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MORPHOLOGY OF THE LYMPH NODES IN BOTTLENOSE DOLPHIN *(TURSIOPS TRUNCATUS)* AND STRIPED DOLPHIN *(STENELLA COERULEOALBA)* FROM THE ADRIATIC SEA

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Morphology of the lymph nodes was examined in six bottlenose dolphins *(Tursiops truncatus)* and three striped dolphins *(Stenella coeruleoalba)* from the Adriatic Sea. All animals had been found dead in nature. One group of the nodes was taken from the tracheal branching area and was marked as bifurcational lymph node, and the other group was taken from the mesenteric root and was marked as mesenteric lymph node. Microscopic analysis showed that the lymph nodes in both dolphin species were surrounded by a connective tissue capsule comprising smooth muscle cells. The parenchyma of the mesenteric and bifurcational lymph nodes in bottlenose dolphin was divided into the peripherally situated cortex with the lymphatic nodules and diffuse lymphatic tissue, and the centrally situated medulla structured of the medullary cords separated by the medullary sinuses. These lymph nodes structurally correspond to the lymph nodes in the majority of terrestrial mammals. The mesenteric lymph node of striped dolphin also had a peripherally situated cortex and a centrally positioned medulla as the majority of terrestrial mammals. In the bifurcational lymph nodes of striped dolphin, there was a central dense lymphatic tissue with the lymphatic nodules and a peripheral less dense lymphatic tissue structured of the cell cords and sinuses. The bifurcational lymph node in striped dolphin resembled porcine lymph nodes and belonged to the inverse lymph nodes.

Key words: Lymph node, bottlenose dolphin, striped dolphin, terrestrial mammals, pig

Lymph nodes are small organs incorporated in the lymphatic system. Their parenchyma comprises highly organised lymphatic tissue consisting of the cortex and medulla. The cortex is structured of the lymphatic nodules and a diffuse lymphatic tissue, and the medulla comprises the cords of the diffuse lym-

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phatic tissue. This typical structure of the lymph nodes is common to the majority of mammals. Volume and distribution of the cortex and medulla of the lymph node tissue may vary significantly between individuals of the same species, depending on the stage of activity and the position of the lymph node. Lymph nodes may vary structurally between animal species as well. Hence, pigs have lymph nodes of atypical, inverse form (Nicander et al., 1993; Raviola, 1994).

Data about the lymph nodes of cetaceans are very scarce. Moskov et al. (1969) described the lymph nodes of common dolphin *(Delphinus delphinis)* and harbour porpoise *(Phocena phocena)*, pointing out their structural similarity with lymph nodes of the pig. Simpson and Gardner (1972) claim that dolphin lymph nodes are typically structured. They described a thin cortex without any mention of the lymphatic nodules in it and gave the details of the dilated sinuses. Romano et al. (1993) believe that the lymph nodes of the beluga *(Delphinapterus leucas)* are not of inverse form. Cowan and Smith (1999) described typical structure of the lymph nodes with incomplete subcapsular sinuses in bottlenose dolphin *(Tursiops truncatus)*.

Given the markedly different descriptions of the lymph nodes in cetaceans, we thought that microscopic examination of some lymph nodes in bottlenose dolphin *(Tursiops truncatus)*, representative of the resident population, and in striped dolphin *(Stenella coeruleoalba)*, an occasional inhabitant of the Adriatic Sea, might be of value.

Materials and methods

The present study included nine animals: six bottlenose dolphins and three striped dolphins. Both species are under the most stringent protection in Croatia (Rules on the Protection of Some Mammals, Law on Nature Protection, Republic of Croatia, 1995). All the studied animals had died in nature and were found in different places along the Croatian Adriatic coast. They were brought to the Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, where external measurements according to Perrin (1975) were carried out. Age assessment by growth-layer groups in the dentine according to Slooten (1991) was also performed.

