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Morbillivirus and herpesvirus in free-ranging bottlenose dolphins in the Canary Islands: a molecular retrospective study

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A retrospective study in free-ranging bottlenose dolphins (*Tursiops truncatus*) was performed by a molecular biological method polymerase chain reaction (PCR). Samples from 35 specimens stranded in Canary Islands from 1997 to 2013 were examined. The PCR analyses were performed on selected tissue samples, upon availability, for the presence of morbillivirus and herpesvirus. In addition, immunohistochemical detection of morbilliviral antigen in tissues was performed on PCR-positive samples. A systemic morbillivirus infection was detected, for the first time in the Canary Islands, in a juvenile bottlenose dolphin stranded in 2005. Sequence analysis of a conserved fragment of the morbillivirus phosphoprotein gene indicates that the virus is closely related to dolphin morbillivirus reported in striped dolphins currently in the Mediterranean Sea. This particular point is extremely important to better understand the epidemiology and transmission of morbilliviruses between cetacean populations. Conventional PCR detected herpesviral DNA in samples from four animals. The PCR positivity was observed in the skin, lung, and brain. Unit of Histology and Veterinary Pathology, Institute for Animal Health, Veterinary School, University of Las Palmas de Gran Canaria, Trasmontaña s/n, Arucas (Las Palmas), Canary Islands 35413, Spain.

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Two cases of morbillivirus infection in white beaked dolphins in the rehabilitation centre SOS-Dolfijn

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The virulence of morbillivirus differs according to host species. In odontocetes morbillivirus causes major epizootics with substantial mortalities in 4 species. In 8 species, including the white-beaked dolphin, morbillivirus has been observed as an incidental infection without it being clear what the virulence was. The admittance of two white-beaked dolphins, with an active morbillivirus infection, into the rehabilitation centre SOS-Dolfijn, provided a rare opportunity to investigate the virulence of morbillivirus in white-beaked dolphins. Both animals were investigated clinically and pathologically. Two forms of infection were diagnosed, an acute systemic infection in case number 1 and a chronic central nervous infection in case number 2. DNA analysis of the morbillivirus demonstrated it was Dolphin Morbillivirus (DMV) which was most closely related to DMV observed in a white-beaked dolphin which stranded in 2007 on the German coast. A moderate polioencephalitis was observed in the cerebrum of case number 2. However no nervous signs were observed. Case number 1 had a subacute systemic infection. No secondary infections were observed in this animal. Both animals died to causes unrelated to their morbillivirus infection. No DMV was detected in two contemporaneously stranded white-beaked dolphins. Stranding rate of white-beaked dolphins did not increase during the period of the stranding of the two DMV infected animals. These data suggest DMV is not highly virulent in white-beaked dolphins.

Fatal autoimmune disease in a 10 year old manatee (*Trichechus manatus manatus*)

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In summer 2012 Zoo Nuremberg received from Zoo Beauval the female manatee Luna. The transfer was recommended by the EEP in order to secure optimal breeding conditions for this female. She had a history of 2 dead calves (2008, 2011). In Nuremberg she twice showed skin lesions, which healed without treatment. In summer 2013 some vaginal discharge was observed. In autumn 2013 marked skin lesions, reduced behavior and feeding were noticed. The animal was moved to the medical pool for a complete examination including skin-, blow-, faeces- and blood samples, ultrasonography and x-rays. The blood tests revealed a slight anemia. There were no signs of pregnancy. The animal was treated with antibiotics. The symptoms progressed dramatically and she died 3 days later. The necropsy showed severe skin lesions with secondary bacterial and mycological infection. The anemia was confirmed and a hydrocephalus was detected. The lesions looked like an autoimmune disease. A close analysis of the Studbook data revealed that also her grandfather died from a suspected autoimmune dermatosis, so that a genetic predisposition for this disease should be taken into consideration.

Environmental monitoring program at the Marine Life Park, Singapore

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The Marine Life Park (MLP) is a new facility on Sentosa Island in Singapore and houses twenty-four (13:11) Indian Bottlenose Dolphins (*Tursiops aduncus*). The various aspects of its artificial environment are diligently monitored to ensure optimal living conditions for the animals. These include water quality, air quality, and pathogen monitoring (specifically *Burkholderia pseudomallei*). The most pressing environmental issue for 2013 was the Southeast Asian haze, wherein Singapore was one of the territories that was most severely affected. Respiratory samplings were done at a higher frequency than usual due to reports of chuffing and mucus expulsion in some of the animals. Results, however, were within normal limits. The haze is said to be a yearly phenomenon, and is allegedly due to seasonal burning of forests in Sumatra for palm oil production. Infrastructure modifications are being planned to provide refuge if haze reach unhealthy levels in the future. Also, the dolphins are currently being trained for "voluntary nebulization" behavior using a custom-made nebulizer. Despite having very low to negligible results for *B. pseudomallei* in the physical environment, the monitoring program is in full effect. No clinical case of melioidosis has been detected in the current dolphin population at MLP.

