IJVS 2020; 15(1); Serial No: 32; Pages: 70-77

Iranian Veterinary Surgery Association

IRANIAN JOURNAL OF VETERINARY SURGERY

Journal homepage: www.ivsajournals.com

ORIGINAL ARTICLE

Computed Tomographic Anatomy of the Lungs, Bronchi and Trachea in the Caspian Pond Turtle (*Mauremys caspica*)

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Received: 5 February 2020
Accepted: 7 April 2020
Online: 7 April 2020

Keywords:

CT Anatomy;
Lungs, Bronchi;
Trachea;
Caspian pond turtle.

Abstract

Objective- The intention of this research is studying the full topographic characteristics of the lower respiratory tract consist of lungs, bronchi, and trachea in Caspian pond tortoise, especially those features that are applicable in clinical examination, and their proximity to other organs.
Design- Experimental study.
Animals- Six female adult Caspian Pont turtles (*Mauremys Caspica*)
Procedures- Siemens Somatom spirit Dual Slice computed tomography (CT) scan machine has been used. The samples were fixed in ventral recumbency to take CT-scan images. In each sample, the CT-scan was done in both fixed limb and neck and extended form. Some morphometric parameters like the volume of the coelomic cavity, right and left lungs, and others were measured through the Syngo MMWP VE40A software.
Results- Trachea passing a short distance in the midline of the neck, and deviated to the left; then, it entered to the coelomic cavity. The tracheal bifurcation site was movable and move to caudal parts with neck flexion. The entrance site of the bronchi to the lungs was fixed. With the consideration of coelomic cavity volume and various parts of that in the neck extended position, the lungs consist of 42.12% of the coelomic cavity and 22.58% in the flexed neck position.
Conclusion and Clinical Relevance- According to the observations, we can say the flexion of the neck leads to some alterations in the tracheal bifurcation site and locating posteriorly to the lungs hilum. As a result, the way of the bronchus changes to inter the lungs. The branches of bronchus in these turtles also are like other reptiles, and it's not similar to a bronchial tree and it contains just limited small branches. The CT-scan is applicable for alive animals, so this is one of the best choices for anatomical studies in animals who are in danger of extinction.

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DOI: 10.30500/ivsa.2020.218718.1210

1. Introduction

Chelonia is an order of reptiles who have carapace. Turtle, tortoise, and terrapin have all been used for various members of this order. The term tortoise uses for terrestrial species that live on the land. Terrapins are kind of amphibians who associated with freshwater, and turtle term is exclusively belongs to species who live in the sea.[1-3]

Scientists did some studies on the anatomy of the turtles. In 2006 Valente *et al*. evaluated the radiographic images of the abdominal cavity and cervical region of the Loggerhead sea turtle. In this study, the radiographic images captured from the dorsoventral view from immature tortoise, and 15 near-adult, that 17 of them were alive, and 13 of them were euthanize. In this study, no sedative drug used.[4] In 2007, Valente *et al*. assessed the computed tomography (CT) scan of the spine and coelomic cavity of sea turtles. In this study, the CT-scan of 12 case of dead and alive tortoise investigated. Moreover, in this study, to limit head and limb movement, Ketamine and diazepam were used. After the CT-scan, the samples fixed with 10% formalin.[5] Saber in 2010 illustrated the anatomical characteristics of the respiratory system in Egyptian tortoise. In this study, they utilize CT-scan, 3D reconstruction, and other prevailing autopsy techniques. In their results, they pointed out the zone of tracheal bifurcation, the entrance of bronchus to the lungs, and the position of the parenchyma of lungs.[6] In 2013, Mans *et al*. studied the impacts of positioning and the extension of the neck on the volume of lungs in CT-scan of Red-eared slide.[7] In 2013, Lim *et al*. evaluated the appearance of lung fibrosis in a kind of 20 years old female Leopard tortoise with symptoms of difficulty breathing, unusual breathing sound, anorexia, and depression, and reported this case through CT-scan method.[8] CT-scan was used in anatomical studies in some animals like sturgeons and turtles.[9-11] Zehtabvar *et al*. in 2014 studied anatomical and CT-scan features of the respiratory system in European pond turtles, and they pointed out some specific points in this study. They mentioned some differences in tracheal length and tracheal bifurcation in another species. Moreover, they discussed the lung's topographic features in two different neck positions with an extended and flexed position. In this study, the general view of the distal site of lungs assessed with CT-scan and gross anatomical methods, and they gathered valuable differences with other species.[12] Asadi Ahranjani *et al*., in 2015, applied three methods at the same time, consists of anatomical methods, radiography, and CT-scan in four Euphrates softshell turtle (*Rafetus euphraticus*) cases natural skeletal system and natural anatomical features investigated. In this study, they could reconstruct the natural skeletal system of this kind of turtles by 3D imaging CT-scan technique.[13]