The bottlenose dolphins studied were marked in the protocol as D41, D72, D80, D87, D96 and D103. Bottlenose dolphin D41 was an about 12 years old, 261 cm long female of 224 kg body mass. Bottlenose dolphin D72 was an about 10 years old, 235 cm long male of 153 kg body mass. Bottlenose dolphin D80 was an about 18 years old, 294 cm long male of 324 kg body mass. Bottlenose dolphin D87 was a 188 cm long female of 79 kg body mass; unfortunately, her age could not be assessed. Bottlenose dolphin D96 was an about 12 years old, 268 cm long female of 169 kg body mass. Bottlenose dolphin D103 was a juvenile male about 186 cm long and of 101 kg body mass.

The striped dolphins studied were marked in the protocol as D74, D79 and D89. Striped dolphin D74 was an about 15 years old, 199 cm long female of 100 kg body mass. Striped dolphin D79 was an about 22 years old, 198 cm long female of 91 kg body mass. Striped dolphin D89 was an about 23 years old, 209 cm long male of 98 kg body mass.

Samples of the lymph nodes were taken for histological examinations. One group of the nodes, taken from the tracheal branching, was marked after *Nomina Anatomica Veterinaria* as bifurcational (tracheobronchial) lymph nodes. Another group was taken from the mesenteric root and designated as mesenteric lymph nodes according to *Nomina Anatomica Veterinaria* (1994). For morphological examination of the lymph nodes, tissue samples were fixed in 4% neutral formaldehyde solution, embedded in paraffin and cut into 6 µm thick slices. The slices were stained with haematoxylin and eosin (HE), by Mallory's method to demonstrate collagen fibres, and with Masson's trichrome stain to differentiate collagen fibres from the smooth muscle cells (Romeis, 1968). The specimens were analysed under Nikon Microphot-FXA light microscope and under Nikon-SMZ-U stereomicroscope.

Results

In all examined bottlenose dolphins, the mesenteric lymph nodes were surrounded by a capsule of dense connective tissue. The trabeculae extended from the capsule towards the interior of the organ. The capsule comprised numerous smooth muscle cells, distributed mostly along the inner edge. Smooth muscle cells occurred in the trabeculae as well. The mesenteric lymph node of all studied animals had a thick capsule, with the exception of the juvenile animal marked D103 that had a thin capsule with a few smooth muscle cells (Fig. 1). The parenchyma of the mesenteric lymph node in all examined bottlenose dolphins was divided into the peripherally located cortex and the centrally positioned medulla. In all animals, the cortex of the mesenteric lymph node was very thick and filled densely with lymphatic nodules and diffusely arranged lymphocytes. The cortex contained mostly secondary nodules with primary ones here and there. Medullary cords projected from the cortex of the mesenteric lymph node towards the centre of the organ. Subcapsular, intermediary and medullary sinuses were well marked. No structural difference could be recorded in the parenchyma of the mesenteric lymph node between the young and the adult bottlenose dolphins.

In all bottlenose dolphins studied, the bifurcational lymph node was surrounded by a dense connective tissue capsule with trabeculae diffusing towards the interior. In bottlenose dolphins the capsule of the bifurcational lymph node was thinner than that of the mesenteric lymph node. Smooth muscle cells were individually distributed within the capsule but towards the subcapsular sinus there was a thin, continued layer of smooth muscle cells (Fig. 2). The cortex of

the bifurcational lymph node in these animals was situated peripherally and was filled mostly with the secondary nodules. In the interior of the organ there was the medulla with medullary cords and medullary sinuses.

In all examined striped dolphins, the mesenteric lymph nodes were surrounded by a thin capsule structured of a dense connective tissue with individual smooth muscle cells. Striped dolphin D89 had a thin layer of smooth muscle cells at the surface of the capsule (Fig. 3). The mesenteric lymph node of all striped dolphins had a thick, peripherally situated cortex with many secondary nodules and diffuse lymphatic tissue, as well as a well-formed trabecular network. The medulla was structured of the medullary cords and medullary sinuses. Subcapsular and intermediary sinuses could be recorded.

