Research and characterization of selected pathogens of cutaneous and mucocutaneous lesions in cetaceans from the Brazilian coast

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Prof. Dr. José Luiz Catão Dias

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RESUMO

SACRISTÁN YAGUE, C. Pesquisa e caracterização de patógenos cutâneos e mucocutâneos selecionados em cetáceos da costa brasileira. [Research and characterization of selected pathogens of cutaneous and mucocutaneous lesions in cetaceans from the Brazilian coast]. 2017. 181 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2017.

Cetáceos são sentinelas do ambiente marinho, atualmente ameaçados por diversos fatores, principalmente antropogênicos. Os processos de pele e mucosas externas são os mais facilmente identificados, bons indicadores do estado de saúde em cetáceos. Lesões cutâneas e mucocutâneas já foram amplamente relatadas em cetáceos de vida livre e de cativeiro, mas pouco se sabe a respeito dos fatores etiológicos envolvidos, evolução das lesões dermatológicas e suas consequências sistêmicas. Vírus são os agentes mais comumente envolvidos em lesões cutâneas e mucocutâneas, especialmente os herpesvírus (HV), associados a lesões de morfologias variáveis em mucosas, e poxvírus dos cetáceos (Cetacean Poxvirus – CePV), principalmente associados a lesões de pele “tatuagem” ou “anel” características. Agentes fúngicos também podem causar doenças dermatológicas em cetáceos, como por exemplo a paracoccidioidomicose ceti, caracterizada por lesões esbranquiçadas elevadas e proliferativas, causada por leveduras não-cultiváveis de Paracoccidioides brasiliensis (ordem Onygenales). Apesar de mundialmente reportados, a ocorrência desses agentes etiológicos em cetáceos do Atlântico Sul ainda é pouco compreendida. O objetivo desse estudo foi identificar e caracterizar patógenos cutâneos e mucocutâneos selecionados (HV, CePV e P. brasiliensis) de cetáceos brasileiros de vida livre e desenhar métodos diagnósticos mais sensíveis para sua detecção. Todos os animais estudados encalharam ao longo da costa brasileira, entre 2005 e 2015, exceto por três botos-cor-de-rosa que foram fisicamente imobilizados e liberados após a coleta de amostras. Para atingir tais objetivos, empregamos técnicas moleculares e histológicas, e ocasionalmente de imunohistoquímica e microscopia eletrônica. A presença de HV e CePV foi avaliada, respectivamente, em amostras cutâneas e de mucosa oral e genital de 115 espécimes, e amostras de pele de 113 indivíduos; enquanto a presença de membros da ordem Onygenales foi avaliada em quatro espécimes que apresentavam lesões macroscópicas compatíveis. Amostras de pele ou de mucosa oral de quatro animais foram positivas para a PCR de HV: uma lesão ulcerada de pele de coloração esbranquiçada de um boto-cinza (Sotalia guianensis), uma amostra de tecido lingual de um golfinho-pintado-do-Atlântico (Stenella frontalis), lesões ulcerativas e amostras de pele saudável de um cachalote-anão (Kogia sima), e uma lesão proliferativa de pele em boto-vermelho-boliviano
(Inia boliviensis). Os primeiros três animais estavam infectados com alphaherpesvírus. Uma sequência mais similar com gammaherpesvírus foi obtida da lesão proliferativa de pele do boto-vermelho-boliviano. A sequência do boto-vermelho-boliviano possivelmente pertence a um novo gênero de gammaherpesvírus. Ademais, todas as outras amostras de tecidos disponíveis dos especímenes HV-positivos, à parte de pele e mucosa oral, também foram avaliadas por técnicas de PCR e histológicas. Uma sequência diferente de alphaherpesvírus foi encontrada no estômago e em um linfonodo mesentérico do cachalote-anão. Achados microscópicos em dois animais HV-positivos (dermatites proliferativas em boto-vermelho-boliviano e boto-cinza) eram compatíveis com HV. CePV foi identificado em lesões de pele do tipo “tattoo” de um golfinho-nariz-de-garrafa (Tursiops truncatus) e de um boto-cinza por meio de técnicas moleculares estabelecidas, e observação de partículas de poxvírus por microscopia eletrônica. Animais CePV-positivos apresentavam degeneração balonosa epidérmica e ocasionais inclusões intracitoplasmáticas anfófilas ou eosinófilas compatíveis com CePV. Motivos aminoácidos específicos para todos os CePVs também foram identificados, reforçando a sugestão de um novo gênero, chamado Cetaceanpoxvirus.

