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Case Report

Cetacean Morbillivirus in a Risso's Dolphin (*Grampus griseus*) that Stranded in the West Coast of Luzon Island, Philippines

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Abstract

Background: Cetacean morbillivirus (CeMV) is a highly infectious virus of whales, dolphins, and porpoises and is one of the major viral pathogens of cetaceans. It can infect a wide range of cetacean species and has caused mass mortalities in several parts of the world in the past 30 years. The virus causes immunosuppression that often results in secondary viral, bacterial, and parasitic infections (including *Toxoplasma gondii*) in an infected animal. On 10 January 2019 a Risso's dolphin stranded in Morong, Bataan, Philippines with recurring diarrhea. The dolphin succumbed three months later while in rehabilitation. **Methodology**: Necropsy, histopathology, serology, and PCR were carried out to determine the cause of death and other clinical signs. **Results**: Gross necropsy showed that the animal died due to intestinal volvulus. Serology revealed CeMV antibody titers at ≥1:512 via CeMV virus neutralization, as well as IgG antibody against *T. gondii*. No CeMV RNA was detected. **Conclusion**: To the best knowledge of the authors, this is the first documented case of CeMV infection in a cetacean from the Philippines. The absence of reports of mass mortalities that can be attributed to CeMV in the Philippines suggests that the virus is likely endemic in the region.

Keywords

cetacean morbillivirus (CeMV), gastrointestinal toxoplasmosis, *Toxoplasma gondii*, Risso's dolphin, entanglement

1. Introduction

Cetacean morbillivirus (CeMV) is a highly infectious viral disease [1 – 2] belonging to the genus Morbillivirus and Family Paramyxoviridae [3]. It is one of the major viral pathogens of cetaceans [4] because it infects a wide range of species [5] and has caused several mass mortalities in several parts of the world in the past 30 years [1][6 - 7].

CeMV was first documented during an outbreak in the Spanish coast of the Mediterranean Sea that killed thousands of striped dolphins *Stenella coeruleoalba* from 1990 to 1992 [8]. It was later determined that the first evidence of CeMV activity was in the

Indian River Lagoon, USA in 1982 [9] and the first massive CeMV epizootic occurred on the east coast of the USA between 1987-1988. The event killed over 50% of the in-shore Atlantic bottlenose dolphin population [10-11]. There are currently five described strains of CeMV [5]. The first three recognized strains - dolphin morbillivirus (DMV) [8], porpoise morbillivirus (PMV) [3], and pilot whale morbillivirus (PWMV) [12-13]; and the novel beaked whale morbillivirus (BWMV) [12], and Guiana dolphin morbillivirus (GDMV) [2][14].

CeMV is believed to be transmitted through inhalation of aerosol droplets as well as direct and indirect contact [2][5]. The virus causes immunosuppression among infected cetaceans and causes respiratory and nervous system problems [1][15-16]. Further, the infection often leads to secondary viral, bacterial, fungal, and parasitic infection [1] [17]. Classic pathological lesions of the infection include bronchointerstitial pneumonia, meningoencephalitis, and lymphoid depletion [1][5][7][18]. However, these are often not present in CeMV cases, or are masked by lesions caused by opportunistic pathogens [19].

Virus isolation is the gold standard for a definitive diagnosis for CeMV. However, histology, immunohistochemistry, and serology have been used to screen and confirm the disease [1-2]. Recent serological and epidemiological investigations, including stranding reports, revealed that the virus is widespread globally and have been detected in the Mediterranean [20-21], Atlantic coast of the US and Gulf of Mexico [22], North Atlantic [7], South Atlantic [23], Southeastern Pacific [24], Southwestern Pacific [25], Central Pacific [26], Western Australia [7], Indian Ocean, and West Pacific [23].

Toxoplasma gondii is an intracellular coccidian parasite of mammals with wild and domestic cats as its only known definitive hosts [27-30]. It is transmitted by ingestion of oocysts in food or water, or by transplacental infection with tachyzoites [31]. Infection with this parasite is prevalent worldwide and has been reported in many species of mammals, including marine mammals [32-34]. Serologic surveys in the coasts of the United States and western Mediterranean show that infection with *T. gondii* among cetaceans is common [33][35-36]. Clinical Toxoplasmosis has already been documented in several cetacean species – Guiana dolphin (*Sotalia guianensis*) [37], spinner dolphin (*Stenella longirostris*) [38], Atlantic bottlenose dolphin (*Tursiops truncatus*) [39], striped dolphin (*Stenella coeruleoalba*) [15]; beluga whales (*Delphinapterus leucas*) [40], and Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) [41], including in a Risso's dolphin (*Grampus griseus*) [31]. This paper reports a case of CeMV infection that is seropositive for *T. gondii* in a Risso's dolphin that stranded in the west coast of Luzon, Philippines.

