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**Anatomy of the lower respiratory tract in domestic birds, with emphasis
on respiration**

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With 16 figures

Short title: Avian respiration

Abstract

This manuscript describes the anatomy of the lower respiratory tract in domestic bird species including the chicken and pigeon. The here-described anatomical structures play a major role avian respiration, which is fundamentally different from respiration in mammals. During in- and expiration a continuous caudocranial airflow is present within the tertiary bronchi of the *Paleopulmo*, while the *Neopulmo*, which is only present in phylogenetically recent species, is characterized by tidal respiration. Various anatomical structures and aerodynamic mechanisms have been described in an attempt to explain the proposed mechanism of respiration. The air sac system that is essential for avian respiration usually comprises an unpaired clavicular air sac and paired cervical, cranial and caudal thoracic, and abdominal air sacs. The latter are by far the larger and are interwoven with the abdominal organs.

Key words: Bird; Anatomy; Lower respiratory tract; Respiration

36 ANATOMY OF THE LOWER RESPIRATORY TRACT

37 **Lungs**

38 *Configuration*

39 The pinkish lungs of birds lie dorsally in the thorax and extend between the first ribs and
40 the kidneys. In comparison with mammals, the avian lungs are relatively small and less
41 elastic. A transverse section through the thorax shows the triangular shape of the lungs
42 that are characterized by a broad medial side and an acute lateral border (Fig. 1). The
43 dorsal surface of the lung shows indentations formed by the vertebral ribs (Fig. 2).
44 Ventrally, the lungs rest on the horizontal septum (Fig. 1).

45 In phylogenetically recent bird species, such as the chicken and pigeon, each lung
46 consists of two compartments. As its name suggests, the paleopulmo (*Paleopulmo*) is
47 phylogenetically the older compartment. It lies craniodorsal and medial to the neopulmo
48 (*Neopulmo*). It is the larger compartment as it accounts for approximately 75% of the
49 lung volume in most bird species (King and McLelland, 1975). It should be noticed that
50 the distinction between the paleopulmo and the neopulmo is of functional nature and
51 cannot be made by means of gross anatomical dissection. The functional characteristics
52 of both pulmonary compartments will be elaborated below during the description of the
53 bronchial and parabronchial systems.

54

55 *Bronchial system*

56 The left and right primary bronchi (*Bronchus primarius*) stem from the tracheal
57 bifurcation and pass through the lungs from craniomedially to caudolaterally, i.e. from
58 the paleopulmo to the neopulmo. After leaving the respective lung caudally through the

59 *Ostium abdominale*, they continue into the respective abdominal air sac (Fig. 2). As a
60 result, the primary bronchus can be divided into a cranial extrapulmonary part (*Pars*
61 *extrapulmonalis*) and an intrapulmonary part (*Pars intrapulmonalis*). The extrapulmonary
62 part of each primary bronchus is composed of 16-18 incomplete cartilaginous rings that
63 open towards the median plane. It is lined by a pseudostratified ciliary epithelium that
64 contains some goblet cells. A smooth muscle layer can be observed underneath. The
65 intrapulmonary part of the primary bronchus, which is also known as *Mesobronchus*,
66 travels along the ipsilateral pulmonary artery and vein near the ventral side of the lung
67 (Figs. 1 and 2). Halfway its intrapulmonary trajectory, the mesobronchus widens, forming
68 a *Vestibulum*. Its membranous wall is supported by cartilage up to this point (Nickel et
69 al., 1977). The mesobronchus also contains a smooth muscle layer (Barnas et al., 1978).

70 Several secondary bronchi (*Bronchi secundarii*) branch off from the
71 mesobronchus (King and McLelland, 1975). The secondary bronchi that branch within
72 the paleopulmo can, in accordance with the topography of this pulmonary compartment,
73 be grouped as the medial bronchi. The secondary bronchi of the neopulmo are
74 accordingly termed the lateral bronchi (King and McLelland, 1975; König et al., 2007).
75 Furthermore, both groups contain bronchi that travel either in ventral or in dorsal
76 direction. Consequently, medioventral, mediodorsal, lateroventral and laterodorsal
77 secondary bronchi can be distinguished (King, 1979).