Nesse estudo também foram desenvolvidas novas técnicas de PCR convencional e real-time com SYBR® Green, significativamente mais sensíveis do que os métodos atualmente disponíveis em literatura. Um boto-cinza, inicialmente negativo segundo os métodos de PCR previamente conhecidos foi diagnosticado positivo para CePV por meio das novas técnicas aqui descritas. Leveduras refratáveis (4−9 μm de diâmetro) foram observadas à microscopia sob a forma de lesões de pele granulomatosas moderadas e necróticas em quatro golfinhos-nariz-de-garrafa, e pela primeira vez, em um abscesso muscular (esse último um indício do potencial invasivo desse agente). Leveduras de Onygenales sp. foram identificadas em lesões de pele por meio de imunohistoquímica e uma sequência de P. brasiliensis mais semelhante (100% de identidade de nucleotídeos) àquela descrita em golfinhos de Cuba do que a casos de humanos e mamíferos descritos no Brasil, foi encontrada em lesões de pele de um dos especímenes. Esse estudo relata a primeira identificação molecular de HV em cetáceos da América do Sul e em golfinhos de rio no mundo. Além disso, descrevemos a primeira amplificação de CePV e P. brasiliensis em odontocetes da América do Sul, conferindo a etiologia desse tipo de lesões. Quatro das cinco novas sequências de HV identificadas são possivelmente novas espécies, provisoriamente chamadas Delphinid HV-10, Kogiid HV-2, Kogiid HV-3 e Iniid HV-1.

Palavras-chave: Patologia. Dermatologia. Herpesvírus. Poxvírus. Paracoccidioides brasiliensis.
ABSTRACT

SACRISTÁN YAGUE, C. Research and characterization of selected pathogens of cutaneous and mucocutaneous lesions in cetaceans from the Brazilian coast. [Pesquisa e caracterização de patógenos cutâneos e mucocutâneos selecionados em cetáceos da costa brasileira]. 2017. 181 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2017.

