

Figure. Comparison of the prevalence of *Leishmania infantum* as tested by PCR and ELISA and of other infections compulsorily tested in 431 blood donors in Fortaleza, state of Ceará, northeastern Brazil. HBV, hepatitis B virus; HCV, hepatitis C virus; HTLV, human T-cell lymphotropic virus; Tc, *Trypanosoma cruzi*; Tp, *Treponema pallidum*.

determine whether recipients of blood from donors who are PCR positive and/or leishmanial antibody positive become infected with *L. infantum*. Persons with advanced AIDS or other immunosuppressive conditions seemingly would be at greatest risk for VL.

In Brazil, legislation requires that all blood for transfusion be tested for *T. cruzi*, hepatitis B and C, *T. pallidum*, human T-cell lymphotropic virus types 1 and 2, and HIV-1 and -2. As additional information becomes available, screening for *L. infantum* also might be advisable to reduce the possibility of the recipient becoming infected, developing VL, and possibly being a reservoir of infection in the community (10), particularly in Ceará and other regions where the prevalence of *L. infantum* infection is high.

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Morbillivirus and Pilot Whale Deaths, Canary Islands, Spain, 2015

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To the Editor: Four strains of cetacean morbillivirus (CeMV; family *Paramyxoviridae*, genus *Morbillivirus*) have been detected in the global cetacean population: porpoise morbillivirus (1), dolphin morbillivirus (2), pilot whale morbillivirus (PWMV) (3), and Longman's beaked whale morbillivirus (4). In addition, 2 novel

CeMV sequences or strains isolated from the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) and the Guiana dolphin (*Sotalia guianensis*) have been recently reported in the Southern Hemisphere (5,6).

Pilot whales are known to be susceptible to 2 strains of CeMV, PWMV, and dolphin morbillivirus (3,7,8). Only 2 deaths of whales have been reported to be caused by PWMV: 1 long-finned pilot whale (*Globicephala melas*) (3) and 1 short-finned pilot whale (*G. macrorhynchus*) (8). We report deaths of 3 short-finned pilot whales caused by PWMV in the northeastern Atlantic Ocean along the coast of the Canary Islands, Spain.

During mid-January–May 2015, a total of 3 whales (animals 1, 2, and 3) were found dead along the coasts of the Canary Islands (Table). Complete standardized necropsy was performed for all whales. Tissue samples from animals 1 and 2 were collected and fixed in 10% neutral-buffered formalin for histologic and immunohistochemical analyses (online Technical Appendix Figure, <http://wwwnc.cdc.gov/EID/article/22/4/15-0954-Techapp1.pdf>). Immunohistochemical analysis was performed (brain, intestine, lymph nodes, lung, kidney, adrenal gland, uterus, ovary, testis, and spleen) by using a monoclonal antibody against the nucleoprotein of canine distemper virus (CDV-NP; VMRD, Inc., Pullman, WA, USA) (7). Samples of lung, pulmonary lymph nodes, larynx, laryngeal tonsil, intestine, spleen, and brain were frozen (–80°C) for virologic analysis.

Grossly, the most remarkable findings in animal 1 were severe suppurative rhinitis, with clogged nasal passages by the accumulation of large quantity of purulent material, otitis media, sacculitis, and laryngitis. Severe diffuse epithelial hyperplasia and hyperkeratosis was observed along the

upper respiratory tract and keratinized stomach. Animal 2 had severe proliferative dermatitis and cheilitis, and severe, suppurative, laryngeal tonsillitis. Animal 3 had advanced autolysis, which precluded pathologic analysis.

Histologically, moderate, multifocal, bronchiointerstitial pneumonia, severe suppurative tonsillitis and systemic lymphoid depletion were identified in animals 1 and 2. Severe nonsuppurative meningoencephalitis with neuronal and glial cell degeneration and necrosis, microgliosis and syncytial cells were observed in animal 2.

Immunohistochemical analysis showed morbillivirus antigen in the bronchiolar epithelium, type 2 pneumocytes, and alveolar multinucleate cells. Syncytia from lymph nodes, laryngeal tonsil, spleen, and intestine also showed positive immunolabeling for morbillivirus. Epithelial tropism caused by the virus was suggested by identification of viral antigen in epithelia of the lung, larynx, keratinized stomach, intestine, kidney, urinary bladder, epididymis, and endometrial glands. In addition, intense immunolabeling was detected in neurons (soma, dendrites, axon hillock, and axons) and glial cells, primarily throughout the cerebral gray matter of animal 2.