78 The medioventral secondary bronchi (*Bronchi medioventrales*) are the first four
79 secondary bronchi that branch off from the mesobronchus, at the level of the vestibulum.
80 As their name suggests, they are responsible for the air supply of the medioventral part of
81 the lung, which entirely consists of paleopulmo. These bronchi are also known as the

82 ventrobronchi (Nickel et al., 1977). The first ventrobronchus has a direct connection with
83 the cervical air sac through a stout branch. In addition, it supplies the clavicular air sac
84 and the cranial thoracic air sac by means of indirect connections formed by tertiary
85 bronchi or parabronchi. The second ventrobronchus is connected by parabronchi to the
86 clavicular and cranial thoracic air sacs. The third ventrobronchus has direct connections
87 with these air sacs. The fourth ventrobronchus has indirect connections with the cranial
88 and caudal thoracic air sacs (King and McLelland, 1975) (Fig. 2).

89 The mediodorsal secondary bronchi (*Bronchi mediodorsales*), usually eight in
90 number, diverge from the dorsal wall of the caudal segment of the primary bronchus.
91 Since they traverse the paleopulmo in dorsal direction, they are also designated by the
92 term dorsobronchi (Nickel et al., 1977). These secondary bronchi have no connections
93 with the air sacs, but have indirect connections with the medioventral secondary bronchi
94 through a series of parallel, cranioventrally running parabronchi (King and McLelland,
95 1975). The plane in which the parabronchi of the medioventral and mediodorsal
96 secondary bronchi anastomose is called the *Planum anastomoticum* of which the *Linea*
97 *anastomotica* is superficially visible (King, 1979).

98 Six lateroventral secondary bronchi (*Bronchi lateroventrales*), also known as the
99 laterobronchi (Nickel et al., 1977), arise from the ventral wall of the caudal part of the
100 primary bronchus and run into the *neopulmo*. The first and second laterobronchi have
101 indirect and direct connections with the caudal thoracic air sac. The last three to four
102 laterobronchi have direct connections with the abdominal air sac (King and McLelland,
103 1975) (Fig. 2). In addition, parabronchial anastomoses are present between the

medioventral and lateroventral secondary bronchi, laterally extending the *linea anastomotica* (King, 1979).

Finally, the laterodorsal secondary bronchi (*bronchi laterodorsales*) leave the caudal segment of the primary bronchus at its lateral wall. They most often comprise five larger bronchi that are located in the cranial part of the *neopulmo* and numerous (approximately 25) smaller bronchi that are situated more caudally (King and McLelland, 1975). The lateroventral and laterodorsal secondary bronchi anastomose by means of irregularly assembled, well-branched parabronchi.

Parabronchi

The parabronchi represent numerous anastomosing small tubes that leave the secondary bronchi. Consequently, these structures can be called tertiary bronchi. However, since some parabronchi directly branch from the main bronchus, the term parabronchi should be preferred (Nickel et al., 1977). Near the clavicular, cranial and caudal thoracic and abdominal air sacs, several parabronchi unite into larger ducts that form recurrent or indirect connections with these air sacs. Such parabronchi are known as *saccobronchi* or *bronchi recurrentes* (Nickel et al., 1977).

Parabronchi present a uniform diameter of 0.5 to 2 mm, according to the species (Schummer, 1973; King and McLelland, 1975) (Fig. 3). As a result, they occupy more than half of the lung volume (Nickel et al., 1977). The lumen is lined by a single squamous epithelium that contains surfactant producing cells. The smooth muscle cells that are located below the epithelium adjust the diameter of the lumen that presents numerous small sacculations, called *Atria*. These are delineated by ridge-like *Septa*

(Nickel et al., 1977; König et al., 2007) (Fig. 4). The atria lead to tapered funnels (*Infundibula*) that conduct the air into small tubes or air capillaries (*Pneumocapillares*) (King, 1979). Since these branch and interconnect with each other, a fine network with 3-10 μm wide meshes is formed (Fig. 5). The air capillaries are lined by a single layer of epithelial cells that resemble the mammalian pneumocyte type I, and are surrounded by an extensive network of blood capillaries (Maina, 2006) (Fig. 6). The efficiency of the gas exchange is enhanced by the transverse directions of the air and blood flows, a physiological process known as the cross-current effect (Liem et al., 2001).

