

Is cetacean morbillivirus a threat for Amazon river dolphins?

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SUMMARY

Cetacean morbillivirus (CeMV) is a high transmissible pathogen that causes epizootic outbreaks with high mortality in cetacean populations worldwide. Its investigation may appear a feasible strategy in populations management, especially of endangered species like the Amazon river dolphin (*Inia geoffrensis*). We aim to investigate the exposure of 581 individuals inhabiting Sustainable Development Reserve Mamirauá (RDSM), Amazonas, Brazil, to CeMV. Dolphins were sampled annually between 1999 and 2020 and serum stored at -20°C. This investigation may unveil epidemiologic aspects of CeMV through the past exposure to morbilliviral disease in the RDSM population and will aid for adequate population management.

Project Description

The monitoring of health parameters of aquatic mammals is key to biodiversity conservation in Amazon and becomes even more urgent when it is related to endangered species, such as the Amazon river dolphin (*Inia geoffrensis*). As a food chain top predator with long lifespan, the species can be considered as sentinels of the aquatic environment. Free ranging cetaceans have been successfully used as environmental sentinels in long-term health monitoring programs in the coast of the US (Hall et al., 2007; Reif et al., 2008; Schaefer et al., 2009; Wells et al., 2004). It has been shown that the environmental degradation has a detrimental effect not only on ecosystems and biodiversity, but also may promote the emergency of diseases with epizootic characteristics (Bossart, 2011). One of the most important viral diseases of aquatic mammals is caused by Cetacean morbillivirus (CeMV; family Paramyxoviridae).

Currently, CeMV represents the most concerning pathogen for wild cetaceans given its high infectious characteristic and lethality. The transmission is thought to occur mostly horizontally by inhalation of aerosolized virus. Typical lesions caused by this virus include encephalitis, bronchointerstitial pneumonia, and lymphoid depletion leading to immunosuppression. Cetaceans that survive the acute disease may develop fatal secondary infections and chronic encephalitis (Van Bresse et al., 2014). CeMV infections resulted in unusual die-offs of bottlenose dolphins (*Tursiops truncatus*), Guiana dolphins (*Sotalia guianensis*), striped dolphins (*Stenella coeruleoalba*) among others (Groch et al., 2018; Mira et al., 2019; Stone et al., 2011). Currently, five different morbillivirus strains have been reported in cetacean worldwide (Van Bresse et al., 2014). The Guiana dolphin morbillivirus (GDMV) strain has been identified in Brazil, causing the death of >200 Guiana dolphins in Rio de Janeiro state (Groch et al., 2018).

The exposure of a determined population to CeMV can be investigated by the presence or absence of serum antibodies against the viral antigen (Gregory D. Bossart et al., 2010; Stone et al., 2011), providing crucial information on epidemiology for the management of wildlife populations. Although very important, long-term serology monitoring of free ranging cetaceans is still very limited, as it requires a complex and expensive capture and release logistics, and depends on behavioral characteristics of the species. In the present study, we aim to investigate the exposure to CeMV of a wild population of Amazon river dolphin inhabiting Sustainable Development Reserve Mamirauá (RDSM), Amazonas, through the survey of neutralizing antibodies against CeMV. A capture-release program for monitoring

of the Amazon river dolphin population has been carried out for 21 years. A total of 704 dolphins have been marked and sampled until now, which made available 681 serum samples of 581 individuals for the present study. Some individuals were sampled in different occasions along the years allowing a seroepidemiologic survey in a longitudinal scale.

We aim to determine the prevalence of pathogen-specific antibodies (i.e. the proportion of seropositive animals with antibody titers above an established threshold) and gain insights into cetacean morbilliviral occurrence and how the disease may have progressed along the years. Furthermore, serum samples from other localities in Amazon, e.g. Rio Madeira and Rio Negro, are available for analysis in a future phase of the project, and might provide insights on disease spreading among Amazon river dolphin populations. The analysis of Amazon river dolphin serum samples will unveil epidemiologic aspects of CeMV through the past exposure to morbilliviral disease in the RDSM population, Amazonas, Brazil. We believe that our results will serve as a baseline reference for other dolphin populations in the Amazon region and will aid for adequate population management.

