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NOTE

Pathology

Extralobar pulmonary sequestration in two pinniped species

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ABSTRACT. Two cases of extralobar pulmonary sequestrations from a walrus (*Odobenus rosmarus*) and a Steller sea lion (*Eumetopias jubatus*) are described in the present study. Grossly, an independent, soft unilocular cystic mass was found within the abdominal cavities of both animals, adherent to the diaphragm in *O. rosmarus* and attached to the cardia of the stomach in *E. jubatus*. Histopathologically, the cysts were lined by pseudostratified ciliated columnar epithelium with abundant goblet cells, while the wall comprised of glands, hyaline cartilage, bronchiole- and alveolus-like structures, smooth muscles, and large, well-developed elastic and muscular arteries. The pinniped cases presented are exceptionally rare and to the best of the authors' knowledge, marks the first descriptions of this congenital anomaly in wildlife.

KEY WORDS: congenital anomaly, cystic mass, ectopic lung, extralobar pulmonary sequestration, pinniped

Pulmonary sequestration is a congenital lung anomaly thought to result from reduplication during embryonic development or an accessory bronchopulmonary tissue deriving from a supernumerary lung bud [9]. It is defined as a mass of non-functioning, incompletely differentiated pulmonary tissue independent of the tracheobronchial tree and pulmonary arteries [4]. Pulmonary sequestrations may manifest as either extralobar or intralobar types, where the extralobar types are distinct and separate from the other lung lobes, and intralobar types found within the lung or the visceral pleura [12]. In the veterinary literature, various terms, such as ectopic lung, accessory lung and pulmonary choristoma, have been used interchangeably to describe the same lesion, but confusion in this terminology has resulted in some authors referring to a different lesion with the same terms. Nevertheless, the occurrence of true extralobar pulmonary sequestrations in animals is exceptionally rare and to the best of the authors' knowledge, it has only been accounted in a few cattle [3, 5, 14] and one dog [7]. The present report describes two cases of extralobar pulmonary sequestration in a walrus (*Odobenus rosmarus*) and a Steller sea lion (*Eumetopias jubatus*), first accounts of this condition in wildlife.

The first case is an 11-year-old female *O. rosmarus*, which was caught around Wrangel Island, Russia, in the Chukchi Sea (71°15′N, 180°00′E) as a pup and kept at a captive facility in Japan for 3,746 days. The animal had been experiencing lack in appetite for over a period of two months before eventual death followed. At necropsy, the animal measured 233 cm by straight line length (from tip of nose to tip of tail with animal on its back), and a soft unilocular cystic mass measuring approximately 10 cm in diameter was found in the abdominal cavity, adherent to the diaphragm and adjacent to the abdominal aorta (Fig. 1). The cyst was completely surrounded in peritoneum-like membrane without any communication with other organs. A cut through the cyst resulted in highly viscous condense milk-like material flowing out (Fig. 1, inset). No other apparent gross lesions or anomalies were detected.

The second case is a 1-year-old male wild *E. jubatus* measuring 203 cm by straight line length. The sea lion was shot off Rebun Island, Hokkaido, Japan, in the Sea of Japan (45°29′01″N, 140°58′03″E) for population control purposes under the

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"Comprehensive countermeasure project for fishery damage prevention", supported by the Fisheries Agency of Japan. Age was determined by counting dentinal growth layers in the right upper canine according to standard techniques [2]. A soft unilocular cystic mass measuring 7.5 × 7 × 8 cm within the abdominal cavity, attached to the cardia of the stomach was the only anomaly and significant finding at necropsy (Fig. 2). As with the first case, the cystic mass was covered in peritoneum-like membrane and lacked communication with the stomach. The cystic mass was fixed in formalin intact and later revealed to have a lipoma-like appearance and texture at cut surface (Fig. 2, inset).