Air sacs

Definition

The air sacs of birds (*Sacci pneumatici* or *Sacci aerophori*) are large, pneumatized, avascular structures that have connections with the bronchial system, but are not involved in gas exchange. However, they are invaluable for the respiration in birds since they act as bellows that generate a cranially oriented unidirectional airflow through the air capillaries of the paleopulmo, both during inspiration and expiration (Nickel et al., 1977). In addition, they support the sound production in many bird species. Furthermore, the air sacs regulate the body temperature, lower the volumetric mass density and redistribute the weight during flying and swimming (Schummer, 1973; Vollmerhaus and Sinowatz, 1992). Their walls are transparent and mainly consist of a single squamous epithelium that is supported by a thin layer of fibrous and elastic connective tissue that can be locally supplemented by muscle fibres (Nickel et al., 1977). However, patches of ciliated epithelium are also present (Bezuidenhout, 2005). Neuroepithelial bodies that function as

mechano- and/or chemoreceptors are distributed in the walls of the air sacs. Since these have connections with the vagal nerve, it is hypothesized that the air sacs influence respiration (Kubke et al., 2004).

The total number of twelve primordial air sacs that are distributed throughout the avian body, from the neck to the pelvic cavity, is in most bird species reduced by fusion. Only *Lari* and *Ciconiiformes* possess pairs of cervical, lateral clavicular, medial clavicular, cranial thoracic, caudal thoracic and abdominal air sacs. Amongst the domestic bird species, the highest degree of reduction is present in the turkey (*Meleagris gallopovo*). The number of air sacs in this species is seven (King and Atherton, 1970). Pigeons and chickens possess nine air sacs (Fig. 7). These include the single clavicular air sac and the paired cervical, cranial thoracic, caudal thoracic and abdominal air sacs (Fig. 8). Most air sacs have one or two direct connections with the primary or a secondary bronchus and several indirect or recurrent connections with the parabronchi (King, 1966; King and McLelland, 1975).

The description of the air sacs below is based on the observations made on corrosion casts of chickens that were previously described by Casteleyn and co-workers (2010).

Clavicular air sac

The single, but bilaterally symmetrical clavicular air sac (*saccus clavicularis*) is located cranioventral to the heart and lungs (Fig. 8). It is almost completely enclosed by the thoracic girdle and the sternum (Fig. 7). Due to the presence of numerous diverticula, the clavicular air sac presents a complex appearance (Fig. 9). Generally, this air sac shows a

cranial part (*pars cranialis*) and a caudal part (*pars caudalis*). The cranial part can be recognized by the central spheroidal compartment (*pars centralis*) that is situated ventral to the trachea, syrinx, oesophagus and the large arteries and veins arising from the heart, and that is bilaterally flanked by a conical diverticulum (*pars cranialis sinister et dexter*) that lies lateral to the trachea and points in cranial direction. The caudal compartment displays an intrathoracic part and a portion that lies almost entirely extrathoracic. The intrathoracic part contains the *diverticulum subcardiacum* (syn. *subcordale*) and the *diverticulum supracardiacum* (syn. *supracordale*), located ventral and dorsal to the heart, respectively. The part that lies mainly extrathoracic presents three diverticula. These include the coracoidal, axillary and humeral diverticula. The conical coracoidal diverticulum (*diverticulum coracoidale*) is located adjacent to the subcordal diverticulum and pneumatizes the coracoid bone, hence its name. The large spheroidal axillary diverticulum (*diverticulum axillare*) surrounds the shoulder joint and consists of a lateral extrathoracic and a medial intrathoracic fragment. Finally, the humeral diverticulum (*diverticulum humerale*) enters the humerus by its *foramen pneumaticum*.

The clavicular air sac is linked to the lung by direct and indirect connections. The indirect connection involves the parabronchi of the first and second medioventral secondary bronchi (*ventrobronchi* I and II) that are located in the cranioventral part of the lung. The direct connection with the third medioventral secondary bronchus (*ventrobronchus* III) is known as the *ostium claviculare* and can be seen near the hilus of the lung (King, 1966; Nickel et al., 1977) (Figs. 2 and 3).

