Universitat Autònoma de Barcelona

ANATOMICAL DESCRIPTION OF THE COELOMIC CAVITY ORGANS USING RADIOGRAPHY, ULTRASONOGRAPHY, AND COMPUTED TOMOGRAPHY IN HEALTHY VEILED CHAMELEONS (Chamaeleo calyptratus) AND PANTHER CHAMELEONS (Furcifer pardalis)

ADRIÁN MELERO JURADO

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Memoria para optar al grado de Doctor presentada por

ADRIÁN MELERO JURADO

Director:

Jaume Martorell Monserrat

Departament de Medicina i Cirurgia Animals

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El Doctor **JAUME MARTORELL MONSERRAT**, Profesor Agregado de Universidad de las Áreas de conocimiento de Medicina y Cirugía Animal de la Facultad de Veterinaria de la Universitat Autònoma de Barcelona,

HACE CONSTAR,

Que la memoria titulada "**ANATOMICAL DESCRIPTION OF THE COELOMIC CAVITY ORGANS USING RADIOGRAPHY, ULTRASONOGRAPHY, AND COMPUTED TOMOGRAPHY IN HEALTHY VEILED CHAMELEONS (*Chamaeleo calyptratus*) AND PANTHER CHAMELEONS (*Furcifer pardalis*)",** presentada por Adrián Melero Jurado para la obtención del grado de Doctor en Veterinaria por la Universidad Autònoma de Barcelona, ha sido realizada bajo mi dirección y, considerándola satisfactoriamente finalizada, autorizo su presentación para que sea juzgada por la comisión correspondiente.

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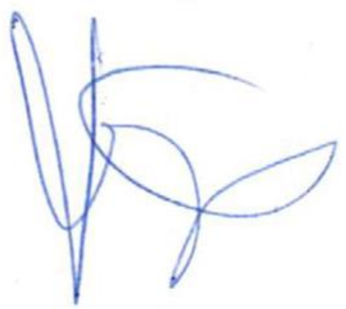

Firmado: Jaume Martorell Monserrat

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1. ABSTRACT

Veiled chameleon (*Chamaeleo calyptratus*) and Panther chameleon (*Furcifer pardalis*) are the most popular chameleons over the world, and consequently, two of the most frequently species attended in veterinary practice.

The most commonly used imaging techniques in diagnostic protocols in herpetology include radiography, ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI). However, few studies about imaging description in reptiles have been published.

The objectives of this prospective anatomic study were to develop imaging techniques for these species and to describe the normal anatomy of the coelomic organs using radiography, US and CT scan.

Seventeen healthy veiled chameleons (7 males and 10 females) and fifteen healthy panther chameleons (13 males and 2 females) were included in the study. Animals were considered to be healthy on the basis of the results of a complete physical examination and coprology testing.

The imaging study was performed in sedated chameleons after the administration of alfaxalone (Alfaxan®, Crawley, UK) 4-6 mg/kg IV in the ventral vein of the tail. A right lateral and dorsoventral radiographic views were performed with a mammography cassette plate in all animals. Then, an ultrasound was performed in right lateral recumbency using a linear 15 to 18-MHz transducer. Finally, a CT examination was performed with a 16-slice helical CT scanner in sternal recumbency. Post-mortem study of one chameleon of each species were used to clarify and illustrate coelomic anatomy and to assess imaging findings.

The results of the current study suggest that the different imaging techniques (radiography, ultrasonography and computed tomography) allow the visualization of the liver (including caudal vena cava and hepatic veins), gallbladder, stomach, intestines, gonads, fat bodies and kidneys in healthy veiled and panther chameleons. The urinary bladder was identified using US and CT examination. The spleen, pancreas and adrenal glands were identified in post-mortem studies, but could not be visualized with any imaging technique.

In conclusion, this study provides a guide of the normal imaging anatomic features of the coelomic organs in veiled chameleons and panther chameleons. Findings can be used as a reference for future research studies or for examinations of clinically ill patients.

2. RESUMEN

El camaleón velado (*Chamaeleo calyptratus*) y el camaleón pantera (*Furcifer pardalis*) son dos de las especies de camaleón más populares en el mundo, y en consecuencia, dos de las que más frecuentemente se atienden en la consulta veterinaria.

Las diferentes técnicas de diagnóstico por imagen suelen incluirse de forma rutinaria en los protocolos diagnósticos en medicina herpetológica, entre ellas la radiografía, la ecografía, la tomografía computerizada y la resonancia magnética. Sin embargo, los estudios publicados sobre descripción anatómica mediante técnicas de imagen son escasos, y la interpretación de estas últimas se basa en la experiencia propia del clínico o bien por extrapolación de otras especies ya estudiadas.

Por este motivo, los objetivos de este estudio anatómico prospectivo fueron desarrollar protocolos de diagnóstico por imagen en estas especies y describir la anatomía de los órganos de la cavidad celómica en animales sanos, mediante el uso de radiografía, ecografía y tomografía computerizada.

Se incluyeron diecisiete camaleones velados (7 machos y 10 hembras) y quince camaleones pantera (13 machos y 2 hembras) sanos en base a los resultados del examen físico general y del estudio coprológico. El estudio se realizó en camaleones sedados tras la administración de alfaxalona (Alfaxan®, Crawley, UK) 4-6 mg/kg IV en la vena ventral de la cola. Una vez sedados, se realizó un estudio radiográfico con dos proyecciones (lateral derecha y dorsoventral) con chasis de mamografía. Seguidamente, se realizó un estudio ecográfico completo de la cavidad celómica mediante sonda lineal de 15 a 18-MHz en decúbito lateral derecho. Finalmente, se realizó una tomografía computerizada helicoidal con un scanner de 16 cortes en decúbito esternal. Se realizó el estudio post mortem de un ejemplar de cada especie por tal de esclarecer, ilustrar y evaluar los hallazgos de imagen.

Los resultados obtenidos sugieren que las diferentes técnicas de imagen estudiadas permiten la visualización del hígado (incluyendo la vena cava caudal y las venas hepáticas), la vesícula biliar, el estómago, los intestinos, las gónadas, los cuerpos grasos y los riñones en camaleones velados y pantera. La vejiga de la orina solo pudo identificarse mediante ecografía y tomografía. El bazo, el páncreas y las glándulas adrenales fueron identificados en los estudios post mortem pero no pudieron ser visualizados mediante ninguna de las técnicas de imagen.

En conclusión, este estudio proporciona una guía de las características anatómicas normales de los órganos celómicos mediante técnicas de diagnóstico por imagen en camaleones velados y camaleones pantera. De esta forma, los hallazgos pueden ser utilizados como referencia para exámenes de pacientes enfermos o para futuros estudios de investigación.

3. INTRODUCTION

Chameleons are a group of saurian with great interest among biologists, veterinarians and herpetology enthusiasts for their aesthetics, their variability between species and their peculiar anatomical and physiological characteristics. However, the way to a good maintenance and reproduction in captivity is being very slow due to the specific environmental and dietary requirements.

This long and slow way travelled in the last 20 years towards its correct maintenance in captivity has been closely related to the evolution of its medicine, as well as to the demand generated by receiving quality and specialized veterinary care.

Hence the need in recent years to conduct scientific studies that focus their attention on the study of these species.

The present study is about two of the most popular species of chameleons maintained in captivity in Europe and the rest of the world: the Yemen or veiled chameleon (*Chamaeleo calyptratus*) and the panther chameleon (*Furcifer pardalis*).

3.1. General description

Chameleons share many anatomical and physiological characteristics with all reptiles. Reptiles are ectothermic: they cannot generate their own body heat and they need external sources to regulate their body temperature. Some metabolic heat is produced but the poor insulation due to the lack of fur and body fat means this heat cannot be retained.[1,2]

Reptiles require a range of temperatures to be able to thermoregulate (known as the preferred optimum temperature zone (POTZ)). It is usually within a range of 20–38ºC, but it can vary by 4–10ºC, depending on the species. Within this range, reptiles reach the preferred body temperature (PBT) which is the optimum temperature for the functioning of the reptile metabolism (e.g., healing, digestion, reproduction, or immunocompetence).[2-5]

The reptile metabolism is slower than mammals and it is also influenced by other factors such as diet and predation behaviour. Active predators, such as chameleons, have higher metabolic rates and, as they feed daily, expend their energy maintaining their gut functions all the time.[6,7]

Veiled chameleon (*Chamaeleo calyptratus*)

Veiled chameleon (*Chamaeleo calyptratus*) lives in the humid coastal lowlands, coastal slopes, and high plateaus of the southwest corner of the Arabian Peninsula in Yemen and Saudi Arabia. He is an arboreal lizard and prefers to live in bushes and shrubs.[8,9,10]

The veiled chameleon is characterized as an aggressive, brightly coloured chameleon (Figure 3.1.1.). Mature males have a larger casque (head crest or helmet) than females. They are born with tarsal spurs which makes sexing them very easy. Male body length can reach 55cm and the female's length is about 35-40cm, but females are heavyly bodied.[10]

The body is very laterally flattened and the body drawing is variable but usually consists of stripes of green and yellow coloration.

The average life expectancy is usually about 5 years for both sexes, reaching their sexual maturity at 5-6 months of age.[8]

Like other species, they do not usually drink from a container. A drip or artificial rain system should be provided, or water sprayed on the leaves and branches at least three times a day. The humidity should be maintained around 50%.

They require direct UVB / UVA light, either in the form of sunlight or in the case of keeping them in captivity, with a UVB spotlight suitable for reptiles. The photoperiod should range between 12-14h of light and 10-12h of darkness.[11]

They are one of the few species of chameleons which can tolerate wide temperature ranges, though they prefer to live in a temperature range of 24-26ºC maximum daytime and about 18-20ºC at night, keeping also a hot point of maximum 32-35º inside the terrarium so that the chameleon can be heated if desired.[8] These temperatures are based on the mean temperatures along the year in Yemen.

Panther chameleon (*Furcifer pardalis*)

Panther chameleon (*Furcifer pardalis*) are originally from the island of Madagascar. Their distribution in the island is very wide, and they also inhabit nearby islands and islets, as well as in Mauritius and Reunion, where they have been introduced and naturalized (they are called 'Reunion Endormi, lendormi or zendormi').[8,12]

Originally, the panther chameleon lived in the leafy forests, but the destruction of the habitat due to massive logging and deforestation for agricultural purposes have caused them to adapt to other environments, preferring open forests and clearings with low and medium-sized thickets, farmland, gardens, coffee plantations, and are common even in coastal dunes. They can be found in altitudes ranging from sea level to 1200m.[8,12]

There are many chromatic patterns, known as "phases" or "localities" in panther chameleons. This fact is due to the wide distribution in malgache areas and the isolation between populations, separated by natural barriers and without genetic exchange (Figure 3.1.2.)

Males can reach approximately 55cm, but do not usually exceed 45cm. The size of the females is smaller, around 30-35cm.[8] Life expectancy is usually 4 years, being lower for females (about 3 years), and a maximum of 6 years for males.[8]

The proper environmental humidity should be around 60-90%, depending on the locality of each chameleon, since within the island of Madagascar can be quite variable. In captivity, it is achieved thanks to artificial rain systems or spraying them with water several times a day.

As in veiled chameleons, they require direct UVB / UVA light. The photoperiod should range between 12-14h of daylight and 10-12h of darkness.[12]

The POTZ (based on the mean temperature along the year in Madagascar) should range from 24-26ºC of maximum daytime and about 16-18ºC as night temperature. A maximum heat point (around 30-32ºC) should be provided inside the terrarium.[8,12]

Both species are mainly insectivores, but veiled chameleons are one of the few chameleons that also enjoy the taste of plants. They are adapted to eating leaves of plants especially when they cannot get a source of liquid water during the dry seasons.[12]

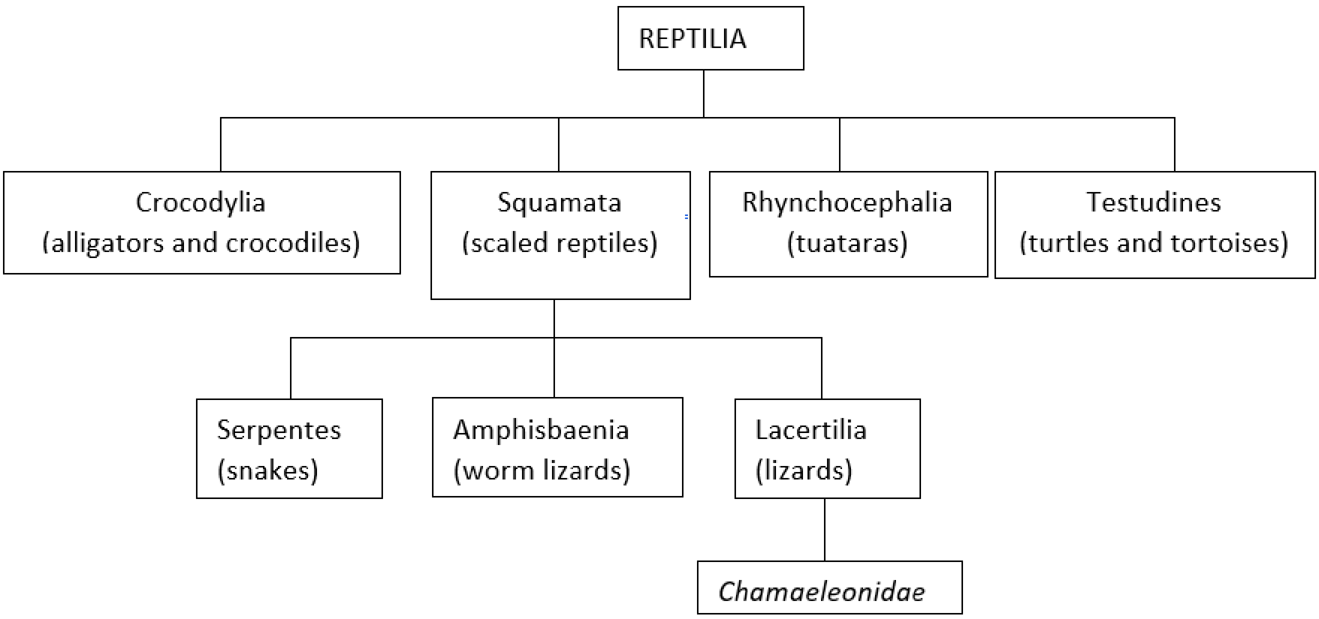
FIGURE 3.1.1. Image of an adult male of veiled chameleon (*Chamaeleo calyptratus*).

FIGURE 3.1.2. Photograph showing the Sambava locality of Panther chameleon (*Furcifer pardalis*)

3.2. Taxonomy

Chameleons belong to the reptilia class, subclass diapsida, by the presence of two holes (temporal fenestra) in each side of their skulls. They are classified in the squamata order; this order, in turn, is divided into three sub-orders: lacertilia (lizards), serpentes (snakes) and amphisbaenia (worm lizards). The family of Chamaeleonidae belongs to the lacertilia sub-order (Table 3.2.1).[13]

The family Chamaeleonidae was divided into two subfamilies: Brookesiinae and Chamaeleoninae.[14] Under this classification, Brookesiinae included the genera Brookesia, Rhampholeon and Rieppeleon; while Chamaeleoninae included the genera Bradypodion, Calumma, Chamaeleo, Furcifer, Archaius, Nadzikambia, Kinyongia and Trioceros.[8,15]



ALGORITHM 3.2.1. Taxonomy of the family Chamaeleonidae.

3.3. Coelomic anatomy and physiology of the family Chamaeleonidae

Musculoskeletal system

Reptiles have a non-ossified movable skull and the cartilaginous tissue allows a great mandibular laxity in order to avoid scaping of the prey. Chameleons have lost their mandibular laxity; however, they have a powerful tongue with a similar function.

The laterally flattened body of chameleons and prehensile feet enable them to keep their centre of gravity over a narrow support base. The chameleon's feet are zygodactyl, with toes fused together and opposed in groups of two and three. The tail is prehensile, non-regenerable and without autotomy. These features allow them to maintain their centre of gravity on a narrow support base.[13,16]

Integument

They have relatively thick, protective and keratinized dry skin with ectodermal scales formed by folding of the epidermis and outer dermal layers.

The skin contains few glands. Many lizards have femoral or precloacal pores. These are not true glands but rather are invaginations of the skin that produce a waxy secretion for territorial marking and social communication. They tend to be larger and more developed in mature males.[13] Chameleons do not have these structures.

The different skin colour patterns are due to the presence of multiple types of pigment cells in the dermis of the skin known as chromatophores. Chameleons are able to undergo rapid colour changes due to hormonal and neurologic control mechanisms of the chromatophores to alter their size and positioning within the dermis.[13]

Under the skin, they have an insulating lipid layer that prevents dehydration, which has allowed them to adapt to terrestrial life. In addition, the skin is also innately involved in the synthesis of vitamin D_3.[13,16]

Cardiovascular system

Chameleons have a three chambered heart, with left and right atria and a single ventricle. The ventricle is divided into 3 chambers: the cavum arteriosum, the cavum venosum and cavum pulmonale. Blood leaves the heart through the pulmonary artery arising from the cavum pulmonale and the two aortic arches arising from the cavum venosum.

In this way they can pump blood to the lungs or other parts of the body, facilitating thermoregulation or diving. Thus, the three-chambered heart function is similar to that of a four-chambered heart.[13]

Chameleons have renal portal system. It has been well documented in the literature for its parenteral therapeutic implications. Venous circulation from the tail, and little from the hind limbs, routes directly to the kidneys via renal portal system. The injection of drugs into the caudal half of the body could undergo a first-pass excretion from the kidney before entering the systemic circulation.[13,16]

Blood pressure is important for maintaining good tissue irrigation. Unlike other classes of animals, the control of baroreceptors is not temperature dependent.

The spleen is an important extramedullary haematopoietic organ. In chameleons, it lies in the dorsal mesentery, close to the greater curvature of the stomach and pancreas, as in mammals. However, the spleen-to-bodyweight ratio is the smallest of any vertebrates. It appears to be primarily lymphopoietic and no blood storage capacity has been reported in reptiles. The predominant splenic lymphocyte is the T-cell.[17,18]

Respiratory system

The upper respiratory tract is formed by the choana (two long openings located in the palate and communicating with nasal cavity and nares). The glottis is located caudally, just at the base of the tongue and it is normally closed except during inspiration and exhalation. The trachea has incomplete rings and bifurcates near the level of the heart.[13,16]

The lower respiratory tract is formed by the lungs, which are divided into interconnected chambers by a few large septa, and there is a membrane that connects to the pericardium. Chameleons have hollow, smooth-sided, finger-like projections on the margins of their lungs similar to air sacs. These are not used in gas exchange and allow increasing their lung volume by up to 40% in order to intimidate predators. Some chameleons also have an accessory lung lobe that projects from anterior trachea cranial to their forelimbs. This may fill with secretions in case of infection, resulting in swelling of the ventral neck.[13,16]

Digestive system

The lips of chameleons have flexible skin but are not movable. The teeth are acrodont (not replaced except in very young animals), which are attached to the biting edges of the jaws without sockets. The tongue is mobile and telescopic; the hyoid apparatus and the accelerator muscle allow to propel it and capture prey.[13]

The gastrointestinal tract of insectivorous reptiles is simple. The oesophagus is short, thin-walled, and enters the stomach (tubular, simple and divided into fundic and pars pylorica regions) on the left side of the coelom. The intestine is very short, which flows into the cloaca. The cloaca is divided into 3 parts: the coprodeum (which collects faeces), the urodeum (which collects urine and receives the sexual structures) and the proctodeum (the final chamber before the vent).

The liver is encapsulated, bilobed and possesses gall bladder. The right lobe is the larger of the two lobes. The gall bladder is located between the two lobes and has an important role in fat digestion. Bile emulsifies fat and allows it to be absorbed in the intestine. Biliverdin is the main biliary pigment; the chameleons do not produce bilirubin due to the lack of the enzyme biliverdin-reductase.[13,16]

The pancreas in chameleons is a small elongated structure that lies along the mesenteric border of the duodenum.[13]

Reproductive system

Chameleons are oviparous and they have breeding seasons determined by photoperiod cycles, temperature, rainfall and availability of food.[8,13] In males, a corresponding fluctuation is seen in testicular size.[16]

Mature males have bilateral hemipenal bulges at the base of the tail. These are an invagination of the cloacal wall with the ability to externalize and retract during and after copulation thanks to the hemipenis retractor muscle.[13,16] Some species of chameleons also have head structures as horns, crests, and plates that are lacking in females.[8] Mature males of veiled chameleons have a larger casque (head crest or helmet) than females.

They have paired testes, provided with epididymis and vas deferens. The testes are located cranially to the kidneys, with the right testis positioned more cranially than the left. There is no urethral structure.[13,16]

Females have paired ovaries and oviducts, which terminate at the urodeum of the cloaca. The ovaries are located attached to the peritoneum along the ventral surface of each kidney. In chameleons, the ovary can extend cranially between the two lungs due to the increased size and composition depending on age and time of year in relation to breeding season.[13,16]

Urinary system

In chameleons, the kidneys are metanephric, paired, symmetric, elongated, slightly lobulated, and flattened laterally.[13,16] The kidneys are located in the caudodorsal coelom, and the caudal poles are located within the pelvic canal, extending to the base of the tail.[19]

Chameleons kidneys lack a renal pelvis and also lack the loop of Henle in the nephrons.[19] Thus, they are unable to concentrate urine above that of plasma. Chameleons excrete insoluble uric acid, which allows water conservation. Uric acid offers the advantage that it is excreted with minimal water loss; but unlike mammals, it is excreted by renal tubules, so dehydration does not slow down their excretion.[19] As such, if uric acid increases in blood due to dehydration or kidney disease, a uric gout may occur.

