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Short communication

An outbreak of lethal adenovirus infection among different otariid species

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ABSTRACT

An outbreak of fatal fulminant hepatitis at a Japanese aquarium involved 3 otariids: a California sea lion (*Zalophus californianus*), a South African fur seal (*Arctocephalus pusillus*) and a South American sea lion (*Otaria flavescens*). In a span of about a week in February 2012, 3 otariids showed diarrhea and were acutely low-spirited; subsequently, all three animals died within a period of 3 days. Markedly increased aspartate amino transferase and alanine amino transferase activities were observed. Necrotic hepatitis and eosinophilic intranuclear inclusion bodies in liver hepatocytes and intestinal epithelial cells were observed in the South American sea lion on histological examination. Otarine adenovirus DNA was detected from the livers of all three animals by polymerase chain reaction and determination of the sequences showed that all were identical. These results suggest that a single otarine adenovirus strain may have been the etiological agent of this outbreak of fatal fulminant hepatitis among the different otariid species, and it may be a lethal threat to wild and captive otariids. This is the first evidence of an outbreak of lethal adenovirus infection among different otariid species.

1. Introduction

In marine mammals, adenoviruses have been isolated from wild cetaceans, including a sei whale (*Balaenoptera borealis*) (Smith and Skilling, 1979), two bowhead whales (*Balaena mysticetus*) (Smith et al., 1987) and a beluga (white whale, *Delphinapterus leucas*) (De Guise et al., 1995). These viruses were isolated from gastrointestinal samples, and the correlation between disease and these viruses in cetaceans remains unclear. Meanwhile, in pinnipeds (families *Otariidae*, *Phocidae* and *Odobenidae*), adenovirus particles have been reported in the livers of stranded California sea lions (*Zalophus californianus*) with hepatitis in California (Britt et al., 1979; Dierauf et al., 1981), and a novel adenovirus, otarine adenovirus 1, has been isolated (Goldstein et al., 2011). Although the origin was not clear, walrus adenovirus and fur seal adenovirus were used in serological study of Hawaiian monk seal (*Monachus schauinslandi*) (Aguirre et al., 2007). In humans and terrestrial animal species, adenoviruses generally have a narrow host range, but the host range and pathogenicity of adenoviruses in marine mammals, as well as the morbidity and mortality resulting from these diseases, remains poorly understood.

In this study, we describe an outbreak of fatal acute fulminant hepatitis in an aquarium in Japan that affected three different otariid species – a California sea lion, a South African fur seal (*Arctocephalus pusillus*) and a South American sea lion (*Otaria flavescens*). Based on liver function test, histological examination and genetic analysis, we determined that the outbreak of fatal fulminant hepatitis among these different otariid species was due to infection by a single otarine adenovirus strain.

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2. Materials and methods

2.1. Animals and samples

In a span of about a week in February 2012 at an aquarium in Mie Prefecture, Japan, three otariids, a California sea lion, a South African fur seal and a South American sea lion, showed diarrhea and were acutely low-spirited; subsequently, all three animals died within a period of 3 days. First, on 12 February 2012, clinical symptoms of diarrhea were observed in a South African fur seal, and it died 6 days later. On the next day, a South American sea lion and a California sea lion showed diarrhea and died during the following day (Table 1). Before the appearance of clinical symptoms, these three animals had been clinically healthy, and no otariid species had been introduced into the aquarium since 2007. Serum was collected from each animal, and serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were measured using an automated biochemical analyzer TBA-c16000 (Toshiba Medical Systems, Tochigi, Japan). Tissue samples were collected from the South American sea lion for histological examinations; tissue samples were fixed in 15% neutral buffered formalin, embedded in paraffin, cut into 3-μm sections, and stained with hematoxylin and eosin. DNA was extracted from the livers of all three animals using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.2. Polymerase chain reaction (PCR)

The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was amplified as an internal control and assay of the quality of the extracted DNA using GAPDH primers (Beineke et al., 2004). To detect adenoviral DNA, PCR was conducted using the adenovirus consensus primers for the pol gene (Wellehan et al., 2004). PCR was also performed using primers for the adenoviral hexon (Thomson et al., 2002) and canine adenovirus 1 (CAV-1) and -2 (Hu et al., 2001) genes, and PCR with primers specific to otarine adenovirus (Goldstein et al., 2011) was also conducted. PCR mixtures (20 μl) contained 10 μl of GoTaq Green MasterMix (Promega, Madison, WI, USA), 10 pmol of each primer, and 2 μl of extracted DNA.

2.3. Sequences and phylogenetic analyses

PCR products of the pol and hexon gene amplifications were applied to direct sequencing using an ABI 310 DNA sequencer (Applied Biosystems, Foster City, CA, USA) with a BigDye Terminator ver. 3.1 Cycle Sequencing kit (Applied Biosystems). Sequencing was performed in both directions for verification. Deduced amino acid sequences were aligned with other adenovirus sequences using the CLUSTAL W program within MEGA5.05 (Tamura et al., 2011). Phylogenetic trees were constructed by the maximum likelihood method with the Jones–Taylor–Thornton model, uniform rates for sites, complete deletion treatment for gaps and missing data, and Nearest-Neighbor-Interchange search heuristic method within MEGA5.05. The confidence of each branch in the phylogeny was estimated with bootstrap values calculated from 1000 replicates.

3. Results

3.1. Liver function test and histological examination

Liver function tests revealed markedly increased AST and ALT activities (Table 1), which suggest fulminant hepatitis. Histological examinations of the South American sea lion showed necrotic hepatitis and eosinophilic intranuclear inclusion bodies in the liver hepatocytes (Fig. 1) and intestinal epithelial cells, suggesting the possibility of adenovirus infection.

3.2. Sequencing and phylogenetic analyses

Fragments of the expected size and sufficient quality were produced by GAPDH amplification, indicating that DNA extracted from the livers of the three animals was of sufficient quality. Neither CAV-1 nor -2 was detected in the PCR using primers for CAV. Adenovirus DNA was amplified from livers of the South American sea lion and California sea lion by consensus PCR and consensus nested PCR,

Table 1
Characteristics and clinical data for 3 otariids that died in this outbreak.

Animal species	Date of birth	Sex	Date symptoms appeared	Date of death	Clinical symptoms	AST (IU/L)	ALT (IU/L)	AST/ALT
South African fur seal *Arctocephalus pusillus*	3 June, 1987	Female	12 February, 2012	18 February, 2012	Diarrhea Low-spirited	7390	3531	2.1
South American sea lion *Otaria flavescens*	30 July, 1986	Male	19 February, 2012	20 February, 2012	Diarrhea Acutely low-spirited	12590	438	28.7
California sea lion *Zalophus californianus*	16 June, 1987	Female	19 February, 2012	20 February, 2012	Diarrhea Acutely low-spirited	Not determined	Not determined	

AST, asparate amino transferase; ALT, alanine amino transferase.