In both cases, tissue samples of the cysts were fixed in 10% neutral buffered formalin and routinely processed for histopathology. Additionally, in the case of *O. rosmarus*, representative tissue samples (liver, spleen, kidney, heart, lung, pancreas, esophagus, diaphragm, small and large intestines and ovaries) were collected and processed likewise. Sections were stained with hematoxylin and eosin (HE), alcian blue pH 2.5 (AB), elastica van Gieson (EVG) and Watanabe's method for reticulum along with further immunohistochemistry by a monoclonal mouse anti-alpha smooth muscle actin (αSMA) (dilution 1:1,000; clone 1A4, Dako, Glostrup, Denmark) antigen. For immunohistochemistry, deparaffinization, antigen retrieval, immunohistochemical labeling with 3,3'-diaminobenzidine chromogen, and counterstaining with Meyer's hematoxylin were performed with the Histofine Simple Stain MAX-PO system (Nichirei Biosciences Inc., Tokyo, Japan). Tissue sections in which the primary antibodies were replaced by phosphate buffered saline served as negative controls [10].

Microscopic examinations revealed similar histological features of the cysts in either case. The cysts were lined by pseudostratified ciliated columnar epithelium with abundant AB-positive goblet cells, while the contents were proteinaceous mucus. Structures of glands, hyaline cartilage and smooth muscles surrounded the cysts, of which represented a trachea-like arrangement (Fig. 3). The outer layer of the cyst walls comprised of hyaline cartilage, glands, bronchiole- and alveolus-like structures, discontinuous smooth muscles, and large, well-developed elastic and muscular arteries. Bronchiole-like structures were lined by epithelium identical to that of the cyst, surrounded by glands and hyaline cartilage (Fig. 4). In the case of *O. rosmarus*, basophilic mucus, proteinaceous material and foamy macrophages accumulated within the lumen of the prominent alveolus-like structures (Fig. 5). On the other hand, these alveolus-like structures were less distinct especially with HE in *E. jubatus*, but were pronounced through reticular stain (Fig. 6). Immunopositivity to the anti-αSMA antibody confirmed the identity of smooth muscles, and EVG stain clearly distinguished abundant elastic fibers in the large arteries. Small foci of inflammatory cell infiltration were also noted in the interstitial tissues. The cystic masses were diagnosed as extralobar pulmonary sequestrations on the basis of gross and microscopic observations. No other significant histological changes were detected in *O. rosmarus* where representative tissues were available for examination.

The morphological features found in the two cases were similar to those previously reported as extralobar pulmonary sequestrations in animals [3, 4]. We confirmed the cystic masses as independent, moderately differentiated lung structures lacking any communications to the respiratory tracts or the digestive system, encapsulated in their own peritoneum/pleura-like membrane. Although, aberrant arteries, the other important diagnostic criterion for detecting this condition in humans [9], were unidentified during necropsy in either case, since the cysts were located in the abdominal cavity, systemic arteries and not pulmonary arteries were thought to have supplied the masses. Additionally, stroma of the masses in both cases included well-developed, thick-walled, elastic and muscular arteries, which most likely is an indication of systemic vascular supply. In the case of *O. rosmarus*, the cyst was located next to the abdominal aorta, which may be an indication that it received blood supply directly from the aorta, similar to the vast majority of extralobar pulmonary sequestration cases seen in humans [12].

Differential diagnoses included a bronchogenic cyst and bronchopulmonary foregut malformation. Bronchogenic cysts are benign congenital malformations that are lined by ciliated pseudostratified columnar epithelium with focal areas of cartilage, smooth muscle and seromucinous glands within their walls, histologically resembling pulmonary sequestrations [8]. The only difference between the two conditions is that pulmonary sequestrations include alveolar structures, whereas bronchogenic cysts are limited to bronchiolar structures. The term bronchopulmonary foregut malformation describes sequestrations that maintain communication with the gastrointestinal tract [6]. Attachment to the cardia in the case of *E. jubatus* resembled a bronchopulmonary foregut malformation at first sight, but closer examination from the stomach mucosa denied this potential diagnosis. The two pinniped cases both had alveolar structures without any communication to other organs and hence were confidently diagnosed as extralobar pulmonary sequestrations.

