ARTERIAL AND VENOUS VASCULARIZATION OF THE LUNG IN AMERICAN MINK (NEOVISON VISON)

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ABSTRACT

This paper presents a comprehensive anatomic overview of normal pulmonary vascularization in American mink (Neovison vison), with an emphasis on venous and arterial peculiarities as regards its course and potential disruptions. The study is designed as macro-morphological and vascular network analysis of the lungs in fifteen minks of different age and gender. Dissection is conducted along with the injection and corrosion method in order to clearly visualize the vascular topology, bronchial tree, arterial and venous trees and their interconnections. The specimens exhibit consistence in the arterial branching pattern with negligible range of alterations. It was noticed that upon leaving the right ventricle of the heart, the pulmonary trunk divides ventrally from the site of tracheal bifurcation. The divisions were identified as the left and right pulmonary arteries, which then ramified in the left and right lung, respectively. Left a. pulmonalis further divides into two major branches: ramus lobi cranialis and ramus lobi caudalis, while the right a. pulmonalis gives five major branches for the lobes in the right lung. Total of five pulmonary veins leave the pulmonary lobes and enter the left atrium of the heart. Mink has a distinct niche in biomedical research, proving as a suitable model to enhance the understandings of the various diseases. Gained insights are valuable as reference values for examination of the vasculature in other Mustelidae species, recognition of potential deviations and vascular remodeling due to respiratory diseases.

Keywords: Corrosion casts, lung lobes, pulmonary artery, pulmonary veins
INTRODUCTION

The mink is a medium size member of the order carnivore and *Mustelidae* family. The family involves two living species of mink, American mink (formerly referred to as *Mustela vison*) and European mink (*Mustela lutreola*) since the third species, sea mink (*Neovison macrodon*) rapidly became extinct. Reclassification within the family *Mustelidae* yielded American mink separately to belong to its own *Neovison* genus, while European mink remained to be a member of *Mustela* genus (Lopez-Giraldez et al., 2005). Despite the fact that before the divergence they taxonomically were parts of the same genus, there are some differences between these two mink species. The main differences lie in the body size, tail length and white markings on chin and lip surface, justifying that the American mink is bigger in size and exhibits longer tail. American mink proved as a naturally efficient hunter and an aggressive predator having high prey drive (Poole and Dunstone, 1976). Diverse range of prey includes rodents, rabbits, fish and waterfowl (Day and Linn, 1972). It is capable of attacking prey larger than themselves, unlike its European counterpart whose diet includes some vegetation.

The American mink is a mustelid whose native range is North America and Canada, but throughout the twentieth century, the populations were established across Europe (Smal, 1988) and Asia. Its typical habitats include areas with dense vegetation and rocky cover located near streams, rivers or lakes, where they can dig their burrows, and abandoned dens of other animals as well. Mink has proved as a suitable predictive model subject for comparative biomedical research, where they have been successfully applied with the aim to deepen the knowledge and develop adequate treatments. In that manner, they were used as models of human diseases in the field of toxicology (Calabrese et al., 1992), encephalopathy (Marsh, 1972), muscular dystrophy (Hegreberg et al., 1976) and sinonasal and airway inflammation (Kirkeby et al., 2017). The investigation of Aleutian disease and correlation between its manifestations in mink and in human is given in (Jepsen et al., 2009). Due to the human interest for fur industry, minks have become available for many researches as dissection models and in the experimental surgery (Katica and Hadžiomerović, 2020).

Although research literature devoted to mammals appears as fairly voluminous, studies oriented towards mink’s (*Neovison vison*) pulmonary vascularization are less documented. Several studies were conducted regarding mink vascular system including investigation of the hepatic veins (Hadžiomerović et al., 2016) and renal blood vessels (Mrvić et al., 2017). The investigations of the macroscopic anatomy and vasculature of the lungs are available for many domestic and wild animals including dog, fox, goat, wild boar, cat, lynx and pig (Voyevoda et al., 1992; Roos et al., 1999; Cabral et al., 2001; Oliveira et al., 2001; Laakkonen and Kivalo, 2013; Vandecasteele et al., 2015).

