

# Computed Tomography and 3D Reconstruction of the Respiratory Organs of the Egyptian Tortoise (*Testudo kleinmanni*)

**A.S.Saber and Basma M. Kamal**

Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Sadat city Branch, Menoufiya University, Egypt

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## Abstract

This study is designed to identify, describe and illustrate the topography of the respiratory organs of the Egyptian tortoise (*Testudo kleinmanni*) using a digital camera and computed tomography. This may aid in obtaining complete data on dissection procedures, the topography and the initial section outlines and the safety of access during surgical procedures. The core of the present study is a series of photographs showing stages of the dissection. A detailed description of the dissection method and anatomy of the exposed organs are also given. Thirteen wild adult live tortoises, *Testudo kleinmanni*, (three males and ten females) were collected from Marsa Matroh and Cairo governorates with an age ranging from 15-19 years, weight between 430–450 gm, and carapace, and plastron lengths 10.5-11cm and 9.5-9.8 cm respectively. Computed tomography provides detailed information on the respiratory system, the location of the coelomic structures with respect to the carapace (the upper shell) and the vertebrae, and allows a comparison of cross-sectional

anatomic views obtained with the digital camera with images obtained via computed tomography sections of the coelomic structures.

## Key Words

Egyptian Tortoise, respiratory organs, Computed tomography (CT), 3D construction.

## Introduction

Chelonians (refers to the order shelled reptiles), are the most ancient of all living reptiles on the earth having evolved over 200 million years ago. They predate dinosaurs and their primeval shelled appearance has made them popular and fascinating pet with none of the sinister connotations of snakes.

Evans, (1986) reported that North America has a quarter of the world's chelonians while Europe has only two species of fresh-water turtle and three terrestrial species. Australia has only aquatic side necks species.

Of all reptiles, chelonians live the longest, (over 50 years). Larger species like the Galapagos tortoise (*Geochelone nigra*) have been recorded to live for over 150 years. (Bellairs, 1969; Pough et al., 1998, 2002).

Chelonians have a dramatic ability to flex their cervical vertebrae and are classified into two suborders according to their ability to move the head into their shell. The Pleurodira or side neck turtles have three main points of neck flexure so that the neck forms a "s" shape. They are unable to retract their head inside their shell, instead placing it sideways. The Cryptodira or hidden-neck turtles have two points of neck flexure that form a horizontal "s" shape bend, allowing the head to be completely withdrawn inside the shell. The Cryptodira includes the majority of chelonian species (11 families). Some species such as the snapper turtle and sea turtles have lost the ability to hide their head in their shell (Bellairs, 1969; Hoffstetter & Gasc, 1970; King, 1996).

Boyer and Boyer (2006) mentioned that the true tortoises, testudinidae, consist of 14 extant families, 12 genera with 46 living species found throughout the tropic, subtropic, and temperate zones of the world. The Egyptian Tortoise is one of the least known Mediterranean tortoises in its natural history and captive maintenance. Breeding outside its natural zone of distribution

has rarely been recorded. It is known as the "Egyptian Dwarf tortoise" because its very small size.

*Testudo kleinmanni* can usually be found in semi-desert and coastal regions of the Near and Middle east. And is traditionally found throughout the arid deserts around the Mediterranean Sea, extending from Israel to Libya.

This study is designed to identify, describe and illustrate the topography of the respiratory organs of the Egyptian tortoise (*Testudo kleinmanni*) using a digital camera and computed tomography. This may aid in obtaining complete data on dissection procedures, the topography and the initial section outlines and the safety of access in surgical procedures. The core of the present study is a series of photographs showing stages of the dissection. A detailed description of the dissection method and anatomy of the exposed organs are also given.

## Materials and Methods

### **1-Animals used in the study**

Thirteen wild adult live tortoises, *Testudo kleinmanni*, (three males and ten females) were collected from Marsa Matroh and Cairo governorates with an age ranging from 15-19 years, weight from 430–450 gm, and carapace and plastron lengths 10.5-11cm and 9.5-9.8 cm respectively. Computed tomography was used to provide detailed information on the respiratory sys-

tem, the location of the coelomic structures with respect to the carapace (the upper shell) and the vertebrae, and to compare cross-sectional anatomic specimens that were obtained by using the digital camera to images obtained via computed tomography sections of the coelomic structures.

