

ORIGINAL ARTICLE

CT-based 3D reconstruction and basic anatomical analysis of the 3D anatomy of the air sac system in domestic birds

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Abstract

The complex anatomy of the avian respiratory system makes it necessary to broaden our knowledge using modern imaging and reconstructual possibilities. The visualization of these structures can be used for clinical situations, in research or as teaching aids in veterinary education. For this we generated 3D models from diagnostic imaging data (computed tomography [CT] scans) of birds. We describe in detail a repeatable method of animal preparation for scanning, data handling and image analysis. CT scans with varying slice thickness and resolution were obtained in prone and supine body positions to analyse air sac morphology and volume changes relative to posture or sexual dimorphism in birds. The resulting data were prepared and analysed using a reconstructual software (3D Slicer) based on manual and semi-automatic labelling and subsequent 3D models of the air sac system were created. The terminology employed has been referenced from the *Nomina Anatomica Avium, Second Ed.*

KEYWORDS

3D slicer, air sac 3D reconstruction, air sac morphology, air sac volume, bird anatomy, bird respiration, CT reconstruction

1 | INTRODUCTION

1.1 | Air sacs in birds

The functional anatomy of the respiratory tract and air sacs in birds is well documented based on traditional anatomical preparatory methods in ostriches (*Struthio camelus*) (Bezuidenhout et al., 1999), sulphur crested cockatoos (*Cacatua galerita*) (Jaensch et al., 2002), chickens (*Gallus domesticus*) (Casteleyn et al., 2010, 2018; Kobienia, 2008), turkeys (*Meleagris gallopavo*) (Cover, 1953; King & Atherton, 1970; Ragab & Reem, 2016), mallard ducks (*Anas platyrhynchos*) (Demirkan et al., 2006), geese (Onuk et al., 2013) and a variety of other species (Duncker, 1971; Taylor et al., 1962). There are significant variations in the anatomy of air sacs among different bird species (Casteleyn et al., 2018; King & Atherton, 1970; Ragab & Reem, 2016).

Air sacs in birds act like bellows, forcing air through the virtually rigid, gas-exchanging portion of the lungs (Maina, 2009). Airflow is continuous and unidirectional due to the presence of aerodynamic valving (Maina, 2009). They are arranged in different areas of the body connected via the bronchial system to the parabronchi in the lungs where the gas exchange occurs. The structure of the bronchial system and the air sacs differs among the avian species.

Functionally, the air sacs are divided into the cranial (cervical, clavicular, and cranial thoracic) and the caudal (caudal thoracic and abdominal) groups (Scanes & Dridi, 2022; Sereno et al., 2008). In turkeys (*Meleagris gallopavo*) the cervical air sac fuses with the pars lateralis of the clavicular sac to form the cervicoclavicular air sac (King & Atherton, 1970; Ragab & Reem, 2016).

The air sac volumes vary with posture (Malka et al., 2009; Nevitt et al., 2014), nutritional (Andrássy-Baka, Romvári, Milisits, et al., 2003;

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Andrássy-Baka, Romvári, Sütő, et al., 2003) and reproductive status. There is a remarkable difference in the anatomy of the thoracoabdominal cavity between the male and female birds in the breeding phase (Scanes & Dridi, 2022). This may influence the air flow through the lungs during anaesthesia and during various life phases of the animals.

1.2 | Methods for studying the respiratory system

Commonly used methods for studying the morphology of the avian air sac system are dissection (Cover, 1953; Duncker, 1971; Gilbert, 1939), casting (Casteleyn et al., 2010, 2018; Demirhan et al., 2006; King & Atherton, 1970; Ragab & Reem, 2016; Taylor et al., 1962), plastination (Kobienia, 2008), diagnostic imaging (Faillace et al., 2021; Krautwald-Junghanns et al., 1993; Krautwald-Junghanns, Schuhmacher, & Sohn, 1998; Malka et al., 2009; Schwarz et al., 2016) or the combination of these methodologies (Krautwald-Junghanns, Valerius, et al., 1998; Petnehazy et al., 2012). Of these, dissection and casting processes can alter the appearance of the air-filled cavities. The chemicals used as fixatives during dissection can result in shrinking tissue matter. As a result, it can be difficult to reconstruct the complicated spatial relations of a notoriously complex system.

1.3 | Casting of the respiratory system

Filling the respiratory system with low boiling point metal or resin was a phenomenal leap forward in describing the morphology of the air sacs. The first moulds were fabricated by simply pouring the casting material into the trachea and opening small holes throughout the body (Gilbert, 1939). When implementing this method, the trapped air cannot be completely removed, resulting in incorrect volumetric data (Taylor et al., 1962). To avoid this phenomenon, a vacuum was introduced to remove the air from the body (Bezuidenhout et al., 1999; Duncker et al., 1964; Duncker & Schlüter, 1964). The pitfall of this method is that the amount of the resin used for filling the air sacs is dependent upon the individual's experiences in performing the casting and only few studies describe the positioning of the animals during the process (Dubach, 1981). To fully comprehend this dependency on human judgement, a comparison was made (Krautwald-Junghanns, Valerius, et al., 1998) to examine the difference between the volumes gained with computed tomography (CT) and casting. According to their findings, the amount of resin used for casting was dependent upon the individual's subjective decision. A further problem encountered is that only dead animals are used for casting, and data of those were compared to the volumes measured on anaesthetised, supine positioned birds.

1.4 | New possibilities with diagnostic imaging (CT)

In contrast, diagnostic imaging gives the possibility to collect a large amount of data regarding the respiratory system of healthy or diseased

birds allowing reconstruction for further processing and studying. In the past 30 years, CT has become an important tool for human and veterinary diagnostics and research. CT is a useful tool for performing in vivo measurements such as body composition analysis (Andrássy-Baka, Romvári, Milisits, et al., 2003; Andrássy-Baka, Romvári, Sütő, et al., 2003; Milisits et al., 2015; Romvári et al., 2004), bone density and deformity measurements (Charuta et al., 2016; Donkó et al., 2018; Tatara et al., 2016), air sac volumetry (Hawkins et al., 2013; Krautwald-Junghanns et al., 1993; Krautwald-Junghanns, Schuhmacher, & Sohn, 1998; Malka et al., 2009; Nevitt et al., 2014) and for detecting other pathological changes in birds (Schwarz et al., 2016). CT data enables the calculation of respiratory system volumes in human and veterinary medicine (Brinkschulte et al., 2013, 2014; Nardelli et al., 2015; Giacomini et al., 2018; Köhler et al., 2021; Lawson et al., 2021).

Although several publications describe the cross sectional anatomy of a variety of bird species visualized by CT (Krautwald-Junghanns et al., 2010; Orosz, 1992; Petnehazy et al., 2012; Veladiano et al., 2016), few detail the anatomy and 3D reconstructions of CT data regarding the respiratory system of birds (Faillace et al., 2021; Lawson et al., 2021; Malka et al., 2009; Ponganis et al., 2015; Schachner et al., 2021).

Air flow analyses in the respiratory system of birds have been performed using cross sectional (Maina & Woodward, 2009) and CT data highlighting the aerodynamic and heat exchange function of the nasal passages (Bourke & Witmer, 2016), and aerodynamic valving during inspiration in the bronchial system of the *Struthio camelus* (Maina, 2009).

There are only a few publications where parts of (Nevitt et al., 2014), or the whole respiratory system, of the bird is present utilizing 3D reconstruction data obtained with CT (Schachner et al., 2021). These models can serve as a base for the evaluation of respiratory apparatus morphology (Lawson et al., 2021; Malka et al., 2009; Nevitt et al., 2014) and air flow analysis (Bourke & Witmer, 2016).

The aim of this study is to demonstrate the application of CT in modelling, visualization and understanding of the lower respiratory tract of two domestic (galliform) and one exotic (anseriform) species utilizing different scanning resolutions and positioning of the animals. We also intend to introduce basic reconstruction techniques and highlight the difficulties in identification of the various regions of the avian air sacs when performing analysis based on 3D models.

2 | MATERIALS AND METHODS

2.1 | Animals

2.1.1 | In vivo analysis

The live animal studies were approved by the University of Kaposvár and the Somogy County Agricultural Administration Office Food Safety and Animal Health Directorate (SOI/31/01537-1/2017 (KA-2306)).

Study 1: We reconstructed air sacs from CT data (slice thickness of 2 mm) of Broad Breasted turkeys, (*Meleagris gallopavo*) scanned in both, prone and supine positions.

Four commercial line turkey toms at the age of 16 weeks were procured from a local hatchery and used solely for this study.

Study 2: This study examined how air sac morphology correlates with sexual dimorphism. A total of four chickens (*Gallus domesticus*) at the age of 22 weeks, two cocks and two laying hens, were scanned (slice thickness = 0.6 mm) during a control study for body composition analysis.

2.1.2 | Specimen scanning

Study 3: We aimed to show the effect that higher resolution ($200 \times 200 \times 200 \mu\text{m}$ voxel size) has on the reconstruction quality. Specifically, a male mallard duck (*Anas platyrhynchos*) specimen was scanned at Parrish Creek Veterinary Hospital & Diagnostic Center (86°N 70°W Centerville, UT, 84014, USA).

2.1.3 | Preparation of the animals for the in vivo CT examinations

Study 1: On the day prior to scanning, the turkeys were transported to the Institute of Diagnostic Imaging and Radiation Oncology, Kaposvár University, for acclimatization with restricted feed yet unlimited water access for the day of the study. Each turkey underwent a physical examination by a research veterinarian (the main author) to assess for clinical signs of abnormalities that may affect the morphology of the respiratory system.