Cetaceans are sentinels of the marine environment, currently threatened by many factors, mainly anthropogenic. The most easily identified compromising conditions are those affecting the skin and external mucosae - good indicators of the cetacean’s health status. Cutaneous and mucocutaneous lesions have been extensively reported in wild and captive cetaceans, but little is known about the involved etiological factors, evolution of the dermatological lesions and their systemic consequences. Viruses are the most commonly involved agents in cutaneous and mucocutaneous lesions, especially herpesviruses (HV) and, associated with skin and mucosal lesions with varying morphologies, and cetacean poxviruses (CePV), mainly associated with characteristic “tattoo” or “ring” skin lesions. In addition, fungal agents are also recognized as causative agents of dermatological disease in cetaceans, especially in the process known as paracoccidioidomycosis ceti, observed as raised proliferative whitish lesions, caused by non cultivable yeast of Paracoccidioides brasiliensis (order Onygenales). Despite being reported worldwide, the occurrence of these etiological agents in southern Atlantic cetaceans is still poorly understood. The goal of this study was to identify and characterize selected cutaneous and mucocutaneous pathogens (HV, CePV and P. brasiliensis) of free-ranging cetaceans from Brazil, and to design more sensitive diagnostic methods for their detection. All the studied cetaceans stranded along the coast of Brazil, between 2005 and 2015, except three wild riverine dolphins that were physically contained and released after sample collection. In order to achieve our goals, we employed molecular, histopathological, and occasionally immunohistochemical and electron microscopy techniques. The presence of HV and CePV was evaluated in cutaneous, and oral and genital mucosal samples from 115 specimens and skin samples from 113 individuals, respectively; whereas the presence of members of the genus Onygenales sp. was evaluated in four specimens presenting macroscopic compatible lesions. Skin or oral mucosal samples from four animals were HV PCR-positive: a whitish ulcerated skin lesion from a Guiana dolphin (Sotalia guianensis), a lingual sample from an Atlantic spotted dolphin (Stenella frontalis), ulcerative lesions and healthy skin samples from a dwarf sperm whale (Kogia sima), and a proliferative skin lesion from a Bolivian river dolphin (Inia boliviensis).
The tree first animals were infected with alphaherpesvirus. A sequence more similar to gammaherpesvirus was obtained from the Bolivian river dolphin's proliferative skin lesion. The Bolivian river dolphin sequence could possibly be a member of a new gammaherpesvirus genus. Additionally, all other available tissue samples from HV-positive specimens, aside from skin and oral mucosa, were also tested by PCR and histologically evaluated. A different alphaherpesvirus sequence was found in the stomach and in a mesenteric lymph node of the dwarf sperm whale. Microscopic findings in two HV-positive animals (chronic proliferative dermatitis in Bolivian river dolphin and Guiana dolphin) were compatible with HV. CePV was identified in “tattoo” skin lesions of an Atlantic bottlenose dolphin and a Guiana dolphin by established molecular methods, and poxviral particles were observed by electron microscopy. CePV-positive animals presented epidermal ballooning degeneration and occasionally small, pale eosinophilic or amphophilic intracytoplasmic inclusions, compatible with CePV. Specific amino acid motifs for all CePV were also identified, reinforcing the suggestion of the new Cetaceanpoxvirus genus. We also designed novel SYBR® Green real-time and conventional CePV PCR methods significantly more sensitive than those currently available in the literature. An additional Guiana dolphin, previously negative based in established PCR methods was diagnosed positive for CePV through these new techniques. Refractile yeasts (4−9 μm in diameter) were observed under light microscopy in mild granulomatous and necrotic skin lesions of four Atlantic bottlenose dolphin, and for the first time, in a skeletal muscle abscess (the former possibly indicating the invasive potential of the agent). Onygenales sp. yeasts were identified in skin lesions by immunohistochemistry and a sequence of *P. brasiliensis*, more similar (100% nucleotide identity) to the one described in an Atlantic bottlenose dolphin from Cuba than to human or any other terrestrial mammals cases in Brazil, was obtained from the skin lesion of one of the specimens, confirming the etiological agent of these type of lesions. Herein we report the first molecular identification of HV in South American cetaceans and in riverine dolphins worldwide. This study also describes the first amplification of CePV and *P. brasiliensis* in odontocetes from South America. Four of the five novel herpesvirus sequences herein identified are possibly novel species, tentatively named Delphinid HV-10, Kogiid HV-2, Kogiid HV-3 and Iniid HV-1.
The ecosystem health could be defined as the lack of signs of ecosystem distress, its resilience (defined as the ability to recover rapidly and complexly from an injury), and/or the absence of risk or threats towards the ecosystem’s composition, structure and/or function (RAPPORTS, 1995). In order to maintain biodiversity, preserve healthy ecosystems is necessary (HILTY; MERENLENDER, 2000).

One of the most used methods to assess ecosystem health is the measurement of the effects of a phenomenon or substance of interest over a species used as a sensor, known as indicator species - usually invertebrates, but also of animals of upper trophic level (HILTY; MERENLENDER, 2000; CARIGNAN; VILLARD, 2002; HEINK; KOWARIK, 2010).

Cetaceans are important indicator species, used as sentinels of marine and riverine environments due to their long life spans, high position at the food chain, storage ability of their large fat deposits (e.g., anthropogenic pollutants) and shared sensitivity to certain pathogens (e.g. *Toxoplasma gondii*), toxins and chemicals with humans (REDDY et al., 2001; WELLS et al., 2004; MOORE et al. 2008; BOSSART, 2011; WISE et al., 2009; GIBSON et al., 2011). The study of resident populations, e.g., Atlantic bottlenose dolphin (*Tursiops truncatus*), Guiana dolphin (*Sotalia guianensis*), beluga whale (*Delphinapterus leucas*), provides additional information on specific geographical areas of the marine environment (DE GUISE; LAGACÉ; BÉLAND, 1994; SIMÕES-LOPES; FABIAN, 1999; AZEVEDO et al., 2017). Cetaceans are charismatic aquatic megafauna species that arise very strong human empathy, easily observed at their natural habitat (BOSSART, 2011), subject of a growing whale and dolphin watching tourism. Cetacean-associated economical activities also include hunting, either for commercial or livelihood purposes (REEVES, 2002; TRYLAND et al., 2014; DA SILVA JÚNIOR, 2017), a commonly controversial issue, also strongly related with cultural values.