Structurally, the bifurcational lymph node of striped dolphin essentially differed from the mesenteric lymph node (Fig. 4). There was a strong trabecular network running through the centre of the lymph node with a dense lymphatic tissue that contained the secondary nodules and diffusely arranged lymphocytes around it. The intermediary sinus was situated between the trabeculae and a dense lymphatic tissue. A less dense lymphatic tissue structured of the cell cords and sinuses was situated peripherally (Fig. 5). A very thin capsule with smooth muscle cells here and there surrounded the bifurcational lymph node in all striped dolphins studied. On the surface of the lymph node efferent lymphatic vessels could be seen (Fig. 6). The possible morphology of bifurcational lymph node in striped dolphins is demonstrated by the schematic diagram (Fig. 7).

Discussion

The structure and distribution of the cortex and medulla in the mesenteric and bifurcational lymph nodes of all studied bottlenose dolphins corresponded to those found in the majority of terrestrial mammals. The lymph nodes of terrestrial mammals are surrounded by a dense connective tissue capsule with a few elastic fibres. It is a known fact that humans (Leeson et al., 1988) and ruminants (Nicander et al., 1993) have smooth muscle cells as well. The trabeculae extended from the capsule towards the interior of the organs where they branched and mutually anastomosed. The space between the capsule and the trabeculae was filled with dissimilarly distributed lymphatic tissue. It has been shown that in the majority of terrestrial mammals there are two areas that are clearly separated, a peripherally situated cortex and a centrally positioned medulla. The cortex of a lymph node is organised into an outer cortex consisting of a network of reticular cells and reticular fibres with the primary and secondary lymphatic nodules, and a deep cortex consisting of diffuse lymphatic tissue. The centre of the lymph node comprised medullary cords arising from the lymphatic tissue of the deep cortex, separated by medullary sinuses (Belisle and Saint-Marie, 1981; Nicander et al., 1993; Junqueira

et al., 1995). Between the capsule and the surface of the outer cortex there was a subcapsular sinus for direct receipt of the afferent lymph from the afferent lymphatic vessels. Intermediary (trabecular) sinuses, parallel with the trabeculae, connected that sinus with the medullary sinuses. In the majority of mammals, lymph nodes are known to have afferent and efferent lymphatic vessels. The approach of the afferent lymphatic vessels (vas lymphaticum afferens) is on the convex side of the lymph node, opposite the hilus, from where they pass through the capsule and deliver the lymph to the subcapsular sinus. From the subcapsular sinus the lymph passes to the intermediary and medullary sinuses. The efferent vessels (vas lymphaticum efferens) collect the lymph and leave the lymph node in the hilar region (Heath and Perkins, 1989; Banks, 1993; Junqueira et al., 1995).

In their description of the mesenteric, periaortic, cervical and parotid lymph nodes in the Atlantic bottlenose dolphin *(Tursiops truncatus)*, Hawaiian spinner dolphin *(Stenella longirostris)*, common dolphin *(Delphinus delphis)*, Amazon river dolphin *(Inia geoffrensis)* and pilot whale (*Globicephala* sp.), Simpson and Gardner (1972) arrived at the conclusion that their structure was typical of the lymph nodes seen in the majority of terrestrial mammals. Romano et al. (1993) came to a similar finding in their study of the lymph nodes of the cervical, thoracic and abdominal area in beluga *(Delphinapterus leucas)*, and Cowan and Smith (1999) concluded the same about the structure of lymph nodes of bottlenose dolphin.