Cervical air sacs

The paired cervical air sac (*saccus cervicalis*) is composed of a central, a thoracic and a cervical part (Fig. 10). The single, conical central part (*pars centralis*) lies dorsal to the trachea at the level of the caudalmost cervical vertebra. It is bilaterally flanked by the cranial part of the clavicular air sac. The paired thoracic segment (*pars thoracica*) extends craniocaudally from the penultimate cervical vertebra to the third thoracic vertebra. Finally, the tubular cervical segment (*pars cervicalis*) comprises a bilateral transverse duct (*ductus intertransversarius*) that reaches the atlas and is accompanied by the vertebral artery and vein. These structures run through the osseous transverse canal (*canalis transversarius*) that is situated within the openings in the transverse processes (*foramina transversaria*) of the consecutive cervical vertebrae. From each of both ducts, supramedullar diverticula (*diverticula supramedullaria*) arise that protrude into the intervertebral openings (*foramina intervertebralia*) between two consecutive cervical vertebrae. These protrusions of the cervical air sac lie dorsal to the spinal cord, against the vertebral arches. In addition to the supramedullar diverticula, several vertebral diverticula (*diverticula vertebralia*) arise from the transversal ducts and envelop the cervical vertebrae.

The cervical air sac has a direct connection with the first medioventral secondary bronchus (*ventrobronchus* I) through the *ostium cervicale*. This connection is located at the cranial pole of the lung, at the level of the pulmonary artery (King, 1966) (Fig. 2).

Cranial thoracic air sacs

The paired cranial thoracic air sac (*saccus thoracicus cranialis*) lies ventral to the lungs and caudal to the subcordal diverticula of the clavicular air sac (Fig. 11). It resides in the

subpulmonal cavity (*cavum subpulmonale*) that is encased by the body wall and the oblique and horizontal septa (Nickel et al., 1977).

Direct and indirect connections between the cranial thoracic air sac and the first, second and fourth medioventral secondary bronchi (*ventrobronchi* I, II and IV) are located at the ventral side of the lung. In addition, the cranial thoracic air sac has a direct connection with the third medioventral secondary bronchus (*ventrobrochus* III) through the *ostium thoracicum craniale*, which is situated at the hilus of the lung (King, 1966, Nickel et al., 1977) (Fig. 2).

Caudal thoracic air sacs

The paired caudal thoracic air sac (*saccus thoracicus caudalis*) is positioned caudal to the lungs and dorsocaudal to the cranial thoracic air sacs within the subpulmonal cavity (Fig. 11). Moreover, these air sacs are situated lateral to the cranial portions of the abdominal air sacs.

At the caudoventral side of the lung, the fourth medioventral bronchus (*ventrobronchus* IV) and first lateroventral bronchus (*laterobronchus* I) have indirect connections with this air sac. The latter secondary bronchus also has a direct connection with the caudal thoracic air sac together with the second lateroventral bronchus (*laterobronchus* II) through the *ostium thoracicum caudale* (King, 1966) (Fig. 2).

Abdominal air sacs

The voluminous paired abdominal air sac (*saccus abdominalis*) extends bilaterally from the caudal side of the lungs to the pelvic cavity (Fig. 8). These air sacs are covered by the

abdominal wall and intertwined with the abdominal organs (Fig. 12). As a consequence, corrosion casts of the abdominal air sacs present impressions of abdominal organs, such as the kidneys at the dorsal side of either abdominal air sac, the liver, the proventricle, gizzard and spleen at the ventral side of the left abdominal air sac, and the intestines at the ventral surface of the right abdominal air sac (Fig. 13).

Both abdominal air sacs present three dorsal diverticula. The bilateral iliolumbar diverticula (*diverticula iliolumbalia*) have their origin at the level of the sixth intercostal space and are situated in the lumbosacral canal. The perirenal diverticula (*diverticula perirenalialia*) surround the separate renal lobes (Fig. 14). Finally, the acetabular diverticula (*diverticula acetabularia*) encompass the hip joints (Fig. 15). At the ventral side of the left abdominal air sac, pronounced gastric diverticula (*diverticula gastrica*) can be observed cranial and medial to the gizzard (Fig. 14).

