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ANATOMY OF STRUCTURES ASSOCIATED WITH THE LOWER RESPIRATORY TRACT OF THE NORTH AMERICAN OPOSSUM (*Didelphis virginiana*)

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ABSTRACT

This study documents the macroscopic anatomy of the associated structures of the lower respiratory tract of the North American opossum (*Didelphis virginiana*). Cranial deep cervical, cranial mediastinal and tracheobronchial lymph nodes drain the lower respiratory tract. Vascularization of the lungs is via the bronchial artery a branch of the bronchoesophageal artery. The bronchial artery divides into right and left bronchial arteries which follow the branches of the bronchial tree. The pulmonary arteries divide into pulmonary lobar arteries which follow the bronchial division. Pulmonary lobar veins from each lobe of the right and left lungs join to form three pulmonary veins (right, left and middle). The three pulmonary veins join to form a common pulmonary venous trunk that opens into the left atrium of the heart. Sympathetic innervation to the lungs comes from the ipsilateral sympathetic trunks through the mediastinal pleura and pulmonary ligaments as thoracic splanchnic nerves. Parasympathetic innervation to the lungs is via branches from the ipsilateral vagus nerves. The right and left phrenic nerves are formed by components of ventral branches of cervical nerves 2 - 6 which pass through the cervical (C_2-C_4) and brachial (C_5-C_6) plexuses.

Key words: Pulmonary lymph nodes; Vagus and phrenic nerves; North American opossum (*Didelphis virginiana)*

INTRODUCTION

For twenty-five years, the North American opossum has been used in biomedical research in the United States. The extensive use of this marsupial in research has yielded numerous papers in the area of embryogenesis [10,14,16] and yet there have been relatively few anatomical descriptions of macroscopic anatomy. Two sources provide a detailed description on the origin and development of the lymphatic system of the North American opossum [9,18]. Wade and Neely [17] and McClure [11] each briefly describe the pulmonary vasculature while Bernard et al. [3] work provides a brief description of the bronchial artery. The goal of the present study is to provide a complete description of the structures associated with the respiratory system of the North American opossum.

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MATERIAL AND METHODS

Fourteen (7 females and 7 males) North American opossums (*Didelphis virginiana*) were used to study structures associated with the lower respiratory tract. Three of these opossums were used to produce tracheobronchial vascular casts and one was used for a whole body arterial injection. The tracheobronchial vascular casts were produced by injection of latex into the pulmonary vasculature followed by injection of silicone into the airways [6,7]. Prior to arterial injection, the opossum was embalmed with 10% buffered formalin. Twenty-four hours after embalming, latex was injected into the common carotid artery to aid visualization and dissection of the lower respiratory tract vasculature.

RESULTS

Pulmonary Vasculature and Bronchoesophageal Artery

The pulmonary trunk (truncus pulmonalis) bifurcates into right and left pulmonary arteries (arteria pulmonalis dextra et sinistra). The right and left pulmonary arteries cross the ventral surface of their respective principal bronchi (bronchus principalis) and curve laterally then dorsally to

attain a position dorsal to each bronchus. As the right and left principal bronchi (bronchus principalis dexter et sinister) divide into lobar bronchi (bronchi lobares), the right and left pulmonary arteries divide into branches that follow the bronchial division. The right pulmonary artery divides into a cranial lobar branch (ramus lobi cranialis), a middle lobar branch (ramus lobi medii), a caudal lobar branch (ramus lobi caudalis) and an accessory lobar branch (ramus lobi accessorii). The left pulmonary artery divides unevenly into 3 branches. Two smaller branches arise from the left pulmonary artery to supply the cranial and caudal parts of the cranial lobe. The large, third branch of the left pulmonary artery enters the caudal lobe as the caudal lobar branch and sends branches along the caudal lobar bronchial division. In general, the lobar arteries course along the dorsal surface of the lobar bronchi with the exception of the accessory lobar artery. This artery passes between the middle and caudal lobar bronchi of the right lung (pulmo dexter) to course along the ventral surface of the accessory lobar bronchus.