2. Case Presentation

2.1. History

An adult male Risso's dolphin with a nylon fishing line entangled around its peduncle stranded on a beach in Morong, Bataan on 10 January 2019. It was negatively buoyant and had a body condition score of 2 following Joblon et al. (2014) [42]. The nylon fishing line, which penetrated through the skin and blubber was removed, revealing deep wounds on the peduncle and leading edge of the tail flukes. This likely affected the swimming and hunting of the dolphin as the condition worsened over time. A large, oval, deep fresh wound, likely sustained from a cookie-cutter shark bite was also noted on its right abdomen. This suggests that the animal may have become a vulnerable target.

The dolphin was immediately transported to Ocean Adventure, a marine mammal park near the stranding site for rehabilitation. Diarrhea was observed but direct fecal smears were negative for helminth ova and protozoa. Co-amoxiclav (10 mg/kg PO BID), Ciprofloxacin (15mg/kg PO BID), silymarin (600 mg PO BID), vitamins, and electrolytes were administered with water by gastric intubation. The wounds on the peduncle, tail flukes, and abdomen were treated topically with antibiotic ointment (Polymyxin B sulfate + Bacitracin zinc + Neomycin sulfate). The diarrhea initially resolved after two days and the dolphin's condition improved significantly within a week. The dolphin occasionally displayed aerial behaviors such as breaching and bowing a few weeks later. There were no behavioral indications of neurological and respiratory

problems. However, the diarrhea became recurrent for the remainder of its rehabilitation. The animal ceased to feed on 11 April 2019 and succumbed the following day.

2.2. Necropsy

Routine necropsy revealed intestinal volvulus as the immediate cause of its death. Tissue samples were collected and stored in 10% buffered formalin for histopathologic processing and examination. Unfortunately, the brain was not collected due to logistical difficulties. The tissues were stained using the standard hematoxylin-eosin staining technique. Villous fusion and atrophy were noted in the intestinal epithelium (A and B). Pulmonary congestion, edema, and consolidation (C), and splenic congestion (D) were also observed.