Molecular detection of CeMV was performed by using a 1-step reverse transcription PCR for a 426-bp conserved region of the phosphoprotein gene (7). All tested samples from animals 1 and 2 and a laryngeal tonsil sample from animal 3 showed positive PCR results. Because co-infections with herpesvirus and morbillivirus were observed during morbillivirus epizootics in seals in 1988 and dolphins in 2006–2007, we also tested the same tissue for herpesvirus by conventional nested PCR (9). Herpesvirus DNA was detected in all samples from animal 1 except

Table. Characteristics for 3 short-finned pilot whales stranded along the Canary Islands, Spain, 2015*

| Animal no., age/sex | Total body length, cm | Weight, kg | Date stranded | Location | Decomposition code | Main pathologic findings | IHC-positive result | PCR-positive result |
|---------------------|-----------------------|------------|---------------|---------------|--------------------|---|---|--|
| 1, J/F | 202 | 119 | Jan 14 | Fuerteventura | Moderate autolysis | Dermatitis, suppurative rhinitis, paranasal sinusitis, otitis media, air sacculitis, laryngitis, laryngeal tonsillitis, BIP, hyperkeratotic gastritis, lymphoid depletion | Lung, larynx, laryngeal tonsil, lymph nodes, spleen, adrenal gland, keratinized stomach, intestine, uterus, ovary | Lung, larynx, pulmonary lymph node, laryngeal tonsil, spleen, intestine |
| 2, C/M | 168 | 75 | May 15 | Fuerteventura | Fresh | Dermatitis, cheilitis, suppurative laryngeal tonsillitis, BIP, encephalitis | Lung, laryngeal tonsil, lymph nodes, spleen, kidney, testis, epididymis, intestine, adrenal gland, urinary bladder, brain | Lung, pulmonary lymph node, laryngeal tonsil, intestine, brain |
| 3, A/M | 448 | 900 | May 20 | Tenerife | Advanced autolysis | Advanced autolysis | None | Laryngeal tonsil |

*IHC, immunohistochemical analysis; J, juvenile; BIP, bronchiointerstitial pneumonia; C, calf; A, adult. Tissues that were positive for herpesvirus and morbillivirus simultaneously are indicated in bold.

lung, although no specific lesions compatible with this infectious agent were observed.

A pool containing all morbillivirus-positive PCR amplicons for animals 1 and 2 (GenBank accession nos. KT006289 and KT006290), a PCR amplicon for the brain sample from animal 2 (GenBank accession no. KT006291), and a PCR amplicon for the larynx from animal 3 were sequenced. A BLAST search (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) showed that amplified samples were nearly identical to reference PWMV sequences (GenBank accession nos. AF200817 [3] and FJ842381 [8]). The sequence obtained from animal 3 was too short and degenerated to be accurately classified as CeMV, although it showed high homology with PWMV and porpoise morbillivirus.

It has been proposed that pilot whales might be enzootically infected with CeMV (10). These whales might be responsible for maintaining and transmitting CeMV over long distances or to other odontocetes. No die-offs have been observed in these species. However, an outbreak of a lethal morbillivirus infection in long-finned pilot whales caused by a dolphin morbillivirus strain occurred in the Mediterranean Sea during the end of October 2006–April 2007 (7).

Results of this study support the previous hypothesis that pilot whales have a species-adapted morbillivirus but indicate that lethal infections are not as rare as previously believed (3). The tropism of the virus in these cases, the high number of multinucleated syncytial cells, and the severity of the lesions resemble the acute systemic symptoms observed in dolphins infected with morbillivirus (2). Thus, pilot whales in the northeastern Atlantic Ocean could be at risk for infection, especially in one of the main pilot whale-watching regions between La Gomera and Southern Tenerife Islands in the Canary Islands, which has >700,000 visitors each year.

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Serogroup-specific Seasonality of Verotoxigenic *Escherichia coli*, Ireland