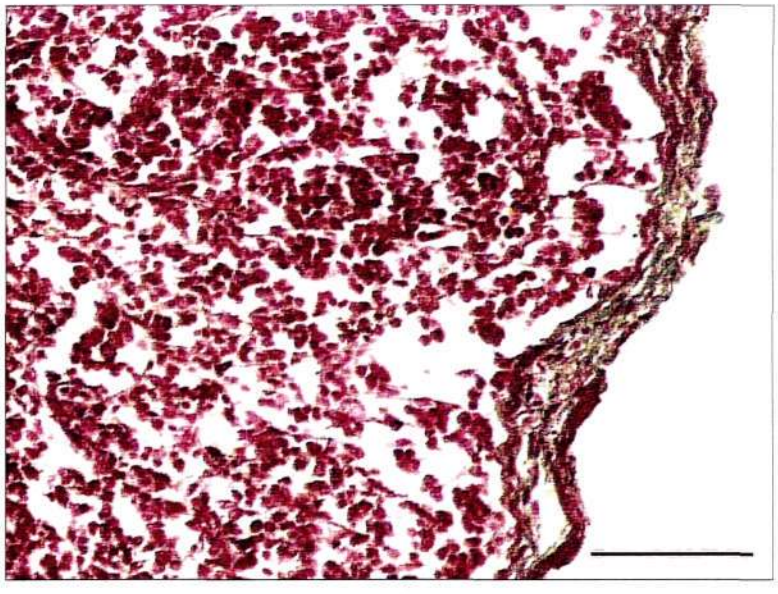

Fig. 1. Mesenteric lymph node. Bottlenose dolphin *(Tursiops truncatus)* D103, juvenile dolphin. The thin connective tissue capsule comprises individual smooth muscle cells. There is cortex of the lymph node with a subcapsular sinus below the capsule. Masson's trichrome stain; 20 × 2.5; bar scale = 100 μm

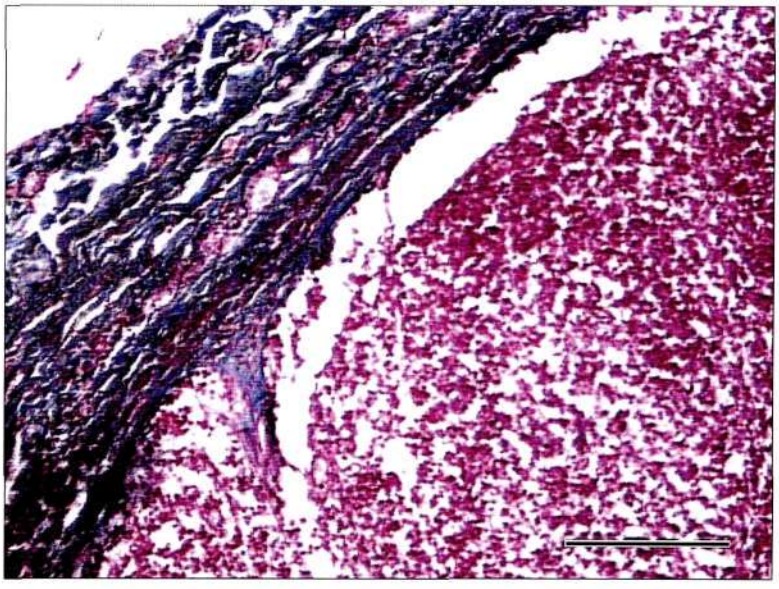

Fig. 2. Bifurcational lymph node. Bottlenose dolphin D87, female. Thin and continuous line of smooth muscle cells is situated along the inner edge of the connective tissue capsule. Masson's trichrome stain; 10 × 2.5; scale bar = 200 μm