Two clades are recognized in the unranked Cetacea taxon: Odontoceti (dolphins, porpoises and toothed whales) and Mysticeti (baleen whales), respectively comprised of by ten and four families (Box 1).
Box 1 - Clades (Odontoceti and Mysticeti), families and cetacean species.

| CLADE | FAMILY | SPECIES |
|-------|--------|---------|
| Odontoceti | Physeteridae | sperm whale (*Physeter macrocephalus*) |
| | Kogiidae | pygmy sperm whale (*Kogia breviceps*)
| | | dwarf sperm whale (*K. sima*) |
| | Ziphiidae | e.g., Hector’s beaked whale (*Mesoplodon hectori*)
| | (beaked whales) | |
| | Platanistidae | Indian river dolphin (*Platanista gangetica*) |
| | Iniidae | e.g. Bolivian river dolphin (*Inia boliviensis*)
| | (pink river dolphins) | |
| | Lipotidae | baiji (*Lipotes vexillifer*) |
| | Pontoporiidae | Francisca/toninha (*Pontoporia blainvillei*) |
| | Monodontidae | beluga (*Delphinapterus leucas*)
| | (belugas and narwhals) | narwhal (*Monodon monoceros*) |
| | Delphinidae | e.g., Atlantic bottlenose dolphin (*Tursiops truncatus*)
| | (true dolphins) | |
| | Phocoenidae | e.g., Burmeister’s porpoise
| | (true porpoises) | (*Phocoena spinipinnis*) |
| Mysticeti | Balaenidae | e.g., southern right whales (*Eubalaena australis*)
| | (right whales and bowhead whale) | bowhead whale (*Balaena mysticetus*) |
| | Neobalaenidae | pygmy right whales (*Caperea marginata*) |
| | Eschrichtiidae | grey whale (*Eschrichtius robustus*) |
| | Balaenopteridae | e.g., Bryde’s whale (*Balaenoptera brydei*)
| (rorquals) | |

All of these families are part of a larger group, the order Cetartiodactyla, which also comprises the Artiodactyla (e.g., suborders Suina [pigs and boars], Tylopoda [camels and llamas], and Ruminantia [deer, giraffes and cows]) (TRUJILLO et al., 2010; GRAVENA et al., 2014; COMMITTEE ON TAXONOMY, 2016). The divergence between cetaceans and their terrestrial ancestors occurred approximately 53 Million years ago (ARNASON; GULLBERG; JANKE, 2004). Since then, these organisms have adapted to an obligate aquatic life cycle through special adaptive mechanisms,
such as echolocation (odontocetes), filter-feeding (mysticetes), presence of a
vestigial pelvis girdle, loss of hind limbs at the end of the fetal development, and
other modifications in organs, such as kidneys, lungs, gonads, internal ears and skin
(e.g., absence of sebaceous or apocrine glands) (ROMMEL; LOWENSTINE, 2001;
THEWISSEN et al., 2006; DINES et al., 2014; PYENSON, 2017). A high number
of cetacean species is present in Brazil, a megadiverse country, with at least 48
riverine, coastal or pelagic described cetacean species (LODI; BOROBIA, 2013;
GRAVENA et al., 2014; CYPRIANO-SOUZA et al., 2016). This rich diversity is
promoted by three main marine currents: Malvinas/Falklands, North Brazil, and
Brazil, and two riverine basins (Amazonas and Tocantins), creating different
ecosystems (LODI; BOROBIA, 2013).

The study of wildlife health, through the evaluation of the etiology, occurrence
and prevalence of diseases, is a very important branch of conservation management,
not to mention the application of such processes as sensitive indicators of
anthropogenic impacts (DEEM; KARESH; WEISMAN, 2001). Cetacean health
studies may have even further significance, especially when it comes to raising the
general public’s awareness about the deterioration of the marine environment
(BOSSART, 2011).