The aim of this paper is twofold. First is to provide novel overall insight in normal pulmonary vascular patterns in mink, since numerous studies of the pulmonary vasculature consider the lung arteries, lung veins and bronchial arteries as separate topics. Exposure of complex structure of normal vasculature emerges as powerful for the use of mink as an animal model for studying the pulmonary hypertension, and is suitable for establishment of an appropriate therapeutic approach. Second is to expose macro-morphological features of the lungs and trachea. Comparative analysis of the findings yielded by the obtained conclusions and analogue studies of other mammals found in modern relevant literature is given.
MATERIAL AND METHODS

Minks of both sexes were obtained from a fur farm where the animals were euthanized according to the law, while carcasses remained undamaged (Official Gazette of B&H No. 34/02). Collected samples included fifteen adult minks (ten males and five females), mean body weight 1.55 ± 0.7 kg. The samples were subjected to preparation in the following way: dissection is performed preceding the drying and corrosion techniques. The five lung samples were exposed to the drying process in order to determine the anatomical structure and form of the lungs. Upon removal of the lungs from the thoracic cavity, the process of successive purification involved light water rinse. The lungs were further attached to a source of compressed air for a 2-3 days period to finish the process of drying of the lungs.

Through application of the acrylic monomer (Interacryl cold - Interdent, Slovenia), a powder and solvent form were mixed in 1:2 proportions, and the tracheobronchial impression of the lungs of four animals was established. The mass was previously colored with yellow pigment (Biodur, AC53). The injection method was conducted by using an automatic infusion pump (NE-1000, USA) with the injection pressure set to 99 ml/h. The injected preparation was maintained on ambient temperature for 24 hours, so the polymerisation process could be carried out. Afterwards, the samples were immersed into 30% hydrochloric acid (HCl) solution at room temperature for five days. The immersion completely corroded the lung tissue, while the tracheobronchial impression remained intact.

Next, the three-dimensional (3D) structure of the pulmonary blood vessels was thoroughly examined. By using the method of monomer injection, pulmonary vascularization of six animals were studied. Prior to injection, the prepared monomer was colored with red (Biodur, AC50) for the arteries and blue pigment (Biodur, AC52) for the veins, respectively. The impression of the pulmonary artery was obtained by opening the right ventricle of the heart and attaching cannula at the beginning of a. pulmonalis. In order to clearly visualize branches of the pulmonary veins, the left atrium of the heart was opened and filling with polymer was performed. Under pressure, the polymer filled out the pulmonary veins down to the smallest segments. As a final step of the method, the corrosion of the pulmonary tissue in acid (30% HCl) was conducted. Terminology used in this paper is fully compliant with the current anatomical terminology – Nomina Anatomica Veterinaria (NAV, 2017).

RESULTS

Thorax of Neovison vison is bounded by the ribs, vertebrae and sternum and takes the shape of a pointed, wide-based cone. Cranial part of the thorax narrows towards apertura thoracis cranialis. Caudal part, oriented towards apertura thoracis caudalis, represents the base of the pointed cone, and as such is wide and voluminous. The lungs (Figure 1) are of a typical polylolobular shape and lie in the cavum pectoris section of the thorax. The position of the heart (Figure 1) in Neovison vison is slantwise and quite horizontal. Measured values for the lung weight are 38±0.7 g in male minks, and 25±0.8 g for the female minks. The base of the heart lies cranially and a bit to the right, while the apex is ventrocaudal and entirely to the left. The highest value of the heart weight is measured for male minks (15.1±0.4 g), while the heart of the female minks weighs less more than 60% (9.7±0.2 g). Air dried specimens allowed us to investigate macroscopic
anatomy and lobar division. The lung of a mink is, similarly as in the most of mammals, clearly divided into the separate lobes by deep interlobar fissures. The right lung consists of four lobes: *lobus cranialis*, *lobus medius*, *lobus caudalis* and *lobus accessorius*, while the left lung has only two lobes, *lobus cranialis* and *lobus caudalis* (Figure 1). The trachea connects the upper respiratory airways to the lungs via the tracheobronchial tree. As depicted in Figure 2, the trachea diverges into two principal bronchi (*bronchus principalis dexter et sinister*) at its bifurcation dorsal to the base of the heart. Bronchi are directed towards the hili of the lungs. The principal bronchi give off smaller branches, the left lung has two lobar bronchi for its two lobes, while the right lung bronchi splits into four branches. The lobar bronchi branches give off four major segmental bronchi which radiate dorsally, ventrally, medially and laterally into the parenchyma.

The pulmonary trunk leaves the right ventricle of the heart in order to supply blood to the lungs. Shortly upon exiting, situated ventrally from trachea bifurcation, the trunk splits into two terminal branches, right and left pulmonary arteries, which then get ramified in the left and right lung. Left *a. pulmonalis* (Figure 3), which is 1 cm long, divides into two major branches, *ramus lobi cranialis* and *ramus lobi caudalis*. The caudal branch of the left pulmonary artery gives a small branch for the lower part of the cranial lobe. Right *a. pulmonalis* gives five major branches for the lobes in the right lung. The cranial ramus courses cranially, while the second branch that ramified...
in the lower part of the cranial lobe is directed laterally. The next branch which divides from the right pulmonary artery ramified in the right middle lobe. The remaining two branches are directed toward the right caudal and accessory lobes where they terminate.