In the veterinary field, reports of extralobar pulmonary sequestrations have been restricted to a few cattle [3, 5, 14] and a single case of a dog [7]. Thomson described 25 of these cattle incidents, where 56% were found within the abdominal cavity, 12% within the thoracic cavity and 32% with a subcutaneous distribution [14]. Meanwhile, around 65% of extralobar pulmonary sequestrations in humans are found in the left hemithorax, typically between the lower lobe and the diaphragm [12]. This suggests that there could be a discrepancy between the tending site in occurrence of this condition among the medical and veterinary medical fields, although much further epidemiological research in the veterinary side is needed. The two current cases were both intra-abdominal masses and hence followed the general predilected site of occurrence among animals.

There is little doubt that extralobar pulmonary sequestrations are of congenital origin, often asymptomatic and incidentally noticed during routine physical examinations [12]. The majority of intralobar types, however, are acquired secondary to infection and probably the consequence of repeated chronic pulmonary inflammation [12]. The pinniped cases were clearly extralobar types and thought to be of congenital origin, especially in the 1-year-old *E. jubatus*. In the case of *O. rosmarus*, the age of the animal was 11 years and hence not in the stage of her earliest life. As the cystic mass was attached to the diaphragm, this could have been a possible source of minor respiratory distress, which potentially may have led to the animal's chronic inappetence. Although more than 65% of human patients with extralobar pulmonary sequestration are known to have co-existing congenital defects [12], there

