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LETTER TO THE EDITOR

Neuronal and astrocytic involvement in striped dolphins (*Stenella coeruleoalba*) with morbilliviral encephalitis

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Summary. – Dolphin morbillivirus (DMV), a highly pathogenic agent, may cause peculiar, "brain-only" forms of infection (BOFDI), in which viral antigen and/or genome is found exclusively in the brain from striped dolphins (*Stenella coeruleoalba*). These BOFDIs show morphopathological similarities with subacute sclerosing panencephalitis and old dog encephalitis (ODE) in measles virus-infected patients and in canine distemper virus-infected dogs, respectively. The brain tissue from 3 BOFDI-affected striped dolphins was investigated by means of double labelling-indirect immunofluorescence (DL-IIF) and ultrastructurally, in order to characterize the DMV-targeted neuronal and non-neuronal cell populations, along with the associated submicroscopic findings. Viral colonization of calbindin-immunoreactive (IR) and nitric oxide synthase-IR neurons was detected in the cerebral parenchyma from the 3 DMV-infected dolphins under study, associated with nuclear (chromatin) and cytoplasmic (mitochondrial) ultrastructural changes. Furthermore, a limited viral targeting of brain astrocytes was found in these animals, all of which exhibited a prominent astrogliosis/astrocytosis. To the best of our knowledge, those herein reported should be the first submicroscopic pathology and neuropathogenetic data about BOFDI in striped dolphins. In this respect, the marked astrogliosis/astrocytosis and the low viral colonization of brain astrocytes in the 3 DMV-infected dolphins under investigation are of interest from the comparative pathology and viral neuropathogenesis standpoints, when compared with ODE-affected dogs, in whose brain a non-cytolytic, astrocyte-to-astrocyte infectious spread has been recently documented. Further studies aimed at characterizing the complex DMV-host interactions in BOFDI-affected striped dolphins are needed.

Keywords: dolphin morbillivirus; striped dolphin; Stenella coeruleoalba; encephalitis; neurons; astrocytes

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Abbreviations: Ab = antibody; BOFDI = "brain-only" form of dolphin morbillivirus infection; CALB = calbindin; DL-IIF = double labelling-indirect immunofluorescence; DMV = dolphin morbillivirus; FITC = fluorescein isothiocyanate; IHC = immunohistochemistry, immunohistochemical; mAb = monoclonal antibody; MeV = measles virus; N = nucleoprotein; NOS = nitric oxide synthase; ODE = old dog encephalitis; poAb = polyclonal antibody; RT-PCR = reverse transcriptase PCR; SSPE = subacute sclerosing panencephalitis

A small fraction (8–20 out of 1 million) of measles virus (MeV)-infected humans have been reported to develop a peculiar neurologic disease form called "subacute sclerosing panencephalitis" (SSPE) (1, 2), sharing a number of neuropathological and neuropathogenetic similarities with "old dog encephalitis" (ODE) in canine distemper virus (CDV)-infected dogs (*3, 4*).

Dolphin morbillivirus (DMV), a highly pathogenic agent responsible for at least 5 unusual mortality events among Mediterranean cetaceans, may also cause peculiar, "brain-only" forms of infection (BOFDI), in which im-

