

Epidemiological analysis of Morbillivirus in cetaceans in the Canary Archipelago

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Abstract

From 1999 to 2023, 30 cetaceans of various species stranded on the coasts of the Canary Islands exhibited microscopic lesions compatible with Cetacean morbillivirus (CeMV) infection, confirmed through immunohistochemical and/or molecular techniques. This study aimed to analyze prevalence and compare findings with other regions, assess temporal distribution, seasonal incidence, species affected, age-group susceptibility, disease presentation, and strain diversity. As a result, the prevalence of CeMV-positive cetaceans stranded on the Canary Islands coast during this period was 3.56%, which is relatively low compared to similar studies in other regions. CeMV infection cases were recorded in most years of the study period, with notable exceptions in 1999-2001, 2003-2004, 2014, 2017, and 2023. Some of these periods were associated with outbreaks in the close Mediterranean Sea. A higher incidence of CeMV was observed during the spring and winter seasons, with 30% and 27% of CeMV-positive strandings occurring during these periods, respectively. Eight different cetacean species were infected by CeMV during the study period in the Canary Islands. The short-finned pilot whale (*Globicephala macrorhynchus*) and the striped dolphin (*Stenella coeruleoalba*) had the highest number of positive cases. An age susceptibility was noticed, since juveniles or subadults comprised the majority (63%) of CeMV-infected animals that stranded. Despite no reported outbreaks, most CeMV-infected cetaceans in the Canary Islands presented with the acute systemic form of the disease, predominantly affecting striped dolphins. Two distinct strains of CeMV circulate in Canary Islands waters: DMV is widespread among various cetacean species, while PWMV has been associated with an unusual mortality event and chronic localized encephalitis in short-finned pilot whales. These findings provide insights into the prevalence, seasonal patterns, species susceptibility, and strain diversity of CeMV in the Canary Islands over a 25-year study period.





Introduction

Theoretical fundamentals

There is growing concern among veterinary and human health disciplines about the importance of the link between health and environmental conditions, particularly in the context of species conservation (Deem et al., 2001). The connections between human health, animal health, and the environment in which they live are well known and have recently been termed "one health, one medicine." Habitat loss, overexploitation, human alteration of ecosystems, pollution, and pathogens are increasingly important factors for species conservation (Lafferty & Gerber, 2002). In the marine environment, human impacts have rapidly devastated populations, species, and ecosystems, as evidenced by the decline in populations of the Amazon river dolphin (*Inia geoffrensis*) and the Tucuxi (*Sotalia fluviatilis*) in Brazil, or the Vaquita (*Phocoena sinus*) in Mexico. The latter, a small porpoise endemic to the northern part of the Gulf of California, is on the brink of extinction partly due to illegal fishing operations in protected marine areas within Mexico's Gulf of California.

In the context of pathogens, these can act as drivers of environmental change, causing the extinction of endangered species, altering the proportions of predators, prey, competitors, or recycling organisms necessary for the proper functioning of ecosystems, and affecting habitats already threatened by fragmentation and climate change. Over the last 20 years, emerging infectious diseases (EIDs) have become a complex and serious concern with consequences for human, animal, and environmental health on a global scale (Bossart, 2007, 2011; Bossart et al., 2014). Factors contributing to the increased incidence of EIDs include microbial adaptation, host immune dysfunction, human population expansion and consequent environmental degradation, climate change that causes shifts in zoonotic vectors, and the negative synergistic effects of EIDs with other infectious and non-infectious diseases.

Cetaceans are considered sentinel species, meaning their health reflects the broader state of marine environments many species have long life spans, are long-term coastal residents, feed at a high trophic level, and have large blubber stores that can serve as depots for anthropogenic chemicals and toxins (Bossart, 2011). The utility of marine mammals as ecosystem sentinels is a function of their ecological diversity and the variability inherent in marine ecosystems. The presence and impact of morbillivirus in



cetacean populations can serve as an indicator of ocean health, highlighting changes in marine ecosystems that might also affect other marine life and human activities.

Cetacean morbillivirus (CeMV) is a highly infectious virus affecting marine mammals, particularly cetaceans such as dolphins, whales, and porpoises. Studying morbillivirus in cetaceans contributes to our understanding of disease ecology and the evolution of pathogens. It helps scientists learn how the virus spreads, adapts, and persists in marine environments. This knowledge is essential for developing effective management and mitigation strategies to protect cetacean populations.

Research on Cetacean morbillivirus infections informs conservation policies and management practices. It provides the scientific basis for creating and implementing measures to monitor, prevent, and respond to outbreaks, ensuring the protection of vulnerable cetacean species.



Background and status

Morbilliviruses (genus *Morbillivirus*, subfamily *Paramyxovirinae*, family *Paramyxoviridae*, order *mononegavirales*) are responsible for severe mortality and morbidity in humans and other animal species including marine mammals. Morbilliviruses consist of a non-segmented linear single-stranded RNA genome of negative polarity comprising about 15.702 base pairs (bp). This genus comprises seven species that, in general, are highly infectious, can be transmitted by the respiratory route, cause profound immune suppression predisposing to opportunistic infections, and tend to cause large outbreaks in previously unexposed populations (De Vries et al., 2015). Carnivore morbilliviruses are very neurotropic and can lead to long-term persistent infections in the central nervous system (Barrett, 1987). The agent causing measles in humans and primates is the prototype morbillivirus (Blixenkron-Moller et al., 1994; Rima et al., 2005; Rubio-Guerri et al., 2013; Van Bresseem et al., 2014) (Table1).

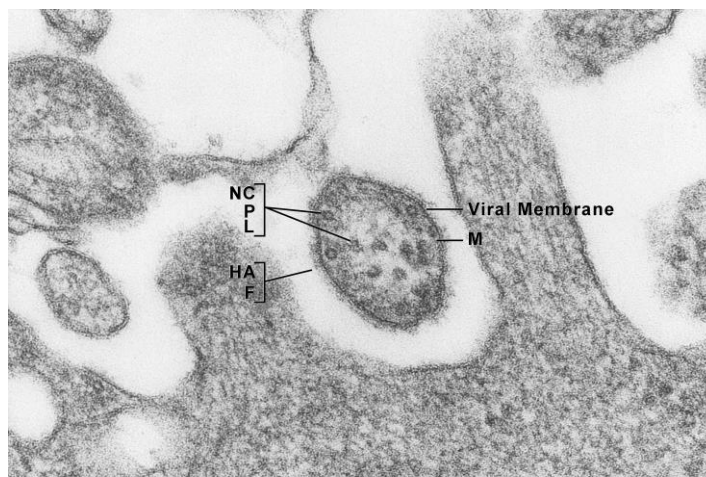
Morbillivirus species	Natural host spectrum	Associated diseases
Measles virus (MV)	<i>Humans</i> (primates)	Measles; giant cell pneumonia; sclerosing panencephalitis; inclusion body encephalitis.
Rinderpest virus (RPV)	<i>Artiodactyla</i>	Cattle plague.
Peste-des-petits ruminants virus (PPRV)	<i>Artiodactyla</i>	Pneumoenteritis of sheep and goats.
Cetacean morbillivirus (CeMV)	<i>Cetacea</i>	Distemper.
Canine distemper virus (CDV)	Order: <i>Carnivora</i> Family: <i>Canidae</i>	Distemper; osteodystrophy; old dog encephalitis (ODE).
Feline morbillivirus (FeMV)	Order: <i>Carnivora</i> Family: <i>Felidae</i>	Distemper.
Phocid distemper virus (PDV)	<i>Pinnipeda</i>	Distemper.

Table 1: Morbilliviruses - Taxonomy, host spectrum and clinical impact. International Committee on Taxonomy of Viruses (ICTV).

The virions of the *Paramyxoviridae* family exhibit a pleomorphic structure, ranging in diameter between 150 to 250 nm (Blixenkron-Moller et al., 1994; MacLachlan & Dubovi, 2017; Van Bresseem et al., 2014) and are composed of a lipid envelope formed by glycoproteins, which include the matrix protein (M), the fusion protein (F), and either the hemagglutinin protein (H) or the hemagglutinin-neuroaminidase protein (NH).



Additionally, they have a nucleocapsid that is spike-shaped and helically symmetrical, containing the viral RNA along with the nucleoprotein (N), the phosphoprotein (P), and the RNA-dependent RNA polymerase (L) (Figure 1).



NC = Nucleocapsid; P = Phosphoprotein; L = Large protein;
HA = Hemagglutinin; F = Fusion protein; M = Matrix protein

Figure 1. Measles virus observed by electron microscopy (Centers for Disease Control and Prevention's, Public Health Image Library).

Four viruses belonging in the Morbillivirus genus are responsible of significant causes of disease and mortality in marine mammals, involving canine distemper virus (CDV) in seals and polar bears (Follmann et al., 1996; Garner et al., 2000; Grachev et al., 1989), cetacean morbillivirus (CeMV) affecting mainly dolphins and whales, but exceptionally detected in seals (in a Mediterranean monk seal (*Monachus monachus*) and in a captive harbor seal (*Phoca vitulina*)) (Mazzariol et al., 2013; Petrella et al., 2021) and in wild Eurasian otters (*Lutra lutra*) (Padalino et al., 2019) and phocine distemper virus (PDV) in pinnipeds (Duignan et al., 2014). Based on the hemagglutinin gene sequence, PDV, like CDV (canine distemper virus), is composed for a single lineage in which they are closely related with a wild-type strains circulating in the North Atlantic (Duignan et al., 2014).

Cetacean morbillivirus has caused several outbreaks of lethal diseases and has resulted in cetacean strandings in odontocetes and mysticetes around the world, including harbour porpoises (*Phocoena Phocoena*), striped dolphins (*Stenella coeruleoalba*), bottlenose dolphins (*Tursiops truncatus*) and short- and long-finned pilot whales (*Globicephala* spp.). CeMV was first recognized as a pathogen of marine mammals following epidemics among harbor porpoises and striped dolphins in Europe in the late 1980s and early 1990s. Since then, infection has been recognized in a wide range of odontocetes and some



mysticetes globally (Van Bressem et al. 2014) with at least four well characterized strains and two different genetic lineages of CeMV currently proposed: the CeMV-1 lineage and the CeMV-2 lineage (L. A. Dierauf & D Gulland, n.d.; Van Bressem et al., 2014).

The CeMV-1 includes a) porpoise morbillivirus (PVM); b) dolphin morbillivirus (DMV), firstly isolated from Mediterranean striped dolphins; c) pilot whale morbillivirus (PWMV), recovered from a long- finned pilot whale (*Globicephala melas*) stranded in USA; and d) a fourth strain named beaked whale morbillivirus (BWMV) initially detected in a Longman's beaked whale (*Indopacetus pacificus*) stranded in Hawaii (West et al., 2015). Van Bressen et al. proposed the terminology **CeMV-1** for the “old” **northern hemisphere lineage** that includes DMV, PMV, PWMV and BWMV, and **CeMV-2** for the “new” **southern hemisphere lineage**, consisting of virus strains originally detected in a Guiana dolphin (*Sotalia guianensis*) from Brazil (Groch et al., 2014) and in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) from Western Australia (Stephens et al., 2014). It has been recently reported a novel morbillivirus strain detected in a Fraser's dolphin (*Lagenodelphis hosei*) stranded in Hawaii (West et al., 2021)

The first recognized marine mammal morbilliviral epizootic occurred in 1987-1988 along the Atlantic coast of the United States of America (USA). Over half of the coastal population of bottlenose dolphins may have perished (Lipscomb et al., 1994; Schulman et al., 1997). In 1988, thousands of harbor seals and a few harbor porpoises died due to morbilliviral outbreaks in northwestern Europe (Jensen et al., n.d.; Lipscomb et al., 1994). Later in the western Mediterranean, a different outbreak killed thousands of striped dolphins (Domingo et al., 1995). In 1993-1994, another epizootic affected bottlenose dolphins in the Gulf of Mexico (Kennedy, 1998). Recently, morbilliviral infections have been observed in cetaceans in the Pacific, where 6 of 18 common dolphins stranded along the beaches of southeastern California tested showed positive antibodies to DMV. Which indicates a present proximity of common dolphin infection form the Northern Hemisphere on Pacific's Ocean with morbillivirus disease (Krafft et al., 1995; Reidarson et al., 1998). Taubenberger and colleagues reported a case of lethal morbilliviral infection in a long-finned pilot whale, phylogenetically related to, but distinct from, the other cetacean morbilliviruses, PMV and DMV. Recently, unusual mass-mortality events have been reported in Rio de Janeiro, Brazil between November 2017 and March 2018, affecting Guiana dolphins (Groch et al., 2020), and Indo- Pacific bottlenose dolphins from Westerns Australia (Stephens et al., 2014).



