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Comparative Study of Major Blood Vessels Outgoing from and Incoming to the Heart in Two Snake Species, Boa *Boa constrictor* L. (Boidae) and Corn Snake *Pantherophis guttatus guttatus* (L.) (Colubridae)

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Abstract: The purpose of this study is to examine and compare blood vessels outgoing from and incoming to the heart and lungs in two snake species, *Boa constrictor* and *Pantherophis guttatus*. These snake species are raised as pets and often require veterinary care. Two female snakes were used, in which the major arterial and venous blood vessels were followed through precise macrodissection and radiography following injection of barium sulphate in the dorsal aorta. Significant differences were confirmed in the branching of the left and right aorta in the comparison between the two individual species. The variation of the carotid artery and the ramification from it were followed in the boa, while in the corn snake, the highly developed right jugular vein was observed.

Key words: reptiles, boa, corn snake, common carotid artery, jugular vein.

Introduction

Reptiles are widespread and their share as home-grown animals increases immensely, especially snakes of the families Boidae and Colubridae (O'Malley 2005). The cardiovascular system of snakes is highly peculiar and, on the basis of the heart and lungs topography, three main types have been recognised. The first type is arboreal, with heart located at a point one-third to one-fourth of the body length (Lillywhite 1988, Kik & Mitchell 2005) and lungs with short vascular portions behind heart and long saccular regions, which lack blood vessels involved in gas exchange (Lillywhite 1988). The second type occurs in terrestrial snakes and is characterised by heart near to the body mid-length and lungs with somewhat vascular region near the heart. The third type is characteristic for sea snakes where heart is in the body midpoint and lungs consist almost entirely of vascular tissue (Lillywhite 1988). Jensen et al. (2014a) studied the anatomy and physiology of hearts of squamate reptiles in general and emphasised on the unique features of pythonid snakes and varanid lizard hearts that endow them with mammal-like blood pressures. Except in pythons and varanid lizards, the squamate heart has a highly consistent design including a disproportionately large right side (systemic venous), probably due to prevailing pulmonary bypass (intraventricular shunting). Knowledge of the development, location and topography of the arterial and the venous blood vessels is critical for manipulations, interventions and surgeries in these animals. Snakes have the ability to divert blood from the pulmonary to the systemic blood supply through a shunt from right to left, which increases pulmonary resistance by vaso-

constriction of the pulmonary arteries. This physiological feature may lead to issues in anaesthesia of reptiles, but also is useful in diving, apnoea, chronic pneumonia and lung damage. Thus blood can be directed in areas of the body where it is needed, causing an increase in body temperature. The shunt from left to right stabilises oxygen content in the blood of reptiles (Cadie 1988, Cheek & Richards 2003, O'Malley 2005, Kardong 2008, Jensen et al. 2014a). The pulmonary artery branches in reptiles with two functional lungs arising from the cavum pulmonale and carries deoxygenated blood to the lungs (Kik & Mitchell 2005). All these adaptations are possible owing to the inlet and outlet great vessels of the lungs and heart in reptiles. Typical example studied by Jensen et al. (2014b) is the caval vein myocardium, which provides the sinus venosus functions as a chamber. Blood from the precaval, postcaval and hepatic veins drains into the sinus venosus, a muscular structure located on the dorsal surface of the right atrium (Kik & Mitchell 2005). During atrial diastole, blood drains from sinus venosus to the right atrium. The right atrium of snakes can be larger than the left (Girling & Hynes 2004). Jensen et al. (2003) studied the growth of structures and chambers of the formed heart, two aortas and pulmonary vein and changes in morphology in the model organism corn snake (*Pantherophis guttatus guttatus*). These changes were traced back the development and annotated through interactive 3D pdfs. In the Burmese python (*Python morulus bivittatus*), compartment of the heart, right and left aortic arch, pulmonary trunk and veins with two dimensional echocardiographic cross sections has been reported by Schilliger (2005). The cardiac measurements and description of cardiac anatomy were performed in red-tailed boas (*Boa constrictor constrictor)* using a real-time B-mode ultrasonography (Conceição 2014). Rodrigues (2015) found a negative relationship between body size and heart rate in the *Boa constrictor* and reinforced the influence of related metabolic characteristics.