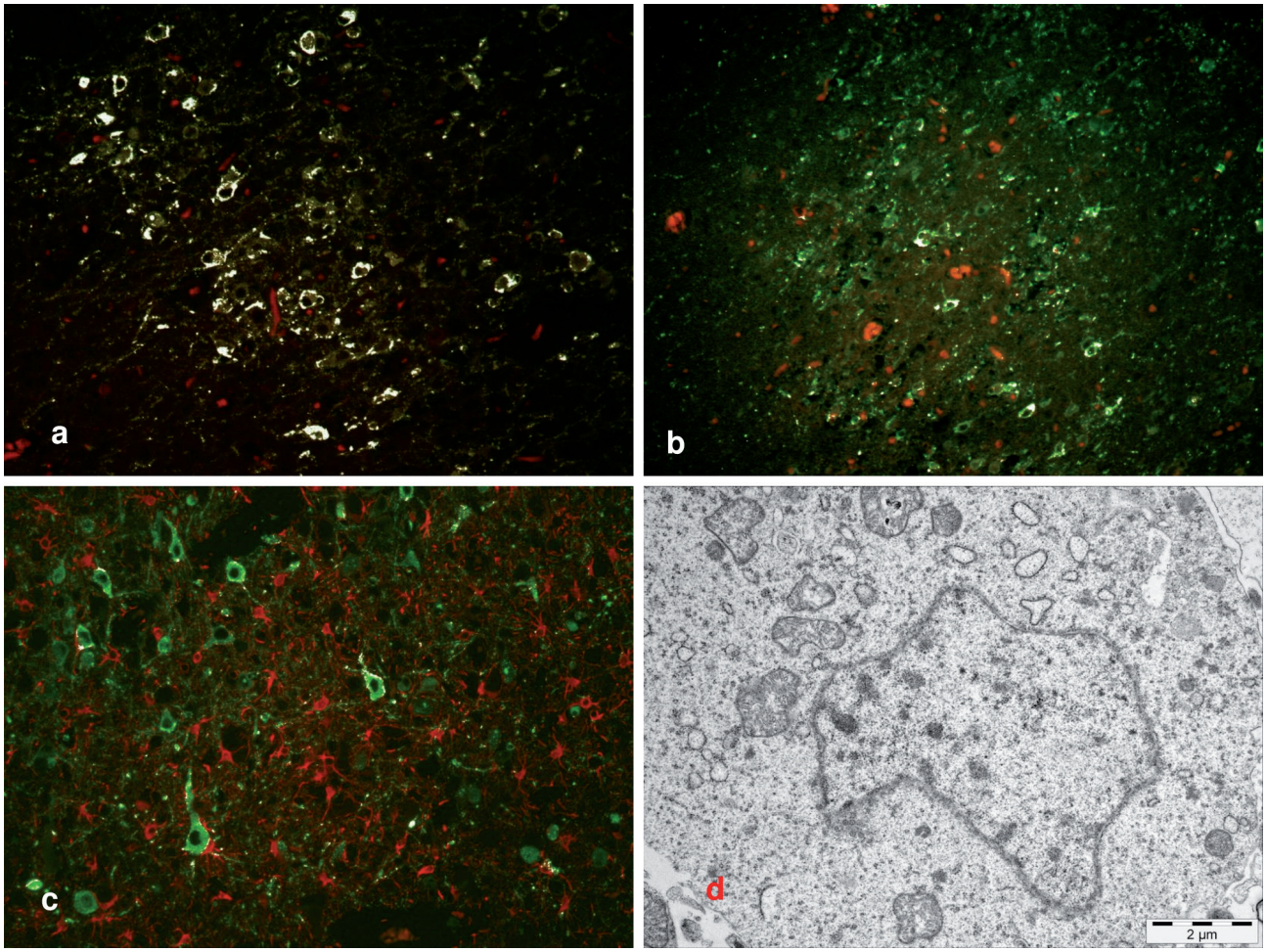

Fig. 1

Striped dolphin (*Stenella coeruleoalba*)

(a) Brain tissue from an adult animal affected by a "brain-only" form of DMV infection (BOFDI). A prominent colocalization (which is expressed as a white signal, obtained by means of the *ImageJ* software) of morbilliviral antigen immunolabelling is shown within calbindin (CALB)-immunoreactive (IR) neurons. Double labeling-indirect immunofluorescence (DL-IIF), Texas Red and fluorescein isothiocyanate (FITC) fluorochromes; 20x objective. **(b)** Brain tissue from the same animal depicted in Fig. 1-a. A less pronounced colocalization (which is expressed as a white signal, obtained by means of the *ImageJ* software) of morbilliviral antigen immunostaining is shown within nitric oxide synthase (NOS)-IR neurons, as compared to CALB-IR cells (Fig. 1-a). DL-IIF, Texas Red and FITC fluorochromes; 20x objective. **(c)** Brain tissue from a BOFDI-affected animal (young individual). Despite a prominent astrogliosis/astrocytosis, a very limited colocalization (which is expressed as a white signal, obtained by means of the *ImageJ* software) of morbilliviral antigen immunolabeling is shown within astrocytes (which are marked in red, while neurons are marked in green). DL-IIF, Texas Red and FITC fluorochromes; 20x objective. **(d)** Brain tissue from the same animal depicted in Fig. 1-c. Transmission electron microscopy shows variably sized, shaped and damaged mitochondria with swollen *cristae* inside a brain cell's cytoplasmic compartment**.** Uranyl acetate and lead citrate staining (bar = 2 microns).

munohistochemical (IHC) and/or biomolecular (RT-PCR) evidence of viral antigen and/or genome is found exclusively in the brain tissue from infected striped dolphins (*Stenella coeruleoalba*) (*5–8*).