The right, middle and left pulmonary veins, which return blood from the lungs to the left atrium (atrium sinistrum) of the heart (cordis), are formed by the pulmonary lobar veins (Fig. 1). The pulmonary lobar veins lay along the ventral surface of the corresponding lobar bronchi except for the accessory pulmonary lobar vein which is dorsal to the accessory lobar bronchus. The right pulmonary vein is formed by the right cranial pulmonary lobar vein (venae pulmonalis lobi cranialis dextra) and the right middle pulmonary lobar vein (venae pulmonalis lobi medii). In addition, an extra vein from the cranial part of the right caudal lobe may contribute to the right pulmonary vein. The right caudal pulmonary lobar vein (venae pulmonalis lobi caudalis dextra) and the accessory pulmonary lobar vein (ramus lobi accessorii) join to form a middle pulmonary vein. The left pulmonary vein is formed by a branch from the cranial part of the cranial lobe and a branch from the caudal part of the cranial lobe which join the left caudal pulmonary lobar vein (venae pulmonalis lobi caudalis sinistri) (Fig. 1). The right, left and middle pulmonary veins join to form a common pulmonary venous trunk that opens into the left atrium of the heart.

On the right side of the mediastinum, the bronchoesophageal artery (arteria bronchoesophagea) originates from the right, third dorsal intercostal artery (arteria intercostales dorsales) (Fig. 2). The bronchoesophageal artery courses ventrally through the mediastinum across the right side of the esophagus and divides into a bronchial artery (ramus bronchalis) and a common trunk that divides into the esophageal artery (ramus esophageus) and an artery that supplies the right side of the trachea, right vagus nerve (nervus vagus) and right tracheobronchial lymph node (lymphonodi tracheobronchales dexter) (Fig. 2). Before the bronchial artery divides into right and left bronchial arteries, a common branch is given off which passes through the hilus of the right lung (hilus pulmonis) and divides to follow the cranial and middle lobar bronchi and their branches (Fig. 2). After this, the bronchial artery continues caudoventrally through the middle mediastinal pleura (mediastinalis pleura) and divides caudal to the tracheal bifurcation (bifurcatio tracheae) into branches to the right and left lungs (pulmo sinister) (Fig. 3a). The right bronchial artery courses ventrally toward the hilus of the lung through the mediastinal pleura, medial to the right vagus nerve. The right bronchial artery enters at the hilus caudal to the right principal bronchus, divides, and follows the branches of the caudal lobar bronchus. The left bronchial artery continues through the mediastinum to the left side of the thoracic cavity (Fig. 3a and 3b). Caudal to the left principal bronchus it anastomoses with a mediastinal artery (rami mediastinales) and continues ventrally toward the hilus of the left lung. Here the left bronchial artery enters the lung and sends branches to the left bronchial division. The mediastinal artery originates from the caudal surface of the aortic arch (arcus aortae) at the second intercostal space (spatium intercostale). After the anastomosis of the left bronchial artery and the mediastinal artery, a small artery arises from the left bronchial artery just prior to its passage through the hilus of lung. This small artery continues in a caudal direction through the mediastinal pleura and divides into two branches which parallel the right and left vagus nerves (Fig. 3a).

Lymph Nodes of the Lower Respiratory Tract

Cranial deep cervical lymph nodes (lymphonodi cervicales profundi craniales) are present lateral to the laryngotracheal junction and ventral to the longus capitis muscle (Fig. 4). Each

Figure 1. Ventral view of tracheobronchial vascular cast. Right pulmonary vein (R), middle pulmonary vein (M), left pulmonary vein (L), right cranial pulmonary lobar vein (RC), right middle pulmonary lobar vein (RM), extra vein from the caudal lobe contributing to the right pulmonary vein (E), right caudal pulmonary lobar vein (RCd), accessory pulmonary lobar vein (A), venous return from the cranial part of the left cranial lobe (CCr), venous return from the caudal part of the left cranial lobe (CCd), left caudal pulmonary lobar vein (LCd), left atrium (LA), junction of common pulmonary trunk with left atrium (arrows), accessory pulmonary lobar artery (PA), transected accessory lobar bronchus (*).