Anaesthesia was used to prevent movement during the scanning. A ventilation mask connected to a calibrated vaporizer (Dräger Vapour 19.3) was positioned over the head of the animals. The induction was made using 5 vol% of isoflurane (Abbott Laboratories, Abbott Park, IL, USA) and 1.5 L/min. of oxygen. Once the birds became unconscious, the isoflurane level was lowered to 1.6 vol% while the oxygen flow remained at 1.5 L/min. No intubation was performed during the experiment.

Positioning: The animals first were placed in sternal recumbency with the neck and the legs extended, and the wings folded in a normal position. A SIEMENS SOMATOM Definition Flash CT (2×128 slices multislice scanner; Siemens AG, Erlangen, Germany) was used for the imaging procedures. Transverse slices from the head to the end of the tibiotarsus were made. Scanning parameters: 6 s exposure time, 120 kV, 80 mAs, collimation 0.6, pitch 0.6 (table travel per rotation), spiral scanning mode, and 2 mm slice thickness. Following reconstruction, the resulting voxel size was: $0.64 \times 0.64 \times 2$ mm. The reconstruction algorithm was B 30f.

The animals were scanned using the same scanning parameters in a supine position as well to depict the differences in the air sac morphology between sternal and supine positions.

Following scanning, the animals were transported back to their cage and recovered under veterinary supervision.

Study 2: Preparation of the chickens for scanning was based on the same handling and anaesthetic procedures implemented for the

turkeys in Study 1. The animals were aged 22 weeks on the day of the study. A SIEMENS SOMATOM Definition Flash AS+ (128 slices multislice scanner; Siemens AG, Erlangen, Germany) was used in support of the imaging protocol. Transverse slices from the head to the end of the tibiotarsus were made in sternal position. The following parameters were used for scanning: 12 s exposure time, 120 kV, 40 mAs, collimation 0.6, pitch 0.6 (table travel per rotation), spiral scanning mode, and 0.6 mm slice thickness. Following reconstruction, the voxel size measured: $0.35 \times 0.35 \times 0.35$ mm. The reconstruction algorithm was U 90u. The middle of the FOV was centred to the midline of the thoracoabdominal cavity.

2.1.4 | Preparation of the cadaver for the CT examination

Study 3: A male mallard duck cadaver was scanned in a head first, sternal position with the head and neck extended and the wings folded beside the trunk. An Epica Vimago scanner was utilized. Transverse slices from the beak to the end of the trunk were made with the following parameters: 7 s exposure time, 80 kV, 60 mA, and 0.2 mm slice thickness. After reconstruction, the resulting voxel size was: $0.2 \times 0.2 \times 0.2$ mm.

Scanning parameters for the different studies are listed in Table 1.

2.2 | Data processing and 3D reconstruction

2.2.1 | Technical description

The resulting DICOM data were processed with the 3DSlicer (www.slicer.org, v. 4.11 stable release) open access software.

Basic segmentation:

1. We imported the CT series with the *DICOM browser* module and set the window level (WL) to -400 Hounsfield Unit (HU) and the window width (WW) to 2300 HU for basic anatomical visualization.
2. Following the window settings procedure, we cropped the series in the *Crop Volume* module resulting in isotropic voxels for the subsequent reconstructions.
3. The resulting volume was opened in the *Segment Editor* module and a segment was created for the air containing volumes of the respiratory tract and another segment for the bones of the body. During the *Threshold* effect operation, the range was set between -1024 and -860 HU and applied to only select the air containing voxels. While using the *Island* effect *Keep selected island* option, only the parts of the air sac system were retained. In the next step, the threshold was set between 280 and 2800 HU and the bones were segmented. The resulting segments were visualized in 3D with the *Show 3D* feature and used as a comparison model during the manual segmentation procedure (Figure 1).

TABLE 1 Scanning parameters used in the different studies.

Study	Species	Sex	Position	Tube current (mA)	Tube voltage (kV)	Pitch	Reconstructed slice thickness (mm)	Reconstruction algorithm	Resulting voxel size
Study 1	<i>Meleagris gallopavo</i>	Male	Sternal	80	120	0.6	2	B30f	0.64 × 0.64 × 2 mm
		Male	Supine	80	120	0.6	2	B30f	0.64 × 0.64 × 2 mm
Study 2	<i>Gallus gallus domesticus</i>	Male	Sternal	40	120	0.6	0.6	U 90u	0.35 × 0.35 × 0.35 mm
		Female	Sternal	40	120	0.6	0.6	U 90u	0.35 × 0.35 × 0.35 mm
Study 3	<i>Anas platyrhynchos</i>	Male	Sternal	60	80	1	0.2	NA	0.2 × 0.2 × 0.2 mm

Detailed segmentation:

1. We accessed the cropped series using the *Segment Editor* module, added labels to the parts of the respiratory-air sac system and identified them in full accordance with the *Nomina Anatomia Avium* Second Ed. (Baumel)
2. There was no fixed WW/WL set for the reconstruction as visualization of the different septa underwent continuous changes throughout which we frequently modified the setting to achieve optimal results.
3. We used the *Paint* effect with the *Editable intensity range* option set between -1024 and -860HU to effectively segment only the air-containing voxels.
4. We initiated the segmentation with the abdominal air sac. If air containing voxels inside the intestines were selected, they were deleted at the end of the procedure using the *Islands* effect, *Remove selected island* option by selectively clicking and removing them. The scanning resolution enabled the display of the fine membranes between different portions of the air sac system. While tracking them, we labelled the compartments with the corresponding colours defined from the outset. The different anatomical regions were compared with the uniform 3D model we created at the beginning of the basic segmentation.
5. Regarding reconstructing the skeletal system, we used the *Threshold* effect set between 210 and 2800HU. The air-containing voxels between 1024 and -860HU inside the bones were reconstructed as well, to identify the diverticula inside of them.
6. Following completion of the labelling, we created 3D models to examine the segmentation results and made refinements if and where needed.
7. While utilizing the labels, we calculated the volume and surface in reference to the separate parts aligned with the *Label statistics* module.
8. The results for the 3D models were saved in appropriate formats (vtk, stl) for further processing. The results for the volumes and surfaces were saved in a csv format.

3 | RESULTS

3.1 | Reconstruction results for anatomical modelling

In Study 1, our aim was to make a clear anatomical identification of the different parts of the air sac system in which we followed the thin membranes between each compartment.

This enabled us to recognize the borders and identify a clear separation between the air sacs in the following regions:

1. Abdominal and caudal thoracic air sacs, caudal to the lungs (Figures 2a and 2b).
2. Caudal thoracic and cervicoclavicular air sacs at the middle of the trunk ventral to the lungs (Figure 3)

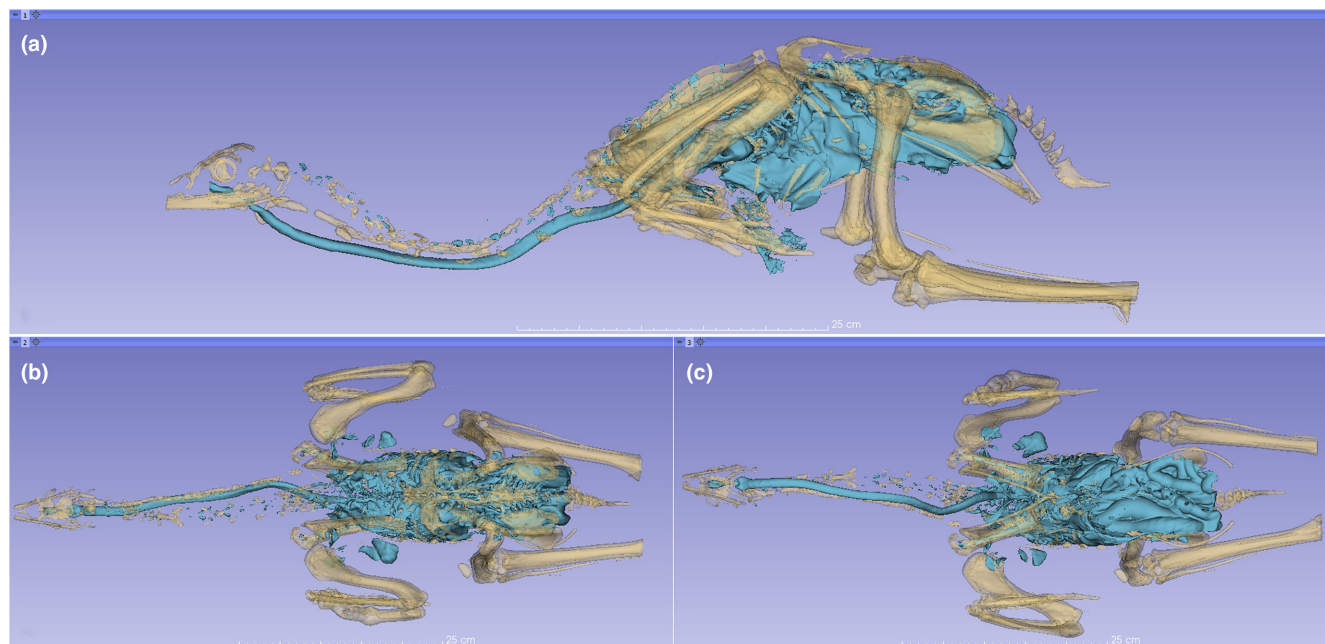


FIGURE 1 Simplified 3D model after reconstructing the air containing voxels of the respiratory apparatus only (between -1024 and -840 HU) and the skeleton (HU range: 280 – 2800) in the case of a domestic turkey. Osseous structures are yellow, air-filled regions are light blue. The 3D model of the skeleton was made separately and its opacity has been decreased in order to get a better overview of the air sacs. (a) lateral; (b) dorsal and (c) ventral view.