Chameleons have thin-walled bladder. The urinary waste flows through the ureter into the urodeum of the cloaca before entering the urinary bladder (or colon in species that lack a urinary bladder). As discussed above, the kidneys cannot concentrate urine; therefore, the colon, urinary bladder, and cloaca have an important role in water reabsorption. Active ion transport and passive water absorption occur in the wall of the colon, while the bladder also reabsorbs water and sodium, but secretes potassium and urates.[19]

Fat bodies

The fat bodies are paired structures that lie adjacent to the kidneys and gonads in the caudal celomic cavity. The size of the fat bodies can provide a general assessment of the body condition of the patient. Some reptiles from temperate climates use these structures to provide yolk for the first clutch of eggs after the winter. Males show similar cycles but have smaller fat bodies than females. Their main function is fat storage.[13,20]

Endocrine system

The endocrine system is comprised by numerous glands. The gonads are responsible for the production of sex hormones. Their production is influenced by photoperiod, temperature, and seasonal cycles. The pituitary gland has the same functions as in other vertebrates, producing adrenocorticotropin, prolactin, and follicle-stimulating hormone.[13,16]

The pineal gland, closely associated with the parietal eye, detects the presence and absence of light, and produces an important hormone in daily and seasonal cycles, the melatonin. This gland converts photic stimuli into neuroendocrine messages and may play a role in thermoregulation. Some lizards have a more superficial parietal gland or third eye, which has a lens, cornea, and retina and is located just beneath the skin in the parietal foramen at the junction between the parietal and frontal bone. Although it does not form images, it is thought to sense changes in the intensity and wavelength of light and may aid thermoregulatory shuttling. Crocodiles and chameleons lack parietal eye.[13,16]

Chameleons, like mammals, also have thyroid and parathyroid glands. The thyroid glands maintain and stimulate metabolism under pituitary control. They also play an important role in normal ecdysis and growth. Thyroid morphology varies between species and can be paired, bilobed or unpaired; the commonest is the bilobed organ with an isthmus over the trachea, as in mammals. In reptiles, the parathyroid glands are found near the thymus or ultimobranchial bodies and not with the thyroids. The parathyroid glands control plasma calcium and phosphorous levels, with similar mechanisms as mammals

Adrenal glands are located in the mesorchium or mesovarium and have the same functions as in other vertebrates. These are yellow or red in colour and lie retroperitoneally in crocodiles and chelonians and closely adherent to the gonads in lizards and snakes. In lizards, they always lie dorsal to the gonads and lie asymmetrically with the right cranial to the left.[13,16]

The pancreas has the same endocrine and exocrine functions as in other animals. In lizards it has three parts: one extending towards the gall bladder, one towards the duodenum, and one towards the spleen. Insulin and glucagon control glucose levels.[13,16]

3.4. Current importance of diagnostic imaging in reptiles

Diagnostic imaging techniques are important tools for the morphologic and functional evaluation of internal organs and the diagnosis of reptilian diseases.[21]

These techniques generally have been underused in reptile medicine due to the relatively small amount of information published regarding imaging techniques and interpretation, the paucity of veterinary radiologists skilled in diagnostic imaging of reptiles, the poor tissue contrast and the overlapping structures in techniques such as radiography, and the lack of clear guidelines for normal and abnormal imaging parameters of these species.[22]

Fortunately, these conditions are improving: the volume of published information on imaging of reptiles is increasing, the newer imaging methods, CT and MRI are being increasingly utilized for diagnostic imaging evaluation procedures for reptile patients and are providing information not attainable from radiographic studies. Currently, there are diagnostic and therapeutic measures available that were not possible a few years ago to implement reptile and amphibian patients.[23]

These advances have led owners to expect and demand more targeted and competent diagnostic testing for their animals.

3.4.1. Radiography

Radiography is commonly used as a diagnostic test to further evaluate the health of reptile patients. It is the most commonly used imaging technique in reptiles to assess the respiratory tract, gastrointestinal tract, urinary tract, and skeletal system. Specific metabolic disorders (e.g. metabolic bone disease in lizards and chelonians) as well as infectious diseases can also be diagnosed using radiographic techniques.[21] Although the consulted literature cites several times that this technique is underused in reptile clinical practice compared with its use in dogs and cats, after the review it can be observed that in most cases radiography is part of the diagnosis. However, the inherent tissue contrast of the coelom and the lack of accessibility to reference material are some important factors that can affect its use and must be considered.[22,24]

<u>Equipment</u>

The radiographic equipment used in reptile medicine is similar to that needed in small animals. To enable the use of short exposure times, x-ray machines with a performance of at least 200 mA and a voltage range between 40 kV and 100 kV are recommended for reptile and amphibian patients.[23]

Films or film-screen combinations utilized in mammals are also useable in reptiles. High definition mammography films are very suitable to evaluate small animals as the detail resolution is much better than the standard film-screen combinations.[23]

The use of new digital radiographic systems allows picture adjustment. This can make the diagnosis much easier and reduces the number of X-ray shots due to poor quality images, reducing radiation exposure of the patient.[23]

Restraint and positioning

The different imaging positions utilized for diagnostic evaluation of reptile species varies significantly between lizards, snakes, and chelonians. The two standard positions for the radiographic investigation of lizards are dorsoventral and lateral. The three standard projections used in chelonians are dorsoventral, lateral, and craniocaudal.[21-27]

Correct positioning of the patient is important to obtain good diagnostic images and to evaluate normal gross morphology of internal structures. Chameleons can be restrained successfully in both positions, with ropes, adhesive tape or radiographed in radiolucent boxes. (Figure 3.4.1.1) Very weak animals can also be radiographed dorsoventrally without being restrained. Manual restraint is achieved by holding the hindlegs of the animal against the tail base with one hand; the other hand should hold the forelegs together alongside the neck. Invasive procedures and the nature and disposition of some species (eg., venomous snakes and large lizards) may require sedation or general anaesthesia to facilitate imaging and prevent injuries to patient and handler.[21,27]

3.4.2. Ultrasonography

Ultrasonography is an important non-invasive diagnostic tool in reptile medicine and allows visualization and interpretation of normal anatomy and texture of visceral organs. It is a very important technique for the detection of soft tissue abnormalities, such as cysts, abscesses and neoplasia, and free fluid within the coelomic cavity; and it is very useful for evaluation of the reproductive status of reptiles. Ultrasonography is also routinely used for the collection of biopsy samples and aspirates from visceral organs, intracoelomic masses, and fluids.[21,28]

FIGURE 3.4.1.1. Panther chameleon restrained with adhesive tapes during the radiographic examination.

Equipment

Small reptiles require high resolution equipment and 7.0 to 12.0 MHz transducers with small footprints. For large reptile species such as large chelonians or crocodilians, 5.0 MHz or 3 MHz transducers are recommended to ensure a satisfactory depth of ultrasound wave penetration. Linear, convex or sector transducers can be used, and they are chosen depending on the reptile size and the structure examined.[21,28-30]

Application of coupling gel is recommended to facilitate good contact with the transducer. Alternately, submergence of the body part being imaged in warm water will improve contact and image quality.[28,30]

Restraint and positioning

Most reptiles can be imaged with manual restraint alone, but the animal should be sedated or anaesthetized in order to avoid transducer damage and prevent injuries to patient and handler, especially in venomous animals, large and aggressive reptiles, and stressful patients.[21,30]

In lizards, there are three main approaches: the ventral approach, the renal approach from the dorsal body wall, and the heart approach from the cranial or axillary region. The ventral coupling site is the main approach to examine the majority of the organs in the body cavity.[30]

Most lizard species are positioned in dorsal recumbency. However, in flattened species such as chameleons, the examination is performed with the animal positioned in lateral recumbency.

In snakes, the approach is usually along the ventral body wall in the area delineated by the ventral scales.[29,30]

Finally, in chelonians, only the prefemoral and cervicobrachial areas are suitable as coupling sites, therefore very small transducers must be used on most patients. Most internal organs can be identified through the prefemoral windows (Figure 3.4.2.1.).[29,30]

FIGURE 3.4.2.1. Transducer position in the prefemoral window during ultrasonography in a yellow-bellied slider (*Trachemys scripta scripta*). prefemoral window.

Chelonians are positioned in ventral recumbency or slightly inclined for an ultrasonographic study. They can be placed on a container so that their legs hang in the air. The sedation or anaesthesia of the animals are usually required due to the difficulty or inability to extend the limbs.[28,30]

3.4.3. Computed tomography (CT)

Computed tomography (CT) is a three-dimensional radiographic procedure that uses thousands of fast projections taken with a radiograph tube that spins around the patient.[31]

CT provides two-dimensional or three-dimensional reconstructed images and is particularly good for supplying detail of hard structures (bones, eggs) or airways. It avoids the overlapping of different structures (organs, shells, or scales) as happens in the conventional radiographic studies.[31] CT scan allows to evaluate the vasculature (visualized with a radiopaque contrast injection), the skeletal system (metabolic bone diseases, shell fractures in chelonians, mandibular and/or maxillary abscesses in lizards and osteomyelitis of the spine in snakes), respiratory tract, reproductive system, liver, spleen and kidney diseases and neoplasia in reptiles.[32-38]

Equipment

The scanners used are usually those designed for human medicine, however, the examination parameters (slice thickness, pitch, anatomical region to be imaged, processing algorithm, contrast medium type, dose, rate of infusion, and timing of imaging relative to contrast administration) must be adapted depending on the device (2-, 8-, 16-, or 64-slice CT) and the reptile patient. Multiplanar reconstructions (MPR) are the display of transverse images in sagittal and dorsal planes with the ability to triangulate a lesion on all three views.[31,39,40]

Finally, an important advantage of the CT is the duration of the procedure. It usually lasts a maximum of approximately 90 seconds, which is very short in comparison to other imaging modalities such as MRI.[39]

Restraint and positioning

Reptiles need to be immobilized to avoid artifacts caused by movement. The animals can be fixed on special tables or the patients' legs are taped into the shell in the case of chelonians. However, anaesthesia is usually needed in very active chelonians, lizards, and snakes. In weak and stuporous reptiles, CT-scan can be performed without anaesthesia.[31,39,40]

The animals are positioned in sternal recumbency (Figure 3.4.3.1.). Transverse views are the commonly used planes for CT examinations. But in chelonians and lizards, transverse, sagittal and dorsal planes are usually reconstructed to evaluate the images. [31,39,40]

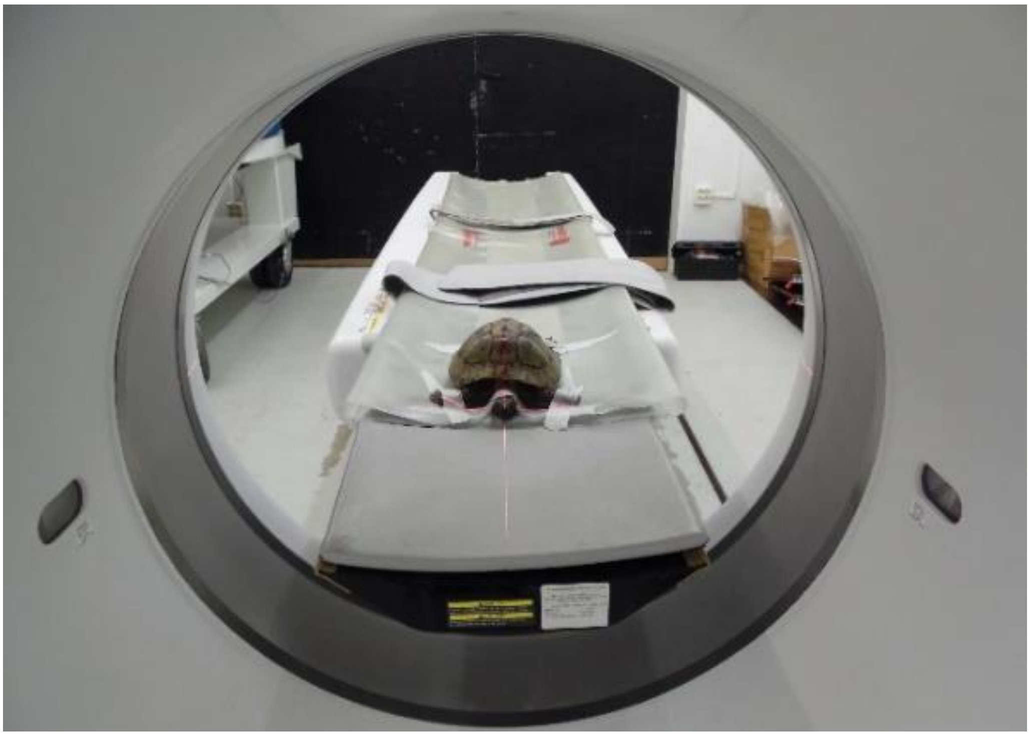


FIGURE 3.4.3.1. Photograph showing a Greek tortoise (*Testudo graeca*) positioned in sternal recumbency during the CT examination.

4. OBJECTIVES AND HYPOTHESIS

The objectives and hypothesis of the current study are:

General objective:

1. To describe the coelomic organs using radiography in healthy veiled and panther chameleons.

Specific objective:

2. To provide a radiology protocol, appearance and reference biometry in healthy veiled and panther chameleons.

General objective:

3. To describe the coelomic organs ultrasonography in healthy veiled and panther chameleons.

Specific objective:

4. To provide an ultrasound protocol, appearance and reference biometry in healthy veiled and panther chameleons.

General objective:

5. To describe the coelomic organs using CT scan in healthy veiled and panther chameleons.

Specific objective:

6. To provide a CT protocol, appearance and reference biometry in healthy veiled and panther chameleons.

Hypothesis

'The imaging techniques assessed (radiography, ultrasonography and computed tomography) allow to identify and evaluate the most coelomic organs in veiled and panther chameleons.

5. STUDIES

5.1. STUDY I

Radiographic appearance of the coelomic cavity organs in healthy veiled chameleons (*Chamaeleo calyptratus*) and panther chameleons (*Furcifer pardalis*)

INTRODUCTION

Radiography is commonly used as a diagnostic test to further evaluate the health of a reptile patient; it is the most commonly used imaging technique in reptiles to assess the respiratory tract, gastrointestinal tract, urinary tract, and skeletal system. Specific metabolic disorders as well as infectious diseases can also be diagnosed using radiographic techniques. However, this technique is underused in reptile clinical practice compared with its use in small animals.[22,24] The lack of anatomic references is one of the most important factors that can affect its use and must be considered.

Veiled chameleon (*Chamaeleo calyptratus*) and panther chameleon (*Furcifer pardalis*) are the most popular chameleons in some Europe countries, and consequently, two of the most frequently species attending veterinary practices.

However, to the author's knowledge, the description of normal radiographic anatomy has not been performed comprehensive in the veiled chameleon (*Chamaeleo calyptratus*) and panther chameleon (*Furcifer pardalis*).

Therefore, the objectives of this study were to develop a reference guide for imaging interpretation and to describe the normal radiographic appearance of the coelomic organs in both species.

MATERIAL AND METHODS

The aim of this prospective anatomic study is to describe the radiographic appearance to coelomic organs of chameleons. Nineteen veiled chameleons (9 males and 10 females) and seventeen panther chameleons (14 males and 3 females) from a pet shop facility were used in the present study. Animals were healthy on the basis of results of a complete physical examination and coprology.

The animals were housed in groups of two or three in separate terrariums equipped with UVB lights and heat bulbs during 3 days. The environmental temperature was approximately 28-30ºC during the day, and 22-24°C at night. Humidity was provided with a watering artificial rain system for a minute every hour, 8 hours a day. The lighting cycles were of 14 hours of light and 10 hours of darkness. The animals were fasted for 2 days prior to diagnostic imaging to empty the gastrointestinal system.

Radiography: the radiographic study of the coelomic cavity was performed in each animal by 1 Diplomate of the European College of Veterinary Diagnostic Imaging (ECVI), 1 ECVI resident and 1 Diplomate of the European College of Zoological Medicine (ECZM) or 1 ECZM-Herpetology resident. All the animals were acclimated in standard conditions for these species as it has been described before. The animals were used to performed other anatomical studies using ultrasonography and computed tomography.

The imaging study was performed in sedated chameleons after the administration of alfaxalone (Alfaxan®, Crawley, UK) 4-6 mg/kg IV. They were restrained in dorsoventral and right lateral recumbency using adhesive tapes.

Images were obtained with an indirect digital radiography system or computed radiography (CR) APR-VET-32KW-T380 SEDECAL, S.A X-ray machine using a mammography cassette plate (CP1M200, 18x24, Konica Minolta) and revealing with a Digitizer Konica Minolta (Regius Model 170). All studies were performed using the same radiographic parameters: 40 KVp, 5 mAs, 160 mA, 0.032 s. For each animal, the radiographic study lasted 2 to 4 minutes. Visibility, radiodensity, location, delimitation and size were noted for each organ and subsequently described, except for the heart, which will be treated in a separate study. All measurements and ratio calculations were performed by a European College of Zoological Medicine herpetology resident using image analysis software (Centricity PACS-IW 3.7.3.9, GE Healthcare).

The decisions for subject inclusion were made by an ECZM-Diplomate and an ECZM-Herpetology resident, who practice exclusively in zoological medicine. All decisions were based on consensus opinions at the time of the procedure.

Vertebral Kidney Index: in order to assess the evaluation of the kidneys size, a ratio between kidney and lumbar vertebral body length was calculated.

Post-mortem study of one chameleon of each species were used to clarify and illustrate coelomic anatomy and to assess imaging findings. The study protocol was reviewed and approved by the "Comissió d'Experimentació Animal-Generalitat de Catalunya" CEA-OH/9203/1.

Statistical analysis: The approach of boundary limits was performed using an individual confidence interval. These upper and lower reference limits were assessed by individual

95% confidence intervals of means (95% CI). Additionally, description of measures of anatomic structures were performed by individual 95% confidence intervals of means. The upper and lower reference limits were assessed results will be shown using median ± standard deviation (SD). Correlation between body weight, testes and kidneys were calculated with Spearman's rho correlation coefficients (ρ) and associated P values. Values of $P \le 0.05$ were considered significant. All statistical analyses were selected and completed using standard software (*SPSS Statistics, version 25.0*) by a biostatistician professor of the School of Medicine.

RESULTS

From all the initial animals, seventeen veiled chameleons (7 males and 10 females) and fifteen panther chameleons (13 males and 2 females) were included in the imaging study. One veiled chameleon and one panther chameleon were excluded because the clinical examination revealed dyspnoea and an intracoelomic mass, respectively. One veiled chameleon and one panther chameleon were excluded due to the presence of kidney mineralization observed in the ultrasonographic examination. Mean ± SD weight was 102.0 ± 16.8 g for veiled chameleons and 141.8 ± 20.1 g for panther chameleons (both ranges, 75 to 175 g); all chameleons were around 8-18 months of age at the time of the study.

The radiographic study allowed the visualization of the lungs, aorta, liver, caudal vena cava, gallbladder, oesophagus, stomach, intestines, gonads, fat bodies and kidneys in veiled chameleon (Figure 5.1.1. and 5.1.2.) and panther chameleon (Figure 5.1.3. and 5.1.4.).

Lungs: the lungs were visualized in all chameleons as radiolucent sac-like structures located dorsal to the liver against the dorsal wall of the coelomic cavity in the lateral view. In the same view, the aorta could be observed as a soft tissue band overlapped with de lung in the dorsal coelom, running parallel to the spine. In the both views, the lungs could observe that they were extended caudally, occupying a big portion of the celomic cavity.

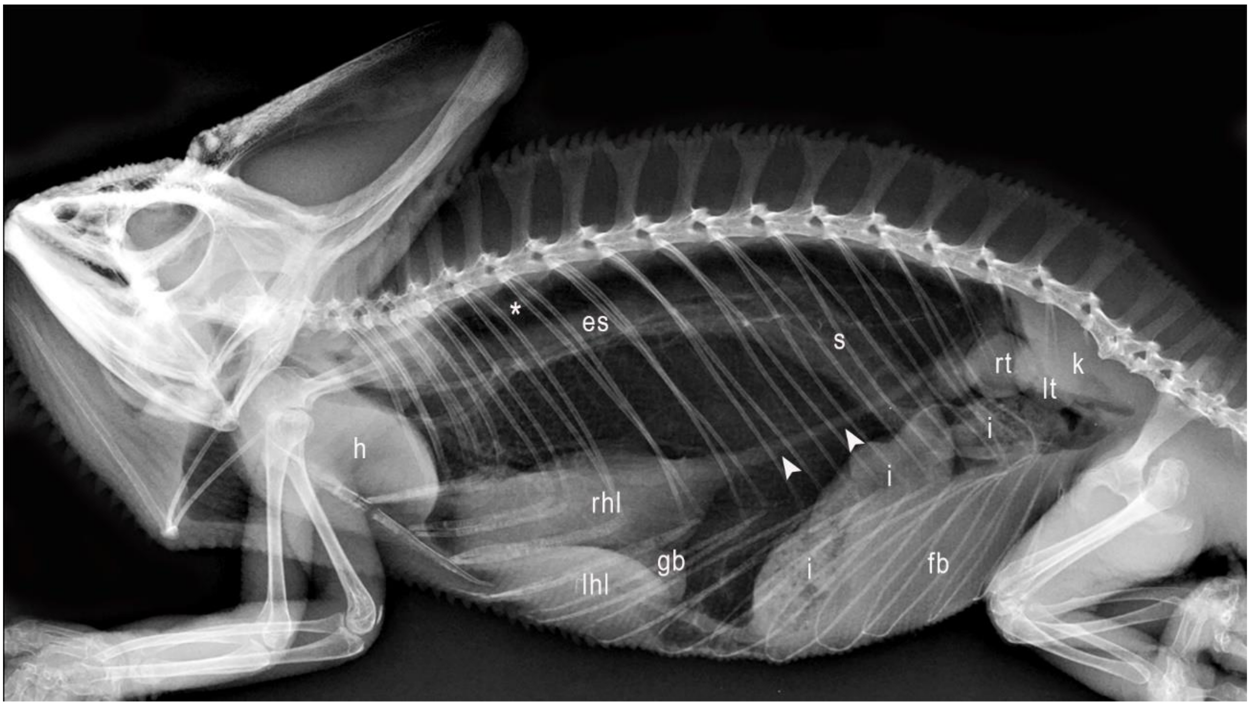


FIGURE 5.1.1. Right lateral view in a veiled chameleon (*Chamaeleo calyptratus*). h – heart, es – oesophagus, rhl – right hepatic lobe, lhl – left hepatic lobe, gb – gallbladder; s – stomach, i – intestines; r – kidney, rt – right testicle, lt – left testicle, fb – right fat body, * – aorta, arrowheads – caudal vena cava.

Liver: the liver was visualized in all chameleons, and it was located in the cranioventral coelom, delimited cranially by de cardiac silhouette. Dorsally, it was delimited by the lungs and caudally by the intestinal package and fat bodies. It was possible to differentiate between right and left hepatic lobes. In the lateral view, the right lobe was well defined, visualized as a triangular shape structure of soft tissue opacity, with smooth marginated borders. It represented the gross volume of liver parenchyma and the left lobe was located in ventral position, overlapping with the ventral portion of the right hepatic lobe. The left hepatic lobe was appreciated like a fusiform soft tissue opacity with a summation effect due to the superimposition. In the dorsoventral view, this hepatic lobe could be visualized well defined as an oval soft tissue opacity in the cranial coelom. It was located at the left side of the vertebral column without overlapping with right hepatic lobe parenchyma.

FIGURE 5.1.2. Dorsoventral view in a veiled chameleon (*Chamaeleo calyptratus*). h – heart, rhl – right hepatic lobe, lhl – left hepatic lobe, i – intestines, rt – right testicle.

The caudal vena cava was seen as a tubular soft tissue band running on the coelomic cavity from the anatomic area of the renal silhouette to the caudodorsal border of the right hepatic lobe. It crossed the region of gastric silhouette.

In veiled chameleons, mean right lobe measured 2.68 ± 0.42 cm in length and 1.90 ± 0.31 cm in high; the left lobe was 2.34 ± 0.36 cm in length and 0.91 ± 0.15 cm in high. In panther chameleons, right lobe measured 3.04 ± 0.32 cm in length and 1.82 ± 0.24 cm in height; the left lobe was 2.23 ± 0.25 cm in length and 0.91 ± 0.17 cm in height. The width of the lobes could not be determined due to the lack of differentiation between them in the dorsoventral view.

There was a significant positive correlation of the right hepatic lobe length ($\rho = 0.89$, $P < 0.001$) and left hepatic lobe length ($\rho = 0.83$, $P < 0.001$) with body weight in veiled chameleon. In panther chameleons, there was also positive correlation of the right hepatic lobe length ($\rho = 0.78$, $P = 0.002$) and left hepatic lobe length ($\rho = 0.62$, $P = 0.014$) with body weight.

