachial approach.

LITERATURE CITED
Figure 1: Exploration échographique de la cavité coelomique chez les ophidiens: illustration schématique de la topographie des organes internes (d'après Chiodoni et al., 1992).

A: trachée
B: thymus
C: thyroïde
D: cœur
E: Vestige du poumon gauche
F: poumon droit
G: oesophage
H: sac à air
I: foie
J: estomac
K: vésicule biliaire
L: rate
M: pancréas
N: intestin grêle
O: testicule
P: glande surrénale
Q: canal déférent
R: côlon
S: rein
T: uretère
U: cloaque
V: glandes parfumées
W: hémipénis
**Figure 1: Ultrasonographic exploration of the coelomic cavity in Serpentes: schematic illustration of internal organ topography (from Chiodoni et al., 1992).**

<table>
<thead>
<tr>
<th>A: trachea</th>
<th>M: pancreas</th>
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</thead>
<tbody>
<tr>
<td>B: thymus</td>
<td>N: small intestine</td>
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<tr>
<td>C: thyroid</td>
<td>O: testicle</td>
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<tr>
<td>D: heart</td>
<td>P: adrenal gland</td>
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<tr>
<td>E: vestige of left lung</td>
<td>Q: ductus deferens</td>
</tr>
<tr>
<td>F: right lung</td>
<td>R: colon</td>
</tr>
<tr>
<td>G: oesophagus</td>
<td>S: kidney</td>
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<tr>
<td>H: air sac</td>
<td>T: urethra</td>
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<tr>
<td>I: liver</td>
<td>U: cloaca</td>
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<tr>
<td>J: stomach</td>
<td>V: scent glands</td>
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<tr>
<td>K: gallbladder</td>
<td>W: hemipenis</td>
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<tr>
<td>L: spleen</td>
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