The ever-increasing importance of turtles as a pet animal, and their numerous presence of them in wildlife, the number of cases that come to clinics for an analysis of health situation, and clinical examinations of this animals have been increased dramatically. But unfortunately, the anatomical constructions of the turtles and the movement restriction of limbs in their shell, the comprehensive examination of them in clinics, are arduous. Thus, clinical technicians have to access reliable references to reach a better knowledge of their anatomical features. The respiratory system is one of the most critical organs in the body, and the correlated diseases are common in them, especially in pet turtles. As a result, because of the complicated structure of shell in them, the exact examination of them is hard and related to another paraclinical methods like diagnostic imaging by which all can access a rational image of a general anatomical structure of live respiratory system.

Caspian pond turtles (*Mauremys caspica*) is one of the noteworthy species in Iran and the word, although there are limited pieces of evidence about anatomical features in exotic animals through radiographic studies. *Mauremys caspica* belongs to the *Geoemydide* family. Carapace length of this species leads to 23 cm, and the color of this structure is olive green or olive-brown. Caspian tortoise frequently habitats in Iran are north, west and southwest provinces, including Gilan, Mazandaran, east and west Azarbayjan, Kordestan, Kermanshah, Ilam, Khouzestan, Lorestan, and Fars.[14]

According to the similar studies in species like European pond turtle and the applying of CT-scan method for anatomical studies and assessing the results of these studies, CT-scan has been chosen as the best way for anatomical studies; therefore, in this study, we tried to trace the respiratory system of this species through CT-scan method. In this method, the organs studied while they are working and the animal is alive, and after the examination, they have returned to their habitat. The intention of this research is studying the full topographic characteristics of the lower respiratory tract consist of lungs, bronchi and trachea in Caspian pond turtles, especially those features that are applicable in clinical examination, and their proximity to another organs. It seems that this technique can provide us numerous valuable knowledge about the typical situation of the lower respiratory system in Caspian pond tortoise. Also, there would be lots of diversity between various species, and this study could play an essential role as a reference for other research. Moreover, it could be utilized for therapy goals.

2. Materials and Methods

Samples

Six female adult Caspian Pont turtles (*Mauremys caspica*) with the weight 675 ± 3.22 g and length of 23 ± 1.42 cm studied. The samples kept in proper condition for one week to be accustomed to the environment and came back to the general situation. In this phase, Black Sea sprat fish (*Clupeonella cultriventris*) has been used for feeding target to the samples.

CT Study

Siemens Somatom spirit Dual Slice CT scan machine has been used. After the transfer of each sample to the radiology department at the small animal hospital (Faculty of Veterinary Medicine, University of Tehran), the samples were anesthetized by injection of ketamine (10%, Alfasan 25 mg/kg, intramuscular), and diazepam (10 mg / 2 ml Caspian, 1 mg/kg, intramuscular).[15] The samples were fixed in ventral recumbency to take CT-scan images.[16,17] In each sample, the CT-scan was done in both fixed limb and neck and extended form. Turtles were fixed with leucoplast sticks for this position. Specific CT-scan factors were used to study each part, which was the appropriate width (WW) and window level (WL) for each region, and in the result section, pointed out. The factors were rotation time, 0.8 s; slice thickness, 1 mm; reconstruction interval, 0.5–1 mm; pitch, 1.5; X-ray tube potential, 130 kV; and X-ray tube current, 100 mA.[11,12]

Lung and bones window was used to scrutinize the main points. For the 3D reconstruction of images, various patterns were used, and different CT-scan images took which was mentioned in the result part.

Morphometric Study

The volume of the coelomic cavity, right and left lungs, and also the length of the trachea and right and left bronchi, height, and width of the right and left lung was measured through the syngo MMWP VE40A software. The measured parameters were analyzed with SPSS 16 and sample t-test ($p < 0.05$); moreover, the volume of right and left lung both in extending and flexing position were measured.

3. Results

CT Study: Extended Head and Neck Position

Trachea and bronchus. After deliberation of images, we concluded that the trachea started from glottis at the central line and ventral of the pharynx, and it extended to the ventral. Trachea passing a short distance, and deviated to the left; then, it entered to the coelomic cavity. On the left side, the trachea located between second and third ribs, and in the middle of coracoid bone, it divides into two branches, which is left and right bronchi (Figure 1).