Morbillivirus-infected dolphins are rarely observed alive, but in cases that have been described, they may have many tremors, and may be in a poor nutritional state with low lipid reserves and there is often a heavy accumulation of ectoparasites (L. a Dierauf et al., 2001). In pinniped, clinical signs resemble canine distemper in dogs, and include pyrexia, serous or mucopurulent ocular and nasal discharges, coughing, mucosal cyanosis, and dyspnea. Neurological signs include depression, lethargy, dead tremors, convulsions, and seizures. Subcutaneous tissues in the cervical and thoracic regions may become emphysematous and prevent animals from diving. Pregnant females may abort (Van Bresse et al., 2014). In both cetaceans and pinnipeds, treatment is supportive, and there is not vaccine against these morbilliviruses. Morbillivirus infects lymphoid tissues, leading to immunosuppression and a higher risk of secondary infections. Vaccinations using a commercially available modified live virus canine distemper (CDV) vaccine or a killed CDV vaccine have not been conducted in North America. This is partly due to the lack of a readily available safe and effective vaccine other than the modified live virus CDV, as well as the absence of a perceived need for vaccination. Immunizing free-ranging marine mammals remains controversial and challenging to implement effectively (“CRC Handbook of Marine Mammal Medicine, Second Edition,” 2002).

Morbillivirus has a predilection for lymphocytes, epithelial cells, and neurons. Viral replication in lymphoid organs can result in immunosuppression, and predispose the animals to opportunistic bacterial, fungal protozoal or other parasitic infections. Lymphoid depression also induces the development of other typical lesions produced by this virus, non-suppurative bronchointerstitial pneumonia and lymphocytic depletion in lymphoid organs. The most observed lesion associated with the central nervous system is non-suppurative encephalitis (Mazzariol et al., 2018).

Histologically, CeMV infection is primarily associated with lesions of the respiratory, lymphoid, and central nervous systems. It is common to see interstitial pneumonia (by necrosis of type I pneumocytes), serofibrinous exudation into alveoli, proliferation of type II pneumocytes and formation of large syncytia in the alveolar and bronchiolar air spaces (Lipscomb et al., 2001). Briefly, bronchointerstitial pneumonia with congestion, edema, serofibrinous exudation into alveoli, proliferation of type II pneumocytes, and syncytia are seen histologically. Syncytia and inclusions (intracytoplasmic and intranuclear) are more common in cetacean than pinniped lung tissue. The central nervous system (CNS), particularly the cerebrum, can also be impacted. Encephalitis is characterized by neuronal



necrosis, gliosis, perivascular cuffing, and demyelination with astrocytosis and syncytia. Acute infection results in significant lymphoid depletion. In two cases of morbilliviral dermatitis in pinnipeds, syncytia were prominent in the epidermis, follicular epithelium, and sebaceous glands, along with eosinophilic intracytoplasmic inclusions (Schulman et al., 1997) In summary, lymphadenitis, ulcerative stomatitis, nonsuppurative encephalitis, ophthalmitis, serosanguinous fluid in the thorax and/or abdomen, fibrosis of the lung, eosinophilic intracytoplasmic and intranuclear inclusion bodies, may be seen in the skin, gastrointestinal tract, respiratory tract, urogenital tract, or CNS.

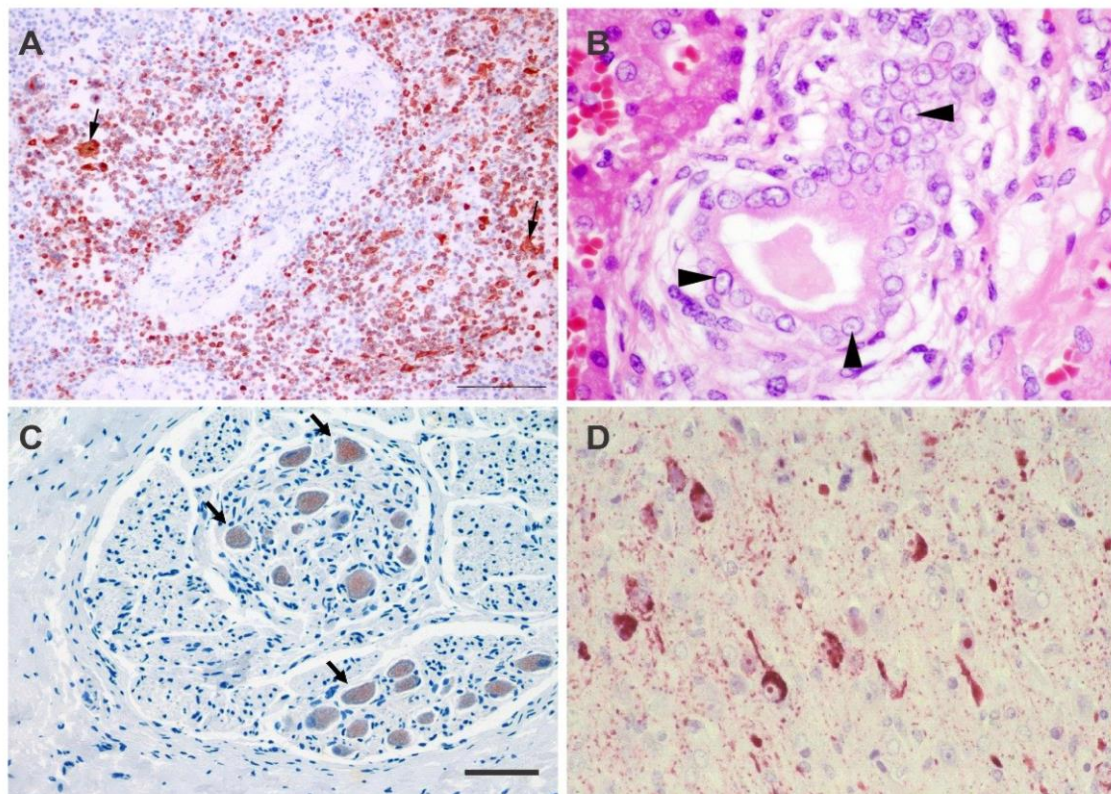


Figure 2: Histopathological findings of morbilliviral infections in dolphins: (A) Bottlenose dolphin (*Tursiops truncatus*), Canary Islands, Spain (2005): Positive immunoperoxidase staining of morbilliviral antigen in lung lymph node cells. (B) Common bottlenose dolphin (*Tursiops truncatus*), USA (2014): Eosinophilic intranuclear inclusions in liver biliary epithelial cells. (C) Guiana dolphin (*Sotalia guianensis*) São Mateus, Brazil (2010): Immunohistochemical staining of morbilliviral antigen in stomach neuronal cytoplasm. (D) Striped dolphin (*Stenella coeruleoalba*), Latium, Italy (1993): Strong immunohistochemical labeling in brain cortical neurons and viral inclusion bodies. Each case involved the avidin-biotin peroxidase technique with various counterstains. (Van Bresseem et al., 2014)

Diagnosis relies on identifying characteristic histopathological lesions and is further confirmed by immunocytochemistry and electron microscopy. Viral RNA can be detected in both fixed and fresh tissue using a PCR-based assay. Immunocytochemistry is a technique used to determine the presence and specific levels of CeMV cellular proteins on histological sections (Terio et al., 2018; Van Bresseem et al., 2014).



Real time RT-PCR (rtRT-PCR) is designed for the analysis and detection of various CeMV genes, offering quick and dependable identification of different CeMV strains. This test identifies various strain types but is particularly sensitive to DMV and PMV (Van Bresseem *et al.*, 2014). Additionally, an antigen capture enzyme-linked immunosorbent assay (ELISA) can be used on tissue homogenates, isolating the virus in primary or secondary kidney cells can be challenging unless the carcass is fresh. Paired serum samples showing a rising antibody titer or a single serum sample with a high IgM titer can also be used to confirm morbillivirus infection (Barrett *et al.*, 1995; Van Bresseem, Van Waerebeek, *et al.*, 1998).

The main differential diagnosis of CeMV is made with *Herpesvirus sp.* Specifically Phocine Herpesvirus Type-1 (PhHV-1). PDV, PhHV-1 and Influenza all lead to pneumonia in pinnipeds, but each has distinct histopathological characteristics. PDV results in bronchointerstitial pneumonia, PhHV-1 leads to interstitial pneumonia and the Influenza virus A causes bronchial pneumonia. While bacteria and parasites can also cause pneumonia, their presence does not rule out a concurrent viral infection. Accurate diagnosis is crucial to confirm a viral cause in any case (“CRC Handbook of Marine Mammal Medicine, Second Edition,” 2002).

Epidemiology of morbillivirus infections illustrates the episodic and contagious nature of the infections. Large, unusual mortality vent related to cetacean morbillivirus have occurred in many areas on repeated occasions (Duignan *et al.*, 1996; Kemper *et al.*, 2016; Lipscomb *et al.*, 1994)). Morbilliviruses are highly infectious and require large populations of susceptible individuals to persist as there is no carrier state, and infection confers lifelong immunity. The transmission by droplet and aerosol in the respiratory route, and direct contact with infectious tissues or fluids. Furthermore, venereal and vertical transmission is possible, via milk and in utero, which is highly suspected to occur in cetacean species (West *et al.*, 2015). Morbillivirus transmission is thought to occur mostly by horizontal dissemination after inhalation of aerosolized virus shed by infected individuals the close association of animals in cetacean pods makes transmission within a group through aerosols highly effective. The migratory behavior of cetaceans promotes transmission between groups (Rowles *et al.*, 2011).

There are five stages of pathogenesis and clinical course of morbillivirus infections in cetaceans.



- 1.1 Acute systemic infection is generally associated with severe multifocal diffuse interstitial bronchopneumonia. Therefore, there may be strong ICH staining in the lungs, lymphoid organs and other tissues, that is the main variable in extent between individual cases. (Domingo et al., 1992; Duignan et al., 1992; Kennedy et al., 1991; Lipscomb et al., 1994; Schulman et al., 1997; Stephens et al., 2014; Stone et al., 2011).
- 1.2 Subacute systemic disease appears in animals that survive the acute stage of infection may succumb to opportunistic infections, such as *Toxoplasma gondii*, herpesviruses, *Photobacterium damsela* and fungi, as a consequence of deep immunosuppression. While some of the lesions could be obscured by the inflammatory response to opportunistic pathogens. Often non-suppurative, demyelinating meningoencephalitis is localized focally distributed, this might be a feature of subacute infection. Immunohistochemistry and RT-PCR are useful for confirmation of the diagnosis of morbilliviral infection in these cases (Domingo et al., 1992; Stephens et al., 2014)
- 1.3 Chronic systemic infection appears when animals survive the acute and sub-acute manifestations of infection and later succumb to secondary infections acquired as a result of viral immunosuppression. Commonly these animals have poor body condition, and the proximate cause of death may be multifactorial. Typical lesions of viral CeMV infection can be distinguished by ICH in some lymph nodes and lungs, and viral RNA can be quantified by RT-PCR. (Lipscomb et al., 1994; Taubenberger et al., 1996)
- 1.4 Chronic, localized CeMV encephalitis, this presentation is characterized by the presence of lesions and virus only in the brain. This pathogenesis was consistently observed in *Stenella coeruleoalba* after epidemics 2006-2008 in the Mediterranean Sea (Domingo et al., 1995; Mazzariol et al., 2013; Sierra et al., 2014; Soto et al., 2011).
- 1.5 Subclinical infection remains speculative. It consists of a seropositive response developing high titers against DMV when they survived. The individuals who have been checked (*Delphinus delphis*) on southern California coast (Reidarson et al., 1998) had mild lymphocytic meningoencephalitis and its brain was positive for morbillivirus RNA by RT-PCR.