Total of 5 pulmonary veins leave the pulmonary lobes and enter the left atrium of the heart. Two larger and one smaller vein enter the heart cranially. The aforementioned larger veins (v. pulmonalis lobi cranialis dextri et sinistri) (Figure 3) transport the blood from the cranial lobes of the right and left lung, while the third, smaller branch (v. pulmonalis lobi medii) comes from the right side and transfer the blood from the right middle lobe. The caudal lobes are drained by v. pulmonalis lobi caudalis sinistri and v. pulmonalis lobi caudalis dextri forming the left and right caudal confluence, respectively. Various numbers of smaller branches originate from V. pulmonalis lobi caudalis sinistri (Figure 3), ending in the left cranial lobe. The vein of the accessory lobe - v. pulmonalis lobi accessorii is formed by the dorsal and ventral segmental vein of the same size. On its path, it is flowing into the right caudal lobar vein. The 4-6 ventral and 6-8 dorsal segmental veins flow into the caudal lobar vein, and the ventral ones follow the corresponding segmental bronchi. The bronchovascular relation is the same in the proximal and distal parts of

**Figure 2** Corrosion cast of the bronchial tree – dorsal aspect. 1. trachea, 2. bronchus principalis dexter et sinister, 3. bronchi lobaes craniales, 4. bronchi lobaes caudales, 5. bronchi lobaes medii, 6. bronchi lobaes accessorii.
the lobe. The arteries closely follow the bronchi and are in close relationship with them, lying within the segments. However, the corresponding veins lie between the subsegments and segments, draining two neighbouring segments. The veins do not directly follow the bronchi and arteries, they are separated from them and independent.

Both of the pulmonary arteries, left and right originate from truncus pulmonalis, at the site of the tracheal bifurcation. The left pulmonary artery crosses dorsally the bronchus of the left cranial lobe and reaches the main left bronchus. It follows the left bronchus laterally and enters the left caudal lobe at the hilus. The corresponding vein lies in the hilus, more medial and ventral to the bronchus. The left pulmonary artery generates a branch for the cranial segment of the left cranial lobe.

At the root of the lobe, the branch is placed near the segmental bronchus. The corresponding vein follows the segmental bronchus, while the artery crosses it. Further, the left pulmonary artery bends in an arch over the vein of the cranial segment of the left cranial lobe. Upon entering the left caudal lobe, the left pulmonary artery spans from the lobar bronchus to margo obtusus. On its path towards the base of the lobe, the artery subdivides into branches which follow the segmental bronchi. Five ventrolateral branches are considerably longer and larger than the dorsomedial seven branches.

The right pulmonary artery crosses ventrally the bronchus of the right cranial lobe and reaches the main right bronchus. It follows the right bronchus laterally through the hilus of the right caudal lobe. The right pulmonary artery generates a branch

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**Figure 3** Corrosion cast of bronchial tree and blood vessels of lung – ventral aspect. Yellow – bronchial tree, red – vv. pulmonales, blue – a. pulmonalis

1. trachea, 2. a. pulmonalis sinistra, 3. a. pulmonalis dextra, 4. v. pulmonalis lobi cranialis sinistri, 5. v. pulmonalis lobi caudalis sinistri, 6. v. pulmonalis lobi cranialis dextri, 7. v. pulmonalis lobi caudalis dextri, 8. v. pulmonalis lobi medii, 9. v. pulmonalis lobi caudalis dextri
which courses from the ventral side of the bronchus to the right cranial lobe. This segmental branch extends dorsally from the corresponding vein. Its root is located far from the segmental bronchus, but crosses it from the medial side on its course. Then, it follows the bronchus close upon the dorsal side towards the lobar apex. The endings of this branch closely follow the corresponding subsegmental bronchi and supply the cranial segment of the right cranial lobe. Immediately next to this branch, the right pulmonary artery produces another smaller segmental branch for the caudal segment of the right cranial lobe, which crosses the vein and extends together with the segmental bronchus. This branch further subdivides into two smaller branches, superior and inferior.

**DISCUSSION AND CONCLUSIONS**

In the present study, it is of interest to emphasize that identical phenomena were observed as the outcomes of the morphologic and vascularization analyses in all samples. Therefore, there was no need to correct gender bias in collected data. The scientific data is lacking the results regarding the lungs of the mink. Various research exploited the vascularization topic, but to the best of the authors’ knowledge, normal pulmonary vascularization of mink wasn’t described.