The Health Concept comprises not only the absence of disease, but also the
interaction among environmental, biological and social parameters that influence the
organisms’ adaptation to environmental changes and populations’ resilience
(STEPHEN, 2014). Due to ethical and logistic issues, the most common way to
assess the health status of free-ranging cetacean populations is through the study of
stranded animals. For instance, the first identification of morbillivirus in cetaceans
was performed in stranded dead striped dolphins (Stenella coeruleoalba) (DOMINGO
et al., 1990; GERACI; LOUNSBURY, 2005). Other alternative methods adapted to
the study of cetaceans’ health include exhaled breath analysis and skin biopsies
(NOREN; MOCKLIN, 2012; RAVERTY et al., 2017). These studies have clarified
many of the current cetacean morbidity and mortality causes, such as: (1) fishing
interaction and competition, (2) hunting, (3) pollution: debris, heavy metals, organic
pollutants (e.g., the high of neoplasm prevalence in the St. Lawrence River’s beluga
whale [Delphinapterus leucas] population in Canada, associated with polycyclic
aromatic hydrocarbons, and reproduction impairment in odontocetes from European
waters associated with polychlorinated biphenyls), (4) ship collision and acoustic
pollution, (5) biotoxins, (6) the effects of climate change (e.g., oceanic acidification, alterations in the food chain), and (7) infectious diseases (DE GUISE; LEGACE; BÉLAND, 1994; MARTINEAU et al., 2002; WRIGHT et al., 2007; FERNÁNDEZ et al., 2008; PARSONS et al., 2008; MARIGO et al., 2010; KAPLAN et al., 2013; LITZ et al., 2014; JEPSON; DEAVILLE; LAW, 2016; UNGER et al., 2016).

Diseases can affect wild population’s reproductive trends, survival and dispersal (GULLAND; HALL, 2006). Initially, wildlife infectious diseases were only valued when the wildlife-livestock interface was involved or when considered potential zoonoses. Nevertheless, the concern over their impact and threat to the biodiversity is currently increasing (DASZAK; CUNNINGHAM; HYATT, 2000; DE CASTRO; BOLKER, 2004). One must also consider the differences between the terrestrial and aquatic environments. In the marine environment, some diseases may spread at a faster rate than on the terrestrial environment, as seen in certain viral epizootics reported in fish (Australian pilchard, *Sardinops sagax neopilchardus*) by herpesvirus (10,000 km/year), and phocids and cetaceans by morbillivirus (3,000 km/year) (MCCALLUM; HARVELL; DOBSON, 2003; WHITTINGTON et al., 2008).

Diseases in cetaceans, including of several emerging pathogens, e.g., morbillivirus, poxvirus, herpesvirus, *Brucella ceti*, *T. gondii*, *Paracoccidioides brasiliensis*, have been increasingly reported (ESPERON; FERNANDEZ; SANCHEZ-VIZCAINO, 2008; VAN BRESSEM et al., 2009; BELLIERE et al., 2011; GONZALES-VIERA et al., 2013; GROCH et al., 2014; SIMEONE et al. 2015; VILELA et al., 2016). However, assessing cetacean diseases’ spatiotemporal trends, as observed in other marine species, such as corals, is very challenging (SIMEONE et al., 2015). Cetaceans are long-live animals that generally produce only one calf per year; therefore, infectious diseases that compromise reproductive success and/or fertility (e.g., brucellosis, toxoplasmosis, sarcocystosis by *Sarcocystis neurona*) could negatively impact cetacean populations (MILLER et al., 1999; JARDINE; DUBEY, 2002; BARBOSA et al., 2015). In addition, physiological stress - increasingly associated with anthropogenic impact over the marine and riverine environments - can impair the cetacean immune system, increasing its susceptibility to infectious diseases (WRIGHT et al., 2007; REIF et al., 2009; JEPSON; DEAVILLE; LAW, 2016).
The study of infectious diseases affecting the skin and oral and genital mucosa of cetaceans is considered especially relevant for several reasons: (1) the skin is directly evaluated through visual assessment, which is sometimes the only or the main available resource in field studies; (2) although infectious cutaneous and mucosal diseases are usually not fatal, and often self-limiting, they may serve as entry routes for other pathogens; (3) several systemic diseases cause cutaneous and oral mucosal alterations (e.g., herpesvirus, erysipelas); and (4) cutaneous and oral and genital mucosal alterations may be good indicators of cetaceans’ health status (BOSSART; EIMSTAD, 1988; SCHULMAN; LIPSCOMB, 1999; PETTIS et al., 2004; BOSSART et al., 2008; REIF et al., 2009; VAN BRESSEM et al., 2009; SIERRA et al., 2014). Cutaneous and mucocutaneous alterations have been observed both in wild and in captive cetaceans, since the 50’s (SIMPSON; WOOD; YOUNG, 1958); however, most of the time, the involved etiological agents were not identified (MALDINI et al., 2010; FURY; REIF, 2012; GROCH, 2014).