Approach and objective

In this study, our primary objective was to document the epidemiologic prevalence of CeMV in Canary Islands stranded cetaceans. The purpose of this bibliographic and retrospective research is to review the available and contrasted information about CeMV infection, from 1999 to 2023, to compile the cases for epidemiological analysis considering various susceptibility factors, such as host species, age, sex, stranding date and location, and the CeMV strain detected, when possible. Finally, we aimed to compare our results with findings reported in both nearby and distant geographical regions.

Material and methods

The Canary Islands stranding network records every stranded cetacean, documenting its biological characteristics and pathological findings. A retrospective study was undertaken, compiling necropsy reports of stranded cetaceans in the Canary archipelago from 1999 to December 2023. During this 25-years period, postmortem examinations were conducted following international protocols (Kuiken, 1991; “Report of IWC Workshop on Marine Debris: The Way Forward1,” 2021).

During necropsies, representative samples from all the major organs and lesions were collected and consecutively processed routinely, being fixed in 10% neutral buffered formalin, and embedded in paraffin, sectioned at a thickness of 5 micrometers and stained with hematoxylin and eosin (H&E) for histopathological analysis. Additional samples were collected and kept frozen at -80°C for molecular studies. The information about each stranding (date, location with coordinates and type) and decomposition code (grade 1: extremely fresh carcass; grade 2: fresh carcass; grade 3: moderate decomposition; grade 4: advanced decomposition and grade 5: mummified or skeletal remains) as well as life history data (species, age category (fetus/neonate/calf, juvenile/subadult and adult), sex and body condition) was systematically recorded (“Report of IWC Workshop on Marine Debris: The Way Forward1,” 2021). The nutritional status (good, moderate, poor or emaciated) was based on the anatomical parameters (marked bony process and prominent bones through the skin), the convex or concave aspect of the dorso-axial muscular mass and the presence and distribution of fatty tissue in several organic locations. All the animals were identified by CET number, a laboratory reference, species, and stranded date and location.

The Anatomopathological Reports (APRs) of stranded cetaceans in the Canary Islands are performed by the "Red Canaria de Varamientos de Cetáceos (RCV)," established by



the University Institute of Animal Health and Food Security (IUSA) at the University of Las Palmas de Gran Canaria (ULPGC). We look for key words, such as bronchointerstitial pneumonia, non-suppurative meningoencephalitis, inclusion bodies, type II pneumocyte hyperplasia, lymphoid depression, among others (to find those animals with a high probability of infection by CeMV) within the APRs, available on the IUSA computer for consultation. Once those cases were detected, the results were corroborated with available information regarding immunohistochemical and molecular techniques (molecular laboratory, IUSA).

For immunohistochemistry, tissue samples were incubated with a mouse monoclonal antibody against the nucleoprotein antigen of CDV (CDV-NP MAb, VMRD Inc) at 1:200 dilution, as previously described (Groch et al., 2020).

For molecular analysis, frozen samples were thawed and mechanically macerated for subsequent simultaneous extraction of DNA and RNA using a QuickGene R Mini 80 nucleic acid isolation instrument with the DNA Tissue Kit S (QuickGene, Kurabo, Japan) according to the manufacturer's instructions with some modifications: an RNA carrier (Applied Biosystems™, Thermo Fisher Scientific, Waltham, MA, USA) was added during the lysis step, as previously published (Sacristán et al., 2015).

Molecular detection of CeMV was performed using one or more of three polymerase chain reaction (PCR) methods. (Barrett et al., 1993; Groch et al., 2020; Sacristán et al., 2015; Sierra, Zucca, et al., 2014). A negative control (non-template) and an amplification-positive control (known cetacean morbillivirus RNA previously obtained in our laboratory) were included in each DNA/RNA extraction and PCR protocol.

PCR amplicons were purified (Real Clean spin kit (REAL®, Durviz, S. L., Valencia, Spain)) and sequenced by the Sanger method. The BLAST algorithm (www.ncbi.nlm.nih.gov/blast/Blast.cgi) was then used to compare the obtained amplicons with similar sequences available in GenBank.

In addition, information about CeMV infection in stranded cetaceans from the Canary Islands is also published in peer-reviewed research articles, original research contributions (PhD thesis; Arbelo et al., (2013); Díaz-Delgado et al., (2018); Díaz-Santana *et al.*, (2018); Zucca (2017). The search was carried out in institutional repositories (e. g. AccedaCRIS) and directories of scientific publications and high-impact journals, such as PubMed, by using relevant keywords related to our research topic. We



also used Boolean Operators (AND, OR, NOT) to combine keywords effectively. We consecutively browsed through the search results and selected articles that were relevant to our study to further review abstracts to determine if the articles provided the information we looked for, to finally accessed to the full-text articles for more detailed descriptions. All the articles were properly cited.

Results

Eight hundred and forty-one animals stranded in the Canary Islands and were submitted to a standardized necropsy during the period of study. From these, 30 animals were identified as confirmed infected by CeMV (3.6%), which included cetaceans stranded in Gran Canaria (n= 8), Fuerteventura (n=8), Tenerife (n=8), Lanzarote (n=5) and La Graciosa (n=1). Thirty-two cetaceans (3.8%) were additionally identified as suspected cases of CeMV infection without complementary analysis (IHC and/or PCR).

All the biological information and stranding data for the 30 confirmed CeMV-positive cetaceans in compiled in supplementary table 2. Specifically, supplementary table 2 contains the CET number, the laboratorian reference, the sex, the age-category, the stranding date (S.D.) and location (S.L.), the nutritional status (B.C.), stranding condition (dead/alive; S.C) and the decomposition code (D.C). A brief description of the organs where the viral agent was detected, the method of diagnosis and the disease presentation form (subclinical infection/subacute systemic disease/ acute systemic disease/ localized chronic encephalitis/ chronic systemic infection) considering the lesions at macroscopic and microscopic diagnosis is gathered in supplementary Table 3.



1. Microscopic lesions observed.

The initial criterion for suspecting CeMV infection in cetaceans is the presence of characteristic lesions (neurologic, pulmonary, and lymphatic) as indicated by the disease's presentation form. However, animals were only classified as CeMV-positive based on positive results from immunohistochemistry and/or molecular techniques. Molecular analyses are crucial for identifying the specific CeMV strains responsible for the infections. Out of the 841 cetaceans stranded in the Canary Islands during the study period (1999-2023), 30 (3.57%) were confirmed to be infected by one of two different CeMV strains, DMV and PWMV. Considering the hallmark lesions described in CeMV-positive cetaceans in the literature, we observed that non-suppurative meningoencephalitis (Figure 3) and lymphoid depletion (Figure 4) were present in 66.6% of the CeMV-infected animals from our study, respectively, while bronchointerstitial pneumonia was only present in the 43.3% of the cases (Figure 5) (Table 4).

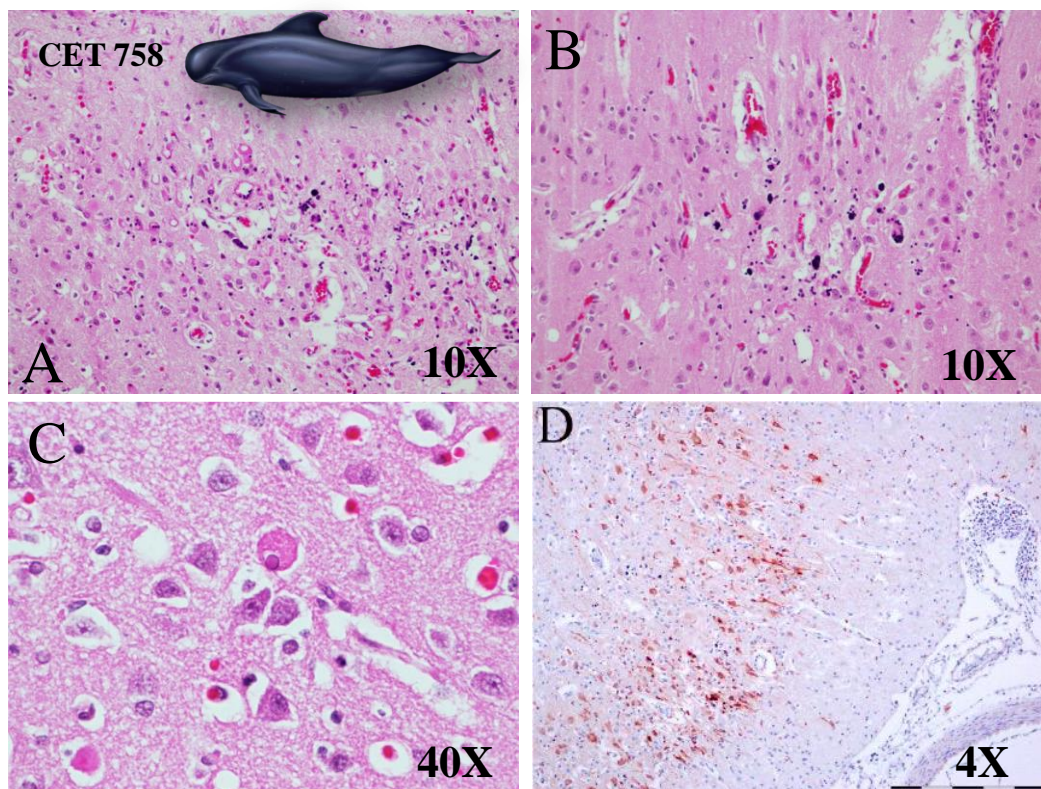


Figure 3. Central Nervous System, H&E (A-C) and IHC (D). Acute necrotizing encephalitis with syncytia formation. CET 758, *Globicephala macrorhynchus*.

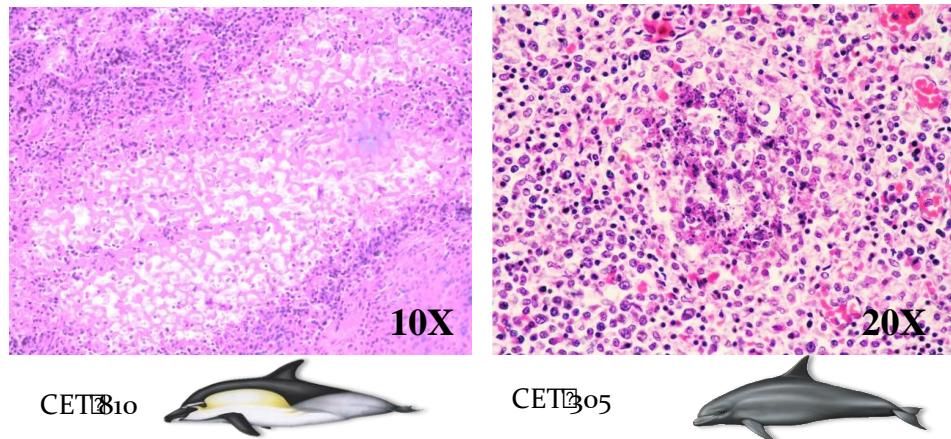


Figure 4. Lymphoid system, H&E. Lymphoid depletion and edema and germinal center necrosis in *Delphinus delphis* and *Tursiops truncatus*.