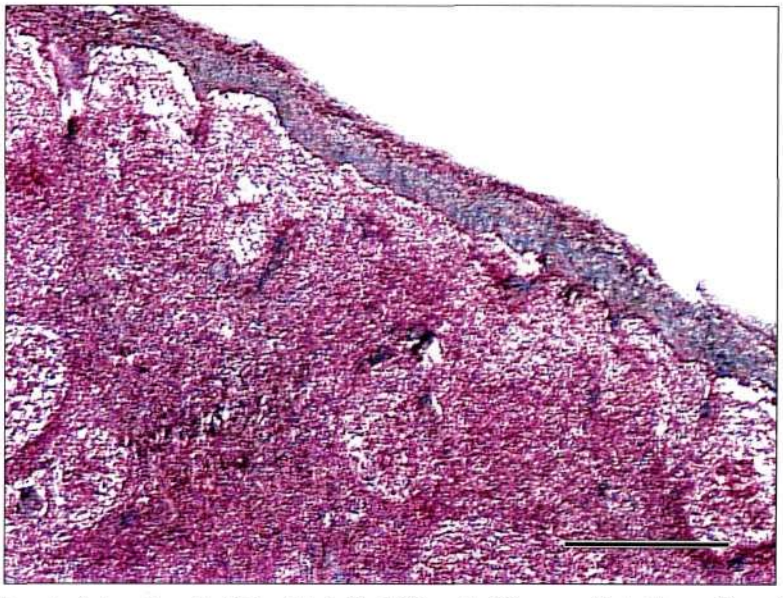

Fig. 3. Mesenteric lymph node. Striped dolphin D89, male, 23 years old. At the surface of the thin connective tissue capsule a layer of smooth muscle cells appears. The cortex of the lymph node, with secondary nodules and diffuse lymphatic tissue, is located peripherally. Masson's trichrome stain; 4 × 2.5; scale bar = 500 μm

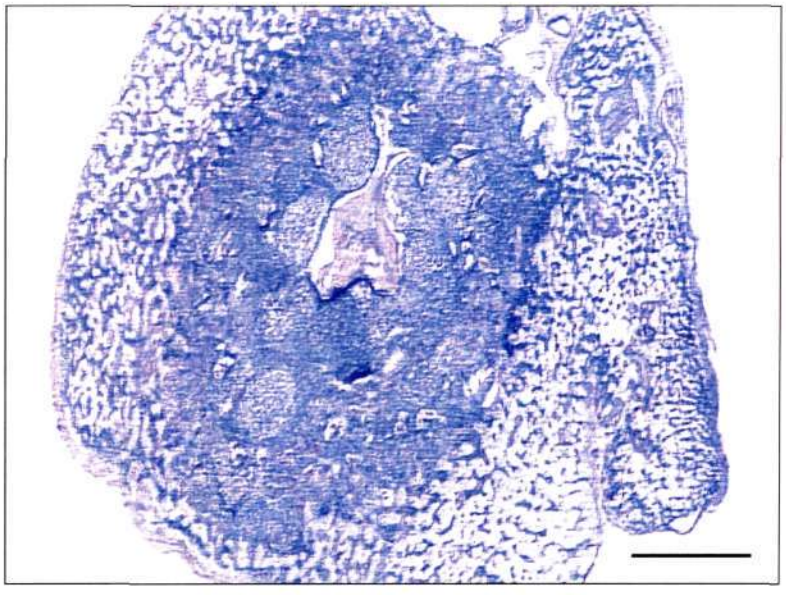

Fig. 4. Bifurcational lymph node. Striped dolphin D89, male, 23 years old. Centrally situated trabeculae are surrounded by dense nodular tissue. Less dense lymphatic tissue is situated peripherally. Haematoxylin and eosin (HE), stereomicroscope, 3 × 1; scale bar = 1 mm

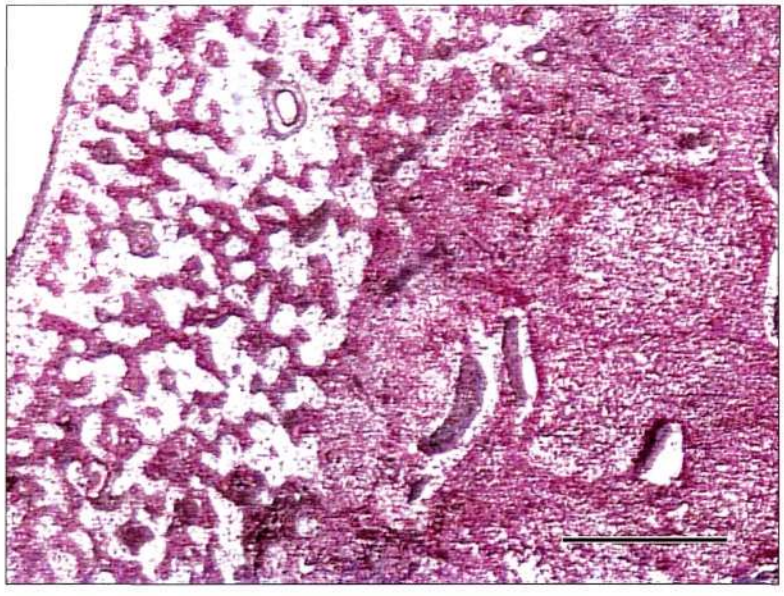

Fig. 5. Bifurcational lymph node. Striped dolphin D89, male, 23 years old. Central position of dense lymphatic tissue with secondary nodules and diffusely distributed lymphocytes. Around the dense nodular tissue there is a less dense lymphatic tissue with cell cords and sinuses. Masson's trichrome stain; 4 × 2.5; scale bar = 500 μm