The right bronchus from the division site moves caudoventrally from the ventral of the neck until it reaches the right side of the body. Finally, it enters the right lung caudodorsally in the space between the third and fourth ribs (Figure 1). The left bronchus in the division site move caudodorsally, and in the area between third and fourth ribs, it enters to the left lung (Figure 2). The entrance site of the left bronchus is located more caudally than the right one.

Lungs. Lungs consist of two dorsal and ventral surfaces. The ventral surface is in the proximity of viscera, and the dorsal has located in the vicinity of the carapace. In general, each lung consists of four interrelated and interconnected spaces. In the evaluations of the lungs, we concluded that the cranial part consists of more parenchyma, and the caudal region, which was the fourth space, had a more significant lumen. In the cranial part, an apex of the lung also observed (Figure 2).

The lungs had three borders or edges consist of lateral, medial, and cranial. The cranial side located behind the pectoral zone and in the joint site of the scapula, coracoid, and acromion. In other words, in front of the first rib begins and moves toward caudal to the sixth rib. The caudal part of lungs was not narrowed down. The caudal segment of lungs based on the amount of right and left hind limb twitch was variable and different. In all samples, the lungs were ended before the sixth rib. The apex of the cranial part also located between the medial and cranial edge and deviated toward the ventral. It situated in the proximity of the first trunk vertebra in the medial surface of the scapula (Figure 1). The lateral side of the lungs was somewhat concave (Figure 2).

In each lung, there was a central main bronchus. The internal structure of the lungs is plexus, which is divided into limited parts through uncompleted walls (Figure 2). The way of division of bronchus was not the same in different samples. The main central bronchus was continued to the end and the caudal part of the lungs (the fourth part), and in this pathway, three branches stemmed from that for other sections (Figure 2).

CT Study: Flexed Head and Neck

Trachea and bronchus. The study of CT-scan pictures in flexed head and neck position proved that the trachea move from the middle and ventral site toward the left side and locate in left and lateral side of the neck and at space of fourth and fifth ribs it divided into left and right bronchi. The entrance location of bronchus to the lungs is fixed, and it is just like the extended position, the left bronchus enters to the left lung craniodorsally, and right bronchus enter to

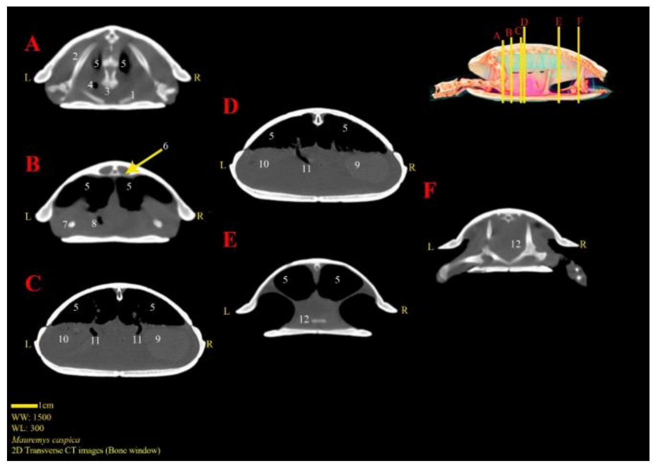

Figure 1. Transverse CT-scan images (bone window) of Caspian pond turtle in extended head and neck position. In 3D image the section sites has been marked. 1. Clavicle, 2. Scapula, 3. Cervical vertebrae, 4. Trachea, 5. Lung, 6. Caudal border of the 2nd rib, 7. Coracoid bone, 8. Tracheal bifurcation, 9. Liver, 10. Stomach, 11. Bronchus, 12. Pelvic girdle.

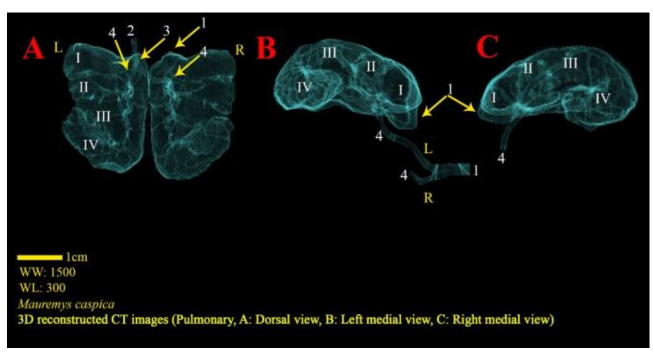

Figure 2. 3D reconstructed CT images of the lung with pulmonary pattern, Caspian pond turtle in extended neck and limb position, the four parts of the lung marked by roman numbers. 1. Apex of the lung, 2. Trachea, 3. Tracheal bifurcation, 4. Bronchus.

the right lung cranioventrally, and it passes under the neck and moves craniodorsally (Figure 3).

