



# Herpes simplex-like infection in a bottlenose dolphin stranded in the Canary Islands

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**ABSTRACT:** A bottlenose dolphin, stranded in the Canary Islands in 2001 exhibited non-suppurative encephalitis. No molecular detection of cetacean morbillivirus (CeMV) was found, but a herpesviral-specific band of 250 bp was detected in the lung and brain. The sequenced herpesviral PCR product was compared with GenBank sequences, obtaining 98 % homology (p-distance of 0.02) with *Human herpesvirus 1* (herpes simplex virus 1 or HSV-1). This is the first report of a herpes simplex-like infection in a stranded dolphin.

**KEY WORDS:** Bottlenose dolphin · Herpesvirus · *Herpes simplex virus* · Infectious diseases · Zoonosis

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## INTRODUCTION

Herpesviral infections are very common in a wide variety of hosts, mainly vertebrates, including birds, reptiles and mammals (McGeoch et al. 2006). Herpesviruses are double-stranded DNA viruses, with an icosahedral form and a genome length that varies from 120 to 230 kbp (Roizman 1996).

There are few reports of herpesviral infections in marine mammals. Most reports are morphological, where the diagnosis is based on gross pathology (Baker 1992), histopathology and immunohistochemistry (Kennedy et al. 1992) or transmission electron microscopy (TEM) (Martineau et al. 1988, Van Bressem et al. 1994). So far, no cetacean herpesviruses have been isolated with cell culture; thus, molecular diagnostic tools are essential to establish their taxonomic classification. Amplification of conserved DNA regions within the herpesvirus polymerase gene (Van-Devanter et al. 1999) and terminase gene (Hargis et al. 1999) has been possible using universal PCRs. Further sequencing of the resultant amplicons has provided new reports of alphaherpesvirus in the bottlenose dolphin *Tursiops truncatus* (Blanchard et al. 2001, Manire et al. 2006, Smolarek-Benson et al. 2006), and gammaherpesvirus in bottlenose dolphin, Risso's dolphin *Grampus griseus*, the dwarf sperm whale *Kogia*

*simia* and Blainville's beaked whale *Mesoplodon densirostris* (Saliki et al. 2006, Smolarek-Benson et al. 2006).

The Canary Islands are a privileged coastal system in terms of cetacean diversity and abundance, with more than 30 cetacean species (Stephanis & Urkiola 2007). This is due to their strategic location in the migratory routes and their oceanographic characteristics. The short continental shelf of the islands favours a close approach to the coast and the establishment of resident populations such as bottlenose dolphin, the short-finned pilot whale *Globicephala macrorhynchus* and the sperm whale *Physeter macrocephalus*. As a consequence, cetaceans can be observed close to the coast and are exposed to industrial, agricultural and urban sewage sources.

A retrospective study was performed to detect herpesviral sequences in banked tissue of stranded dolphins in the Canary Islands. The present report describes the first case of a herpes simplex-like infection in a bottlenose dolphin.

## MATERIALS AND METHODS

**Samples.** An adult male bottlenose dolphin stranded dead on Tenerife (Canary Islands) in 2001 was analyzed in this study. Gross pathology showed emacia-

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tion and the main histopathology finding was mild, non-suppurative encephalitis. The lung showed verminous pneumonia. Samples from brain, liver and lung were frozen at  $-80^{\circ}\text{C}$  until further analysis. In order to determine the presence of viral agents related to encephalitis in marine mammals, an RT-PCR for morbillivirus and a PCR for herpesvirus were performed on brain, liver and lung samples.

**RNA and DNA extraction.** Prior to nucleic acid extraction, 1 g of tissue was manually macerated in 10 ml of sterile phosphate-buffered saline (PBS) and subsequently centrifuged at  $900 \times g$  for 15 min. Supernatants from the macerates were collected. RNA extraction was carried out from a 100  $\mu\text{l}$  macerated sample using the TriPure Reagent (Roche Diagnostics) method, following the manufacturer's instructions. DNA extraction was performed with High Pure Template Preparation Kit (Roche Diagnostics), following the manufacturer's instructions. Extracted DNA was eluted in 50  $\mu\text{l}$  of sterile water.

