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Anatomical features of ossa cordis in the Steller sea lion

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ABSTRACT. Irregular triangular cartilage or bone fragments are sometimes found in the fibrous triangle of the heart. Ossa cordis and/or cartilago cordis has been demonstrated in various terrestrial animal species. Regarding marine mammals, sperm whales lack heart bones, and there have been no studies on bones or cartilage in pinniped hearts. Therefore, we examined the ossa cordis and/or cartilago cordis of the Steller sea lion. Eleven Steller sea lion hearts were examined morphologically and histologically. Before dissection, some hearts were imaged by CT to confirm the presence of ossa cordis or cartilago cordis. As a result, ossa cordis-like fragments were confirmed in four adults and one pup. All of the fragments were found at the right fiber triangle, and one adult had ossified tissue, including adipose tissue in the bone marrow cavity. The ossa cordis probably support the aorta because they surround the aorta as in other terrestrial animals. Steller sea lions can dive to a few hundred meters, but they need to rest on land frequently. Hence, their ossa cordis help maintain heart function during the tachycardia that occurs upon repeated surfacing and movements on land after diving in water.

KEYWORDS: cardiac skeleton, cartilago cordis, ossa cordis, Steller sea lion

The heart functions as a pump that circulates oxygen throughout the body by pumping blood; therefore, the heart is constantly under a heavy load. In adult cows weighing 600–700 kg, the heart pumps blood at a rate of 70 times per minute [12], and the normal venous blood pressure is constantly maintained at 130–180 mmHg [13]. There are left and right fibrous rings around the left and right atrioventricular orifices; these rings bear cartilago cordis or ossa cordis in some terrestrial species [18]. The ossa cordis is a basic structure in most ruminants [2, 7], and the cartilago cordis is found in farm animals such as horses and pigs [10]. In cattle, the two ossified left and right ossa cordis have the shape of an irregular flat triangle [10], the right ossa cordis is larger and attaches to the right semilunar valve, and the left ossa cordis attaches to the left semilunar valve and is smaller than the right ossa cordis. In contrast, buffaloes and sheep have only one ossa cordis [2]. Ossa cordis are also found in otters, which have three to five ossa cordis scattered on the annulus fibrosus [4].

The ossa cordis is thought to play a role in stabilizing contraction and relaxation of the heart by preventing excessive valve dilation [2, 5]. Furthermore, it supports the valve of the aorta, pulmonary ostium and atrioventricular orifice by electrically separating the atrium and ventricle to harmonize heart contraction [2, 5]. Duran *et al*. [3] proposed new functions of the cartilaginous foci in Syrian hamster hearts as pivots resisting the mechanical tensions of cardiac motion. However, it was reported that heart disease may also be associated with the development of ossa cordis, as observed for idiopathic myocardial fibrosis in chimpanzees [14]. Marine mammals include nonlanding species (i.e., cetaceans) and landing species (i.e., pinnipeds), and their cardiac workloads may be different. Cetaceans and pinnipeds are known to reduce their heart rate (bradycardia) during diving [6, 8]. It is not clear whether there is a relationship between diving and ossa cordis development, but odontocete sperm whales are known to lack ossa cordis and central fibrous bodies [9].

Unlike cetaceans, the Steller sea lion (*Eumetopias jubatus*, SSL), a pinniped, lives in the water but often rests on the shore [16]. Furthermore, the SSL is the largest pinniped of the order Carnivora and extensively inhabits the North Pacific coast [16]. The SSL

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exhibits sexual dimorphism, with males up to 330 cm in length and weighing approximately 1,120 kg and females up to 270 cm in length and weighing approximately 350 kg [16]. The Asian population of SSLs breeds in a rookery in Russian waters from late May to early July. SSLs can dive to depths greater than 200 m, but they regularly forage at approximately 20 m [11]. A previous report noted that the SSL develops bradycardia after the initiation of diving [8]. During bradycardia, the SSL heartbeat decreases to as low as 40% compared to that at rest [8], which is less than that (64%) recorded for the northern elephant seal (*Mirounga angustirostris*) [1] and that (20%) reported for California sea lions (*Zalophus californianus*) [17]. In this study, we determined the presence or absence of ossa cordis and cartilago cordis and studied their anatomical features in a pinniped, the SSL.