The sex of the animals in this study was determined by the tail length which is longer in males than females, the presence of a vulval opening in females, and the total body length of females, which is longer than in males.

## **2- Anesthetic Technique**

Each animal was immobilized with succinylcholine (0.8 mg.kg<sup>-1</sup>) and euthanized by intracardiac overdose of sodium pentobarbitone. (Barboza, 1995) For CT examination, three adult live females were anaesthetized with a combination of ketamine (15 mg/kg) and diazepam (0.5 mg/kg) injected intra-muscularly in both the fore and hind limbs. It required about 30 min for complete anesthesia after injection (Valente et al., 2006).

## **3- Instruments and fixatives used**

Digital camera, titanium saw, scalpel blades, handles, blunt probes, toothed forceps, un-toothed forceps, scissors, 80% ethanol, and 10% neutral buffered formalin solution.

## **4- Anatomical consideration**

The dissection was begun by removing the plastron (the upper shell) using the saw and making a cut through the skin of the neck. This was then extended

laterally, and the cut continued around the axillary regions near the plastron until reaching the pelvic limbs. Care should be taken to avoid cutting into the body cavity because the skin and muscles near the hind limbs are thin, so the cut should follow the plastron's caudal margin.

By removing the plastron, the acromion processes and the long triangular coracoid processes on each side of the body became clear. Then we rotated the acromion and coracoid cranially to separate the muscles from the peritoneum. After removing the shoulder girdle we exposed the viscera by breaking the attachments of the scapula to the carapace. Then we cut the shoulder muscles to free it from its attachment to the shell and neck.

## **5-Computer-assisted radial tomography Three-dimensional reconstruction**

The CT was performed on three live tortoise females. No intravenously or orally administration contrast material was used, and all tortoises were kept in ventral recumbency for the examination. The live *Testudo* were carefully kept wet prior to the scan and anaesthetized with 0.5 mg/kg diazepam injected intra-muscularly into both fore and hind limbs. Complete anesthesia was achieved after 30 minutes after injection (Valente, et al. 2006b).

Settings for the computed tomography (CT) image technique were as follows: 120 kVp, 200MA. Image acquisition time was approximately 30 sec. The field of view was 12cm long, 7cm wide.

In volume rendering with no cuts the settings were: KV 120, mA N/A Rot 1.50s/HE 8.4mm/rot 7.0mm/2.0sp W=508 L=51.

In volume rendering no cut showing Air structures the settings were W=456 L=787. For coronal and sagittal CT reconstruction the setting were W=119 L=949.

One millimeter thick contiguous views were obtained using a bone setting. Window width = 3.500 Hounsfield units; window level=500 Hounsfield units). The soft tissue settings were: window width = 400 Hounsfield units; window level=66 Hounsfield units.

Computed tomography of the coelomic structures was obtained by a multi - detector row CT scanner (Asteion, Toshiba Medical, Japan) using the following parameters: 120 kVp, 200 mA, 16·1 mm detector configuration and a 512 × 512 matrix. The field of view ranged from 12 - 14 cm and total examination time was from 10 - 15 s. volumetric reconstruction of image sections with a 1 mm slice width and interval of 0.8 mm was performed. Although the original MDCT sections were taken using 1 mm thickness, better images were obtained after manipulation and adjusting to sections of 4 mm. Multiplanar reformatted images and 3D volume-rendered images were generated on a Vitrea computer workstation (Vitrea version 3.0.1., Vital Images). We used bone and parenchymal filters to improve the contrast between the different structures.

The terminology applied to the anatomical structures corresponded to that of the Electronic Nomina Anatomica Veterinaria (2005) whenever possible; in addition, some terms were reviewed by Wyneken (2001).

## Results

The pulmonary system is composed of the glottis, trachea, bronchus to each lung and the right & left lung (fig 1).

### *Glottis*

The glottis is located at the base of the tongue in the rostral middle portion of the oropharynx. It leads directly into the trachea.

### *Trachea*

The trachea consists of sixteen complete cartilaginous rings. It is flexible and bifurcates quickly into a pair of primary bronchi. Each bronchus consists of extra and intrapulmonary parts. The trachea passes ventral to the esophagus as a single structure from the level of the second to the fourth cervical vertebrae where it bifurcates (fig 2).