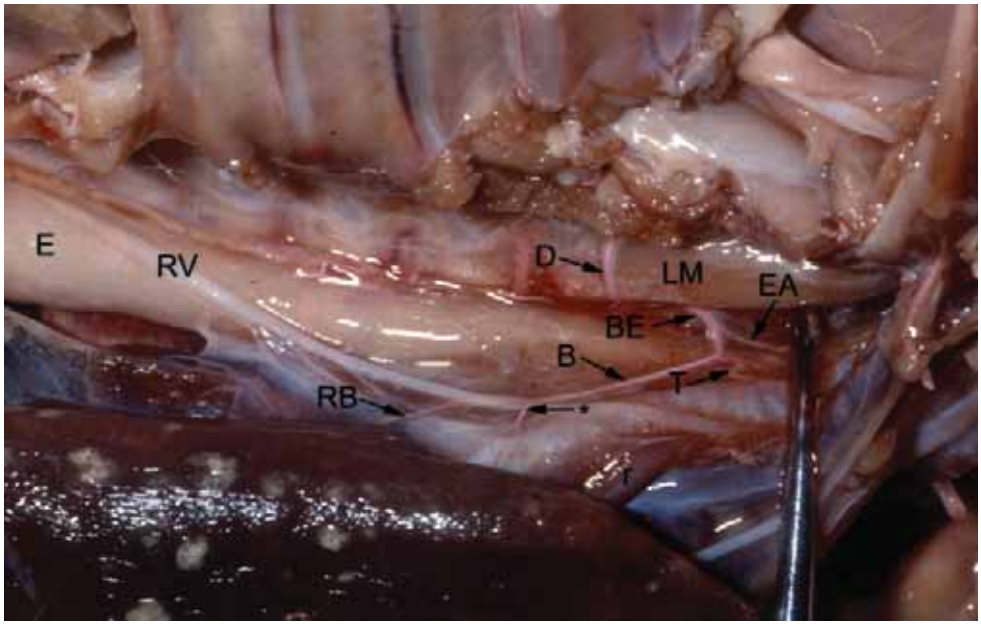

Figure 2. Right lateral view of dorsal mediastinal structures (ribs reflected dorsally). Bronchoesophageal artery (BE), third dorsal intercostals artery (D), esophagel artery (EA), tracheal branches (T), bronchial artery (B), common branch to right cranial and middle lobar bronchi (*), right bronchial artery (RB), right vagus nerve (RV), esophagus (E), right tracheobronchial lymph node (r), longus colli muscle (LM).

lymph node is oval and is 4.31 ± 0.9 mm wide by 11.78 ± 1.3 mm long. Cranial mediastinal lymph nodes (lymphonodi mediastinales craniales) and tracheobronchial lymph nodes are present along the thoracic trachea. The two cranial mediastinal lymph nodes, which lie cranial to the base of the heart (basis cordis) in adipose tissue, are located respectively one each on the right and left ventrolateral surfaces of the trachea at the level of the first to second intercostal spaces (Fig. 5). The cranial mediastinal lymph nodes measure 4.9 ± 0.65 mm in width and 17 ± 0.86 mm in length. The right and left phrenic nerves (nervus phrenicus) course across these lymph nodes. Three tracheobronchial lymph nodes are located in the region of the tracheal bifurcation at the level of the third to fourth intercostal spaces (Fig. 6). The right and left tracheobronchial lymph nodes (lymphonodi tracheobronchales bifurcationis sinister) are 4.84 ± 0.62 mm wide and 9.54 ± 1.69 mm long, oval shaped and lay on the lateral side of the right and left principal bronchi respectively. The middle tracheobronchial lymph node (lymphonodi tracheobronchales bifurcationis medii) is the largest of the three lymph nodes. It is oval shaped and measures 6.3 ± 0.57 mm in width and 14.6 ± .54 mm in length. It is located in the angle of the tracheal bifurcation.

