



## NOTE

Pathology

# Intrapancreatic accessory spleen in a harbor porpoise (*Phocoena phocoena*)

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**ABSTRACT.** The occurrence of accessory spleens in cetacean species is high yet confirmed reports of intrapancreatic accessory spleen, a congenital malformation, remain undescribed. The current study provides the gross, microscopical, histochemical and immunohistochemical features of an intrapancreatic accessory spleen in a harbor porpoise (*Phocoena phocoena*). Grossly, a 17 × 18 × 9 mm well-demarcated, dark brown to red mass expanded the left pancreatic lobe. Microscopically, this mass consisted of mature splenic tissue interspersed with exocrine pancreatic acini. Intrapancreatic accessory spleens should be considered in the list of differential diagnoses for intrapancreatic nodular lesions in cetaceans.

**KEY WORDS:** cetacean pathology, developmental anomaly, heterotopia, intrapancreatic accessory spleen

Accessory spleens are one of the most frequent congenital splenic anomaly in both humans and animals [11, 18]. They may be single or multiple and can be located anywhere in the abdominal cavity, with the most common location in humans being the splenic hilum, followed by the pancreatic tail [19]. Intrapancreatic accessory spleens (IPAS) are typically incidental findings during surgery for other conditions but can pose a clinical challenge via multiple imaging techniques, mimicking hypervascular and neuroendocrine tumours [17]. While IPAS are not uncommon in humans [15], they are seldom described in the veterinary literature, including only a few cases in nonhuman primates [8, 12], pigs [10, 16], rabbits [1, 6], and dogs and cats [13].

In a variety of cetacean species, the prevalence of accessory spleens is a remarkably common finding, and function as compensatory lymphoid organs to splenic activity has been suggested in respect to diving adaptation [5]. A condition suggestive of IPAS has been described in the bottlenose dolphin (*Tursiops truncatus*) but detailed histological and immunohistochemical descriptions are lacking [2, 3]. We describe the gross, microscopical, histochemical and immunohistochemical features of an IPAS in a harbor porpoise (*Phocoena phocoena*).

A 139.5 cm-long (measured from the tip of maxilla to fluke notch), subadult male harbor porpoise was found stranded dead on the coast of Otaru, Hokkaido, along the Sea of Japan (43°12'54.7"N, 140°55'16.1"E) on 24 February 2017. The animal was retrieved the following day and necropsy was performed within 24 hr of discovery. The porpoise was in good body condition (blubber thicknesses: 38 mm, dorsal; 35 mm, lateral; and 34 mm; perpendicular along the level of the umbilicus). Tissue samples of liver, spleen, accessory spleen, kidney, heart, lung, thyroid gland, pancreas, adrenal gland, thymus and lymph nodes of the pulmonary hilum, mediastinal, gastric, mesenteric, and hepatic hilum were collected and fixed in 10% neutral buffered formalin. The samples were processed routinely, embedded in paraffin-wax, sectioned at 5 μm, and stained with hematoxylin and eosin for microscopic examination. Selected sections of the intrapancreatic spleen, normal spleen and pulmonary hilar lymph node of the same animal were additionally stained with Masson's trichrome (MT) for collagen fibres, Watanabe's silver impregnation for reticulum and thiosemicarbazide-periodic acid methenamine silver (TSC-PAM) for visualization of the basement membrane.

On gross examination, a 17 × 18 × 9 mm well-demarcated, dark brown to red mass expanded the left pancreatic lobe. On cut surface, the intrapancreatic mass had numerous 1–2 mm diameter white foci (Fig. 1). This mass did not show any continuity with the spleen. Other gross findings included: multiple 1 to 2 cm diameter accessory spleen-like nodules throughout the mesentery and omentum; mild biliary trematodiasis (*Campyla oblonga*); and mild pulmonary nematodiasis (Pseudaliidae). There was no evidence of abdominal trauma.