The extra-pulmonary part of the bronchus consists of 45 rings and is situated external to the pulmonary parenchyma. The entrance of the bronchi into the corresponding lungs was identified as two circular areas at the level of the 1<sup>st</sup> dorsal vertebra.

### *Bronchi*

The bronchus is un-branched, and there is neither bronchial tree nor alveoli. The lung parenchyma is organized into thin-walled trabeculae that sur-

round niches. The niches contain shallow open box-like acini or foveolar spaces which are deeper than they are wide, (Fig 3).

### **Lungs:**

They are large, multi-chambered structures extending caudally from the level of the nuchal region for about 6.5cm - 8.5 cm (Fig 2,3). They are located dorsally in the coelomic cavity and attached to the carapace and vertebral column via the pulmonary ligament. Ventrally, the left lung is attached to the stomach via the gastro-pulmonary ligament, while the right lung is attached to the right lobe of the liver via hepatopulmonary ligament. Caudally, the lung is covered by the peritoneum that overlies the kidney and adrenal glands and is adjacent to the gonads; the medial border of each lung is firmly attached by fibrous connections to the dorso-lateral surfaces of the vertebral column. The lungs develop dorsal and dorso-lateral dilatations.

In tortoises, the diaphragm is absent.

### **Computed tomography and 3D reconstruction**

Only the soft tissue window was used to capture the images for this study. The craniocaudal survey of the adult tortoise was done in the ventral recumbency position. The CT images were obtained in transverse, dorsal, sagittal and ventral planes (Scout image, fig 4). The morphology of the lungs, bronchi and pulmonary blood vessels could be distinguished. The central intrapulmonary bronchus was clearly seen in the transverse sections. Each central

bronchus extended dorsally and longitudinally into the lung, and had numerous airways extending from it. The lungs were not lobed. The lung field in the transverse plane was in the range of 0.34 mm to 0.100 mm; in the ventral plane 0.4 mm to 0.75mm; in the sagittal plane and paramedian plane from 0.4 mm to 0.86 mm. The lung appears black due to its nature of being filled with air (Figs 5- 19).

In three dimensional reconstruction the lung appears very large occupying approximately 75 % of the carapace length and the two primary bronchi appear while entering the lung tissue (Fig 20, 21).

### **Trachea and esophagus**

The trachea and esophagus were easily recognizable. The trachea was observed ventral and to the right of the esophagus and maintained this position from the level of the second to the fourth cervical vertebra (Fig 20, 21). Multiplanar CT reconstructions, in different planes of scan (soft tissue window) of the body showed division of the trachea into two primary bronchi, one for each lung. The tracheal bifurcation was seen at the level of the 2nd to 4th cervical vertebrae. The extra and intrapulmonary parts of the bronchi were identified clearly. The external part of the left bronchus crossed ventrally to the esophagus. The central intrapulmonary bronchus was clearly seen in the transverse sections. Each central bronchus extended dorsally and

longitudinally into the lung (**Fig 16-19**).

The trachea appears very clear in transverse section from 0.18mm to 0.27 mm and then rapidly bifurcate into two primary bronchi from the cut 0.29 mm to 0.55 mm.

## Discussion

The results of this study agrees with those of Wyneken (2001), who stated that the respiratory system of the tortoise is composed of the glottis, trachea, a bronchus to each lung and the right & left lungs. The airway begins at the glottis, which is located at base of the tongue in the rostro- middle portion of the oropharynx that leads directly into the trachea. The glottis and its muscles are supported by the hyoid apparatus. In general, *Testudo kleinmanni* is similar to that described by Harris (1992) in *Testudo*, Perry (1989) in terrestrial tortoises and Gans & Hughes (1967) in *Testudo graeca*.

Christopher and Hernandez-Divers (2003) and Valente, et. al., (2007) in *Caretta caretta* mentioned that the trachea has complete cartilaginous rings. It is flexible and bifurcates into a pair of primary bronchi between the mid-cervical region and level of the heart, depending on the species. Each bronchus then curves laterally and enters the lungs. The central bronchus extends throughout the length of the lungs. To the contrary, in the spe-

cies *Testudo kleinmanni* studied here the trachea is very short, extending only to the level of the second to the fourth cervical vertebrae. This may be related to the fact that in the *Testudo* the neck vertebrae flex vertically, allowing the head to be drawn straight back within the shell. Moreover, in this species the most cranial position of the carina allows breathing even when the head and neck are withdrawn. (Pecor, 2003).