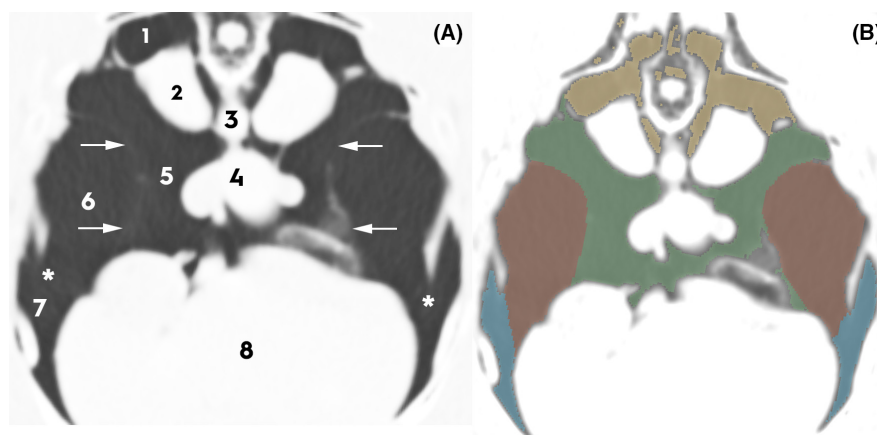


FIGURE 2A The septa between the abdominal (arrows), thoracic and cervicoclavicular air sacs (asterisks) in a 16-week-old turkey at 2 mm slice thickness. A, Before and B, after manual segmentation. 1, Perirenal diverticula of the abdominal air sac; 2, kidney; 3, descending aorta; 4, testicle; 5, abdominal air sac; 6, thoracic air sac; 7, cervicoclavicular air sac (intrathoracic diverticulum) and 8, liver. The WW/WL setting was shifted for better visualization of the septa between the air sacs.

3. The extrathoracic diverticula of the cervicoclavicular air sac; conical central part (cranial to the heart), humeral (if it was present), axillary, subscapular and pectoral diverticulum (Figure 4).

There were locations where a clear separation was very difficult to visualize due to the complexity of septal borders:

1. The horizontal septum between the intrathoracic part of the cervicoclavicular air sac and the caudoventral surface of the lung was not visible on all scans. The clear separation between the cardiac diverticula (cervicoclavicular air sac) and the bronchial

system at some points was not always possible due to the blurry appearance of the septum (Figure 5).

2. The intrathoracic part of the cervicoclavicular air sac: Separating the cardiac and sternal diverticula. The borders between the two air filled cavities at the cranial part of the heart (atrial level) were not clear on all slices. There was an abundance of small compartments to objectively assign either to the cardiac or to the sternal diverticulum.

As a result, we have a detailed 3D model of the respiratory system and air sacs of the examined bird species (Figures 6a–6c).

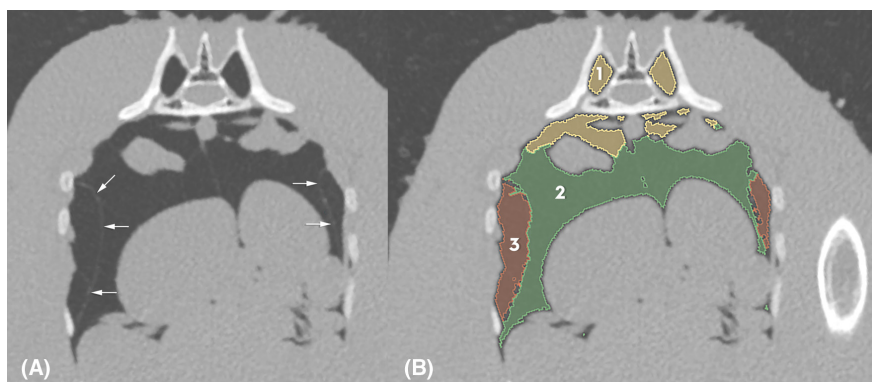


FIGURE 2B The septa between the abdominal and thoracic air sac in the cock at $0.35 \times 0.35 \times 0.35$ mm isovoxel size. A, Before and B, after manual segmentation. 1, Perirenal diverticula of the abdominal air sac; 2, abdominal air sac and 3, caudal thoracic air sac. At smaller voxel size the identification of the septa were easier but the signal to noise ratio (SNR) decreased at the air containing regions.

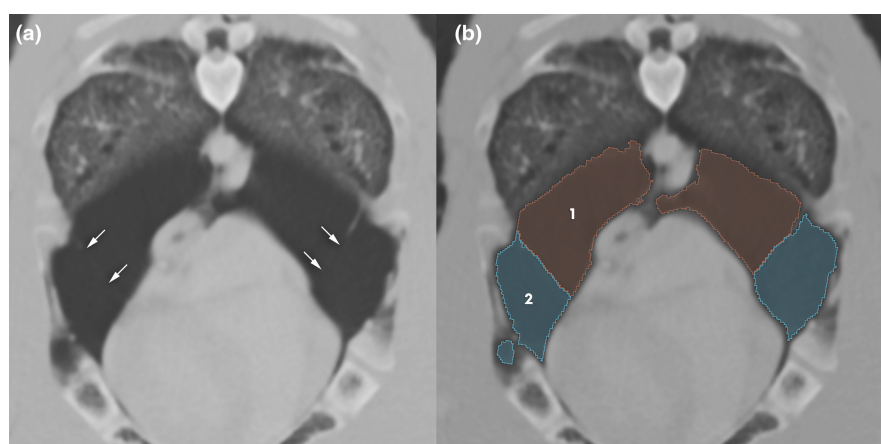


FIGURE 3 The septa between the thoracic and the cervicoclavicular air sacs (arrows) in turkey at 2 mm slice thickness. (a), Before and (b), after manual segmentation. 1, Thoracic air sac and 2, cervicoclavicular air sac (intrathoracic diverticulum).

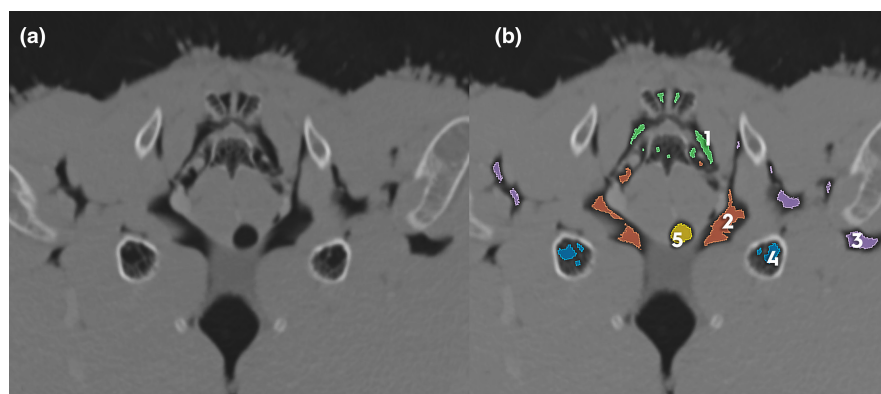


FIGURE 4 The extrathoracic diverticula of the cervicoclavicular air sac, cranial to the heart, before (a) and after (b) manual segmentation. 1, Cervical part of the cervicoclavicular air sac; 2, cranioventral diverticulum of the cervicoclavicular air sac; 3, axillary and subscapular diverticula; 4, coracoid diverticula in the coracoid bone and 5, trachea. In this turkey the humerus was filled with soft tissue density volume.

3.1.1 | Trachea

The thick wall of the trachea and the air inside facilitated easy reconstruction. The walls of the proximal part of the two main bronchi at the level of the syrinx, where they are surrounded by the clavicular air sac between the pulmonary arteries, were too thin for the semi-automatic segmentation, therefore, this area required manual labelling. After crossing over the horizontal septum, they develop into the atria in which they are not distally discernible.

In the mallard duck, we separated the portions of the syrinx, bulla and tympanum at the resolution used for scanning ($0.2 \times 0.2 \times 0.2$ mm) (Figure 7).

3.1.2 | Cervical air sac

The cervical air sacs were the smallest in volume and the most divided among all birds. They originated from the anterior half of the lungs and exist as small compartments on the 3D models adjacent to the cervical vertebrae. As seen on CT scans, they appear as dark areas at the transverse canal and in the vertebral canal (supramedullary diverticulum) surrounding the medulla spinalis. In the turkey, the cervical air sac fused with the lateral part of the clavicular sac to form the cervicoclavicular air sac. In this species it is divided into diverticula vertebrales and diverticula interpulmonaris. The latter was situated ventral to the crest of the notarium, between the two lungs.

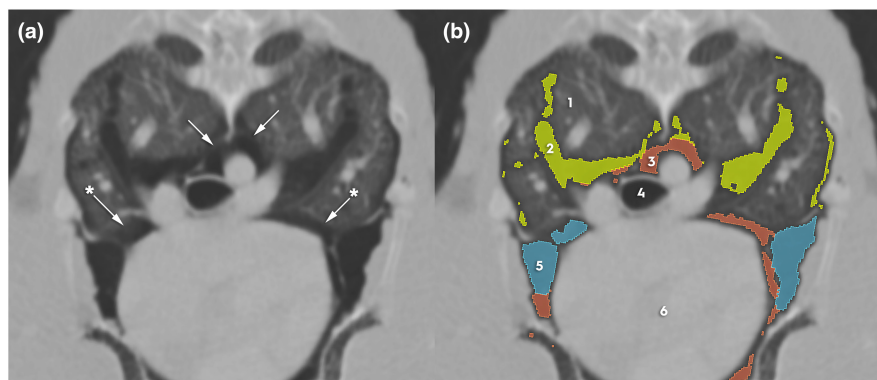


FIGURE 5 Transverse scan of a turkey and manual segmentation at the level of the heart. Before (a) and after (b) manual segmentation. 1, Lung; 2, bronchial system inside the lung; 3, cardinal diverticula of the intrathoracic part of the clavicular air sac; 4, oesophagus (filled with air); 5, heart and 6, sternal diverticulum of the intrathoracic part of the clavicular air sac. The arrows with the asterisks pointing at locations where the transverse septum was not clearly identifiable. Dorsal to the heart where the septum was originating from the ventral crest of the notarium it was well visible.