Assessment of the levels of polycyclic aromatic hydrocarbons and organochlorine contaminants in bottlenose dolphins (*Tursiops truncatus*) from the Canary Islands, the Eastern Atlantic Ocean.

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The concentrations of 57 Persistent Organic Pollutants (POPs) were determined in free-ranging and stranded bottlenose dolphins (*Tursiops truncatus*) from the Canary Islands. 64 blubber biopsies were collected from 2003 to 2011 and also blubber and liver of 27 dolphins stranded from 1997 to 2011. Median of total PCBs and DDTs were 30.8 ppm and 24.2 ppm lipid weight (lw), respectively, in biopsies, and 27.6 ppm and 23.2 ppm (lw), respectively, in blubber of stranded. Among PCBs, the highly chlorinated PCB 180, 153 and 138 were the predominant congeners, and the dioxin-like PCBs median levels were of 4.6 ppm (lw) in live specimens and 2.9 ppm (lw) in blubber of stranded dolphins. All the samples showed detectable values of any of the 16 PAHs studied. Phenanthrene was the major compound followed by pyrene and naphthalene. In free-ranging, the median value for total PAHs was 13.6 ppm (lw) while median PAH values in stranded was 0.8 ppm (lw). According to our results, concentrations of POPs are at toxicologically relevant levels in the bottlenose dolphins. The present information represents the first study of pollutants in free-ranging cetaceans from the Canary Islands and increases the data of stranded already reported from the studied area.

Possible allergic reaction in Bottlenose Dolphin (*Tursiops truncatus*): A case report

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An adult female Bottlenose Dolphin (*Tursiops truncatus*) and two of her offspring showed dermatological symptoms in their abdomen in spring 2013. An allergic reaction was suspected because of the unusual high pollen content in the air and the dolphin pool at that time. Symptoms included pruritus, marked reddish areas and little wheals which increased in size over a short period of time. Diagnostic tools included blood and biopsy samples, swabs cultures from the wheals, intradermal tests and response to treatment.

Cultures proved negative to pathological bacterial or fungal growth. Blood analyses were inconclusive, not showing any abnormal values. Biopsy sample showed hyperplasic dermatitis. Intradermal test proved difficult to evaluate and further research is warranted to set range values for specific *Tursiops truncatus* Ig G levels against different reagents. Methylprednisolone response proved effective at different doses in the three animals, being the highest dose 0, 2 mg/Kg SID 10 days, and the lowest 0,02 mg/Kg SID 3 days.

Herpesvirus infection associated with tubulo-interstitial nephritis in a Blainville's beaked whale (*Mesoplodon densirostris*)

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Here we described a novel alphaherpesvirus associated with tubulo-interstitial nephritis in an adult male beaked whale (*Mesoplodon densirostris*) stranded in the Canary Islands. The animal, which showed a poor body condition, was found alive near shore on Tenerife Island, and died soon after beaching. Necropsy started 8 hours postmortem (code 1-2) and routine sampling for histological, immunohistological, electron-microscopy (EM), bacteriological and virological studies were carried out. Membranous glomerulonephritis with multifocal lymphoplasmacytic interstitial nephritis, multifocal interstitial and tubuloepithelial necrosis with presence of intranuclear inclusion bodies in tubuloepithelial cells were the main pathological findings. In the immunohistological study, HV antigen was only detected in the kidney, in which immunopositivity was clearly found in the intranuclear inclusions bodies of tubuloepithelial cells. Ultrastructurally, intranuclear inclusion bodies labeled immunohistologically, corresponded to HV particles. The HV DNA detection was conducted by a pan nested HV polymerase chain reaction (PCR). Positivity was observed in lung and kidney tissues. The same 181 bp (60 aa) sequence was obtained from the kidney and lung samples, and a 692 bp (230 aa) sequence from the kidney [GenBank accession number JN863234]. With the phylogenetic analysis it was shown that the sequence obtained in this study is a novel herpesvirus.

Traumatic intra-/interspecific interactions as a cause of mortality of stranded cetaceans in the Canary Islands

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From December 1999 to April 2013, a total of 574 cetaceans were stranded in the Canary Islands and a systematic and comprehensive pathological study was carried out in 405 specimens. Some cases showed severe traumatic injuries due to anthropogenic activities related to fisheries and vessel traffic but also to natural causes such as interactions between animals. A retrospective study revealed the presence of lesions related to intra- or interspecific in 31 (7.65%) out of 405 cases (including 13 different species). The most common observed lesions were hemorrhages (found in 29 out of 31; 93.55%), tooth marks in skin (18 out of 31; 58.06%) and fractures (14 out of 31; 45.16%), mainly located in the thorax but also on the head. Six out of 31 animals (19.35%) showed multiple fractures, sometimes bilateral, and other discrete lesions distributed along the body suggesting a repeated impact (on more than one direction) (Dunn et al., 2002). Final diagnosis was highly consistent with interaction between animals in 20 out of 31 (64.51%) cases. Other etiologies of trauma were not completely ruled out in 8 (25.81%) cases and the cause of death remained inconclusive in 3 cases (10%), due to advanced decomposition of the carcasses.