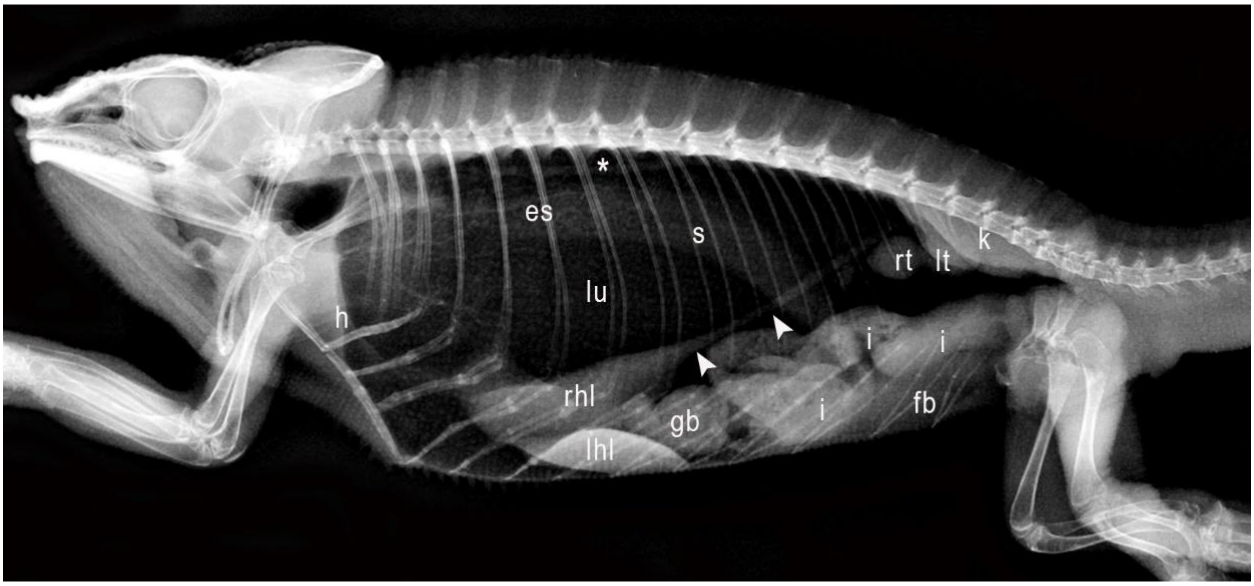


FIGURE 5.1.3. Right lateral view in a panther chameleon (*Furcifer pardalis*). h – heart, es – oesophagus, rhl – right hepatic lobe, lhl – left hepatic lobe, gb – gallbladder; s – stomach, i – intestines; k – kidney, rt – right testicle, lt – left testicle, fb – right fat body, * – aorta, arrowheads – caudal vena cava.

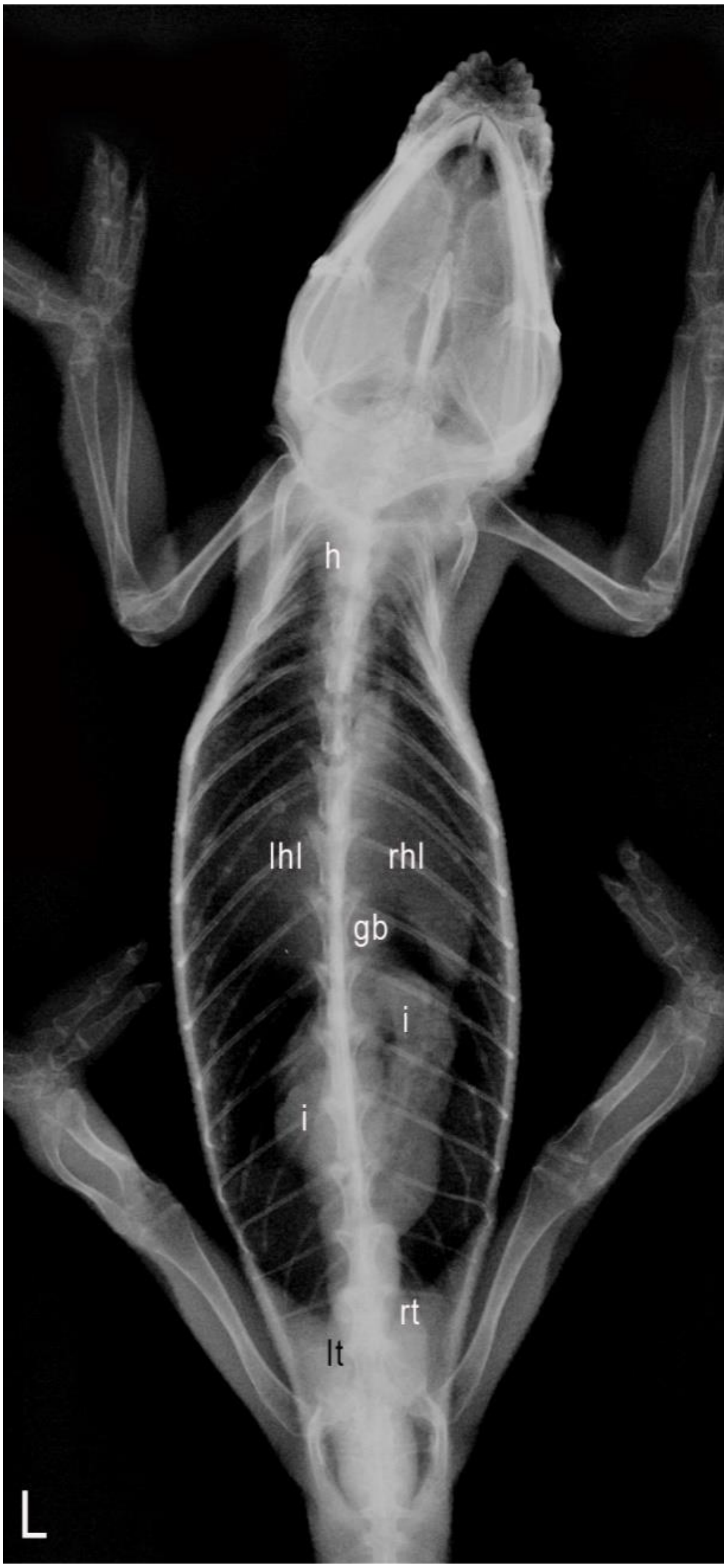


FIGURE 5.1.4. Dorsoventral view in a veiled chameleon (*Chamaeleo calyptratus*). h – heart, rhl – right hepatic lobe, lhl – left hepatic lobe, gb – gallbladder, i – intestines, rt – right testicle.

Gallbladder: the gallbladder was found in the ventral aspect of the medium coelom in 8 of 17 (47%) veiled chameleons and 5 of 15 (33.3%) panther chameleons in lateral view, overlapped between the two hepatic lobes as well defined, round to ovoid, soft tissue opacity structure, protruding caudally. It was delimited craniodorsally by liver parenchyma and caudally by intestines and fat bodies. In dorsoventral view, gallbladder could just be identified in one panther chameleon (figure 5.1.4.).

In veiled chameleon, mean gallbladder length was 0.90 ± 0.19 cm and 0.63 ± 0.17 cm in width. In panther chameleon, the gallbladder measured 1.22 ± 0.26 cm in length and 0.78 ± 0.21 cm in width.

Gastrointestinal tract: it was possible to distinguish different components of the gastrointestinal tract in all animals. In the lateral view, the oesophageal silhouette was visualized in the cranial coelom as a horizontal soft tissue band parallel to spine. Caudally it continued with the stomach, located in the medium coelom, ventral to the spine and dorsally to caudal vena cava.

The stomach acquired an oblique disposition craniodorsal to ventrocaudal with mixed opacity depending on the content (soft tissue if liquid or food present or even empty; gas opacity bubbles). On the lateral view, when the stomach was moderately distended with food, the position was more parallel to the vertebral column showing a globoid shape (resembling a wine boot) and it was slightly more widened than the oesophagus. In the dorsoventral view, it was possible to defined it superimposed to the column, with a “coma” shape. The most cranial and widened portion were located to the right side of the coelom. The tail of the coma, corresponding to the pylorus region, was found to the left side.

When the stomach was empty, resulted more difficult to marked the limits between the oesophagus and the stomach because both organs showed a soft tissue opacity and were observed as a band that lies ventrally and parallel to the vertebral column on the lateral view. Only the caudal aspect of that band acquired a caudoventral disposition corresponding to the stomach (similar to a hockey stick). The stomach was identified in all the chameleons on the lateral view and only visible on the dorsoventral projection when it was empty or slightly distended, superimposed to the vertebral column.

The intestines were found in the caudoventral coelom, delimited cranial by liver and lungs and caudodorsal by kidneys and pelvis. In both views, they were seen as irregular structures but with smooth, undulated margins and mixed opacity, soft tissue and gas opacity bubbles. In the lateral view, a fat opacity structure was seen superimposed in the caudoventral aspect.

Gonads: the gonads could be visualized in all veiled chameleon males (7/7, 100%), in 3 of 10 (30%) veiled chameleon females, in 10 of 13 (76.9%) panther chameleon males, and in all panther chameleon females (2/2; 100%) examined. The testes were localized in the caudodorsal part of the coelom, ventrally to the lumbar spine region, delimited caudal by the kidneys, cranial by the stomach and lungs and ventral by de intestines and fat bodies. The testes were found as well-defined, rounded and smooth margins soft tissue opacity structures, similar in size between them. The right testicle was seen more cranially than the left, superimposed to the caudal aspect of the right testicle in the lateral view. On the dorsoventral view, they were also well identified, in the caudal coelom, the right testicle was slightly more cranial and in the right side related to the vertebral column and the left teste was caudally and left side. In veiled chameleons, the mean dimensions of the testes were 0.93 ± 0.09 cm in length and 0.81 ± 0.11 cm in width for the right testicle and 0.90 ± 0.11 cm in length and 0.80 ± 0.11 cm in width for the left testicle. In panther chameleons, mean right testicle was 0.90 ± 0.11 cm in length and 0.75 ± 0.07 cm in width and 0.85 ± 0.09 cm in length and 0.72 ± 0.09 cm in width for the left testicle. The width of the testes could not be determined due to the overlapping of adjacent structures in the dorsoventral view.

There was no correlation between body weight and right or left testicle length ($\rho = 0.14$, $P = 0.76$; and $\rho = 0.14$, $P = 0.76$; respectively) in veiled chameleon. In panther chameleon, no correlation was also found between body weight and right testicle length ($\rho = 0.39$; $P = 0.30$) and left testicle length ($\rho = 0.57$; $P = 0.14$).

In the sexually active females, the ovaries could occupy the caudal two-third of the coelom cavity and they could efface the rest of abdominal organs. They were identified as multiple oval to rounded soft tissue opacity structures depending on the follicles maturation stage (Figure 5.1.5.). Eggs could be seen with a mineral opacity shell in the final stage of development (Figure 5.1.6.).

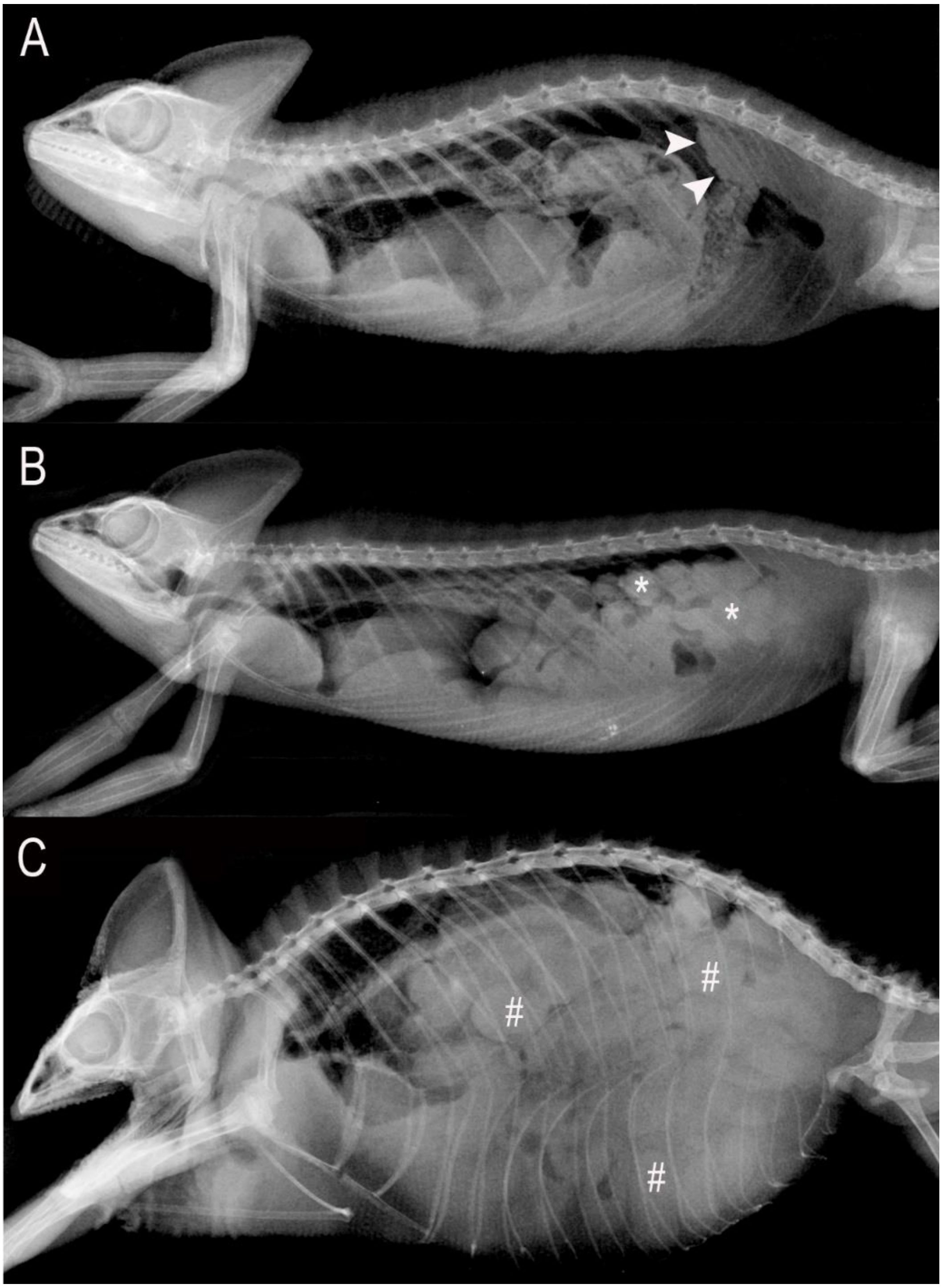


FIGURE 5.1.5. Radiographic images of the different stages of ovarian follicles development in veiled chameleon (*Chamaeleo calyptratus*). A) Image of the ovary in the initial stage of oogenesis (arrowheads). B) Active ovary with spherical vitellogenic follicles (*). C) Image of the unshelled eggs (#) in the oviduct.

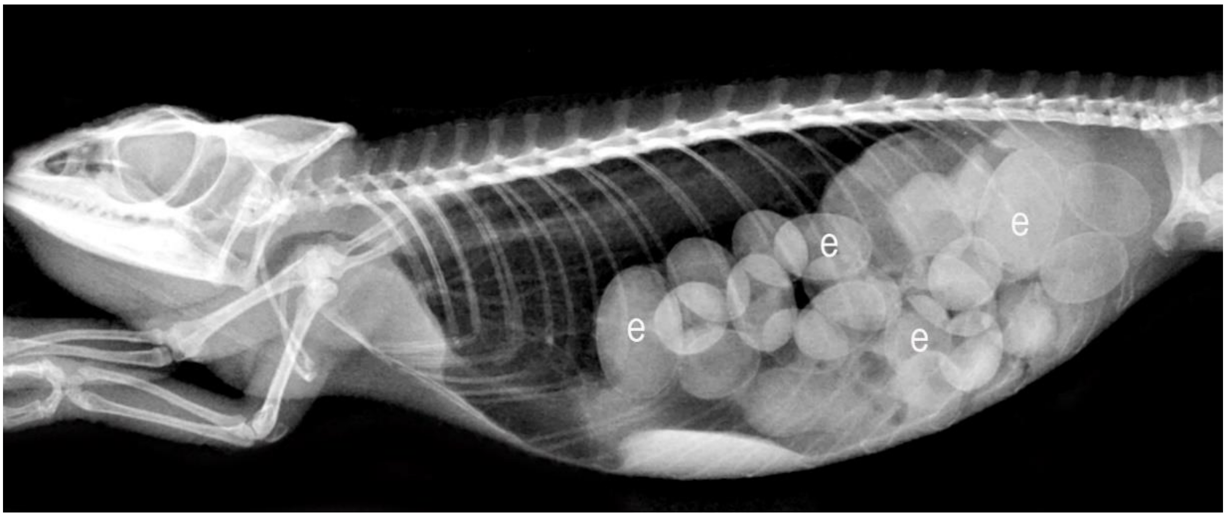


FIGURE 5.1.6. Right lateral view of a gravid panther chameleon (*Furifer pardalis*) with shelled eggs (e).

The appearance was similar to a "bunch of grapes". The limits of the ovarian anatomic region were well delimited, ventral by intestines and fat bodies, cranial by lung and stomach and caudal by the kidneys. However, the inactive ovaries were identified as ill-defined, irregular region of soft tissue opacity, overlapping with the intestines and the kidneys.

The diameter range of the well-defined follicles was 0.54 to 0.74 cm in veiled chameleons and 0.46 to 0.67 cm in panther chameleons. The eggs size range was 0.72 to 0.91 cm in width and 0.93 to 1.41 cm in length in veiled chameleons. In panther chameleons, this range was 0.62 to 0.81 cm in width and 0.96 to 1.05 cm in length.

Fat bodies: the fat bodies were ubicated on the same location as intestines, overlapping with it, slightly more ventral, as a fusiform, not well-defined, fat opacity structures. The fat bodies could be visualized in all animals, but it could not be possible to measure them accurately due to the overlapping with adjacent structures.

Kidneys: the renal silhouette was localized on the caudodorsal coelom as a fusiform soft tissue structure. Dorsally, they were delimited and embedded in the lumbar region, contacting with the spine. Ventrally, it was delimited by the intestines and cranioventrally by the gonads. The kidneys could not be identified as two separate structures and the caudal limits could not be identified due to the overlapping of the caudal poles with the pelvic canal. The renal silhouette measurements were 2.28 ± 0.24

cm in length and 0.55 ± 0.08 cm in high in veiled chameleons; in panther chameleons, they were 2.55 ± 0.23 cm in length and 0.63 ± 0.08 cm in high.

There was a strong positive correlation between renal silhouette ($\rho = 0.85$; $P < 0.001$) and body weight in veiled chameleons. In panther chameleons, there was also positive correlation ($\rho = 0.60$; $P = 0.02$). The ratio kidney length/vertebra length was 4.06 ± 0.16 in veiled chameleons and 4.10 ± 0.19 in panther chameleons.

Post-mortem study: radiographic localizations of the spleen, kidneys, adrenal glands, pancreas, liver, caudal vena cava, hepatic veins, portal vein, gallbladder, stomach and intestine, gonads, and urinary bladder were confirmed on necropsy for both species.

DISCUSSION

Results of the current study suggest that liver, caudal vena cava, gallbladder, stomach, intestines, gonads, fat bodies and kidneys can be visualized using radiography of the coelomic cavity in healthy veiled and panther chameleons.

In the present study, the animals were fasted for 48 hours prior to diagnostic imaging in order to avoid the overlapping of the coelomic structures with the gastrointestinal content.

In the present study, animals were sedated to perform the radiographic study to help minimize the stress of handling and the risk of injuries and accidents. In addition, the sedation allowed to reduce radiation exposure to the animal handler.

The radiographic studies were performed using a mammography cassette plate in order to obtain good quality images. The two imaging positions utilized for the radiographic investigation of chameleons were dorsoventral and lateral. Due to the vertical flattened anatomical conformation of the chameleons, the lateral view was the best position to identify the majority of the coelomic structures. In contrast, species such as bearded dragons have a horizontal flattened anatomical conformation and, for them, the dorsoventral view is more useful.[27]

As in other species such as *Eublepharis macularius* or *Physignatus concincinus*, the spleen, pancreas, adrenal glands and urinary bladder were identified in post-mortem studies, but could not be visualized radiographically in any animal.[23,27] Visceral overlapping, similar radiopacity to adjacent structures or the small size of these organs could be suggestive reasons why the identification was not possible. While not visible in healthy animals, these organs may be visible radiographically in animals with pathology that affects size or parenchymal structure, such as green iguana with urolithiasis or corn snake with pancreatitis.[22,41]

The **lungs** were identified in all animals. In anesthetized patients the respiratory volume was reduce and the lungs were collapsed. For this reason, the evaluation of the lungs is better in the lateral view because in the dorsoventral view the chameleons are flattened when they are anesthetized. The authors recommend radiographic lung evaluation in conscious animals if it does not involve a risk or stress for the patient.

The **gallbladders** could be seen only in some animals of the study probably because of the small size. The size of the gallbladder can vary depending on the degree of contraction, the composition of the diet, and even the length of fasting.[42] The gallbladder could be identified only in the lateral view because the visceral overlapping and similar radiopacity to adjacent structures in dorsoventral view. The content of the gallbladder had soft tissue opacity in all the chameleons. The normal appearance could vary in pathological cases. For example, a mass effect or mineralization in the gallbladder projection area have been observed in cases of cholecystitis or biliary abscess.[43,44]

The **different parts of the gastrointestinal tract** could be identified in all animals, such as oesophagus, stomach and intestines. The presence of gastric material or gas contributed to a gross variability in shape and disposition of the gastric silhouette, due to the degree of distention. The different segments of the intestine could not be differentiated without barium contrast barium as published in box turtles and bearded dragons.[45,46]

In comparison with bearded dragon, which show a horizontal flattened anatomical conformation, the anatomical location of the stomach in these species is described as J-shape on the DV view, with the stomach located on the left side of the body, with the small curvature oriented toward mid- line. On our specimens, due to their vertical flattened anatomical conformation, the gross of the stomach is identified on the mid-line on the DV view and comparable less elongated with a coma-shape.[45]

The **gonads** were easily identified in sexually active animals. The testes were well identified in both radiographic views. This allowed to differentiate them, given that the right teste was visible always cranially on the dorsoventral view. In other species such as turtles, snakes, and most lizards' testes cannot be identified, although they may become large during breeding season.[47]

The testes were similarly in size in each individual and there was no difference between the two species evaluated. There was no evidence of differences in size or appearance of the testes along the study, even among animals of different body weight in different seasons. The fact that testes did not appear to vary in size for different seasons could suggest that these species are sexually active throughout the year when kept in captivity. Therefore, the best chance of visualization of the testes is in chameleons, whereas testes in turtles, snakes, and most lizards remain invisible, although they may become large during breeding season.[47]

The ovaries were clearly identified in sexually active females and the appearance was similar to those in other species.[47] The inactive or immature ovaries were difficult to locate. The large size of ovarian follicles difficult the evaluation of the coelomic organs in breeding females because overlapped the adjacent structures. In the female specimens included in this study, different stages of folliculogenesis could be identified. It was more accurate make a count of mineralized eggs because of a well-defined thin mineral opacity wall as it has been previously described in green iguana.[24,27] It was possible to measure the length and width of them in both views. Define the margins and take precise measurements of the follicles was more complicated due to its soft tissue opacity with overlapping between them.