MATERIALS AND METHODS

A total of eleven SSL hearts were analyzed, including hearts from the carcasses of 2 male pups (neonates), 3 subadults (3 males) and 6 adults (5 males and 1 female) provided by the "Preventing Fisheries Damage Management Project" (https://www.jfa.maff.go.jp/j/press/sigen/140806.html), which protects against harmful organisms and is associated with the Fisheries Agency of Japan (Table 1). The SSLs were roughly classified as pups (SSL1, 2), subadults (SSL3–5) and adults (SSL6–11) according to their age. Whole SSL hearts were fixed in 10% neutral buffered formalin solution after harvest. The ossa cordis-like spicules were separated from the fiber triangle after confirming their presence or absence. The harvested spicules were washed and bleached with an agent containing sodium hypochlorite and sodium hydroxide. The spicules were measured with a caliper to determine their maximum length, maximum width and maximum thickness. Furthermore, cleaned spicules were prepared for stripping, and then paraffin sections with a thickness of 4 μm were prepared for histological examination. The tissue structure was analyzed by microscopic examination after hematoxylin-eosin (HE) staining. The microscopic examination was outsourced to a pathology inspection company, NORTH LAB Inc. (Sapporo, Japan). In addition, a CT examination was performed for the last four hearts to detect ossa cordis at the Rakuno Gakuen University Animal Medical Center under imaging conditions of 120 kV, 93 mA and a 2.50 mm thickness. The examination results were compared with the data from cows and otters.

RESULTS

In this study, we analyzed the ossa cordis/cartilage cordis of the SSL heart.

Anatomical findings

Ossa cordis-like spicules were found at the right fibrous triangles of the hearts (Fig. 1) and collected from the hearts of four adult males (SSL06, SSL07, SSL09 and SSL11) and one male pup (SSL01) among the eleven SSLs examined. These ossa cordis-like spicules were not attached to the semilunar valves. The ossa cordis-like spicules in SSL09 and SSL11 had hard bodies, but those in SSL01, SSL06 and SSL07 had a relatively soft texture. One ossa cordis-like spicule was found in each of three SSLs (SSL01, SSL09 and SSL11), but two pieces were found in the same area at the opening of the right fibrous triangles of SSL06 and SSL07. The spicule from SSL01 was the smallest among those found in the five animals, measuring 0.5 cm long, 2.7 cm wide and 0.2 cm

Table 1. Steller sea lion (SSL) individual information

Specimen ID	Sex	Weight (kg)	Body length (cm) *	Sexual maturity
SSL01	Male	135	198	Neonate
SSL02	Male	150	182	Neonate
SSL03	Female	210	233	Adult
SSL04	Male	225	276	Subadult
SSL05	Male	250	290	Subadult
SSL06	Male	380	242	Subadult
SSL07	Male	650	302	Adult
SSL08	Male	790	320	Adult
SSL09	Male	900	366	Adult
SSL10	Male	900	363	Adult
SSL11	Male	1,000	398	Adult

*Body length (total length) is the length from the snout to the hindlimb. The classification of maturity was based on the Steller Sea Lion Resource Survey in 2019, Japan Fisheries Research and Education Agency "Reiwa 1st year Steller sea lion resource survey" (http://hnf.fra.affrc.go.jp/H-jouhou/todo/todo_r01.sec.pdf).

