Non-Purulent Meningoencephalomyelitis of a Pacific Striped Dolphin (*Lagenorhynchus obliquidens*). The First Evidence of Morbillivirus Infection in a Dolphin at the Pacific Ocean around Japan

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ABSTRACT. On March 22, 1998, a mature, male, hyposthenic Pacific striped dolphin (*Lagenorhynchus obliquidens*) was stranded at Aoshima Beach in Miyazaki prefecture, Japan. A necropsy performed 14 hr after death revealed mild diffuse congestion and edema of the leptomeninges and mild pulmonary atelectasis. Histopathologically, non-purulent inflammatory were observed throughout the cerebrum, thalamus, midbrain, pons, medulla oblongata, and spinal cord. Hematoxylin and eosin stain revealed no viral inclusion bodies. Immunohistochemistry using a monoclonal antibody against nucleoprotein of canine distemper virus (CDV-NP) revealed a number of CDV-NP-positive granular deposits in the cytoplasm and cell processes of the degenerating or intact neurons. The present paper is a first report of spontaneously occurred morbillivirus infection in a dolphin at the Pacific Ocean around Japan.—KEY WORDS: dolphin, encephalomyelitis, morbillivirus.

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Morbillivirus infection in aquatic mammals was first described in seals and porpoises at the end of the 1980s [1, 10, 11, 14, 15]. Grachev *et al.* [10] reported outbreaks of morbillivuris infection in thousands of Bikal seals (*Phoca sibirica*) in 1987 and 1989. In 1988, Osterhaus and Vedder [15] discovered evidence of morbillivirus infection in thousands of harbor seals (*Phoca uitulina*), and a few harbor porpoises (*Phocoena phocoea*). Subsequently, a severe outbreak of morbillivirus infection occurred in striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea at the beginning of the 1990s [4–6]. Recently, several serological or epidemiological investigations revealed that morbillivirus infection of marine animals such as pinnipeds, dolphins, and whales, is not limited to European waters [1], but is more widespread, including Greenland [3], Antarctica [2], the northeast coast of the United States [8], eastern and Arctic Canada [9], the western Atlantic [7], the Gulf of Mexico [12, 13], and the Southeast Pacific area around central Peru [16]. In spite of this evidence of world-wide aquatic morbillivirus contamination, there is no reports of spontaneously occurring morbillivirus infection or serological evidence in marine animals in the Pacific Ocean around Japan.

In the present paper we describe the pathological features of a Pacific striped dolphin (*Lagenorhynchus obliquidens*) stranded at Aosima Beach in Miyazaki, Japan, and present preliminary evidence of dolphin morbillivirus infection. To our knowledge, this is the first report of spontaneously occurring morbillivirus infection in a dolphin at the Pacific Ocean around Japan.

Case history: On March 22, 1998, a stranded Pacific striped dolphin (*Lagenorhynchus obliquidens*) was found by a veterinarian at Aoshima Beach in Miyazaki prefecture, Japan. This dolphin was a mature male, approximately 140 cm in length and was severely hyposthenic. Soon after being discovered, the case was died at the beach and presented to our laboratory.

Necropsy findings: The necropsy was performed 14 hr after death. The carcass was in good nutritional condition with mild autolysis. Grossly, there were no traumatic lesions over the whole skin. The lungs showed mild diffuse congestion and multifocal atelectasis. In the stomach, there were 20 nematodes, 5 to 15 mm in length, but no food contents. In the brain, there was moderate diffuse hyperemia of the subdural area, and mild diffuse subleptomeningeal edema (Fig. 1).

Histopathology: Tissue samples from each organ were fixed in 10% formalin and routinely processed for paraffin sections. A total of 36 brain samples were taken from the brain. Paraffin sections were routinely stained with hematoxylin and eosin (HE) and some selected brain sections were also stained with Luxol fast blue (LFB). For detection of morbillivirus antigen, a monoclonal antibody against nucleoprotein of canine distemper virus (1:100, CDV-NP MAb, VMRD Inc., U.S.A.) was purchased. A monoclonal antibody against glial fibrillary acidic protein (1:20, GFAP MAb, Dako, Denmark) was also used for visualization of astrocytes. Immunohistochemistry was performed using an Envision Polymer reagent (Dako), and the reaction products were visualized using 3'3-diaminobenzidine (DAB, Sigma, U.S.A.). Before immunostaining, hydrate-autoclave pretreatment was done at 118°C for 5 min, to enhance the immunoreactivity of the antigen in formalin-fixed paraffin sections. As positive controls for CDV-NP immunostaining, paraffin sections from the cerebrum and cerebellum of a Pug dog which had typical lesions of canine distemper with distinct cytoplasmic and intranuclear inclusion bodies, were used.