MATERIAL AND METHODS

Animal capture and sampling

Blood samples were collected from 1999 to 2020 during annual capture-release campaigns of Projeto Boto at RDSM, Amazonas. Briefly, the dolphins were captured using an ethically approved seining technique, moved to a shallow beach and transported by boat to a nearby floating station (da Silva and Martin, 2000). Blood was drawn from the superficial caudal peduncle vessel, transferred into tubes with no anticoagulant, allowed to clot in ambient temperature, and centrifuged for serum separation.

Sample storage, survey and transportation

Serum was kept frozen at -20°C from the time of sampling in the field to present. Two ml from each sample will be separated for the present study. Some individuals were resampled along the years in intervals that varied from one to 10 years. The samples are currently stored at the Aquatic Mammals Laboratory at the National Institute of Amazonian Research, Manaus, Brazil, and will be sent to the Wildlife Comparative Pathology Laboratory at University of São Paulo for further analysis. The samples will be properly packed with dry ice and transported by airplane.

Laboratory analysis

GDMV cell culture is currently been performed in the University of São Paulo aiming to establish a virus neutralization test (VNT) for CeMV screening in Brazil (Groch KR, personal communication). Briefly, once the virus is successfully propagated through cell culture passages, the cell culture supernatant will be collected and used as a source with a known titer of the virus. Dog SLAM-tag Vero cells will be grown in 96-well culture plates and incubated with serial dilutions of the serum sample and a known titer of the virus present in the cell culture supernatant. Infectivity will be identified by the presence of a cytopathic effect (formation of syncytium) on target cells by direct microscope observation. Neutralizing titer will be defined as the highest dilution of serum at which the virus is neutralized in 50% of the wells.

IMPORTANCE OF THE STUDY FOR SPECIES BIOLOGY AND CONSERVATION

Amazon river dolphin populations have been suffering anthropogenic disturbance for many years. Examples of these activities include entanglement ('bycatch') in fishing gear, agriculture expansion, mining, dams constructions, port development, mining, ship traffic among others (da Silva et al., 2018).

Also, in the recent years these dolphins have been deliberately killed in different regions of Amazon to serve as bait for piracatinga (*Calophysus macropterus*) fishery, which led to a significant decline of populations (Brum et al., 2015).

In the vast territory of Amazon, it is nearly impossible to record stranding and cause of death of dolphins since the area has a very low human population density and the extremely high extension of water courses preclude a systematic monitoring. Also, the presence of aquatic predators such as caimans or necrophagous fishes may damage the carcasses and prevent them to strand. For this reason, the causes of death and the number of dolphins that die due to infectious diseases is poorly understood. The search for serum antibodies against infectious and parasitic diseases of living animals may appear a feasible strategy to investigate the health status of free-ranging cetacean populations. Seroprevalence of antibodies against toxoplasmosis, brucellosis, and leptospirosis (Sánchez-sarmiento et al., 2018; Santos et al., 2011) have been investigated in Amazon river dolphins, however, no study has been performed on the highly contagious and potentially lethal morbilliviruses. Amazon river dolphins are currently classified as endangered by the International Union for Conservation of Nature's Red List of Threatened Species (IUCN) (da Silva et al., 2018). All information on the species and the potential threats to its survival need urgent attention. Considering that CeMV is a high transmissible pathogen, that causes epizootic outbreaks with high mortality, investigation on the exposure of Amazon river dolphins to CeMV is of great importance to predict the occurrence of CeMV and species survivorship.

Long term monitoring of serum antibodies against infectious diseases allows the prediction on how the disease occur in different scenarios along the years (Rowles et al., 2011). In the present study, serum of wild dolphins sampled along 21 years may grant us a valuable opportunity to do a retrospective investigation on the exposure of Amazon River dolphins to CeMV. The possibility of monitoring and predicting the occurrence of diseases in free ranging animals is directly related to the management strategies for their conservation. In this context, the present study aims to investigate the occurrence of the morbillivirus in the free ranging Amazon river dolphins inhabiting Sustainable Development Reserve Mamirauá, Amazonas, and to identify early warning indicators of potential long-term health problems at the population level. Also, it will provide baseline information for future studies in different populations of Amazon river dolphins inhabiting the vast area of Amazon.