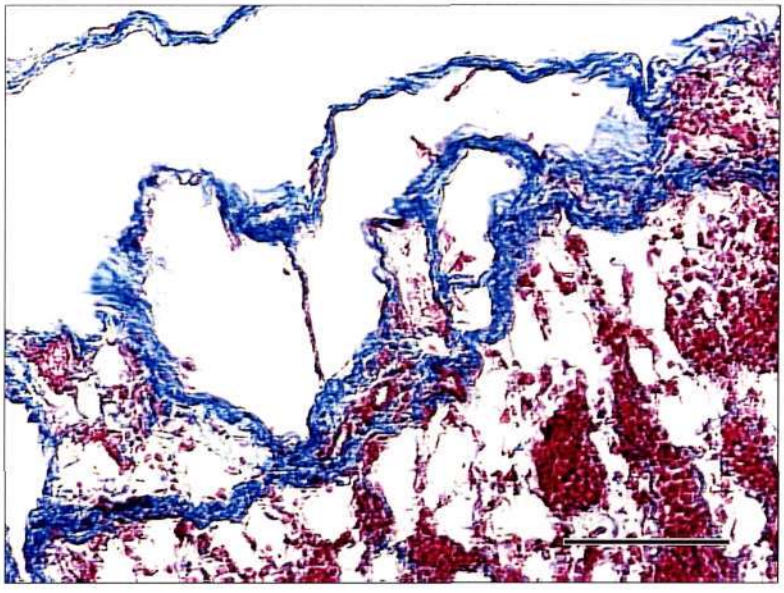

Fig. 6. Bifurcational lymph node. Striped dolphin D74, female, 15 years old. Efferent lymphatic vessels at the surface of the inverse lymph node. Mallory's stain; 20 × 2.5; scale bar = 100 μm

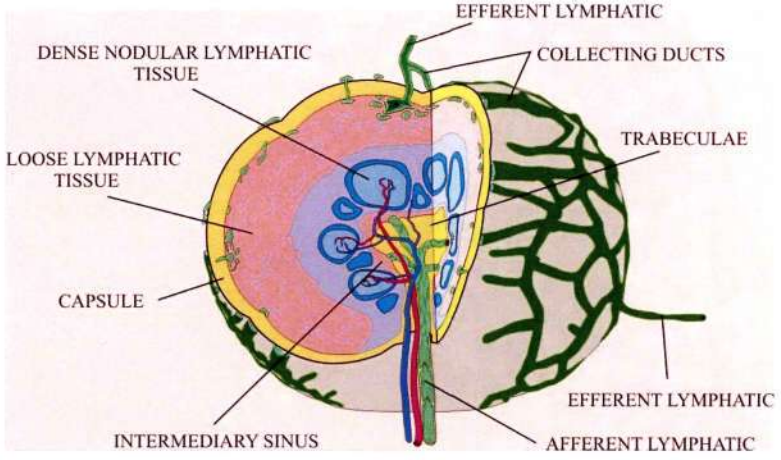

Fig. 7. Schematic diagram. An afferent lymphatic vessel enters a bifurcational lymph node of striped dolphin within a trabecula. Trabeculae are surrounded by dense nodular lymphatic tissue which is similar in appearance to the cortical tissue of lymph nodes in the majority of terrestrial mammals. Between the dense nodular tissue and the trabeculae an intermediary sinus is located. Superficial to the dense nodular tissue, a less dense lymphatic tissue which has similar appearance to medullary tissue seen in the majority of terrestrial mammals, is located. From the afferent lymphatic vessels the lymph flows through the intermediary sinus into the dense nodular tissue. Lymph then enters a tissue that is analogous to the medulla, and through collecting ducts it flows into the efferent lymphatic vessels

Appearance and position of sinuses within the mesenteric and bifurcational lymph nodes of the bottlenose dolphins examined in this study were as in the lymph nodes of the majority of terrestrial mammals, which is contrary to the statement that subcapsular sinuses in the bottlenose dolphin are incomplete (Cowan and Smith, 1999).