Histopathological examinations revealed mild to moderate diffuse non-suppurative inflammation in the brain of the

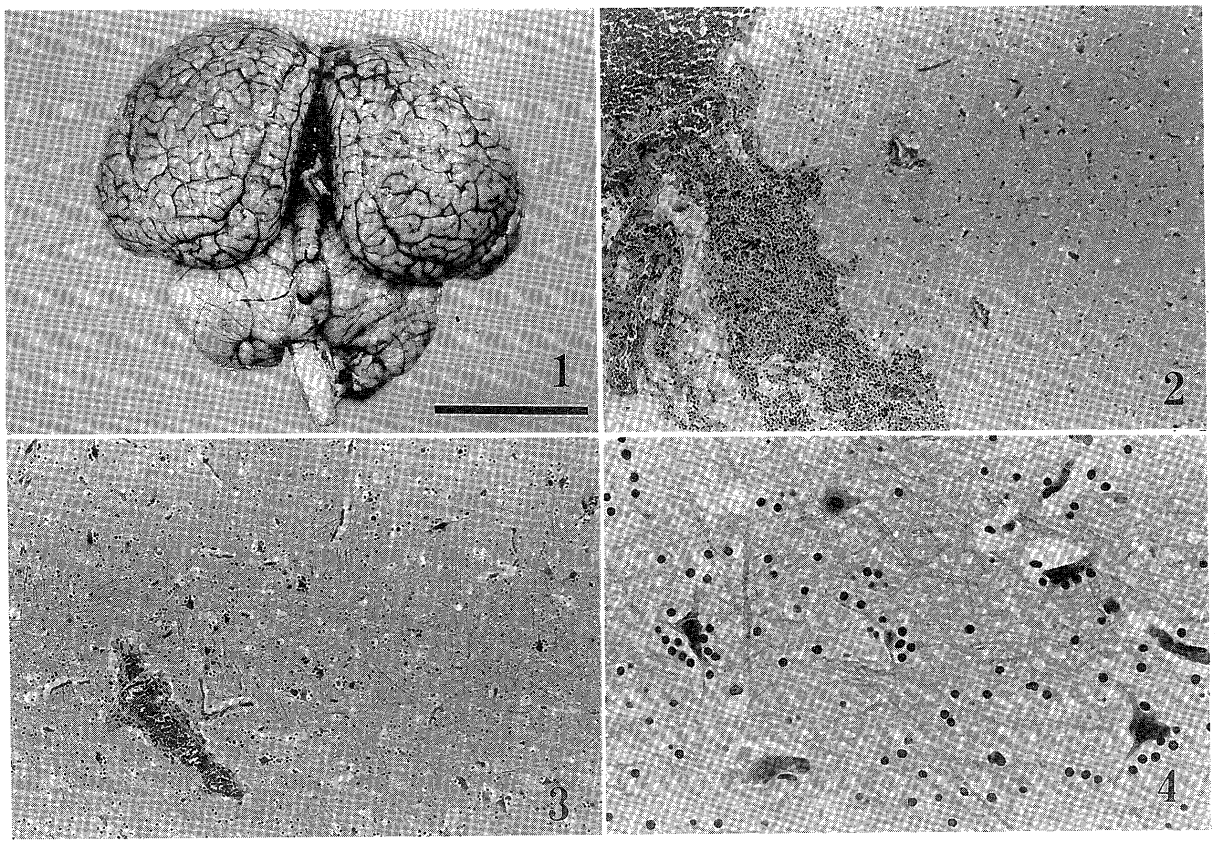
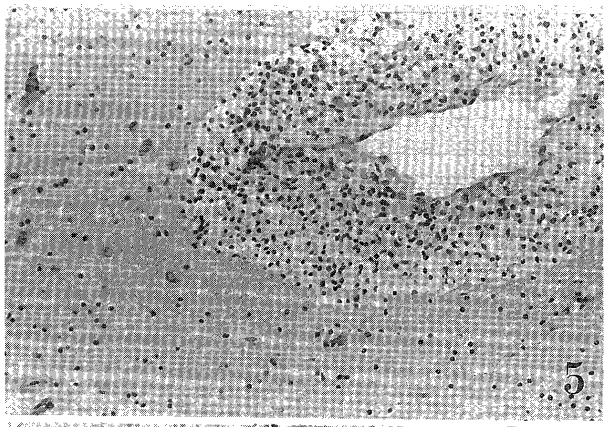
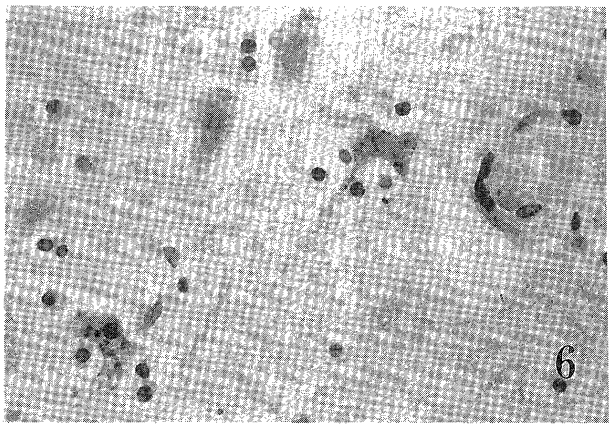