**PCR.** Molecular detection of cetacean morbillivirus (CeMV) was performed by 1-step RT-PCR, which amplifies 426 bp within a conserved region in the phosphoprotein gene (Reidarson et al. 1998). Two negative controls and a positive control of dolphin morbillivirus (DMV), MUC strain, provided by T. Barrett (Institute for Animal Health, Pirbright Laboratory, UK) were included.

A universal nested PCR previously developed (VanDevanter et al. 1996) that amplifies a conserved region within the polymerase gene of the *Herpesviridae* family was applied. To prevent carryover contamination, 2 negative controls were added for each reaction, one for DNA extraction and the other for PCR reaction. A positive control of *Human herpesvirus 1* strain F, was provided by S. Gómez-Sebastián (National Institute for Agriculture and Food Scientific Research and Technology, INIA, Madrid, Spain). The expected size of the amplicon for herpesvirus ranged between 215 and 315 bp.

Products of both PCRs were electrophoresed in 2% agarose gels, stained with Sybr<sup>®</sup> Green. The specific bands were excised and sequenced. Purification of DNA products in positive samples was performed using a GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences), following the manufacturer's instructions. Sequencing was done in triplicate using an ABI Prism 3100 sequencer (Applied Biosystems).

Sequenced products were compared with sequences available in GenBank

using the BLAST search. The sequence obtained was aligned using the Clustal W software. Phylogenetic analysis was performed using MEGA 4.0 software (Tamura et al. 2007). Neighbor-joining was employed to infer the tree topology. The reliability of the trees was tested by bootstrapping 1000 replicates generated with a random seed. The matrix distances were calculated based on p-distance. The consensus tree is represented in Fig. 1.

## RESULTS AND DISCUSSION

A specific band was not observed in the RT-PCR for morbillivirus. In contrast, a herpesviral specific band of 250 bp was detected in lung and brain. The product was sequenced and compared with GenBank published sequences, obtaining 98% homology (p-distance of 0.02) with *Human herpesvirus 1* (herpes simplex virus 1 or HSV-1). Other similarities occurred with *Human herpesvirus 2* (herpes simplex virus 2 or HSV-2) and positive control (F strain of HSV-1), with p-distances of 0.13 and 0.14, respectively. The PCR method used in this study for herpesvirus detection was developed from a conserved fragment of herpesvirus polymerase gene, and the sequences from this region have been found to be unique to each herpesvirus species (VanDevanter et al. 1996). In addition, this PCR method has been used to detect and classify novel sequences of herpesvirus in different hosts such as

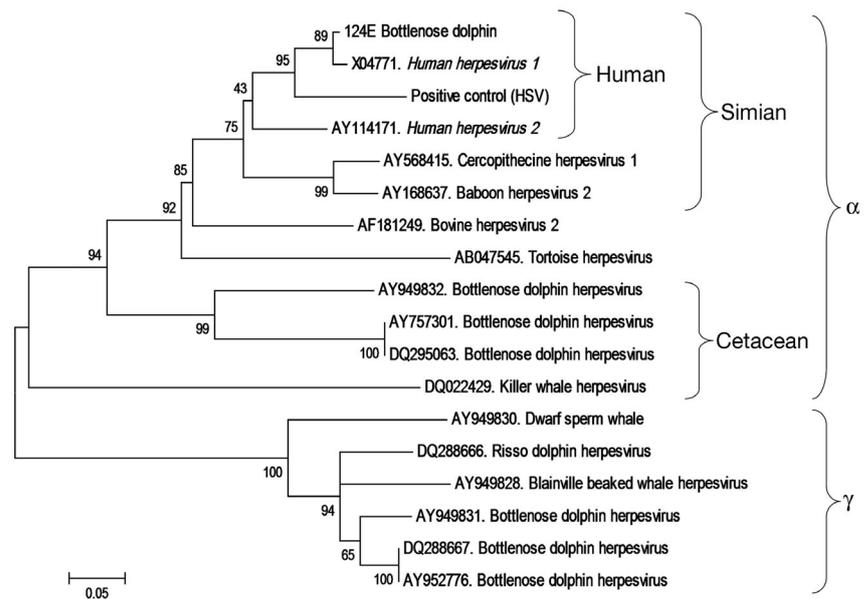


Fig. 1. Neighbor-joining phylogram of 18 selected sequences from cetacean alpha- and gammaherpesvirus and other phylogenetically related alpha-herpesviruses. The name of the sequence shows the GenBank Accession Number and host species. The GenBank Accession Number of the isolate of this study: EU003440