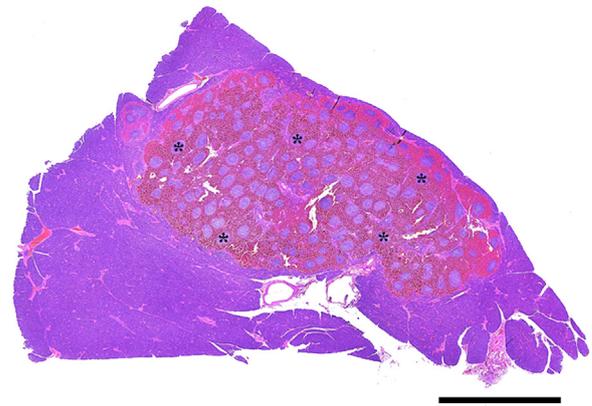
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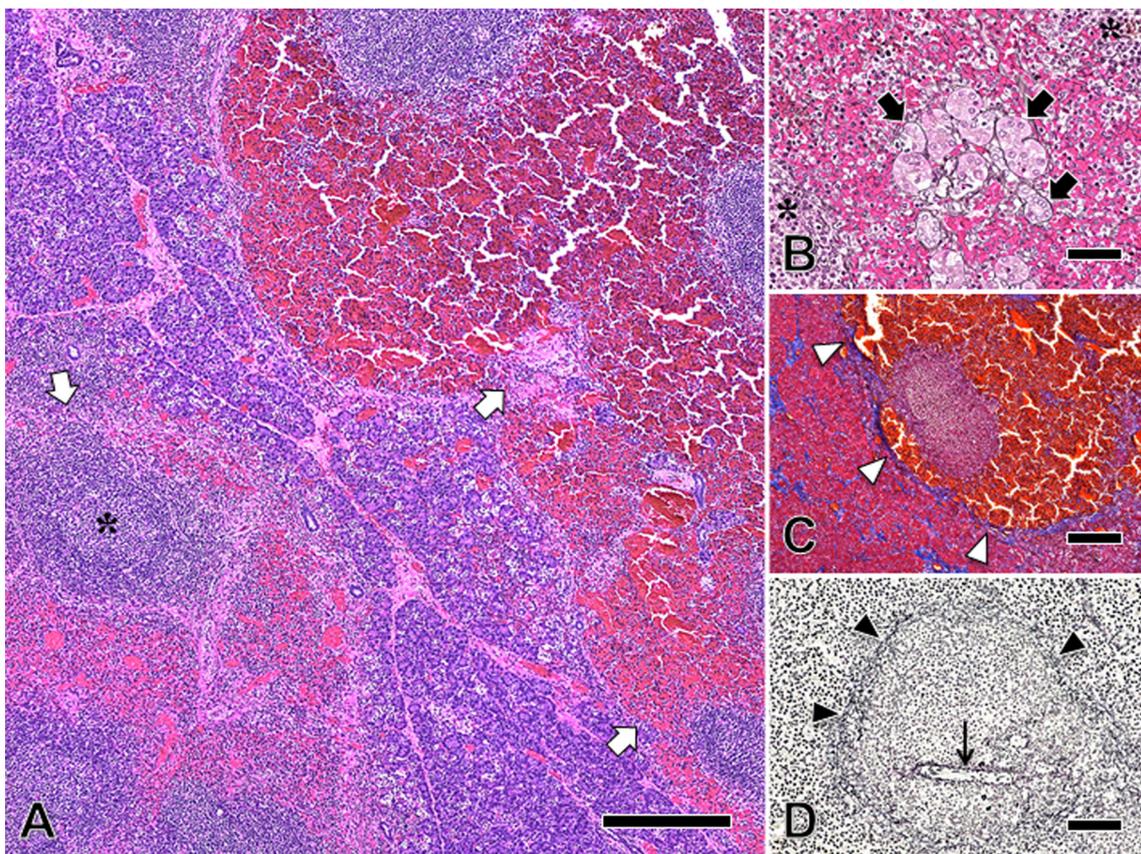
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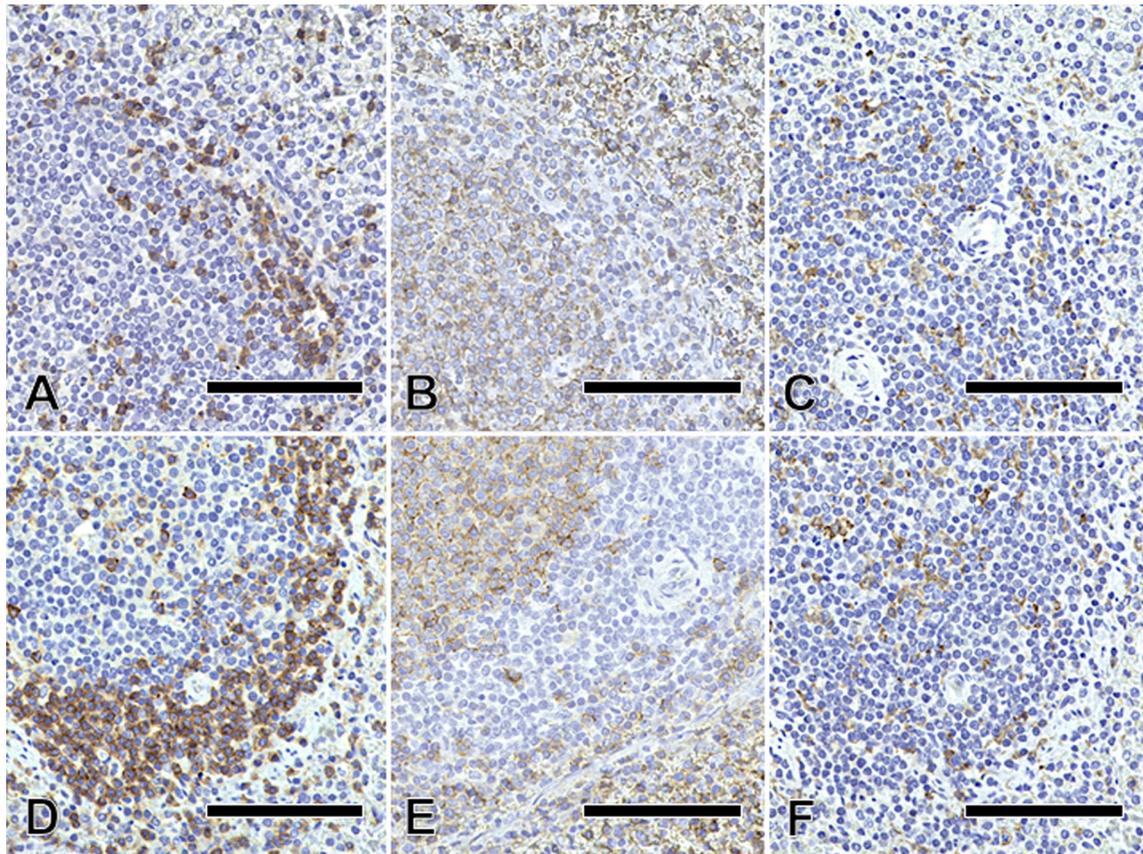
**Fig. 1.** *Ex situ* left lateral view of the pancreas of a harbor porpoise (*Phocoena phocoena*). A dark brown to red mass with numerous white foci (arrows) expands the left pancreatic lobe. Bar=10 mm.