Lungs. The flexion of the head and neck led to the contraction of the lungs, and changes emerged in the edges of the lungs. Therefore, the cranial part of the medial side of the lungs located apart from each other. The apex generally eliminated, and the lateral edges got bulgier. The lungs began precisely after the first rib and continued a bit after the sixth rib (Figure 3). The caudal part of the lungs, in comparison to the extended position, was compacted. (Figure 4) In this state, four parts of the lungs were less distinguishable. The height of the lungs decreased in this position. (Figure 5).

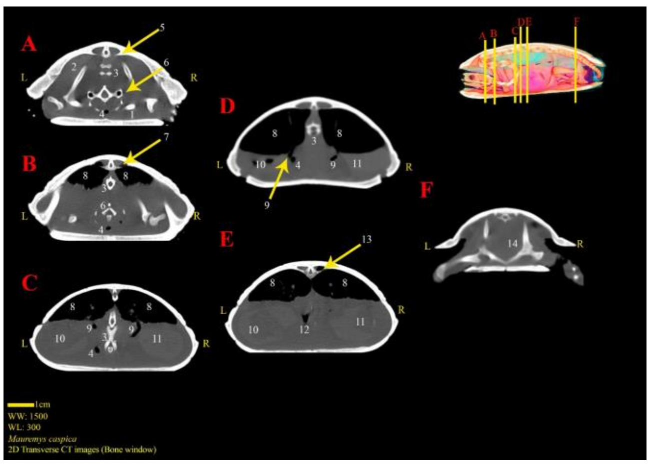

Figure 3. Transverse CT-scan images (bone window), Caspian pond turtle in flexed head and neck positioning, in 3D image the section site has been marked. 1. Clavicle, 2. Scapula, 3. Cervical vertebrae, 4. Trachea, 5. 1st rib, 6. Skull, 7. Caudal border of the 2nd rib, 8. Lung, 9. Bronchus, 10. Stomach, 11. Liver, 12. Tracheal bifurcation, 13. 4th rib, 14. Pelvic girdle.

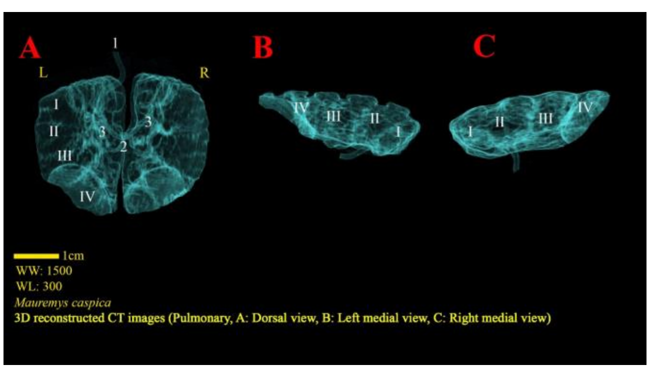

Figure 4. 3D reconstructed CT images of the lung with pulmonary pattern, Caspian pond turtle with flexed head and limb positioning, four parts of the lung has been marked with roman numbers. 1. Trachea, 2. Tracheal bifurcation, 3. Bronchus.

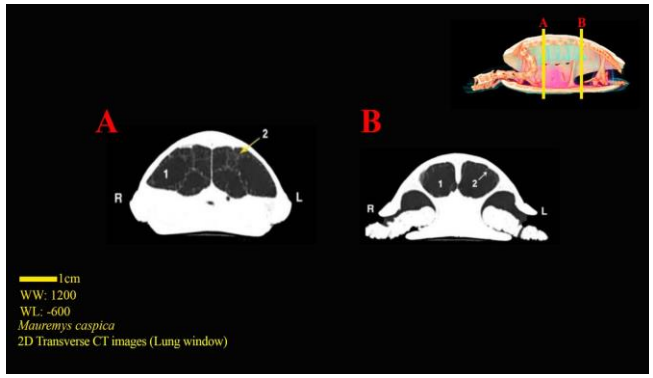

Figure 5. Transversal CT-scan images (lung window) in Caspian pond tortoise, in 3D image the section sites has been marked. 1. Lung lumen, 2. Lung parenchyma.