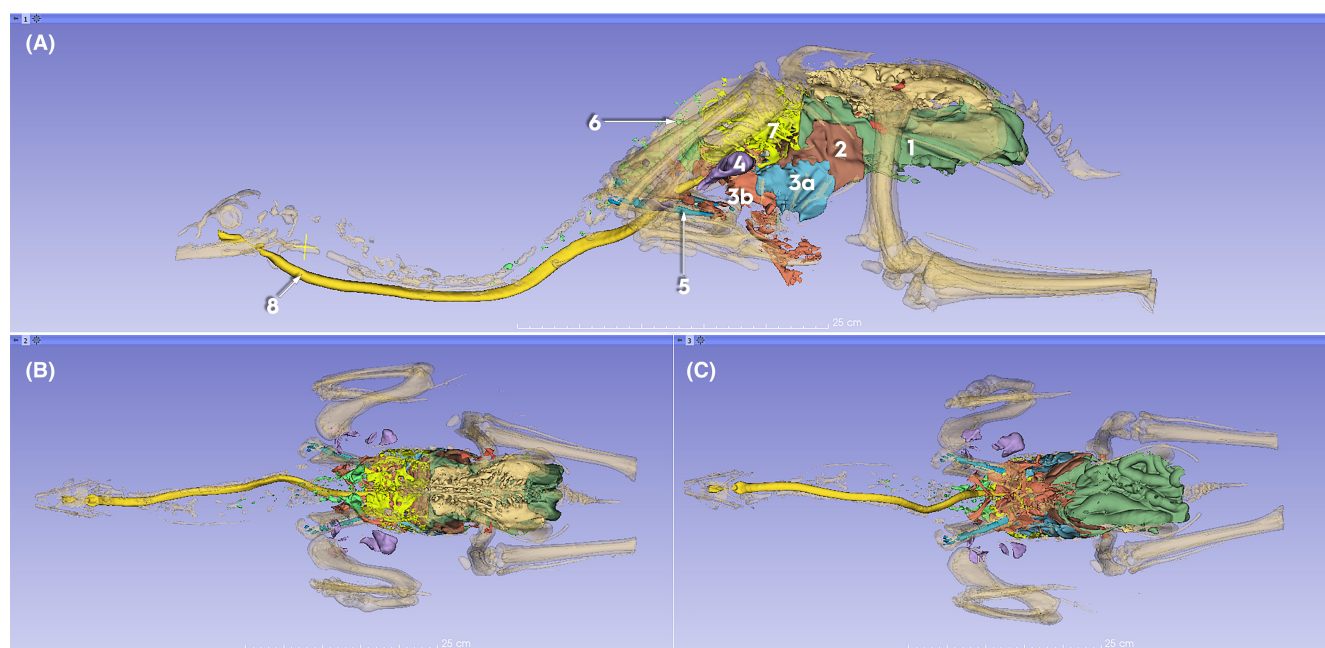


FIGURE 6A Detailed 3D reconstruction of the air sac system of a 16-week-old turkey tom (scanned in sternal recumbency) after detailed manual segmentation of the different parts. The opacity of the skeletal model has been increased. 1, abdominal; 2, thoracic; 3a and 3b, intrathoracic diverticulum of the clavicular part of the cervicoclavicular air sac (sternocardiac diverticulum); 4, axillary diverticula of the cervicoclavicular; 5, coracoid diverticula of the cervicoclavicular; 6, cervical part of the cervicoclavicular air sac; 7, lung and 8, trachea. A, Lateral; B, dorsal and C, ventral view.

3.1.3 | Clavicular air sac

The main portion of the clavicular air sac lies at the cranial part of the thoracoabdominal cavity surrounding the main vessels arising from the heart (left and right cranial vena cava, main arteries for the head and wings), the trachea, oesophagus, and the medial part of the sternocoracoideus muscles. We labelled the main parts of this complex system, specifically, the central part, humeral diverticula, coracoid diverticula and axillary diverticula (Figures 4 and 6a).

There was a difference in the level of bone pneumatization between the turkeys, laying hens and the mallard duck. Among

turkeys, the coracoid was consistently filled with air and in one animal the humerus contained bone marrow. While among chickens, the coracoid was always filled with bone marrow and the humerus contained air in all the animals we scanned. In the mallard duck, both the coracoid and the humerus were filled with bone marrow.

The sternum in turkeys contained small air-filled cavities at the base and in the lamina of the keel of the sternum, whereas only in the base of the keel in laying hens. There was no sign of air-containing compartments in the mallard ducks' sternum.

The axillary diverticula of the clavicular air sac surrounded the muscles lateral to the coracoid and the shoulder joint, and its small

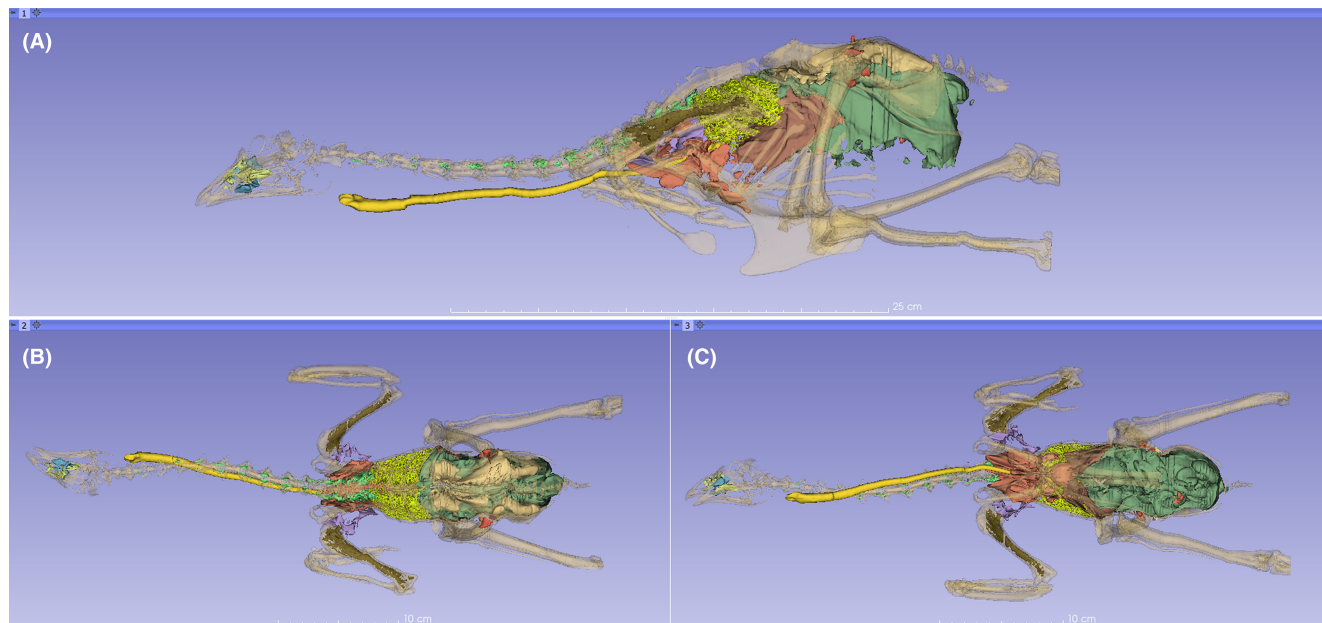


FIGURE 6B Detailed 3D model of the air sac system from the scan of the 22 weeks old cock (scanned in sternal recumbency) after detailed manual segmentation. The opacity of the skeletal model has been decreased. There is a remarkable artefact at the abdominal air sac and the tibiotarsus caused by breath movements. 1, abdominal; 2, cranial and caudal thoracic; 3, intrathoracic diverticula of the clavicular; 4, axillary diverticula of the clavicular; 5, humeral diverticula of the clavicular air sac; 6, lung; 7, trachea and 8, cervical air sac Colour codes for the head-brown, nasal cavity; blue, infraorbital sinus. A, Lateral; B, dorsal and C, ventral view.

compartments surrounded the base of the scapula in chickens, though not in turkeys. In all examined species, there was a remarkable portion of this cavity on the side of the trunk under the skin, ventral to the caudal edge of the latissimus dorsi muscle.

3.1.4 | Thoracic (cranial and caudal) air sacs

The caudal thoracic air sac is absent in turkeys. The thin membrane between the cranial thoracic and caudal thoracic air sacs was detectable only in the mallard duck. The border between those compartments arises at the origination of the costoseptalis muscle and the thin membrane which extends from the dorsolateral to ventromedial direction, inserting at the pericardium and the liver (Figure 3). The cranial thoracic air sacs are positioned between the thoracic wall and the proximal part of the heart and distally lateral to the left and right lobes of the liver. They do not extend to the midline since the caudal thoracic air sac occupies this space from the base of the heart.