Innervation of the Trachea, Lungs and the Diaphragm

The lower respiratory tract of the North American opossum receives innervation from the sympathetic trunks (truncus sympathicus) and the vagus nerves. The right and left sympathetic trunks lay behind the costal pleura (costalis pleura) lateral to the thoracic vertebral bodies (corpus vertebrae thoracicae). The sympathetic trunks cross the lateral surfaces of the dorsal intercostal vessels (arteriae intercostalis dorsalis) to reach their ipsilateral cervicothoracic ganglion (ganglion cervicothoracicum). The right and left cervicothoracic ganglia each lay deep within the groove formed medially by the ipsilateral longus colli muscle (musculi longus colli) and laterally by ribs (costae) one and two and their associated intercostal muscles (musculi intercostales). The sympathetic trunk continues from the ventral margin of the cervicothoracic ganglion and bifurcates into cranial and caudal loops of the ansa subclavia. The ansa subclavia joins the vagus nerve to form the vagosympathetic trunk (truncus vagosympathicus). From the ganglia of the sympathetic trunk of each side, thoracic splanchnic nerves (nervus splanchnicus) course ventrally through the mediastinal pleura (mediastinalis pleura). Near the hilus, they deviate laterally into the pulmonary ligaments (ligamentum pulmonale) and enter each lung.

As the right vagus nerve crosses the ventral surface of the subclavin artery (arteria subclavia), the right recurrent laryngeal nerve (nervus laryngeus recurrens) leaves the vagus nerve and curves dorsomedially to continue cranially along the right side of the trachea. The left recurrent laryngeal nerve leaves the left vagus nerve near the aortic arch and curves medially then cranially around the aortic arch to continue cranially along the left side of the trachea. After the right and left recurrent laryngeal nerves are dispatched, the ipsilateral vagus nerves continue caudally along the dorsal aspect of the thoracic trachea. Caudal to the principal bronchus of the right and left lungs, three to six vagal branches leave each vagus nerve (Fig. 3b). These vagal branches course through the pulmonary ligament to the hilus of each lung. Caudal to the hilus of the lungs, the right and left vagus nerves continue caudally along the dorsal surface of the esophagus. Approximately 10.0 mm from the diaphragm the left vagus nerve divides into dorsal and ventral branches which course caudally along the esophagus. The right vagus nerve remains undivided. Approximately 6.0 mm from the diaphragm, the right vagus nerve and the dorsal branch of the left vagus nerve join along the dorsal esophageal surface to form the dorsal vagal trunk (truncus vagalis dorsalis). The ventral branch of the left vagus nerve continues caudally along the ventral esophageal surface as the ventral vagal trunk (truncus vagalis ventralis).

Coursing medial to the right and left lungs are the ipsilateral phrenic nerves. The right and left phrenic nerves are formed by components of the ventral branches (rami ventrales) of cervical nerves (nervi cervicales) 2 through 6 which pass through the cervical and brachial plexuses. The contribution of cervical nerves 2, 3 and 4 to the phrenic nerve pass through the cervical plexus. The contribution of cervical nerves 5 and 6 to the