The **fat bodies** could be identified in all animals, but their margins could not be well identified because the overlapping with the adjacent intestinal structures. A good tool to evaluate the fat bodies is the ultrasonography.[47]

Only the cranial poles of the **kidneys** could be evaluated radiographically, in comparison with necropsy. The caudal poles of the kidneys were located within the pelvic canal, extending to the base of the tail, hindering the visualization of this portion, as has been published in other species and using ultrasonographic examination in chameleons.[3]

In the dorsoventral view, the overlapping with the adjacent structures (vertebral column) did not allow to evaluate the renal silhouette. Only it could be identified in the lateral view. If there would be nephromegaly, it could be visualized and displacing other close structures such as colon or testes, as it has been described in green iguana (*Iguana iguana*).[3] Mineralization of the kidneys were not identified by radiographic study but it was observed by ultrasonographic examination in two apparently healthy animals, which were excluded from the study.[48]

No references were found regarding vertebral kidney index; therefore, a ratio between kidney length and lumbar vertebral body length was calculated in the current study to provide an objective measure of the renal size. This measure was called Vertebral Kidney Index. The results of the study indicated that a normal reference value for vertebral kidney index is 4.06 ± 0.16 in veiled chameleons and 4.10 ± 0.19 in panther chameleons. A higher index result will suggest nephromegaly.

The main limitation of this study was the lack of the contrast study of the gastrointestinal tract. It would have allowed to describe and identify the whole normal gastrointestinal anatomy such as bearded dragons and eastern box turtles.[45,46]

In addition, the health status of the animals was determined by physical examination and coprological study, but no blood tests were performed. Haematology and biochemistry would have been necessary to obtain more information about the clinical status of the chameleons because the common presence of a subclinical diseases is not uncommon.[13] However, they were not performed in the present study because the owner of chameleons did not permit blood sampling.

In conclusion, this study describes the normal radiographic anatomic features of coelomic structures for veiled chameleons and panther chameleons. Findings can be used as a reference for future research studies or for examinations of clinically ill patients. The radiographic protocols used in the current study were similar to those described for other reptiles.[45,46] However, authors found that most organs could be evaluated in the right lateral view in these chameleons.

The authors also recommend fasting at least 48 hours before the radiographic study because it facilitates the visualization of coelomic organs avoiding the overlapping with distended gastrointestinal structures.

5.2. STUDY II

Ultrasonographic appearance of the coelomic cavity organs in healthy veiled chameleons (*Chamaeleo calyptratus*) and panther chameleons (*Furcifer pardalis*)

Vet Radiol Ultrasound. 2020; 61(1): 58-66

INTRODUCTION

Veiled chameleons (VC) live in the humid coastal lowlands, coastal slopes and high plateaus of the southwest corner of the Arabian Peninsula. Panther chameleon (PC) is originally from the island of Madagascar. The distribution in the island is very wide, and their variations in colour depend on the different subpopulations. Both species are mainly insectivores, but veiled chameleons also eat plants especially when they cannot get a source of liquid water.[15,21,22,28,49,50] In the author's experience, these are the most popular chameleons in Spain, and consequently, two of the most frequent species presenting in veterinary practice.

Intracoelomic diseases are common in these species.[3] Many studies have been published describing normal anatomical characteristics using different imaging tests in reptile species.[51–54] Ultrasonography has been recommended as a method for evaluating reptiles with suspected intracoelomic diseases.[22,28] However, based on our review of the literature, descriptions of normal ultrasonographic anatomy for the veiled chameleon (*Chamaeleo calyptratus*) and panther chameleon (*Furcifer pardalis*) have not been previously published.

The objectives of the current study were to develop an ultrasound protocol for evaluating the coelomic cavity in these species and describe the normal ultrasonographic appearance of the coelomic organs in both species.

MATERIAL AND METHODS

The study was a prospective, anatomic design. Nineteen veiled chameleons (nine males and 10 females) and 17 panther chameleons (14 males and three females) from a pet shop facility were recruited for use in the study. Animals were considered to be healthy on the basis of results of a complete physical examination and coprology. The decisions for subject inclusion or exclusion were made by a European College of Zoological Medicine-Diplomate and a European College of Zoological Medicine-Herpetology resident, both of whom practiced exclusively in zoological medicine. The owner of the pet shop facility approved use of animal data for research purposes. The study protocol was reviewed and approved by the "Comissió d'Experimentació Animal-Generalitat de Catalunya" CEA-OH/9203/1.

The animals were housed in groups of two or three in separate terrariums equipped with ultraviolet-Blights and heat bulbs during 3 days. The ambient temperature was approximately 28-30◦C during the day, and 22-24◦C at night. Humidity was provided with a watering artificial rain system for a minute every hour, 8 h a day. The lighting cycles were of 14 h of light and 10 h of darkness. The animals were fasted for 2 days prior to diagnostic imaging to empty the gastrointestinal system.

Ultrasonography: Ultrasonography of the coelomic cavity organs was performed in each animal by one diplomate of the European College of Veterinary Diagnostic Imaging, and all images were evaluated by one European College of Veterinary Diagnostic Imaging diplomate and one European College of Veterinary Diagnostic Imaging resident, and one diplomate of the European College of Zoological Medicine and one European College of Zoological Medicine herpetology resident. All imaging decisions were based on consensus opinions at the time of the procedures. Ultrasonography was performed in sedated chameleons after the administration of alfaxalone (Alfaxan®, Crawley, UK) 4-6 mg/kg IV. They were restrained in right lateral recumbency by an assistant (Figure 5.2.1.).

FIGURE 5.2.1. Photograph showing the position (right lateral recumbency) of a panther chameleon for coelomic ultrasound. An 18-MHz linear transducer is shown (arrow). It is recommended to use large amount of ultrasound gel to improve the visualization of the image. The transducer should not exert excessive pressure on the patient.

Tempered ultrasound gel was applied immediately prior to examination. Images were obtained with an 18-MHz linear transducer attached to a commercially available ultrasound system (Mylab 70®, Esaote; Genova, Italy). Sagittal and transverse still images of each coelomic organ were obtained as well as video capture of the coelomic structures.

Measurements were made by a consensus of the all observers on ultrasonographic images by means of electronic callipers. For each animal, the ultrasonographic examination lasted 15 to 20 min. Visibility, echogenicity, location, delimitation, and size were noted for each organ (except for the heart) and recorded on a bodymap (Figure 5.2.2.).

Post-mortem examination: One chameleon of each species was euthanized, and a post-mortem examination was performed by the European College of Zoological Medicine diplomat and European College of Zoological Medicine herpetology resident. Photographs of coelomic structures were acquired and compared with ultrasonographic findings.

Body Fat Corporal Index: In order to assess the fatty status, body length and fat body length were measured for all animals using callipers (Mylab 70®, Esaote; Genova, Italy). A ratio of these values was calculated and defined as “body fat corporal index.”

All measurements and ratio calculations were performed by one European College of Zoological Medicine herpetology resident using image analysis software (Centricity PACS-IW 3.7.3.9, GE Healthcare).

Statistical analysis: All statistical analyses were selected and completed by a biostatistician professor of the School of Medicine at the authors’ institution, using commercially available software (SPSS Statistics, version 25.0). The approach of boundary limits was performed using an individual confidence intervals approach. These upper and lower reference limits were assessed by individual 95% confidence intervals of means. The results will be shown using median ± SD. Correlation among body weight, testes, fat bodies, and kidneys were calculated with Spearman’s rho correlation coefficients (ρ) and associated P-values. Values of $P \leq .05$ were considered significant.

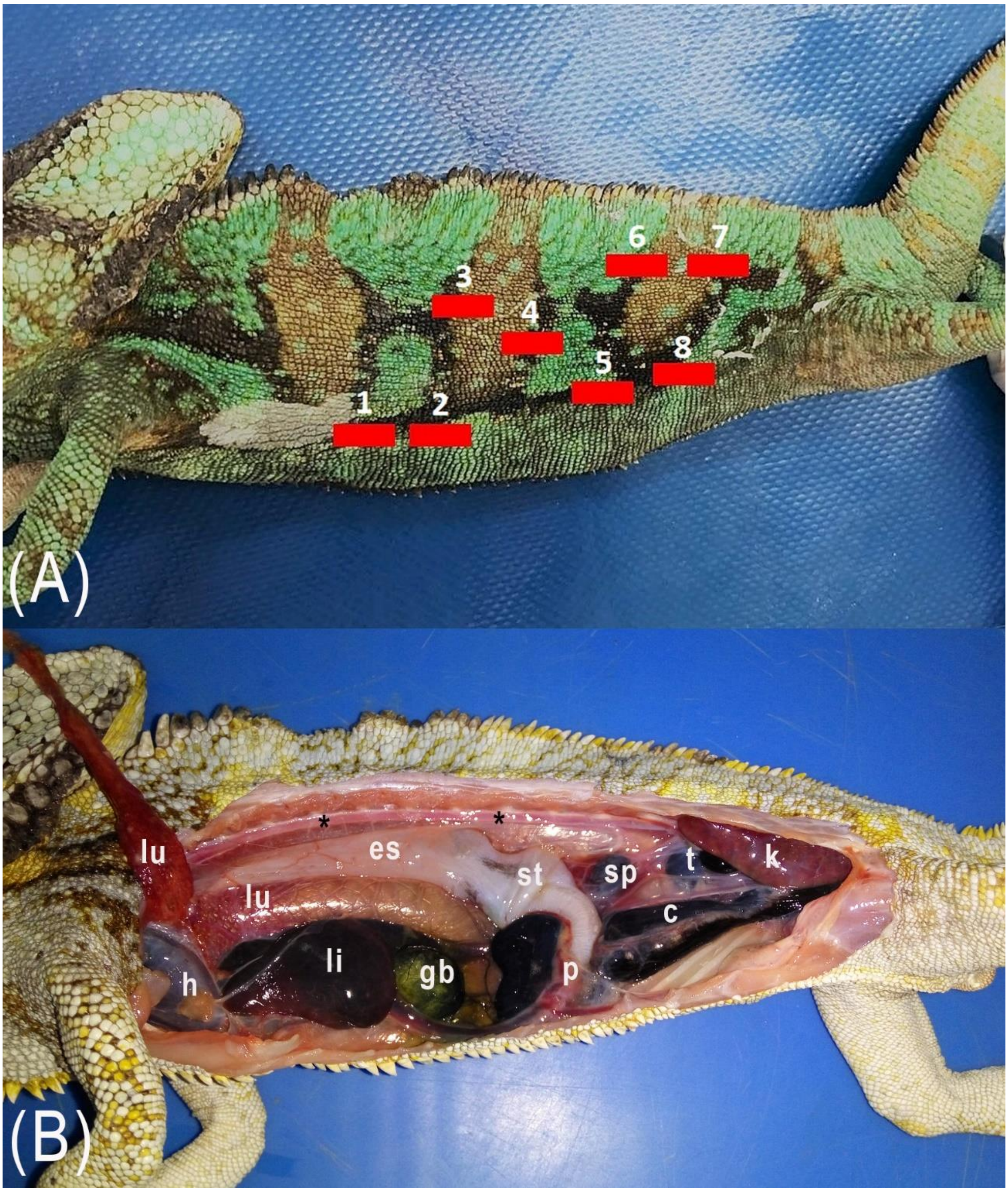


FIGURE 5.2.2. Location of coelomic organs in veiled chameleons (Chamaeleo calyptratus) and panther chameleons (Furcifer pardalis). A, Figure depicting approximate transducer positions and orientations used during ultrasonography to approach the celomic organs. 1, Liver; 2, Gallbladder; 3, Stomach; 4, Small intestine; 5, Colon and fat bodies; 6, Gonads; 7, Kidneys; 8, Urinary bladder. B, Photograph illustrating the appearance of coelomic organs at necropsy. lu, lungs; h, Heart; es, Esophagus; st, Stomach; li, Liver; gb, Gallbladder; p, Pancreas; sp, Spleen; t, Testes; k, Kidney; c, Colon; *, Aorta. The ultrasound beamwas approximately perpendicular to the skin surface for all positions except for position number 7, in which the transducer was angled toward the spine.

RESULTS

A total of 17 veiled chameleons (seven males and 10 females) and 15 panther chameleons (13 males and two females) met final inclusion criteria for the study. One veiled chameleon and one panther chameleon were excluded because of clinical examination findings consistent with dyspnoea and an intracoelomic mass, respectively.

One veiled chameleon and one panther chameleon were excluded due to ultrasonographic findings consistent with kidney mineralization.

Among the animals included in the final study sample, the mean ± SD weight was 102.0 ± 16.8 g for veiled chameleons and 141.8 ± 20.1 g for panther chameleons (both ranges, 75-175 g). Confirmed animal ages ranged from 8 to 18 months at the time of the study.

During the ultrasound protocol, the transducer was placed perpendicular to the skin surface to evaluate the majority of coelomic organs. However, when the kidneys were studied, the transducer was angled toward the spine. Image quality was best when a large amount of ultrasound gel was used and when transducer pressure on the animal was minimized.

The use of different transducer positions and orientations allowed visualization of the liver; caudal vena cava; hepatic veins; portal vein; gallbladder; stomach; small and large intestine; gonads; fat bodies, kidneys, and, when distended, urinary bladder.

Liver: the liver was visualized in all chameleons, and it was located in the cranioventral coelom, delimited cranially by the cardiac apex. This organ was best visualized when the transducer was placed in a sagittal position and oriented perpendicularly to the ventral surface of the cranial aspect of the coelomic cavity (transducer position 1; Figure 5.2.2.).

Dorsally, the liver was delimited by the lungs, and caudally by intestinal package and fat bodies. The ventral surface of the liver was delimited by the ventral coelomic wall.

The liver parenchyma was isoechoic to the adjacent fat bodies with a slightly more granular echotexture and it was incompletely bilobed (Figure 5.2.3.). Mean liver length measured 2.35 ± 0.33 cm and 1.07 ± 0.20 cm in width in veiled chameleons and 2.47 ± 0.26 cm and 0.91 ± 0.15 cm in panther chameleons.

The intrahepatic portion of the caudal vena cava could be visualized in 10/17 (58.8%) and 2/15 (13.3%) panther chameleons. It was the largest hepatic vessel and had an anechoic lumen with hyperechoic walls, and it was located through the right hepatic lobe. Its intrahepatic diameter in Veiled Chameleons measured 0.14 ± 0.04 cm. It only could be visualized in two panther chameleons, with a diameter of 0.08 and 0.11 cm. The hepatic vein with echogenic walls could be seen entering the caudal vena cava from the left hepatic lobe in seven of 17 veiled chameleons and six of 15 panther chameleons (Figure 5.2.3.).

The portal vein could be visualized in six of 17 (40%) veiled chameleons and two of 15 (13.3%) panther chameleons, with an anechoic lumen and hyperechoic walls (Figure 5.2.3.). It was located at the caudoventral aspect of the liver and entering the right hepatic lobe to the left of the gallbladder. Its diameter in veiled chameleons measured 0.08 ± 0.02 cm. In the two panther chameleons, the diameters of the portal vein were 0.05 and 0.1 cm, respectively. There was a significant positive correlation (ρ = 0.77, P <0.001) of the hepatic length with body weight in veiled chameleons, unlike the panther chameleons, where there was no significant correlation (ρ = 0,3, P = 0.27).

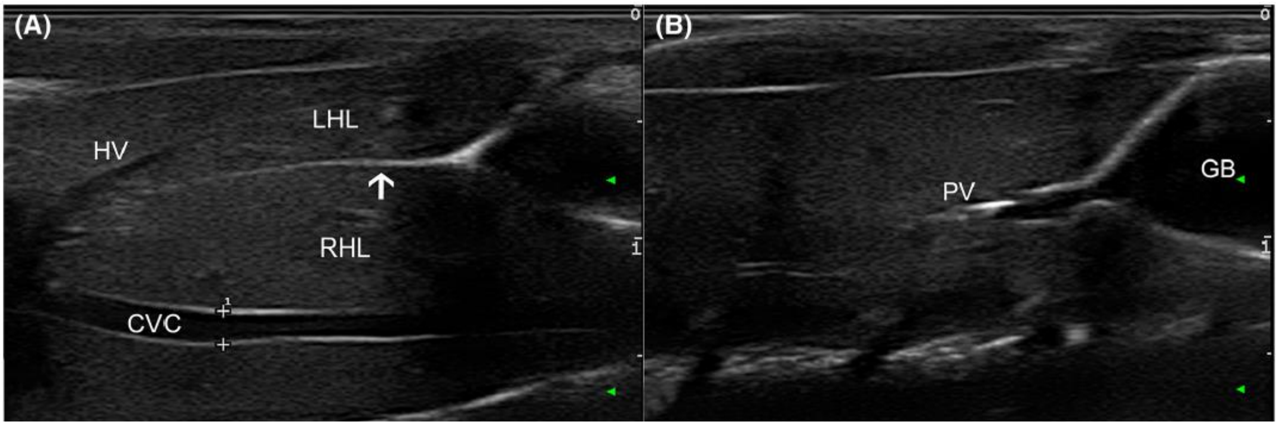


FIGURE 5.2.3. Longitudinal ultrasonographic image of the liver with an 18-MHz linear transducer. A, Intrahepatic portion of the caudal vena cava (CV) in a veiled chameleon. The left hepatic lobe (LHL) is delimited fromthe right (RHL) by a fine hyperechoic line (white arrow). A large hepatic vein (HV) can be seen entering the caudal vena cava from the left hepatic lobe. B, The portal vein (PV) is located at the caudoventral aspect of the liver and entering the right hepatic lobe to the left of the gallbladder (GB). The content is anechoic with hyperechoic walls.

Gallbladder: the gallbladder was located in the ventral aspect of the medium coelom—in the caudoventral border of the liver, between the two lobes—slightly lateralized to the right side of the body in all examined veiled chameleons (17/17; 100%) and in 14 of 15 (93.3%) panther chameleons (transducer position 2; Figure 5.2.2.). It was delimited cranially by the liver and caudally by the intestinal package and fat bodies.

The gallbladder was seen ultrasonographically as a round to oval structure with anechoic content and a thin hyperechoic wall (Figure 5.2.4.). In veiled chameleons, mean gallbladder length was 0.73 ± 0.12 cm, width was 0.43 ± 0.09 cm, and wall thickness was 0.04 ± 0.00 cm. In panther chameleons, the gallbladder measured 0.61 ± 0.12 cm in length, 0.38 ± 0.07 cm in width, and its wall was 0.03 ± 0.00 cm in thickness.

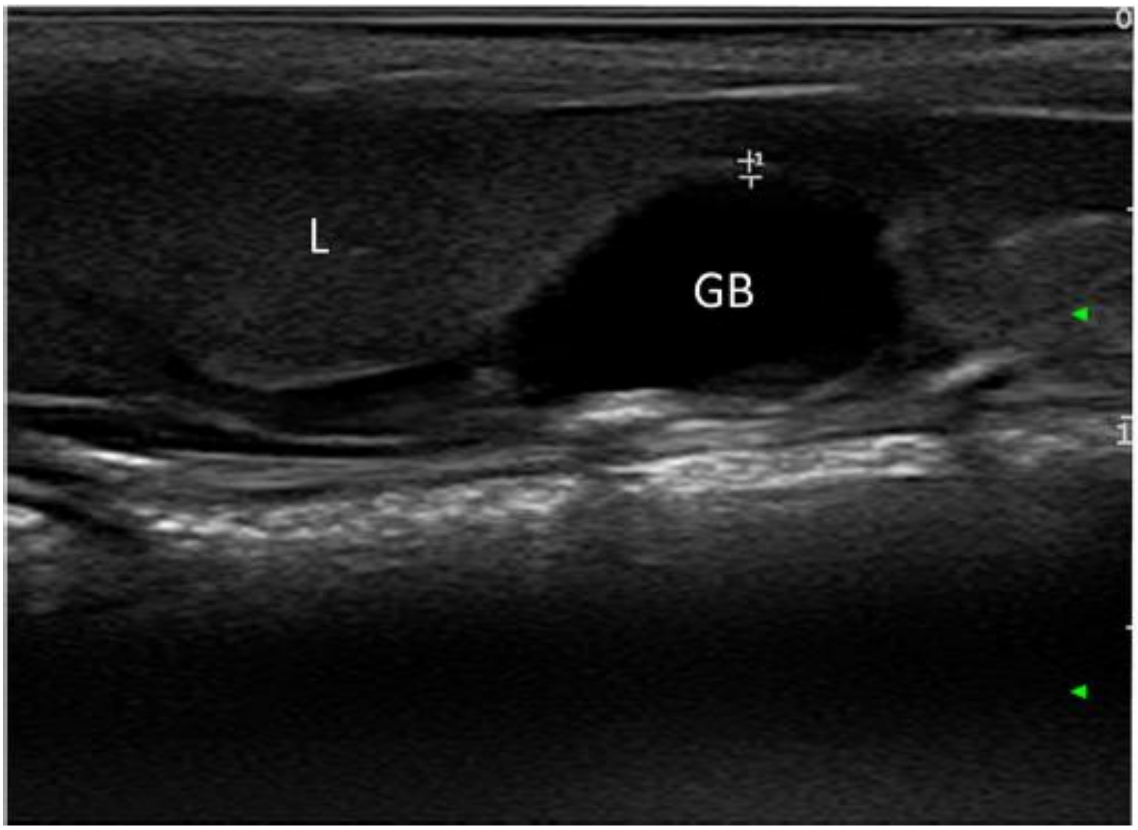


FIGURE 5.2.4. Sagittal ultrasonographic image of the gallbladder (GB) with an 18-MHz linear transducer in a panther chameleon. It is an oval structure with anechoic content delimited with a thin hyperechoic wall. It is located caudoventrally to the liver margin (L)vena cava from the left hepatic lobe. B, The portal vein (PV) is located at the caudoventral aspect of the liver and entering the right hepatic lobe to the left of the gallbladder (GB). The content is anechoic with hyperechoic walls.

Gastrointestinal tract: the **stomach** was seen as a tubular structure located in the mid coelom, ventral to the spine and dorsal to the liver and caudal vena cava (transducer position 3; Figure 5.2.2.). It was difficult to evaluate stomach properly due to artifacts generated by lung air. The hyperechoic wall of the stomach was visualized in six of 17 (35.3%) veiled chameleons and six of 15 (40%) panther chameleons. The stomach wall measured 0.06 ± 0.01 cm in thickness in veiled chameleons and 0.07 ± 0.01 cm in panther chameleons and the wall layering could be seen (Figure 5.2.5.). Differentiation between the oesophagus and stomach was not possible unless the stomach was full of contents.

The small intestine was seen as a tubular structure with a wall with alternating hyper- and hypoechoic layers with similar stratification to what is seen in mammals. Up to five layers could be seen. They could be identified as the interface between contents and mucosa, the mucosa, submucosa, muscle, and serosa. The small intestine was located in the caudoventral coelomic cavity, delimited cranially by liver and lungs, and caudodorsally by the kidneys and pelvis (transducer position 4; Figure 5.2.2.). The small intestine was visualized in four of 17 (23.5%) veiled chameleons and three of 15 (20%) panther chameleons.

