

## Aspiration Pneumonia as a Cause of Neonatal Death in Three Captive Bottlenose Dolphins (*Tursiops truncatus*)

Manami MAKARA<sup>1)</sup>, Akinori SHIMADA<sup>1)\*</sup>, Natsuko KAWAMURA<sup>1)</sup>, Toshiyuki MURASE<sup>2)</sup> and Takehito MORITA<sup>1)</sup>

<sup>1)</sup>Departments of Veterinary Pathology and <sup>2)</sup>Veterinary Microbiology, Tottori University, Tottori 680–8553, Japan

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**ABSTRACT.** Neonatal weakness of calves is one of the common reproductive-related problems in captive cetaceans; clinical signs can be observed in the first few hours after delivery. Three 3-day-old bottlenose dolphins died with history of weakness since birth. Pathological study demonstrated purulent bronchopneumonia associated with prominent bacterial colonies and foreign substances in alveoli, suggesting aspiration pneumonia as a cause of neonatal weakness and resultant death of the three calves.

**KEY WORDS:** aspiration pneumonia, bottlenose dolphin, *Staphylococcus aureus*.

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Neonatal weakness of calves is one of the common reproductive-related problems in captive cetaceans; clinical signs can be observed in the first few hours after delivery [5]. The causes of reproductive-related problems including weakness and resultant death in bottlenose dolphins, however, are not fully understood because of lack in laboratory examinations including pathology [4].

The purpose of the present study was to describe the pathological findings of the lung lesions in three weak calves of bottlenose dolphin.

Three 3-day-old bottlenose dolphins, two females (case 1: 132 cm in length, 28 kg, case 2: 129 cm in length, 27 kg) and one male (case 3: 124 cm in length, 28 kg), died with history of weakness since delivery. There were no signs of dystocia in parturition of all animals. The animals were from different cows of different aquariums and received no treatment after birth. The cows had no history of reproductive-related problems including still birth, weak calf and poor maternal care before. The environment for the animals in the aquariums appeared to be kept in general condition because no episodes of occurrence of illness associated with poor environment were reported in these aquariums. The clinico-pathological findings, obtained in one calf (case 3), were within normal range except for slightly increased number of leukocytes (case 3:  $125 \times 10^2/\mu\text{L}$ ). The animals were brought to the Department of Veterinary Pathology, Tottori University for the pathological diagnosis. After complete necropsy, tissues from the lung, brain, heart, kidneys, liver, spleen, stomach, intestines, thymus, thyroid and adrenal glands were collected and fixed in 10% formalin, routinely processed, and sliced tissues were embedded in paraffin.

Sections of approximately 3  $\mu\text{m}$  thick were cut and stained with hematoxylin and eosin (HE), Gram's stain (Hucker-Conn method). Immunohistochemistry was carried out with the use of anti-*Staphylococcus aureus* antiserum (a kind gift from Dr. M. Kubo, National Institute of

Animal Health, Japan) as a primary antibody and streptavidin-biotin kit (Dako, Japan).

Macroscopically, small to large reddish brown foci of hemorrhage were observed in the lung of three calves. No stomach contents were detected in all animals. There were no significant lesions in other organs examined.

Histopathologically, pulmonary lesions consisted of acute hemorrhage, infiltration of neutrophils and slightly eosinophilic fibrous foreign materials mixed with bacterial colonies (Figs. 1–3). The bacterial colonies consisted of Gram's stain positive and negative organisms. Immunohistochemistry demonstrated that majority of the microorganisms in the lung lesions were positive with the anti-*Staphylococcus aureus* primary antibody (Fig. 4). The organisms were negative when the primary anti serum was substituted with phosphate-buffered saline.

Studies on diseases in the captive cetaceans reported bacterial pneumonia to be a common cause of death [3, 6]. In pinnipeds, bacterial respiratory disease is often accompanied by coughing, whereas in cetaceans most coughing occurs in animals with upper respiratory disease. In addition, an increased respiratory rate is often associated with respiratory disease in pinnipeds, but in dolphins is not readily observed until a significant portion of the lung field is damaged [2]. Thus, the clinical symptoms characteristic to respiratory disease in other animals are not readily encountered in dolphins; the present three calves showed acute hemorrhagic suppurative bronchopneumonia but no distinct signs of respiratory failure were associated also. Because of lack in the clinical data sufficient for the diagnosis of suppurative pneumonia, the present three calves did not receive treatment with antibiotics. Use of antibiotics with a broad spectrum is to be considered for treatment of weak calves without distinct symptoms of bacterial pneumonia.

*Staphylococcus aureus* has been prominent pathogenic isolates in bacterial pneumonia of captive cetaceans; less frequent but common isolates tend to be Gram-negative organisms [2]. In the purulent pneumonia of the present

\* CORRESPONDENCE TO: SHIMADA, A., Department of Veterinary Pathology, Tottori University, 4–101 Minami, Tottori 680–8553, Japan.