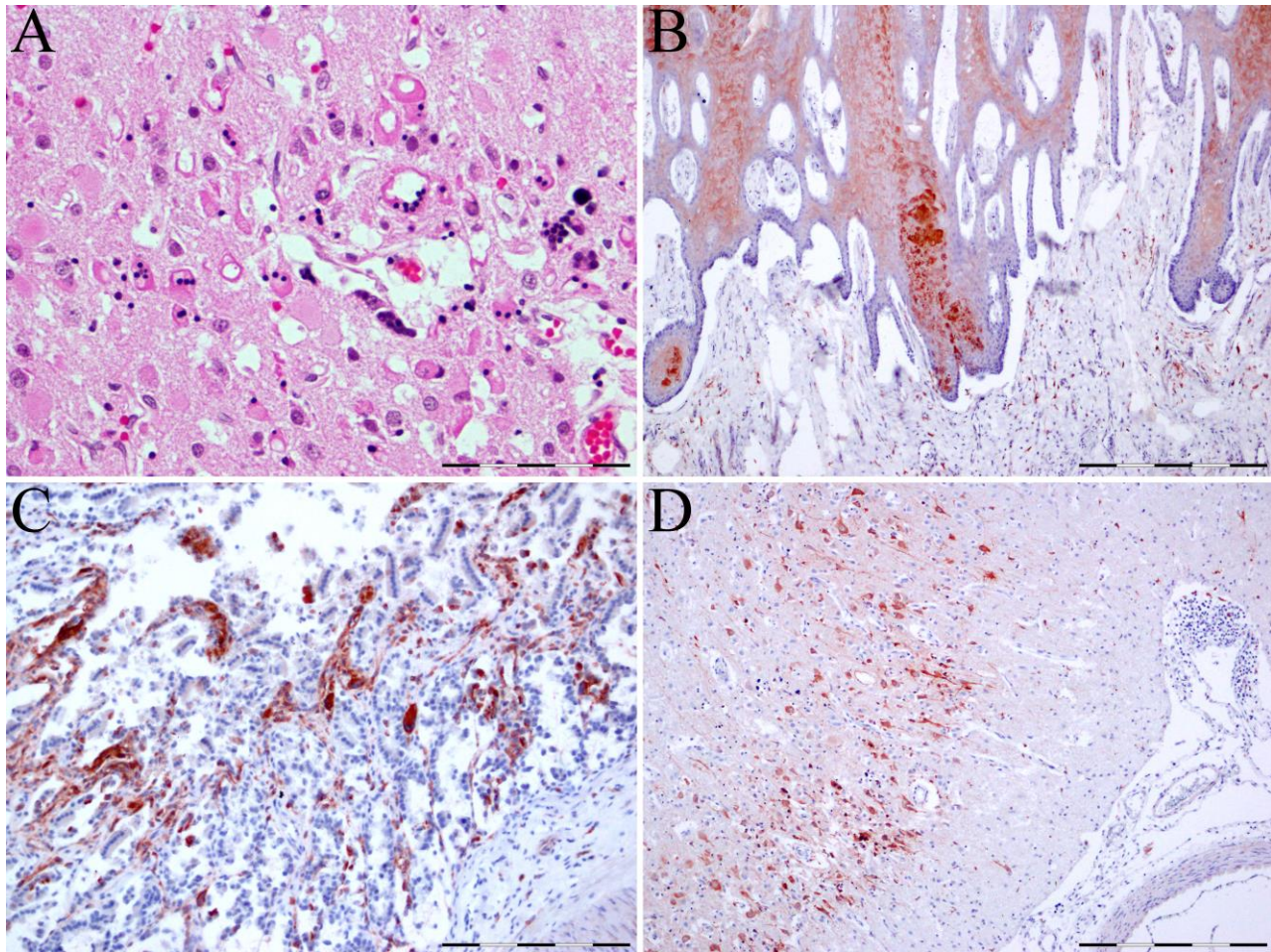
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Morbillivirus and Pilot Whale Deaths, Canary Islands, Spain

Technical Appendix



Technical Appendix Figure. Microscopic images of tissue samples from 2 short-finned pilot whales (*Globicephala macrorhynchus*) from the eastern Atlantic Ocean stranded along the Canary Islands, Spain, 2015. A) Brain of animal 2 showing degenerate neurons with cytoplasmic vacuolation, eccentric nuclei, and chromatolysis. Nuclear pyknosis is present in degenerating necrotic neurons (hematoxylin and eosin stained). Scale bar = 50 μ m. B) First compartment of the stomach of animal 1 showing intense immunoperoxidase staining of morbillivirus in cytoplasm and nuclei of hyperplastic epithelial cells of the keratinized stomach. Clusters of neutrophils were present at different layers of the hyperkeratotic epithelium (avidin–biotin–peroxidase stained and Harris hematoxylin counterstained). Scale bar = 200 μ m. C) Intestine of animal 1 showing intense immunoperoxidase staining of morbillivirus in cytoplasm and nuclei of epithelial and syncytial cells

(avidin–biotin–peroxidase stained and Harris hematoxylin counterstained). Scale bar = 100 μm . D) Brain of animal 2 showing intense immunostaining (neurons, glial cells, and neuronal processes) mainly confined to gray matter of cerebral cortex (avidin–biotin–peroxidase stained and Harris hematoxylin counterstained). Scale bar = 200 μm .