Van Soldt et al. (2015a) examined the pulmonary left-right asymmetry on the adult and developmental morphology of the lung and pulmonary arteries in the snakes *Python curtus breitensteini*, *Pantherophis guttatus guttatus*, *Elaphe obsoleta spiloides*, *Calloselasma rhodostoma* and *Causus rhombeatus* using gross dissection, MicroCT scanning, Amira segmentation and 3D reconstruction. These authors classified three type of lungs and pulmonary arteries in snakes: right and tracheal lungs with right pulmonary artery which is bifurcated into anterior and posterior branches; right lung and artery with vestigial left lung with faveoli or lacking faveoli as left pulmonary artery absent or minute; right and left lung with bifurcated pulmonary trunk into right and left branches descending on dorsal side of the heart.

A study of the venous vascular system that reveals the presence or variations of major veins is of great importance for the veterinary practice as collecting blood in snakes is performed by puncture of the ventral coccygeal vein and the palatine vein or by accessing the jugular vein in an anesthetised animal (Redrobe & McDonald 1999, O'Malley 2005). The above studies on the circulatory system of reptiles and in particular of snake species have a major scale and the contribution to the discovery of variations of arteries and especially veins in them has substantial significance for both zoomorphology and veterinary practice. Different authors repeatedly described the number and type of developed common carotid arteries as well as their branches compared between the four families of snakes: Boidae, Colubridae, Elaphidae and Viperidae (Bellairs 1969, McDowell 1975, Cadie 1988, Pough et al. 1998, O'Malley 2005, Vitt & Caldwell 2013).

In this article, we report our comparative results of the anatomical and radiographic examination of blood vessels outgoing from and incoming to the heart and lungs in two snake species, *Boa constrictor* and *Pantherophis guttatus*.

Materials and Methods

Animals: two female snakes were used, one was a young and immature 2.5 year old boa *Boa constrictor* Linnaeus, 1758 (Squamata: Boidae) and the other was a 4.5 year old corn snake *Pantherophis guttatus* (Linnaeus, 1766) (Squamata: Colubridae). The owners of the first snake noticed non-specific aggressive behaviour and torticollis pointing towards nervous disorder; treatment was given without improvement, and the outcome was fatal. The second snake had lost its appetite, showed apathy and lethargy again, the assigned treatment was unsuccessful and lead to lethal outcome. Due to nonspecific pre-mortem symptoms, these animals were admitted at the University Clinic of the Faculty of Veterinary Medicine, University of Forestry, Sofia, for expert autopsies. Precise macrodissections were performed, which did not clearly identify visible pathological changes.

Native dissection of blood vessels*:* through routine dissection of the main arterial and venous vessels, their anatomical topography location was followed in both snake species. For better mapping and visibility of the arteries, barium sulphate was in-

jected in advance. Identifiable anatomical structures were marked with the help of anatomical nomenclature (Schaller 2007) and documented with a digital camera (Fine pix C20, 12M pixels, Fujifilm, China).

Radiography (*post mortem* arteriography): it was performed after injection of contrast material (barium sulphate, $BaSO_4$) in the dorsal aorta of the reptiles. We mixed the contrast medium in advance with tap water until the liquid substrate resembled milk texture. Following the introduction of the solution, vessels were ligated. The corpses of the snakes were captured by X-ray machine – Eickemeyer® Vet, model E 7239X in standard orthogonal projections – dorso-ventral and lateral images, in which arteries were very well visualised on radiographs. The available macrodissection results were clarified, supplemented and compared.

Results

By virtue of macrodissection and subsequent radiography of the boa and the corn snake we were able to compare the blood vessels leaving the right and left aorta of both species, which were significantly different.

Starting at cavum venosum of the heart ventricle, the two aortas begin on left and right aortic arch (Fig. 1, 4). In both snake species, the left aorta is larger (Fig. 1, 2, 3), while in the corn snake from it branched: a large left common carotid artery and significantly reduced right one (Fig. 3). In contrast, the left common carotid artery is much smaller and is the only branch of the left aortic arch in the boa (Fig.1, 4).

The smaller right aorta in the corn snake gives the vertebral artery (Fig. 3), while in the boa it gives the large right common carotid artery and the vertebral artery (Fig. 1, 4); these vessels were also identified on radiographs and recognised as the main arteries supplying the cranial third of the body and the head (Fig. 5). Intercostal arteries were observed as branches of the dorsal and the right aorta and the vertebral artery (Fig. 2).

Starting at cavum pulmonale of the heart ventricle, the pulmonary trunk begins, which in the boa gives the left and right pulmonary arteries (Fig. 1). In the corn snake, it gives only the right pulmonary artery due to a strong reduced left lung.