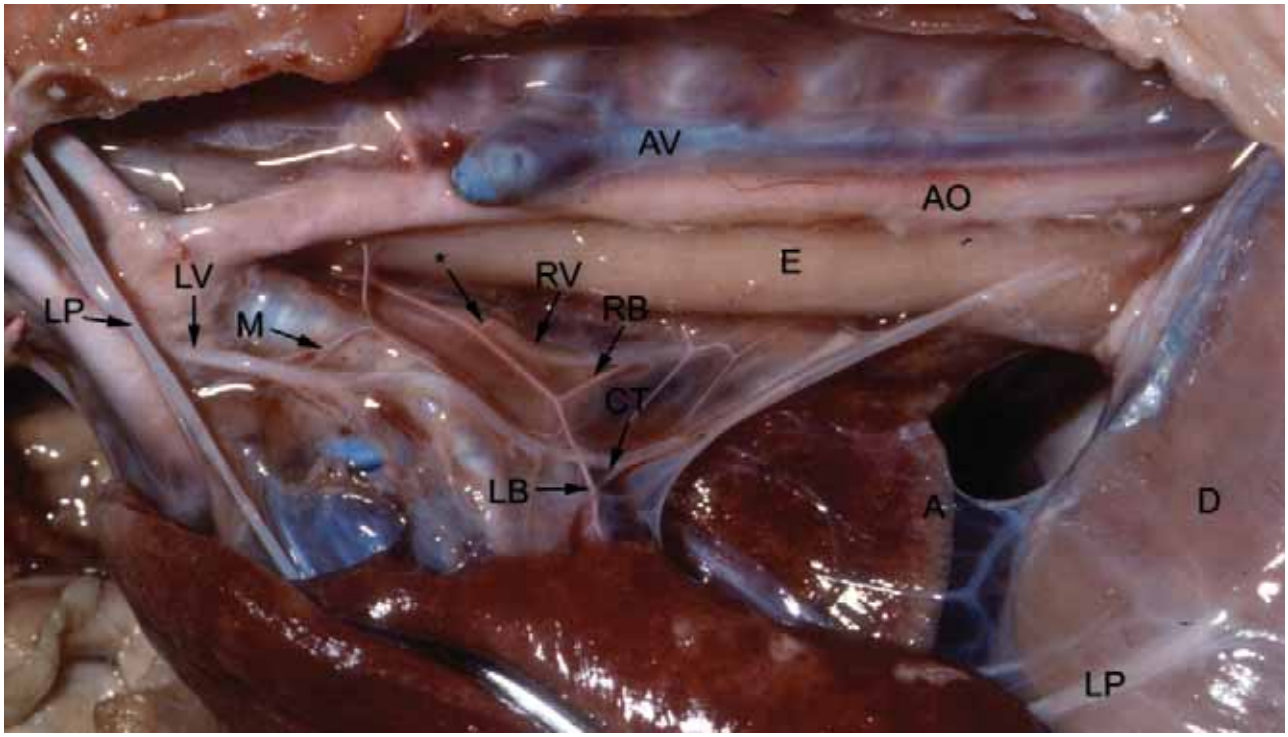

Figure 3a. Left lateral view of dorsal mediastinal structures (dorsal aspect of left lung pulled ventrally). Left bronchial artery (LB), right bronchial artery (RB), common branch to right cranial and middle lobar bronchi (*), mediastinal artery (M), common trunk for small artery from left bronchial artery that divides to follow right and left vagus nerves (CT), left vagus nerve (LV), right vagus nerve (RV), accessory lobe of right lung (A), left phrenic nerve (LP), diaphragm (D), esophagus (E), transected azygous vein (AV), aorta (AO).