Murray (1996) in terrestrial tortoises and Perry (1989) in *Testudo* reported that the medial border of each lung is firmly attached via fibrous connections to the dorsolateral surfaces of the vertebral column. And this agrees with our results in *Testudo kleinmanni*.

Christopher and Hernandez-Divers (2003) in chelonian and Dunckers (1978, 1979, and 2004) in terrestrial tortoises denoted that the lungs are large, multi-chambered structures with many septa and that they are rich in parenchyma. Chelonian lungs are structured differently than the mammalian lungs. The bronchus is unbranched, so there is no bronchial tree. There are no alveoli. In the *Testudo kleinmanni* of this study, the parenchyma was less developed than that in the sea turtle, a finding which is supported by Gans (1969) in the snapping turtle *Chelydra serpentina*, Perry (1998) in *Testudo* and Mosley (2005) in *Testudo*.

*Testudo* is characterized by the ability to retract the head and legs into the shell which possesses a high and curved carapace. The constructional prerequisite is the available volume within the carapace, which became easily disposable by the development of lateral and ventral non-respiratory lung dilatations. Thereby the primarily homogeneously partitioned multi-cameral

Duncker (1978, 1979) has provide a concise discussion of the architectural and structural requirements for a turtle to completely retract its neck:

*Testudo* is characterized by the ability to retract the head and legs into the shell, which possesses a higher, curved carapace. The constructional prerequisite is the available volume within the carapace, which became easily disposable by the development of lateral and ventral non-respiratory lung dilatations.

Thereby the primarily homogeneously partitioned multi-cameral lung became heterogeneously partitioned into densely subdivided respiratory portions and large dilatations lacking respiratory functions, All the lung dilatations facilitated the construction of a high curvature of the carapace, which enables tortoises, to roll back onto their feet after falling on their back, which is important for survival on land .This description is strongly supported by the results of this study .

We have verified that multi detected computed tomography (MDCT) is a useful tool to identify the position of the esophagus and its relationship with the trachea and bronchi. This could help clinicians to know the actual relation of the organs to each other.

One of the most important clinical contributions of CT examination is to describe the relation of the pulmonary system to other organs. Valente, et. al. (2007) explained in a study applying CT to sea turtles that each central bronchus extended dorsally and longitudinally into the lung and had numerous airways extending from it. The lungs were not lobed, and the pulmonary parenchyma was strongly reticulated. These finding agreed with ours in *Testudo kleinmanni*.

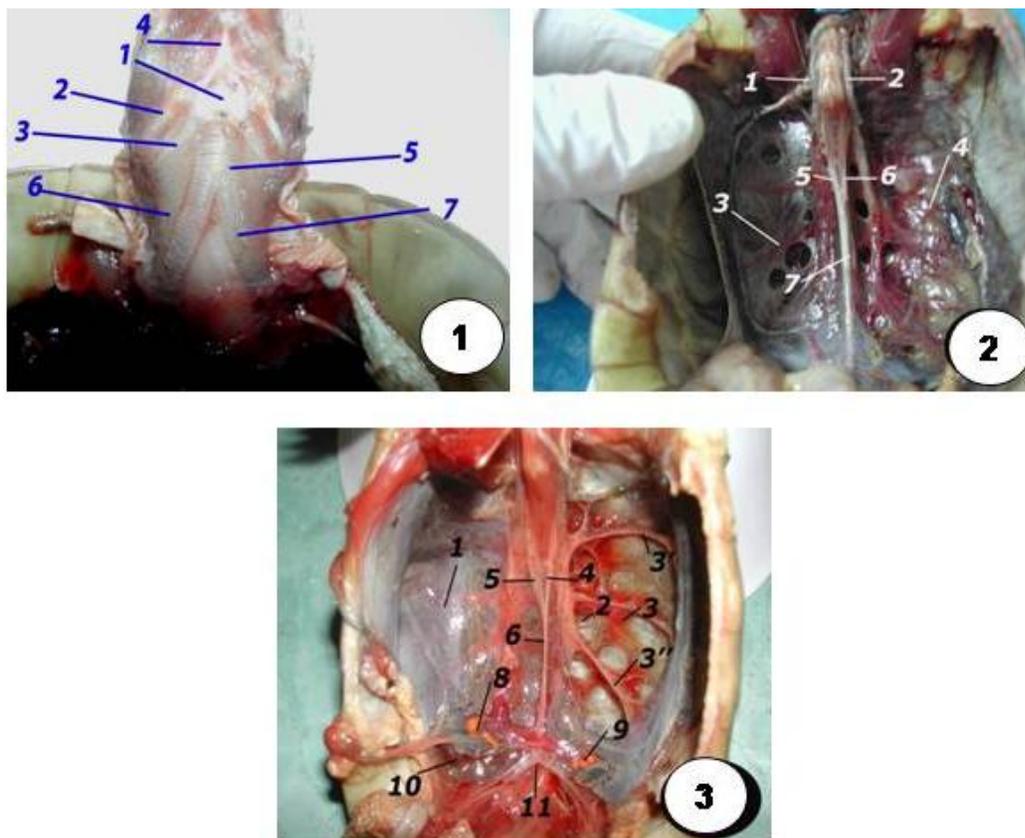
The general morphology of the respiratory tract of *Testudo kleinmanni* of this work and the position of the carina appears to be slightly more cranial than in the sea turtle in which the trachea bifurcates after coursing a relatively short distance down the neck (Murray, 2006). The difference in the position of carina in this case seems to be related to the morpho-functional characteristic of the neck observed in different groups of the Testudines.

