DISEASES OF STRANDED PACIFIC ISLAND MARINE MAMMALS

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ABSTRACT

The University of Hawaii Health and Stranding Lab located at Marine Corps Base Hawaii (MCBH) is the only entity in the Pacific Islands region that responds to strandings, conducts necropsy and cause of death investigations, archives tissues and performs research to identify and evaluate threats to Pacific Island cetaceans. This project focuses on increasing our understanding of infectious diseases in the Pacific Island region by investigating the emerging diseases caused by circovirus, morbillivirus, herpesvirus and toxoplasmosis infections in cetaceans.

Beaked whale circovirus was initially detected in a Longman's beaked whale a decade after stranding in Maui in 2010. This case represented the first known circovirus infection in a marine mammal world-wide and led to PCR screening of archived tissues and the identification of ten additional host cetacean species across the Pacific Basin. Current project objectives include an investigation into tissue tropism of circovirus, sensitivity of viral detection by qPCR and additional screening of stranded individuals. Project progress to date includes a preliminary examination of traditional and qPCR results from the same tissue extracts that were found to be consistent in most cases. Among tissue types, PCR detection of viral DNA was investigated in an expanded suite of tissue types for known positive cases. Detection was found to be most successful in infected individuals in lymphoid organs, followed by brain. The virus was identified in four additional host species for the first time as part of this project, which increases the known host species of beaked whale circovirus from 11 to 15 cetacean species. New host species include the Risso's dolphin, rough-toothed dolphin, pygmy killer whale and killer whale. qPCR positive results were obtained from three to four replicates of five of all tissues tested, indicating the need for multiple replicates for diagnosis as uneven distribution of viral DNA was observed in cetacean tissues infected with circovirus. We completed circovirus testing of samples of all Cuvier's beaked whales in our tissues archives and detected an overall circovirus prevalence of 50% across the Pacific with the presence of this emerging disease identified for the first time from stranded beaked whales in Wake Island and Guam. Testing all samples of false killer whale tissue in our archive for circovirus suggests a 50% positivity rate in this species.

Positive beaked whale circovirus cases included animals with co-infections that led to death such as morbillivirus, *Brucella* and toxoplasmosis. This project includes an examination of pathologies in the published literature typical of morbillivirus, herpesvirus and circovirus to aid in understanding negative health impacts in cetaceans. A systematic literature review of relevant journal articles published within the last 20 years was conducted for circovirus, cetacean morbillivirus and cetacean herpesvirus. Porcine circovirus is the best studied circovirus, followed by avian circovirus and canine circovirus, although circovirus has been detected in multiple mammals and fish. Virus tropism, defined as preferred tissues for infection, varies by strain, but virions are found consistently in macrophages in lymphoid tissue. Cetacean morbillivirus has been

shown to persist in lymphoid tissue, macrophages and B cells and cause changes in immune function leading to immune depression. Cetacean alpha herpesvirus infects neurons, but can be found in other organs and cetacean gamma herpesvirus is typically associated with genital, proliferative skin lesions.

Serological diagnosis of *Toxoplasma* has been conducted in a subset of archived tissues from previously stranded cetaceans to investigate pathogen exposure rate. We completed testing of tissue fluid, serum or aqueous humor in 35 individuals to date that represented spinner dolphins, bottlenose dolphins, striped dolphins and false killer whales and detected antibodies against *Toxoplasma gondii* in five spinner dolphins. Liver and kidney meat juice were found to be the most reliable tissue type for *Toxoplasma* serological testing. Positive serology results for spinner dolphins indicate an overall exposure rate of 21% to this parasite that is known to have resulted in mortality of spinner and bottlenose dolphins in the main Hawaiian Islands.

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INTRODUCTION

Background: Beaked whale circovirus: Obtaining better understanding of an emerging disease

Infectious disease poses a significant threat to Pacific Island cetaceans. Circovirus, morbillivirus and toxoplasmosis represent viral and parasitic diseases now known to infect cetaceans in the Pacific Islands, with morbillivirus and toxoplasmosis responsible for cetacean mortalities (West et al. 2013; West et al. 2021; Landrau-Giovanetti et al. 2022). Circovirus was first identified in a marine mammal world-wide from a stranded Longman's beaked whale from Maui and this novel circovirus was named beaked whale circovirus (BWCV) (Landrau-Giovanetti et al. 2020). With prior funding support from NAVSEA, our laboratory detected beaked whale circovirus using traditional PCR testing in 10 additional cetacean host species across the Pacific, including from beaked whale strandings in American Samoa and in the Commonwealth of the Northern Mariana Islands (Clifton et al. 2023).

The identification of a high prevalence of BWCV in a number of host cetacean species from diverse regions of the Pacific basin has led to important questions about this emerging disease in cetaceans. It is currently unknown if BWCV is an opportunistic pathogen replicating in tissues without clinical significance or if it contributed to disease being that the initial case was complicated by co-infections with morbillivirus and herpes virus (West et al. 2013). In pigs, three different strains of circovirus are recognized, each with characteristic disease. Porcine circovirus 1 is rare and not considered pathogenic. Porcine circovirus 2 is widespread and linked to a wasting syndrome in pigs, as well as systemic disease involving major organ systems and reproductive disease that includes fetal abortions. Recently, a distinct porcine circovirus 3 has been identified that is associated with cardiac lesions. A preliminary examination of histopathology reports for the seven individuals that tested positive for BWCV by PCR found myocardial lesions in four animals. Further work is needed to understand the potential clinical significance of BWCV on the heart and other organs as well as the likelihood of systemic infection occurring. Expansion of cetacean tissue testing to include atrial and ventricular heart tissues as well as any other available tissues from positive cases will aid in our understanding of pathogen distribution.

Despite obtaining sequencing confirmation of circovirus from at least one tissue in each stranded individual deemed positive for circovirus by traditional PCR testing (Clifton et al. 2023), PCR gel electrophoresis results were not necessarily consistent when repeating analysis of the same sample extract in some animals. We believe that this is likely due to inconsistent amplifications that may occur when using traditional PCR if a low viral load is present. Quantitative polymerase chain reaction (qPCR) instrumentation has increased sensitivity and quantitatively measures the viral load present, providing a means to determine the viral load in sample extracts that yielded inconsistent circovirus results using traditional PCR. Information on viral load in circovirus positive animals is anticipated to provide valuable information both in better assessing the clinical impact of the pathogen on an individual cetacean and for the interpretation of future circovirus test results.

The pathogenicity of BWCV is currently unknown but future work will focus on describing pathology that is either common or unique to circoviruses. Circoviruses have long been well recognized for their impact on mortality in the pet trade and agricultural industries, causing

Psittacine beak and feather disease in parrots, as well as porcine respiratory diseases complex and post-weaning multi-systemic wasting syndrome in pigs (Crowther et al. 2003; Fogell et al. 2018; Rose et al. 2012). Circovirus infections do not always result in a pathogenic response, though many of the strains across various species can cause negative health impacts (Gavier-Widen et al. 2012). Disease due to pathogenic circovirus strains includes necrosis and inflammation in the brain, lung, liver, heart, spleen, intestine, and lymph tissues (Bexton et al. 2015; Rampin et al.2006; Seo et al. 2014; Woods and Latimer, 2000; Yang et al. 2015). These viruses are frequently associated with respiratory illnesses (Chen et al. 2021; Lin et al. 2011; Seo et al. 2014) and several wasting diseases (Gavier-Widen et al. 2012; Seo et al. 2014; Yang et al. 2015). Circoviruses have been directly linked to reproductive failure and mortality in fish, birds, and swine, often in newly hatched or young offspring, although these outcomes can be found in infected juveniles and adults as well (Grasland et al. 2013; Lőrincz et al. 2011; Woods et al. 1993; Yang et al. 2015). There is also the potential for indirect negative impacts to infected hosts, with lymphoid depletion observed in cases of chronic circovirus infections that may indicate immune suppression (Mao et al. 2017; Palinski et al. 2017; Yang et al. 2015). Co-infections by other viruses and bacteria have been documented in both mammals and birds found to be infected with circoviruses (Dal Santo et al.2020; Lagan Tregaskis et al. 2020; Zaccaria et al. 2016; Zhen et al. 2021). We have reported a high degree of co-infections in the cetaceans tested positive for circovirus to date (Clifton et al. 2023), further supporting the need to systematically examine the pathology described in the literature that is associated with circoviruses for comparison to that observed in infected cetaceans. We also anticipate that a systematic examination of the cetacean literature for pathological findings associated with cetacean morbillivirus and herpesvirus will aid in the interpretation of the source pathogen responsible for abnormal findings that are observed in co-infected cetaceans.

Background: Toxoplasma Serology Testing to Evaluate Disease Exposure

Toxoplasmosis is the most significant disease threat facing endangered Hawaiian monk seals in the main Hawaiian Islands, responsible for the deaths of at least 15 known seals. Fatally disseminated toxoplasmosis has also been determined as the cause of death in three spinner dolphins that have previously stranded in Hawaiian waters and was responsible for the death of a stranded bottlenose dolphin in 2023 (Migaki et al. 1990; Landrau-Giovannetti et al. 2022; West, unpublished data). We project that the three confirmed cases of fatally disseminated toxoplasmosis in spinner dolphins equates to the deaths of at least 60 spinner dolphins in Hawaiian waters based on low carcass recovery rates. Despite toxoplasmosis being identified is a significant threat to Hawaiian marine mammals, no information is available on the exposure of any Hawaiian cetaceans to *Toxoplasma*. Studies of *Toxoplasma* antibody prevalence have been conducted in stranded cetaceans from other regions of the world where this parasite represents a significant health risk in order to better understand exposure. Serology based studies indicate that *T. gondii* infection is frequent in at least three dolphin species (striped dolphins, bottlenose dolphins and common dolphins) in the Mediterranean Sea (Bigal et al. 2018; Cabezon et al. 2004; Di Guardo et al. 2011). In Russian beluga whales, 11.5% were positive for *Toxoplasma* antibodies (Alekseev et al. 2017). Additionally, positive antibody titers were evident among a number of stranded cetaceans in the Philippines, including the Fraser's dolphin, spotted dolphin, rough-toothed dolphin, Bryde's whale and in pygmy killer whales (Obusan et al. 2019).