The caudal thoracic air sacs start at the base of the heart and exhibit a cranially directed conical shape. A precise identification of their opening was not possible in the scans of the mallard duck, nor the laying hens. These paired air sacs bulge cranially between the base of the heart, liver and cranial thoracic air sacs and run along an oblique path to the lateral thoracic and abdominal walls.

In the mallard duck, the caudal border of the caudal thoracic air sac was located behind the gizzard on the left side. The border

between the caudal and cranial thoracic air sac was running at such a steep angle it appeared blurry on the CT scans due to the partial volume effect. The overall shape of these compartments was more elongated in the duck than the other species examined in this study.

Among laying hens, a clear separation between the cranial and caudal thoracic air sacs was not possible. This compartment was the smallest among the air sacs in chickens.

3.1.5 | Abdominal air sacs

They are connected to the caudal surface of the lungs on both sides and extend up through the caudal end of the thoracoabdominal cavity. Their diverticula (perirenal, femoral and iliolumbar) were all identifiable on the scans. The thin membrane between the left and right air sacs was detectable in all examined birds.

Among turkeys, all three divisions of the kidneys were surrounded dorsally by the perirenal diverticula. This cavity extended caudally into the depression of the obturatorius internus muscle found in the caudal recess of the renal fossa. Small serous duplicatures were identifiable at all divisions of the kidneys including the dorsal side as suspensory elements between the organ and the bony pelvis. The femoral diverticulum was found under the proximal portion of the iliotibialis muscle between the femoral head and antitrochanter. Among hens, the kidneys were found more dorsally to the renal fossa of the pelvis due to the enlarged volume of the abdominal

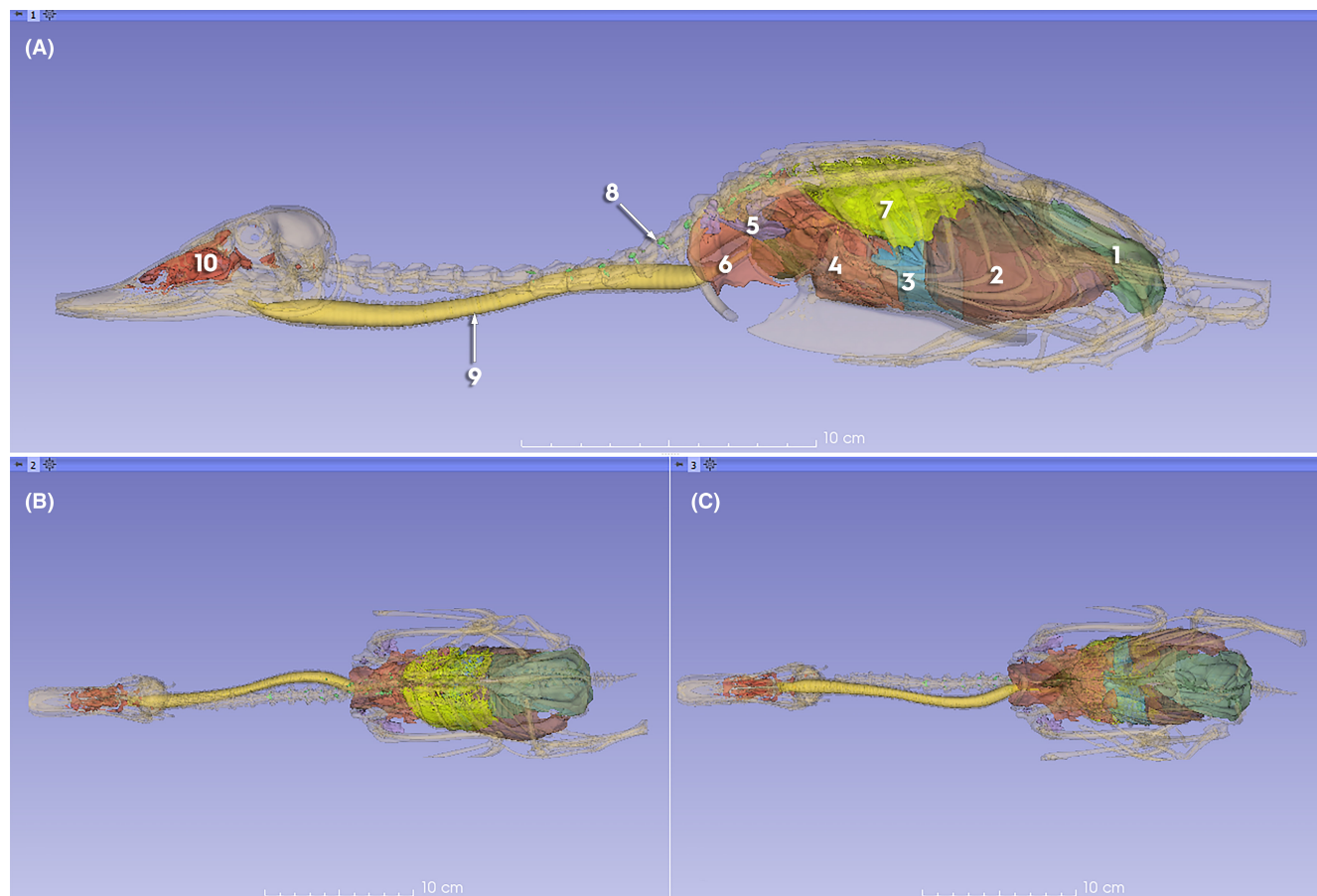


FIGURE 6C 3D model of the air sacs, the trachea and the air filled cavities of the head from the scan of the male mallard duck. 1, abdominal; 2, caudal thoracic; 3, cranial thoracic air sac; 4, intarthoracic diverticula of the clavicular air sac; 5 and 6, extrathoracic diverticula of the clavicular air sac; 7, lung; 8, cervical air sac; 9, trachea and 10, nasal and infraorbital cavity. A, Lateral; B, dorsal and C, ventral view.

viscera and fat. Therefore, the perirenal diverticula appeared more asymmetric in hens than in turkeys.

3.1.6 | Air containing compartments of the head

The resulting smaller voxel in the case of the hens and the mallard duck made it possible to obtain a basic visualization of the paranasal sinuses and the nasal cavity. With regards to the chickens, the bone reconstruction of the entire skull was not possible at this resolution (Figures 8a and 8b).

3.1.7 | Difference between the scanning results in sternal and supine position among turkeys

There was a remarkable difference in the appearance of the abdominal air sac when scanning the turkeys in dorsal recumbency compared with the sternal position. Due to the repositioning of the abdominal viscera in dorsal recumbency, the volume of the abdominal air sacs considerably decreased and the uniformity regarding the compartment became fragmented. The perirenal diverticula dorsal

and the side of the renal divisions disappeared as the organs in the coelomic cavity changed their position and pushed the kidneys towards the bony pelvis. A smaller compartment at the caudal renal division remained open in both positions.

The visceral reposition affected the thoracic air sac volume less as the part of the caudal air sac system (Figures 9a and 9b). Using the *Label statistic* module of the *3D Slicer*, one can calculate the degree of volume changes. The small number of scanned animals did not make it possible to compile a detailed statistical analysis. In our study, we focused on the anatomical description of this effect.

3.1.8 | Impact of sexual dimorphism on the air sac morphology

The appearance of the caudal air sac group (caudal thoracic and abdominal) was affected by active genital organs.

Among cocks, the testicles are found at the cranioventral pole of the kidneys and ventral to the caudal surface of the lungs. In our study, they compressed the caudal part of the thoracic air sac, pushing it forward while occupying a large volume at the

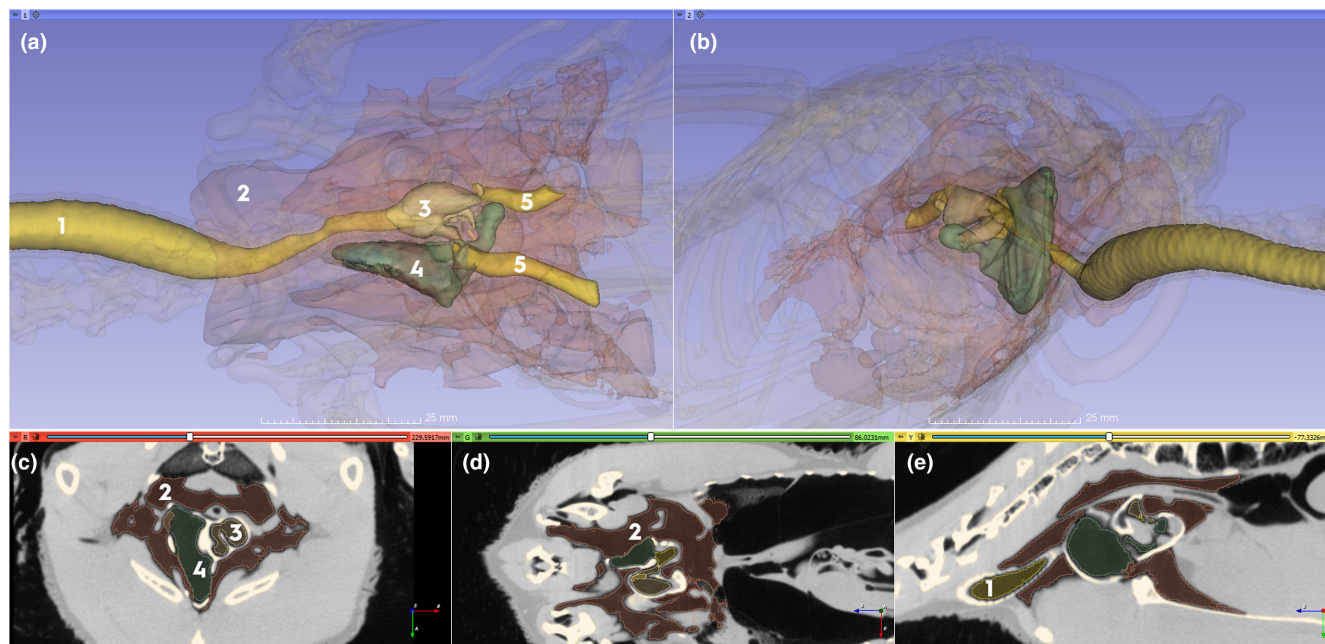


FIGURE 7 3D reconstruction of the syrinx from the male mallard duck series. The position and the surrounding structures can be seen on the three main planes of the CT series. 1, trachea; 2, clavicular air sac; 3, tympanum; 4, bulla syringealis and 5, main bronchi. (a) Dorsal, (b) right cranio-lateral view of the 3D model, (c) transverse, (d) horizontal, (e) sagittal plane of the CT series. The opacity of the skeletal and the clavicular air sac model have been increased.

