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Short communication

New recognition of *Enterovirus* infections in bottlenose dolphins (*Tursiops truncatus*)

Hendrik H. Nollens [a,b,*], Rebecca Rivera [b], Gustavo Palacios [c], James F.X. Wellehan [a], Jeremiah T. Saliki [d], Shannon L. Caseltine [e], Cynthia R. Smith [f], Eric D. Jensen [f], Jeffrey Hui [c], W. Ian Lipkin [c], Pamela K. Yochem [b], Randall S. Wells [g], Judy St. Leger [h], Stephanie Venn-Watson [f]

[a] *Marine Mammal Health Program, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA*
[b] *Hubbs-SeaWorld Research Institute, San Diego, CA 92109, USA*
[c] *Center for Infection and Immunity, Columbia University, New York, NY, USA*
[d] *College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA*
[e] *Receptor Logic, Ltd., c/o School of Pharmacy, TTUHSC, 718 Pine Street, Abilene, TX 79601, USA*
[f] *Navy Marine Mammal Program Foundation, San Diego, CA 92106, USA*
[g] *Chicago Zoological Society, c/o Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, USA*
[h] *SeaWorld, San Diego, 500 SeaWorld Drive, San Diego, CA 92109, USA*

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ABSTRACT

An enterovirus was cultured from an erosive tongue lesion of a bottlenose dolphin (*Tursiops truncatus*). The morphology of virions on negative staining electron microscopy was consistent with those of enteroviruses. Analysis of 2613 bp of the polyprotein gene identified the isolate as a novel enterovirus strain, tentatively named bottlenose dolphin enterovirus (BDEV), that nests within the species *Bovine enterovirus*. Serologic evidence of exposure to enteroviruses was common in both free-ranging and managed collection dolphins. Managed collection dolphins were more likely to have high antibody levels, although the highest levels were reported in free-ranging populations. Associations between enterovirus antibody levels, and age, sex, complete blood counts, and clinical serum biochemistries were explored. Dolphins with higher antibody levels were more likely to be hyperproteinemic and hyperglobulinemic.

1. Introduction

The enteroviruses (EV) are members of the genus *Enterovirus* within the family *Picornaviridae*. Enteroviruses were originally distinguished from these other picornaviruses by their physical, chemical and antigenic characteristics. Current state-of-the-art virus identification and characterization of EV consist largely of sequencing of the viral genome and pairwise genomic sequence alignments. The recognized members of the genus *Enterovirus* consist of the human enteroviruses A–D, poliovirus, porcine enteroviruses A and B, Simian enterovirus A, and bovine enteroviruses A and B (Stanway et al., 2005; Zell et al., 2006). The EV genome contains only one open reading frame (ORF) encoding a large polyprotein which is subsequently cleaved to give the various viral proteins. Genetic distance analyses using the immunodominant VP1 capsid coding region of the EV genome appear to mirror the antigenic relatedness more closely (Oberste et al., 1999). It is commonly accepted that VP1 nucleotide identities of greater than 75% suggest that isolates are serologically identical.

* Corresponding author at: University of Florida, SACS, College of Veterinary Medicine, P.O. Box 100126, Gainesville, FL 32610, USA.
Tel.: +1 619 225 4393; fax: +1 619 226 3944.
E-mail addresses: NollensH@mail.vetmed.ufl.edu, nollensh@vetmed.ufl.edu (H.H. Nollens).

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Most EV enter the host via the oral route and establish infections in the small intestine. EV can be recovered in the acute phase of the infection from the saliva and throat swabs. Most EV infections do not appear to cause clinical disease, but associated clinical signs can include fever, gastrointestinal disease, meningitis, myocarditis, inflammatory myopathies, abortion, and mucocutaneous blisters (Pallansch and Roos, 2007). High levels of neutralizing antibodies are typically generated following EV infection, which usually result in life-long immunity to clinical disease (Minor et al., 1981). This robust antibody response is, in part, responsible for the high prevalence of EV-specific antibodies in the human population (Melnick, 1996).

Here, we report on the isolation and characterization of an enterovirus isolated from an erosive tongue lesion in an adult female Atlantic bottlenose dolphin (*Tursiops truncatus*). The amino-terminal portion of the predicted viral polyprotein was used to determine the phylogenetic classification of the bottlenose dolphin enterovirus (BDEV). The prevalence and clinical relevance of BDEV-like infections in dolphins were assessed in a retrospective serologic survey of wild, stranded, and managed collection dolphin populations.