Fig. 1. Gross lesion of the brain. The leptomeningeal area shows mild diffuse congestion and edema. Bar=4 cm.
Fig. 2. Severe accumulation of lymphocytes in the leptomeninges and perivascular cuffs in the cerebral cortex. HE stain. × 25.
Fig. 3. Degenerating neuronal cells with neuronophagia and perivascular infiltration of lymphocytes in the lamina multiformis and subcortical white matter. HE stain. × 63.
Fig. 4. Microglial accumulation around degenerating neuronal cells representing neuronophagia in the subcortical white matter. HE stain. × 250.

dolphin. The most obvious lesions were perivascular aggregates of lymphocytes in the leptomeninges, and gray and white matter (Fig. 2). In addition, GFAP positive astrocytes with prominent acidophilic cytoplasm and hyperchromatic nuclei, were also accumulated in the perivascular area. In the subleptomeningeal area of these lesions, mild diffuse edematous changes of the neuropil was also recognized. These lesions were widely distributed in almost all of the brain sections examined. Scattered neuronal degeneration, neuronophagia, a small number of glial nodules, and mild gliosis were also frequently seen in the cerebral cortex, thalamus, nuclei of the brainstem and spinal gray matter, but not in the cerebellar cotex. In the cerebrum, these neuronal changes were most dominant in the lamina multiformis of the cortex and subcortical white matter (Fig. 3). Neuronal degeneration was characterized by shrunken neuronal cells with intensely acidophilic cytoplasm and chromatolytic or pyknotic nuclei. Around these degenerating neuronal cells a number of microglial cells had accumulated, representing neuronophagia (Fig. 4). HE staining, revealed no distinct viral inclusion bodies in any region, although very rarely, the cytoplasm, but not the nuclei of the large motor neurons in the brainstem and spinal cord contained slightly eosinophilic granular material. In the white matter of the cerebrum, there was mild to moderate proliferation of reactive astrocytes with plump eosinophilic cytoplasm. In some perivascular areas, there were accumulated foamy macrophages containing a large number of lipid granules (Fig. 5). Immunohistochemical examination with CDV-NP MAb revealed the presence of CDV-positive neuronal cells, although the number was very small in comparison with that in canine distemper brain sections used as a positive control. In the dolphin cerebral cortex, a few CDV-positive neurons were located mainly in the lamina multiformis of the cortex and subcortical white matter, corresponding to the location of neuronal degeneration and neuronophagia. In these areas, CDV-positive granular deposits were detected in the cytoplasm and axons of morphologically intact or degenerating neurons with accumulated microglial cells (Fig. 6). In the nuclei of these CDV-positive neurons, viral antigens were not detected. CDV-positive neurons were seen more frequently





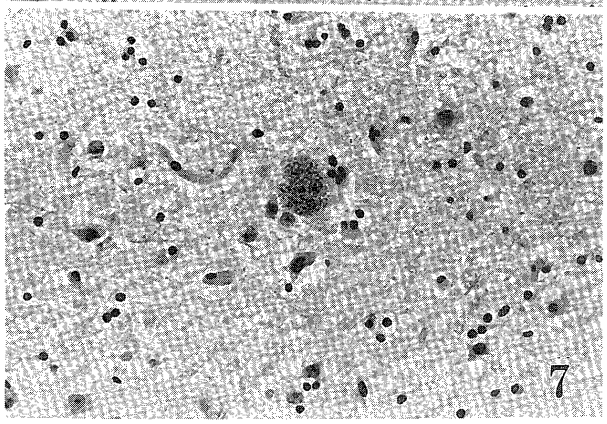


Fig. 5. Severe perivascular accumulation of gitter cells containing a large amount of lipid granules and mild proliferation of reactive astrocytes. HE stain. × 94.
Fig. 6. Granular deposits of viral antigens in the cytoplasm of degenerating neurons. Immunostaining for CDV-NP. Counter stain with hematoxylin. × 400.
Fig. 7. A protozoal cyst mimicking toxoplasma with mild microglial reaction in the cerebral cortex. HE stain. × 250.