Cutaneous and mucocutaneous lesions are usually benign, although they may occasionally be fatal, and present different distributions, characteristics, sizes and presentations (unique or multiple lesions). The main etiological agents known to affect cetacean’s skin, oral and genital mucosa are viruses (e.g., herpesvirus, poxvirus and papillomavirus) and fungal agents (P. brasiliensis) (SWEENEY; RIDGWAY, 1975; VAN BRESSEM; VAN WAEREBEEK; RAGA, 1999; VAN BRESSEM et al., 2009; ESPERON et al., 2012; VILELA et al., 2016). Herpesviruses present a wide distribution and affect most animal species (PELLETT; ROIZMAN, 2007). The first cases of herpesviral infection in cetaceans were reported in the 80’s by electron microscopy (MARTINEAU et al., 1988; BARR et al., 1989). Since then, with the advent of molecular techniques based on the employment of universal primers, novel sequences related to cutaneous and mucocutaneous processes have been described (SMOLAREK-BENSON et al., 2006; VAN ELK et al., 2009; SIERRA et al., 2014). In cetaceans, herpesviruses may cause skin, oral and genital lesions, occasionally characterized by loss of pigmentation or proliferative nodules (VAN BRESSEM et al., 1994; HART et al., 2012). This latter manifestation of the disease is currently surrounded by controversy on whether they are caused by gamma-herpesvirus or by papillomavirus (REHTANZ et al., 2012).

Papillomaviruses cause tumors (papillomas) - mainly in the oral and genital mucosa - in several cetacean species (LAMBERTSEN et al., 1987; REHTANZ et al.,
2006; VAN BRESSEM et al., 2007; RECTOR et al., 2008). Large oral papillomas may affect feeding, whereas genital papillomas could compromise reproduction (VAN BRESSEM; VAN WAEREBECK; RAGA, 1999; VAN BRESSEM et al., 2009).

Poxviruses have been reported in cetaceans since the late 70’s (FLOM; HOUK, 1979; GERACI; HICKS; ST AUBIN, 1979; BRACHT et al., 2006), associated with characteristic skin lesions presenting melanic margins and a stippled interior, known as “tattoo”-lesions (GERACI; HICKS; ST AUBIN, 1979; BRACHT et al., 2006).

Paracoccidioidomycosis ceti, previously known as lobo’s disease, lacaziosis, lacaziosis-like disease, lobomycosis or lobomycosis-like disease, is a cetacean cutaneous disease firstly identified in the 70’s (MIGAKI et al., 1971), caused by *P. brasiliensis* non-cultivable yeasts of the order Onygenales (ROTSTEIN et al., 2009; ESPERON et al., 2012; UEDA et al., 2013; VILELA et al., 2016), closely related to *Lacazia lobo*, the etiological agent of lacaziosis in humans (VILELA et al., 2016). Clinical signs include chronic cutaneous, well demarcated, firm, proliferative, ulcerative or verrucous, whitish- to grayish colored, and occasionally pink lesions (MIGAKI et al., 1971).

In Brazil, papillomatosis and poxvirus-like lesions have been reported, respectively, in a rough-toothed dolphin (*Steno bredanensis*) (GONZALES-VIERA et al., 2011; GONZALES-VIERA et al., 2012) and in Guiana dolphins (GONZALES-VIERA et al., 2012). Skin lesions caused by yeast similar to those observed in paracoccidioidomycosis ceti cases were also described in the country in 1993, in an Atlantic bottlenose dolphin (SIMÕES-LOPES et al., 1993), and suggestive macroscopic lesions have been observed in an Atlantic bottlenose dolphin population (DAURA-JORGE; SIMÕES-LOPES, 2011; VAN BRESSEM et al., 2015) and in Guiana dolphins (VAN BRESSEM; SANTOS; OSHIMA, 2009).