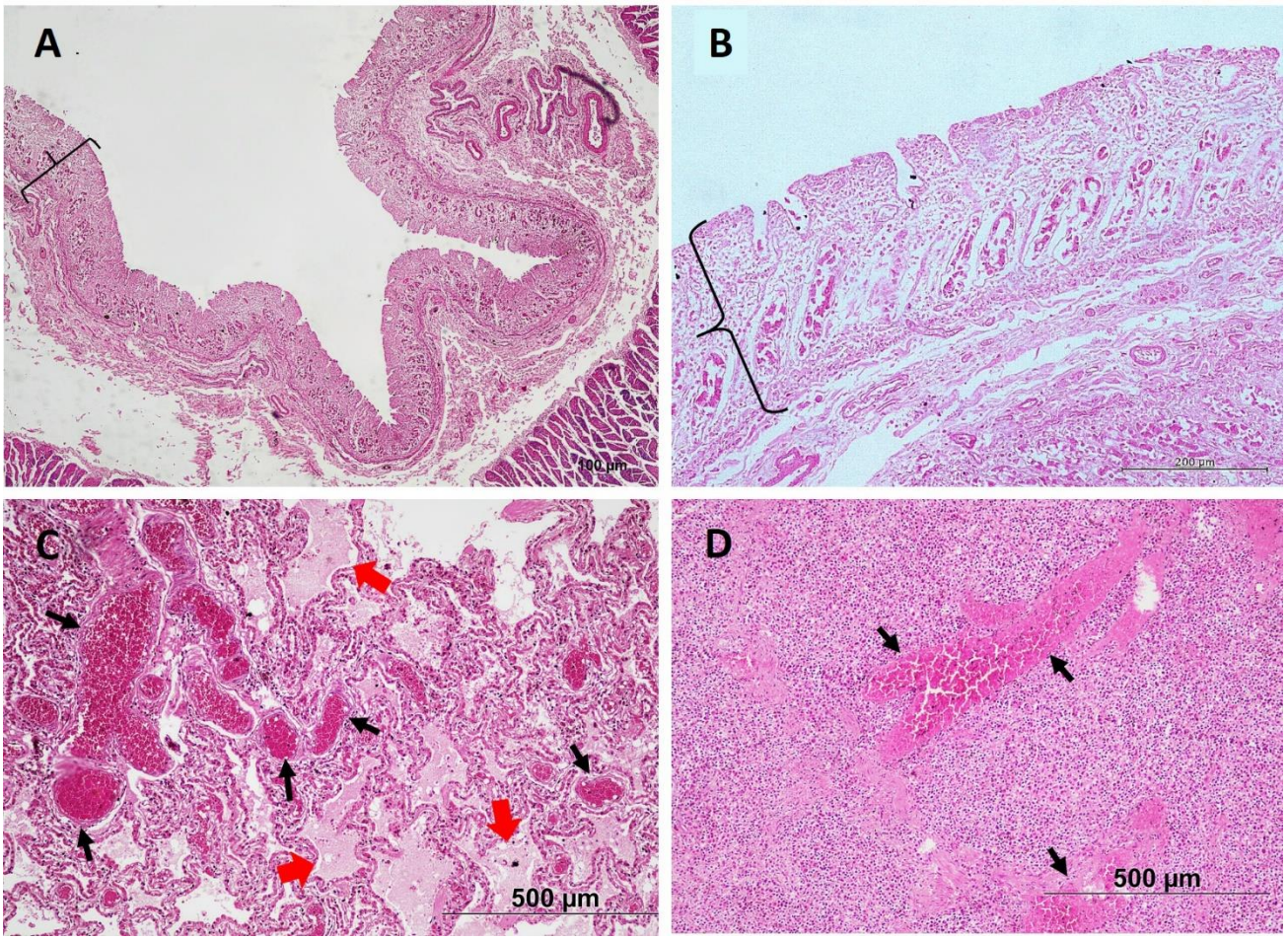

Fig. 1. Histopathological findings of the visceral organs show lesions in the intestine, lungs, and spleen. (A) and (B) Entire segment of intestinal epithelium showing villous fusion and atrophy (bracket). (C) Consolidated section of the lung with congestion (black arrows) and edema (red arrows). (D) Section of the spleen with congestion (black arrows).

2.3. Serology

Serum samples were submitted to the Athens Veterinary Diagnostic Laboratory of the College of Veterinary Medicine, University of Georgia, USA and to the Microbial Ecology for Terrestrial and Aquatic Systems Laboratory of the Institute of Biology, College of Science, University of the Philippines Diliman to screen for CeMV and *T. gondii* respectively. The serology revealed a CeMV antibody titer of ≥1:512 via CeMV virus neutralization (VN), as well as IgG antibodies against *T. gondii* using Toxocell Latex Agglutination Test (LAT: BIOKIT Manufacturing Company, Barcelona, Spain).

2.3. Polymerase Chain Reaction (PCR)

Whole blood and liver samples were also screened for CeMV via PCR at Athens Veterinary Laboratory. No CeMV RNA was detected in both samples.

3. Discussion

This is the first report of CeMV detection in the Philippines. In the West Pacific, CeMV was detected in a Pacific striped dolphin in Japan [43] and a pygmy sperm whale in Taiwan [44]. It has been hypothesized that CeMV has circulated for decades among cetaceans inhabiting the waters of Taiwan and neighboring areas. The Philippines and Taiwan are only separated by the narrow Luzon Strait resulting in shared species (and likely overlapping populations) of marine mammals between the two countries.

Risso's dolphins are known to be susceptible to CeMV infection [2]. In a study conducted by Sierra *et al.* in 2017, PCR results revealed a 16.6% prevalence of the virus among stranded Risso's dolphins in the Canary Islands between 1994 to 2015. Similarly, three Risso's dolphins had high positive serological titers for CeMV in a retrospective study conducted among stranded dolphins along the California coast from 2000 to 2015 [45]. In the case presented herein, the seropositivity to CeMV of Risso's dolphin in this case was confirmed through the VN test, considered as the most reliable assay for the detection of CeMV antibodies due to its high sensitivity and specificity [2].