The abdominal air sac is directly connected with the primary bronchus through the *ostium abdominale*, which is located at the caudal border of the lung. Ventral to this connection, direct connections are present between the abdominal air sac and the last three to four lateroventral secondary bronchi. These connections are supplemented by an indirect connection from the second lateroventral secondary bronchus (*laterobronchus* II) (King, 1966) (Fig. 2).

RESPIRATION

Respiratory movements

The air sacs are of the utmost importance for respiration in birds. Functionally, the air sacs can be grouped in the cranial and caudal air sacs. The cranial air sacs comprise the

cervical, clavicular and cranial thoracic air sacs, whereas the caudal thoracic and abdominal air sacs belong to the caudal group (Fitzgerald, 1969).

Inspiration in birds results from the negative pressure that is created in the thoracic cavity by the action of several muscles (King and McLelland, 1975). The most important inspiratory muscles are the *mm. intercostales externi*, *mm. levatores costarum* and the *m. costosternalis* (Baumel et al., 1990). When these muscles contract, the ribs are displaced craniolaterally. Consequently, the sternum is pushed in cranioventral direction. Indeed, small articulations between the ribs (except the first pair) and the corresponding *ossa sternocostalia* allow the sternum to move (Liem et al., 2001). This results in a lateral and ventral displacement of the abdominal wall. The *m. longissimus dorsi*, that lifts the pelvis, has an additional effect (Baumel et al., 1990). Since the air sacs are partly connected with the ribs, they will expand during inspiration and consequently be filled with air (Bretz and Schmidt-Nielsen, 1971; Bezuidenhout, 2005).

Expiration is the result of external pressure that is exerted on the air sacs. The decreasing volume of the thoracic cavity is partly caused by the contraction of muscles and partly due to the elasticity of the tissues (King and McLelland, 1975). The most important expiratory muscles are the external and internal oblique and transverse abdominal muscles, and the tail muscles (i.e. the *m. caudofemoralis* and the *mm. pubocaudales externus et internus*) (Baumel et al., 1990).

Mechanism of avian respiration

The respiration in birds can be split up in four phases, i.e. two cycles of inspiration and expiration. First, birds that solely possess the *paleopulmo* are considered. During

inspiration (phase 1), the larger volume of air travels through the primary bronchus towards the caudal air sacs, without filling the lung, but expanding these air sacs as a result of the negative pressure created by the inspiratory muscles. A small volume of air travels through the parabronchi of the *paleopulmo* towards the cranial air sacs (Düring and Elemans, 2016). During the subsequent expiration (phase 2), the air present in the caudal air sacs is pressed into the parabronchi of the dorsal secondary bronchi by the action of the expiratory muscles, thus ventilating the lung from caudally to cranially. During the next inspiration (phase 3), the air within the lung is sucked into the cranial air sacs, as a negative pressure is established again. Finally, in the next expiration stage (phase 4), the air within the cranial air sacs is evacuated via the primary bronchus into the trachea and the other upper respiratory pathways. It should be noticed that two respiratory cycles are run before an inhaled air particle leaves the body again. In other words, during each inspiration, both phases 1 and 3 are executed, whereas phases 2 and 4 are run simultaneously during each expiration. As a result, the respiratory tissue is ventilated continuously in caudo-cranial direction, both during inspiration and expiration (König and Liebich, 2001).

In species possessing a *neopulmo*, only part of the inhaled air passes through the primary bronchus into the abdominal air sac, whereas the other part travels in cranio-caudal direction through the parabronchi of the *neopulmo* during phase 1 (inspiratory phase of the first cycle). As a result, the air that is present in the caudal air sacs at the end of the first phase has partly been deoxygenized in the *neopulmo*. In the second phase (expiratory phase of the first cycle), the air is expelled from the caudal air sacs and passes again through the parabronchi of the *neopulmo*, albeit now in caudo-cranial direction.