In the sinus venosus of the right atrium in snake's inflow: caudal vena cava, right and left jugular vein (Fig. 2). In the boa, both jugular veins are almost equal with, where the right one being slightly bigger (Fig. 1, 2), while in the corn snake the difference between them is essential, as the left one is marginally larger (Fig. 3). Oxygenated blood from

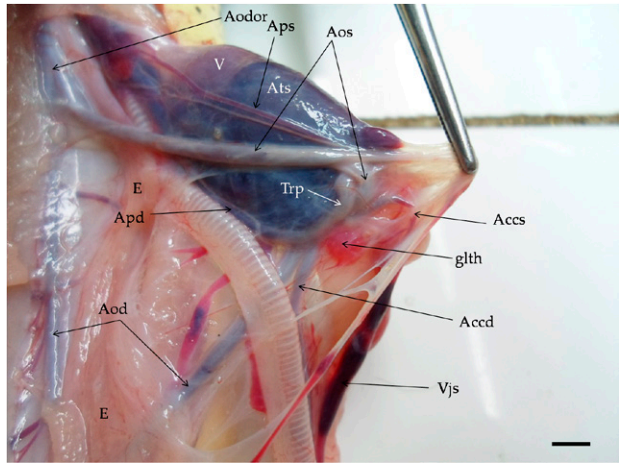

Fig. 1. Heart and blood vessels of *Boa constrictor*. Abbreviations: glth – thyroid gland; E – esophagus; V – ventricle; Ats – left atrium; Aod- right aortic arch; Aos – left aortic arch; Aordor – dorsal aorta; Trp – pulmonary trunk; Aps – left pulmonary artery; Apd – right pulmonary artery; Accs – left common carotid artery; Accd – right common carotid artery; Vjs – left jugular vein. Scale bar 0.8 mm.

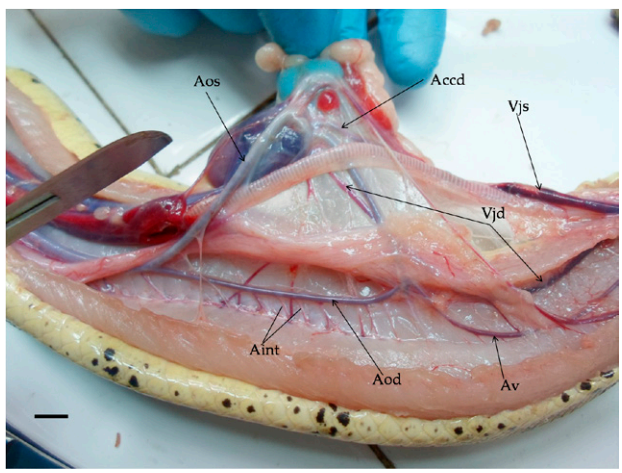

Fig. 2. Heart and blood vessels of *Boa constrictor*. Abbreviations: Aod – right aortic arch; Aos – left aortic arch; Accd – right common carotid artery; Vjs – left jugular vein; Vjd – right jugular vein; Av – vertebral artery; Aint – intercostal arteries. Scale bar 0.5 mm.

the lungs enters the left atrium where in the boa, the left and right pulmonary veins are observed, while in the corn snake again just the right one has been developed.

Discussion

Topography location of the heart and lungs of our specimens of boa and corn snake corresponds fully to the data published by Lillywhite (1988). The heart of the corn snake is located at a point one-third of the body; lungs are with short vascular portions and long

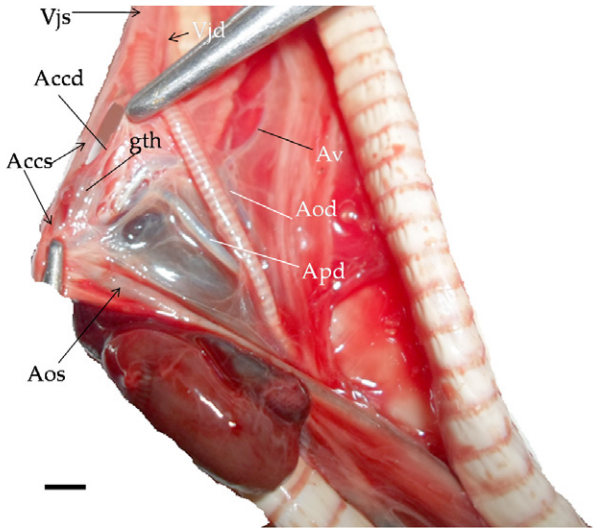

Fig. 3. Heart and blood vessels of *Pantherophis guttatus.* Abbreviations: glth – thyroid gland; Aod – right aortic arch; Aos – left aortic arch; Accs – left common carotid artery; Accd – right common carotid artery; Vjs – left jugular vein; Vjd – right jugular vein; Av – vertebral artery; Apd – right pulmonary artery. Scale bar 0.4 mm.