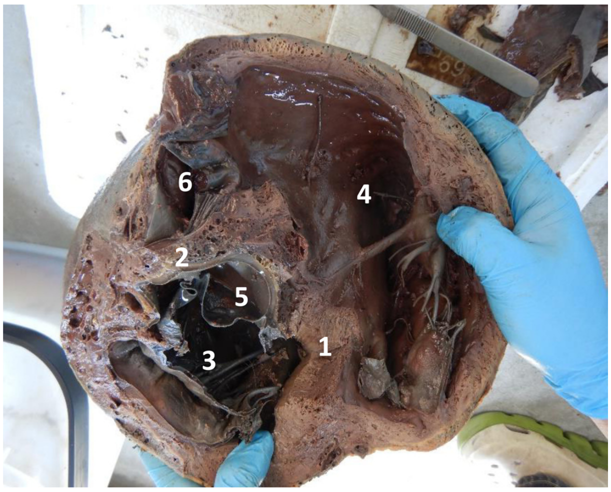

Fig. 1. Cross-section of the heart of the adult male Steller sea lion (SSL) 11. The atrium was removed, and the ventricle was exposed in the cross-section. A sponge-like structure was recognized in the left fibrous triangle. 1: right fibrous triangle, 2: left fibrous triangle, 3: left ventricle, 4: right ventricle, 5: aortic valve and 6: pulmonary valve.

thick (Fig. 2). In SSL06, the smaller spicule was 0.5 cm in length, 1.6 cm in width and 0.3 cm in thickness, and the larger spicule was 0.6 cm in length, 2.5 cm in width and 0.2 cm in thickness (Fig. 2). The smaller spicule of SSL07 was 1.5 cm long, 1.3 cm wide and 0.2 cm thick, and the large spicule was 1.3 cm long, 3.5 cm wide and 0.3 cm thick (Fig. 2). The spicule of SSL09 was 5 cm in length, 3.5 cm in width and 0.9 cm in thickness (Fig. 2). The spicules of SSL11 were found at the right fibrous triangles (Fig. 1) and were 1.6 cm in length, 4.2 cm in width and 0.7 cm in thickness (Fig. 2). The shape of the obtained spicules was irregular, but the spicule of SSL09 was slightly triangular in shape. The size of the spicules increased as the animals grew, and the bone structure developed (Fig. 3 and Table 2).

Fig. 2. The sizes of five ossa cordis-like spicules found in this study. The ossa cordis-like spicules increased in size with age to adulthood.