Due to the collapse of the primary bronchus during expiration (see further), no air that is expelled from the caudal air sacs can pass through the primary bronchus to directly leave the body. The *neopulmo* is thus characterized by a tidal, bidirectional and discontinuous respiration. Inactive birds present only neopulmonic ventilation. Simultaneously with phases 1 and 2, phases 3 and 4 are run, but these have no influence on the ventilation of the *neopulmo*. In rest, the difference in air pressure between the medioventral and mediodorsal secondary bronchi is too low to allow ventilation of the *paleopulmo*. During activity, the air that travels in caudocranial direction through the *neopulmo* enters the parabronchi of the *paleopulmo* before leaving the animal through the trachea. A scheme of the respiration in birds is presented in Fig. 16.

The enigmatic (uni)directional airflow

Three-dimensional reconstructions of the avian respiratory system and computer simulations have confirmed the hypothesis of Hazelhoff (1951) that a cranially oriented unidirectional airflow is present in the *paleopulmo* during both inspiration and expiration (Maina, 2006; König et al., 2007; Moyes and Schulte, 2008; Salomon and Krautwald-Junghanns, 2008; Maina et al., 2009). For many decades, researchers have puzzled over the potential mechanisms that could be responsible for this unidirectional airflow. Although the existence of anatomical valves has been hypothesized (Brandes, 1924; Bethe, 1925; Dotterweich, 1930; Vos, 1934), they have never been observed (Dotterweich, 1936; King, 1966; Duncker, 1971; Jones et al., 1981). Instead, structures that can adjust the diameter of the airways have been detected (Maina, 2002). Just cranial to the branching of the first medioventral secondary bronchus, a protrusion is present in

the wall of the primary bronchus that is larger in the case of quiet respiration compared to high frequency breathing (Wang et al., 1992). This *segmentum accelerans* might be composed of erectile tissue since it contains numerous blood vessels (Maina, 2002). When the tissue swells, the air is deviated from the openings of the secondary bronchi and flows directly to (the *neopulmo* and) the caudal air sacs (Schied et al., 1972).

Since the *segmentum accelerans* is lacking in some species, such as the ostrich (*Struthio camelus*), other mechanisms might play a role in the establishment of the unidirectional airflow (Maina, 2006). Any change in the airflow can be caused by adjustments of the entire diameter of the bronchi. The subepithelial tissue of the extrapulmonary part of the primary bronchus is rich in neuronal tissue that is sensitive to changes in the composition of the air (Bower et al., 1978). In addition, the smooth muscle cells surrounding the openings of the parabronchial atria react to changes in the carbon dioxide concentration of the air (Molony et al., 1976; Barnas et al., 1978). Finally, dynamic compression could play a role. In principle, the diameter of the primary bronchus depends on the difference between the interior air pressure and the exterior pressure that is exerted by the caudal thoracic air sac that is situated caudoventral to the lung. As such, the primary bronchus, especially the part caudal to the *vestibulum* that is devoid of cartilage, can collapse during expiration, forcing the air from the caudal air sacs into the paleopulmonic parabronchi (after having passed through the *neopulmo* when present), while the air from the cranial air sacs cannot flow caudally in the primary bronchus but can only flow cranially and leave the lung via the trachea (Brown et al., 1995).

Schied and co-workers (1972) have demonstrated that the airflow remains unidirectional in fixated lungs, which implicates that dynamic changes in the airways are not solely responsible for the unique flow pattern in birds. In addition, the diameter of the airways fails to present any change during physiological respiration (Jones et al., 1981), which contradicts the principle of dynamic compression. As a result, the unidirectional airflow through the *paleopulmo* seems to be the result of a pressure disequilibrium between the cranial and caudal air sacs (Brackenbury, 1971, 1972; Maina et al., 2009). During inspiration, both the cranial and caudal air sacs are filled with air as a result of the negative pressure created by the inspiratory muscles. However, at any moment during inspiration, the pressure in the caudal air sacs is slightly higher compared to that in the cranial air sacs. As a matter of fact, the caudal air sacs and especially the abdominal air sacs fill prompter as they lie in line with the primary bronchus (Keuthe, 1988). This results in a caudo-cranial unidirectional airflow through the paleopulmonic tertiary bronchi during inspiration. During expiration, the pressure exerted upon the voluminous caudal air sacs is much larger in comparison with the cranial air sacs. As a consequence, a caudo-cranial unidirectional airflow is established in the *paleopulmo* (Brackenbury, 1972). Thus, differences in air pressure between the air sacs and the velocity of the airflow determine the amount and distribution of the air that flows through the bronchi. This principle is called aerodynamic valving (Keuthe, 1988). In brief, the air is prevented to flow directly into the cranial air sacs during inspiration, whereas direct outflow of air through the primary bronchus is avoided during expiration (Düring and Elemans, 2016).