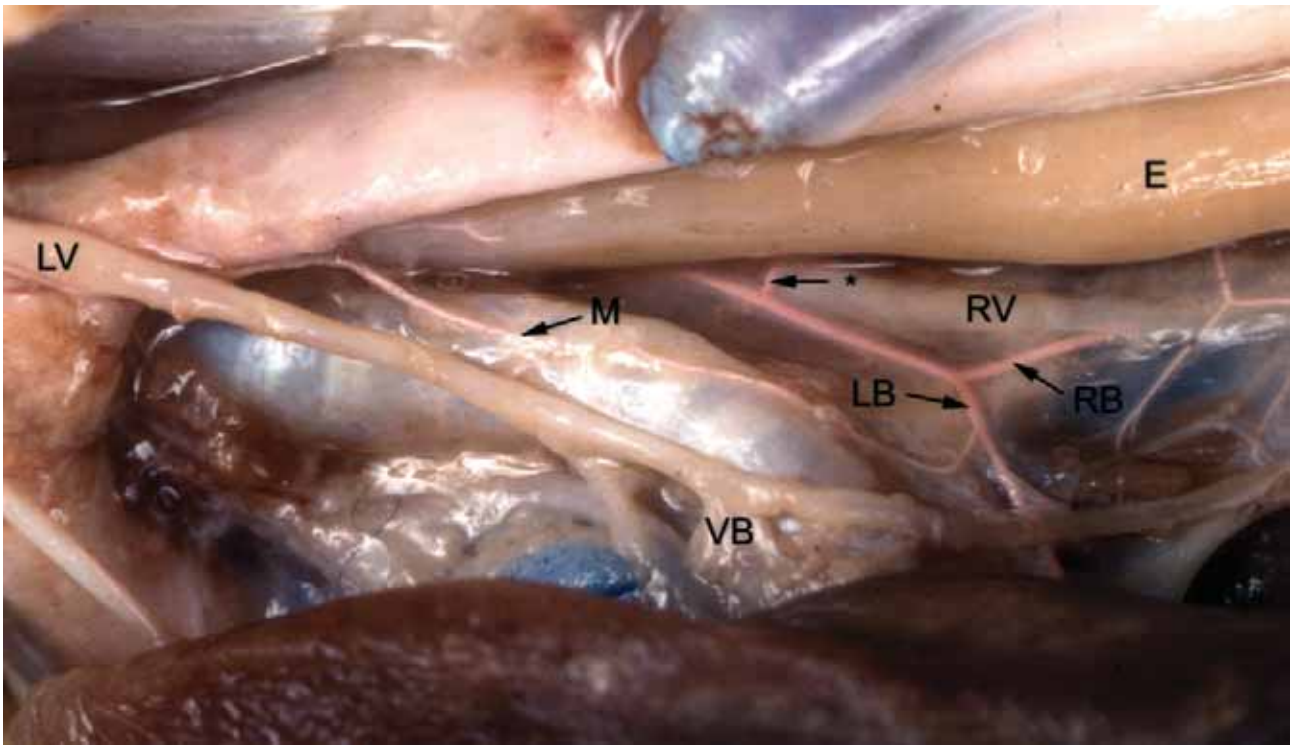

Figure 3b. Close up of lateral view of dorsal mediastinal structures (dorsal aspect of left lung retracted ventrally). Vagal branches (VB) from left vagus (LV) to lung, mediastinal artery (M), common branch to right cranial and middle lobar bronchi (*), left bronchial artery (LB), right bronchial artery (RB), right vagus nerve (RV), esophagus (E).

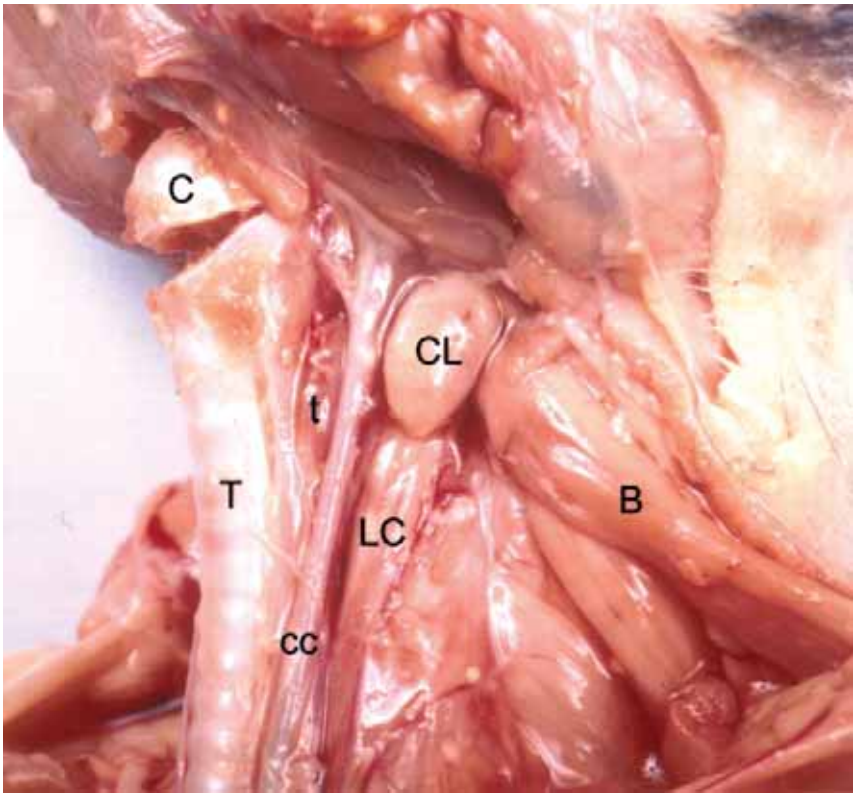

Figure 4. Left ventrolateral view of cranial cervical region. Cranial deep cervical lymph node (CL), transected laryngeal cartilage (C), common carotid artery (cc), thyroid gland (t), brachiocephalicus muscle reflected (B), longus capitis muscle (LC), cervical trachea (T).