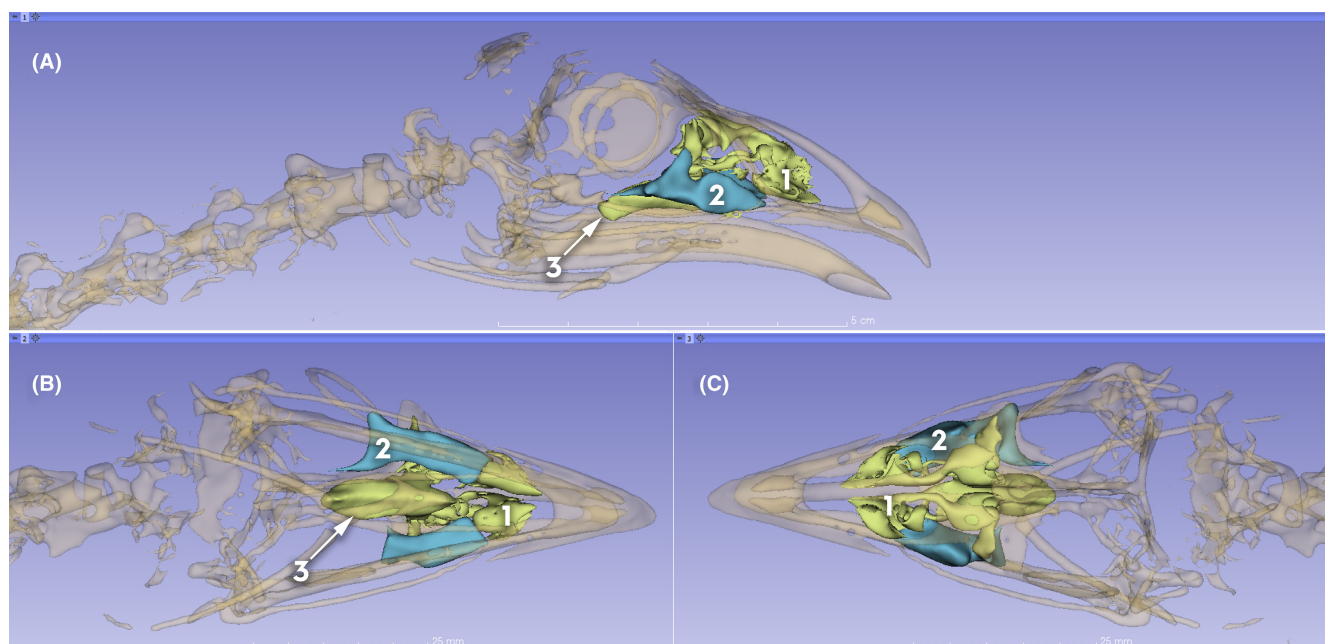


FIGURE 8A Basic 3D reconstruction of the air containing areas of the nasal cavity and the paranasal sinuses in the chicken. 1, Nasal conchae; 2, infraorbital sinus; 3, choana. A, Right lateral; B, ventral; C, dorsal view of the head.

cranioventral side of the abdominal air sac. On the scans, we noted the size difference between the right and left testicle, the latter being larger as it is described in birds (Figure 10, left testicle marked with '+').

The space occupying effect of the genital organs was more pronounced among the two laying hens we scanned in this study

(Figure 11). The animals were in active laying phase, so follicles were detected in the ovarium and eggs forming along other parts of the oviduct, in which one egg with a hard shell was discovered in the uterus. On the scans and in the 3D reconstructions, the appearance of the egg was distorted at the caudal pole due to spiral artefact and respiratory movements. The thoracic air sac volume was

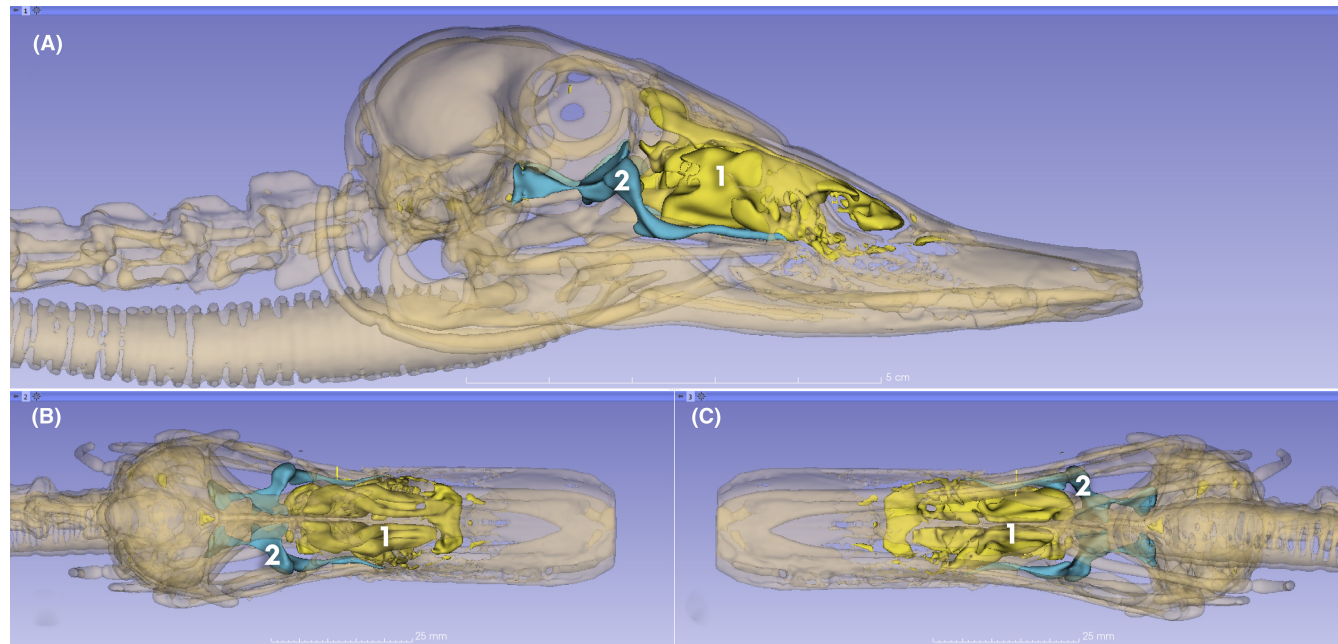


FIGURE 8B Basic 3D reconstruction of the air containing areas of the nasal cavity and the paranasal sinuses in the mallard duck. 1, nasal conchae; 2, infraorbital sinus. A, Right lateral; B, ventral and C, dorsal view of the head.

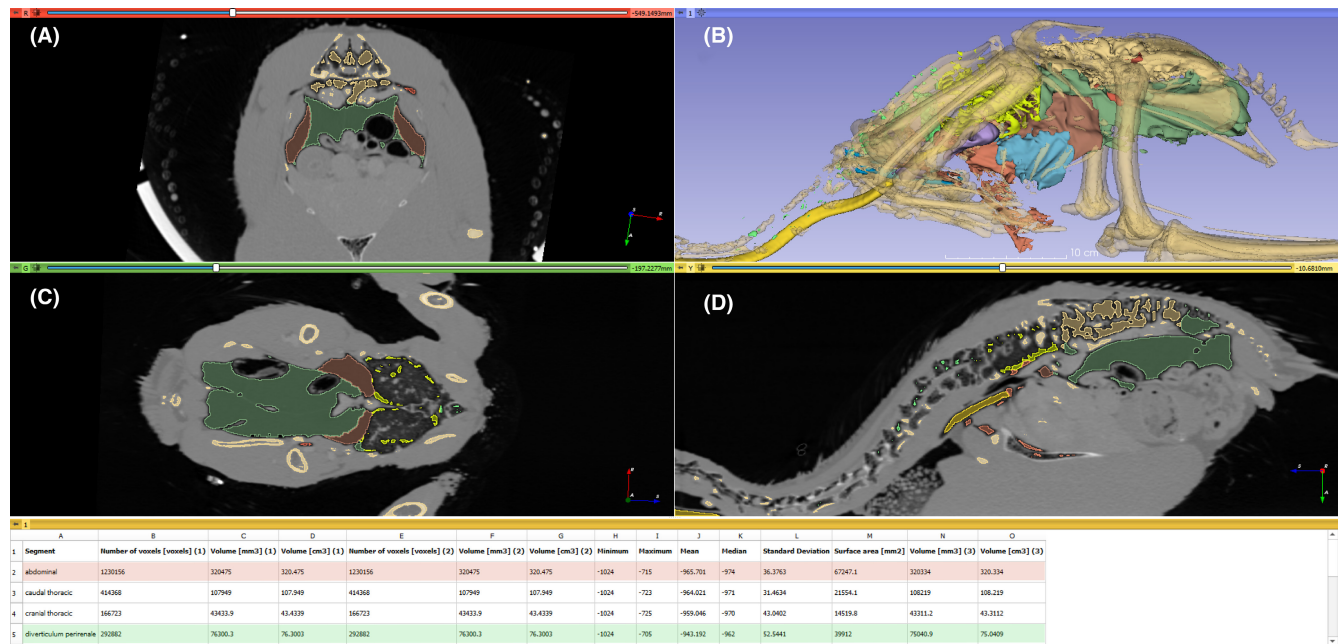


FIGURE 9A 3D reconstruction and detailed volumetric data in a prone position scanned turkey. The numeric values of the abdominal air sac and the perirenal diverticula are highlighted with red and green.

considerably reduced due to an enlarged left ovary, which contained follicles. The oviduct pushed the abdominal air sac dorsally.