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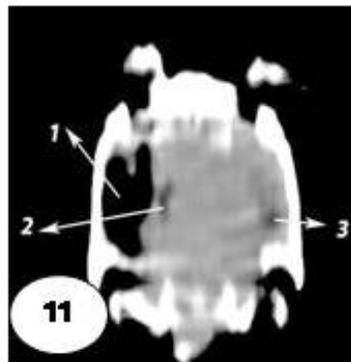
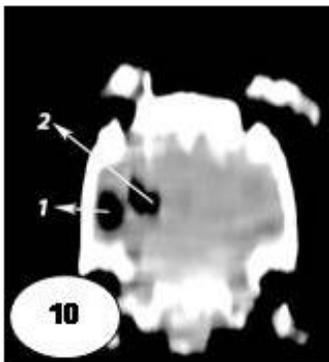
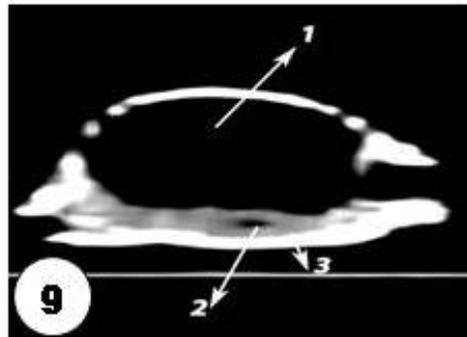
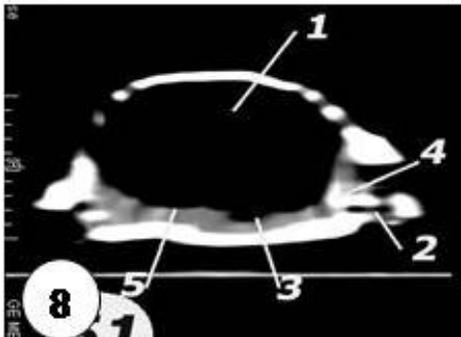
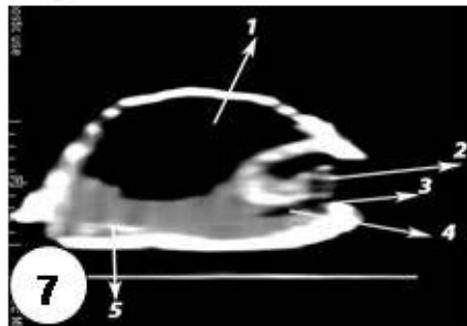
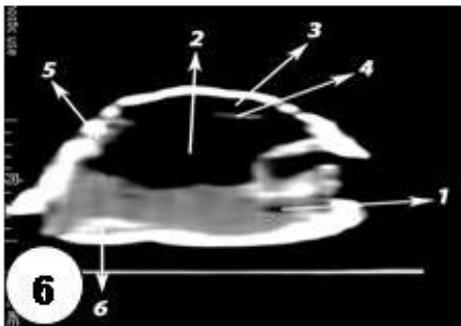
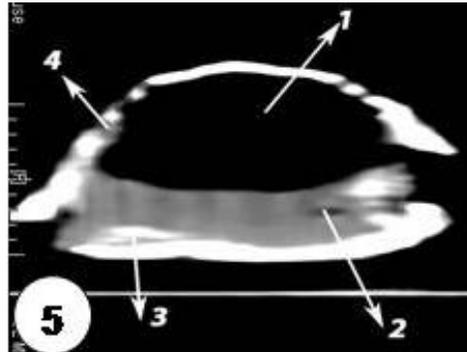
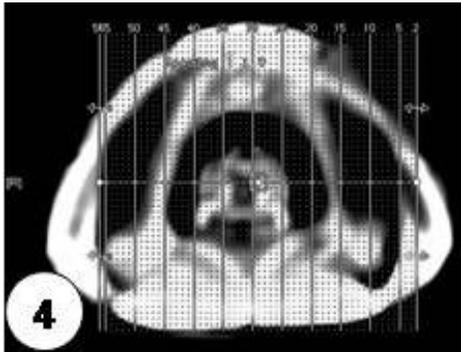
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**Fig (1):** Ventral view of the neck region of the *Testudo kleinmanni* showing: 1 Hyoid (cartilage and bone), 2 Hyoid horn (largely bony), 3 Hyoid horn (cartilage), 4 Lingual process (cartilage), 5 Trachea, 6 Esophagus, 7 Left bronchus.