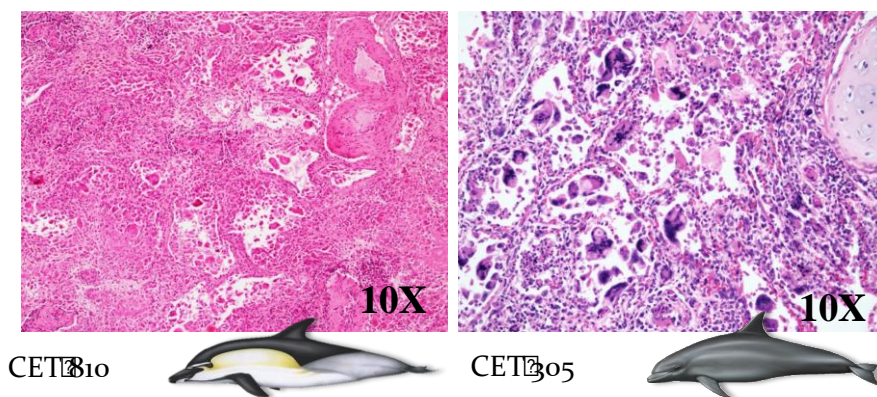


Figure 5. Lungs, H&E. Bronchointerstitial pneumonia with type II pneumocytes hyperplasia and intraalveolar syncytia in *Delphinus delphis* and *Tursiops truncatus*.

<i>Morphological diagnosis</i>	Total	%
<i>Bronchointerstitial pneumonia</i>	n=13/30	43.3%
<i>Non-purulent meningitis/encephalitis/meningoencephalitis</i>	n=20/30	66.6%
<i>Lymphoid depletion</i>	n=20/30	66.6%

Table 4: Percentage of the main morphological diagnoses observed in CeMV-positive cetaceans from our study.

2. Distribution of confirmed CeMV-positive animals by years.

An average annual incidence of 0.0298 CeMV-positive animals was calculated (Figure 6). So, accordingly, the infection presentation's prevalence in stranded and necropsied cetaceans in the Canary Islands during the period of study is 2.98 %, with a higher incidence in the period from 2011 to 2020, where the highest proportion of CeMV-positive cetaceans were found (Figure 7).

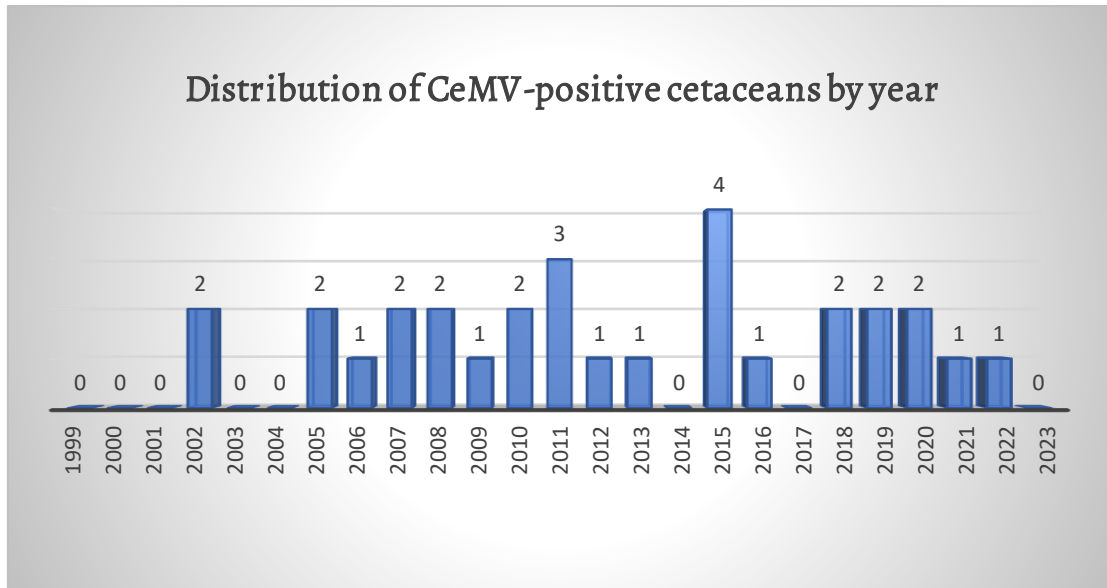


Figure 6: Distribution of CeM-positive stranded cetaceans by years.

In addition, the annual incidence has been obtained (Figure 4): 1999 = 0 (n=0/21), 2000 = 0 (n=0/19), 2001 = 0 (n=0/27), 2002 = 0.062 (n=2/32), 2003 = 0 (n=0/16) 2005 = 0.087 (n=2/23, 2004 = 0 (n=0/32) , 2005 = 0,0869 (n=2/23), 2006 = 0.033 (n= 1/30), 2007 = 0.064 (n=2/31), 2008 = 0.04 (n=2/50), 2009 = 0.034 (n=1/29), 2010 = 0.04 (n=1/25), 2011 = 0.086 (n=3/35), 2012 = 0.026 (n=1/39), 2013 = 0.028 (n=1/36), 2014 = 0 (n=0/40), 2015 = 0.097 (n=4/41), 2016 = 0.014 (n=1/73), 2017 = 0 (n=0/34), 2018 = 0.024 (n=2/84), 2019 =0.02 (n=2/100), 2020= 0.026 (n=2/78), 2021 =0.013 (n= 1/78), 2022= 0.011 (n= 1/91) y 2023=0 (n=0/38).

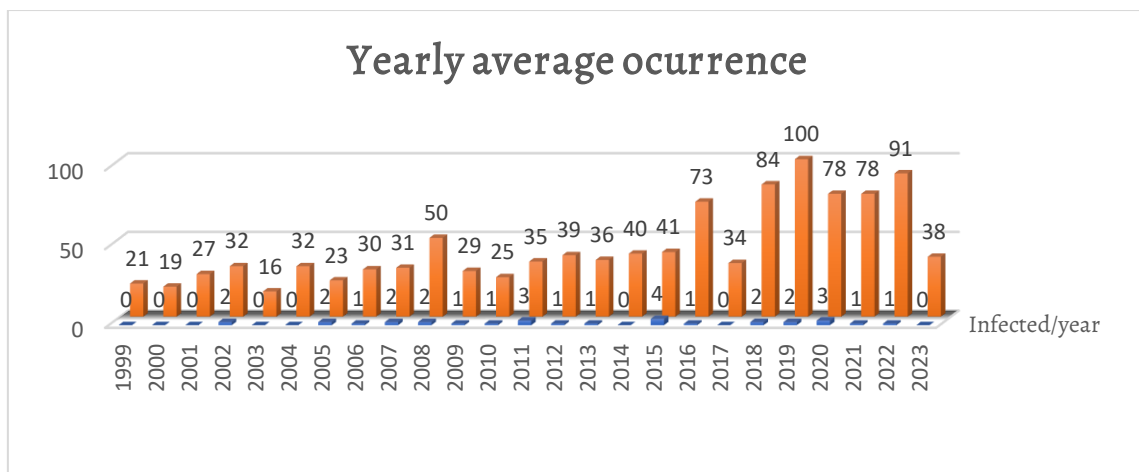


Figure 7: Yearly average occurrence of confirmed CeMV-positive cetaceans in the Canary Islands (1999-2023).

Regarding the season in which confirmed CeMV-positive cetaceans were found, we observed the highest prevalence of infection in the spring, with 30% of the affected



individuals (n=9) recorded during this time. This was followed by winter with 27% of the cases (n=8), autumn with 23% (n=7) and summer with 20% (n=6) (Figure 8).

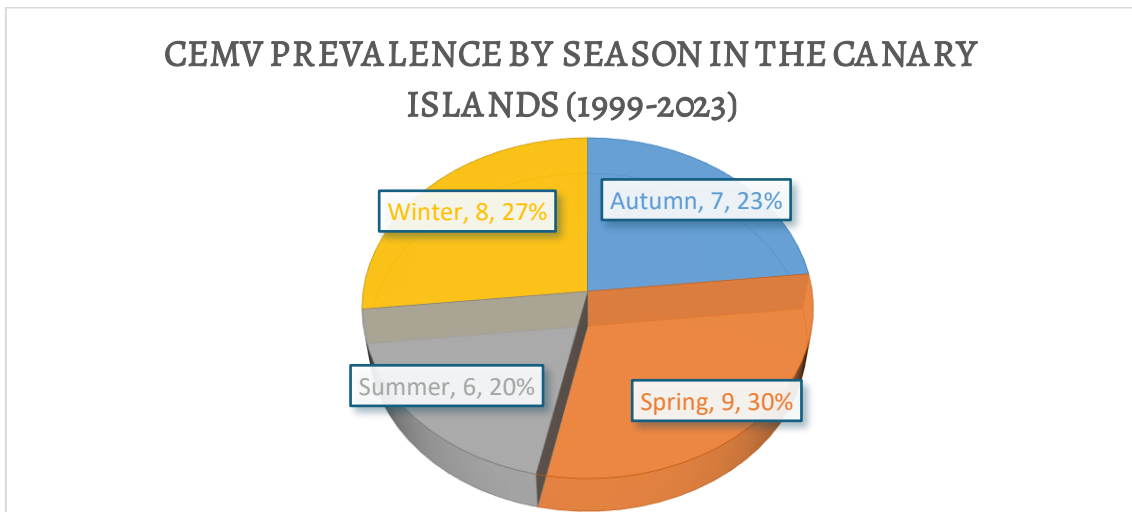


Figure 8: Prevalence of confirmed CeMV-positive cetaceans in the Canary Islands (1999-2023) by seasons (Spring, Summer, Autumn and Winter).

3. Prevalence of CeMV by species.

As previously mentioned, out of the 841 cetaceans stranded and necropsied during the study period, 30 were confirmed to be infected with CeMV, belonging to 8 different species: Atlantic spotted dolphin (*Stenella frontalis*), short-finned pilot whale (*Globicephala macrorhynchus*), bottlenose dolphin (*Tursiops truncatus*), common dolphin (*Delphinus delphis*), striped dolphin (*Stenella coeruleoalba*), Cuvier's beaked whale (*Ziphius cavirostris*), Risso's dolphin (*Grampus griseus*) and Fin whale (*Balaenoptera physalus*) (Figure 9).

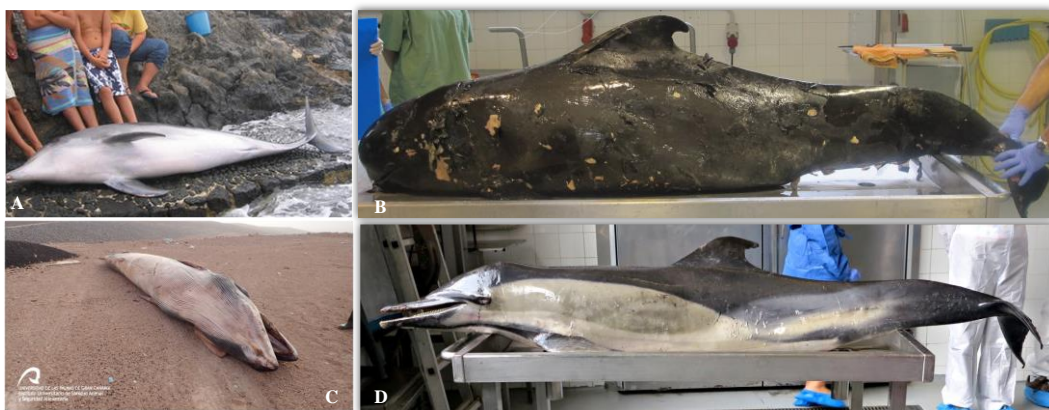


Figure 9. CeMV-positive cetaceans stranded in the Canary Islands from 1999-2023. A) CET 305, *Tursiops truncatus*. B) CET 739, *Globicephala macrorhynchus*. C) CET 1062, *Balaenoptera physalus*. D) CET 810, *Delphinus delphis*.



Taking into account the number of animals of each of the 8 CeMV-positive species that stranded in the archipelago in these 25 years, the percentages of CeMV-positive cetaceans by species were: Atlantic spotted dolphin with 3% (n=1/166), short-finned pilot whale with 30% (n=7/60), bottlenose dolphin with 7% (n=2/67), common dolphin with 7% (n=2/92), striped dolphin with 40% (n=8/176), Cuvier's beaked whale with 3% (n=1/50), Risso's dolphin with 7% (n=2/26) and fin whale with 3.33% (1/30) (Figure 10). So, the prevalence of infection by this viral pathogen is highest in the species *Stenella coeruleoalba* (40%), followed by *Globicephala macrorhynchus* (30%) and *Grampus griseus* (7%) and *Delphinus delphini* (7%) in the same proportion.