in the brainstem and gray matter of the spinal cord. In glial cells, ependymal cells or endothelial cells, CDV antigens were not detected. Among 36 brain regions examined, only one protozoal cyst, approximately 30 um in diameter, containing a large number of bradyzoites, was found in serial sections of the parietal cortex (Fig. 7). Around the protozoal cyst, there was very mild microglial reaction. Apart from this section, no other protozoal organisms were detected. Based on these findings in the brain, "non-purulent meningoencephalomyelitis due to morbillivirus infection with a non-lesional protozoal cyst" was diagnosed.

Among visceral organs other than the brain, significant lesions were found only in the spleen, lymph nodes, and intestines. Moderate to severe lymphoid follicular depletion with deposition of slightly eosinophilic, homogeneous hyaline material was commonly observed. Immunohistochemical examination using CDV-NP MAb, revealed no viral antigens in these organs or in the lungs, liver, and kidney.

The most characteristic lesion in the present case was scatted neuronophagia with lesional CDV-NP antigens in the degenerating neurons distributed in the cerebral cortex, nucleus of the brainstem and spinal gray matter, although there were no visible inclusion bodies. Perivascular cuffing by lymphocytes was also seen frequently in almost all the brain sections examined. Demyelination and malacic necrosis in the white matter have been reported to be typical features of acute to subacute dolphin morbillivirus encephalitis [4]. Although these lesions were not present in this case, there was mild diffuse astrocytosis and occasional perivascular accumulation of foamy macrophages, suggesting subacute to chronic injury of the white matter. In addition, there were no lesions or viral antigens of morbillivirus infection in the visceral organs other than the brain. Acute dolphin morbillivirus infection is well known to cause systemic involvement consisting mainly of interstitial bronchopneumonia and lymphoid depletion in the lymph nodes and spleen with distinct inclusion bodies and viral antigens. In this case, lymphoid follicular depletion was present in the spleen, lymph nodes, and large intestine, but there were no viral inclusions or antigens. We supposed that these lymphoid depletion might be the non-specific change induced by severe persistent stress. From these findings our dolphin was considered to be suffering from chronic persistent morbillivirus infection localized in the CNS. Similar observations of chronic dolphin morbillivirus infection limited to the CNS have been reported by Domingo *et al.* [5]. Among 84 necropsied dolphins found in the Mediterranean sea from 1990 to 1994, 6 had mild to severe CNS-located morbillivirus infection. In these cases, there was no evidence for systemic infection immunohistochemically. HE staining revealed no viral inclusion bodies in half, and demyelination was detected in only two brains. It was considered that these lesions were represented

chronic CNS-localized morbillivirus infection, similar to human subacute sclerosing panencephalitis (SSPE) due to morbillivirus infection [5]. In contrast, the pathological features in the present case were quite different from those in SSPE because of the lack of demyelination and glial scars in the white matter, and the absence of distinct inclusion bodies. However, the wide-distribution of the brain lesions consisting of neuronal degeneration with neuronophagia or diffuse gliosis in the white matter may be represent some conformity with those of human SSPE.

Among the brain sections examined, a protozoal cyst mimicking that of toxoplasma was found in the brain cortex with very mild histological changes. Serial sections of the brain region revealed no other protozoal cysts or tachyzoites. In addition, no protozoal organisms were found in any other organs including the lymph nodes, spleen, and lungs, which have been shown to be commonly involved in toxoplasmosis in dolphins [6]. Moreover, toxoplasma infection can cause necrotizing inflammation in the lungs and lymph nodes as well as non-suppurative encephalitis [6]. These findings, including previous data, suggest that the protozoal infection in our dolphin was sporadic or secondary, and did not contribute to the pathogenesis of diffusely distributed brain lesions consisting of neuronal degeneration.

To clarify the situation of morbillivirus contamination among marine mammals in the Pacific Ocean around Japan, further serological examinations or viral isolation will be needed. Although this paper is a single case report of spontaneously occurring dolphin morbillivirus infection, we suggest the possibility of mass contamination with the virus in this area. In conclusion, this paper has presented preliminary immunohistochemical evidence of morbillivirus infection in dolphins, suggesting the contamination of aquatic mammals by the virus even in the Pacific Ocean around Japan.

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