Since no information has been hitherto published in this regard, we used double labelling-indirect immunofluorescence (DL-IIF) and transmission electron microscopy on the brain tissue from 3 BOFDI-affected striped dolphins (2 adults and 1 young individual), in order to characterize the neuronal and non-neuronal cell populations targeted by the viral pathogen, along with their submicroscopic changes. Evidence of DMV antigen and/or genome exclusively in the brain parenchyma from the 3 dolphins under study had been previously obtained by means of *ad hoc* IHC and RT-PCR investigations, as reported elsewhere (*6, 9*). A commercially available murine monoclonal antibody (mAb) recognizing a highly conserved epitope of the nucleoprotein (N) antigen of CDV was employed as primary Ab in the aforementioned

IHC and DL-IIF investigations; to this aim, suitable positive (previously ascertained cases of morbilliviral infection among free-ranging cetaceans) and negative (omission of primary Ab and/or utilization of brain sections from dolphins infected by unrelated pathogens) control tissues were included in each IHC run (*6, 9*). The neuronal and non-neuronal cell populations colonized by DMV in the brain tissue from the herein investigated dolphins were assessed by means of 3 commercially available Abs, namely an anti-calbindin (CALB) murine mAb, an anti-nitric oxide synthase (NOS) rabbit polyclonal (poAb), and an anti-glial fibrillary acidic protein (GFAP) rabbit poAb. Appropriate, commercially available goat anti-mouse and anti-rabbit immunoglobulins were additionally used as secondary Abs, with fluorescein isothiocyanate (FITC) and Texas Red being employed as fluorochromes for DL-IIF studies. Finally, the anti-CALB and anti-NOS Abs were also "validated", by means of Western blot analysis, for their use on striped dolphins' brain.

A prominent viral colonization of CALB-immunoreactive (IR) neurons (Fig. 1a) and, to a lesser extent, also of NOS-IR neurons (Fig. 1b), was found in the brain parenchyma from the 3 DMV-infected dolphins under study, independently of their age. Furthermore, although a prominent astrogliosis/astrocytosis was detected in all of these animals, a very limited viral colonization of their brain astrocytes was simultaneously observed (Fig. 1c). Ultrastructural evidence of nuclear chromatin fragmentation and clumping as well as of nuclear membrane damage, along with variably sized, shaped and altered mitochondria, were also observed in several brain cells (Fig. 1d).

Worthy to be mentioned, no direct (histological, immunohistochemical, ultrastructural, microbiological and biomolecular), nor indirect (serological) evidence of coinfection(s) by neurotropic pathogens other than DMV, both viral (such as *Herpesvirus*) and non-viral (such as *Toxoplasma gondii* and *Brucella* spp.), was found in the 3 cetaceans under investigation.

To the best of our knowledge, those herein reported should be the first ultrastructural pathology and neuropathogenetic data available for BOFDI in striped dolphins. In this respect, the marked astrogliosis/astrocytosis, coupled with the very limited DMV colonization of astrocytes in the 3 dolphins under study, appear to be of interest both from the comparative pathology and the viral neuropathogenesis standpoints. Indeed, in a sharply different manner from what was seen in our DMV-infected animals, the prolonged persistence of CDV in the brain parenchyma from ODE-affected dogs has been recently ascribed to a non-cytolytic, astrocyte-to-astrocyte viral spread through a hitherto undefined glial cell receptor (*10*). Furthermore, as far as human SSPE is concerned, a number of gene mutations associated with an increased viral spread capability have been reported in its causative MeV strains/genotypes (*1, 2, 11*). Since studies of this kind are not available for cetaceans, great caution would be needed before claiming that BOFDI-affected striped dolphins may be considered a "comparative neuropathology and viral neuropathogenesis model" for the study of SSPE in MeV-infected patients.

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