saccular regions, a pattern classified accordingly as typical for arboreal snake. Blood vessels leaving the two aortas are described in details in the garter snake *Tamnophis elegans terestris* (Colubridae) by Atwood (1916) and were used as a benchmark for comparison with the vessels of the studied snakes. We observed that the left and right aortic branches received blood from the cavum venosum, which matched description by Kik & Mitchell (2005). Development, number and type of carotid arteries were compared among four snake families: Boidae, Colubridae, Elaphidae and Viperidae (Bellairs 1969d, McDowell 1975, Cadie 1988, Pough et al. 1998, O`Malley 2005, Vitt & Caldwell 2013). In the family Colubridae, which includes the garter and corn snake, only the left common carotid artery is developed and the right one is weak and rudimentary (vestigial), supplying with blood only the thyroid gland and it is denoted as a glandular branch (Atwood 1916). In the garter snake, the right aortic arch is branched into the strong left and the weak right common carotid arteries, the vertebral and two intercostal arteries, where the left aorta has no branches (Atwood 1916). The difference in this corn snake is only in the branching of these vessels, namely that the left common carotid artery and the rudimentary right one are branches of the left aortic arch and vertebral artery comes from the right aortic arch. Like the snakes of the family Colubridae, snakes of the family Elaphidae, which are closely related, have a single left common carotid artery

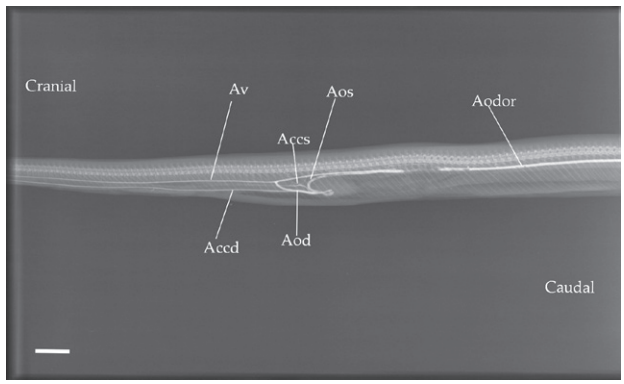

Fig. 4. Lateral radiography of the cranial third of *Boa constrictor*. Abbreviations: Aod- right aortic arch; Aos – left aortic arch; Aordor – dorsal aorta; Accs – left common carotid artery; Accd – right common carotid artery; Av – vertebral artery. Scale bar 2.5 cm.

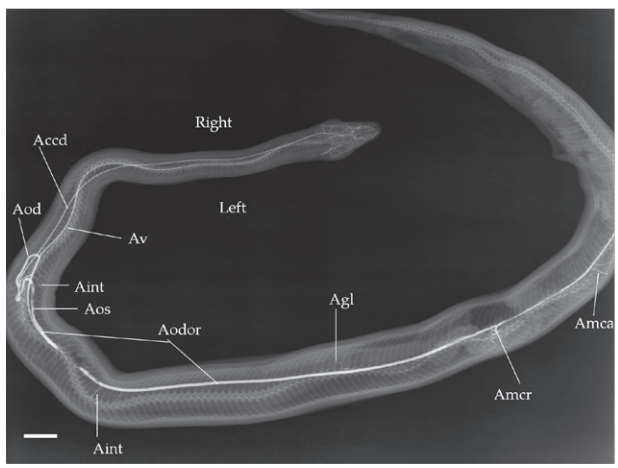

Fig. 5. Dorsoventral radiograph of *Boa constrictor*. Abbreviations: Aod- right aortic arch; Aos – left aortic arch; Aordor – dorsal aorta; Accs – left common carotid artery; Accd – right common carotid artery; Av – vertebral artery; Aint – intercostal artery; Agl- gastrolienal artery; Amcr – cranial mesenteric artery; Amca – caudal mesenteric artery. Scale bar 2.5 cm.