Although the existence and nature of subclinical infection was deemed to be speculative by Van Bressem *et al.* in 2014., the many reports of CeMV positive cases without the classical pathological findings do indicate that subclinical CeMV infection is probable [1]. In the study conducted in the Canary Islands, CeMV was detected in the mesenteric lymph node and brain of a Risso's dolphin but no typical lesions were observed [1]. Four sperm whales (*Physeter macrocephalus*) that mass-stranded in Italy were positive for DMV via immunohistochemistry and RT-PCR, but did not exhibit pathognomonic lesions [46]. Three seropositive common dolphins that stranded along the coast of southern California also did not have the characteristic lesions of the disease [4][47]. Similarly, a striped dolphin from the central coast of California that tested positive in RT-PCR did not have CeMV lesions [45]. In this particular study, it was reported that there could actually be more RT-PCR positive animals that were missed since only those with lesions were normally screened for viral detection. Perhaps the most substantial evidence for subclinical infection is that of the study conducted by Bossart *et al.* in 2010. They found evidence of DMV infection among the Atlantic bottlenose dolphins in the Indian River Lagoon after the 1987 to 1988 DMV epizootic, with 10.1% of the population born after that period seropositive for the virus. This indicates that viral transmission and subclinical infections are occurring even without mass mortalities happening. Moreover, cetaceans that have been endemically infected with CeMV are potential reservoirs and vectors. Transmission likely occurs through the inhalation of aerosolized virus but vertical transmission was also reported. Vertical transmission takes advantage of the reduction in immune response efficiency occurring during the pregnancy of a cetacean [48].

Opportunistic infections of *T. gondii* are known to occur in CeMV-infected animals [7][45][47-48]. Among previous reports, one general observation in CeMV-infected cetaceans is the presence of widespread lymphoid cell depletion, which leads to severe immunodeficiency and (consequently) secondary microbial infections. Examples of these infections include those that are caused by Herpesvirus and *T. gondii*, neurotropic pathogens associated with cerebral lesions similar to those observed in CeMV-affected

cetaceans [48]. In the case of this Risso's dolphin, lymphoid depletion, one of the hallmarks of CeMV infection, was not observed, suggesting that the *T. gondii* infection may not be a consequence of the CeMV infection but by other factors. While the dolphin tested seropositive for IgG against *T. gondii*, this cannot conclusively confirm chronic or acute infection in the absence of avidity and other corroborating tests.

T. gondii is a zoonotic coccidian parasite of cats that can infect all other mammals as intermediate hosts [28-29]. Although the infection of the parasite is prevalent in both animals and humans worldwide [32], clinical cases of Toxoplasmosis are uncommon [49]. Toxoplasmosis is usually triggered by immunosuppression [18][27-28][50]. Serologic surveys of wild common bottlenose dolphins in the US revealed that *T. gondii* infection is common [36]. In the Philippines, *T. gondii* was also detected among stranded dolphins via serologic and PCR assays in the studies conducted by Obusan et al. in 2015 [51] and 2019 [52], and De Guzman et al. in 2020 [53]. Antibodies against, as well as target genes of the parasite were detected in several individuals of cetacean species, including three of the five Risso's dolphin sampled.

The intestinal volvulus in the present case may have been caused by recurring diarrhea since chronic gastrointestinal disease is apparently a predisposing factor for this condition in cetaceans [54]. The cause of the diarrhea, however, cannot be determined.

4. Conclusions

This paper reports and confirms the presence of CeMV in a Risso's dolphin in the Philippines. Further, the subclinical nature of the CeMV infection in this case suggests that there could be reservoir individuals, populations, or species that can serve as vectors for transmitting the virus [23][55]. Morbilliviruses generally cause heavy mortalities when introduced in naïve populations as observed among cetacean populations in the Mediterranean Sea and Northwestern Atlantic [44]. The absence of reports of mass mortalities that can be attributed to CeMV in the Philippines suggests that the virus is likely endemic in the region. Retrospective epidemiological study of the archived tissue specimens and surveillance studies are recommended to determine the prevalence of the virus in and around the Philippine waters. Genotyping of CeMV that may be detected in future studies is likewise recommended to determine the strain that is circulating in the region.

Author Contributions

Conceptualization, Methodology, and Investigation - L.J.A.S., A.T.G. and M.C.M.O; Writing - Original Draft, L.J.A.S.; Writing – Review & Editing, L.J.A.S., M.C.M.O., C.S.T., and L.V.A. Resources, M.C.M.O.; Visualization, C.S.T.

Ethics Approval and Consent to Participate

Not applicable

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Conflict of Interest

The authors declare no conflict of interest.

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