Serological diagnosis of toxoplasmosis takes advantage of the long-term persistence of specific antibodies in serum following exposure to the parasite. Antibody testing of archived stranded specimen tissues in Hawaii will provide a means to determine the prevalence of *Toxoplasma* exposure among Hawaiian cetacean species. This is valuable information that can be used to evaluate the probability that pathogen exposure leads to death. Antibody prevalence in wildlife species where sample storage conditions are challenging (eg. wolverines, caribou) suggests that *Toxoplasma* antibody prevalence can be reliably measured from matrices besides blood serum, with meat juice from the heart possibly being advantageous when measuring low antibody levels (Bachand et al. 2018; Sharma et al. 2019). A large number of testing methodologies including various agglutination tests, immunofluorescence testing, Western blot and enzyme-linked immunosorbent assays (ELISA) have been widely used to detect *Toxoplasma* antibodies in animals with ELISA believed to be the most reliable, practical and economical (Liyanage et al. 2021).

METHODS

Circovirus testing objectives addressed during calendar year 2023 are listed below:

Circovirus: Expand tissue testing for BWCV in positive animals to include 4-5 heart locations, all archived lymph nodes described by location and other available organ samples to evaluate pathogen distribution. Tissue sample screening could include up to 20 additional tissues in the case of at least one of the BWCV positive individuals.

Circovirus: Conduct qPCR testing of sample extracts screened for BWCV to measure the viral load present among all positive and negative tissues and among the tissues of individual animals.

Circovirus: Conduct qPCR testing for circovirus in order to determine viral load when this pathogen is present in of a suite of tissues from an additional 20 individuals where selection is not targeted by health status. This will allow for an assessment of the possible presence of BWCV in healthy cetaceans without significant pathology.

Tissue selection was based on availability from previously stranded cetaceans and included 3-15 frozen tissue types chosen from lung, liver, spleen, skin, kidney, brain, pancreas, muscle, heart locations, blubber, spinal cord, adrenal, and various lymph nodes. Brain and lung samples from the first BWCV case were used as positive controls during screening. DNA was extracted from each sample using Qiagen DNeasy Blood and Tissue Kits (Qiagen, Germantown, Maryland) according to the manufacturer's protocol. The DNA concentration of each extract was quantified using Qubit dsDNA Broad-Range Assay Kits and a Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts).

Traditional PCR was carried out following the protocol descibed by Clifton et al. 2023. Briefly, the BWCV forward primer 5' CTTCAGATTCCCCGTCAAGA 3' and BWCV reverse primer 5' GTCTCCCCACAATGGTTCAC 3' were used with an initial denaturation at 94°C for five minutes, 40 cycles of denaturing at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension step at 72°C for five minutes.

The polymerase chain reaction (PCR) protocol (Clifton et al. 2023) was adapted for quantitative PCR (qPCR) using QuantStudio 3 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Waltham, Massachusetts). Nuclease-free water was used as a negative control to assess primer dimerization and result quality. All traditional PCR products and qPCR products with positive amplification curves were examined by gel electrophoresis on a 1 % agarose gel.

Visible bands at 400bp in size indicated the likely presence of BWCV. Products from all suspected positive cases were prepared for DNA sequencing using QIAquick PCR and Gel Cleanup Kits (Qiagen, Germantown, Maryland) and sequenced using the reverse primers at the Advanced Studies in Genomics, Proteomics, and Bioinformatics lab at the University of Hawai'i. DNA sequences were analyzed using BioEdit Sequence Alignment Editor and NCBI BLAST nucleotide database.

Project objectives associated with examining the published literature to characterize pathology associated with circovirus, morbillivirus and herpesvirus are listed below:

Circovirus: Examine pathological findings associated with circoviruses in the published literature to create a comprehensive reference table of descriptions for identifying common and unique pathology that may be of clinical significance in cetaceans.

Examine pathological findings associated with morbillivirus and herpesvirus in the published literature to create a comprehensive reference table of descriptions for identifying common and unique pathology that can be compared to cetacean circovirus co-infection cases.

Systematic literature reviews with a focus on the published literature describing original research articles, case studies and review articles were conducted on circovirus infection in birds and mammals. Published articles focused on disease syndromes and pathological changes observed in affected animals and detection of the virus in tissues. The only prior publications on circovirus in cetaceans were led by our research group. Published articles were organized into a literature review table format that includes the name of article, authors, year, publication, species, disease syndrome or pathological findings, methods, and a brief summary of findings.

Similar to our literature review on circovirus in birds and mammals, we reviewed the published literature describing original research articles, case studies and review articles on morbillivirus and herpesvirus infection in cetaceans. This literature review included descriptions of pathological change and viral effects on the immune system and how this may contribute to co-infections. Published articles were again organized into a literature review table format that includes a focus on morbillivirus or herpes virus, the name of article, authors, year, publication, species, disease syndrome or pathological findings, methods, and a brief summary of findings.

***Toxoplasma* serology objective addressed during calendar year 2023 is as follows:**

Toxoplasma: Test a suite of tissue sample fluids in 50 previously stranded cetaceans for Toxoplasma by ELISA to estimate exposure rates and prevalence of this parasite among Pacific Island cetaceans.

Previous validations have indicated that meat juice – the fluid released upon excision of animal tissues – provides a reliable matrix for detection of *Toxoplasma* antibodies in other wildlife species. Meat juice was collected from thawed frozen samples of the following tissue types from stranded cetaceans: adrenal, brain, heart, kidney, lung, liver, and muscle, as well as a variety of lymph node types (anal, hilar, marginal, mediastinal, mesenteric, and prescapular). In addition to meat juice, samples of serum and aqueous humor were analyzed when available. Samples were diluted (meat juice 1:2, serum and aqueous humor 1:10) using solutions from a commercially available kit (TOXOS-MS-2P-I59, Innovative Diagnostics, Grabels, France) and analyzed in duplicate per the manufacturer's protocol. Bovine serum positive controls were analyzed in each assay, in addition to the kit's internal controls, to ensure accuracy of the results. Final optical density of processed samples was analyzed at 450 nm (Biotek HTX Synergy, Agilent Technologies, Santa Clara, California, USA).

RESULTS

Sensitivity of Circovirus Detection in Tissues Tested by both PCR and qPCR

To compare the sensitivity of viral detection by molecular methods and investigate viral load in tissues that previously tested positive by traditional PCR, five tissues (one per animal) were used in a replicate trial via qPCR. The selected tissues for replicate qPCR trials were initially tested using traditional PCR and confirmed as BWCV positive by genetic sequencing. Five replicates per tissue were prepared using the known positive DNA extract. Amplification of viral DNA was observed in three of the five tissue samples tested by qPCR (Table 1). Of the three tissues that tested positive by qPCR, amplification was only observed in 3-4 of the replicates. All replicates that demonstrated amplification using qPCR techniques were confirmed as BWCV by genetic sequencing. In one case four replicates of hilar lymph node tested positive, and in the other cases, three replicates of brain tissue and three replicates of mesenteric lymph node had detectable levels of circovirus DNA by qPCR (Table 1). Number of cycles at the time of detection ranged between 34 and 38 cycles in the three animals where positives were detected by qPCR in contrast to 16 cycles in the positive control sample from the initial Longman's beaked whale circovirus case. This suggests that viral loads of BWCV are substantially lower in the three animals positive by PCR compared to a relatively higher viral load in the positive control sample.

Circovirus Organ Tropism Detected by PCR and qPCR

Fifteen cases in the UH Health and Stranding Lab were identified as positive for BWCV by Clifton et al. (2023) using traditional PCR for viral DNA detection. Five additional cases were identified as positive by traditional PCR as part of the current project. A comprehensive suite of tissues were tested to investigate viral organ tropism in the five newly identified BWCV cases. Samples of lymph nodes were the most common tissues that contained detectable levels of viral DNA (n=4). In the case of the other positive individual the only positive tissue of a suite of tissues tested was brain.

As part of this project, the list of tissues screened from the 15 positive cases described in Clifton et al. (2023) has been expanded to include available heart locations (ventricle, atria, pericardium, aorta, or pulmonary artery and vein) and lymph nodes from various locations across the body

(Table 2). Screening for BWCV to date includes an expanded suite of tissues from positive individuals with additional positive tissues identified in five cases. Samples of right atrium, right ventricle and lymphoid tissue tested positive for a Blainsville's beaked whale first identified as positive in Clifton et al. (2023). Circovirus DNA was detected in samples of spleen and lymph node in five cases, and in samples of brain, muscle and pancreas in each of one case (Table 2).

Circovirus Detection in Novel Host Species

Clifton et al. (2023) discovered 10 new host species for BWCV beyond the first Longman's beaked whale case. To date, circovirus screening efforts targeted additional species in an effort to determine if BWCV is present in other cetacean host species. Species that had not been previously tested, or where only minimal individuals have been previously tested and/or species represented by only a few individual animals in our tissue archive were selected for screening. Excluding cases that had no viable tissue samples and those already tested negative by Clifton et al. (2023) led to the screening of two pygmy killer whales, two Risso's dolphins, one killer whale, and four rough-toothed dolphins (Table 3). Suspect positives for BWCV included the two pygmy killer whales, one Risso's dolphin, the killer whale, and two rough-toothed dolphins. Genetic sequencing is pending to confirm these cases as BWCV positive.

Circovirus Prevalence in Cuvier's Beaked Whales

We completed Circovirus screening for all Cuvier's beaked whales (*Z. cavirostris*) in the Health and Stranding Lab archive. Clifton et al. (2023) demonstrated a 60% prevalence in individuals from across the Pacific Island region. Four additional Cuvier's beaked whales were tested for the presence of BWCV by qPCR (Table 4). Two of these cases are suspected positive for BWCV, and genetic sequencing is pending to confirm. KW2019002 from Guam and KW2020012 from Wake Island demonstrated potential detection in the kidney; a tissue which has shown detection in 8 of 15 cases from Clifton et al. (2023). If confirmed by sequencing, BWCV has a 50% prevalence in stranded Cuvier's beaked whales (5/10) not only in the Hawaiian Islands, but across the Pacific Island region. Only one tissue was tested in one individual without detection due to limited and poor sample condition (KW2015010, muscle).