In Brazil, studies of infectious agents related to cetacean cutaneous and mucocutaneous lesions are scarce, with limited histopathological descriptions and complete absence of immunohistochemical and/or molecular identification.
The aim of this study was to determine the infectious etiology of cutaneous and mucocutaneous lesions in cetaceans from Brazil, through the analyses of a decade of sample collections (2005-2015). To the authors’ knowledge, this is the first study to molecularly detect and identify selected emerging infectious pathogens – herpesvirus, poxvirus and *Paracoccidioides brasiliensis* – affecting this clade in Brazil, and in the case of herpesvirus and *P. brasiliensis*, also in South America.

The available literature particularly documents the connection between herpesvirus activation and immunosuppression, which could also be caused by the agent itself. Poxvirus is also considered a potentially immunosuppressant agent to other vertebrate species. Therefore, the presence of cutaneous “tattoo” lesions caused by poxvirus, proliferative whitish and verrucous lesions associated with *P. brasiliensis*, and proliferative or whitish lesions potentially related with herpesvirus could be used as health indicators, even though viral immunosuppression is not well established in cetaceans.

Among the novel herpesvirus sequences we described, three of them – both sequences from dwarf sperm whale and especially the one from the Bolivian river dolphin – greatly differ from previously known sequences. The macroscopic and microscopic findings observed in the Guiana dolphin and the Bolivian river dolphin are similar to previously reported herpesvirus skin lesions, although the marked herpesvirus genetic divergence observed in the Bolivian river dolphin requires further research. On the other hand, the herpesvirus detected in dwarf sperm whale’s skin lesions could have been an incidental finding, related to systemic infection. Future studies, employing techniques such as electron microscopy, amplification of longer fragments or the complete genome and/or viral culture are necessary to correctly classify these novel herpesviruses, which in the case of the Bolivian river dolphin could possibly lead to a new genus within the *Gammaherpesvirinae*.

We identified characteristic cetacean poxvirus amino acid motifs, providing new evidence to further support its inclusion in a new genus, *Cetaceanpoxvirus*. In order to do so, one requires either cell culture or sequencing of longer fragments - preferably of the complete genome - to establish the type species. Detailed and
comprehensive anatomopathological studies are required to further understand this agent’s impact on cetaceans.

The relative stability observed in the odontocete cetacean poxvirus allowed us to design novel real-time and conventional PCR techniques. Unfortunately, it was not possible to design new methods for the other studied agents, due to the high variability between the herpesviruses and limited number of described *P. brasiliensis* sequences. The newly designed techniques are highly sensitive and more efficient in diagnosing these agents in cetaceans when compared to those currently available in the literature.

Upon histopathology and immunohistochemistry, we detected Onygenales yeasts in raised, verrucous and whitish cutaneous lesions of four Atlantic bottlenose dolphins, and in muscular tissue of one specimen; this latter finding indicates this agent’s invasive potential. Subsequently, *P. brasiliensis* was identified as the etiological agent of a yeast-associated cutaneous lesion in the latter specimen’s, similar to those previously reported in cetaceans from other latitudes. We confirmed the role of *P. brasiliensis* as an etiological agent of this type of lesion, previously attributed, without any molecular or immunohistochemical diagnostic support, to *Lacazia lobo*.

*P. brasiliensis* is responsible for the most relevant human systemic mycosis in South America, the paracoccidioidomycosis. Comparative studies between human and dolphin paracoccidioidomycosis cases are fundamental to further clarify this agent’s cycle and pathology. The zoonotic potential of cetacean-infecting yeasts is still not fully understood.

Finally, we believe that further studies are necessary to provide new sensitive tools to diagnose these agents, understand their cycle and associated pathological processes, their zoonotic potential, and clarify the natural history of these agents and their hosts, the potential impact of cutaneous and mucocutaneous lesions in cetaceans - specially in endangered species and populations - and their role as health indicators of marine and riverine environments.
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