Intestines were more easily identified when they had food content, although the different segments could not be distinguished. The small intestine wall was 0.09±0.01 cm in thickness in veiled chameleons and 0.09 ± 0.01 cm in panther chameleons.

Finally, the colon was seen in 13 of 17 (76.5%) veiled chameleons and 11 of 15 (73.3%) panther chameleons as a circular structure with hypoechoic layer and echoic content in a transversal approach. It was located between the fat bodies in the caudoventral aspect of the coelomic cavity (transducer position 5; Figure 5.2.2.) and delimited dorsally by kidneys and gonads and ventrally by the coelomic wall. The content of the colon varied among animals; hyperechoic particles of the chyme with a moderate amount of fluid, gas, or both were observed. It was not possible to identify the transition between the small intestines and colon, probably because the small size. The colon wall was 0.1 ± 0.01 cm in thickness in veiled chameleons and 0.1 ± 0.01 cm in panther chameleons. The mean diameter was 0.55 ± 0.08 cm in veiled chameleons and 0.62 ± 0.14 cm in panther chameleons.

Gonads: the gonads could be visualized in all male veiled chameleons (7/7; 100%), five of 10 (50%) female veiled chameleons, 10 of 13 (76.9%) male panther chameleons, and in all female Panther Chameleons (2/2; 100%) examined. In some animals, it was difficult to identify the gonads because of the presence of abundant gas within the gastrointestinal tract.

The testes were located in the caudodorsal part of the coelom, in the lumbar region, delimited ventrally by the intestinal package and fat bodies, caudally by the kidneys and cranially by the lungs and stomach.

The right testicle was located more cranial than the left (transducer position 6; Figure 5.2.2.). The testes were oval, symmetrical, well-defined and homogeneous structures with hypoechoic and coarse echotexture in relation to the adjacent fat bodies (Figure 5.2.6.). In Veiled Chameleons, the mean dimensions of the testes were 0.90 ± 0.08 cm in length and 0.71 ± 0.10 cm in width for the right testicle and 0.90 ± 0.16 cm in length and 0.68 ± 0.12 cm in width for the left testicle. In panther chameleons, mean right testicle was 0.74 ± 0.12 cm in length and 0.56 ± 0.07 cm in width and 0.83 ± 0.14 cm in length and 0.59 ± 0.10 cm in width for the left testicle. There was no evidence of

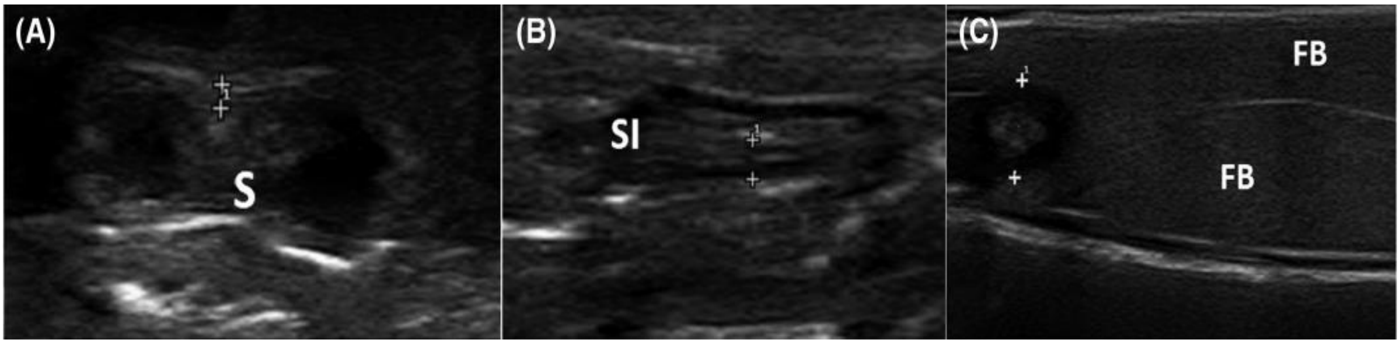


FIGURE 5.2.5. Sagittal ultrasonographic images of the gastrointestinal tract with an 18-MHz linear transducer in a veiled chameleon. A, Pyloric portion of the stomach (S) with hyperechoic wall and thin layers (between callipers). B, The small intestine (SI) is a tubular structure with a wall alternating hyper-hypoechoic layers similar to the wall layering in mammals. C, The colon (between callipers) is a circular structure with hypoechoic layer and echoic content. It was located between the fat bodies (FB) in the caudoventral aspect of the coelom. The fat bodies are well-defined, tubular, and

differences in size or appearance of the testes along the study; even among animals of different body weight in different seasons.

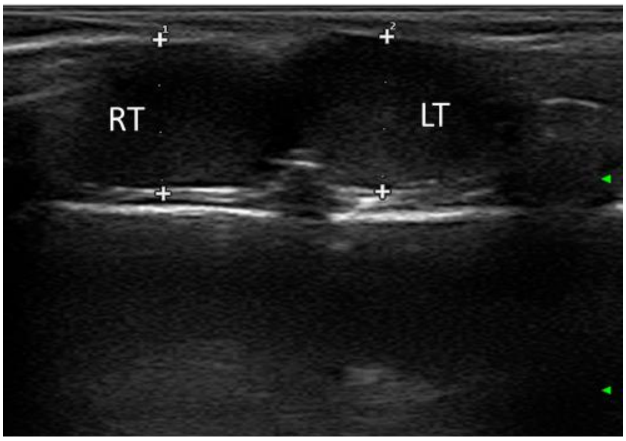


FIGURE 5.2.6. Sagittal ultrasonographic image of the testes with a 18-MHz linear transducer in a male panther chameleon. The testes are located in the sublumbar region as oval, symmetrical, well-defined, hypoechoic, homogeneous structures. Right testicle (RT) is cranially to the left (LT).

There was no correlation between body weight and right or left testicle length (ρ = 0.25, P = 0.589; and ρ = -0.1, P = 0.819; respectively) in veiled chameleons. In panther chameleons, no correlation was also found between body weight and right testicle length (ρ = 0.3; P = 0.392). However, there was a weak positive correlation between body weight and left testicle length (ρ = 0.651; P = 0.03).

In the sexually active females, the ovaries could be identified just cranially to the kidneys as clusters of multiple target structures with hyperechoic periphery and hypoechoic central area (corresponding to follicles), ranging in diameter from 0.33 to 0.92 cm in veiled chameleons. When eggs were seen they appeared as ovoid structures with hyperechoic wall and hypo/anechoic content (Figure 5.2.7.). In female panther chameleons, active ovaries could not be visualized.

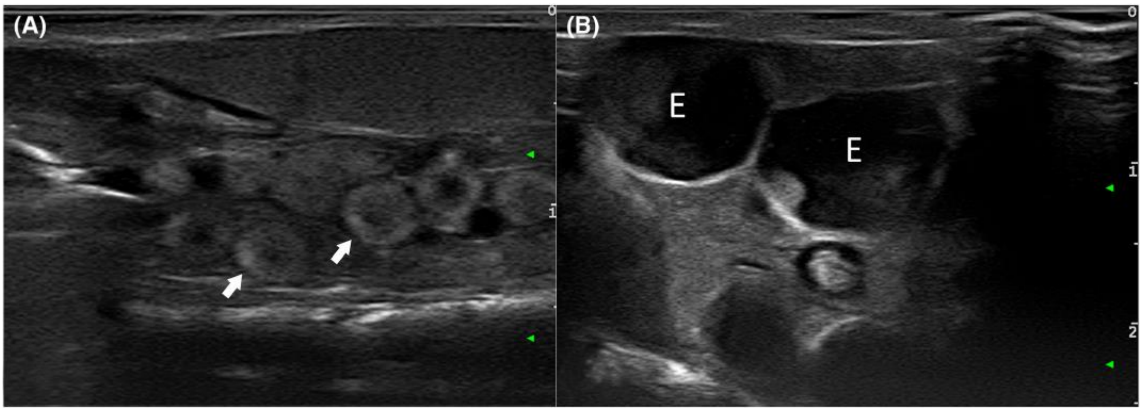


FIGURE 5.2.7. Sagittal ultrasonographic images of the ovaries with an 18-MHz linear transducer in a veiled chameleon. A, In the sexually active females, the ovaries are identified as clusters of multiple target structures (follicles) with hyperechoic periphery and hypoechoic central area (arrows). B, developed eggs (E) are visible as ovoid structures with hyperechoic wall and hypo/anechoic content.

Fat bodies: fat bodies were located in the cranioventral part of the coelom (Figure 5.2.8.), delimited cranially by liver and lungs and caudodorsally by the kidneys and pelvis (transducer position 5; Figure 5.2.2.).

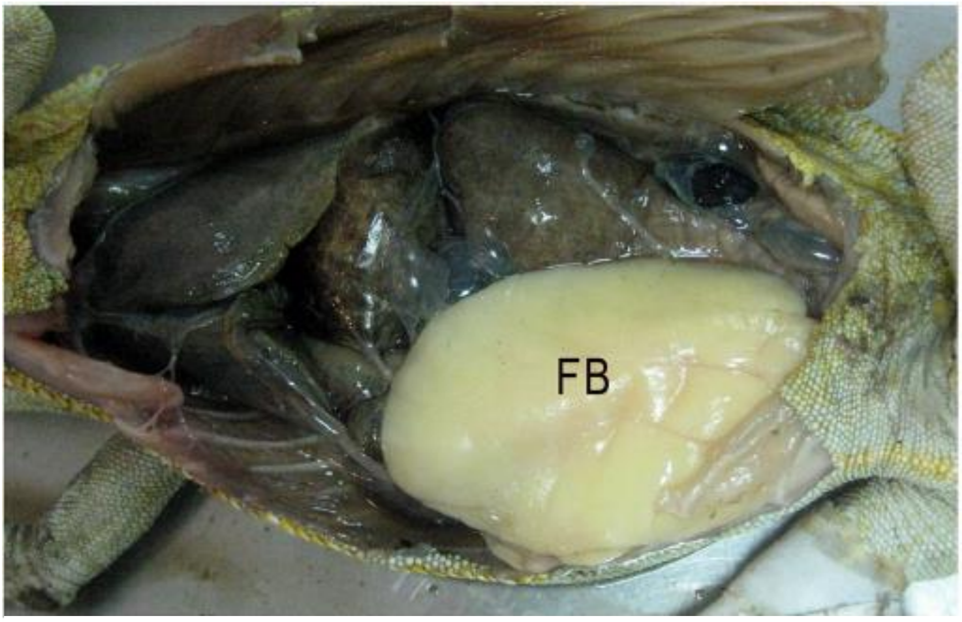


FIGURE 5.2.8. Fat bodies (FB) location in the necropsy of veiled chameleon.

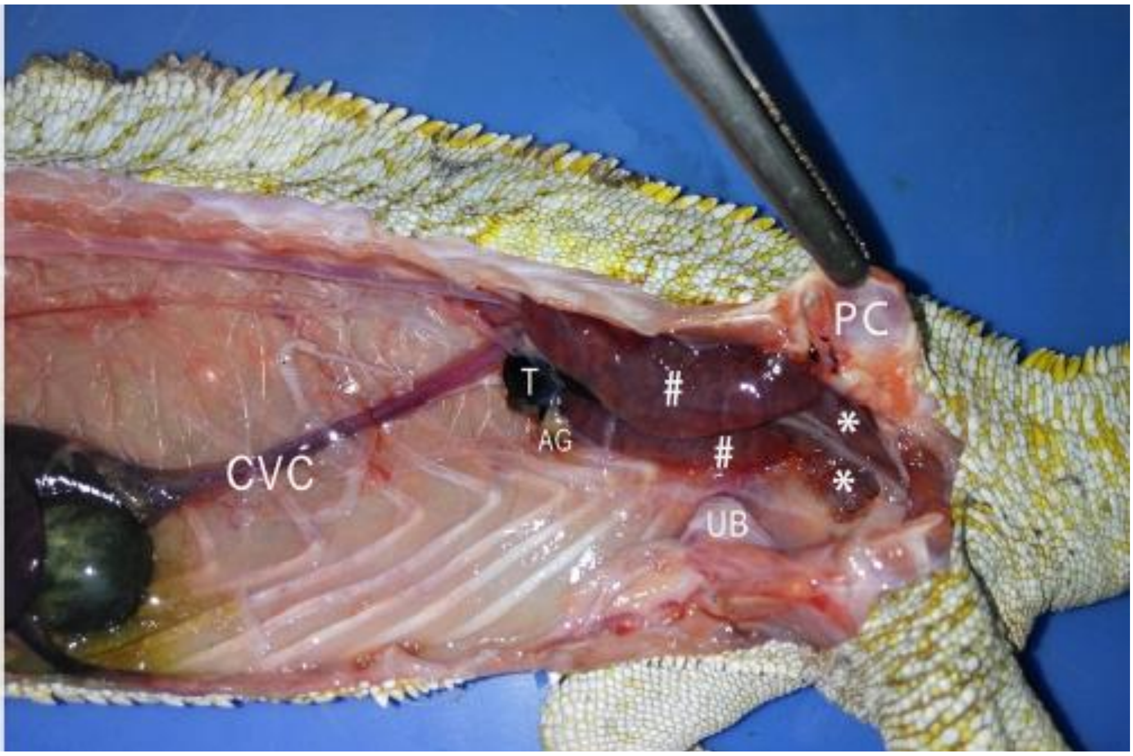


FIGURE 5.2.9. Photograph of the post-mortem study of a veiled chameleon. The cranial pole of the kidneys (#) was clearly identified during the ultrasonography. The caudal portions of the kidneys (*) are located into the pelvic canal, hindering the visualization of these portion in the ultrasonography. When the pelvic canal (PC) is dissected, the caudal pole remains exposed. The caudal vena cava (CV), testes (T) and adrenal glands (AG) can also be observed.

The fat bodies could be visualized in 15 of 17 (88,2%) veiled chameleons and in all (100%) panther chameleons. These appeared as well-defined, tubular, and symmetrical structures with homogeneous echogenicity. Fat bodies contour was delimited by a longitudinal thin hyperechoic line (Figure 5.2.5.). The main vessels related to fat bodies belong to the kidney (renal veins branches), but they could not be identified during ultrasonography. The fat bodies measurements were included in Table 1.

There were significant positive correlations between right ($\rho = 0.631$; $P = 0.012$) and left ($\rho = 0.683$; $P = 0.005$) fat body length and body weight in veiled chameleons. In panther chameleons, there was no correlation between body weight and right fat body length ($\rho = 0.33$; $P = 0.266$) and left fat body length ($\rho = 0.36$; $P = 0.178$). The ratio body length/fat body length was 0.17 ± 0.02 in veiled chameleons and 0.12 ± 0.02 in panther chameleons.

Table 5.2.1. Values of fat body and kidney measurements using a 18-MHz linear transducer in veiled and panther chameleon.

		VEILED CHAMELEON		PANTHER CHAMELEON	
		LENGHT (cm)	WIDTH (cm)	LENGTH (cm)	WIDHT (cm)
FAT BODIES	Right	2.33 ± 0.38	0.51 ± 0.10	2.03 ± 0.35	0.45 ± 0.09
	Letf	2.33 ± 0.3	0.48 ± 0.07	1.90 ± 0.33	0.37 ± 0.08
KIDNEYS	Right	2.31 ± 0.24	0.42 ± 0.06	2.61 ± 0.27	0.50 ± 0.06
	Left	2.29 ± 0.26	0.42 ± 0.05	2.64 ± 0.26	0.55 ± 0.05

Fat bodies: n = 15 in veiled chameleons and n = 15 in panther chameleons. Kidneys: n = 17 in veiled chameleons and n = 15 in panther chameleons.

Kidneys: the kidneys were seen by placing the transducer in a longitudinal prepubic location—in the caudodorsal coelom; parallel to the spine and perpendicular to the skin (transducer position 7; Figure 5.2.2.). The visualization of the kidneys was improved when the transducer was angled 45◦ toward the spine rather than a perpendicular approach. The cranial poles of the kidneys were clearly seen in all animals (approximately 60% of total kidney length), but the caudal pole was located within the pelvic canal, extending to the base of the tail, hindering the visualization of this portion (Figure 5.2.9.). In the images obtained with the technique described above, the kidneys were delimited dorsally by the spine, ventrally by the intestinal package, cranially by the gonads, and caudally by the pelvis. They appeared as hyperechoic, symmetrical, and fusiform paired structures with fine echotexture, similar to the fat bodies. Between the kidneys, a tubular structure with anechoic lumen could be observed, corresponding to the aorta (Figure 5.2.10). Kidney mineralization was observed in two apparently healthy animals, which were excluded from the study. The kidney measurements are included in Table 5.2.1. The aorta diameter was measured at the cranial portion of the kidney. It was 0.13 ± 0.02 cm and 0.12 ± 0.02 cm in veiled and panther chameleons, respectively.

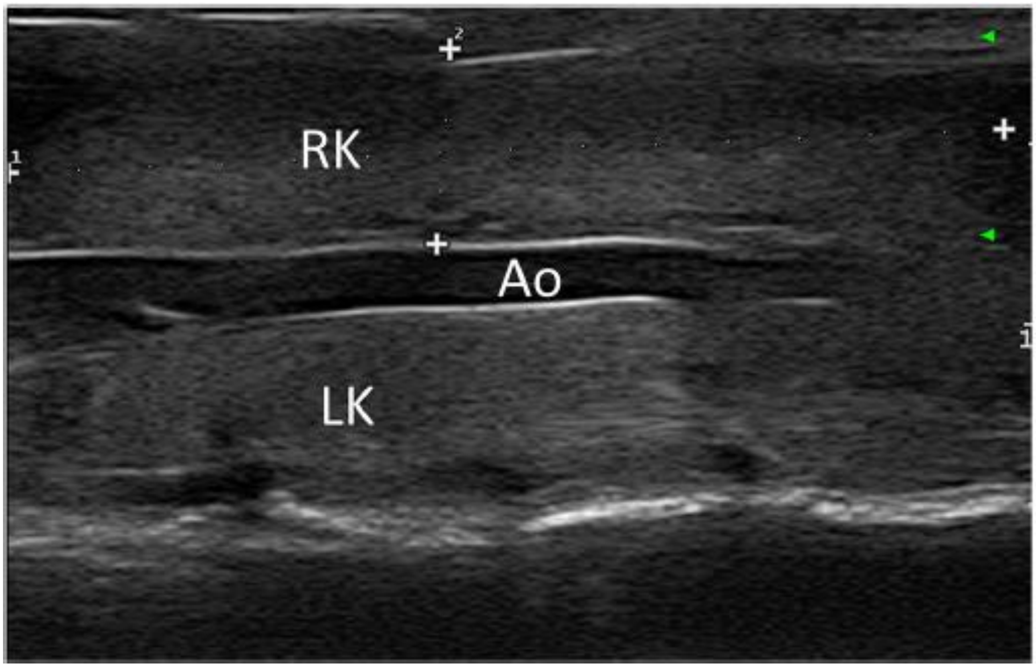


FIGURE 5.2.10. Sagittal ultrasonographic image of kidneys with an 18-MHz linear transducer in Veiled Chameleon. The right (RK) and left kidneys (LK) appear as hyperechoic, symmetrical, and fusiform paired structures with fine echotexture, similar to the fat bodies. Between the two kidneys, a tubular structure with anechoic lumen can be observed, corresponding to the aorta (Ao).

There was a strong positive correlation between right ($\rho = 0.818$; $P < 0.001$) and left ($\rho = 0.789$; $P < 0.001$) kidney length and bodyweight in veiled chameleons. In panther chameleons, there was mild correlation between body weight and right ($\rho = 0.5$; $P = 0.05$) and left kidney length ($\rho = 0.51$; $P = 0.05$).

Urinary bladder: the urinary bladder was seen in 11 of 17 (64.7%) veiled chameleons and 13 of 15 (86.7%%) panther chameleons in direct apposition with the body wall as a rounded anechoic structure delimited by a thin hyperechoic wall. Irregular, mobile, and hyperechoic structures with acoustic shadow, the urate salts, were visible within the lumen in nine of 17 (52.9%) veiled chameleons and seven of 15 (46.7%) panther chameleons (Figure 5.2.11.). The urinary bladder was located in the caudoventral coelom, delimited craniodorsally by the fat bodies and ventrally by the body wall (transducer position 8; Figure 5.2.2), and it measured 0.64 ± 0.14 cm in width and 0.93 ± 0.18 cm in length in veiled chameleons and 0.53 ± 0.30 cm in width and 0.65 ± 0.15 cm in length in panther chameleons. The wall of the urinary bladder measured 0.04 ± 0.00 cm in veiled chameleons and 0.03 ± 0.01 cm in panther chameleons.

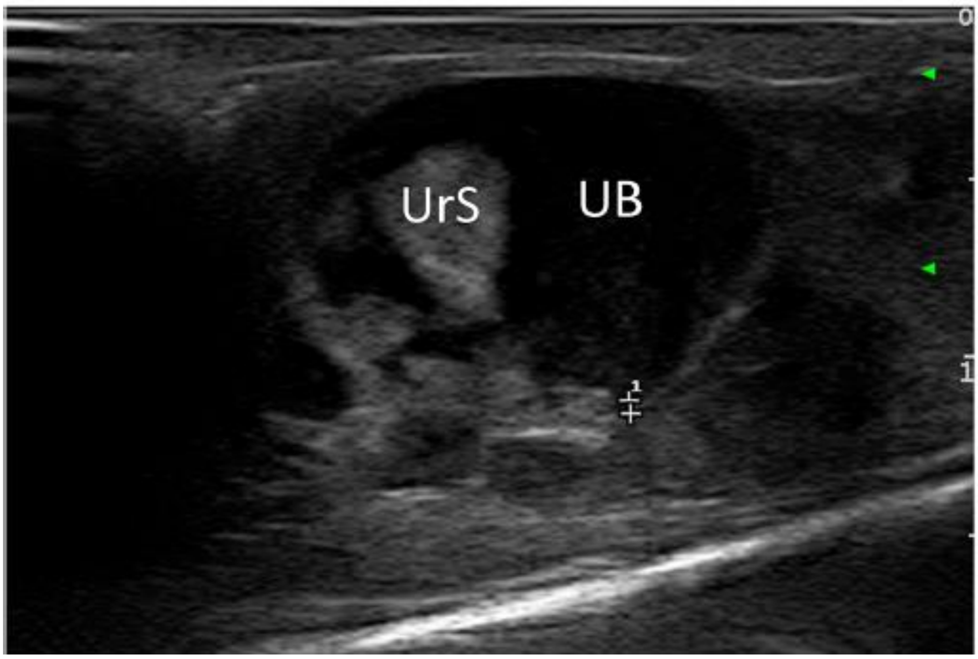


FIGURE 5.2.11. Sagittal ultrasonographic image of urinary bladder (UB) with an 18-MHz linear transducer in veiled chameleon. The UB is located caudal to fat bodies as an anechoic structure delimited by thin hyperechoic wall. In this case, a hyperechogenic structure with mild acoustic shadow is observed, corresponding to urate salts (UrS)

Coelomic cavity: a small amount of hypoechoic or anechoic free coelomic fluid could be observed in two of 17 (11.8%) veiled chameleons and two of 15 (13.3%) panther chameleons. This fluid was located between the liver and body wall and adjacent to the fat bodies and colon. The pancreas, adrenal glands, and spleen could not be visualized during ultrasonographic examinations for any of the animals.