3.2 | Simplified reconstruction

According to the afore-mentioned problems encountered while aiming for a clear anatomical separation, we created a simplified

reconstruction technique regarding the turkey, based on the functional separation of the air sacs.

1. No diverticula (including the perirenal, femoral, axillary, etc.) were separated during reconstruction of the air sacs.
2. No distinction was made between the thoracic and abdominal air sacs (those were assigned to the caudal group). The cervicoclavicular air sac represented the cranial group (Figure 12).

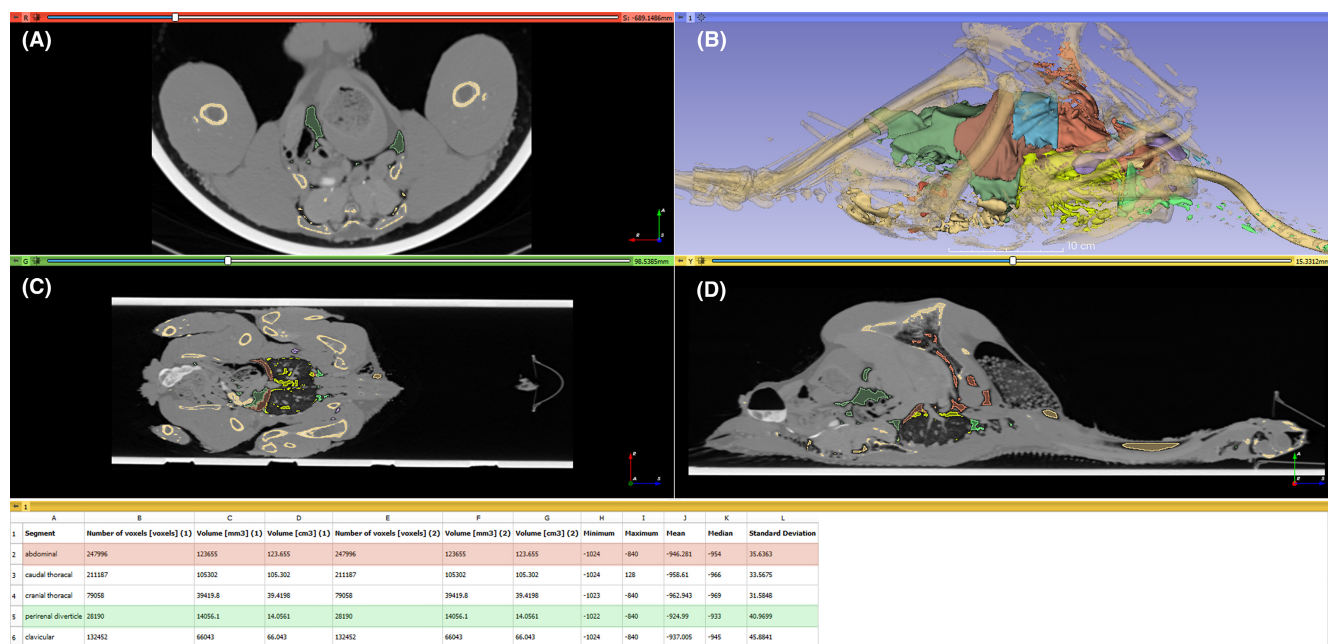


FIGURE 9B 3D reconstruction and detailed volumetric data the same turkey scanned in supine position. The numeric values of the abdominal air sac and the perirenal diverticula are highlighted with red and green. Those are the most affected volumes by the different positioning of the birds.

We separated the trachea from the primary bronchus at the point where the two bronchi were clearly separating distal to the syrinx.

overview regarding skeletal pneumatization ratios among various (larger body size) bird species.

4 | DISCUSSION

4.1 | Possibilities and benefits of using computed tomography in analysing avian anatomy

CT and the software used in data analysis (Fedorov et al., 2012) offer improved possibilities for the researcher to study and to precisely calculate the volume, partial volumes and different proportions of this complex, air-filled system (Lawson et al., 2021; Schachner et al., 2021). The animals can be scanned multiple times, in different nutritional or sexual states (Andrássy-Baka, Romvári, Milisits, et al., 2003; Andrássy-Baka, Romvári, Sütő, et al., 2003), and in various positions (Hawkins et al., 2013; Malka et al., 2009; Nevitt et al., 2014).

At the same time, live animal scanning bears its limitations. The animals must be anaesthetised during examination, breathing artefacts can occur, as depicted in our models (Figure 7b right tibiotarsus). If a better resolution is aimed for, UHR (ultra-high-resolution scanning) will require a higher dose of radiation with an elapsed scanning time, limiting the possibility of repeated examinations. The resolution and voxel size we used in our study is not comparable to what could have been achieved using micro-CT. Therefore, in consideration of our results, we can only show the pneumatization of larger bones yet no particular analysis is possible regarding the postcranial skeletal pneumaticity and bone structure (Fajardo et al., 2007; Moore, 2021). Our results still offer a method and an

4.2 | Comparing our results with the literature about air sac anatomy

Cover (1953) described nine air sacs in the turkey, namely the anterior thoracic (unpaired), posterior thoracic, thoraco-cervical, lesser abdominal and greater abdominal. Rigdon (1957) identified a large clavicular or cervical sac, a pair of thoracic sacs and two paired abdominal air sacs, a small and a large one. King and Atherton (1970) and Ragab and Reem (2016) identified the cervicoclavicular sac, medial clavicular sac, cranial thoracic sacs and abdominal sacs. Our 3D model created from our scans is similar to the casts published by Cover (1951), Rigdon et al. (1958) and Ragab and Reem (2016). The resolution we used in our studies was sufficient to generate models of the air sacs, the main diverticula connected to them and the overall shape of the main bronchial system. However, it was not enough to study some crucial branching of the secondary bronchi (lateroventral) specifying their arborization and the connection to a specific air sac. Therefore, we were not able to decide whether the air sacs of the caudal group consist of a caudal thoracic and abdominal or a lesser and greater abdominal air sac in turkey. We identified the air sacs of the caudal group according to the NAA (Baumel et al., 1993) as thoracic and abdominal air sac.

In the chickens and the duck we found similar air sac anatomy as described in the literature (Akester, 1960; Demirkan et al., 2006; Goodchild, 1970; King & Payne, 1962). Interestingly among laying hens, a clear separation between the cranial and caudal thoracic air sacs was not possible.

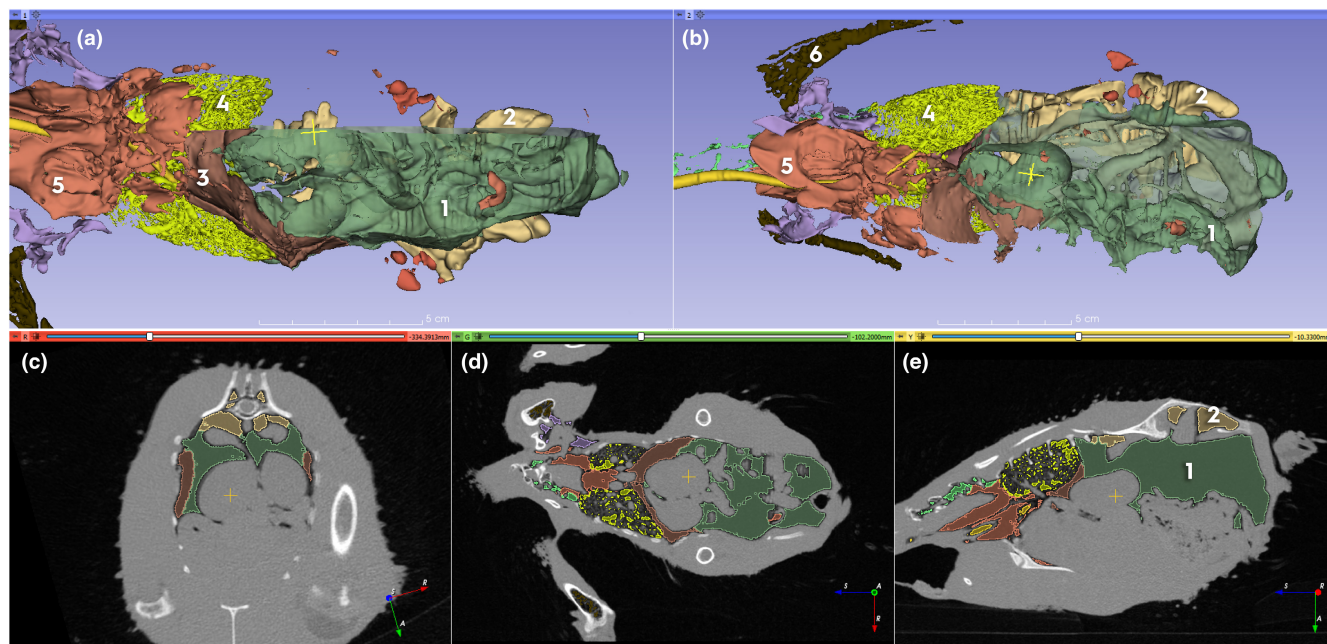


FIGURE 10 3D reconstructions and CT scans of the trunk of a cock. 1, Abdominal air sac; 2, perirenal diverticula; 3, thoracic air sac; 4, air in the lungs; 5, clavicular air sac and 6, air in the humerus. (a) Ventral view; (b) ventrolateral view of the 3D model; (c) transverse; (d), horizontal and (e) sagittal view of the CT scan. The 3D model of abdominal and the thoracic air sac has been cut in the sagittal plane as seen on (e) for better visualization of the left testicle's impression.