**Fig (2):** Ventral view of the *Testudo kleinmanni* lung after removal of all visceral organs to expose the lung; 1 Right bronchus, 2 Left bronchus, 3 Right lung, 4 Left lung, 5 Right aorta, 6 Left aorta, 7 Dorsal aorta .

**Fig (3):** Ventral view of the *Testudo's* coelomic cavity after removal of the digestive and genital systems, showing: 1 Right lung covered with pleura, 2 Left lung free from pleura, 3, 3", and 3" lung non-respiratory dilation, 4 Left aorta, 5 Right aorta, 6 Dorsal aorta, 7 Costal artery, 8 Right adrenal gland, 9 Left adrenal gland, 10 Right kidney, 11 distal branch of dorsal aorta.



**Fig (4):** Craniocaudal view of the adult *Testudo kleinmanni* in ventral recumbency describing different planes at which CT images were obtained as transverse, dorsal, sagittal, and ventral plane (Scout image).

**Fig (5):** Multiplanar CT reconstructions, Paramedian sagittal scan at **0.27 mm** (soft tissue window) of the body showing: 1 Lung, 2 primary bronchus, 3 Pubic bone, 4 Kidney.

**Fig (6):** Multiplanar CT reconstructions, Paramedian sagittal scan at **0.29 mm** (soft tissue window) of the body showing the vertebral column (dorsal vertebrae) between the two lung fields 1 Primary bronchus, 2 Right lung, 3 left lung, 4 dorsal vertebrae (thoracic), 5 kidney, 6 Pubic bone.