The parenchyma of the mesenteric lymph node in the striped dolphins studied was different from that of the bifurcational lymph node. The distribution of cortex and medulla in the mesenteric lymph node corresponded to that seen in the lymph nodes of the majority of terrestrial mammals, whereas the bifurcational lymph node is known to be of the inverse form, structurally resembling porcine lymph nodes. Pigs have atypical lymph nodes (Nicander et al., 1993; Banks, 1993), with an inverse dense lymphatic tissue containing lymphatic nodules and structurally resembling cortical tissue in the lymph nodes of other animal species, and a less dense lymphatic tissue that visually resembles the medulla of a typical lymph node. Porcine lymph nodes have no hilus, thus the afferent lymphatic vessels enter at various surface points, pass through the centralised trabeculae, branch, and run into the intermediary sinus. The lymph then passes through the sinuses of lymphatic tissue and the collecting ducts bring it to the surface of the lymph nodes, from where it enters the efferent lymphatic vessels (Spalding and Heath, 1987).

Irrespective of the fact that the lymph nodes in whales are structurally typical, Simpson and Gardner (1972) mention some nodes the cortex of which does not contain any nodules. Moskov et al. (1969) concluded that in common dolphin *(Delphinus delphis)* and harbour porpoise *(Phocena phocena)* the form of lymph node resembling the porcine lymph node was more common. Lucić (2002) also assumed that some lymph nodes in striped dolphin from the Adriatic Sea may be of inverse structure.

In our study, the lymph nodes of bottlenose dolphin and striped dolphin were surrounded by a capsule structured of a dense connective tissue. From the capsule the trabeculae extended towards the interior. Within the capsule there were smooth muscle cells. Smooth muscle cells were reported to be distributed along the interior edge of the capsule in the lymph nodes of beluga (Romano et al., 1993). Our study showed that in the lymph nodes of bottlenose dolphin smooth muscle cells were distributed all over the capsule, with the majority concentrated along a thin line by the interior edge. In striped dolphin, occasionally occurring smooth muscle cells were individually distributed within the capsule of mesenteric and bifurcational lymph nodes. Given the fact that in one striped dolphin the mesenteric lymph node had a layer of smooth muscle cells along the surface of the capsule, it may be assumed that the deviation was a matter of that individual animal.

Cowan and Smith (1999) classified lymph nodes in bottlenose dolphins into four groups: somatic, visceral, lymph nodes related to the respiratory tract,

and lymph nodes along the aortal arch. Visceral nodes have a thick capsule with many smooth muscle cells. The mesenteric lymph node is an example of a visceral node with many intermingled smooth muscle cells that extend through the trabeculae, too. Cowan and Smith (1999) thought that visceral nodes were contractile organs that played an active role in circulation and filtering of the lymph. On the other hand, lymph nodes related to the respiratory tract had a thinner capsule with less smooth muscle cells. Our results corresponded to these findings in the case of mesenteric and bifurcational lymph nodes in bottlenose dolphin. All studied lymph nodes of striped dolphins had a thin capsule.

Based on the above findings it may be concluded that the capsule of the studied lymph nodes in both species contains smooth muscle cells. In the mesenteric and bifurcational lymph nodes of bottlenose dolphin smooth muscle cells are concentrated mostly in a thin line along the interior edge of the capsule. In striped dolphin there are fewer smooth muscle cells and they are individually distributed in the capsule of the mesenteric and bifurcational lymph nodes.

Moreover, the lymph nodes of bottlenose dolphins have a typical distribution of cortex and medulla within the lymph node parenchyma, as is the case with the majority of terrestrial mammals. On the other hand, in addition to the typically structured lymph nodes, the striped dolphin has their inverse forms as well.

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