Circovirus in False Killer Whales

We completed screening for BWCV in archived false killer whales during calendar year 2023, which included the endangered insular main Hawaiian Islands individuals, additional tissues from a pelagic false killer whale by-caught outside of the Exclusive Economic Zone and an individual stranded in the Northern Mariana Islands (Rota) where a limited necropsy was conducted (Table 6). We detected additional positive tissues when expanding the number and types of tissues tested in the pelagic false killer whale described in Clifton et al. (2023). We also detected an additional three false killer whales that were positive for BWCV. These include an endangered insular individual that stranded in Molokai in 2010 with significant pathological findings in the adrenal gland and other organs, a decomposed endangered insular false killer whale stranded on Maui and an individual from Rota, Northern Mariana Islands. The endangered insular false killer whale stranded on Molokai has been confirmed positive for BWCV by genetic sequencing while sequencing is pending for the Maui and Rota stranding events.

Literature review of pathologies associated with circovirus, morbillivirus and herpesvirus

We prepared a literature review table with 21 recent references of published original research articles, case studies and review articles describing circovirus infections in birds and mammals (Table 6). Disease syndromes have been characterized in birds (Beak and Feather Disease, Pigeon Ill thrift). Disease can be acute leading to sudden death or chronic associated with beak deformity, feather loss, and wasting. Circovirus particles and viral assemblies were observed consistently in the cytoplasm of macrophages in lymphoid organs of affected birds, and epithelial cells in animals with Beak and Feather disease. Coinfections have been documented with Beak and Feather disease. The best studied circoviruses are porcine circoviruses I, II and III. Porcine circovirus I is considered apathogenic. Infections with porcine circovirus II led to a wasting syndrome and destruction of lymphoid tissues with virus found in macrophages in depleted lymphoid follicles. Coinfections with pseudorabies virus (herpes virus) and mycoplasma are recorded and included cases with multiorgan inflammation. Porcine circovirus III causes inflammation in multiple organs including a vasculitis, myocarditis, dermatitis and nephropathy syndrome in piglets. Virus was detected in lesions, in macrophages and histiocytes of lymphoid organs, and endothelial cells in cases of vasculitis by in-situ hybridization and by qPCR of tissue samples. Virus was not consistently detected in affected tissues (lung, kidney) and immune-mediated inflammation was suggested.

We similarly selected and reviewed 20 peer reviewed articles published within the last 15 years that focus on morbillivirus and herpesvirus infections in cetaceans (Table 7). Morbillivirus has been recognized as a cetacean pathogen of significant concern for more than 20 years and has caused epizootic mortality events in dolphins. Observed lesions include meningitis, encephalitis, interstitial pneumonia and changes in lymphoid organs consistent with lymphoid necrosis and depletion. Morbillivirus infection has been linked to suppression of the immune system documented by changes in lymphoid cell populations and cytokine expression. Persistent infection of morbillivirus is documented by viral RNA in lymphoid cells. Cytoplasmic and intranuclear eosinophilic inclusion bodies are detected in infected cells by histopathology. Co-infections by *Brucella*, *Toxoplasma*, circovirus, herpesvirus, fungal organisms and parasites are common and contribute to disease and/or lead to death. In a study of CeMV infection in dolphins, viral neurotropism was observed in striped dolphin and bottlenose dolphins and pronounced lymphoid depletion and lung disease in Guiana dolphins. Herpesviruses are DNA viruses that cause lifelong infection, and viruses persist in neurons and macrophages and B-cells in lymphoid tissues. Alpha herpesvirus and gamma herpesvirus infections have led to death of cetaceans. Clinical and sometimes fatal Herpesvirus infection combined with other pathogens such as morbillivirus and circovirus suggests the significance of immunosuppression caused by morbillivirus and potentially circovirus. Herpesviruses cause characteristic intranuclear inclusions that are observed by histopathology. While alpha herpesviruses are more commonly associated with encephalitis, and gamma herpesvirus with integumentary lesions, in the genital area, viral tropism is not consistent.

Toxoplasma Serology Testing

To date, 35 individuals that represent four different species have been tested for presence of *T. gondii* antibodies (Table 8). Species include bottlenose dolphins (n=4), false killer whales (n=1), spinner dolphins (n=24), and striped dolphins (n=6). A total of 94 samples have been tested for

Toxoplasma antibodies in one to four tissue types per animal. Five individuals were found to have *T. gondii* antibodies present. All five animals were spinner dolphins that stranded between 2007 and 2016. Tissue types that were positive for antibodies included kidney (n=5), liver (n=3), lung (n=2), and mediastinal lymph node (n=1) meat juice. This subset of data collected suggests a potential exposure rate of 21% among Hawaiian spinner dolphins, with an overall exposure rate among all species of cetaceans tested of 14%.

DISCUSSION

Circovirus PCR and qPCR Testing and Literature Review of Pathologic Findings

Beaked whale circovirus is an emerging disease that has been detected in over ten cetacean host species across the Pacific basin (Clifton et al. 2023). Despite obtaining sequencing confirmation of circovirus from at least one tissue in each new stranded individual deemed positive for circovirus by traditional PCR testing (Clifton et al. 2023), repeated Circovirus DNA testing of the same sample extracts showed inconsistent results between traditional and qPCR amplificationPreliminary findings suggest inconsistent amplificationby traditional PCR if the viral load in the tissue is low. qPCR instrumentation has increased sensitivity and quantitatively measures the viral load present, providing a means to determine the viral load in sample extracts that yielded inconsistent circovirus results using traditional PCR. We will continue qPCR testing efforts as part of this project and increase our focus on lymphatic tissue testing as suggested from our findings to date to investigate organ trophism.

One objective of this project was to review literature describing pathology and tissue tropism of circovirus in other species in order to better understand the impact of this virus on cetacean health. While circovirus has consistently been shown to infect macrophages in lymphoid tissues in other species, the overall effect of this infection is not clear given that co-infections are common (Chen et al. 2021; Opriessnig et al. 2012, 2007). However, based on information from the literature and our test results to date, we will focus further testing on lymphoid tissues, especially medullary regions where macrophages are abundantly found. Other tissues for additional testing include kidney samples of circovirus positive Cuvier's beaked whale (100% positivity) and heart, where myocarditis has been confirmed by histopathology. Porcine circovirus III causes nephropathy and mononuclear myocarditis and viral DNA was demonstrated in renal tubular epithelial cells and myofibers in the heart by *in situ* hybridization on formalin fixed tissue samples (Cobos et al. 2022). Canine circovirus was associated with hemorrhagic enteritis and the virus was detected in fecal samples (Gomez-Betancourt et al. 2023; Anderson et al. 2017; Li, et al. 2013). We plan to expand PCR testing to include fecal samples from dead stranded animals which also has the potential for application to live animals. Viral shedding may indicate enteric tropism of BWCV even though enteric disease is rarely identified in cetaceans due to the difficulty of obtaining samples from the gastrointestinal tract of stranded cetaceans that are fresh enough for histopathological examination.

Another literature review of recent publications that describe the pathology of morbillivirus and herpesvirus in cetaceans adds to the understanding of the role of morbillivirus in immunosuppression in chronically infected animals (Diaz-Delgado et al. 2019). Immuno-depletion and presence of viral inclusions in cytoplasm and nuclei, and syncytia are characteristic of morbillivirus infections in lymphoid tissues (Groch, 2020, Diaz-Delgado, 2019, DiGuardo et al.

2016). The SLAM molecule on lymphoid cells has been identified as viral receptor (Ohishi et al. 2019; DiGuardo 2018). Co-infections in morbillivirus positive cetaceans are common and indicative of immunosuppression and involve opportunistic pathogens such as herpesvirus, *Toxoplasma* and fungal pathogens (Sierra et al. 2020; Groch et al. 2017; Soto et al. 2012). Alpha herpesvirus infection has led to significant mortality of cetaceans in the Mediterranean causing encephalitis and persistence in neurons (Sierra et al. 2020), but PCR has demonstrated the virus in other tissues including lymphoid organs (Sierra et al. 2022; Vargas-Castro 2021). Gamma herpesvirus has been demonstrated in macrophages and epithelial cells of cetaceans and is mostly associated with proliferative skin lesions in the genital area (Sierra et al. 2022). If both circovirus and morbillivirus are detected in macrophages in depleted lymph nodes, and pathological findings show poor body condition and opportunistic disease then this would indicate profound immunosuppression. Next steps in this project include greater examination of circovirus DNA in lymphoid tissues of PCR positive cases, and consideration of co-infections and overall pathological findings to better understand the impacts of this emerging disease.

Future work as part of this project includes *Toxoplasma* antibody testing of additional stranded cetacean individuals and species to better understand exposure rates and if Hawaiian cetaceans have implied immunity to this parasite. We will also conduct cetacean morbillivirus antibody testing which will signify the first effort to investigate seroconversion in Hawaiian cetaceans.

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Table 1. Cycle of amplification during a 40-cycle qPCR trial of tissues confirmed as BWCV positive by traditional PCR. ND - no detection, NT - not tested.

ID	Species	Tissue	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
KW2010005	*I. pacificus*	Lung (control)	16	16	NT	NT	NT
KW2010019	*P. crassidens*	Brain	35	35	35	ND	ND
KW2022020	*P. electra*	LN: L marginal	ND	ND	ND	ND	ND
KW2023005	*T. truncatus*	LN: mesenteric	ND	ND	ND	ND	ND
KW2023011	*S. longirostris*	LN: L hilar	ND	34	34	34	34
KW2023012	*S. longirostris*	LN: mesenteric	38	38	ND	38	ND

Table 2. Expanded qPCR screening results for fifteen individuals previously identified as positive for beaked whale circovirus by Clifton et al. (2023). Positive tissues listed below have been confirmed by genetic sequencing. LN - lymph node, R/L - right/left.