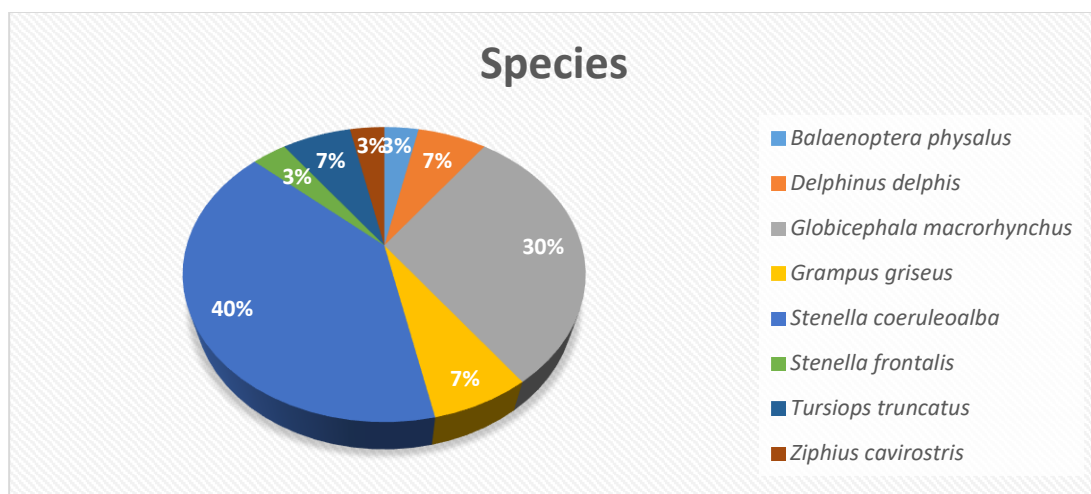


Figure 10: Prevalence of Cetacean *Morbillivirus sp.*-positive animals among the 8 species in which the virus was detected.

Additionally, there are still 32 stranded cetaceans with morphological diagnoses highly indicative of CeMV infection, but without confirmation by complementary analyses at this time. These 32 cetaceans belongs to any species for which CeMV has been previously detected in the Canary Islands, except for the species *Steno bredanensis* (Figure 11).

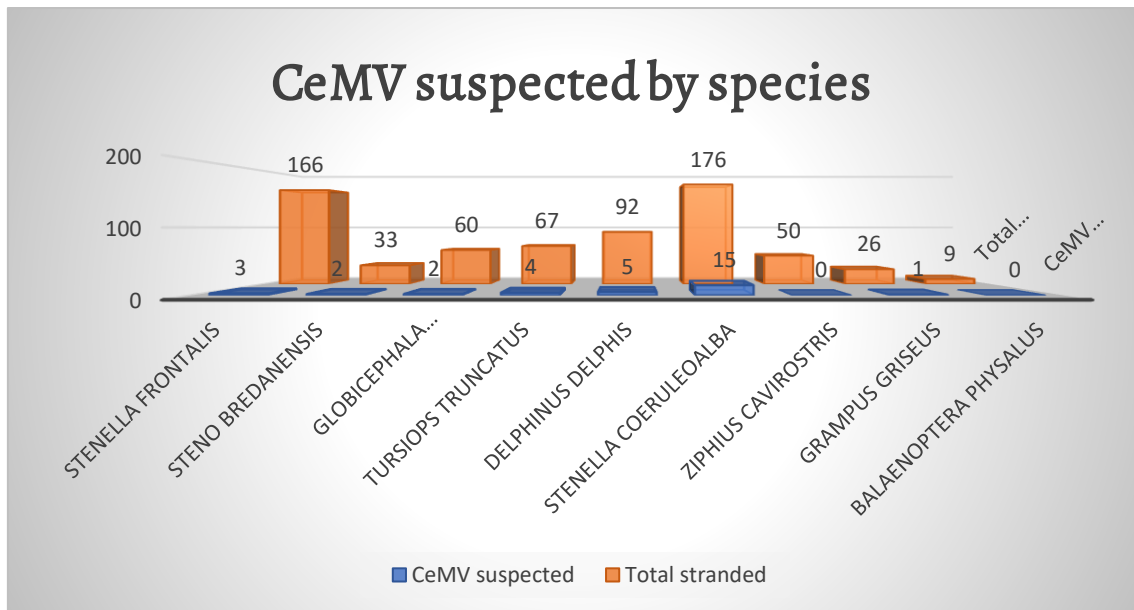


Figure 11: Prevalence of the suspected infection by species, comparing the total amount necropsied.

4. Prevalence of CeMV by sex.

Males represented 57% of the CeMV-positive cetaceans in the Canary archipelago during the period study (n=17/30), while females represented a slightly smaller percentage, being the 43% (n=13/30 (Figure 12).

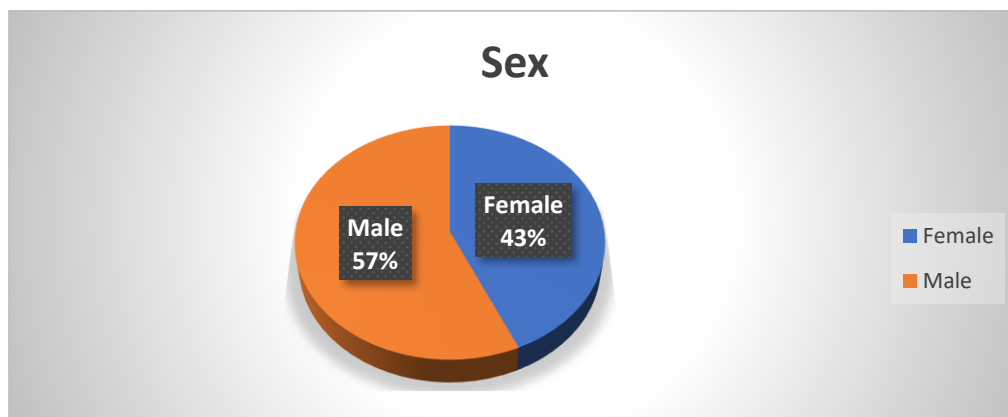


Figure 12. Prevalence of the CeMV infection by sex.

5. Prevalence of CeMV by age.

Most CeMV-positive cetaceans were juveniles/subadults, 63% (n=19/30), followed by adults, accounting for 20% of the animals (n=9/30) and finally calves, accounting for 2% (n=2/30 (Figure 13).

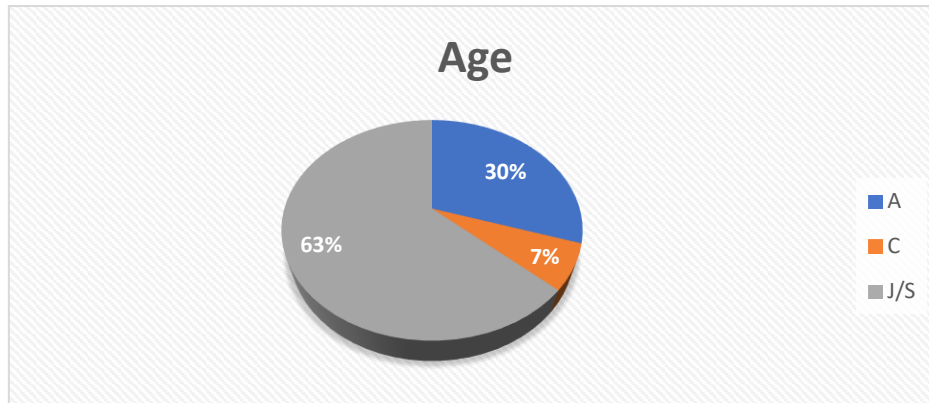


Figure 13: Prevalence of CeMV infection by age.

6. Prevalence of CeMV by nutritional status and decomposition code.

Most of the animals infected with morbillivirus, in our study, were in poor or moderate body condition, representing the 33.33% (n=10/30) of the CeMV-positive cetaceans, respectively. CeMV-positive cetaceans in good body condition represented the 20% of the cases (n=6/30), while only the 6% of the animals infected by CeMV were in very poor body condition at the time of stranding (n=2/30). Subsequently, the infected animals were in good body condition. The remaining individuals had very poor/undetermined nutritional status, with 6% of the cases (n=2/30), respectively (Figure 14).

About the degree of decomposition at the time of necropsy, they were mostly found in moderate autolysis, (code 3) 70% (n=21/30), 20% (n=6/30) had an advanced degree of autolysis (code 4), and the remaining 10% (n=3/30) were fresh (code 2) (Figure 14).

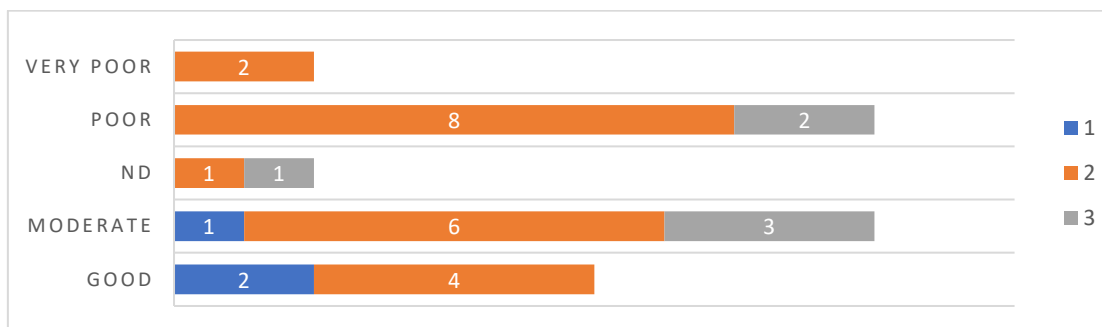


Figure 14: Prevalence of CeMV by nutritional state and decomposition code (1, 2 and 3).

7. Distribution of CeMV by stranding location.

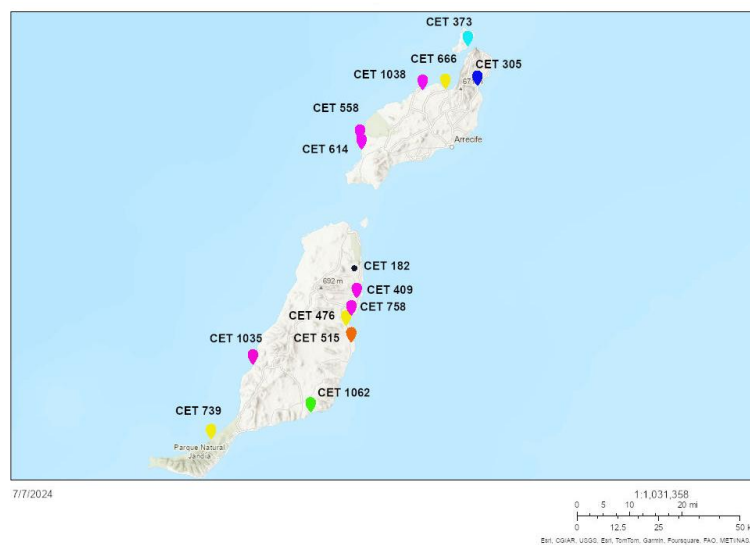
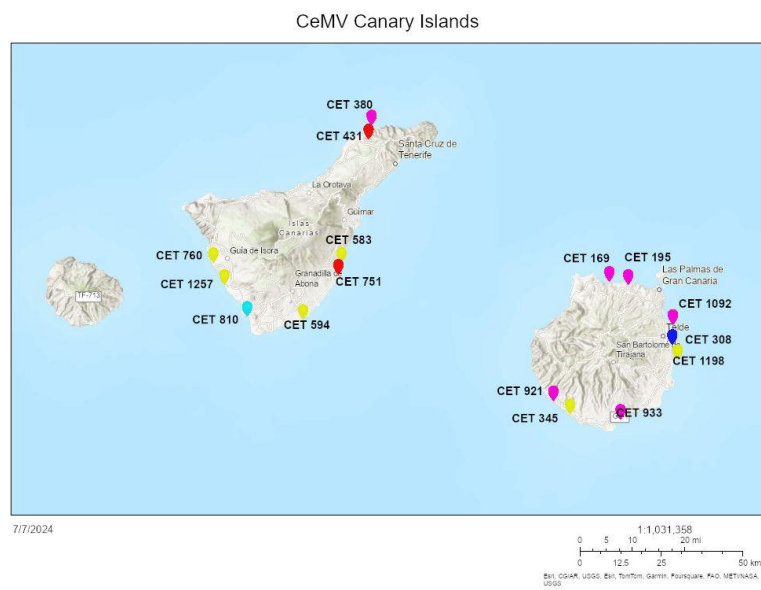
All the cases that have been studied were found dead on the sea or stranded along the coast of the islands from this Archipelago. Most animals were found in Gran Canaria (26,6%), Fuerteventura (26,6%) and Tenerife (26,6%). The remaining 20,2% of the



animals were distributed between the islands of Lanzarote (16,6%) and La Graciosa (3,3%) (Figure 15).