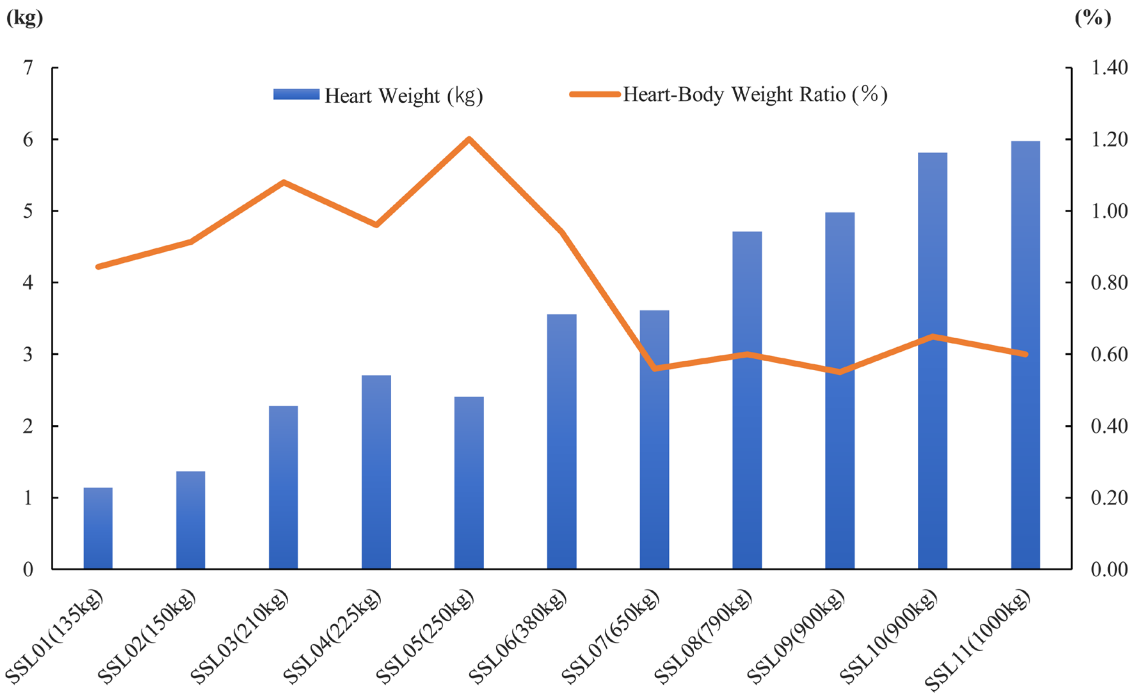

Fig. 3. Heart weight and heart-body weight ratio in Steller sea lions (SSLs). The weight of the heart increased with the weight of the body. Ossa cordis-like spicules were found in SSL01, SSL06, SSL07, SSL09 and SSL11. These SSLs were classified as pups (SSL1, 2), subadults (SSL3–5) and adults (SSL6–11) according to their estimated age.

Table 2. Characteristics of the obtained Steller sea lion (SSL) ossa cordis/cartilago cordis

ID	Age group	Length (cm)†	Width (cm)†	Thickness (cm)†	Tissue	Number
SSL01	Pup	0.5	2.7	0.2	Cartilage and connective tissue	1
SSL02	Pup	–	–	–	–	–
SSL03	Subadult	–	–	–	–	–
SSL04	Subadult	–	–	–	–	–
SSL05	Subadult	–	–	–	–	–
SSL06‡	Adult	S: 0.5, L: 0.6	S: 1.6, L: 2.5	S: 0.3, L: 0.2	Cartilage and connective tissue	2*
SSL07‡	Adult	S: 1.5, L: 1.3	S: 1.3, L: 3.5	S: 0.2, L: 0.9	Cartilage and connective tissue	2*
SSL08	Adult	–	–	–	–	–
SSL09	Adult	5	3.5	0.87	Cartilage	1
SSL10	Adult	–	–	–	–	–
SSL11	Adult	1.6	4.2	0.7	Bone and fatty marrow	1

†Maximum length of the spicules in each direction. ‡S indicates shorter spicules, and L indicates longer spicules. *Probably split during dissection.

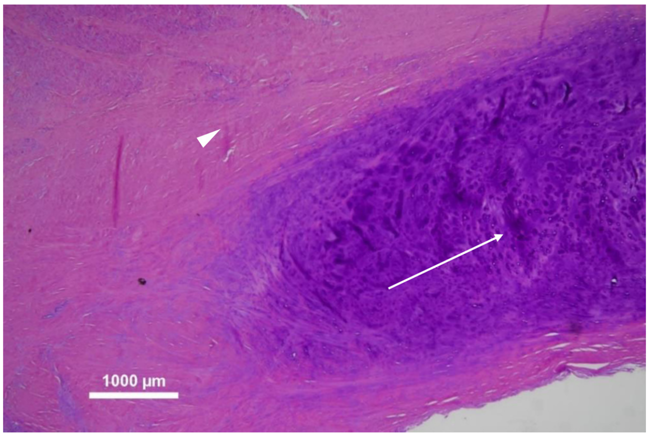

Fig. 4. Histopathology of the cartilago cordis of the adult SSL09. The cartilago cordis-like spicules include purple-stained hyaline cartilage (long arrow) and muscle fibers (arrowhead). Hematoxylin eosin staining.