ID	Species	Positive Tissues	Negative Tissues
KW2007005	*S. longirostris*	-	Blubber, muscle, GI content, spinal cord
KW2008008	*Z. cavirostris*	Pancreas	R/L lung, blubber, skin, LN: anal
KW2008009	*S. longirostris*	Muscle	Blubber, skin
KW2010012	*M. densirostris*	R atrium, R ventricle, LN: bronchial	L ventricle, LN: mesenteric
KW2011008	*P. macrocephalus*	LN: tracheobronchial	R/L atrium, R/L ventricle
KW2011016	*Z. cavirostris*	-	LN: mesenteric, prescapular, venous plexus
KW2015007	*Z. cavirostris*	-	R/L atrium, R/L ventricle, LN: R lung, mesenteric, tracheobronchial, intestinal, stomach, colonic
KW2015013	*S. longirostris*	-	R/L atrium, R/L ventricle, LN: R/L marginal, sublumbar, mesenteric, L prescapular, tracheobronchial
KW2017007	*G. macrorhynchus*	-	R/L atrium, LN: axillary, hepatic, L lung, L prescapular,

			pulmonary, R/L submandibular,
KW2017008	*G. macrorhynchus*	-	R/L atrium, R/L ventricle, LN: R axillary, hilar, R/L marginal, prescapular, submandibular
KW2018001	*L. hosei*	-	R/L atrium, R/L ventricle, pericardium, LN: anal, L prescapular, retroperitoneal
KW2019008	*P. electra*	-	R/L atrium, R/L ventricle, pericardium, LN: aortic, L axillary, L hilar, L lung, L marginal, mesenteric, R/L prescapular
KW2019025	*P. crassidens*	LN: R marginal, mesenteric	LN: anal, R/L prescapular, L submandibular
KW2020007	*S. coeruleoalba*	-	LN: anal, aortic, colonic, diaphragmatic, hilar, R marginal, mesenteric, preaortic, prescapular

Table 3. qPCR screening of stranded individuals belonging to species not recognized as hosts of beaked whale circovirus. LN - lymph node, R/L - right/left, brain - not specified as cerebrum or cerebellum. Prior screening by Clifton et al. (2023) did not detect the presence of BWCV in seven *F. attenuata* and one *S. bredanensis*.

Species	**ID**	**Positive Tissues**	**Negative Tissues**
F. attenuata	KW2009006^{+}	R lung^{+}, R ventricle^{+}, L kidney^{+}	L lung, L ventricle, liver, spleen, cerebrum, cerebellum, LN: mediastinal
	KW2015006^{+}	R/L lung^{+}	Liver, spleen, R atrium, R ventricle, pericardium, cerebrum, cerebellum, LN: mesenteric, marginal
G. griseus	KW2013023	-	R/L lung, R/L ventricle, L atrium, cerebrum, cerebellum, spleen, LN: mesenteric, R marginal
	KW2015004^{+}	Kidney^{+}	R/L lung, L atrium, R ventricle, liver, spleen, cerebrum, cerebellum, LN: L marginal, prescapular, tracheobronchial
S. bredanensis	KW2011021	-	R/L lung, R kidney, spleen, liver, cerebrum, cerebellum, LN: aortic, pancreatic, prescapular
	KW2016001^{+}	R lung^{+}	Heart, cerebellum, lymph node
	KW2016002^{+}	Lymph node^{+}	R lung, heart, cerebellum
S. bredanensis	KW2016017	-	Aqueous humor, brain, muscle

O. orca	KW2008010[+]	Liver[+], spleen[+]	R/L lung, kidney, pancreas, muscle

[+]Suspect positive, genetic confirmation by sequencing is pending.

Table 4. Screening results of all archived Cuvier's beaked whales for beaked whale circovirus by qPCR. LN - lymph node, R/L - right/left.

ID	Location	Positive Tissues	Negative Tissues
KW2008008*	Hawai'i	Lung*, kidney*, LN: mesenteric*, tracheobronchial*, pancreas	Spleen*, liver*, blubber*, LN: anal
KW2011015	Saipan	-	Cerebrum, cerebellum, muscle, lymph node
KW2011016*	Saipan	Lung*, kidney*, spleen*, liver*, LN: mediastinal*	Brain*, venous plexus, LN: prescapular, mesenteric, venous plexus associated
KW2015003*	Guam	-	Brain*, lung*, kidney*, spleen*, liver*, LN: lung*
KW2015007*	American Samoa	Brain*, spleen*, kidney*	Lung*, liver*, R/L ventricles, R/L atria, LN: intestinal, colonic, stomach, R lung, mesenteric, tracheobronchial
KW2015010	Guam	-	Muscle
KW2016005*	Hawai'i	-	Brain*, lung*, kidney*, spleen*, liver*, pancreas*
KW2019002	Guam	L kidney[+]	Cerebrum, L lung, liver, spleen, R adrenal, meninges, L atrium, R ventricle, LN: mesenteric, splenic
KW2020012	Wake Island	Kidney[+]	Cerebrum, cerebellum, L lung, liver, spleen, LN: L marginal, L hilar

*Screening by Clifton et al. 2023

[+]Suspect positive, sequencing pending

Table 5. Screening of all false killer whales beaked whale circovirus by qPCR. LN - lymph node, R/L - right/left, brain - not specified as cerebrum or cerebellum.

ID	Location	Positive Tissues	Negative Tissues
KW2010019	Hawai'i	Brain	Liver, spleen, lung, LN: mesenteric
KW2019025	Hawai'i, outside EEZ	Brain*, lung*, liver*, LN: mediastinal*, mesenteric, marginal	Kidney*, spleen*, LN: anal, R/L scapular, submandibular
KW2013018	Hawai'i	-	Brain, liver, spleen, L lung, LN: L marginal
KW2015015	Hawai'i	-	Brain, Lung, lymphnode
KW2016016	Hawai'i	-	Brain, liver, adrenal, L lung, LN: L marginal
KW2016020	Hawai'i	-	Brain, liver, spleen, L lung, feces, LN: L marginal
KW2019026	Rota	L Lung^{+}	Skin, blubber, muscle, liver, kidney, R/L atria, R/L ventricle
KW2021003	Hawai'i	Liver^{+}	R/L lung, kidney, pancreas, muscle, heart, muscle

*Screening by Clifton et al. 2023

$^{+}$Suspect positive, sequencing pending

Table 6. Circovirus literature review to inform pathologic findings in cetaceans.

Title	Publication	Lit. Type	Pathogen/ Strain	Disease characteristics	Organs involved	Summary	Application	Reference
Role of Canine Circovirus in Dogs with Acute Hemorrhagic Diarrhea	Veterinary Record	Original Research	Canine circovirus	Severe hemorrhagic diarrhea, hypovolemia, tachycardia, stress leukogram with neutrophilic left shift	G.I. system - intestine	Case studies, respond to supportive treatment.	Multiple canine viruses, circovirus more commonly, co-infection with canine parvovirus	Anderson, A., Hartmann, K., Leutenegger, C. M., Proksch, A. L., Mueller, R. S., & Unterer, S. (2017). Role of Canine Circovirus in Dogs with Acute Hemorrhagic Diarrhea. *Veterinary Record*, *180*(22), 542–542. https://doi.org/10.1136/vr.103926
Ultrastructural Identification of Circovirus in the Liver of Saffron Finch (*Sicalis flaveola spp.*)	Int. Journal of Morphology	Original Research	Avian circovirus	Hepatobiliary congestion and necrosis	Liver	Wild caught finches in captivity, sudden death, necropsied, negative staining, and embedding of lung, liver, intestine. Circovirus spherical particles, non enveloped, <20nm	Electronmicroscopic identification of circovirus	Catroxo, M. H., Martins, F. A. M. C. R. P., Melo, A. N., Milanelo, L., Petrella, S., Fitorra, L., & Petri, S. B. S. (2011). Ultrastructural Identification of Circovirus in the Liver of Saffron Finch (*Sicalis flaveola spp.*). *International Journal of Morphology*, *29*, 537–542. https://doi.org/10.4067/S0717-95022011000200039
Investigation of Lethal Concurrent Outbreak of Chlamydiosis and Pigeon Circovirus in a Zoo	Animals	Original Research	Pigeon circovirus	Sudden death (58 birds)	Liver, Bursa of Fabricius, skin, kidney	Cytoplasmic organisms epithelia (Chlamydia). Botryoid cytoplasmic inclusions in histiocytes in B, fabricii	Histopathology of circovirus, inclusions in histocytes in lymphoid organs, co-infection	Chen, W.-T., Teng, C.-A., Shih, C.-H., Huang, W.-H., Jiang, Y.-F., Chang, H.-W., Jeng, C.-R., Lai, Y.-H., Guo, J.-C., Wang, P.-J., Cheng, C.-H., & Chang, Y.-C. (2021). Investigation of Lethal Concurrent Outbreak of Chlamydiosis and Pigeon Circovirus in a Zoo. *Animals*, *11*(6), 1654. https://doi.org/10.3390/ani11061654
Targeted Surveillance Detected Novel Beaked Whale Circovirus in Ten New Host Cetacean Species Across the Pacific Basin	Frontiers in Marine Science	Original Research	Cetacean Circovirus	Multiple, various, asymptomatic	Circovirus PCR: brain tissue was the most consistently positive tissue type (69%), followed by lymph tissue (67%) and lung tissue (64%).	Host species and tissue specificity for PCR screening and further analysis	Species and cases for further investigation, organ selection for further examination	Clifton, C. W., Silva-Krott, I., Marsik, M. G., & West, K. L. (2023). Targeted Surveillance Detected Novel Beaked Whale Circovirus in Ten New Host Cetacean Species Across the Pacific Basin. *Frontiers in Marine Science*, *9*. https://www.frontiersin.org/articles/10.3389/fmars.2022.945289

