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Characterization of a parainfluenza virus isolated from a bottlenose dolphin (*Tursiops truncatus*)

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Abstract

A novel member of the parainfluenza virus family was identified in a bottlenose dolphin with respiratory disease. The case animal was a 19-year old male Atlantic bottlenose dolphin (*Tursiops truncatus*) that presented with signs of respiratory illness, including raspy, foul-odored breaths and cream-colored exudate from the blowhole. Focally extensive pyogranulomatous bronchointerstitial pneumonia with moderate numbers of intralesional yeast organisms was identified on histopathological examination. Other significant microscopic findings included multifocal erosive and ulcerative tracheitis and laryngitis consisting of active laryngeal lymphatic tissue and dilated glands with eosinophilic fluid. The cause of death was attributed to respiratory disease of unknown etiology. In addition to the postmortem isolation of *Candida glabrata* and mixed bacteria from lung tissue, a virus was isolated from two antemortem affected lung aspirates collected over a 2-month period and two postmortem samples (mediastinal lymph node and left lung tissue homogenate). The morphology of the virions on negative staining and transmission electron microscopy was consistent with that of paramyxoviruses. Two genomic fragments, comprising 532 and 419 nucleotides from the open reading frames that code for the viral polymerase and fusion protein, respectively, were amplified by polymerase chain reaction using degenerate primers. Phylogenetic analyses of the two viral RNA segments showed that the isolate comprised a novel virus strain, tentatively named *T. truncatus* parainfluenza virus type 1 (TtPIV-1). The virus is monophyletic with, but genetically distinct from, the various bovine parainfluenza virus type 3 strains. © 2007 Elsevier B.V. All rights reserved.

Keywords: Tursiops; Dolphin; Parainfluenza; Virus; Respiratory; Pathogen

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1. Introduction

Parainfluenza viruses (PIVs) are a group of non-segmented, negative-strand RNA viruses of the family *Paramyxoviridae*. Canine PIV-2, Simian PIV-2 and the Human parainfluenza viruses types 2 (HPIV-2) and 4 (HPIV-4) make up the genus *Rubulavirus*. The recognized members of the genus *Respirovirus* consist of the human parainfluenza viruses HPIV-1 and HPIV-3, Simian virus 10 (SV-10), as well as their murine relative Sendai Virus (SeV) and bovine parainfluenza virus 3 (BPIV-3) (Fauquet, 2005). PIVs appear to share a common evolutionary root, which is reflected in their morphology, antigenic reactivity, genome organization and genomic sequence. All PIVs have pleomorphic, spherical virions that range between 150–200 nm in diameter (Karron and Collins, 2007). Partial antigenic cross reactivity can be detected among all members of the genus (Ito et al., 1987). Their genomes are between 15,100 and 15,600 bp in length and all PIV genomes contain six genes: HN, F, M, N, P and L (Karron and Collins, 2007). Of these, the F gene encodes the fusion protein that is responsible for cell penetration and syncytium formation. The more conserved L gene encodes a large multifunctional protein that has RNA polymerase, capping and methylation activity (Karron and Collins, 2007).

PIVs include some of the most ubiquitous respiratory viral pathogens of infants and children, most of which cause high morbidity involving infections of the upper and lower respiratory tract (Karron and Collins, 2007). The human PIVs can replicate in the epithelial cells that line the entire respiratory tract and are a common cause of pneumonia, bronchiolitis and pharyngitis. Sinusitis and otitis media can occur in some patients (Karron and Collins, 2007). HPIV-1 and HPIV-2 have a particular tropism for the larynx and upper trachea, which commonly results in the croup syndrome. Most human croup patients recover spontaneously after 48–72 h, although in some cases fatal progressive airway obstruction can occur. The murine Sendai virus causes a severe but transient rhinitis and pneumonia in rats and mice (Giddens et al., 1987; Zurcher et al., 1977). Canine PIV-2 and bovine PIV-3 are associated with kennel cough and shipping fever syndromes in dogs and feedlot cattle, respectively. Shipping fever is a multifactorial, multiagent disease

syndrome of which the terminal stage is characterized by a necrotizing, fibrinous pneumonia (Rehmtulla and Thomson, 1981). Similarly, kennel cough is a multiagent respiratory disease syndrome. Dogs infected with canine PIV-2 typically develop a croup-like laryngotracheobronchitis and bronchiolar inflammation (Wagener et al., 1984).

In cetaceans (the group of animals that consists of all whales, dolphins and porpoises), respiratory anomalies are a common cause of disease (Dunn et al., 2001). A limited number of primary respiratory pathogens, however, have been identified in this group of animals. Bronchointerstitial pneumonia is commonly observed in cetaceans infected with cetacean morbillivirus (Duignan, 1999). Three virus isolates belonging to the influenza A subtypes (H1N3, H13N9 and H13N2) have been isolated from whales with hemorrhagic pneumonia (Hinshaw et al., 1986; Lvov et al., 1978). Other infectious organisms such as bacteria and fungi have also been identified as primary respiratory pathogens. *Pasteurella* spp. and *Nocardia* spp. have been isolated from bottlenose dolphins with tracheitis and hemorrhagic bronchopneumonia (Medway and Schryver, 1973; Pier et al., 1970). The most commonly reported mycotic pulmonary infection of cetaceans is pulmonary aspergillosis (Reidarson et al., 2001). In wild and stranded cetaceans, respiratory disease is most often attributed to pseudaliid and crassicaudid parasite infestations (Measures, 2001).

Here, we report on the isolation and morphological and molecular characterization of a parainfluenza virus isolated from an Atlantic bottlenose dolphin (*Tursiops truncatus*) with severe respiratory disease. Nucleic acid sequences of the F and L genes support the classification of the virus as a member of the genus *Respirovirus*. The virus is tentatively named *T. truncatus* parainfluenza virus type 1 (TtPIV-1) and is a suspected primary respiratory pathogen of bottlenose dolphins.

2. Methods

2.1. Clinical case history

The case animal was a 19-year old male Atlantic bottlenose dolphin that presented with signs of respiratory illness, including raspy, foul-odored

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