(Cadie 1988, Pough et al. 1998, O`Malley 2005). This is not the case for the snakes of the family Boidae, where two developed common carotid arteries have been described (Bellairs 1969d, Pough et al. 1998, O`Malley 2005, Vitt & Caldwell 2013). However, in our study, only one right common carotid artery leaving the right aorta was observed (Fig. 4, 5). Similar ramification is described in snakes of the family Viperidae by McDowell (1975) and Cadie (1988), while retention of a right common carotid in the neck of snakes with lesser asymmetry as boids, pythons and *Xenopeltis* is indicated by Bellairs & Underwood (1951) and Farmer (2011), without being categorically or mandatory specified for *Boa constrictor*. In this boa, the left common carotid ar-

tery was weak, supplied the thyroid gland and was a branch of the left aortic arch, which was completely opposite to that observed in the garter snake. From the right aorta before its merging with the left aorta in the dorsal aorta in the boa begin the vertebral artery and 9-10 intercostal arteries, while in the garter snake, they are only two (Atwood 1916). In all three types of snakes where parietal (intercostal) branches dividing from the left aortic arch are not observed.

The cardiopulmonary systems of boids, pythons and *Xenopeltis* are less evolved than those in the majority of the other snakes. These differences include: persistence of two lungs with fewer asymmetry in size than observed in other snakes, retention of a left pulmonary artery, left and right aortae of similar diameters (Bellairs & Underwood 1951, Butler 1895, Farmer 2011). Significant asymmetry in both the development of lungs and in diameters of the aortae is seen in majority of snake species, where the diameter of the left aorta is over three times greater than the right one in some Colubridae and Crotalinae (Ray 1934, Van Bourgondien & Bothner 1969, Farmer 2011). Our study showed similar results: larger left aortic arch in the boa and corn snake. In the families Colubridae, Elaphidae and Viperidae only the right aorta is developed, while the left one is completely rudimentary (Pough et al. 1998, O`Malley 2005, Kardong 2008), as observed also in the corn snake, we identified only the right pulmonary artery and vein. Van Soldt et al. (2015a) confirmed that right lung and pulmonary artery are developed the more evolved snake *Pantherophis guttatus guttatus* and the same authors found vestigial left lung with faveoli and vascularisation from the minute left pulmonary artery in the corn snake. Similar development or rudimentary left lung without vascularised faveoli, absent left pulmonary artery is observed in Colubrinae, Natricinae and Elaphinae (Van Soldt et al. 2015a). The less evolved snakes of the family Boidae have two lungs (Pough et al. 1998, O`Malley 2005, Kardong 2008), where the right one is more developed and the left one can reach 85% of the size of the right one (Mitchell 2003). This was confirmed by Van Soldt et al. (2015a) also in the boa, together with the availability of two (left and right) pulmonary arteries bifurcating by pulmonary trunk and pulmonary veins. Similar development is present in Boinae and Pythoninae (Schilliger 2005, Van Soldt et al. 2015a). Another study explored wall thickness of the two pulmonary arteries in *Python regius* (Van Soldt et al. 2015b). Other type is observed in the Hydrophiinae, Acrochordidae and Crotalinae, where pulmonary trunk continues in the right and separates into an anterior branch toward the tracheal lungs and posterior branch toward the right lung (Van Soldt et al. 2015a). The latter type is not found in the boa and corn snakes studied by us. The last typifications correspond to the published about the Crotalinae (Van Bourgondien & Bothner 1969), Viperidae (Brongresma 1949) and also about snakes with two lungs and associated pulmonary arteries (Brongresma 1951, 1957). Significant differences in the third type were observed in the Viperidae within the dorso-ventral position of the pulmonary vessels (Brongresma 1949).

Many authors describe two jugular veins or precaval veins finishing into sinus venosus in snakes (Kik & Mitchell 2005), while favouring a stronger development of the right one (Atwood 1916, Cheek & Richards 2003, Mader 2006) was confirmed in the corn snake by us. This was insignificant and smaller in our boa: two veins were almost equally developed, where the right one was slightly stronger.

Conclusion

Our paper supports the conclusion that the left common carotid artery is present in snakes of the family Colubridae. In contrast, in the family Boidae, there are developed two common carotid arteries and only the right common carotid artery was observed in the studied boa. This fact can be considered as an anatomical variation or as an evolutionary novelty in the less evolved snakes of the family Boidae. It should be noted that in the corn snake the access to the jugular vein is on the right, while in the boa constrictor it may be bilateral. With this study, we aim to give a boost to in-depth and extensive research of the blood vessels of the different snake families as they represent not only purely anatomical, but also medicinal, zoomorphological and evolutionary interest.

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