2. Materials and methods

2.1. Virus isolation

The case dolphin was a 16-year-old, clinically healthy, female Atlantic bottlenose dolphin that presented with multifocal, small (approximately 1 mm), and erosive tongue lesions. The dolphin was part of a managed collection and was housed in a coastal open ocean water enclosure. No behavioral abnormalities were noted. A throat swab was collected from the case dolphin using a sterile cotton swab and was mailed overnight on ice to a reference laboratory for viral isolation using African green monkey kidney (Vero) cells (Supplemental Materials). One infected Vero cell monolayer exhibiting CPE was processed for negative staining electron microscopy (NEM) (Fig. 1 and Supplemental Materials).

2.2. Molecular characterization and phylogeny

A viral genomic sequence segment was generated through degenerate PCR (Nix et al., 2006). The viral isolate was also studied by using GreeneChip Vr1.5 (Palacios et al., 2007). Hybridized cDNA was eluted from the microarray and was sequenced. The contiguous sequence was obtained by primer walking between these sequences (Fig. 2 and Supplemental Materials: Table 1). Each nucleotide position was sequenced at least three times in each direction. Sequence identity was determined via pairwise comparison with the sequences in GenBank, EMBL and the Data Bank of Japan using TBLASTX (Altschul et al., 1997). Phylogenetic placement was determined for the predicted VP1, VP2, and VP3 proteins individually using maximum likelihood and Bayesian posterior analysis (Fig. 3 and Supplemental Materials).

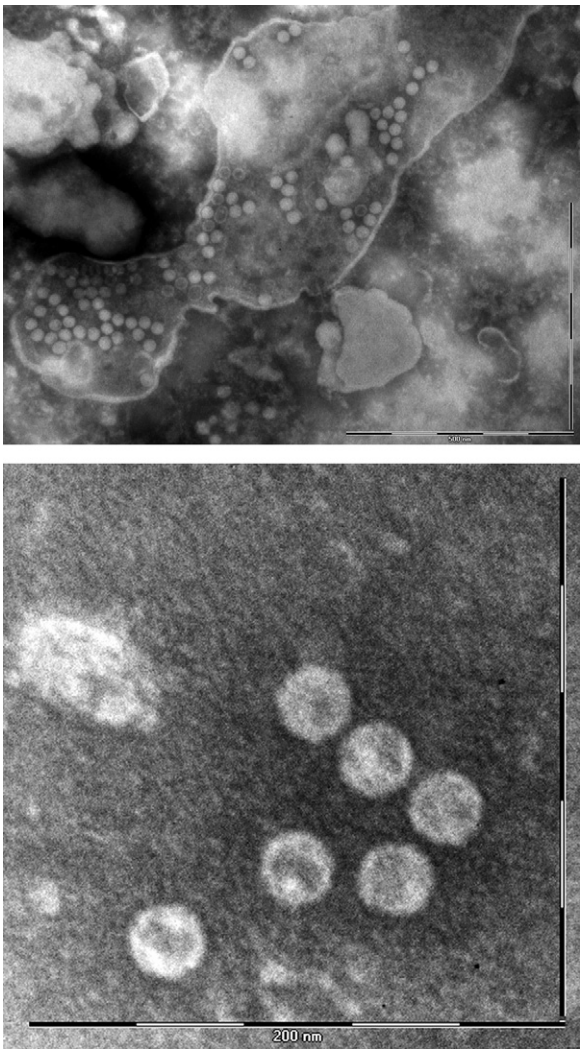

Fig. 1. Negatively stained preparations of the bottlenose dolphin enterovirus BDEV as seen by electron microscopy. Bar = 500 nm (top) and 200 nm (bottom). The size and morphology of the virions were consistent with those of enteroviruses. Empty capsids were occasionally observed.

2.3. ELISA development

Purified BDEV was used as antigen in the whole virus-based indirect ELISA system (Supplemental Materials). The dolphin sera were analyzed in triplicate and a no-serum negative control and one reference serum sample were included on each plate. A biotinylated monoclonal antibody specific for bottlenose dolphin IgG (Nollens et al., 2007) was used for the detection of bound antibodies. The OD_{405} was recorded 60 min after addition of the substrate. For analysis, all results were presented as OD_{405} ratios, defined as the mean OD_{405} of the triplicate readings of the unknown samples divided by the mean OD_{405} reading of the reference sample.

2.4. Serum sample collection

Serum samples were collected from five dolphin populations (Supplemental Materials: Table 2). Population