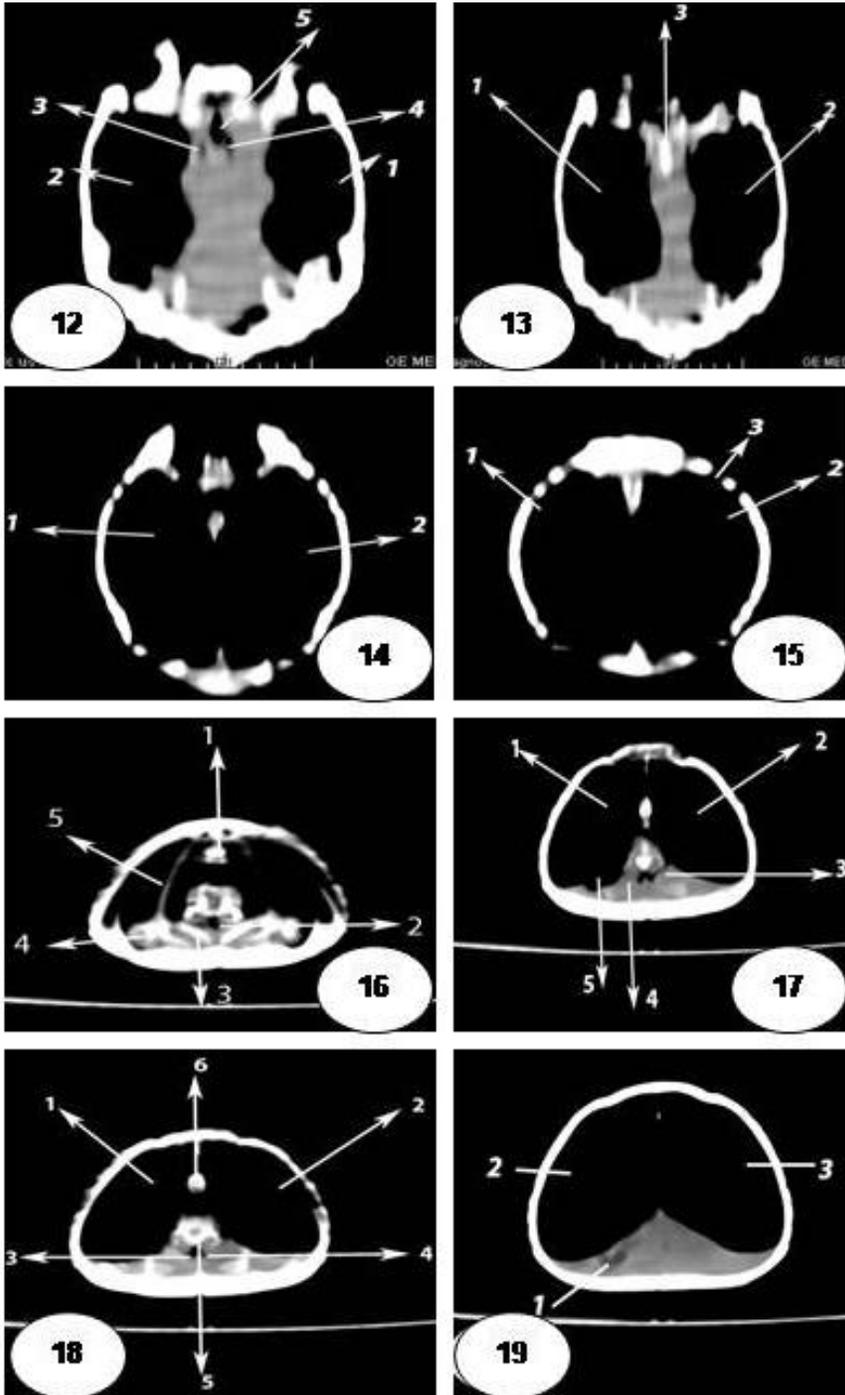
**Fig (7):** Multiplanar CT reconstructions, sagittal scan **0.32 mm** (soft tissue window) of the body showing: 1 lung, 2 Head, 3 Trachea, 4 Bifurcation into two primary bronchi, 5 Pubic bone.

**Fig (8):** Multiplanar CT reconstructions, Paramedian sagittal scan at **0.43 mm** (soft tissue window) of the body showing: 1 Lung, 2 Stomach, 3 Gall bladder.

**Fig (9):** Multiplanar CT reconstructions, Paramedian sagittal scan at **0.47 mm** (soft tissue window) of the body showing: 1 Lung, 2 Trachea, 3 Stomach, 4 First cervical vertebrae, 5 Outer contour of urinary bladder.

**Fig (10):** Multiplanar CT reconstruction at **0.11 mm** (ventral plane – soft tissue window), showing the stomach and it the lung just below it **1 lung, 2 Stomach**

**Fig (11):** Multiplanar CT reconstruction at **0.13 mm** (ventral plane – soft tissue window), showing: 1 Lung, 2 beginning of duodenum, 3 Gall bladder.



**Fig (12):** Multiplanar CT` reconstruction at **0.18 mm** (ventral plane – soft tissue window), showing: 1 Right lung, 2 Left lung, 3 Left primary bronchus, 4 Right primary bronchus, 5 Trachea.

**Fig (13):** Multiplanar CT` reconstruction at **0.21 mm** (ventral plane – soft tissue window), showing the lung field with vertebral column 1 left lung, 2 Right lung, 3 Vertebral column (cervical vertebrae) .

**Fig (14):** Multiplanar CT` reconstruction at **0.32 mm** (ventral plane – soft tissue window), showing the lung field that completely occupy the whole dorsal aspect with vertebral column, 1 Left lung, 2 Right lung.