cetaceans (Blanchard et al. 2001, Saliki et al. 2006, Smolarek-Benson et al. 2006) and reptiles (Govett et al. 2005, Johnson et al. 2005, Wellehan et al. 2005). Due to the high homology found between the sequence obtained in the present study and the sequence for HSV-1 published in GenBank (X04771), it could be concluded that the first may be considered an HSV-like sequence.

On the other hand, p-distances among the sequence obtained from this bottlenose dolphin and others previously described in GenBank for cetacean herpesviruses ranged between 0.45 and 0.66 for alphaherpesviruses and 0.63 and 0.69 for gammaherpesviruses. Most of the molecular reports of cetacean herpesviruses are limited to cutaneous and mucosal infections (Saliki et al. 2006, Smolarek-Benson et al. 2006). Very few data are available about herpesviral systemic infections in cetaceans; however, one case of encephalitis in a harbour porpoise has been reported (Kennedy et al. 1992). The diagnosis of herpesviral encephalitis has been based on immunohistochemistry, showing cross reaction with HSV and bovine herpesvirus. Unfortunately these data are insufficient to identify the viral species implicated in this case. In addition, 2 cases of generalized systemic herpesviral infections in bottlenose dolphins (Blanchard et al. 2001) have been described, and 2 novel sequences from herpesviral polymerase genes were detected. Main pathological findings were observed in the lymphoid system, heart, skin and adrenal glands. These sequences showed a close homology with those described in other bottlenose dolphins with skin diseases (Smolarek-Benson et al. 2006) and therefore they may be considered as the same viral species.

Although it is widely demonstrated that viruses are species-specific, alphaherpesviruses are considered the least specific among the *Herpesviridae* family. Most of the reports about HSV in species other than humans are restricted to non-human primates such as chimpanzees (McClure et al. 1980), gorillas (Heldstab et al. 1981), common marmosets (Mätz-Rensing et al. 2003), saki monkeys (Schrenzel et al. 2003), gibbons (Landolfi et al. 2005) and orangutans (Kik et al. 2005). Only a few descriptions of HSV infections in non-primate hosts are available; they describe infections in a chinchilla (Wohlsein et al. 2002), a rabbit (Grest et al. 2002) and an African pygmy hedgehog (Allison et al. 2002).

The presence of an HSV-like sequence of possible human origin in a bottlenose dolphin has not been previously described. The small continental shelf of the Canary Archipelago favours the proximity of different cetacean populations to the coast, and this predisposes them to greater exposure to pesticides, industrial pollutants and urban wastes. As the human population increases, the environmental release of human micro-

organisms through urban sewage also increases, as has been demonstrated for enterovirus and norovirus (Payment et al. 2001, Haramoto et al. 2006). This could be a potential risk for cetacean populations. In addition, the role of faecal bacteria from Hong Kong's human sewage as the cause of death of stranding dolphins has been described previously (Parsons & Jefferson 2000).

Although an HSV-like sequence has been detected in the brain of a stranded bottlenose dolphin, the role of this virus as an etiologic agent of mild non-suppurative encephalitis has not yet been determined. To resolve this question, immunohistochemical or TEM detection should be performed; unfortunately there was not adequate sample to perform these assays. The presence of herpesviruses in stranded cetaceans from the Canary Islands and their role in cetacean health status and stranding causes will be evaluated in further studies.

*Acknowledgements.* The authors thank R. Cabrera and E. Neves for their technical support. This study was financed by the project of the National Researching Plan, REN 2002-04162-C02-02.

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Editorial responsibility: Michael Moore,  
Woods Hole, Massachusetts, USA

Submitted: September 27, 2007; Accepted: March 6, 2008  
Proofs received from author(s): May 5, 2008