Post-mortem study: ultrasonographic localizations of the spleen, kidneys, adrenal glands, pancreas, liver, caudal vena cava, hepatic veins, portal vein, gallbladder, stomach and intestine, gonads, and urinary bladder were confirmed on necropsy for both evaluated animals (one veiled chameleon and one panther chameleon).

DISCUSSION

Results of the current study suggest that kidneys, liver, caudal vena cava, hepatic veins, portal vein, gallbladder, wall of the stomach and intestine, gonads, and urinary bladder (when distended) can be visualized using ultrasonography of the coelomic cavity in healthy veiled and panther chameleons. We performed ultrasound examinations in lateral recumbency because the vertical flattened anatomical conformation of the chameleons did not permit good quality images in dorsal recumbency.

In contrast, species such as bearded dragons have a horizontal flattened anatomical conformation and, for them, the ventral approach is more useful.[51] The images obtained in right and left lateral recumbency during ultrasonography appeared similar and therefore authors decided to use only right lateral images for the standardized protocol.

The ultrasound beam was placed perpendicular to the skin surface to evaluate the majority of coelomic organs. However, when the kidneys were studied the transducer was angled toward the spine.

In the present study, animals were sedated to perform the ultrasonography to help minimize the stress of handling and the risk of injuries and accidents. As in other species, stress can increase mortality in reptiles.[11] Sedation also reduces respiratory volume. Anecdotally, when the authors of the present study have worked on conscious animals from these species in the past, the amount of gas present in the expanded lungs interfered with the visualization of some of the organs.

The spleen, pancreas, and adrenal glands were identified in post-mortem studies, but could not be visualized ultrasonographically in any animal. Possible reasons could include the small size of these organs (approximately 2-3 mm), similar appearance to adjacent tissues, or visceral superimposition.[51] The spleen was identified as a rounded structure adjacent to the left dorsal aspect of the coelomic wall in the mid portion of the coelomic cavity. The pancreas was closely related to the gastric fundus and the first portion of small intestine. Finally, adrenal glands were located in contact with gonads and close related to the renal veins. While not visible in normal animals, these organs may be visible ultrasonographically in animals with pathology that affects size or parenchymal structure.[41]

The gallbladders were of varying sizes and could be easily seen in all the animals of the study. The size of the gallbladder can vary depending on the degree of contraction, the composition of the diet, and even the length of fasting.[42] The content of the gallbladder was anechoic in all the chameleons; but the echogenicity of intraluminal contents is described to be dependent on bile density or the presence of hyperechoic debris in other reptiles.[51,55]

The different parts of the gastrointestinal tract could be identified in all animals, but the segments of small intestine, as in other species, could not be differentiated.[49] The presence of gas limited the visualization of some coelomic organs. This problem occurred in spite of the fact that all animals were fasted for 2 days prior to performing ultrasonography. Therefore, gastrointestinal gas may be a limitation for using ultrasonography in some clinically affected animals. However, the gastric and intestinal layering was easily observed in the majority of animals. This finding differed from reports in other species such as bearded dragons, in which there was great difficulty in identifying the different gastrointestinal layers.[51]

The gonads were easily identified, especially in mature animals. Although not specifically evaluated in this study, there was no evidence of differences in size or appearance of the testes along the study; even among animals of different body weight in different seasons.

There was no correlation between the body weight and the size of the testes. Although it subjectively appeared that there would be a correlation between left testicle size and body weight, no statistically significant correlation was identified. It is possible that this was due to the small size of the studied population. The fact that testes did not appear to vary in size for different seasons could suggest that these species are sexually active throughout the year when kept in captivity. More studies would be necessary to understand ultrasonographic changes and appearance in the reproductive tract over time.

The ovaries were clearly identified in sexually active females and the appearance was similar to those in other species.[56,57] The inactive or immature ovaries were difficult to locate. The evaluation of the coelomic organs was difficult in breeding females, because the large size of ovarian follicles overlapped the adjacent structures. In these cases, different stages of folliculogenesis could be identified, so the ultrasonography could also possibly be used in the future to evaluate the reproductive status of breeding females.[57]

The size of the fat bodies can vary depending on the diet, the physiological or reproductive status, or even the presence of underlying pathologies. When fat bodies are smaller or absent, causes of energy consumption such as disease, folliculogenesis, and others should be investigated.[20] Fat bodies were identified in 30 of 32 animals of the present study. The echogenicity was similar to the liver and the testes.

No references were found regarding fat bodies and body condition index; therefore, a ratio between body length and fat body length was calculated in the current study to provide an objective measure of the fatty status. This measure was called body fat corporal index. The results of the study indicated that a normal reference value for body fat corporal index is 0.17 ± 0.02 in veiled chameleons and 0.12 ± 0.02 in panther chameleons.

Only the cranial poles and approximately 60% of the kidneys could be evaluated ultrasonographically, in comparison with necropsy. The caudal poles of the kidneys were located within the pelvic canal, extending to the base of the tail, hindering the visualization of this portion, as it has been published in other species.[51,53,56]

In other larger species the caudal pole can be identified by a caudal approach.[49,53,56] The visualization of the kidneys was improved when the transducer was angled 45◦ toward the spine rather than a perpendicular approach.

The presence or absence of the urinary bladder depends on the family and the sexes in reptiles. Unlike other lizards, such as gekkonid lizard *Hoplodactylus pacificus*, in which small bladder is present in the male but is absent in the female, in veiled and panther chameleons, both possess urinary bladder.[53,56,58] In the current study, the urinary bladder was only identified in the chameleons when it was distended; and sometimes irregular, mobile, and hyperechoic structures with acoustic shadow were identified inside, corresponding to urate salts.

One of the limitations of this study was that the anatomic reference information was based on a small sample population. The ranges of animal size and age in our sample should therefore be taken into consideration when applying this information to other animals. The chameleons were considered healthy on the basis of results of a complete physical examination and coprology findings. However, the presence of a subclinical disease is not uncommon.[48] Blood analyses would give more information about the clinical status of the animal. However, they were not performed in the present study because facility's owner did not permit blood sampling.

In conclusion, this study describes species-specific, normal ultrasonographic anatomic features of coelomic structures for veiled chameleons and panther chameleons. Findings can be used as a reference for future research studies or for examinations of clinically ill patients. The ultrasound protocols used in the current study were similar to those described for other reptiles such as bearded dragons and iguanas; however, authors found that most organs were visible with right lateral recumbency in these chameleons. The authors also recommend sedation for ultrasonography of chameleons to help minimize stress and minimize gastrointestinal gas.

5.3. STUDY III

Computed tomography of the coelomic cavity in healthy veiled chameleons (*Chamaeleo calyptratus*) and panther chameleons (*Furcifer pardalis*)

INTRODUCTION

Diagnostic imaging has been included in the diagnostic protocols in reptile medicine for decades. Radiographic and sonographic examinations are well-established diagnostic imaging methods, but the presence of shells, scales and overlying structures have been limiting factors for a correct interpretation in these techniques.[59]

The introduction of computed tomography (CT) has revolutionized the practice of diagnostic imaging in reptiles. The CT scan also allows a better interpretation of the images, especially in the chelonia group, where the carapace can hasten the assessment of the coelomic cavity. There is little information of the normal anatomical characteristics in some species using advanced imaging techniques.[22,40,60] Computed tomography has been used in the evaluation of diseases in the skeletal system, respiratory tract, reproductive tract, liver, spleen and urinary tract in reptiles.[32-38]

Based on the literature review made by the authors, there are no published descriptions of normal CT and macroscopic cross-sectional anatomy of both species of chameleon.

The objectives of this study were to describe CT characteristics of the coelomic organs in healthy patients, and to provide normal reference measurements and attenuation values for each organ in these species.

MATERIALS AND METHODS

Study population: This is an anatomic design prospective study. A total of 36 chameleon from an animal wholesaler were included in this study: 9 males and 10 females veiled chameleons and 14 males and 3 females panther chameleons. Only healthy animals were finally included in the research. A complete physical examination and coprological study were performed by a Herpetology resident and a Diplomate of the European College of Zoological Medicine.

The use of the data in the research was approved by the owner of the animal wholesaler. This study was conducted following the national welfare and protection guidelines of "Comissió d'Experimentació Animal-Generalitat de Catalunya" CEA-OH/9203/1.

The animals were acclimated in terrariums with ultraviolet-B lights and heat bulbs as temperature sources, following the recommendations for these species in previous studies for 3 days.[47]

Celomic ultrasound was performed to describe the appearance of the celomic organs and has been previously published.[47]

Computed tomography: coelomic cavity computed tomography was performed in all the chameleons by a European College of Veterinary Diagnostic Imaging (ECVDI) resident, a senior radiologist or an ECVDI diplomate. All the animals were sedated with alfaxalone 4-6 mg/kg IV (Alfaxan® 20 mg/ml; Dechra, Worcestershire, UK) into the caudal vein.

The CT examinations were performed with a 16-slice helical CT scanner (General Electric Brivo CT 385) in caudocranial direction with the animals positioned in ventral recumbency (Figure 5.3.1.). All examinations were obtained with a slice thickness of 0.625 mm, interval thickness of 0.625 mm, collimation pitch of 0.5625:1, 120 kV, 80mA, field of view of 20 cm and a matrix of 512 × 512. The transverse images were reconstructed (MPR) in sagittal and dorsal planes. CT images were displayed in soft tissue window (Window level: 350; Window width: 40) and in lung window (Window level: -500; Window width: +1500). Coelomic organs height and width were assessed

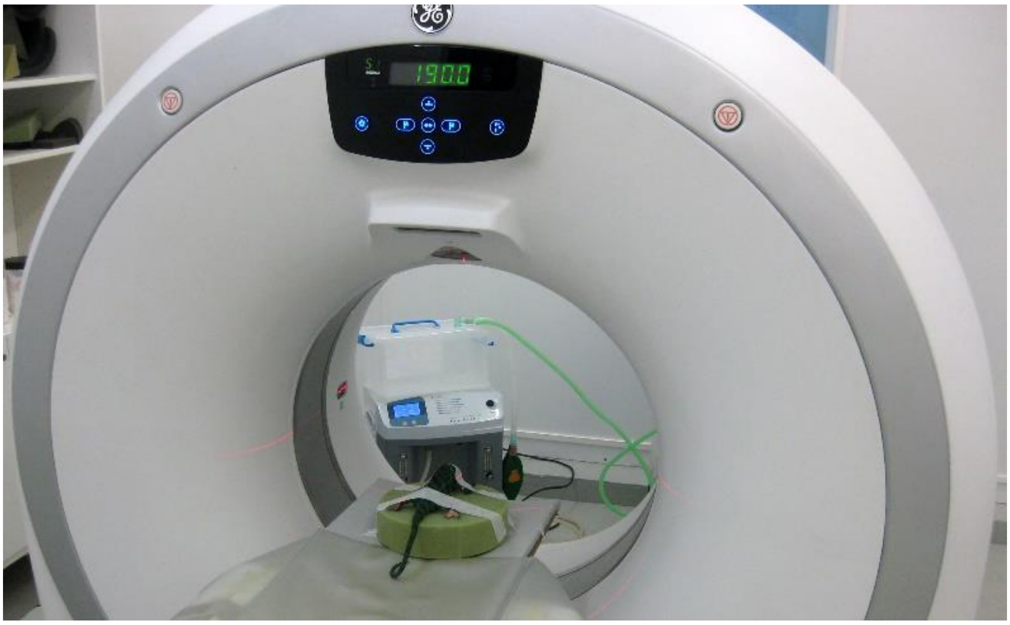


FIGURE 5.3.1. Picture of a panther chameleon (*Furcifer pardalis*) positioned in sternal recumbency during the CT scan.

using the transverse images. Coelomic organs length was assessed using the sagittal and dorsal reconstructions.

Non-ionic iodinated contrast medium (iobitridol, Xenetix® 300 mg/mL, Guerbet, Madrid, Spain) was administered through an intravenous 26-gauge catheter directly into the caudal vein, at a dose of 600 mg/kg. Post-contrast CT was performed at 30 seconds and 1 minute after contrast administration.

All data were transferred to a workstation and analysed using a medical image viewer (RadiAnt DICOM Viewer version 4.6.9, 64-bit).

Organ visibility, location, delimitation, attenuation, and size were registered (data not shown for the heart). Attenuation values of the coelomic organs were measured in Hounsfield units (HU), using a circular/ovoid region of interest (ROI) on the images of every organ following the same protocol previously used in small animals.[61,62] One measurement was performed for each organ in the pre-contrast and post-contrast study. The section with the largest cross-sectional area of tissue was selected on one of the images in transverse or dorsal planes. The ROI included one-third to one-half of the area of the organ in the selected image.

Anatomical post-mortem study: One frozen specimen of each chameleon species was used to illustrate coelomic anatomy and to assess imaging findings. They were sectioned using an electric saw to correspond with the CT images. All sections were cleaned and photographed.

Statistical analysis: An individual confidence interval was used to evaluate the boundary limits. Upper and lower reference limits were obtained by individual 95% confidence intervals of means (95%CI). Additionally, description of measures of anatomic structures were performed by median and their 95%CI and absolute range of them. Pearson correlation coefficients (r) between body weight and testicle length, fat body length, and kidney length were calculated and associated P values. All statistical analyses were performed using standard software (*SPSS Statistics, version 25.0*).

RESULTS

The criteria for the current research included a total of 17 veiled chameleons (7 males and 10 females) and 15 panther chameleons (13 males and 2 females). Four chameleons were excluded due to the presence of some pathologies previously described in another study.[47]

The mean body weight ± SD of the animals included in the study was 102.0 ± 16.8 g for veiled chameleons and 141.8 ± 20.1 g for panther chameleons (range of 75-175 g). The age ranges of the animals were 8-18 months.

The contrast study was successfully performed in 27 out of 32 animals (15 veiled chameleons and 12 panther chameleons). However, accidental contrast extravasation occurred in 3 veiled chameleons and 2 panther chameleons. The contrast remained in the adjacent tissue of the injection site. In the post-contrast study, the contrast enhancement was better observed at 1 minute than 30 seconds after contrast administration.

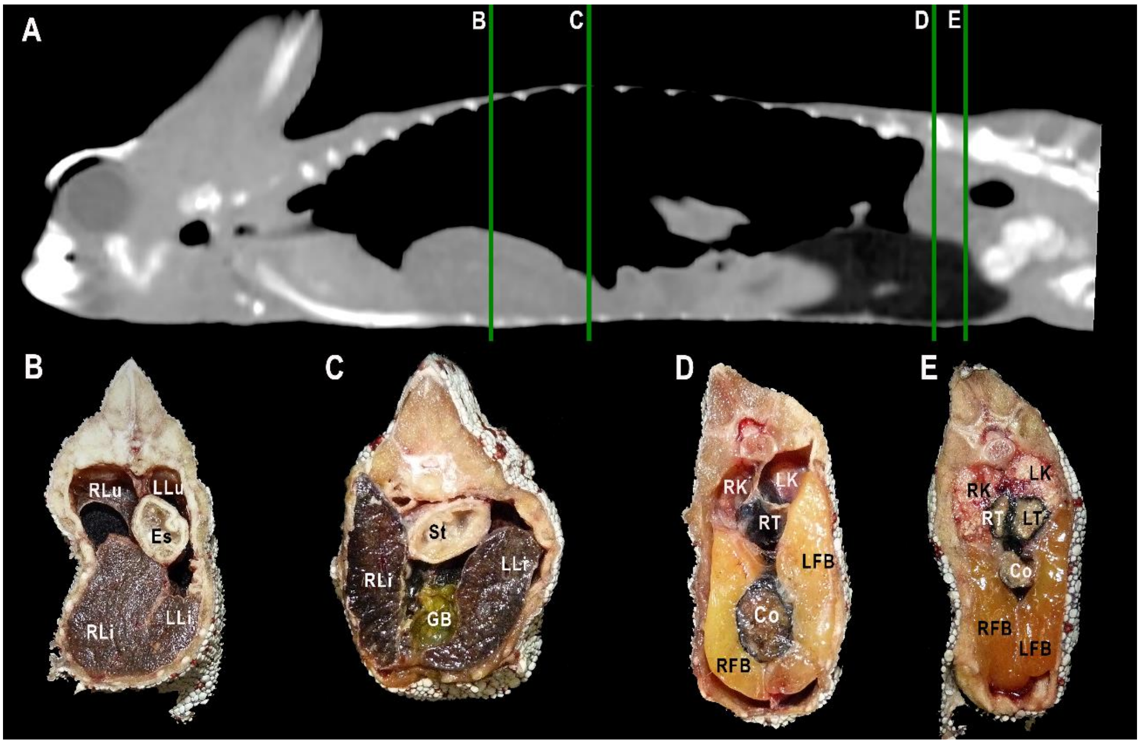


FIGURE 5.3.2. Sagittal pre-contrast CT image displayed in soft tissue window (A) and cranial viewsof gross anatomical sections at the level of the liver (B), gallbladder (C), fat bodies (D) and kidneys (E) in a veiled chameleon (*Chamaeleo calyptratus*). RLu – right lung, LLu – left lung, Es – Esophagus, RLi – right liver lobe, LLi – left liver lobe; ST – stomach, GB – gallbladder; RK – right kidney, LK – left kidney, RT – right testicle, LT – left testicle, RFB – right fat body, LFB – left fat body, Co – colon.

Liver -including caudal vena cava, hepatic vessels, and gallbladder-, stomach, intestines, cloaca, gonads, fat bodies, kidneys and, when distended, urinary bladder were identified in the CT images with the aid of the anatomical sections (Figure 5.3.2.). The location of each organ was the same as that observed in the ultrasound study.[12] Spleen, pancreas, and adrenal glands could not be identified.

Lungs: the lungs were visualized in all chameleons, and they occupied more than two-thirds of the dorsal coelom, depending on their degree of expansion. Because the variable size of the lungs depending on the degree of expansion, no measurements were taken. In the soft tissue window, the lungs were seen as a large air attenuating structure

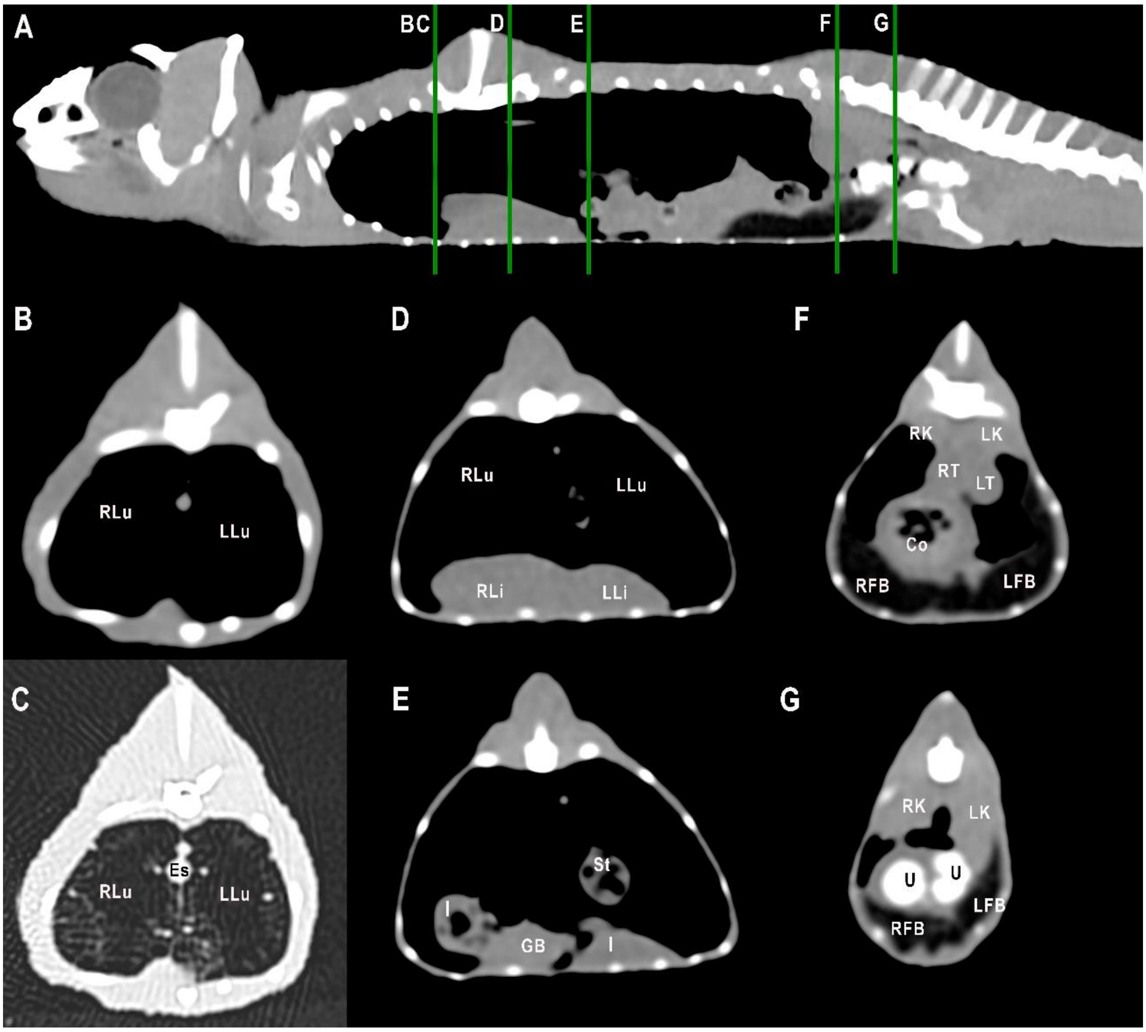


FIGURE 5.3.3. Sagittal pre-contrast CT image displayed in soft tissue window (A) and transverse images displayed in soft tissue (B) and lung windows (C) at the level of the lungs, liver (D), gastrointestinal tract (E), fat bodies (F) and urinary bladder (G) in a panther chameleon (*Furcifer pardalis*). RLu – right lung, LLu – left lung, Es – oesophagus, RLi – right liver lobe, LLi – left liver lobe, St – stomach, I – intestine, GB – gallbladder, RK – right kidney, LK – left kidney, RT – right testicle, LT – left testicle, Co – colon, RFB – right fat body, LFB – left fat body, U – urinary bladder.

(Figures 5.3.3 and 5.3.4). With the lung window, the lung parenchyma in the pre-hilar portion of the lungs appeared as a thin, faintly outlined, homogeneous and hypoattenuating area surrounding an air-filled cavity (Figure 5.3.3); in the post-hilar portion of the lungs, the parenchyma was barely visible. A transverse septum could be observed as a thin, hyperattenuating structure along the lungs. The caudal portion of the gas attenuating structure corresponded with air sacs (Figure 5.3.4).

Liver: The liver was observed in all chameleons as a structure with low soft tissue attenuating values and it was incompletely bilobed. The right lobe was larger than the left one (Figures 5.3.3, 5.3.4., 5.3.5, and 5.3.6). The intrahepatic portion of the caudal vena cava could be visualized in all animals as a hyperattenuating line within the right hepatic lobe in the sagittal plane. Post-contrast, the liver parenchyma enhanced homogeneously, and the hepatic veins and caudal vena cava were better defined from the adjacent parenchyma when they were filled with the contrast.