PROJECT TIMELINE

Sample collection: Blood samples were collected from 1999 to 2020 during annual capture-release campaigns of Projeto Boto.

Sample transportation: The samples will be transported frozen with dry ice from Manaus to the University of São Paulo, São Paulo-SP in December 2020.

Sample analysis: Jan 2021 – June 2021.

BUDGET (TOTAL: 2,000 USD)

Packing and Shipping of samples from Manaus to São Paulo: 200USD

Laboratory reagents and kits: 1,800 USD

- 96-Well Cell Culture Plates: 450 USD
- Barrier (Filter) Tips: 280 USD
- Dubeco` minimun essential media: 260 USD
- Fetal Bovine Serum (FBS): 100 USD
- Trypsin – enzimitc dissociation: 50 USD

- Cell Culture Flasks: 340 USD
- Cell culture antibiotics: 90 USD
- Serological pipette: 150 USD
- Surface disinfectants: 40 USD
- Disposable gloves: 40 USD

OTHER FUNDS AVAILABLE FOR THIS PROJECT

- Research scholarship - National Council for Scientific and Technological Development (CNPq): 14000.00 USD.
- The project “Strategies for health monitoring of aquatic mammals from the Amazon” is founded by the Amazonas Research Foundation (FAPEAM): 10317.00 USD. This project and Projeto Boto support boat expeditions to collect samples in the field and equipment for adequate storage of samples. However, it doesn’t include any specific budgeted to cover serology analysis expenses.

PERMITS

Successive permits were granted to Projeto Boto along the years by IBAMA/SISBIO (Brazilian National Environmental Agency) number 13157–1. Also, the permit number 72503-1 was granted by IBAMA/SISBIO for sample collection, transport and analysis to the project “Strategies for health monitoring of aquatic mammals from the Amazon” which is leaded by the proponent of the present study.

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SHORT CURRICULUM VITAE

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Current employment

Researcher: National Institute of Amazonian Research: Manaus, 06/01/2019 to present
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PhD in Animal Science: March 2011 – Feb 2015

Title: "Use of blubber as matrix to measure sexual steroid levels of humpback whales (*Megaptera novaeangliae*)" – School of Veterinary Medicine and Animal Science – University of São Paulo.

Masters by Research Degree: March 2004 – Sep 2006

Title: "Hematology and blood chemistry of the Amazon river dolphin *Inia geoffrensis* (de Blainville, 1817)" - Integrated Program in Tropical Biology and Natural Resources - National Institute of Amazonian Research

Veterinary Sciences: Aug 1997 – Feb 2002

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Peer Reviewed Publications

- Hematologic profile of Amazon river dolphins *Inia geoffrensis* and its variation during acute capture stress
PLOS ONE: 2019-12-26
DOI: 10.1371/journal.pone.0226955
- Health assessment of juvenile green turtles in southern São Paulo State, Brazil: a hematologic approach
Journal of Veterinary Diagnostic Investigation: 2019-12-17
DOI: 10.1177/1040638719891972
- Successful rehabilitation of *Macronectes halli* and *Macronectes giganteus* in southeastern Brazil
Brazilian Journal of Veterinary Research and Animal Science: 2018-07-26
DOI: 10.11606/issn.1678-4456.bjvras.2018.138436
- Use of a porous cellulose membrane (Membracel®) and moxibustion for severe excoriative injury in a Chilean skua (*Catharacta chilensis*)
Brazilian Journal of Veterinary Research and Animal Science: 2018-06-30
DOI: 10.11606/issn.1678-4456.bjvras.2018.138489
- Feasibility of using humpback whale blubber to measure sex hormones
Journal of Experimental Marine Biology and Ecology: 2017-01
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