CONCLUSIONS

Respiration in birds is fundamentally different from mammalian breathing. During the entire respiratory cycle, a continuous caudo-cranial airflow is present within the tertiary bronchi of the *paleopulmo*. In contrast, the *neopulmo* is characterized by a tidal airflow. Various anatomical structures that play a role in the establishment of the unidirectional airflow through the *paleopulmo* have been described. However, differences in air pressure between the cranial and caudal air sacs seem to be most important.

Conflict of interest statement

None declared.

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Figures

Fig. 1. Cranial views of frozen anatomical cross sections through the thoracic cavity of a pigeon. A: Unmodified specimen, B: Specimen in which the air sacs have been filled with yellow latex rubber. 1 = thoracic vertebra, 2 and 2' = left and right lung, 3 = oesophagus, 4 = tracheal bifurcation, 4' and 4'' = left and right primary bronchi in the respective lungs, 5 in Fig. A = aortic arch, 6 = cranial thoracic air sac (the arrows in Fig. B indicate the subcordal diverticula of the clavicular air sac), 7 = heart, 8 and 8' = left and right supracoracoid muscle, 9 and 9' = left and right m. pectoralis, 10 and 10' = left and right m. latissimus dorsi. Notice the dorsal localisation of the lungs. The arrowhead in Fig. B indicates the presence of latex rubber in the sternum, which is consistent with pneumatisation of this structure. Adapted from Casteleyn et al. (2011)

Fig. 2. Medioventral view of the right lung of a pigeon. The primary bronchus, pulmonary artery and pulmonary vein have been cannulated by the yellow, red and blue tubes, respectively. The tubular structure of the tertiary bronchi is visible at the lung surface. Full white arrowhead = direct connection between the primary bronchus and the abdominal air sac; empty white arrowheads = direct connections between the lateroventral secondary bronchi and the abdominal air sac; arrow = direct and indirect connections between the lateroventral and medioventral secondary bronchi and the caudal thoracic air sac; box = direct and indirect connections between the medioventral secondary bronchi and the cranial thoracic air sac, oval = indirect connections between the parabronchi of the medioventral secondary bronchi and the clavicular air sac; dashed

arrow = direct connection between the third medioventral secondary bronchus and the cranial thoracic air sac; asterisk = direct connection between the third medioventral secondary bronchus and the clavicular air sac; arrow with bulb end = direct connection between the first medioventral secondary bronchus and the cervical air sac. Adapted from Casteleyn et al. (2011)

Fig. 3. Medial view of a corrosion cast of the right lung of a pigeon. A: The primary bronchus (1) (broken near the hilus) gives origin to secondary bronchi (2) from which numerous tertiary bronchi (3) arise. In the pigeon, the latter have a diameter of approximately 0.7 mm. The primary bronchus travels along the pulmonary artery (a) and vein (v) that are not filled with polymer. B: A secondary bronchus (2) gives off tertiary bronchi (3) of which the atria are visible as minuscule protrusions of the cast. Adapted from Casteleyn et al. (2011)

Fig. 4. Stereomicroscopic view of a cross section through the lung of a pigeon. A: Lower magnification showing the primary bronchus (1), two secondary bronchi (2), two blood vessels (arrows) and numerous tertiary bronchi (asterisks). B: Higher magnification of the tertiary bronchi. The wall of each tertiary bronchus (encircled) presents numerous atria (arrow). Adapted from Casteleyn et al. (2011)

Fig. 5. Light microscopic images of the lung of a pigeon. A: Lower magnification showing the primary bronchus (1), the origin of a secondary bronchus (2) and numerous tertiary bronchi (3). Notice the presence of bronchus-associated lymphoid tissue (arrows)

in the wall of the primary bronchus. Several blood vessels (arrowhead) are scattered throughout the lung parenchyma. B: Higher magnification of the lung parenchyma demonstrating the close association between the tertiary bronchi (3) and the blood vessels (arrows). C: High magnification of a tertiary bronchus. Its wall contains smooth muscle tissue (arrowhead). The atria (asterisks) lead to infundibula (longer arrows) that give access to air capillaries (shorter arrows). Adapted from Casteleyn et al. (2011)