Morphologic Study

The difference of length in the right and left lung in an extended position was not meaningful, and the lungs have the same size ($p > 0.05$) (Table 1). The difference in the length of lungs in the flexed position and extend position was not meaningful in statistics. As a result, the flexion of the neck has no effects on the length of the lungs.

The difference in width of the right and left lungs in extended position was not meaningful in statistic measurements, and the lungs have the same size. Therefore, the flexion of the neck does not affect the width of the lungs ($p > 0.05$). The difference of width in lungs in flexed and extended position has no meaningful statistic size; therefore, the flexion of the neck does not affect the width of the lungs ($p > 0.05$).

The difference in the size of the left and right lungs in the neck extended position was not meaningful in statistics, and the lungs have the same height ($p > 0.05$).

The difference of the size of the lungs in neck flexed position and neck extended position was not meaningful in statistics. Therefore, the flexion of the neck leads to a decrease in the height of the lungs ($p > 0.05$).

The difference in the volume of right and left lungs in extended and flexed positions was not meaningful statistically, and the lungs had the same size ($p > 0.05$).

The difference in the volume of lungs in neck flexed and neck extended position was meaningful statistically. As a result, the flexion of the neck affected the volume of the lungs and led to a decrease of them.

The difference between the left and right bronchi length was meaningful statistically, and the right bronchus were longer ($p < 0.05$).

With the consideration of coelomic cavity volume and various parts of that in the neck extended position, the lungs consist of 42.12% of the coelomic cavity and 22.58% in the flexed neck position.

Table1. Morphometric studies results (units are cm and cm^3)

Parameters	Mean±SD (Left)	Mean±SD (Right)
Length of the lung in the extended neck position	6.31±0.23[a]	6.41±0.41[a]
Length of the lung in flexed neck position	6.48±0.22[a]	6.23±0.31[a]
Width of lung in neck extended position	3.43±0.24[b]	3.49±0.24[b]
Width of the lung in the flexed neck position	3.37±0.21[b]	3.30±0.31[b]
Height of the lung in the extended neck position	1.88±0.33[c]	1.91±0.11[c]
Height of the lung in the flexed neck position	0.94±0.34[d]	0.93±0.31[d]
Volume of the lung in the extended neck position	39.91±0.22[e]	41.85±0.13[e]
Volume of the lung in the flexed neck position	21.31±0.34[f]	22.53±0.44[f]
Bronchi length	1.23±0.21[g]	2.31±0.25[h]
Coelomic cavity volume	194.11±0.71	

The different letters indicate a statistically significant difference between the left and right parameters ($p < 0.05$).

4. Discussion

According to studies on the respiratory system, the overall morphology of the respiratory system in *Mauremys caspica* despite all the similarities, it contains differences. According to existed reports, the most differences are related to the trachea and bronchus positioning.[5,12]

In *Testudo kleinmanni* and *Caretta caretta*, the trachea is located ventral and right of the esophagus.[5,6] According to the references and studies, the trachea in *Cryptodia* is too short; the study of Saber and Saber in 2010 confirmed this topic so that the division site of the trachea in *Testudo kleinmanni* is in the proximity of second to fourth cervical vertebrae. This way of tracheal bifurcation locating leads to a decrease of the pressure on the trachea in the flexed head and neck position and provides the breathing condition.[6,18,19]

In sea turtles, the tracheal bifurcation locates more caudally in comparison with Tortoise. In *Caretta caretta*, the tracheal bifurcation in the proximity of the first trunk vertebrae. Still, according to the impotence of sea turtles in the flexion of head and neck, the caudal location of the tracheal bifurcation site doesn't lead to any breathing problem.[5]

Mauremys capica belongs to *Cryptodira* suborder, has the ability of head and neck flexion. In this study, the trachea has considerable length, it begins from the ventral of the pharynx, and it gradually locates in the left side of the neck, and enters to the coelomic cavity; the division of the trachea happened in the left side in front of the first rib. In *Emys orbicularis*, also the trachea in the coelomic cavity and above of the acromion bone in the left side divide to the left and right branches.[16, 19] The cause of the trachea left gravitation and its entrance to the coelomic cavity provide some adaptation to inhibition of trachea locating between the neck and plastron and finally disruption of breathing when the neck and head flexed in this species. This adaptation is visible in *Emys orbicularis*.[11,12]