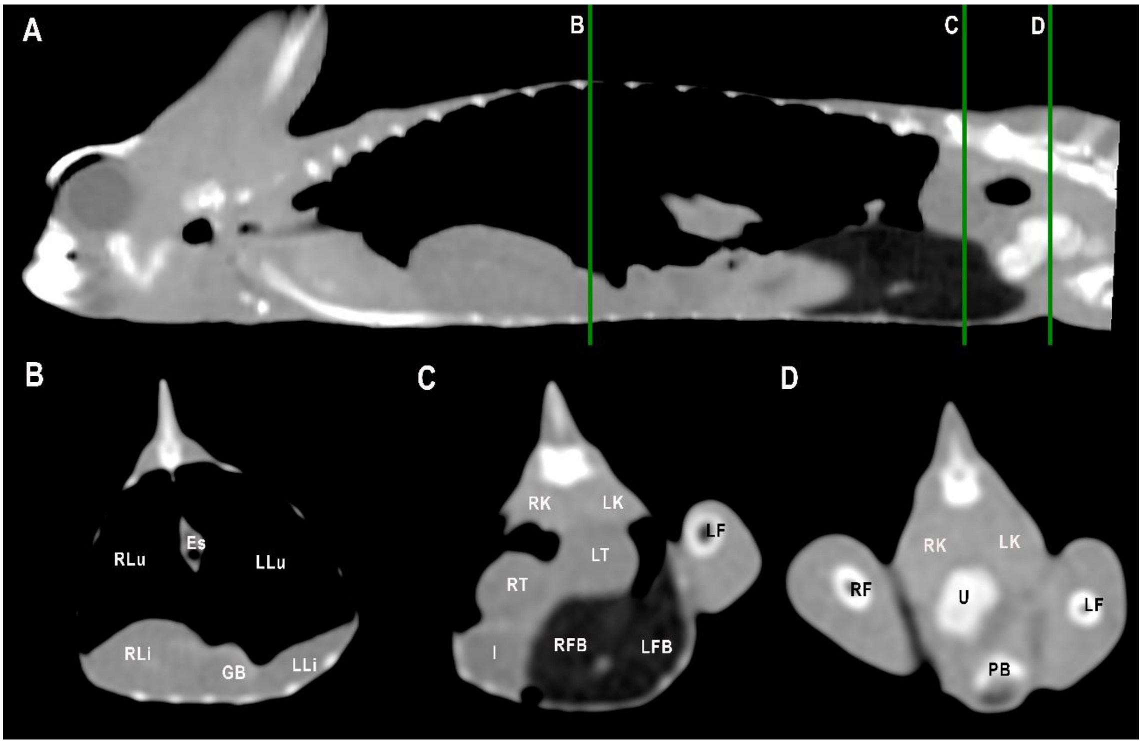


FIGURE 5.3.4. Sagittal pre-contrast CT image displayed in soft tissue window (A) and transverse images displayed in soft tissue window at the level of the gallbladder (B), fat bodies (C) and urinary bladder (D) in a veiled chameleon (*Chamaeleo calyptratus*). RLu – right lung, LLu – left lung, Es – oesophagus, RLi – right liver lobe, LLi – left liver lobe, GB – gallbladder, RK – right kidney, LK – left kidney, RT – right testicle, LT – left testicle, I – intestine, RFB – right fat body, LFB – left fat body, U – urinary bladder, LF – left femur, RF – right femur, PB – pelvic bone.

The liver measurements are included in Table 5.3.1. The pre-contrast attenuation of the hepatic parenchyma was 25.47 ± 4.23 in veiled chameleons and 39.28 ± 11.95 HU in panther chameleons. The post-contrast attenuation was 50.12 ± 8.01 HU and 50.03 ± 5.10 HU, respectively.

There was a significant positive correlation between the right hepatic lobe length (ρ = 0.77, P <0.001) and left hepatic lobe length (ρ = 0.77, P <0.001) and the body weight in veiled chameleon. In panther chameleons, there was also a positive correlation between the right hepatic lobe length and body weight (ρ = 0.62, P = 0.01). However, there was no correlation between the left hepatic lobe length and the body weight (ρ = 0.5, P = 0.05).

Table 5.3.1. Values of liver, testes, fat bodies and kidneys measurements observed in computed tomography in veiled and panther chameleon.

		VEILED CHAMELEON			PANTHER CHAMELEON		
		LENGHT (cm)	WIDTH (cm)	HEIGHT (cm)	LENGTH (cm)	WIDHT (cm)	HEIGHT (cm)
LIVER	Right lobe	2.70 ± 0.42	1.04 ± 0.14	0.99 ± 0.12	2.46 ± 0.30	0.94 ± 0.14	0.72 ± 0.11
	Letf lobe	1.99 ± 0.30	0.78 ± 0.17	0.74 ± 0.12	1.79 ± 0.25	0.75 ± 0.14	0.65 ± 0.14
TESTES	Right	0.90 ± 0.08	0.84 ± 0.04	0.71 ± 0.06	0.74 ± 0.12	0.74 ± 0.12	0.51 ± 0.07
	Letf	0.90 ± 0.08	0.83 ± 0.10	0.73 ± 0.06	0.83 ± 0.14	0.73 ± 0.11	0.51 ± 0.06
FAT BODIES	Right	2.65 ± 0.46	0.64 ± 0.14	0.61 ± 0.15	2.11 ± 0.65	0.45 ± 0.15	0.44 ± 0.12
	Left	2.53 ± 0.40	0.63 ± 0.13	0.62 ± 0.12	1.92 ± 0.67	0.52 ± 0.19	0.39 ± 0.08
KIDNEYS	Right	3.17 ± 0.32	0.35 ± 0.04	0.46 ± 0.07	3.04 ± 0.26	0.45 ± 0.06	0.50 ± 0.06
	Left	3.03 ± 0.33	0.38 ± 0.05	0.45 ± 0.06	2.91 ± 0.22	0.45 ± 0.05	0.50 ± 0.05

Liver: n = 17 in veiled chameleons and n = 15 in panther chameleons. Testes: n = 7 in veiled chameleons and n = 11 in panther chameleons. Fat bodies: n = 15 in veiled chameleons and n = 14 in panther chameleons. Kidneys: n = 17 in veiled chameleons and n = 15 in panther chameleons.

Gallbladder: The gallbladder was observed in all veiled chameleons (17/17; 100%) and in 14 of 15 (93.3%) panther chameleons as a fluid attenuating circular structure (Figures 5.3.3 and 5.3.5) with no visible wall in pre-contrast images. In post-contrast images, the thin wall of the gallbladder enhanced homogeneously, and the content was hypoattenuating in comparison with the liver parenchyma.

Mean gallbladder length was 0.72 ± 0.09 cm, mean width 0.55 ± 0.09 cm, and mean height 0.4 ± 0.07 cm in veiled chameleons. In panther chameleons, the gallbladder measured 0.71 ± 0.12 cm in length, 0.48 ± 0.07 cm in width, and 0.44 ± 0.07 cm in height. Pre-contrast attenuation of the gallbladder was 28.91 ± 2.73 HU in veiled chameleons and 36.99 ± 3.08 HU in panther chameleons. Post-contrast attenuation was 30.25 ± 7.99 HU and 43.00 ± 4.01 HU, respectively.

Gastrointestinal tract: the oesophagus (Figure 5.3.3 and 5.3.5) was located dorsally to the liver, being visible as a tubular structure with soft tissue attenuation followed by the stomach. It was visualized in all animals. The stomach was identified as an elongated

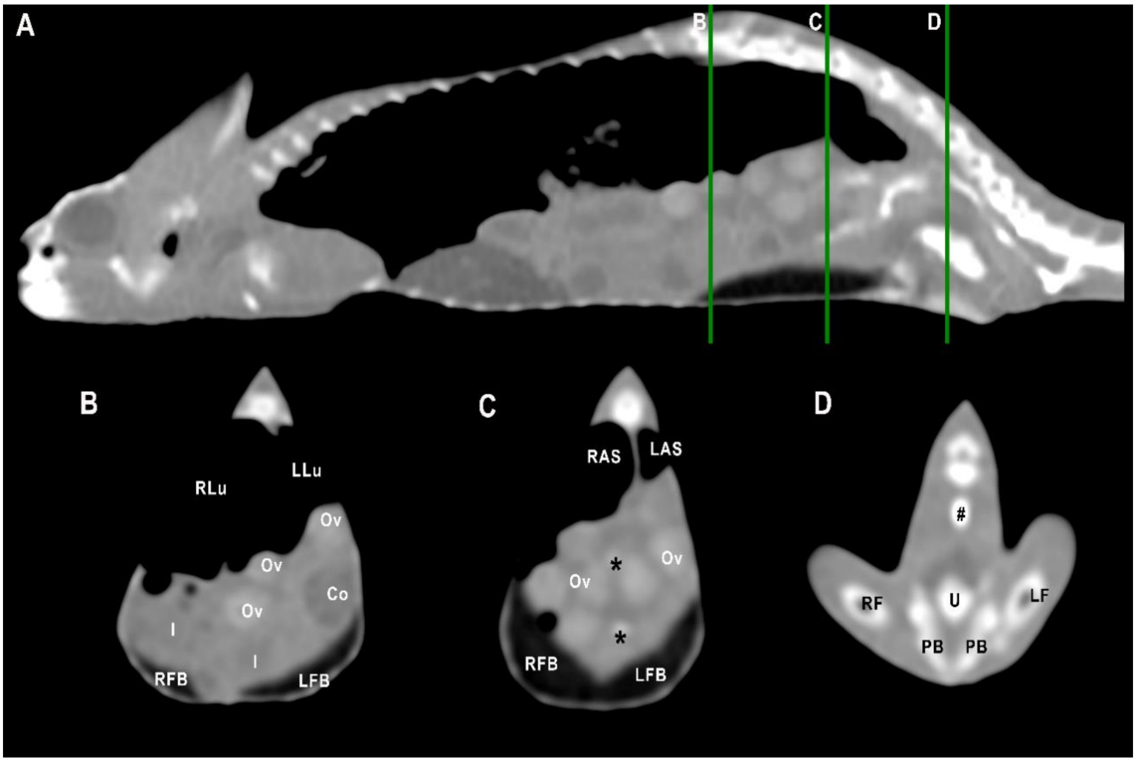


FIGURE 5.3.5. Sagittal post-contrast CT image displayed in soft tissue window (A) and transverse images displayed in soft tissue window at the level of intestines (B), fat bodies and ovaries (C), and urinary bladder (D) in a veiled chameleon (*Chamaeleo calyptratus*). RLu – right lung, LLu – left lung, Ov – ovaric follicles, Co – colon, I – intestine, RFB – right fat body, LFB – left fat body, RAS – right air sac, LAS – left air sac, * – ovarian vessels, # – caudal vein, U – urinary baldder, RF – right femur, LF – left femur, PB – pelvic bone.

and tubular structure with soft tissue attenuation. In all animals it was slightly distended with mixed fluid and gas content (Figure 5.3.3).

The intestines were visualized in all animals. Bowel loops were seen as tubular structures with a soft tissue attenuation wall and small gas bubbles in the lumen (Figures 5.3.3, 5.3.4 and 5.3.5). However, the different intestinal segments could not be easily identified. Only the colon could be differentiated with gas or faeces when it was distended (Figure 5.3.3). In pre-contrast images, the wall intestine attenuation was 29.07 ± 2.30 HU in veiled chameleons and 34.93 ± 3.35 HU in panther chameleons. In post-contrast images, the attenuation was 58.98 ± 8.02 HU and 57.89 ± 3.02 HU, respectively.

Finally, the cloaca was seen in 3 of 17 (17.6%) veiled chameleons and in 2 of 15 (13.3%) panther chameleons as an irregular mineral attenuating structure located in the caudoventral coelom within the pelvic bones. This attenuation is caused by the presence of urate salts located in the most caudal part of the pelvic canal (Figures 5.3.4 and 5.3.5).

Gonads: the testicles could be visualized in all veiled chameleon males (7/7; 100%) and in 11 of 13 (84.6%) panther chameleon males. The testicles were circular, symmetric structures with homogeneous soft tissue attenuation and with mild diffuse enhancement after contrast administration (Figures 5.3.3 and 5.3.5).

The testes measurements are included in Table 5.3.1. In pre-contrast images, the attenuation of testicles was 28.28 ± 3.28 HU in veiled chameleons and 23.20 ± 2.43 HU in panther chameleons. The post-contrast attenuation was 34.02 ± 3.01 HU and 37.58 ± 4.98 HU, respectively.

There was no correlation between body weight and right testicle width and left testicle width in any of the two species.

The ovaries were identified cranial to the kidneys in 4 of 10 (40%) females of veiled chameleon and in all panther chameleon females (2/2; 100%). They could be identified as a cluster of multiple circular structures -the ovarian follicles- with soft tissue attenuation. Mild diffuse enhancement of these structures could be observed after contrast administration (Figures 5.3.4 and 5.3.6). The diameter of the well-defined follicles ranged from 0.19 to 0.4 cm in veiled chameleons and from 0.42 to 0.78 cm in panther chameleons. The eggs were observed as oblong structures with alternating hyper- and hypoattenuating layers (Figure 5.3.6). The centre of the egg had fluid attenuation and the peripheral hyperattenuating line was the shell. The size of the eggs ranged from 0.56 to 0.8 cm in width and from 0.93 to 1.70 cm in length in veiled chameleons. In panther chameleons, this range was 0.60 to 0.7 cm in width and 0.93 to 1.05 cm in length. The pre-contrast attenuation range of ovarian follicles was 25.5-39 HU in veiled chameleons and 12.34-39.60 HU in panther chameleons depending on the stage of follicle and egg development. Post-contrast attenuation range was 31.10-44.25 HU in veiled chameleons and 22.54-55.21 HU in panther chameleons.

Fat bodies: the fat bodies could be visualized in 15 of 17 (88.2%) veiled chameleons and 14 of 15 (93.3%) panther chameleons. They appeared as well defined, fusiform, bilateral, and symmetrical fat attenuating structures (Figures 5.3.3, 5.3.4, 5.3.5 and 5.3.6). They had a wide base in the ventral aspect and were narrow in the apical zone, as a triangle shape. The fat bodies measurements are included in Table 5.3.1. The pre-contrast attenuation of the fat bodies was -107.16 ± 8.28 HU in veiled chameleons and -95.62 ± 11.16 HU in panther chameleons. In post-contrast images, the fat bodies did not show enhancement.

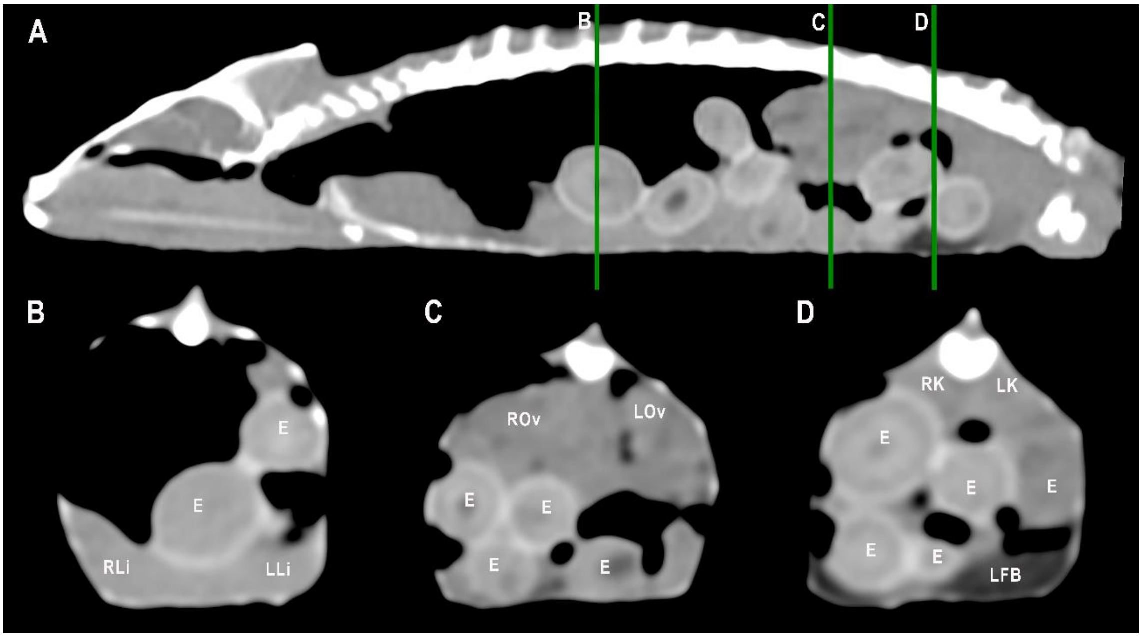


FIGURE 5.3.6. Sagittal pre-contrast CT image displayed in soft tissue window (A) and transverse images displayed in soft tissue window at the level of the liver (B), ovaries (C) and fat bodies (D) in a panther chameleon (*Furcifer pardalis*). E – eggs, RLi – right liver lobe, LLi – left liver lobe, ROv – right ovary, LOv – left ovary, RK – right kidney, LK – left kidney, LFB- left fat body.

In veiled chameleons, there was a positive correlation between body weight and the length of the right and left fat bodies (ρ = 0.774; P = 0.001 and ρ = 0.742; P = 0.002 respectively). In panther chameleons, there was no correlation between body weight and any of the fat body lengths.

Kidneys: the kidneys were clearly visible in all animals. They appeared as lobulated oval and bilateral structures with soft tissue attenuation (Figures 5.3.3, 5.3.5 and 5.3.6). The cranial pole of the kidneys was cranially to the pelvic bones. The caudal pole of the kidneys was seen

within the pelvic canal. The kidneys were most easily identified in the sagittal plane and in the post-contrast studies. The kidneys showed diffuse heterogeneous enhancement after contrast administration.

The kidney measurements are included in Table 5.3.1. The pre-contrast attenuation of the kidneys was 33.84 ± 4.21 HU in veiled chameleons and 44.52 ± 3.75 HU in panther chameleons. The post-contrast attenuation was 55.00 ± 4.00 HU and 62.00 ± 5.00 HU, respectively.

In veiled chameleons, there was a positive correlation between body weight and the length of the right kidney ($P < 0.001$; ρ = 0.899) and the left kidney ($P < 0.001$; ρ = 0.914). In panther chameleons, there was no correlation between body weight and right or left kidney length (ρ = 0.25, P = 0.38; and ρ = 0.29, P = 0.292, respectively).

Urinary bladder: the urinary bladder was seen in 8 of 17 (47.1%) veiled chameleons and in 4 of 15 (26.7%) panther chameleons. It was visualized as an irregular mineral attenuating structure due to the presence of urate salts in the lumen (Figure 5.3.3). A wall could not be visualized. The size of urinary bladder could not be determined due to the poor visualization of the wall. The attenuation range was wide and variable because it depended on the content and amount of urates. The range was 143.34 to 295.18 HU in veiled chameleons and 154.26 to 356.92 HU in panther chameleons. In post-contrast images the urinary bladder could contain variable amounts of contrast depending on the time lapsed after contrast administration.

DISCUSSION

Liver -including caudal vena cava, hepatic vessels, and gallbladder-, stomach, intestines, cloaca, gonads, fat bodies, kidneys and, when distended, urinary bladder can be easily visualized using CT in healthy veiled and panther chameleons.

The spleen, pancreas, and adrenal glands were identified in anatomical post-mortem study, but not in the CT scan in any animal in the current study, probably because the small size of these organs.[47] In other species such as green iguana (*Iguana iguana*) or the black and white tegu (*Tupinambis merianae*) the spleen could be identified in CT as a small, rounded, soft tissue density, partially silhouetting with the stomach. Only in the green iguana the pancreas was identified in CT as a soft tissue density interposed between the small intestinal loops.[63]

In the study, all soft-tissue window transverse images were compared with their corresponding anatomical slice. The sectional anatomy of the coelomic organs in veiled and panther chameleons allows a correct morphologic and topographic evaluation of the anatomic structures, which is a useful tool for the interpretation of the CT images.

All the CT scan examinations were performed in sternal recumbency, as it has been described in other studies in reptiles.[32,36,63] Unlike ultrasound, the recommended positioning of the patient for CT in reptiles is sternal recumbency, regardless of the animal shape, flattened or not, as it has been described in other studies in loggerhead sea turtle (*Caretta caretta),* green iguana or bearded dragon *(Pogona vitticeps).*[38,47,63]

The overlapping of structures in radiology of species with flattened anatomical conformation impairs the correct identification of the coelomic organs. In the current study, CT allowed proper identification of the coelomic structures without artifacts caused by gas from the lungs, air sacs or intestines, as it happens in ultrasonography.[47]

The animals were anesthetized in the current study in order to immobilize them. It also produces stress reduction which is beneficial to reduce mortality in reptiles.[11,39]

The contrast study was successfully performed in 27 out of 32 animals (15 veiled chameleons and 12 panther chameleons). However, in 5 chameleons (3 veiled chameleons and 2 panther chameleons) a small amount of extravasated contrast remained in the tissue adjacent to the injection site due to the small diameter of the coccygeal vein. The presence of contrast in the perivascular soft tissues did not cause any adverse effects and did not hinder the interpretation of the images. In 5 animals the contrast could not be successfully administered due to the small size of the coccygeal vein.

The contrast enhancement was better after 1 minute of the administration due to the low heart rate of these species. These results agree with the study of Nardini G. et al (2014).[34] In green iguana the contrast medium was observed in the caudal vena cava 3 seconds after injection, but not in the aorta. Thirty-six seconds after injection the contrast was visible in the liver parenchyma, and after 600 seconds the contrast was still mildly appreciable in the liver.[34] As expected, the parenchymal organs enhanced after contrast administration, and side effects were not observed in any animal.[34]

The lung parenchyma was described as a homogeneous and hypoattenuating area around an air-filled cavity, similar to other reptiles such as the bearded dragon, black and white tegu, ball python (*Python regius*) and boa constrictor (*Boa constrictor*).[9,15] The transverse septum between the lungs could also be identified; this is in accordance with what has been described in the green iguana.[63] Although lung parenchyma and air sacs should be evaluated using lung window, both structures can be observed with soft tissue window.

The liver was encapsulated, bilobed and the right lobe was the largest of the two lobes. Similarly, in a study in black and white tegu and bearded dragon, a liver lobe extending caudally in a dorsolateral position was evident on the right side of the coelomic cavity.[63]

The gallbladder was easily identified as in previous studies in reptiles.[63] The nutrition of the animals or the existence of bile sediment can produce variations in the gallbladder attenuation. Similar findings have not been found in CT studies of other reptiles in the reviewed literature. However, changes in echogenicity, likely representing the same variations, have also been described using ultrasonography in chameleons and other reptiles.[47,51,55]

A variation in the gallbladder size was observed among the chameleons of the study. This fact can be explained by the composition of the diet, the degree of contraction, and even the length of fasting.[20,47]

The stomach was identified in the craniodorsal coelom when it was distended with an elongated and tubular shape in the chameleons of the study. In contrast, the stomach had C-shaped appearance in the green iguana and the black and white tegu.[63]

The intestines were localized immediately caudoventral to the liver. The intestine segments could not be differentiated due to the similar appearance along the gastrointestinal tract as in bearded dragon and black and white tegu.[63] As in other reptile CT studies, the gastrointestinal stratification could not be differentiated. In dogs and cats, gastrointestinal layers are visible only in 22% of gastrointestinal segments, most often in the stomach and jejunum.[64] However, the recommended tool for a proper evaluation of gastrointestinal layering in chameleons is the ultrasonography.[47]

On the other hand, the colon and cloaca could be identified when distended with content. However, when the colon was empty, the identification was not possible because its attenuation was similar to the adjacent tissues. The cloaca was only identified when it contained urate salts, which were observed as mineral attenuating structures.