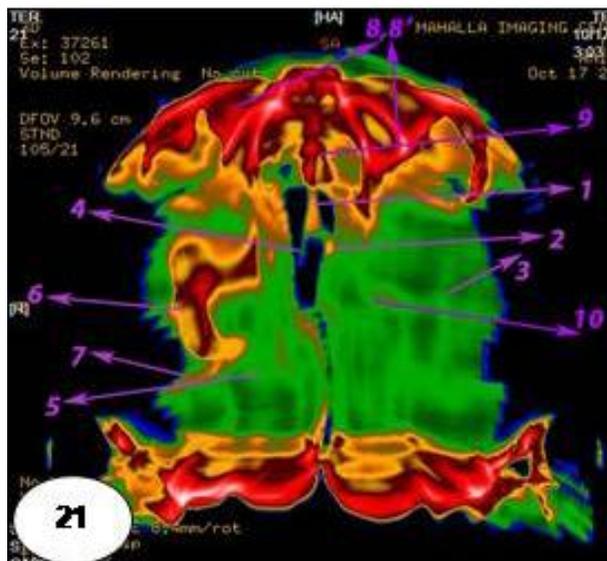
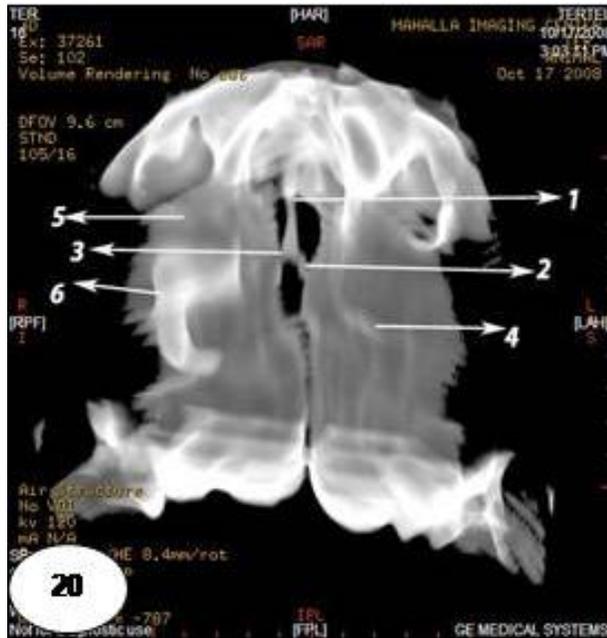
**Fig (15):** Multiplanar CT` reconstruction at **0.42 mm** (ventral plane – soft tissue window), showing the extended lung field, 1 Left lung, 2 Right lung, 3 Hole within the bony structure of carapace .

**Fig (16):** Computed tomography image, at **0.18 mm** (transverse plane – soft-tissue window), showing the trachea and the esophagus (the tortoise head in the picture is retracted to inside the shell) 1 1<sup>st</sup>dorsal vertebrae (thoracic), 2 Trachea, 3 Esophagus, 4 Head of the humerus, 5 Scapula.

**Fig (17):** Computed tomography image, at **0.21 mm** (transverse plane – soft-tissue window), showing the lung field with two primary bronchus 1 Left lung, 2 Right lung, 3 Left primary bronchus, 4 Right primary bronchus, 5 Cervical vertebrae, 6 Dorsal vertebrae.

**Fig (18):** Computed tomography image, at **0.21 mm** (transverse plane – soft-tissue window), showing the lung field with two primary bronchus. 1 Left lung, 2 Right lung, 3 Left primary bronchus, 4 Right primary bronchus, 5 Cervical vertebrae, 6 Dorsal vertebrae.

**Fig (19):** Computed tomography image, at **0.45mm** (transverse plane – soft-tissue window), showing the duodenum, 1 Duodenum, 2 Left lung, 3 Right lung.



**Fig (20)** – 3-D reconstruction (soft tissue window) in ventral recumbency show the extension of the lung field 1 Trachea, 2 Right primary bronchus, 3 Left primary bronchus, 4 Right lung, 5 Left lung, 6 Stomach.

**Fig (21)** – 3-D reconstruction (soft tissue window) of adult *Testudo kleinmanni* female in ventral recumbency, 1 Trachea, 2 Right primary bronchus, 3 Right lung, 4 Left primary bronchus, 5 Left lung 6 Stomach, 7 Duodenum, 8,8' Shoulder girdle, 9 Cervical vertebrae.