The investigation of *Mauremys caspica* images in extension and flexion of neck demonstrate numerous similarities with *Emys orbicularis*. In both species, the entrance site of bronchus to the lungs was fixed, and in the flexion of the neck, the tracheal bifurcation and the path of bronchus to the lungs was changing; the tracheal bifurcation to the caudal of the bronchus entrance to the lungs. Consequently, the bronchus had to move craniodorsally toward the lungs to lead the lungs.[11,12] The coherence between extending and flexing of the neck and the location of the respiratory system evaluated just in *Emys orbicularis* and *Mauremys caspica*, and in the other species of turtles, no studies have been done; also, in sea turtles, this issue is meaningless because of their disability of the neck flexion.[5]

The lungs in *Mauremys caspica,* like other turtles, are even and connected to the carapace. in the ventral of them, a thin membrane and non-muscular is visible, which is named post pulmonary membrane or pseudo diaphragm in references, and it has no active role in breathing action.[1,3]

In summary, a group of muscles plays a role in turtles breathing. The first group is Serratus muscles which are move caudoventrally from the front of the carapace until it leads to the coracoid bone. The second group is abdominal oblique muscles. When these muscles get contracted, the forelimb move outwardly, and the coelomic wall move toward the ventral; as a result, it provides sufficient space for an increase of the lung volume, and the inspiration happens. In expiration, pectoral muscles and transverse abdominal muscles play roles. The contraction of these muscles leads to the return of the forward limb to the shell and move of the wall forward. As a result, the pressure of viscera to the lungs and outflow of the air.[3,19]

According to the mentioned outlines, the post pulmonary membrane has no role in turtles breathing, and it just makes a border between lungs and other visceral members. In Zehtabvar *et al*. 2014 study, this membrane even couldn't separate the lungs from other viscera.[12]

The evaluation of *Mauremys caspica*'s lungs demonstrates that the lung has a plexus structure with a little parenchyma, and in each lung, there is a central bronchus. This feature, besides the absence of a bronchial tree and diaphragm in turtle, leads to resilience and the compaction ability of lungs when the head and neck and limbs are flexed.[7] In references and various studies on *Caretta caretta* and *Testudo kleinmanni*, they mentioned the presence of central bronchus in the lung; but about *Emys orbicularis,* they mentioned that each bronchus contains two dorsal and ventral branches.[5,6] In our study also there was a central bronchus.

There is a report in which they say in limbs and neck flexion time, the volume of lungs decreases to 0.2 of its size.[19] In our study, also by comparison of images in head and neck extended and flexed position we faced the decrease of lungs volume which is related to different causes like such as locating the neck between lungs, the compaction of apex of lungs, their displacement ventrolaterally and also the receding of medial surface of lungs from each other.

Mans *et al*. in 2013 designed a study on *Trachemys scripta elegans*, and they pointed out the impact of physical elements on the volume of lungs, such as the fullness of the digestive system and bladder and the presence of ovulatory follicle before the hatch. The factors, as mentioned earlier, lead to alteration of coelomic cavity pressure, the pressure to the lung, and consequently decrease the volume of that.[7]

According to the observations, we can say that the respiratory system of *Mauremys caspica* is similar to other turtles in the components. The trachea, against the Tortoise and sea turtles and the same as the pond turtle after passing a short path, deviates to the left, and it enters to the coelomic cavity from the left side. The bronchus with much less diameter in comparison with the trachea begins from the proximity of the first trunk vertebrae, and move caudally and inters to the lungs.

The right bronchus concerning the division site in the left side, to inter the right lung paves a longer path in comparison with the left bronchus. The flexion of head and neck lead to some alterations in the tracheal bifurcation and locating posteriorly unto the lungs hilum. As a result, the way of the bronchus changes to inter the lungs. After the entrance of the bronchus to the lungs, they continued in the lungs. The branches of bronchus in these turtles also are like other reptiles, and it's not similar to a bronchial tree. It contains just limited small branches that are used for air circulation to the lung's faveoli.

The lungs located in a space between the shell, pelvic girdle, thoracic girdle, and in the proximity of coelomic viscera. Any change in the head, neck, limb, and inner organs location leads to lungs volume alteration.

CT-scan is an appropriate method for fast evaluation of body structures. This feature cause facilitates the inspection of the skeleton and lungs with numerous details. The CT-scan is applicable for alive animals, so this is one of the best choice for anatomical studies in animals who are in danger of extinction.

Acknowledgment

The authors wish to express their appreciation to everyone that assists us in this study.

Conflict of Interests

None.

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