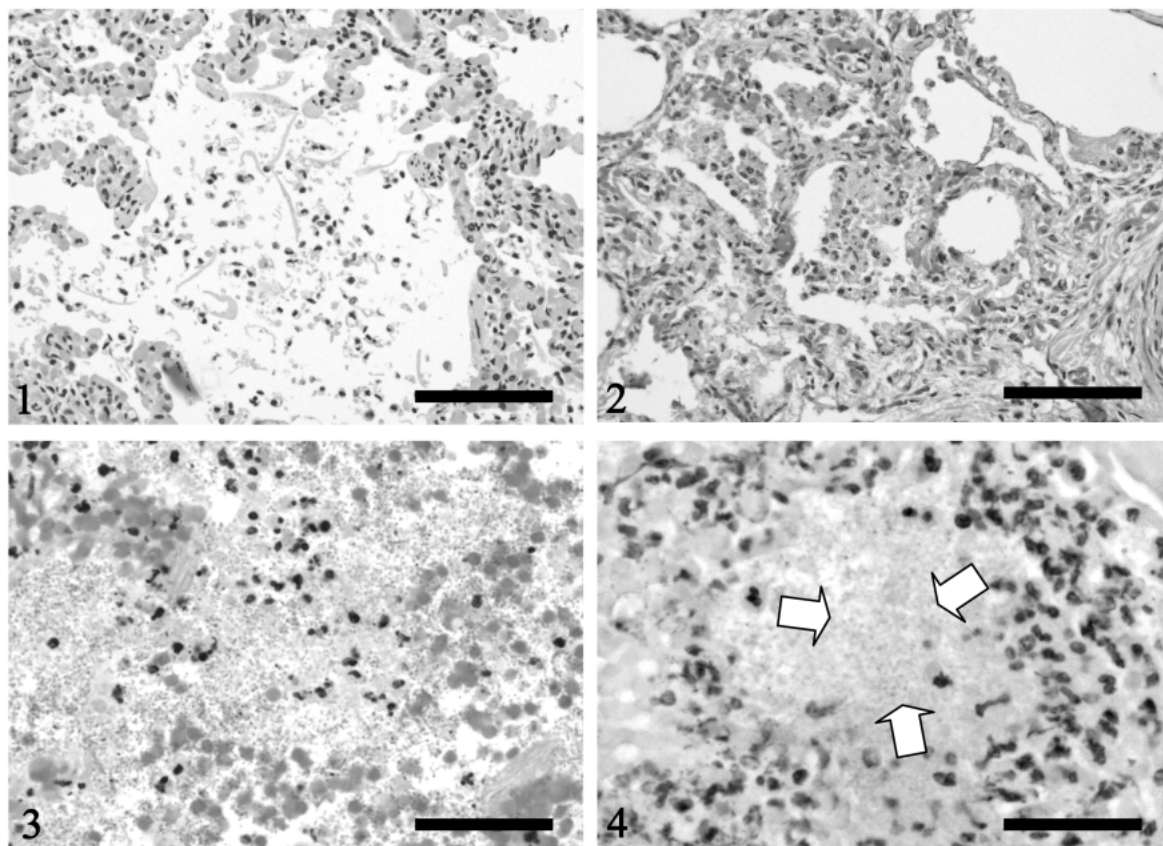


Fig. 1. Histological findings of the lung, showing slight hemorrhage, eosinophilic fibrous foreign materials and inflammatory cells in alveoli. 3-day-old bottlenose dolphin (case 1). Hematoxylin and eosin (HE) stain. Bar = 150  $\mu$ m.

Fig. 2. Histological findings of the lung, showing occasional bacterial colonies and inflammatory cells in alveoli. 3-day-old bottlenose dolphin (case 2). HE stain. Bar = 150  $\mu$ m.

Fig. 3. Histological findings of the lung, showing severe hemorrhage, inflammatory cells and bacterial colonies in alveoli. 3-day-old bottlenose dolphins (case 3). HE stain. Bar = 80  $\mu$ m.

Fig. 4. Majority of the microorganisms (arrows) was labeled positively with the anti-*Staphylococcus aureus* antiserum at 1:3,000 in dilution. 3-day-old bottlenose dolphin (case 3). Immunohistochemistry. Bar = 60  $\mu$ m.

three calves, majority of the microorganisms was also positive for anti-*Staphylococcus aureus* antibody.

A significant inflammatory response was observed in the lung lesions of the present three calves. Therefore, aspiration of the foreign materials might occur in the reproductive tract of cows during parturition or in the pool at early time after birth. Dysphagia and pharyngeal paralysis, which are common causes of aspiration pneumonia in domestic animals [1], may not be responsible for the pulmonary lesions in the three calves, because gross anatomy of dolphins is different from other animals; there is no communication between the pharynx and larynx in dolphins. Exact source of the slightly eosinophilic fibrous foreign materials observed in the bronchioles and alveoli was not determined in this study.

Neonatal weakness and resultant death of calves are common reproductive-related problems in captive cetaceans [5]. The present three calves with signs of weakness were demonstrated to have been suffered from purulent pneumonia

with prominent bacterial colonies and foreign substances in alveoli, suggesting that, in part, aspiration pneumonia may be responsible for neonatal weakness and resultant death observed in these animals. Further study is required to elucidate the exact cause of the aspiration of foreign materials in the weak calves of captive bottlenose dolphins.

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