Figure 15: Stranding location within the Canary archipelago of the CeMV-positive cetacean of this study.



Figures 16-17. Description of the images: (Yellow): *Globicephala macrorhynchus*; (Pink): *Stenella coeruleoalba*; (Red): *Grampus griseus*; (Light blue): *Delphinus delphis*; (Dark blue): *Tursiops*



truncatus; (Orange): *Stenella frontalis*; (Green): *Balaenoptera physalus*; (White): *Ziphius cavisrostris* (Black).

8. Prevalence of CeMV by disease presentation form.

The prevalence for the acute presentation form of the disease was found to be the most frequent in our study, accounting for the 36.6% of the cases (n=11/30), followed by localized chronic encephalitis 30% (n=9/30), chronic systemic infection 20% (n=6/30) and finally subclinical infection 6.6% (n=2/30) and subacute systemic disease 6.6% (n=2/30). The acute form of disease occurred mostly in the period 2015-2020. While localized chronic encephalitis was mostly detected between 2006 and 2013 (Figure 18).

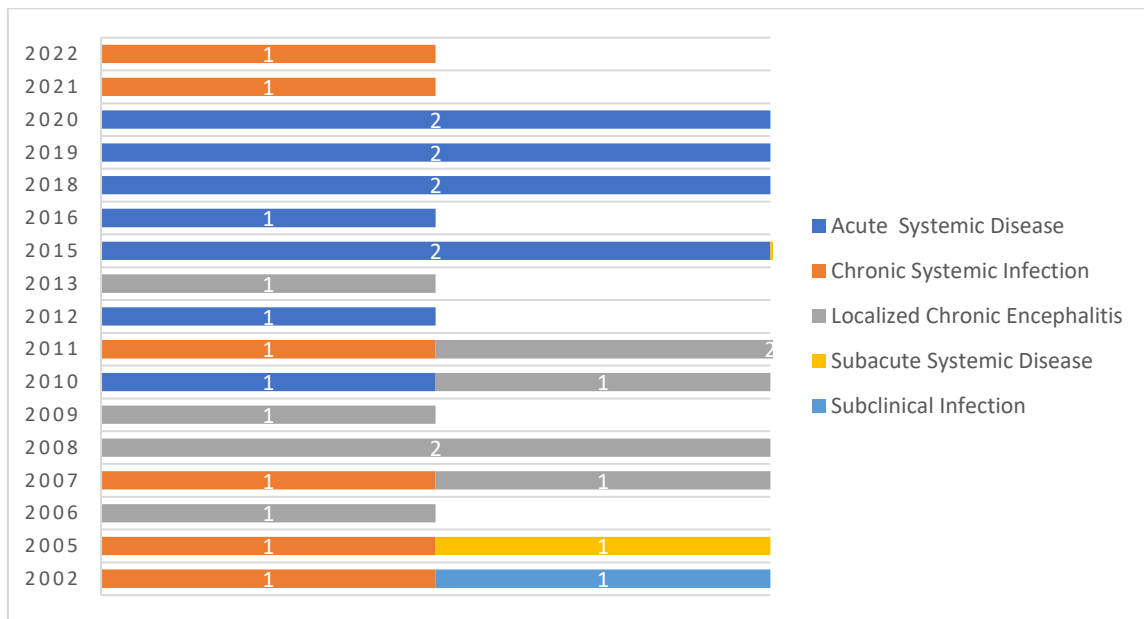


Figure 18: Prevalence of CeMV by disease form.

Evaluating the disease presentation form by species revealed a predilection for the acute systemic form in *Stenella coeruleoalba*. Similarly, chronic localized encephalitis was notably prevalent in *Globicephala macrorhynchus* (Table 5).



Species	Disease presentation form count
<i>Balaenoptera physalus</i>	1
Acute Systemic Disease	1
<i>Delphinus delphis</i>	2
Acute Systemic Disease	1
Localized Chronic Encephalitis	1
<i>Globicephala macrorhynchus</i>	9
Acute Systemic Disease	2
Chronic Systemic Infection	2
Localized Chronic Encephalitis	4
Subacute Systemic Disease	1
<i>Grampus griseus</i>	2
Localized Chronic Encephalitis	1
Subclinical Infection	1
<i>Stenella coeruleoalba</i>	12
Acute Systemic Disease	7
Chronic Systemic Infection	3
Localized Chronic Encephalitis	2
<i>Stenella frontalis</i>	1
Localized Chronic Encephalitis	1
<i>Tursiops truncatus</i>	2
Chronic Systemic Infection	1
Subacute Systemic Disease	1
<i>Ziphius cavirostris</i>	1
Subclinical Infection	1
Total general	30

Table 4: Disease presentation form by species.

The strain most frequently detected by these analytical methods was *Dolphin morbillivirus sp.* (DMV) 56.6% (n=17/30) affecting *Ziphius cavirostris* 5.8% (n=1/17), *Stenella coeruleoalba* 58.82% (n=10/17), *Tursiops truncatus* 11.76% (n=2/17), *Grampus griseus* 11.76% (n=2/17), *Delphinus Delphi* 5.8% (n=1/17) and *Balaenoptera Physalus* 5.8% (n=1/17). *Globicephala macrorhynchus* was also reported to be the only species infected by the *pilot whale morbillivirus sp.* (PWMV) strain with a presentation of 26.6% (n=8/30). The remaining individuals tested positive for morbillivirus infection were found to have unknown/inconclusive results 13.33% (n=4/30), the only individual *Stenella frontalis* in this review was found to have an unknown strain, since it was negative by molecular methods (supplementary Table 3).



Discussion

The primary objective of this retrospective study about morbillivirus infection cases in stranded cetaceans in the Canary Islands from 1999 to 2023 was to identify animals with microscopic lesions and immunohistochemical positive-tests, and/or positive-real-time PCR tests consistent with morbillivirus infection, to further investigate the possible relationship between different parameters among the CeMV-infected animals.

CeMV infection in cetaceans results in a range of gross and microscopic lesions affecting multiple organ systems. More specifically, skin lesions, including ulcers and erosions, especially around the mouth and blowhole; severe pneumonia characterized by firm, consolidated lungs, presence of exudate in the airways and pulmonary congestion, ulcerative lesions in the gastrointestinal tract and swelling and discoloration of the brain, are among the most common macroscopic lesions displayed by infected animals, although they can be also associated to other causes (Kennedy et al., 1991, 1992, 1996; Domingo et al., 1992; Van Bresseem et al., 2014; Groch et al., 2020a). However, certain microscopic lesions are key indicators for CeMV infection, such as those described the respiratory, lymphatic, digestive, and central nervous systems, due to the cytopathic effects of the virus and the host's immune response.

More in detail, pulmonary microscopic lesions range from bronchointerstitial pneumonia with alveolar septal thickening, syncytial cells and inclusion bodies within alveolar spaces and lymphocytic infiltration in the interstitial and peribronchial areas. In our study, bronchointerstitial pneumonia was considered a key lesion of CeMV infection (Domingo et al., 1992; Groch et al., 2020; Kennedy, 1998; Kennedy et al., 1991, 1992; Sierra, Sánchez, et al., 2014; Van Bresseem et al., 2014; Vargas-Castro et al., 2023)), which was present in the 43.3% (11/30) of the CeMV infected stranded cetaceans in the Canary Islands in the period of study (1999-2023), corresponding to the animals that was in the acute systemic disease dead (11/13) and the subacute systemic disease (2/13) stage at the time of dead.

Lymphoid depletion and necrosis in lymph nodes, as well as syncytial cells and viral inclusion bodies have been frequently reported in lymphoid tissues of CeMV infected cetaceans (Kennedy et al., 1991, 1992, 1998; Domingo et al., 1992; Sierra et al., 2014; Van Bresseem et al., 2014; Groch et al., 2020a; Vargas-Castro et al., 2023). In our study, lymphoid depletion was identified as a key indicator of CeMV infection, being present in



66.6% (20/30) of the CeMV infected stranded cetaceans in the Canary Islands in the period of study (1999-2023). Animals with this lesion were classified as: acute systemic disease (11/20), subacute systemic disease (2/20), chronic systemic infection (6/20) and subclinical infection (1/20).

Regarding the CNS, non-suppurative meningoencephalitis, neuronal necrosis and gliosis and the presence of syncytial cells and viral inclusion bodies in neurons and glial cells are the most common reported findings in CeMV infected cetaceans (Kennedy et al., 1991, 1992, 1996; Domingo et al., 1992; Sierra et al., 2014; VanBressem et al., 2014; Sierra et al., Sierra et al., 2016; Groch et al., 2020a; Vargas-Castro et al., 2023). We considered non-suppurative meningoencephalitis as the main brain lesions detected in cases of CeMV infection, being detected in 66.6% of 20/30) of the CeMV infected stranded cetaceans in the Canary Islands in the period of study (1999-2023). Animals with this lesion were classified as: acute systemic infection (3/20), subacute systemic infection (2/20), chronic systemic infection (6/20) and localized chronic encephalitis (9/20).

Microscopic skin lesions have been less frequently described, which consisted of epidermal hyperplasia and necrosis as well as dermal infiltration by lymphocytes and macrophages (Dagleish et al., 2021; Groch et al., 2020) One Cuvier's beaked whale was included in the subclinical stage of the infection because of the detection of DMV DNA in a skin tissue sample without associated lesions (Felipe-Jiménez et al., 2022).

The presence of syncytial cells (multinucleated giant cells) and intranuclear and/or intracytoplasmic viral inclusion bodies (eosinophilic or basophilic) is a hallmark of morbillivirus infections, indicating active viral replication and cell fusion (Domingo et al., 1992; Groch et al., 2020; Kennedy, 1998; Kennedy et al., 1991, 1992; Van Bressemer et al., 2014). So, accordingly, these indicators are only present in the more acute stages of the disease.

Nevertheless, despite very CeMV-suspicious microscopic lesions or in their absence (as in the case of subclinical CeMV infections), a definitive diagnosis should be based on complementary analysis. Previous studies have shown some cases testing positive in RT-PCR, but no typical pathological findings resulting from CeMV infection. This suggests that relying solely on pathological examination results in retrospective studies could lead to misdiagnosis of CeMV infection. Additionally, there may be risks of false-negative



results in certain conditions, such as when less sensitive detection methods are used or in cases of persistent infection with low virus copies (Yang et al., 2016).

The gold standard diagnostic test involves virus isolation with VERO or SLAM cells, which is not always feasible in many circumstances. Instead, a rapid CeMV confirmation and clade determination can be obtained through RT-PCR followed by sequencing. Real-time RT-PCR (rtRT-PCR) targeting different genes of the virus, has been demonstrated to be highly sensitive and specific for different CeMV strains without cross-reacting with other morbilliviruses (Van Bresseem et al., 2014). In our study, a definitive diagnosis was relied on real-time PCR and/or immunohistochemistry, since subclinical and subacute forms of disease do not manifest microscopic lesions of clear relevance, as previously mentioned.