Fig. 6. Scanning electron microscopic views of corrosion casts of the bronchial (A) and vascular systems (B) of a pigeon lung. Fig. A shows the atria (asterisks), infundibula (longer arrows) and air capillaries (shorter arrows) of a tertiary bronchus (3). Fig. B visualizes the capillary meshwork surrounding a tertiary bronchus (3). Adapted from Casteleyn et al. (2011)

Fig. 7. Cranial view of the skeleton of a young chicken demonstrating the topography of the air sacs that are filled with orange polymer. Adapted from Casteleyn et al. (2010)

Fig. 8. Left lateral view of a corrosion cast of the air sacs in a young chicken. Grey = trachea, yellow = cervical air sac (thoracic part), orange = clavicular air sac, blue = lung, red = cranial thoracic air sac, pink = caudal thoracic air sac, green = abdominal air sac. Adapted from Casteleyn et al. (2010)

Fig. 9. Corrosion cast of the clavicular air sac of a young chicken. A: Ventral view showing the cranial part of the air sac that comprises left and right cranial conical

segments (1) which flank the unpaired central segment (2), the paired caudal segment with its intrathoracic supracordal (3') and subcordal diverticula (3''), the intrathoracic (4') and extrathoracic (4'') segments of the axillary diverticulum and the coracoidal diverticulum (5). B: Lateral view showing the extrathoracic segment of the air sac including the axillary diverticulum (4''), the subcordal diverticulum (3'') and the lung (L). The origin of the humeral diverticulum is indicated by the arrow. Adapted from Casteleyn et al. (2010)

Fig. 10. Corrosion cast of the paired cervical air sac. A: Left lateral view of the cervical (1) and thoracic segments (2). The arrow indicates the first rib. B: Right ventrolateral view showing the cranial extension of the conical central segment (asterisk). The arrows indicate their transverse ducts that connect the thoracic and cervical segments. C: Left lateral view showing the left transverse duct (black arrow) within the transverse canal formed and several vertebral diverticula (white arrows) that are located against the vertebral bodies. D: Dorsal view showing the supramedullar diverticula (arrow). Adapted from Casteleyn et al. (2010)

Fig. 11. Left lateral view of a corrosion cast of the thoracic air sacs. The cranial (1') and caudal thoracic air sacs (1'') as well as the subcordal diverticulum of the clavicular air sac (2) and the abdominal air sac (3) are visible. Impressions of the vertebral and sternal ribs are present on the lateral surface of the lung (L) and cranial thoracic air sac (1'), respectively. Adapted from Casteleyn et al. (2010)

Fig. 12. Caudoventral view of the abdominal organs of a young chicken in which the air sacs were filled with polymer. The gizzard (1) and the intestines (2) intertwine with the abdominal air sacs (yellow). Adapted from Casteleyn et al. (2010)

Fig. 13. Corrosion cast of the abdominal air sacs of a young chicken. A: Dorsal view showing the impressions of the cranial, middle and caudal renal lobes (arrows). B: Ventral view showing the impressions of the liver (1), proventricle (2), spleen (3), gizzard (4) and cloaca (5). The arrows indicate the gastric diverticula. Adapted from Casteleyn et al. (2010)

Fig. 14. Left lateral view of a corrosion casts of the abdominal air sacs (1) in a young chicken showing the iliolumbar (2), acetabular (3), and gastric diverticula (4). The renal diverticula are indicated by the arrows. Adapted from Casteleyn et al. (2010)

Fig. 15. Caudodorsal view of a skeleton of a young chicken in which the air sacs are filled with polymer. The acetabular diverticula (arrows) of the abdominal air sacs are clearly visible. Adapted from Casteleyn et al. (2010)

Fig. 16. Schematic representation of the airflow patterns during inspiration and expiration in bird species containing a *Neopulmo*.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

A

B

C

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16