Retrospective Assessment of Porcine Circovirus 3 (PCV-3) in Formalin-Fixed, Paraffin-Embedded Tissues from Pigs Affected by Different Clinical-Pathological Conditions	Porcine Health Mgmt	Original Research	Porcine circovirus 3	Porcine dermatitis and nephropathy syndrome (PDNS), periweaning failure-to-thrive syndrome (PFTS), congenital tremors type AII, reproductive disorders with (peri)arteritis, myocarditis and encephalitis	CNS, kidney, heart, pooled tissues	Retrospective histopathological evaluation of tissues, ISH for Porcine circovirus 3 and qPCR.	ISH assay (RNAscope technology), inflammatory characteristics (myocarditis, (peri)arteritis kidney (30/35), spleen, liver (15/36)	Cobos, À., Sibila, M., Alomar, J., Pérez, M., Huerta, E., & Segalés, J. (2022). Retrospective Assessment of Porcine Circovirus 3 (PCV-3) in Formalin-Fixed, Paraffin-Embedded Tissues from Pigs Affected by Different Clinical-Pathological Conditions. *Porcine Health Management*, *8*(1), 51. https://doi.org/10.1186/s40813-022-00293-8
Mechanisms of Circovirus Immunosuppression and Pathogenesis with a Focus on Porcine Circovirus 2: A Review	Veterinary Quarterly	Review	Porcine circovirus 2	Immunosuppression: lymphopenia, lymphoid cell depletion, altered cytokine production (interferon, pro-inflammatory cytokines). Atrophy and necrosis of lymphoid organs	Immune system, lymphoid organs	Pathologic changes not always apparent, subclinical infection. Increased susceptibility to co-infections	Lymphoid organs testing for circovirus infection, and review of lymphoid organ histological characteristics, potential target for ISH.	Fehér, E., Jakab, F., & Bányai, K. (2023). Mechanisms of Circovirus Immunosuppression and Pathogenesis with a Focus on Porcine Circovirus 2: A Review. *Veterinary Quarterly*, *43*(1), 1–18. https://doi.org/10.1080/01652176.2023.2234430
Canine Circovirus: An Emerging or an Endemic Undiagnosed Enteritis Virus?	Frontiers in Veterinary Science	Review	Canine circovirus	Hemorrhagic gastroenteritis, granulomatous lymphadenitis with lymphocyte necrosis, vasculitis	Lymphoid system, gastrointestinal system, vascular system	Asymptomatic, but PCR positive (11%) -serum samples, fecal samples in dogs with diarrhea (28%)	Pathogenic and apathogenic. Detection in fecal samples in dogs with and without diarrhea. Co-infection with parvo virus	Gomez-Betancur, D., Vargas-Bermudez, D. S., Giraldo-Ramírez, S., Jaime, J., & Ruiz-Saenz, J. (2023). Canine Circovirus: An Emerging or an Endemic Undiagnosed Enteritis Virus? *Frontiers in Veterinary Science*, *10*. https://www.frontiersin.org/articles/10.3389/fvets.2023.1150636
A Review of DNA Viral Infections in Psittacine Birds	Journal of Veterinary Medical Science	Review	Avian circovirus	Feather dystrophy and loss, beak deformity. 60 species of birds. Peracute and chronic form	Epithelium (feather, beak), macrophages-lymphoid organs/Bursa of Fabricius	Description of avian circovirus, intracytoplasmic basophilic inclusions in macrophages, epithelial cells. Immunosuppression	Immunosuppression, basophilic inclusions in macrophages, co-infection	Katoh, H., Ogawa, H., Ohya, K., & Fukushi, H. (2010). A Review of DNA Viral Infections in Psittacine Birds. *Journal of Veterinary Medical Science*, *72*(9), 1099–1106. https://doi.org/10.1292/jvms.10-0022

Circovirus in Tissues of Dogs with Vasculitis and Hemorrhage	Emerging Infectious Diseases	Case study / natural disease	Canine circovirus	Hemorrhagic gastroenteritis, vasculitis, granulomatous lymphadenitis	Immune system, gastrointestinal system, vascular system	Pathological lesions, intralesional positive ISH (macrophages, monocytes) and TEM in lymph node and spleen, concurrent infections (enteric pathogens, babesia conradae, bocavirus)	Vasculitis, enteritis. ISH positive for Circovirus in macrophages/histiocytes of lymph nodes and spleen. Co-infections.	Li, L., McGraw, S., Zhu, K., Leutenegger, C. M., Marks, S. L., Kubiski, S., Gaffney, P., Dela Cruz Jr, F. N., Wang, C., Delwart, E., & Pesavento, P. A. (2013). Circovirus in Tissues of Dogs with Vasculitis and Hemorrhage. *Emerging Infectious Diseases*, *19*(4), 534–541. https://doi.org/10.3201/eid1904.121390
Concurrent Infections are Important for Expression of Porcine Circovirus Associated Disease	Virus Research	Original Research	Porcine circovirus 2	Porcine circovirus associated disease (PCVAD) - respiratory, reproducive, failure-to-thrive, enteric	Respiratory system, reproductive system, gastrointestinal system, enteric system, immune system - co-infections	Lymphoid tissues-virus in macrophages in depleted lymph follicles	Location of virus, co-infections, multi-organ disease (Pseudorabies-herpes virus, mycoplasma)	Opriessnig, T., & Halbur, P. G. (2012). Concurrent Infections are Important for Expression of Porcine Circovirus Associated Disease. *Virus Research*, *164*(1–2), 20–32. https://doi.org/10.1016/j.virusres.2011.09.014
Porcine Circovirus Type 2–Associated Disease: Update on Current Terminology, Clinical Manifestations, Pathogenesis, Diagnosis, and Intervention Strategies	Veterinary Diagnostic Invest.	Reviews paper	Porcine circovirus 2	Postweaning multisystemic wasting syndrome: lymphoid depletion, histiocytes, with PVC2 antigen or DNA by ISH.Subclinical or clinical.	Multisystemic	Lymphoid tissues-virus in macrophages in depleted lymph follicles. Immune modulation for progression to disease.	Location of virus, co-infections, multi-organ disease (PPRSV, mycoplasma, swine influenza, Herpesvirus - Pseuddorabis).	Opriessnig, T., Meng, X.-J., & Halbur, P. G. (2007). Porcine Circovirus Type 2–Associated Disease: Update on Current Terminology, Clinical Manifestations, Pathogenesis, Diagnosis, and Intervention Strategies. *Journal of Veterinary Diagnostic Investigation*, *19*(6), 591–615. https://doi.org/10.1177/104063870701900601
Novel Canine Circovirus Strains from Thailand: Evidence for Genetic Recombination	Scientific Reports	Case study	Canine circovirus	Respiratory disease	Lung, liver, tonsil, lymph node	PCR and sequencing, and ISH of tissues from dogs with respiratory symptoms. Histiocytes in lymph nodes strongly positive. Genomic recombination of circovirus strains. No other pathogens detected.	Respiratory disease	Piewbang, C., Jo, W. K., Puff, C., van der Vries, E., Kesdangsakonwut, S., Rungsipipat, A., Kruppa, J., Jung, K., Baumgärtner, W., Techangamsuwan, S., Ludlow, M., & Osterhaus, A. D. M. E. (2018). Novel Canine Circovirus Strains from Thailand: Evidence for Genetic Recombination. *Scientific Reports*, *8*(1), Article 1. https://doi.org/10.1038/s41598-018-25936-1

Ultrastructural Findings in Lymph Nodes from Pigs Suffering from Naturally Occurring Postweaning Multisystemic Wasting Syndrome	Veterinary Pathology	Original Research	Porcine circovirus 2	Postweaning multisystemic wasting syndrome	Lymph nodes	Enlarged histocytes in lymph nodes, mitochondrial proliferation, dilation of RER and Golgi. Instracytoplasmic inclusions. Some intranuclear inclusions. Lymph node depletion. "Viral factories" viral nucleoprotein arrays.	Histological and ultrastructural changes in lymph nodes and virus infected histiocytes.	Rodriguez-Cariñg, C., & SegalÉS, J. (2009). Ultrastructural Findings in Lymph Nodes from Pigs Suffering from Naturally Occurring Postweaning Multisystemic Wasting Syndrome. *Veterinary Pathology*, *46*(4), 729–735. https://doi.org/10.1354/vp.08-VP-0141-R-FL
Epidemiology and Transmission of Porcine Circovirus Type 2 (PCV2)	Virus Research	Review	Porcine circovirus 2	Postweaning multisystemic wasting syndrome, respiratory disease, dermatitis and nephropathy syndrome, enteritis, reproductive failure	Respiratory system, gastrointestinal system, reproductive system, skin, urinary system	Pathogenic strain of porcine circovirus, long term shedding of virus, direct transmission. Multiorgan disease.	Summary of disease syndromes caused by Porcine circovirus 2, targets organs to examine in cetacean circovirus infection. Consider fecal testing for circovirus.	Rose, N., Opriessnig, T., Grasland, B., & Jestin, A. (2012). Epidemiology and Transmission of Porcine Circovirus Type 2 (PCV2). *Virus Research*, *164*(1–2), 78–89. https://doi.org/10.1016/j.virusres.2011.12.002
Porcine Circovirus Type 2 (PCV2) Infections: Clinical Signs, Pathology and Laboratory Diagnosis	Virus Research	Review	Porcine circovirus 2	PCV2 subclinical infection, systemic disease, lung disease, enteric disease, reproductive disease, dermatitis and nephropathy syndrome. New PCV terminology to unify various disease pathology	Lymphoid lesisons, respiratory system, digestive syustem, lymphocute depletion and inflammation, reproductive system, heart, fetus, skin	Description of porcine circovirus 2, both clinical and subclinical. Proposal of unified terminology	Lymphoid lesions, lymphocyte depletion	Segalés, J. (2012). Porcine Circovirus Type 2 (PCV2) Infections: Clinical Signs, Pathology and Laboratory Diagnosis. *Virus Research*, *164*(1), 10–19. https://doi.org/10.1016/j.virusres.2011.10.007
Current Understanding of the Pathogenesis of Porcine Circovirus 3. Pathogens (Basel, Switzerland)	Pathogens	Review	Porcine circovirus 3	Inflammation and tissue injury, absence of virus in the lesions. Myocarditis, nephritis, vasculitis, dermatitis and nephropathy syndrome, respiratory disease, diarrhea, reproductive failure	Respiratory system, gastrointestinal system, reproductive system, skin, heart, kidneys, vascular system	Description of porcine circovirus 3's clinical and pathological characteristics, novel findings in field and experimental observations	Mechanism of pathogenicity: virus mediated or immune-response mediated. Porcine dermatitis and nephropathy syndrome possibly immune mediated.	Sirisereewan, C., Thanawongnuwech, R., & Kedkovid, R. (2022). Current Understanding of the Pathogenesis of Porcine Circovirus 3. *Pathogens (Basel, Switzerland)*, *11*(1), 64. https://doi.org/10.3390/pathogens11010064