From January 1999 to December 2023, 30 CeMV-positive cetaceans stranded on the coasts of the Canary Islands. Cases of infection were recorded in most years of this period, with the exceptions of 1999-2001, 2003-2004, 2014, 2017, and 2023. Thus, the prevalence of infection detected in terms of the total number of cetaceans stranded on the Canarian coast and necropsied was 3.56% (n=30/841). Various prevalence rates of CeMV have been reported in different locations and species worldwide during periods not associated with unusual mortality events (UMEs). These rates include: 1.81% (1/55) in stranded beaked whales from the Canary Islands (Felipe-Jiménez et al., 2022); 4.25% (2/47) in exhaled breath of humpback whales (*Megaptera novaeangliae*) in Brazil (Groch et al., 2021); 5.73% (16/279) in cetaceans stranded along the Portuguese and Galician (Spain) coasts (Bento et al., 2016); 14.61% (13/89) in cetaceans stranded on the Mediterranean Spanish coast (Catalonia) (Cuvertoret-Sanz et al., 2020); 16.66% (2/12) in Risso's dolphins (*Grampus griseus*) from the Canary Islands (Sierra et al., 2018); 19.68% (37/188) in stranded cetaceans from Italy (Giorda et al., 2022); 1.74% (5/23) in fin whales (*Balaenoptera physalus*) from Italy (Mazzariol et al., 2017); 4.19% (15/62) in cetaceans stranded in Hawaii (Jacob et al., 2016); 27.50% (11/40) in stranded cetaceans from Brazilian waters (Marutani et al., 2022); 60.0% (3/5) in southern right whales (*Eubalaena australis*) from Brazil (Groch et al., 2019); and a prevalence of 31.9% (113/354) between 2018 and 2021 in Italian waters (Vargas-Castro et al., 2023). Eight different species were involved, with most of the infected animals being males (57%) and juveniles or subadults (63%), consistent with previous studies. CeMV infection exhibits a clear species and age



predisposition, significantly affecting certain species like striped dolphins, bottlenose dolphins, and pilot whales (as in our study); although for the other infected species detected in our study (Atlantic spotted dolphin, common dolphin, Fin whale, Risso's dolphin, and Cuvier's beaked whale) also accounted with cases reported in various regions (Vargas-Castro et al., 2023). Young cetaceans are generally more vulnerable to the virus, experiencing higher morbidity and mortality rates. (Domingo et al., 1992; Kennedy et al., 1992; Van Bresseem et al., 2014; Vargas-Castro et al., 2023). Understanding these predispositions helps in monitoring, managing, and protecting cetacean populations from the impacts of CeMV. The distribution of CeMV by stranding location showed a higher incidence in Gran Canaria (26.6%), Fuerteventura (26.6%), and Tenerife (26.6%) compared to the other islands, although they are also the islands accounting for most of the strandings in the Canarian archipelago.

Cetacean morbillivirus (CeMV) is a significant pathogen affecting cetacean populations globally. Its prevalence varies across different regions and species, with several notable outbreaks and ongoing endemic occurrence (Van Bresseem et al., 2014). According to the geographic distribution: in the Northeastern Atlantic and North Sea there have been sporadic morbillivirus infections observed in white-beaked dolphin (*Lagenorhynchus albirostris*) and fine-whale (*Balaenoptera physalus*) from 1990 to 2011. From the date of appearance, the prevalence of DMV-seropositivity was declining over time and becoming an endemic infection in these populations (adult porpoises and dolphins) (Van Bresseem et al., 2001; Van Bresseem, Jepson, et al., 1998). In addition, the coast of Belgium, northern France, England, and Germany in 1900-2000, the morbillivirus systemic disease was not detected in any porpoise that stranded along the coast (Jauniaux et al., 2002; Kennedy et al., 1992). The PWMV strain was detected by RT-PCR in the brain of an individual *Globicephala macrorhynchus* stranded in the Canary Islands, Tenerife, in 1996. It was reported that the infection shown by the short-finned pilot whale was closer to the PWMV strain than to DMV strain. Thus, it was shown that pilot whales play a key role in epidemiology, transmitting them to equidistant geographical regions or other odontocetes (De Stephanis et al., 2008; Fernández et al., 2008). Furthermore, in 2006 there was a mass stranding of 60 long-finned pilot whales on Spanish Mediterranean coast, whose diagnosis was infection by DMV, supporting the theory that these species is also susceptible to the infection with the DMV strain (Fernández et al., 2008; Bellière et al., 2011). In this retrospective study on the Canary Coasts, among the animals of the



species *Globicephala macrorhynchus* affected by morbillivirus disease (n=9/30), the 89% (n=8/9) had the PWMV strain, while one individual (11%, n=1/9) was not tested by RT-PCR.

On the Mediterranean Sea the strain DMV has caused two outbreaks of mass-mortality on *S. coeruleoalba*. One in 1990-1992, and the other in 2006-2008. Between October 2006 and April 2007 at least 27 morbilliviruses infected *Globicephala melas* stranded along the southern Spanish Mediterranean coast and the Balearic Islands. Early July 2007 where detected *S. coeruleoalba* with DMV strain in the Gulf of Valencia. Then the outbreak extended to France and Italy during the following months affecting *Tursiops truncatus*, most of them juveniles, because adults were protected by immunity acquired during past epidemic, 1990-1992 (Keck et al., 2010). The disease presentation varies, there were deaths by the acute infection, ultimately lethal of a chronic systemic CNS form, but the most common single cause of stranding was chronic morbilliviruses encephalitis. An estimated 200 striped dolphins died in the Western Mediterranean, but the total number of deaths remains unknown. *S. coeruleoalba* population density on the westerns Mediterranean coast is high, so the likely transmission was favored by the reintroduced DMV strain causing the new epidemic. It is suspected possibly *G. melas* transmitted the infection to *S. coeruleoalba*. (Fernández et al., 2008; Raga et al., 2008).

Comparing these results with those from our study on the Canary Islands coast, 3 infected cetaceans: a short-finned pilot whale (*Globicephala macrorhynchus*, juvenile/subadult female) stranded on 11/05/2006, a short-beaked common dolphin (*Delphinus delphis*, adult female) stranded on 26/03/2007 and a striped dolphin (*Stenella coeruleoalba*, juvenile/subadult male) stranded on 16/04/2007 seems to be in connection with the epizootic outbreak taken place there (Sierra, Zucca, et al., 2014). All these animals were found stranded during the spring, which is consistent with the period of the Mediterranean outbreak. Additionally, phylogenetical analysis of the DMV strains detected in this striped dolphin (Sierra et al., 2014) and in other stranded in 2008 (Yang et al., 2016) confirmed the correlation with this episode. The mode of presentation of the infection was localized chronic infection for the short-finned pilot whale and the common dolphin, while a chronic systemic infection was diagnosed for the striped dolphin. Since 2011, these large mass mortalities have not recurred, but there have been several isolated cases. There is a high probability that the infection was introduced by animals from the Atlantic.



The new strain does not cause epidemics, although it is more aggressive in affected individuals (Mira et al., 2019; Rubio-Guerri et al., 2018).

Cetacean morbilliviral RNA was detected by RT-PCR in the brain and lung samples from 22 out of 53 *S. coeruleoalba*, one *T. truncatus* and one *B.physalus* stranded along the Italian Tyrrhenian Sea coast during an unusual mortality event in early 2013 (Casalone et al., 2014). In the Canary Islands, a positive pilot whale (*Globicephala macrorhynchus*) was detected in Lanzarote in 2013, during the spring (juvenile/subadult male individual) with chronic localized encephalitis, tested positive to PWMV strain. Accordingly, there seems to be no correlation between these two events.

Keeping on sight the Atlantic coast, in 2013-2014 along the Atlantic coast from New York, through northern Florida, over a 1500 *T. truncatus* died and the majority of the animals tested were diagnosed positive for DMV (Olmstead et al., 2023). Contact between inshore *T. truncatus* and offshore species (*Globicephala sp.*, *S. coeruleoalba*, offshore *T. truncatus*) and false killer whale (*Pseudorca crassidens*) (Duignan, House, Geraci, Duffy, et al., 1995; Duignan, House, Geraci, Early, et al., 1995), in which CeMV is endemic, may have been the sources of infection, producing the 2013-2014 outbreak (Rosel et al., 2009). Around the Canary Coasts, the only CeMV-positive animal detected in this period was a PWMV infected with the PWMV strain.

RT-PCR was used to detect the strain described as beaked whale morbillivirus (BWMV) in a juvenile male *I. pacificus* specimen stranded on Hana, Maui in March 2010. The concurrent diagnosis with cause of death was a traumatic fracture of the maxillary and mandibular bone, concurrent with which chronic encephalitis was detected. This strain was also detected by RT-PCR in tracheobronchial lymph node and spleen of a *Physeter macrocephalus* neonate stranded on the island of Oahu, Hawaii, in May 2011. Circulating CeMV strains in this manner has been demonstrated throughout the North Pacific (Stephens et al., 2014; West et al., 2013). The appearance of this strain in the Canary Islands has not yet occurred during the period studied, they are not correlated events.

Regarding the coast of South America, a new lineage of CeMV was detected in a *Stenella guianensis* calf washed ashore dead at Guiri, Espiritu Santo State in Brazil, November 2010, during morphological examination, lymphoid depletion, interstitial pneumonia and meningoencephalitis were detected. It is suspected that the interaction of this species in this region with *Steno Bredanensis*, *T.truncatus*, *Megaptera novaeangliae*, and *Eubalaena australis* resulted in the infection of the *S. guianensis* calf (Groch et al., 2014;



Martins et al., 2001; Wedekin et al., 2005). Here, in 2010 there was a stranded CeMV-positive cetaceans, but it belongs to the species *Stenella frontalis*, only detected by IHC.

On the coasts of the Asian continent, specifically the Indian coast of Australia, there was an unusual mortality of 25 individuals from a small resident community in western Australia in June 2009. Closely were detected two mortalities in immature offshore *T. truncatus*, in Queensland, Eastern Australia. One of them was stranded on North Stradbroke Islands, 9n 2009, and the other on Fraser Island in 2010. The strain isolated was related to the DMV isolated in Europe and the USA, however, the Western Australian *T. aduncus* had an unusual form of CeMV. Together it is suggested that CeMV-1 and CeMV-2 are widespread in the water of Australasia (Stone et al., 2011, 2012).

Circulation of the virus along the Italian coast is confirmed along its entire length, with a higher prevalence of presentation since 2013, with the highest incidence concentrated in the 2016-2017 biennium, when more than 40% of the subjects tested were infected. The 2019 strains subjected to phylogenetic investigations confirmed that the Atlantic CeMV strain identified in 2016 (Bento et al., 2016; Pautasso et al., 2019) was first tested in bottlenose dolphins, sperm whales, stenellae and common dolphins off the Italian coast (Pautasso et al., 2019).

Pilot whales are susceptible to DMV and PWMV. DMV caused atypical pilot whale deaths in the Mediterranean Sea (Fernández et al., 2008; Raga et al., 2008). By contrast, there were detected 4 cases of PWMV, 1 in New Jersey, USA and the other 3 associated to an unusual mortality event in the Canary Islands, Spain, in 2015 (Sierra et al., 2016). This strain of CeMV (PWMV) was associated with an atypical stranding off the coast of Brazil in 2020 (Costa-Silva et al., 2023). The infected specimens were in poor body condition, of juvenile age, which may be associated with loss of maternal passive immunity (Costa-Silva et al., 2023).

Conclusions

1. The prevalence of CeMV-positive cetaceans stranded on the coast of the Canary Islands from January 1999 to December 2023 (3.56%) is low compared to similar studies in other geographical areas.
2. CeMV infection cases were recorded in most years of the study period, except for 1999-2001, 2003-2004, 2014, 2017, and 2023. Some of these episodes were related to outbreaks in the Mediterranean Sea.