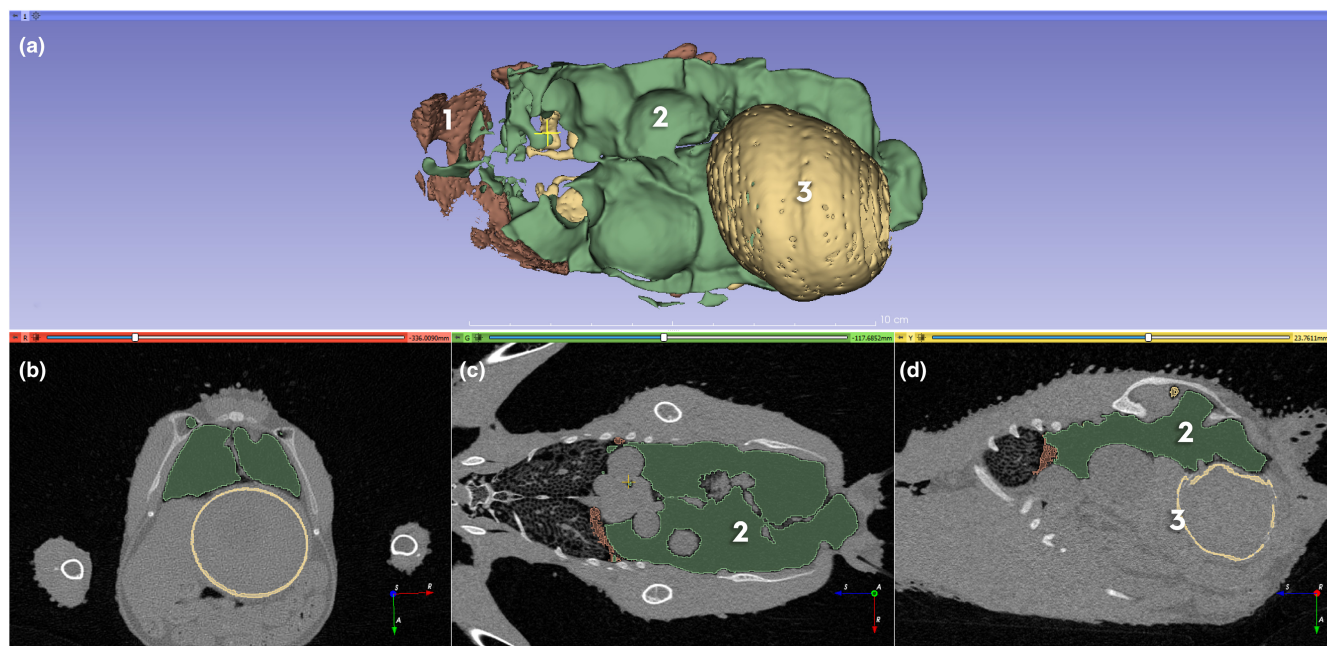


FIGURE 11 3D reconstruction of the caudal air sacs and an egg in a laying hen. 1, Thoracic air sacs; 2, abdominal air sacs and 3, egg shell in the uterus. Spiral artefact seen on the egg shell reconstruction.

4.3 | Suggestions for positioning avian species during lower respiratory imaging with CT

Using a diagnostic imaging method with sufficient resolution, the entire body can be examined and the localization and spreading of various respiratory diseases can be precisely described from a single CT

examination (Schwarz et al., 2016; Veladiano et al., 2016). For radiographic examinations of the coelomic cavity the standard positions are: ventrodorsal supine (VDS), dorsoventral erect (DVE) and lateral (L) (Poland & Raftery, 2019; Zoller et al., 2019). The preferred position for veterinary imaging is the VDS as many birds' keels are often sharp and therefore maintaining a stable sternal positioning is often

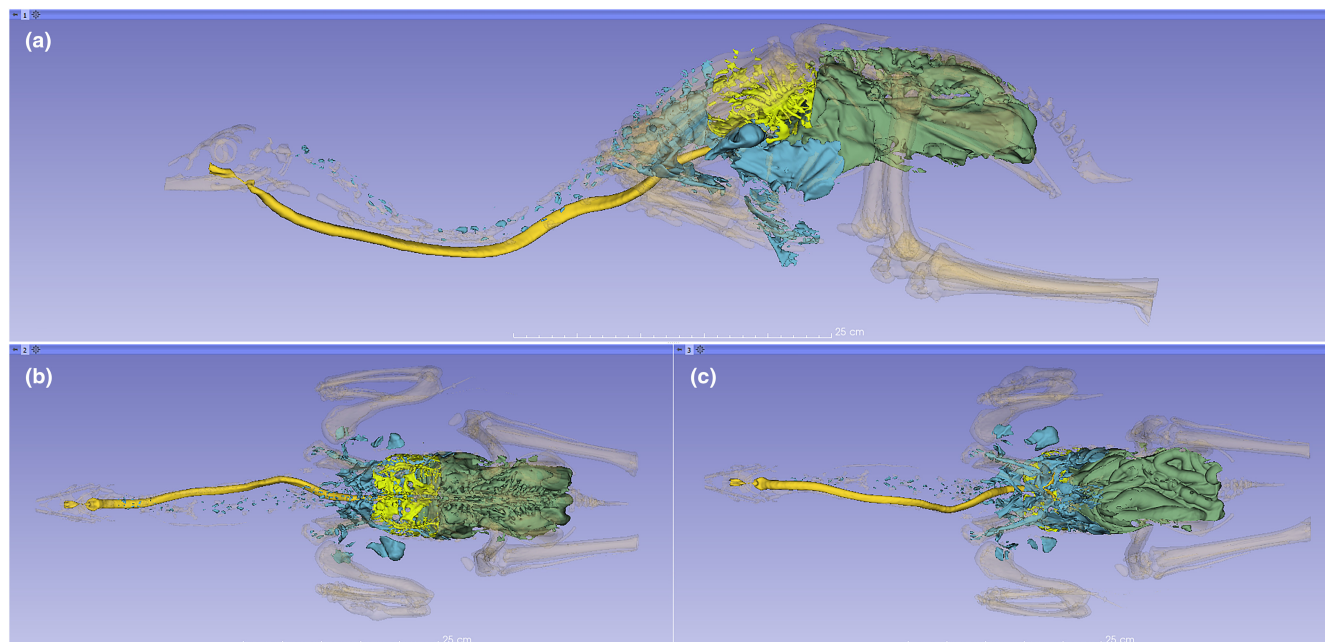


FIGURE 12 Simplified 3D model of the air sac system after combining the air-filled cavities of the caudal and cranial group to visualize the physiological separation of the air sacs. Green: abdominal and thoracic air sac, representing the caudal group. Blue: cervicoclavicular air sac representing the cranial air sac group. Yellow: trachea and lung (a) lateral; (b) dorsal and (c) ventral view.

challenging during a CT scan. The findings of Malka et al. (2009) and Nevitt et al. (2014) were comparable to our results, indicating that the air sac volumes were smallest in dorsal recumbency. In dorsal positioning the shifting of the coelomic organs compress the abdominal air sac as we present in our detailed 3D model, based on the results of the turkey scans (Study 1). Sternal positioning in anaesthesia bears limitations, since anaesthetics may cause muscle relaxation and in ventral recumbency the body weight on the keel may prevent full air sac expansion (Nevitt et al., 2014). According to this the most preferred positioning for an air sac analysis would be a non-anaesthetised animal in standing position (Rivas et al., 2019). A larger bird (turkey, stork, crane, etc.) cannot fit in the gantry in standing position and motion artefacts can occur which could lead to repeated scanning and higher doses of radiation. Therefore, for CT based air sac volumetry analysis in birds our suggestion is to perform the scanning on an anaesthetised, sternally positioned animal.

According to the authors' knowledge no online 3D models are available based on CT data of examined living birds regarding the respiratory system (air sacs). The method used in this study offers basic anatomical references in the identification of the complex air sac system. No statistical analyses were performed regarding the air sac volumes, due to the small sample size of animals examined in our studies. According to our results, we would like to highlight the benefits of utilizing 3D reconstructions of the respiratory systems of birds obtained by CT.

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DATA AVAILABILITY STATEMENT

The basic dataset is available at: (1) <https://doi.org/10.6084/m9.figshare.19722595> (CT scan and 3D models for the turkey), (2) <https://doi.org/10.6084/m9.figshare.19722658> (CT scan and 3D models for the mallard duck) and (3) <https://doi.org/10.6084/m9.figshare.19722739> (CT scan for the laying hen).

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