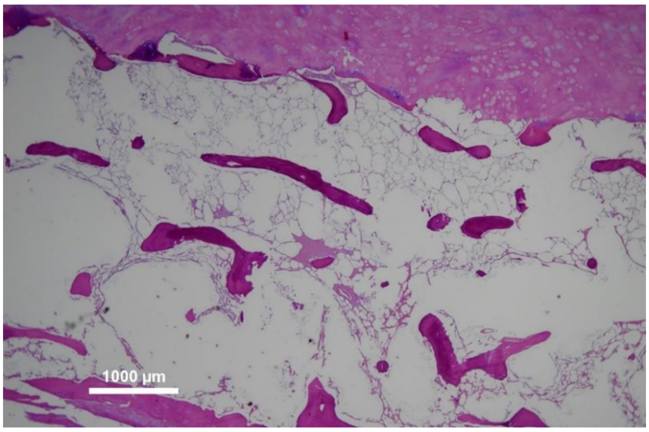

Fig. 5. Histopathology of the ossa cordis of the adult SSL11. The bone marrow cavity was replaced by adipose tissue. Hematoxylin eosin staining.

Histopathological analysis

The histological features of the five spicules indicated that they were ossa cordis (SSL11) and cartilago cordis (SSL01, SSL06, SSL07 and SSL09). The spicule of SSL09 was composed mainly of cartilage tissue, and no obvious bone marrow tissue was detected (Fig. 4). In the spicule of SSL11, fatty bone marrow with abundant adipose tissue at the trabeculae was confirmed (Fig. 5). Abundant cartilage tissue and a small amount of connective tissue were observed in the cardiac bone-like piece found in SSL01 (Fig. 6). In adult individuals, the spicules were composed of a small amount of connective tissue and abundant cartilage tissue (Table 2).

CT analysis

CT examination was performed on the hearts of SSL06, SSL07, SSL09 and SSL11. One large ossa cordis was barely visible in the SSL11 heart, but it was difficult to confirm the clear bone structure.

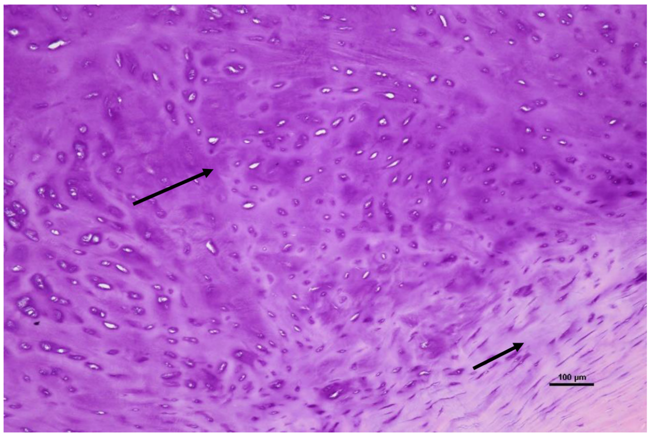

Fig. 6. Histopathology of the cartilago cordis of the neonate SSL01. Cartilage tissue (long arrow) was observed inside the connective tissue (short arrow). Hematoxylin eosin staining. Bar=100 µm.

DISCUSSION

In this study, we demonstrated ossa cordis or cartilago cordis in five of eleven SSL hearts. The SSL ossa cordis and cartilago cordis were distributed in the right fibrous triangle of the heart, surrounding the outflow opening. In addition, the positions were similar to those where ossa cordis were found in cattle (right heart bone) [10], and they were close to the right semilunar cusp of the aortic valve. Therefore, they probably support the aorta, similar to those in other animals. In addition, the ossa cordis obtained from SSL09 were irregularly triangular in shape and were similar to those found in cattle [10]. Since the two spicules of SSL06 and SSL07 were composed of soft tissue, they were difficult to distinguish from the heart muscle tissue. Accordingly, one piece might be separated into two pieces during harvest. Because only one female sample was obtained, it was not possible to detect a sex difference, but all the ossa cordis or cartilago cordis were found in male SSLs.