Normal anatomical imaging of the dog’s thorax is displayed by Rivero et al. (2005), and it should be noted that the lung position regarding the thorax is very similar as the position in mink. The results obtained for dog served as the reference points for comparison with our findings. Left pulmonary artery curves dorsally cranial to the vein from the cranial lobe and bifurcates into two smaller branches for the cranial lobe and a portion that ramifies in the left caudal lobe. The right pulmonary artery runs caudo-laterally, ventral to the lobar bronchi and divides into a branch for the right cranial lobe and 1 cm distally to the origin of this branch, the vessels divide into numerous branches for the lobes of the right lung. The pulmonary veins vary in numbers with the main veins from each lobe (Evans and de Lahunta, 2012). Our study of the mink lung revealed two major branches of the left and five major branches of the right pulmonary artery, while five pulmonary veins entered the left atrium of the heart. The lungs of dog and fox were divided into four lobes on the right and three on the left (Mendoza et al., 2019), while the lynx lung had four lobes on the right and two on the left lung, with the additional subdivision of the cranial lobe by deep interlobar fissure (Laakkonen and Kivalo, 2013). The present study revealed that mink had four lobes on the right and two lobes on the left lung. In pig, venous and arterial branching patterns are comprehensively described (Vollmerhaus et al., 1999; Vandecasteele et al., 2015), outlining that pig is able to meet the requirements for animal model for atrial fibrilation. The tracheal bronchi bifurcate at the level of the heart base into the right and left principal bronchi, which further give off three lobar bronchi of the left and four lobar bronchi of the right lung. The equivalent number of the pulmonary artery branches was documented (Vandecasteele et al., 2015). The present study revealed atypical relation between the number of the lobar bronchi and branches of the pulmonary artery, since the left principal bronchi gives off two and the right principal bronchi gives off four lobar bronchi. The left pulmonary artery divides into two major branches, while the right pulmonary artery ramified into five major branches. Pulmonary vasculature of the goat is discussed by Roos et al. (1999). They described a similar branching pattern of the pulmonary trunk as our study, five branches of the right and three of the left pulmonary artery. These branches supply the individual lobes of the
goat lung which has additional lobes both on the right and left lung, comparing with the mink lung. The benefit of the exposed findings mirrors in contribution to better understanding of the morphological features of the mink lungs and trachea and its pulmonary vasculature, required for the anatomical differentiation with the other laboratory animals. Deep knowledge of normal vascularization is extremely useful for portraying the nature of potential pulmonary vasculature disorders and overcoming the challenges in tissue engineering applications, which is a promising strategy for substituting damaged tissues with functional replacements.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest and they are responsible for all the content of this manuscript.

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Ovo istraživanje predstavlja sveobuhvatni anatomski pregled normalne pulmonalne vaskularizacije američke lasice (Neovison vison) sa naglaskom na venske i arterijske osobitosti u vezi sa njihovim tokom i potencijalnim prekidima. Istraživanje je dizajnirano kao analiza makro-morfoloških i vaskularnih mreža pluća kod petnaest lasica različite starosti i spola. Kako bi se jasno prikazala vaskularna topologija, bronhalno stablo, arterijska i venska stabla te njihova međusobna povezanost, obavljena je dissekcija i primijenjena metoda injiciranja i korozije. Uzorci pokazuju dosljednost u modelu arterijskog grananja sa zanemarivim rasponom razlika. Uočeno je da se pulmonalni trunkus nakon izlaska iz desne komore grana ventralno od bifurkacije traheje. Ogranci su identificirani kao lijeva i desna pulmonalna arterija koje se potom granaju u lijevom i desnom plućnom krilu. Lijeva a. pulmonalis se dalje dijeli na dvije glavne grane: ramus lobi cranialis i ramus lobi caudalis, dok desna a. pulmonalis daje pet glavnih grana za lobuse u desnom plućnom krilu. Ukupno pet pulmonalnih vena izlazi iz plućnih lobusa i ulazi u lijevu pretkomoru. Lasica ima posebnu ulogu u biomedicinskim istraživanjima predstavljajući odgovarajući model za bolje razumijevanje različitih bolesti. Rezultati dobiveni u ovim istraživanjima predstavljaju važne referentne vrijednosti za ispitivanje krvnih žila drugih pripadnika porodice Mustelidae, prepoznavanje mogućih odstupanja i vaskularnih remodeliranja koje uzrokuju različite bolesti.

Ključne riječi: Korozivni odljevi, plućni lobusi, pulmonalna arterija, pulmonalne vene