Canine Circovirus 1 (CaCV-1) and Canine Parvovirus 2 (CPV-2): Recurrent Dual Infections in a Papillon Breeding Colony	Veterinary Pathology	Original Research	Canine circovirus 1 & 2	Sudden death and bloody diarrhea in 5 canines. Segmental crypt necrosis of small intestine and lymphoid follicle depletion in spleen and Peyer's patches.	Lymphoid system, gastrointestinal system, spleen	RT-PCR and immunohistochemistry detected disease in 3 puppies from breeding colony	Crypt necrosis, lymphoid tissue necrosis	Thaiwong, T., Wise, A. G., Maes, R. K., Mullaney, T., & Kiupel, M. (2016). Canine Circovirus 1 (CaCV-1) and Canine Parvovirus 2 (CPV-2): Recurrent Dual Infections in a Papillon Breeding Colony. *Veterinary Pathology*, *53*(6), 1204–1209. https://doi.org/10.1177/0300985816646430
Circoviruses: Immunosuppressive Threats to Avian Species: A Review	Avian Pathology	Review	Avian circovirus - Psittacine, pigeon circoviruses. Human circovirus TorqueTenoVirus-different	Beak and feather disease, Pigeon-illthrift, anorexia	Beak and feather disease - basophiolic cytoplasmic inclusions macrophages,	Ultrastructure, 15-25 nm; TorqueTenoVirus is larger up to 50nm, genome characteristics	Ultrastructure, genomic organization, viral inclusions in macrophages	Todd, D. (2000). Circoviruses: Immunosuppressive Threats to Avian Species: A Review. *Avian Pathology*, *29*(5), 373–394. https://doi.org/10.1080/030794500750047126
Canine Circoviral Hemorrhagic Enteritis in a Dog in Connecticut	Journal of Veterinary Diagnostic Invest.	Case study	Canine circovirus	5-month old, hemorrhagic enteritis, lymphoid necrosis, vasculitis	Multi-organ, vasculitis, lymphoid necrosis, enteritis (sm. Intestine).	Viral particles in endothelial cells and crypts, macrophages in lymph nodes	Virus characteristics and location in endothelial cells and crypts, macrophages	Van Kruiningen, H. J., Heishima, M., Kerr, K. M., Garmendia, A. E., Helal, Z., & Smyth, J. A. (2019). Canine Circoviral Hemorrhagic Enteritis in a Dog in Connecticut. *Journal of Veterinary Diagnostic Investigation*, *31*(5), 732–736. https://doi.org/10.1177/1040638719863102
Reservoirs of Porcine Circoviruses: A Mini Review	Frontiers in Veterinary Science	Review	Porcine circovirus 1, 2, 3	PCV1 apathogenic in pigs, PCV2 Postweaning multisystemic wasting syndrome, PCV3 dermatitis, nephropathy. PCV also interstitial pneumonia, reproductive failure	Multi-organ	Swine diseases. Other species: Enteritis in cattle, seroconversion in rodents, Canine diarrhea. Ticks , shellfish, human (low prevalence)	Widespread, sero conversion in many species. Varying pathogenicity	Zhai, S.-L., Lu, S.-S., Wei, W.-K., Lv, D.-H., Wen, X.-H., Zhai, Q., Chen, Q.-L., Sun, Y.-W., & Xi, Y. (2019). Reservoirs of Porcine Circoviruses: A Mini Review. *Frontiers in Veterinary Science*, *6*, 319. https://doi.org/10.3389/fvets.2019.00319

Table 7. Cetacean morbillivirus and herpesvirus literature review to inform pathologic findings associated with circovirus co-infections.

Title	Publication	Lit. Type	Pathogen/ Strain	Disease characteristics	Summary	Organs involved	Applications	Reference
Presence of herpesvirus in striped dolphins stranded during the cetacean morbillivirus epizootic along the Mediterranean Spanish coast in 2007	Archives of Virology	Original Research	Herpesvirus	Morbillivirus pneumonia, meningo-encephalitis	Mortality episode. 5/8 striped dophin Herpesvirus DNA demonstrated	CeMV characteristic lesions	Coinfection, CeMV mortality, no specific HV lesions.	Bellière, E. N., Esperón, F., Arbelo, M., Muñoz, M. J., Fernández, A., & Sánchez-Vizcaíno, J. M. (2010). Presence of herpesvirus in striped dolphins stranded during the cetacean morbillivirus epizootic along the Mediterranean Spanish coast in 2007. Archives of virology, 155(8), 1307–1311. https://doi.org/10.1007/s00705-010-0697-x
Emerging Viruses in Marine Mammals	CABI Reviews	Review	α-herpesvirus (multiple strains), y-herpesvirus (multiple strains)	Multiple organ necrosis, nephritis, dermatitis, encephalitis. Gamaherpesvirus-genital lesions	Review of emerging viral pathogens in marine mammals	Multiple organs	Co-infections, organ specific testing, strain specific testing	Bossart, G. D., & Duignan, P. J. (2019). Emerging Viruses in Marine Mammals. *CABI Reviews*, *2018*, 1–17. https://doi.org/10.1079/PAVSNNR201813052
Cetacean Morbillivirus: Current Knowledge and Future Directions	Viruses	Case Study	CeMV	bronchointerstitial pneumonia, encephalitis, syncytia, and lymphoid depletion	Review of stranding cases with morbillivirus confirmed	Multiple organs	Coinfection, mortality events	Bressem, M.-F. V., Duignan, P. J., Banyard, A., Barbieri, M., Colegrove, K. M., Guise, S. D., Guardo, G. D., Dobson, A., Domingo, M., Fauquier, D., Fernandez, A., Goldstein, T., Grenfell, B., Groch, K. R., Gulland, F., Jensen, B. A., Jepson, P. D., Hall, A., Kuiken, T., … Wellehan, J. F. (2014). Cetacean Morbillivirus: Current Knowledge and Future Directions. Viruses, 6(12), 5145. https://doi.org/10.3390/v6125145
Short-Finned Pilot Whale Strandings Associated with Pilot Whale Morbillivirus, Brazil	Emerging Infectious Diseases Journal	Case Study	Cetacean pilot whale morbillivirus, alpha herpesvirus (Stenella)	Poor body condition, meningomyelitis, bronchointerestitial pneumonia	3 life stranded animals, PWMV by PCR, 1 co-infection (lung)	Meninges, lung.	Morbillivirus in multiple tissues, alphaherpesvirus in lung	Costa-Silva, S., Sacristán, C., Soares, R. M., Carvalho, V. L., Castilho, P. V., Cremer, M. J., Ewbank, A. C., Duarte-Benvenuto, A., Faita, T., Navas-Suárez, P. E., Vieira, J. V., Pereira, L. G., Alves, C. F., Souza, G. C., Lemos, G. G., Silvestre-Perez, N., Catão-Dias, J. L., & Keid, L. B. (2023). Short-Finned Pilot Whale Strandings Associated with Pilot Whale Morbillivirus, Brazil. *Emerging Infectious Diseases Journal - CDC*, *29*(1). https://doi.org/10.3201/eid2901.221549
Cetacean Host-Pathogen Interaction(s): Critical Knowledge Gaps	Frontiers in Immunology	Review	CeMV, *Herpesvirus, Brucella ceti, Toxoplasma gondii*	Multiple organ disease, immunosuppression, CeMV neuropathy in striped dolphin	Cell receptor for Morbillivirus: lymphotropic Signaling Lymphocyte Activation Molecule" (SLAM/CD150) , neurotropic	Multiple organs	CeMV localization in lymphoid tissue, models of human disease, zoonoses	Di Guardo, G., Centelleghe, C., & Mazzariol, S. (2018). Cetacean Host-Pathogen Interaction(s): Critical Knowledge Gaps. *Frontiers in Immunology*, *9*. https://www.frontiersin.org/articles/10.3389/fimmu.2018.02815