Generally, when hematogenesis is reduced in the bone marrow, the hematopoietic region is replaced by adipocytes, and hematopoietic cells are reduced or eliminated [15]. Therefore, it is speculated that the fatty bone marrow of SSL11 showed evidence of hematogenesis. Similar adipose tissue has been found in the ossa cordis of camels [5] but not in otters (except red bone marrow) [4]. The differences among SSLs are probably due to variation in the age of the individuals sampled and their degree of bone marrow tissue development. As shown in Fig. 4, the cartilago cordis in SSL09 did not include bone marrow but was mainly composed of cartilage tissue. The cartilago cordis detected in pup SSL01 included connective tissue, but no obvious ossification or bone marrow tissue was detected. A previous study revealed ossa cordis in bovine neonates [19], and cartilage dysplasia was found in the hearts of lambs [7]. Even though cartilago cordis was found in one SSL pup, we could not speculate whether the ossa cordis appeared once early in development and then disappeared gradually or grew throughout the individual's lifetime. It is necessary to increase the sample number in order to discuss ossa cordis formation in SSLs. In the present study, pathological changes were not observed in the ossa cordis/cartilage cordis. Previous reports on the ossa cordis in chimpanzees revealed that its formation was related to pathological changes [14]. In this case, an increased collagen level was observed, which was adjacent to the ossa cordis/cartilage cordis. Regarding the SSLs in the present study, neither evidence of collagen proliferation nor immune cells around the ossa cordis/cartilage cordis were found. Therefore, the ossa cordis/cartilage cordis in SSLs might develop without pathological causes.

Regarding the CT images, it was difficult to identify spicules in the SSLs. Three scattered ossa cordis were detected via X-ray in even smaller semi-aquatic mammals, otters [4]. Therefore, those in SSLs were probably less ossified than the ossa cordis in otters.

The texture of the heart is soft, and ossa cordis is absent in sperm whales, which can dive to more than 3,000 m, a high-pressure environment [9]. James *et al*. [9] noted that loss of both ossa cordis and central fibrous bodies might induce electrophysiological and hemodynamic risks if the whale is not under water. In sperm whales, both ossa cordis and central fibrous bodies in the soft heart are degraded for adaptation to buoyant water environments [9]. SSLs are known to develop bradycardia during diving, where the heart rate is 40% lower during diving and returns to tachycardia when ascending [8]. In this way, the heart rate of the SSL changes rapidly due to repeated diving and ascension in relatively shallow waters, in contrast to sperm whales, which also repeatedly dive but to much greater depths, more slowly and with less frequent ascension (once every few hours). However, smaller mammals, such as Syrian hamsters, have ossa cordis, which are under a lower load from their light body weight [3]. Durán *et al*. [3] speculated that the ossa cordis or cartilage cordis function as pivots in resisting the mechanical tension produced by the cardiac pumping motion. According to their theory, a rapid heart rate occurs in otters and SSLs when they emerge on land, and tachycardia reinforces the connective tissue and/or cartilaginous tissue, inducing bone development. Therefore, the ossa cordis of SSLs supports the aorta to provide heart stability. Moreover, the bones may serve as part of the heart skeleton and help maintain the morphology of the heart in order to allow adaptation to the buoyancy-free environment on land. However, the presence of cartilage and bones is not essential for the normal heart capacity of vertebrates. Furthermore, ossa cordis or cartilage cordis differs depending on variation in developmental rate, position, shape and other factors among species and/or among individuals.

In summary, the ossa cordis/cartilago cordis found in SSLs comprises the first report of ossa cordis or cartilage cordis found in pinnipeds. However, their function and development mechanisms are still unknown. Therefore, it will be necessary to further increase the number of samples and to examine the heart function, such as heart rate changes during diving, surfacing and landing.

CONFLICT OF INTEREST. The authors declare no conflict of interest.

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