The testes were clearly observed in mature males of the study. No differences in their measurements were observed throughout the research. This fact could suggest that the breeding season extends throughout the year if the environmental conditions in captivity are favourable. It would be necessary to perform more studies about gonad activity to confirm it.

In females, the ovaries were easily detected in sexually active females and the ovarian description in chameleons was similar to those in other reptiles.[9] The inactive ovaries could not be visualized. The CT images of the active ovaries allowed identification of the different stages of folliculogenesis. When the ovaries were in initial stages of folliculogenesis, the follicles appeared as circular structures with soft tissue attenuation and presented variable sizes. In the final stages of development, eggs were identified as well delimited ovoid structures with an alternating hyper- and hypoattenuating layers.

The appearance of chameleons' follicles and eggs in CT is in general similar to that of CT in bearded dragons or box turtles.[9,65] The CT offers a much larger gray scale and is not limited by superimposition, each follicle and egg is detected and clearly evaluated, except the least developed follicles. In the last phases of development, the eggs could occupy the two-thirds of the coelom, displacing the adjacent structures.

Fat bodies were easily identified because of the negative attenuation value in comparison with the adjacent structures. In general, the chameleons of the study had large fat bodies. The nutritional status, the breeding season or the presence of pathologies could produce variations in its size.[20]

The CT scan facilitated the evaluation of the entire kidneys in the chameleons of the study. Because the caudal poles of the kidneys are in the pelvic canal, they cannot be completely observed using other imaging techniques such as X-ray and ultrasonography. It is well known that CT scan allows the visualization of viscera avoiding the interferences and overlapping of different structures.[47] In the current study, kidney attenuation was similar to the adjacent structures. Therefore, the identification of the kidneys was easier in the sagittal than in transverse plane and after the contrast administration.

Similarly to the cloaca, the urinary bladder was only identified in our study when it was distended with mineral attenuating structures in the lumen, corresponding to urate salts. Although no comments have been found in the literature regarding this finding, to the authors' experience, when the urates were present in the pelvic canal they were located in the cloaca and when they were located more cranial in the coelomic cavity, they were located in the urinary bladder. The measurements of the size urinary bladder could not be performed due to the low contrast between the urinary bladder and adjacent tissues. In addition, the thin wall of the urinary bladder was barely identified. The urinary bladder could contain contrast in different amounts in the post-contrast study depending on the acquisition time of the post-contrast images.

Research limitations were the low number of animals, and the wide range of ages and sizes. The health status of the animals was determined by physical examination and coprological study, but no blood tests were performed because the wholesaler owner did not permit it. Haematology and biochemistry would have been necessary to obtain more information about the clinical status of the chameleons because the common presence of a subclinical diseases.[48]

In conclusion, this study provides a guide of the normal cross-sectional and computed tomographic anatomy of the coelomic cavity in veiled chameleons and panther chameleons. The CT scan may potentially be useful to detect intracoelomic diseases and could be a good tool in forthcoming research in reptiles.

6. GENERAL DISCUSSION

The results of the current study suggest that the different imaging techniques (radiography, ultrasonography and computed tomography) allow the visualization of the liver (including caudal vena cava and hepatic veins), gallbladder, stomach, intestines, gonads, fat bodies and kidneys in healthy veiled and panther chameleons. The urinary bladder was identified using US and CT examination. However, it could not be visualized using radiography.

The animals of the study were fasted for 2 days prior to diagnostic imaging in order to avoid the overlapping of the coelomic structures with the gastrointestinal content.

The animals were sedated to help minimize the stress of handling and the risk of injuries and accidents. In addition, the sedation allowed to reduce radiation exposure to the animal handler.

The positioning of the chameleons during the imaging examinations is slightly different in comparison with other species because of the vertical flattened anatomical conformation.[27] On the one hand, the radiographic examination was performed in dorsoventral and right lateral view. Due to the vertical flattened anatomical conformation the lateral view was the best position to identify the majority of the coelomic structures.

On the other hand, the ultrasonographic studies were performed in lateral recumbency because the vertical flattened anatomical conformation did not permit to obtain good quality images in dorsal recumbency. In contrast, CT scan examinations were performed in sternal recumbency, regardless of the animal shape, as it has been described in other studies in reptiles.[32,36,63]

The spleen, pancreas and adrenal glands were identified in post-mortem studies, but could not be visualized with any imaging technique. Visceral overlapping, similar appearance to adjacent structures or the small size of these organs are suggested reasons why the identification was not possible.

The lung parenchyma could be evaluated with radiography and CT scan in all chameleons. They appeared as air sac-like structures located dorsal to the liver against the dorsal wall of the coelomic cavity. They were extended caudally, occupying a big portion of the coelomic cavity. The transverse septum between the lungs could be identified using CT scan; this is in accordance with what has been described in the green iguana.[63]

During the ultrasonography, the amount of gas present in the expanded lungs of the conscious animals interfered with the visualization of some of the organs. The lung parenchyma could not be evaluated with this technique. The sedation is important because it reduces respiratory volume, and consequently, the gas interference.

The liver was easily identified in all chameleons with the different imaging techniques. It was encapsulated, bilobed and the right lobe was the largest of the two lobes. The best imaging techniques to evaluate the liver parenchyma were ultrasonography and CT scan because radiography did not offer a correct definition of the parenchyma due to the similar radiopacities between coelomic structures.

The gallbladder could be seen only in some animals (8 to 17 veiled chameleons and 5 to 15 panther chameleons) using radiography. On the other hand, it could be easily seen in 17 to 17 veiled chameleons and 14 to 15 panther chameleons using ultrasonography and CT scan. Possible reasons for the difficulty in visualizing the gallbladder would be the small size, the visceral overlapping and similar radiopacity to adjacent structures.

The different parts of the gastrointestinal tract (such as oesophagus, stomach and intestines) could be identified in all animals using the different imaging techniques of the study. The segments of small intestine, as in other species, could not be differentiated with any technique.[49] The colon could be identified when distended with content during ultrasonographic examination and CT scan.

Only the ultrasonography allowed the identification of the gastric and intestinal layers in the majority of animals.

The presence of gas and gastric material limited the visualization of some coelomic organs in the radiographic and ultrasonographic examinations. This problem occurred although all animals were fasted for 2 days prior to performing radiographic and ultrasonographic studies. Therefore, gastrointestinal gas may be a limitation for using these techniques in some clinically affected animals.

Only the CT scan allowed the evaluation of the cloaca in a few animals. It was only identified when it contained urate salts, which were observed as mineral attenuating structures located in the caudoventral coelom within the pelvic bones.

The gonads were easily identified in sexually active animals. The testes were well identified with all imaging techniques. The right teste was visible always cranially to the left teste. The testes were similarly in size in each individual and also there was no difference between the two species evaluated. There was no evidence of differences in size or appearance of the testes along the study, even among animals of different body weight in different seasons; this fact may suggest that these species are sexually active throughout the year when kept in captivity and the testes may persist large in size during breeding season.[47]

The ovaries were clearly identified in sexually active females using the different imaging techniques and the appearance was similar to those in other species.[47,56,57] In these females, radiography, ultrasonography and CT scan allowed to identify the different stages of folliculogenesis.

The inactive or immature ovaries were difficult to locate. The evaluation of the coelomic organs using radiography and ultrasound was difficult in breeding females, because the large size of ovarian follicles overlapped the adjacent structures. The only technique that avoided the interference and the overlapping of the structures was CT scan.

The fat bodies were easily identified using radiography, ultrasonography, and CT scan, but their margins could not be well identified because the overlapping with the adjacent intestinal structures in radiography. The fat bodies were better observed using the CT scan because of the negative attenuation value in comparison with the adjacent structures.

The size of the fat bodies can vary depending on the diet, the physiological or reproductive status, or even the presence of underlying pathologies. Causes of energy consumption such as nutritional deficiencies, the breeding season or the presence of pathologies could produce variations in its size.[20] A ratio between body length and fat body length was calculated using ultrasound to provide an objective measure of the fatty status. This measure was called body fat corporal index.

Only the cranial poles of the kidneys could be evaluated using radiography (lateral view) and ultrasonography, in comparison with necropsy. The caudal poles of the kidneys were located within the pelvic canal, extending to the base of the tail, hindering the visualization of this portion, as has been published in other species.[3,51,53,56]

The evaluation of the entire kidneys could only be performed using CT scan because it allowed the visualization of viscera avoiding the interferences and overlapping of different structures.[47].

A vertebral kidney index (a ratio between kidney length and lumbar vertebral body length) was calculated radiographically to provide an objective measure of the renal size. A higher index result would suggest nephromegaly.

The presence or absence of the urinary bladder depends on the family and the sexes in reptiles. Both veiled and panther chameleons possess urinary bladder.[53,56,58] In the current study, the urinary bladder was only identified using ultrasound and CT scan when it was distended with mineral attenuating structures in the lumen, corresponding to urate salts.

Research limitations were the low number of animals, and the wide range of ages and sizes. The health status of the animals was determined by physical examination and coprological testing only. However, the presence of a subclinical disease is not uncommon.[48] Blood analyses would give more information about the clinical status of the animal. However, they were not performed in the present study because the owner of the chameleons did not permit blood sampling.

7. CONCLUSIONS

1. The designed radiographic protocol could be performed correctly with the use of the cassette-based digital mammography, allowing the observation and measurement of most of the coelomic cavity structures in healthy veiled and panther chameleons.

2. The ultrasonographic protocol allowed to describe and measure the coelomic organs in healthy veiled and panther chameleons positioned in right lateral recumbency. Ultrasonography offered a better visualization of the coelomic structures in comparison with radiography.

3. Computed tomography permitted a three-dimensional evaluation of the coelomic structures avoiding overlapping or air interference as occurred in radiography or ultrasound, respectively. Despite the small size of the animals, this technique allowed a good identification and measurement of the coelomic organs in both species.

4. Finally, some coelomic structures (adrenal glands, spleen and pancreas) could not be visualized using any imaging technique probably because the small size of these organs or the similar appearance with the adjacent structures.

8. REFERENCES

1. Bennett AF, Dawson WR: Metabolism. In: Gans C, Dawson WR, eds. Biology of the reptilia. New York and San Francisco: Physiology A. London: Academic Press; 1976, 5:127–211.

2. Redrobe S. Basic Approach to the Reptile Patient. Paper presented at: 29th World Small Animal Veterinary Association (WSAVA) World Congress; Oct 6-9, 2004; Rhodes, Greece.

3. Barten SL: Lizards. In: Mader D, ed. Reptile medicine and surgery. 2nd ed. St Louis, MO: Saunders Elsevier; 2006;59–77.

4. Pough FH, Janis CM, Heiser JB. The lepidosaurs: Tuatara, lizards and snakes. In: Pough FH, Janis CM, Heiser JB, eds. Vertebrate life. 6th ed. Englewood Cliffs, NJ: Prentice Hall; 2002b;294–341.

5. O'Malley B: General anatomy and physiology of reptiles. In: O'Malley B, ed. Clinical Anatomy and Physiology of Exotic Species. 1st ed. London, UK: Saunders Elsevier; 2005;17-39.

6. Secor SM, Diamond J. Adaptive responses to feeding Burmese Pythons: Pay before pumping. J Exp Biol. 1995; 198:1313–1325.

7. Secor SM, Nagy KA. Energetic correlates of foraging mode of snakes *Crotalus cerastes* and *Masticophis flagellum*. Ecology. 1994; 75(6):1600–1614.

8. Brunetti L, Giandomenico L, Millefanti M. Los Camaleones. 1st ed. Barcelona, Spain: De Vecchi; 2016;1-128.

9. Krysko, K, Enge, K.M. and King, W. The Veiled Chameleon, Chamaeleo calyptratus: a new exotic lizard species in Florida. Flor Scien. 2004; 67(4):249-253.

10. Wilms T, Sindaco R, Shobrak M: *Chamaeleo calyptratus.* The IUCN Red List of Threatened Species, 2012.

11. Rossi JV: General Husbandry and Management. In: Mader D, ed. Reptile medicine and surgery. 2nd ed. St Louis, MO: Saunders Elsevier; 2006;25–41.

12. Jenkins RKB et al: *Furcifer pardalis*. The IUCN Red List of Threatened Species, 2011.

13. Barten S, Simpson S: Lizard Taxonomy, Anatomy, and Physiology. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St Louis, MO: Elsevier; 2019;63-74.

14. Klaver C, Böhme W: Phylogeny and classification of the Chamaeleonidae (Sauria) with special reference to hemipenis morphology. Bonner Zool Monogr. 1986; 22:5-60.

15. Glaw F, Vences M: Chamaeleonidae: Furcifer. In: Glaw F and Vences M, eds. Amphibians and Reptiles of Madagascar. 3th ed. Cologne, DE, Vences & Glaw Verlags GbR; 2007;298-309.

16. O'Malley B: Lizards. In: O'Malley B, ed. Clinical Anatomy and Physiology of Exotic Species. 1st ed. London, UK: Saunders Elsevier; 2005;57-75.

17. Dąbrowski Z, Sano Martins IS, Tabarowski Z et al: Haematopoiesis in snakes (Ophidia) in early postnatal development. Cell Tissue Res. 2007; 328:291–299.

18. Girling SJ, Hynes B: Cardiovascular and hematopoietic systems. In: Girling SJ, Rait P, eds. BSAVA manual of reptiles. 2nd ed. Gloucester, England: BSAVA; 2004;243–260.

19. Holz PH. Anatomy and Physiology of the Reptile Renal System. Vet Clin North Am Exot Anim Pract. 2020; 23(1):103-114.

20. Azeez OI, Meintjes R, Chamunorwa JP. Fat body, fat pad and adipose tissues in invertebrates and vertebrates: the nexus. Lipids Health Dis. 2014; 13:71.

21. Schumacher J, Toal RL. Advanced Radiography and Ultrasonography in Reptiles. Sem Avian Exotic Pet Med. 2001;10(4):162-168.

22. Silverman S: Diagnostic imaging In: Mader D, ed. Reptile medicine and surgery. 2nd ed. St Louis, MO: Saunders Elsevier; 2006;487–488.

23. Pees M: Radiographic investigation. In: Krautwald-Junghanns M, Pees M, Reese S et al. Diagnostic Imaging of Exotic Pets. 1st ed. Hannover, Germany: Schlütersche; 2011;310-333.

24. Holmes SP, Divers SJ: Radiography - General Principles. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St Louis, MO: Elsevier; 2019;486-490.

25. Comolli JR, Divers SJ: Radiography - Snakes. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St Louis, MO: Elsevier; 2019;503-513.

26. Holmes SP, Divers SJ: Radiography - Chelonians. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St Louis, MO: Elsevier; 2019;514-527

27. Divers SJ: Radiography - Lizards. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St Louis, MO: Elsevier; 2019;491-502.

28. Stetter MD: Ultrasonography. In: Mader D, ed. Reptile medicine and surgery. 2nd ed. St Louis, MO: Saunders Elsevier; 2006;665–674.

29. Hochleithner C, Sharma A: Ultrasonography. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St Louis, MO: Elsevier; 2019;543-559.

30. Pees M: Ultrasonography. In: Krautwald-Junghanns M, Pees M, Reese S et al. Diagnostic Imaging of Exotic Pets. 1st ed. Hannover, Germany: Schlütersche; 2011;334-357.

31. Wyneken J: Computed tomography and Magnetic Resonance Imaging Anatomy of Reptiles. In: Mader D, ed. Reptile medicine and surgery. 2nd ed. St Louis, MO: Saunders Elsevier; 2006;1088-1096.

32. Arencibia A, Rivero MA, Miguel I, et al. Computed tomographic anatomy of the head of the loggerhead sea turtle (Caretta caretta). Res Vet Sci . 2006;81:165–169.

33. De Souza JCS, Fernandes THT, De Albuquerque BM, et al. Quantitative computed tomography of healthy adult boas (Boa constrictor). J Zoo Wildl Med. 2018;49(4):1012-1015.

34. Nardini G, Di Girolamo N, Leopardi S, et al. Evaluation of liver parenchyma and perfusion using dynamic contrast-enhanced computed tomography and contrast-enhanced ultrasonography in captive green iguanas (Iguana iguana) under general anesthesia. Vet Res. 2014;10:112.

35. Oraze JS, Beltran E, Thornton SM, et al. Neurologic and computed tomography findings in sea turtles with history of traumatic injury. J Zoo Wildl Med. 2019;50(2):350-361.

36. Pees M, Kiefer I, Thielebein J, Oechtering G, et al. Computed tomography of the lung of healthy snakes of the *species Python regius, Boa constrictor, Python reticulatus, Morelia viridis, Epicrates cenchria*, and *Morelia spilota*. Vet Radiol Ultrasound. 2009;50(5):487-91.

37. Schmidt L, Di Girolamo N, Selleri P. Diagnostic Imaging of the Reptile Urinary System. Vet Clin North Am Exot Anim Pract. 2020; 23(1):131-149.

38. Valente A, Cuenca R, Zamora M, et al. Computed tomography of the vertebral column and coelomic structures in the normal loggerhead sea turtle (*Caretta caretta*). Vet J. 2007;174:362–370.

39. Kiefer I, Pees M: Computed tomography (CT). In: Krautwald-Junghanns M, Pees M, Reese S et al. Diagnostic Imaging of Exotic Pets. 1st ed. Hannover, Germany: Schlütersche; 2011;358-367.

40. Sharma A, Wyneken J: Computed Tomography. In: Divers SJ, Stahl SJ, eds. Mader's Reptile and Amphibian Medicine and Surgery. 3rd ed. St Louis, MO: Elsevier; 2019;560-570.

41. Melero A, Juan-Sallés C, Martorell J. Pancreatitis and Cholecystitis Associated with Cryptosporidium Infection in a Corn Snake (*Panterophis guttatus*). Communication presented at: World Veterinary Association Congress (WVAC); 2018; Barcelona, Spain.

42. Oldham-Ott CK, Gilloteaux J. Comparative morphology of the gallbladder and biliary tract in vertebrates: variation in structure, homology in function and gallstones. Microsc Res Tech. 1997;38(6):571–597.

43. Melero A, Martínez Silvestre A, Espada Y et al. Cholecystitis and biliary abscess associated with bacterial and fungal infection in a bearded dragon (*Pogona vitticeps*). Communication presented at: International Conference on Avian Herpetological and Exotic Mammal medicine (ICARE); 2019; London, UK.

44. Kinney ME, Chinnadurai SK, Wack RF. Cholecystectomy for the Treatment of Mycobacterial Cholecystitis in a Pacific Gopher Snake (*Pituophis catenifer*). J Herp Med Surg. 2013;23(1-2):10-14.

45. Grosset C, Daniaux L, Sanchez-Migallón D, et al. Radiographic anatomy and barium sulfate contrast study of the gastrointestinal tract of bearded dragons (*Pogona vitticeps*). Vet Radiol Ultrasound. 2014;55(3):241–250.

46. Houck EL, Cohen EB, Womble M, et al. Radiographic anatomy and barium sulfate contrast study of the gastrointestinal tract of eastern box turtles (*Terrapene carolina carolina*). Vet Radiol Ultrasound. 2019;60(5):473–484.

47. Melero A, Novellas R, Mallol C, et al. Ultrasonographic appearance of the coelomic cavity organs in healthy veiled chameleons (*Chamaeleo calyptratus*) and panther chameleons (*Furcifer pardalis*). Vet Radiol Ultrasound. 2020;61(1):58-66.

48. Garner MM. Overview of necropsy and necropsy techniques. In: Mader D, ed. Reptile Medicine and Surgery. 2nd ed. St Louis,MO: Saunders Elsevier; 2006:569-580.

49. Raiti P. Non-invasive imaging. In: Girling SJ, Raiti P, eds. BSAVA manual of reptiles. 2nd ed. Gloucester, England: BSAVA; 2004;96–98.

50. Schildger B, Casares M, Kramer M, et al. Technique of ultrasonography in lizards, snakes and chelonians. Sem Avian Exotic Pet Med. 1994;3:147-155.

51. Bucy DS, Guzman DS, Zwingenberger AL. Ultrasonographic anatomy of bearded dragons (*Pogona vitticeps*). J Am Vet Med Assoc. 2015;246:868-76.

52. Valente A, Parga ML, Espada Y, et al. Normal ultrasonographic imaging of the loggerhead sea turtle (*Caretta caretta*). Vet Rec. 2007;161:226–232.

53. Holland MF, Hernandez-Divers S, Frank PM. Ultrasonographic appearance of the coelomic cavity in healthy green iguanas. J Am Vet Med Assoc. 2008;233(4):590-596.

54. Banzato T, Russo E, Finotti L, et al. Ultrasonographic anatomy of the coelomic organs of boid snakes (*Boa constrictor imperator, Python regius, Python molurus molurus*, and *Python curtus*). Am J Vet Res. 2012;73(5):234-245.

55. Martorell J, Espada Y, Ruiz De Gopegui, R: Normal echoanatomy of the red-eared slider terrapin (*Trachemys scripta elegans*). Vet rec. 2004;155:417-420.

56. Prades RB, Lastica EA, Acorda JA. Ultrasonography of the organs of male water monitor lizard (*Varanus marmoratus*, Weigmann, 1834). Philipp J Vet Anim Sci. 2013; 39:247-258.

57. Cojean O, Vergneau-Grosset C, Masseau I. Ultrasonographic anatomy of reproductive female leopard geckos (*Eublepharis macularius*). Vet Radiol Ultrasound. 2018;59:333–344.

58. Beuchat CA. Phylogenetic distribution of the urinary bladder in lizards. Copeia. 1986;2:512-517.

59. Schumacher J. Ultrasound and Computed Tomography Imaging in Reptiles. Communication presented at: World Small Animal Veterinary Association World Congress; 2010; Geneva, Switzerland.

60. Gumpenberger M, Henninger W. The use of computed tomography in avian and reptile medicine. Sem Avian Exotic Pet Med. 2001;10(4):174-180.

61. Mallol C, Altuzarra R, Espada Y et al. CT characterisation of feline adrenal glands. J Feline Med Surg. 2020 22(4):285-291.

62. Bertolini G, Furlanello T, De Lorenzi D, et al. Computed tomographic quantification of canine adrenal gland volume and attenuation. Vet Radiol Ultrasound 2006;47(5):444–448.

63. Banzato T, Selleri P, Veladiano IA, et al. Comparative evaluation of the cadaveric and computed tomographic features of the coelomic cavity in the green iguana (*Iguana iguana*), black and white tegu (*Tupinambis merianae*) and bearded dragon (*Pogona vitticeps*). Anat Histol Embryol. 2013;42(6):453-460.

64. Wisner E, Zwingenberger A. Gastrointestinal tract. In: Wisner E, Zwingenberger A, eds. Atlas of Small Animal CT and MRI. 1st ed. Oxford, UK: Editorial Offices; 2015:538-550.

65. Gumpenberger M. Secrets within the Bony Box – Inside View on Chelonians (An Overview). EAVDI Yearbook 2019. Cambridge, UK: EAVDI Ltd; 2019:29-54.