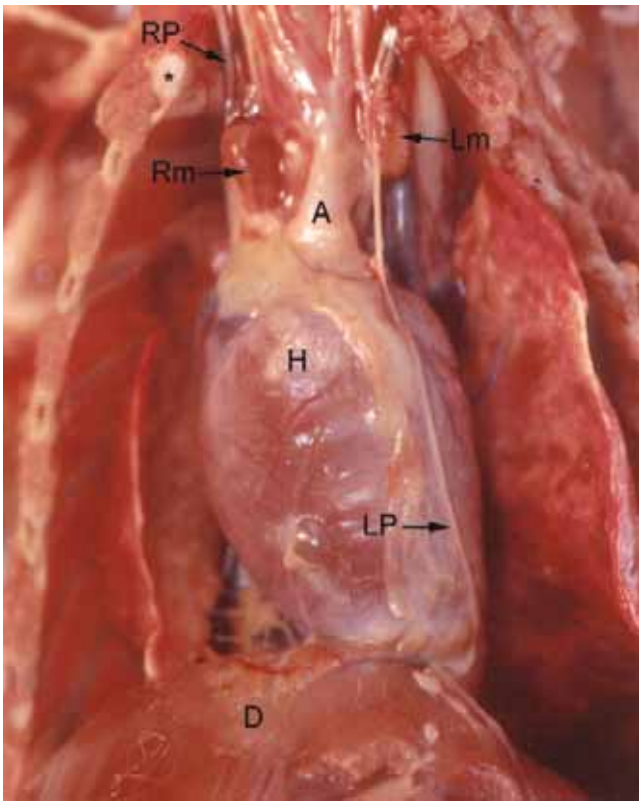

Figure 5. Ventral view of thoracic viscera. Right cranial mediastinal lymph node (Rm), left cranial mediastinal lymph node (Lm), ascending aorta (A), first rib (*), heart (H), diaphragm (D), right phrenic nerve (RP), left phrenic nerve (LP).

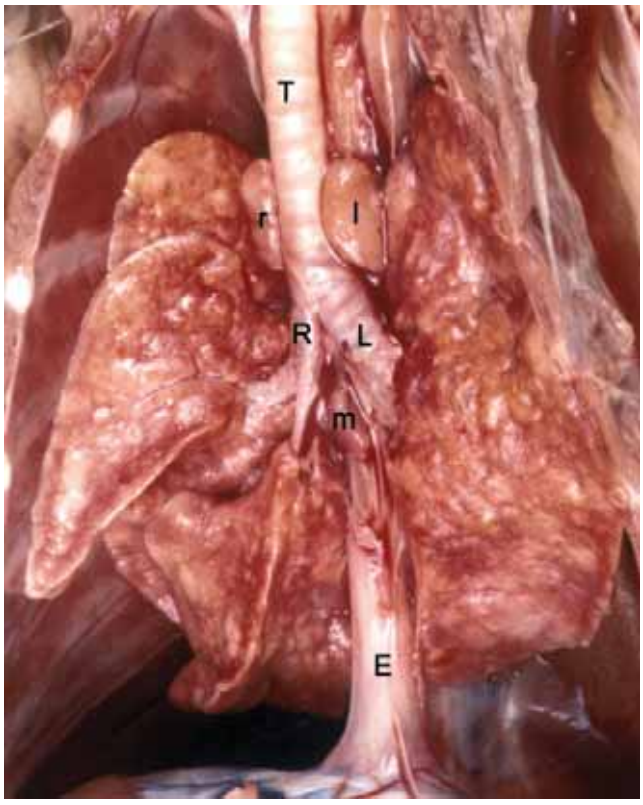

Figure 6. Ventral view of thoracic viscera. Right tracheobronchial lymph node (r), middle tracheobronchial lymph node (m), left tracheobronchial lymph node (l), trachea (T), right principal bronchus (R), left principal bronchus (L), esophagus (E).