					receptor unknown. Effects of toxins. Zoonotic concern			
Cetacean Morbillivirus-Associated Pathology: Knowns and Unknowns	Frontiers in Microbiology	Review	CeMV	Lung, brain lymphoid tissue	Classic disease - lung, brain, lymphoid tissue; vertical transmission; brain-only infection, increased susceptibility during pregnancy immunodepress.	Multiple organs, lymphoid depletion	Nuclear and cytoplasmic inclusions, IHC in lymph, lung, brain. Susceptibility if immunosuppressed.	Di Guardo, G., & Mazzariol, S. (2016). Cetacean Morbillivirus-Associated Pathology: Knowns and Unknowns. *Frontiers in Microbiology*, *7*. https://www.frontiersin.org/articles/10.3389/fmicb.2016.00112
Comparative Immunopathology of Cetacean morbillivirus Infection in Free-Ranging Dolphins from Western Mediterranean, Northeast-Central, and Southwestern Atlantic	Frontiers in Immunology	Original Research	CeMV	Meningoencephalitis with CD3+ (T cells), and CD20+ and PAX5+ (B cells), lymphoid tissues with lymphoid depletion (apoptosis). IFNy in brain. Caspase 3 in lung and brain.	Immunohistochemical (IHC) analyses targeted molecules of immunologic interest: caspase 3, CD3, CD20, CD57, CD68, FoxP3, MHCII, Iba1, IFNγ, IgG, IL4, IL10, lysozyme, TGFβ, and PAX5 in tissues.	CNS, lymphoid organs. Lymphoid hyperplasia in chronically infected animals.	Virus specific changes in lymphoid tissues and CNS demonstrated by IHC targeting specific lymphoid cells. Interplay with viral and host immune factors.	Díaz-Delgado, J., Groch, K. R., Ressio, R., Riskallah, I. P. J., Sierra, E., Sacchini, S., Quesada-Canales, Ó., Arbelo, M., Fernández, A., Santos-Neto, E., Ikeda, J., Carvalho, R. R. de, Azevedo, A. de F., Lailson-Brito, J., Flach, L., Kanamura, C. T., Fernandes, N. C. C. A., Cogliati, B., Centelleghe, C., … Catão-Dias, J. L. (2019). Comparative Immunopathology of Cetacean morbillivirus Infection in Free-Ranging Dolphins From Western Mediterranean, Northeast-Central, and Southwestern Atlantic. *Frontiers in Immunology*, *10*. https://www.frontiersin.org/articles/10.3389/fimmu.2019.00485
Comparative Histopathologic and Viral Immunohistochemical Studies on CeMV Infection among Western Mediterranean, Northeast-Central, and Southwestern Atlantic Cetaceans	PLOS ONE	Original Research	CeMV	Neurotropism in striped dolphin and bottlenose dolphin, lung lesion in Guiana dolphins. Lymphoid depletion	Histopathology of affected tissue, immunolabelling with morbillivirus antigen (IHC)	CNS, lymphoid organs, opportunist infections-endoparasitism, bacterial, fungal, viral	Neuroanatomical location of lesions and virus detection, lymphoid depletion (starry sky), syncytia; broncho interstitial pneumonia	Díaz-Delgado, J., Groch, K. R., Sierra, E., Sacchini, S., Zucca, D., Quesada-Canales, Ó., Arbelo, M., Fernández, A., Santos, E., Ikeda, J., Carvalho, R., Azevedo, A. F., Lailson-Brito, J., Flach, L., Ressio, R., Kanamura, C. T., Sansone, M., Favero, C., Porter, B. F., … Catão-Dias, J. L. (2019). Comparative Histopathologic and Viral Immunohistochemical Studies on CeMV Infection among Western Mediterranean, Northeast-Central, and Southwestern Atlantic Cetaceans. *PLOS ONE*, *14*(3), e0213363. https://doi.org/10.1371/journal.pone.0213363

Molecular identification of a novel gamma herpesvirus in the endangered Hawaiian monk seal (*Monachus schauinslandi*)	Marine Mammal Science	Case Study	y-Herpesvirus	Disease not reported.	Serology - positive to herpesvirus; nasal swab for viral PCR - multiple samples from 122 free and captive, apparently healthy monk seals. 20% positive samples.	Blood, nasal membrane	First detection of y - herpesvirus in Hawai'ian monk seal. Virus not related to other phocine herpesviruses.	Goldstein T, Gulland FMD, Braun RC, Antonelis GA, Kashinsky L, Rowles TK, Mazet JAK, Dalton LM, Aldridge BM, Stott JL: Molecular identification of a novel gammaherpesvirus in the endangered Hawaiian monk seal (Monachus schauinslandi). Mar Mamm Sci. 2006, 22: 465-471. 10.1111/j.1748-7692.2006.00025.x.
The Pathology of Cetacean Morbillivirus Infection and Comorbidities in Guiana Dolphins During an Unusual Mortality Event (Brazil, 2017-2018)	Veterinary Pathology	Original Research	CeMV	Pulmonary edema, ascites, icterus, hepatic lipidosis, multicentric lymph adenomegaly	Gross and histopathology described. Widespread antigen in multiple organs including salivary gland.	Bronchinterstitial pneumonia, lymphoid depletion with lymphocytes carrying virus. Opportunist infections: Toxoplasma, mycosis, brucellosis	Pathological characteristics and location of viral antigen. Coinfections.	Groch, K. R., Díaz-Delgado, J., Santos-Neto, E. B., Ikeda, J. M. P., Carvalho, R. R., Oliveira, R. B., Guari, E. B., Flach, L., Sierra, E., Godinho, A. I., Fernández, A., Keid, L. B., Soares, R. M., Kanamura, C. T., Favero, C., Ferreira-Machado, E., Sacristán, C., Porter, B. F., Bisi, T. L., … Catão-Dias, J. L. (2020). The Pathology of Cetacean Morbillivirus Infection and Comorbidities in Guiana Dolphins During an Unusual Mortality Event (Brazil, 2017-2018). *Veterinary Pathology*, *57*(6), 845–857. https://doi.org/10.1177/0300985820954550
Coinfection of Porcine Circovirus 2 and Pseudorabies Virus Enhances Immunosuppression and Inflammation through NF-κB, JAK/STAT, MAPK, and NLRP3 Pathways	International Journal of Molecular Sciences	Original Research, in-vitro	PCV2 and pseudorabies virus	Porcine kidney cells	Cell culture co-infected with PCV2 and Pseudorabies virus, inflammatory and immune pathways evaluated.	Coinfections modulated IFN-JAK/STAT, downregulated immune pathways more than single infection. Inflammatory responses are mixed.	Cellular inflammatory and immune response to coinfection by PCV2 and Herpes virus	Li, X., Chen, S., Zhang, L., Niu, G., Zhang, X., Yang, L., Ji, W., & Ren, L. (2022). Coinfection of Porcine Circovirus 2 and Pseudorabies Virus Enhances Immunosuppression and Inflammation through NF-κB, JAK/STAT, MAPK, and NLRP3 Pathways. *International Journal of Molecular Sciences*, *23*(8), 4469. https://doi.org/10.3390/ijms23084469
Marine Morbilliviruses: Diversity and Interaction with Signaling Lymphocyte Activation Molecules	Viruses	Review	CeMV, phocine distemper virus	Cetacean species infection history, Mouse model for immune receptor (SLAM)	History of infections of cetaceans, transmission by close contact, initial replication in macrophages/dendritic cells, vertical transmission	SLAM - Lymphocyte activation molecule, transmembrane protein and receptor of CeMV hemagglutinin	Pathogenesis of Morbillivirus, cell receptor, direct transmission route	Ohishi, K., Maruyama, T., Seki, F., & Takeda, M. (2019). Marine Morbilliviruses: Diversity and Interaction with Signaling Lymphocyte Activation Molecules. *Viruses*, *11*(7), Article 7. https://doi.org/10.3390/v11070606

Viral Skin Diseases in Odontocete Cetaceans: Gross, Histopathological, and Molecular Characterization of Selected Pathogens	Frontiers in Veterinary Science	Original Research	Cetacean poxvirus, herpesvirus	Skin disease	Gross and histopathology described. Pox virus in 54.54%, Herpes virus in 43.63% of lesions, CeMV in 1.82%	Skin: Poxvirus-hyperpigmentation tattoo lesion, gamma herpesvirus - flat, raised genital lesions.	Description of skin lesions and etiology (viral pathogens)	Segura-Göthlin, S., Fernández, A., Arbelo, M., Andrada Borzollino, M. A., Felipe-Jiménez, I., Colom-Rivero, A., Fiorito, C., & Sierra, E. (2023). Viral Skin Diseases in Odontocete Cetaceans: Gross, Histopathological, and Molecular Characterization of Selected Pathogens. *Frontiers in Veterinary Science*, *10*, 1188105. https://doi.org/10.3389/fvets.2023.1188105
Herpesviruses: Harmonious Pathogens but Relevant Cofactors in Other Diseases?	Frontiers in Cellular and Infection Microbiology	Review	α, β, y - *Herpesviruses*	Human disease, lifelong infection, severe disease (α and y herpesvirus) in immunodeficient patients	alpha (α), beta (β), and gamma (γ) herpesviruses. Latent and lytic cycles. Pathogen and immune mediated disease.	Coinfections, multiple organs. α in neuronal cells, β and y in macrophages'. B-cells	Persistent infection, severe disease if immunosuppression occurs, co-infections, viral-immune cell interactions	Sehrawat, S., Kumar, D., & Rouse, B. T. (2018). Herpesviruses: Harmonious Pathogens but Relevant Cofactors in Other Diseases? *Frontiers in Cellular and Infection Microbiology*, *8*, 177. https://doi.org/10.3389/fcimb.2018.00177
Histopathological Differential Diagnosis of Meningoencephalitis in Cetaceans: Morbillivirus, Herpesvirus, *Toxoplasma gondii, Brucella sp*., and *Nasitrema sp.*	Frontiers in Veterinary Science	Original Research	Morbillivirus, α - Herpesvirus, *Toxoplasma gondii*, *Brucella sp*., and *Nasitrema sp*.	Classical CNS virus-associated lesions consist of meningeal mononuclear cell infiltrates, lymphoplasmacytic perivascular cuffs, microgliosis, intracytoplasmic and/or nuclear inclusion bodies (INCIBs), and neuronal necrosis and/or associated focal neuronophagia	Detailed description of histopathological lesions in cetaceans with meningo-encpehlitis.	Meninges, brain	Characteristic lesions for each pathogen and pathogen detection. Coinfections common, HV+Brucella, HV+CeMV. CeMV + S. aureus. CeMV-syncytia, intranuclear and intracytoplasmic inclusions. HV- large intranuclear inclusions.	Sierra, E., Fernández, A., Felipe-Jiménez, I., Zucca, D., Díaz-Delgado, J., Puig-Lozano, R., Câmara, N., Consoli, F., Díaz-Santana, P., Suárez-Santana, C., & Arbelo, M. (2020). Histopathological Differential Diagnosis of Meningoencephalitis in Cetaceans: Morbillivirus, Herpesvirus, *Toxoplasma gondii*, *Brucella sp.*, and *Nasitrema sp. Frontiers in Veterinary Science*, *7*, 650. https://doi.org/10.3389/fvets.2020.00650