**Fig. 2.** Subgross details of the intrapancreatic accessory spleen. The intrapancreatic splenic tissue is composed of distinct white (asterisks) and red pulp-like areas, recapitulating a normal spleen. Note the presence of pancreatic tissue fully encircling the accessory splenic tissue. Hematoxylin and eosin. Bar=5 mm.



**Fig. 3.** Histological features of the intrapancreatic accessory spleen. A: Some of the lymphoid follicular structures within red pulp-like areas show evident germinal centers (asterisk). Although the intrapancreatic splenic tissue is generally demarcated from the pancreatic parenchyma by a thin discontinuous fibrous band, the demarcation is ambiguous in some areas (arrows). Hematoxylin and eosin. Bar=300  $\mu$ m. B: Exocrine pancreatic acini have a thin, continuous basement membrane (arrows) and are embedded within the red pulp-like areas deep in the splenic tissue. Note location of acini relative to the two primary follicle-like lymphoid aggregates (asterisks) in the corners. Thiosemicarbazide-periodic acid methenamine silver. Bar=50  $\mu$ m. C: A thin blue collagenous band (arrowheads) is seen between the normal pancreatic parenchyma and intrapancreatic splenic tissue, and delineates the primary follicle-like lymphoid aggregates within. Masson's trichrome. Bar=200  $\mu$ m. D: Delicate fibres of reticulin (arrowheads) delineate primary follicle-like lymphoid aggregates. Note arteriole within the primary follicle-like structure, representing a central arteriole of a spleen. Watanabe's silver impregnation. Bar=100  $\mu$ m.



**Fig. 4.** Immunohistochemical comparisons between the normal spleen (A–C) and intrapancreatic accessory spleen (D–F). A, D: CD3-positive T cells are mainly confined to the mantle zone-like area. CD3. Bars=100  $\mu$ m. B, E: CD20-positive B cells are abundant in the central germinal-like areas. CD20. Bars=100  $\mu$ m. C, F: Ionised calcium binding adaptor molecule (Iba) 1-positive intrafollicular and perifollicular histiocytes are observed in low numbers. Iba1. Bars=100  $\mu$ m.

Microscopically, the intrapancreatic mass was encapsulated by a thin discontinuous fibrous band and contained typical splenic features including white pulp-like (WP) structures interspersed by red pulp-like (RP) areas (Fig. 2). The WP comprised of dense lymphoid follicles with and without germinal centers having often evident mantle zone and somewhat indistinct perifollicular zone (Fig. 3A), and periarteriolar sheath-like structures composed of small lymphocytes. The RP areas contained cordal macrophages forming a reticular meshwork, capillaries and abundant erythrocytes with circulating lymphocytes within wide sinuses that intercalated with non-filtering regions and unsheathed capillaries. Additionally, multifocal exocrine pancreatic acini were embedded in RP areas, for which TSC-PAM stain revealed an intact, continuous basement membrane (Fig. 3B). No evidences of extramedullary hematopoiesis or inflammation were observed. The MT and reticulin stains highlighted the spleen-like fibrovascular support around follicles (Fig. 3C and 3D), diverging from the pattern observed in the lymph node. Additionally, the nodules scattered along the mesentery and omentum were histologically confirmed to be accessory spleens.