phrenic nerve pass through the brachial plexus. As the phrenic nerves course caudally toward the thoracic inlet (apertura thoracis cranialis) they are located medial to the brachial plexus. After passing through the thoracic inlet, the right phrenic nerve inclines over the dorsal aspect of the right atrium (dextrum atrium) to continue along the right side of the caudal vena cava (vena cava caudalis) on its course to the right costal part (pars costalis) of the diaphragm. The right phrenic nerve is anchored by the mediastinal pleura to the parietal pericardium of the heart and the caudal vena cava. The left phrenic nerve, after passing through the thoracic inlet courses, dorsal to the left cranial vena cava (vena cava cranialis). It continues caudally along the left ventral margin of the heart where it is anchored by the mediastinal pleura to the parietal pericardium (Fig. 5). At the apex (apex cordis) of the heart, the left phrenic nerve continues caudodorsally in the caudal mediastinal pleura en route to the left costal part of the diaphragm.

The arterial and nervous supply to the lungs as described was observed in all animals. Additionally, lymph node location and numbers were consistent among all animals.

DISCUSSION

The division of the pulmonary arteries into pulmonary lobar arteries in the North American opossum is similar to that of many domestic mammals as the branching pattern follows that of the bronchi [5,12]. The location of the pulmonary lobar arteries in relation to the bronchi as well as the branching pattern within each lung lobe in the North American opossum is similar to that of the canine [5].

In the North American opossum, pulmonary lobar veins from both lungs join to form three pulmonary veins. These three veins then unite to form a common pulmonary venous trunk before emptying into the left atrium. Previous descriptions of the pulmonary vasculature of the North American opossum report finding a common pulmonary venous trunk in some specimens [11,13,17]. These articles do not describe the vessels which form the common pulmonary venous trunk. Wade and Neely [17] did identify two specimens which had two veins from each lung that joined and formed only two pulmonary veins and in other specimens four pulmonary veins directly entering the left atrium. Hill and Hill [8] and Dowd [4] report that the native cat *(Dasyurus viverrinus)* and the brush-tail opossum *(Trichosurus vulpecula)* also have a common pulmonary venous trunk which is formed by a common right and common left pulmonary vein. Pulmonary venous return as described in marsupials involves some degree of common pulmonary vein formation. These common veins then unite to form a common pulmonary venous trunk. The actual pattern varies slightly among species. We did not find any descriptions which matched that which we observed in the North American opossum.

Concerning blood supply to the lung parenchyma of the North American opossum, Bernard et al. [3] state that either a distinct bronchoesophageal or common bronchial artery originates from the thoracic aorta at the fifth intercostal space. We observed only the former description during this study. In either case, the bronchial artery then divides into right and left branches at the tracheal bifurcation. This division of the bronchial artery described by Bernard et al. [3] is similar to that which we observed in the North American opossum as well as that of many mammalian species [5,12,15]. The anastomosis of the left bronchial artery with a mediastinal artery before the former enters the lung is also seen in the canine [2].

The superior cervical lymph nodes identified by Zimmerman [18] and Kampmeier [9] are likely the cranial deep cervical lymph nodes we observed in the North American opossum. In both cases these lymph nodes are described as lying along the larynx, medial to the cleido-mastoid, cleido-occipital and sterno-mastoid muscle groups. The location of the cranial deep cervical lymph nodes in the North American opossum is similar to those of many domestic carnivores [5,12].

The location of the cranial mediastinal lymph nodes in the North American opossum is similar to the findings of Zimmerman [18] and Kampmeier [9] who refer to these as anterior mediastinal lymph nodes. Kampmeier [9] and Azzali and Didio [1] describe posterior cranial mediastinal lymph nodes in North American opossum and large American opossums of South America (*Didelphis marsupialis* and *Didelphis azarae*). These nodes were not observed in North American opossums utilized in this study.

The three tracheobronchial lymph nodes of the North American opossum are positioned one each

respectively on the lateral side of the right and left principal bronchi and in the angle of the tracheal bifurcation. This positioning is similar that found in many domestic species [5,12,15]. However, in previous reports on the North American opossum [9] and the large American opossums of South America [1], only two bronchial lymph nodes were found. One lymph node was cranial to the tracheal bifurcation and one caudal to the bifurcation.

The innervation to the lung of the North American opossum is unremarkable as it is similar to that of many domestic mammals [5,12].

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