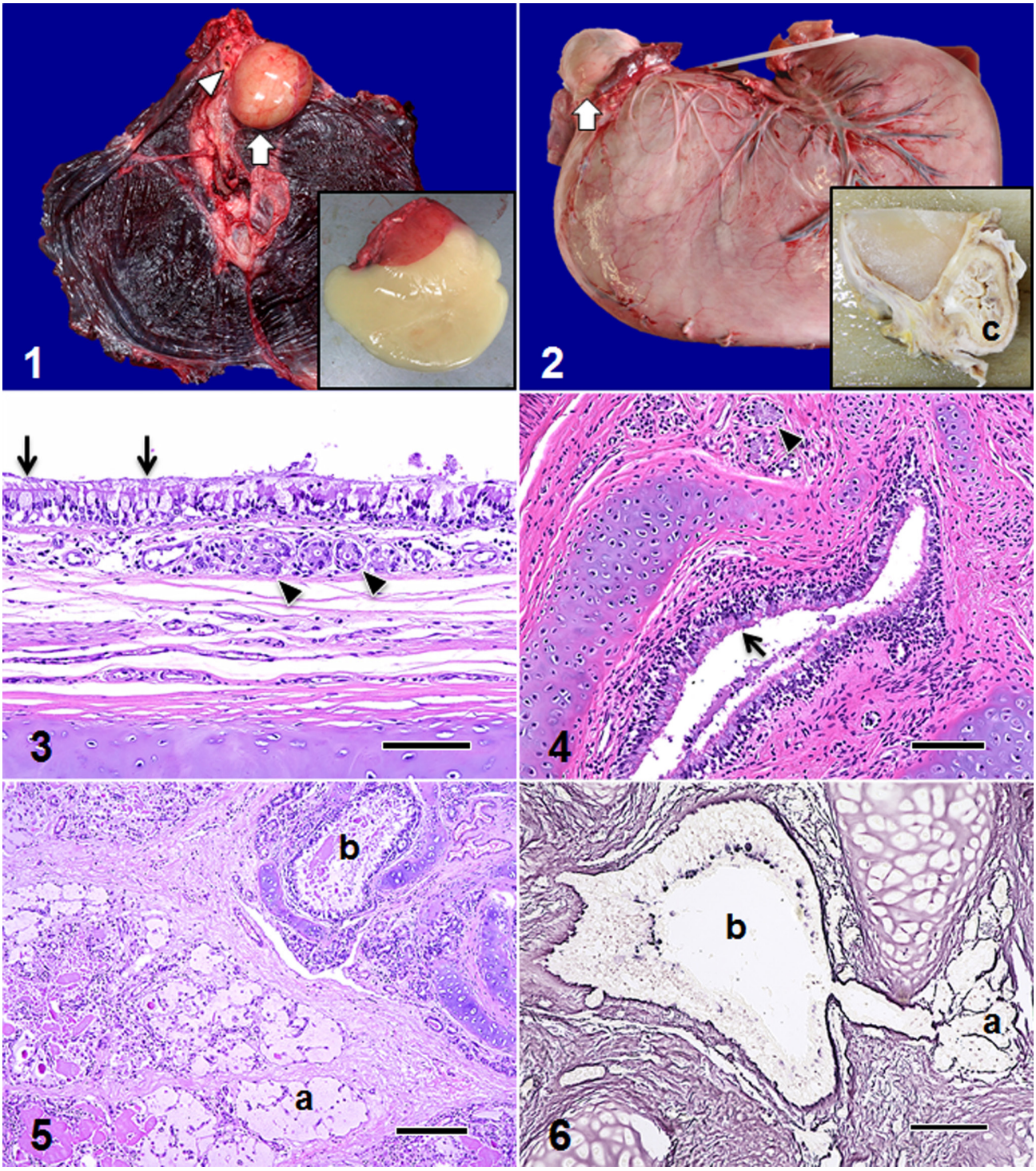

Fig. 1. Unilocular cystic mass (arrow) diagnosed as extralobar pulmonary sequestration, adherent to the diaphragm, adjacent to the abdominal aorta (arrowhead) in *Odobenus rosmarus*. Inset demonstrates the highly viscous condense milk-like material flowing out of the cyst.

Fig. 2. Unilocular cystic mass (arrow) diagnosed as extralobar pulmonary sequestration, adjacent to the cardia of the stomach in *Eumetopias jubatus*. Inset shows the lipoma-like appearance of the post-formalin-fixed cyst at cut surface and its location adjacent to the cardia (c).

Fig. 3. Pseudostratified ciliated columnar epithelium (arrows) with abundant goblet cells lining the cyst. Note the presence of glands (arrowheads) within the submucosa. *O. rosmarus*. Hematoxylin and eosin (HE). Bar=100 μm.

Fig. 4. Epithelium of bronchiole-like structures within the wall of the cyst (arrow) holds identical histological features with epithelium of cyst. The surrounding glands are shown with arrowheads. *E. jubatus*. HE. Bar=100 μm.

Fig. 5. Cyst walls comprised of bronchiole- (b) and alveolus-like (a) structures filled with basophilic mucus and proteinaceous material. *O. rosmarus*. HE. Bar=200 μm.

Fig. 6. Bronchiole- (b) and alveolus-like (a) structures surrounded by hyaline cartilage within the wall of cyst. *E. jubatus*. Watanabe's method for reticulum. Bar=100 μm.

were no other associated anomalies detected in the current cases including anomalies in the intrathoracic lungs.

Congenital anomalies of the respiratory tract, especially of the lung are generally uncommon in all animals [4], and the respiratory tracts of pinnipeds, are no exception. A two year investigation of 34 non-suckling gray seals (*Halichoerus grypus*) found one case of a deformed, flattened trachea [1], a seven year investigation of 210 juvenile northern elephant seals (*Mirounga angustirostris*) reported one case of a focal pulmonary dysplasia [15], and a 20 year investigation of 2,735 preweaned northern fur seal (*Callorhinus ursinus*) pups described a single case of pulmonary bilateral hypoplasia [11]. Therefore, there is no doubt that the two cases of *O. rosmarus* and *E. jubatus* demonstrate extremely rare conditions in pinnipeds. Meanwhile, as marine mammals are known to be particularly vulnerable to the effects of environmental contaminants [13], there is a need for continuous monitoring efforts in the occurrence of congenital anomalies in this group of animals.

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