3. A higher incidence of CeMV has been observed during the spring and winter seasons, with 30% and 27% of the CeMV-positive animals in our study stranding during these seasons, respectively.
4. During the study period in the Canary Islands, eight different cetacean species were infected by CeMV. Similar to previous findings, the short-finned pilot whale (*Globicephala macrorhynchus*) and the striped dolphin (*Stenella coeruleoalba*) had the highest number of positive cases.
5. Most of the animals that stranded with CeMV were juveniles or subadults (63%), which supports previous studies.
6. Despite no reported outbreaks or epizootics in the Canary Islands, during the study period, most of the CeMV-infected cetaceans presented with the acute systemic form of the disease, primarily affecting striped dolphins.
7. Two distinct strains of CeMV circulate in the waters of the Canary Islands. The DMV strain is the most commonly detected among various cetacean species, while the PWMV strain has been linked to an unusual mortality event and is responsible for most cases of chronic localized encephalitis in short-finned pilot whales.

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Annexes

Table 2. Epidemiological data about cetaceans infected with CeMV of Canary Islands.

CET	Species	Sex	Age	S.L.	S.D.	S.S.	B.C.	D.C.
182	<i>Ziphius cavirostris</i>	M	J/S	FTV	24/09/2002	Dead	ND	2
195	<i>Stenella coeruleoalba</i>	M	A	GC	13/11/2002	Alive	Good	1
305	<i>Tursiops truncatus</i>	F	J/S	LZT	18/07/2005	Alive	Moderate	1
308	<i>Tursiops truncatus</i>	F	J/S	GC	16/08/2005	Alive	Good	1
345	<i>Globicephala macrorhynchus</i>	F	J/S	GC	11/05/2006	Dead	Moderate	2
373	<i>Delphinus delphis</i>	F	A	LG	26/03/2007	Alive	Poor	2
380	<i>Stenella coeruleoalba</i>	M	J/S	TF	16/04/2007	Dead	Poor	2
409	<i>Stenella coeruleoalba</i>	F	J/S	FTV	05/02/2008	Dead	Poor	2
431	<i>Grampus griseus</i>	M	J/S	TF	20/04/2008	Alive	Poor	2
476	<i>Stenella coeruleoalba</i>	F	A	FTV	22/01/2009	Dead	Poor	2
515	<i>Stenella frontalis</i>	M	A	FTV	26/02/2010	Alive	Good	2
558	<i>Stenella coeruleoalba</i>	F	A	LZT	10/02/2011	Dead	Good	2
583	<i>Globicephala macrorhynchus</i>	M	J/S	TF	23/07/2011	Alive	Good	2
594	<i>Globicephala macrorhynchus</i>	M	J/S	TF	02/12/2011	Dead	very poor	2
614	<i>Stenella coeruleoalba</i>	M	J/S	LZT	28/04/2012	Dead	Good	2

Supplementary Table 2. Description of abbreviations: CET (number of cetacean), sex (M: male; F: female), age (C: calf, J: juvenile, S: subadult, A: adult), S.L: Stranded Location (FTV: Fuerteventura, GC: Gran Canaria, LZT: Lanzarote, LG: La Graciosa, TF: Tenerife), S.D: Stranded date, S.S: Stranded state, B.C: body condition, D.C: decomposition code (1: very fresh, 2: fresh, 3: moderate autolysis, 4: advanced autolysis and 5: very advanced autolysis).



CET	Species	Sex	Age	S.L.	S.D.	S.S.	B.C.	D.C.
666	<i>Globicephala macrorhynchus</i>	M	J/S	LZT	15/05/2013	Alive	Moderate	2
739	<i>Globicephala macrorhynchus</i>	F	J/S	FTV	14/01/2015	Dead	Moderate	3
751	<i>Grampus griseus</i>	F	A	TF	16/03/2015	Dead	Moderate	2
758	<i>Globicephala macrorhynchus</i>	M	C	FTV	15/05/2015	Dead	Moderate	3
760	<i>Globicephala macrorhynchus</i>	M	A	TF	20/05/2015	Dead	ND	3
810	<i>Delphinus delphis</i>	F	J/S	TF	03/07/2016	Alive	very poor	2
921	<i>Stenella coeruleoalba</i>	M	J/S	GC	06/07/2018	Dead	Poor	3
933	<i>Stenella coeruleoalba</i>	M	J/S	GC	22/09/2018	Alive	Moderate	2
1035	<i>Stenella coeruleoalba</i>	F	J/S	FTV	04/10/2019	Alive	Poor	3
1038	<i>Stenella coeruleoalba</i>	M	A	LZT	29/10/2019	Alive	Poor	2
1062	<i>Balaenoptera physalus</i>	M	C	FTV	22/02/2020	Alive	Poor	2
1069	<i>Stenella coeruleoalba</i>	M	A	GC	13/03/2020	Alive	Poor	2
1092	<i>Stenella coeruleoalba</i>	F	J/S	GC	20/05/2020	Dead	Moderate	2
1198	<i>Globicephala macrorhynchus</i>	F	J/S	GC	02/11/2021	Dead	Moderate	3
1257	<i>Globicephala macrorhynchus</i>	M	J/S	TF	15/09/2022	Dead	Moderate	2

Supplementary Table 2. Description of abbreviations: CET (number of cetacean), sex (M: male; F: female), age (C: calf, J: juvenile, S: subadult, A: adult), S.L: Stranded Location (FTV: Fuerteventura, GC: Gran Canaria, LZT: Lanzarote, LG: La Graciosa, TF: Tenerife), S.D: Stranded date, S.S: Stranded state, B.C: body condition, D.C: decomposition code (1: very fresh, 2: fresh, 3: moderate autolysis, 4: advanced autolysis and 5: very advanced autolysis).



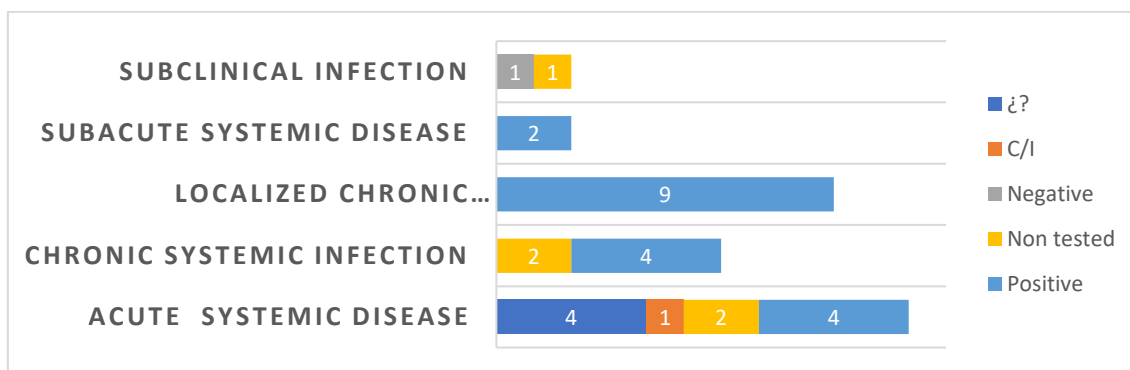
Table 3: Disease presentation form, method of diagnosis and CeMV in stranded cetaceans in the Canary Islands (1999-2023).

CET	Species	Disease presentation form	IHC	PCR	CeMV
182	<i>Ziphius cavirostris</i>	Subclinical Infection	Negative	A	DMV
195	<i>Stenella coeruleoalba</i>	Chronic Systemic Infection	Positive	C/Ñ/R	DMV
305	<i>Tursiops truncatus</i>	Subacute Systemic Disease	Positive	C/R/S	DMV
308	<i>Tursiops truncatus</i>	Chronic Systemic Infection	Positive	C/R	DMV
345	<i>Globicephala macrorhynchus</i>	Localized Chronic Encephalitis	Positive	Non tested	Unknown
373	<i>Delphinus delphis</i>	Localized Chronic Encephalitis	Positive	Negative	Unknown
380	<i>Stenella coeruleoalba</i>	Chronic Systemic Infection	Positive	R	DMV
409	<i>Stenella coeruleoalba</i>	Localized Chronic Encephalitis	Positive	R	DMV
431	<i>Grampus griseus</i>	Localized Chronic Encephalitis	Positive	R	DMV
476	<i>Stenella coeruleoalba</i>	Localized Chronic Encephalitis	Positive	R	DMV
515	<i>Stenella frontalis</i>	Localized Chronic Encephalitis	Positive	Negative/Toxo	Unknown
558	<i>Stenella coeruleoalba</i>	Chronic Systemic Infection	Positive	C/R	DMV
583	<i>Globicephala macrorhynchus</i>	Localized Chronic Encephalitis	Positive	R	PWMV
594	<i>Globicephala macrorhynchus</i>	Localized Chronic Encephalitis	Positive	R	PWMV
614	<i>Stenella coeruleoalba</i>	Acute Systemic Disease	Positive	C/R	DMV
666	<i>Globicephala macrorhynchus</i>	Localized Chronic Encephalitis	Positive	R	PWMV
739	<i>Globicephala macrorhynchus</i>	Subacute Systemic Disease	Positive	Systemic	PWMV
751	<i>Grampus griseus</i>	Subclinical Infection	Non tested	N	DMV
758	<i>Globicephala macrorhynchus</i>	Acute Systemic Disease	Positive	Systemic	PWMV
760	<i>Globicephala macrorhynchus</i>	Acute Systemic Disease	Non tested	G	PWMV
810	<i>Delphinus delphis</i>	Acute Systemic Disease	Positive	C/G/R	DMV



921	<i>Stenella coeruleoalba</i>	Acute Systemic Disease	Positive	C/I/N/Ñ/R/S	DMV
933	<i>Stenella coeruleoalba</i>	Acute Systemic Disease	Non tested	C/R	DMV
1035	<i>Stenella coeruleoalba</i>	Acute Systemic Disease	¿?	A	DMV
1038	<i>Stenella coeruleoalba</i>	Acute Systemic Disease	¿?	C	DMV
1062	<i>Balaenoptera physalus</i>	Acute Systemic Disease	C/I	C/I	DMV
1069	<i>Stenella coeruleoalba</i>	Acute Systemic Disease	¿?	A	¿?
1092	<i>Stenella coeruleoalba</i>	Acute Systemic Disease	¿?	C	¿?
1198	<i>Globicephala macrorhynchus</i>	Chronic Systemic Infection	Non tested	R	PWMV
1257	<i>Globicephala macrorhynchus</i>	Chronic Systemic Infection	Non tested	R	PWMV

Supplementary Table 3. Description of abbreviations: IHC: Immunohistochemical, PCR: Polimerase chain-reaction (A: skin, C: lung, I: liver; Ñ: kidney; S: spleen; N: mesenteric lymph node; R: NCS, cerebrum; G: tonsil; CeMV: cetacean *morbillivirus sp.* (DMV: dolphin morbillivirus, PWMV: pilot whale morbillivirus).



Supplementary Figure 1. Prevalence of CeMV by immunohistochemical studies results.

Viral detection by organs Disease form presentation

	ASD	CSD	LCE	SSD	SI	Grand Total
A	2	0	0	0	1	3
C	2	0	0	0	0	2
C/G/R	1	0	0	0	0	1
C/I	1	0	0	0	0	1
C/I/N/Ñ/R/S	1	0	0	0	0	1
C/Ñ/R	0	1	0	0	0	1
C/R	2	2	0	0	0	4
C/R/S	0	0	0	1	0	1
G	1	0	0	0	0	1
N	0	0	0	0	1	1
Negative	0	0	1	0	0	1
Negative/Toxo	0	0	1	0	0	1
Non tested	0	0	1	0	0	1
R	0	3	6	0	0	9
Systemic	1	0	0	1	0	2
Gran total	11	6	9	2	2	30

Supplementary Table 5: Results of RT-PCR straddle by organs. The table above straddles the PCR results obtained by the form of disease presentation, indicating where viral RNA was found according to the organ studied. The acronyms correspond to: A: skin; C: lung; I: liver; Ñ: kidney; S: spleen; N: mesenteric lymph node; R: CNS (cerebrum); G: tonsil; ASD: acute systemic disease; CSD: chronic systemic disease; LCE: localized chronic encephalitis; SSD: subacute systemic disease; SI: subclinical infection.