Serial sections of the intrapancreatic mass were subjected to immunohistochemistry. Primary antibodies for CD3 (dilution 1:200; rabbit polyclonal, Dako, Tokyo, Japan), CD20 (dilution 1:400; rabbit polyclonal, Thermo Fisher Scientific Inc., Fremont, CA, U.S.A.) and ionised calcium binding adaptor molecule (Iba) 1 (dilution 1:500; rabbit polyclonal, Wako Pure Chemical Ind., Osaka, Japan), were used for immunolabeling T cells, B cells and histiocytes, respectively. Antigen retrieval was performed by microwave heat pretreatment in 0.01 M citrate buffer solution (pH 6.0; 15 min at 97°C) and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide (10 min at room temperature). The detection system Histofine Simple Stain MAX-PO (Nichirei Biosciences Inc., Tokyo, Japan) was applied. The immunoreaction was visualized with 3'3'-diaminobenzidine (Nichirei Biosciences Inc.) chromogen, and tissues were counterstained with Meyer's hematoxylin. Tissue sections in which the primary antibodies were replaced by nonimmune serum of species where primary antibodies were raised served as negative controls [14]. Sections including the same animal's spleen and pulmonary hilar lymph node were used as positive controls. Immunohistochemistry for CD3, CD20 and Iba1 showed cell distribution patterns in the intrapancreatic mass to be histologically indistinguishable from the normal spleen (Fig. 4). Dense follicular aggregations of CD20-positive B cells were surrounded by a thin rim of CD3-positive T cells (Fig. 4B and 4D). Intrafollicular and perifollicular T cells were also observed in low numbers while Iba1-positive histiocytes (presumed follicular and perifollicular dendritic cells) revealed a paucicellular but diffuse distribution (Fig. 4F). No lesions or cytoarchitectural anomalies were noted in the adjacent exocrine or endocrine pancreas. On the basis of the anatomical location,

cytomorphological, histochemical and immunohistochemical features, the lesion was diagnosed as IPAS.

Histologically confirmed reports of heterotopic tissue are uncommon in marine mammals, particularly in cetaceans, in which they are limited to thymic tissues in the parathyroid glands of Ganges river dolphins (*Platanista gangetica*) [9] and a case of kidney tissue in the lung of a common dolphin (*Delphinus delphis*) [4]. Accessory spleens result from failure of the primordial splenic buds fusing within the dorsal mesogastrium during the early stages of human fetal life or from traumatic splenic rupture [19]. Although not uncommon in humans, IPAS is regarded as a rare entity in the veterinary medical field [13, 15]. The condition is benign and largely asymptomatic, but if misdiagnosed by imaging techniques, it may prompt pancreatic resection [11]. The potential clinical relevance of IPAS in harbor porpoises or any other marine mammal is likely low; however, this species is often brought into facilities for rehabilitation purposes or may be part of oceanaria, and should be contemplated as a differential diagnosis for an intrapancreatic mass.

In contrast to the high occurrence of accessory spleens in cetaceans [5], presumed IPAS have only been observed in the bottlenose dolphin to date [2, 3]. The previous reports state that accessory spleens of some bottlenose dolphins were found “embedded” in the pancreas, which most likely is referring to IPAS, but no further histomorphological details and ancillary diagnostic features are available. It is noteworthy that the high prevalence of accessory spleens in cetaceans does not seem to correlate with an increased occurrence of IPAS.

Grossly, differential diagnoses in this case included a hematoma, hemal node, splenopancreatic fusion, hemangioma, hemangiosarcoma or any other highly vascularized tumour growth. Histological features along with histochemistry and immunohistochemistry ruled out these differentials. Despite the fact that there are no official reports of splenopancreatic fusion in marine mammals, this is a consistent (100% occurrence) anatomical variation in the Franciscana dolphin (*Pontoporia blainvillei*) (KRG, JDD, personal observation). In this species, the entire spleen, encapsulated by connective tissue, is fused with the pancreas without any mixture between their cellular elements. Conversely, IPAS in the present case had scattered exocrine pancreatic acini throughout the red pulp areas, suggesting intermingling between the two tissues. The admixing of these tissues has been reported in the human medical field [7], but not in the veterinary literature, and its implication requires further attention.

In summary, the present case provides detailed histological, histochemical and immunohistochemical features of an IPAS in a harbor porpoise. Furthermore, we report the presence of pancreatic acini embedded in the accessory splenic tissue, a novel histomorphological feature in IPAS of animals. Herein, IPAS should be considered in the list of differential diagnoses for intrapancreatic nodular lesions in porpoises.

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