Molecular Characterization of Herpes viral Encephalitis in Cetaceans: Correlation with Histopathological and Immunohistochemical Findings.	Animals: an Open Access Journal from MDPI	Original Research	α-*Herpesvirus*	Meningoencephalitis	12 animals with HV in brain, 28 animals HIV elsewhere. Multiple species (Atlantic dolphins, beaked whale). Histopathology, IHC.	Meninges, brain, choroid plexus (choroiditis)	Perivascular cuffing, intranuclear inclusions, brain - α-herpesvirus by PCR. Genital lesion - y-herpesvirus by PCR. Primers listed.	Sierra, E., Fernández, A., Fernández-Maldonado, C., Sacchini, S., Felipe-Jiménez, I., Segura-Göthlin, S., Colom-Rivero, A., Câmara, N., Puig-Lozano, R., Rambaldi, A. M., Suárez-Santana, C., & Arbelo, M. (2022). Molecular Characterization of Herpesviral Encephalitis in Cetaceans: Correlation with Histopathological and Immunohistochemical Findings. *Animals : An Open Access Journal from MDPI*, *12*(9), 1149. https://doi.org/10.3390/ani12091149
Systemic Herpesvirus and Morbillivirus Co-infection in a Striped Dolphin (Stenella coeruleoalba)	Journal of Comparative Pathology	Case Study	CeMV	Non suppurative encephalitis and system lymphoid necrosis and depletion (spleen, lymph nodes).	Pathology of systemic infection of striped dolphin by HV and CeMV. HV inclusions in lymph node and spleen. PCR pos for pan herpesvirus. Syncytia and brain positive for CeMB.	Brain, lymph node, spleen	Coinfection. CeMV in brain, and lung (IHC). HV in lymph node.	Soto, S., González, B., Willoughby, K., Maley, M., Olvera, A., Kennedy, S., Marco, A., & Domingo, M. (2012). Systemic Herpesvirus and Morbillivirus Co-infection in a Striped Dolphin (*Stenella coeruleoalba)*. *Journal of Comparative Pathology*, *146*(2–3), 269–273. https://doi.org/10.1016/j.jcpa.2011.04.002
Systematic Determination of Herpesvirus in Free-Ranging Cetaceans Stranded in the Western Mediterranean: Tissue Tropism and Associated Lesions	Viruses	Original Research	*Herpesvirus*	Multiple organ inflammatory disease.	Examination of stranded cetaceans. Morbillivirus co-infection 20%. More herpesvirus in neonate, juvenile and fetal. Systemic lesions with α and y-herpesvirus.	Reproductive system, integument, nervous system, kidney, kung, adrenal, lymphoid organs	Viral DNA and RNA detection in multiple organs.	Vargas-Castro, I., Melero, M., Crespo-Picazo, J. L., Jiménez, M. de los Á., Sierra, E., Rubio-Guerri, C., Arbelo, M., Fernández, A., García-Párraga, D., & Sánchez-Vizcaíno, J. M. (2021). Systematic Determination of Herpesvirus in Free-Ranging Cetaceans Stranded in the Western Mediterranean: Tissue Tropism and Associated Lesions. *Viruses*, *13*(11), 2180. https://doi.org/10.3390/v13112180
A Longman's Beaked Whale (*Indopacetus pacificus*) Strands in Maui, Hawai'i, with First Case of Morbillivirus in the Central Pacific	Marine Mammal Science	Original Research	CeMV	Cerebral encephalitis	Examination of Longman's Beaked Whale after stranding on Maui.	Lymph nodes, circulatory system	Novel morbillivirus	West, K. L., Sanchez, S., Rotstein, D., Robertson, K., Dennison, S., Levine, G., Davis, N., Schofield, D., Potter, C., & Jensen, B. (2013). A Longman's Beaked Whale (Indopacetus pacificus) Strands in Maui, Hawai'i, with First Case of Morbillivirus in the Central Pacific. Marine Mammal Science, 29. https://doi.org/10.1111/j.1748-7692.2012.00616.x

Coinfection and Vertical Transmission of Brucella and Morbillivirus in a Neonatal Sperm Whale (*Physeter macrocephalus*) in Hawaii, USA	Journal of Wildlife Diseases	Case Study	*Brucella ceti*, CeMV	Systemic disease	Pneumonia, meningitis, lymphoid depletion	Multiple tissue positive for CeMV, Brucella	Coinfection, vertical - Brucella, CeMV.	West, K. L., Levine, G., Jacob, J., Jensen, B., Sanchez, S., Colegrove, K., & Rotstein, D. (2015). Coinfection and Vertical Transmission of Brucella and Morbillivirus in a Neonatal Sperm Whale (*Physeter macrocephalus*) in Hawaii, USA. *Journal of Wildlife Diseases*, *51*(1), 227–232. https://doi.org/10.7589/2014-04-092
Novel Cetacean Morbillivirus in a Rare Fraser's Dolphin (*Lagenodelphis hosei*) Stranding from Maui, Hawai'i	Scientific Reports	Case Study	CeMV	Systemic disease, non-suppurative meningoencephalitis with syncytia.	Meningitis, pneumonia, lymphoid depletion, portal hepatitis with inclusions in bile duct epithelia	Multiple tissue positive for CeMV by PCR, IHC.	Novel morbillivirus, no co-infections identified.	West, K. L., Silva-Krott, I., Landrau-Giovannetti, N., Rotstein, D., Saliki, J., Raverty, S., Nielsen, O., Popov, V. L., Davis, N., Walker, W. A., Subramaniam, K., & Waltzek, T. B. (2021). Novel Cetacean Morbillivirus in a Rare Fraser's Dolphin (*Lagenodelphis hosei*) Stranding from Maui, Hawai'i. *Scientific Reports*, *11*(1), 15986. https://doi.org/10.1038/s41598-021-94460-6

Table 8. *Toxoplasma gondii* serology screening results.

Species	Common Name	Stranding Date	Positive tissues	Negative Tissues
Pseudorca crassidens	False killer whale	3/11/2021	-	Kidney, liver, lung, muscle, kidney
Stenella coeruleoalba	Striped dolphin	1/2/1997	-	Kidney, liver, lung
Stenella coeruleoalba	Striped dolphin	1/15/2008	-	Muscle
Stenella coeruleoalba	Striped dolphin	6/21/2008	-	Liver, muscle
Stenella coeruleoalba	Striped dolphin	7/6/2012	-	Anal LN, hilar LN, liver, lung (R), mesenteric LN, prescapular LN, muscle
Stenella coeruleoalba	Striped dolphin	3/2/2013	-	Adrenal (L), kidney (L), liver, lung (R), muscle
Stenella coeruleoalba	Striped dolphin	5/4/2016	-	Kidney (L), liver, lung (R), muscle
Stenella longirostris	Spinner dolphin	8/27/1997	-	Lung
Stenella longirostris	Spinner dolphin	9/30/2007	Kidney, lung	Brain, muscle
Stenella longirostris	Spinner dolphin	3/15/2008	Kidney (R), liver	Brain
Stenella longirostris	Spinner dolphin	11/29/2008	-	Liver, lung (R), muscle
Stenella longirostris	Spinner dolphin	12/18/2008	-	Kidney, liver, lung (R)
Stenella longirostris	Spinner dolphin	4/15/2010	-	Liver
Stenella longirostris	Spinner dolphin	8/12/2011	-	Lung (L)
Stenella longirostris	Spinner dolphin	8/30/2011	-	Liver, lung (R)
Stenella longirostris	Spinner dolphin	7/23/2012	-	Kidney (L), lung (R)
Stenella longirostris	Spinner dolphin	8/3/2013	-	Kidney (R), liver
Stenella longirostris	Spinner dolphin	3/10/2014	Kidney (L), mediastinal LN	Aqueous humor, liver, lung (R)
Stenella longirostris	Spinner dolphin	3/10/2014	-	Kidney (R), liver
Stenella longirostris	Spinner dolphin	8/22/2015	Kidney (R), liver, lung	-
Stenella longirostris	Spinner dolphin	5/29/2016	Kidney (R), liver	-
Stenella longirostris	Spinner dolphin	6/4/2016	-	Kidney (R), liver
Stenella longirostris	Spinner dolphin	12/5/2017	-	Kidney (L), liver
Stenella longirostris	Spinner dolphin	12/26/2017	-	Kidney (L), liver
Stenella longirostris	Spinner dolphin	2/26/2018	-	Kidney (R), liver
Stenella longirostris	Spinner dolphin	3/26/2018	-	Kidney (R), liver

Stenella longirostris	Spinner dolphin	8/11/2019	-	Kidney (L), liver
Stenella longirostris	Spinner dolphin	8/16/2019	-	Kidney (R), liver
Stenella longirostris	Spinner dolphin	10/20/2021	-	Kidney (R), liver
Stenella longirostris	Spinner dolphin	7/8/2023	-	Cerebrum, liver, lung (L)
Stenella longirostris	Spinner dolphin	8/4/2023	-	Aqueous humor, heart, kidney (R), liver, lung (R), serum
Tursiops truncatus	Bottlenose dolphin	2/13/2011	-	Aqueous humor, cerebrum, kidney (R), liver, lung (R), mediastinal LN, mesenteric LN, serum,
Tursiops truncatus	Bottlenose dolphin	5/21/2011	-	Aqueous humor, brain, kidney, liver, lung, mesenteric LN, serum
Tursiops truncatus	Bottlenose dolphin	5/9/2020	-	Cerebrum, kidney (L), kidney (R), liver, lung (R), lung (L), mesenteric LN, prescapular LN (L)
Tursiops truncatus	Bottlenose dolphin	5/27/2023	-	Aqueous humor, cerebrum, heart, kidney (L), liver, marginal LN (R), marginal LN (L), mediastinal LN (L), mesenteric LN, lung (R), prescapular LN (L), serum

LN = lymph node

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