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Occurrence of enteropathogens isolated from marine mammals in the coastal regions of Brazil.

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ABSTRACT

The marine ecosystem is recognized as natural habitat of pathogenic microorganisms and infectious diseases caused by these bacteria are dangerous to marine animal's health and could potentially affect several species as well those are endangered. In the other hand the investigations conducted to characterize diseases causative agents and their risks to animal and human health are difficult to carry out, especially due the migratory habits of some marine mammals' species. Aquatic environment is the habitat of microorganisms including *Plesiomonas shigelloides*, *Vibrio* and *Aeromonas* species which are pathogenic to human and animals. In the present investigation we evaluated the occurrence of these pathogens from swabs collected of marine mammals beached or accidentally captured by fishing net in southeastern (RJ) and southern (RS) coastal regions of Brazil. A total of 198 swabs were collected by DEENSP, GEMARS/UFRGS and CECLIMAR institutes and sent to LRNCEB/IOC/FIOCRUZ. The samples were enriched in Alkaline Peptone Water (APW) added with 1% of sodium chloride (NaCl) and APW plus 3% NaCl, incubated at 37°C for 18-24 hours. Following, the samples were streaked onto *Pseudomonas-Aeromonas* Selective Agar Base (GSP Agar) and the suspected colonies were characterized biochemically. The results showed two hundred and twenty two isolates were characterized being *Vibrio alginolyticus, V. parahaemolyticus, V. vulnificus, V. fluvialis Aeromonas veronii* biogrupo *veronii, A. caviae*, *A. hydrophila*, *Aeromonas* sp. and *Plesiomonas shigelloides* the main pathogens isolated. These data appoint the importance of epidemiological surveillance and microbiological monitoring and reinforce the need to implement environment protection programs especially related to endangered cetacean species.

Keywords: skin diseases, marine mammals and aquatic ecosystem.

The aquatic environment represents the habitat of diverse microorganisms some of them with pathogenic characteristics. These bacteria were considered as etiology agent of infectious diseases to human and marine mammals. The human and animal health can be affected by several bacteria but the researches developed to characterize the causative agent of diseases and the potential risk to them are difficult to carry out due the migratory habits of some marine mammals' species (2, 16).

The marine mammal fauna were recognized as an animal group sensitive to environment changes in their habitat. Some bacteria isolated from this group of animals were considered as microbiological indicators parameters that are useful to the monitoring of environment programs. Climate changes caused in the last decades as well the *El Niño* phenomenon has increased the mortality of fishes causing directs effects on the marine mammal's availability of food. The reduction of food resources could forces some species to endeavor a displacement to other areas in order to find food and this behavior could have a negative impact in the life history of the species (12, 19).

The environment pollution caused by industries activities resulted in ecologic disasters with oil and toxic substances in aquatic environment. Also, the increase of human

demography in neighboring protected environment areas cause problems as sewage discharge and minimize the oxygen in the water resulting the mortality of diverse fish's species (3).

The changes in marine environment caused by climate alterations or anthropogenic factors contribute to the occurrence of emergent diseases affecting marine mammals. The Aeromonadaceae family is a group of microorganisms spreading in aquatic ecosystems and much of them are pathogenic to animals and humans. These bacteria are associated to a range of different skin diseases (e.g. furunculosis) in fish as rainbow trout. In this case the main infection site was the dermis represented by open wounds. Currently, there are no doubts about the ability of these bacteria to cause disease, even if present alone. The human infections can present themselves as septicemias and are connected to the ingestion of contaminated seawater or fisheries products by elders, children and carriers of *diabetes mellitus* or chronic hepatic pathologies (4, 5, 7).

Plesiomonas shigelloides is an ubiquitous bacterium, widely distributed in fresh and estuarine water. This microorganism is considered an opportunistic pathogen isolated from normal bacterial flora of healthy animals and also from marine mammals created in aquaculture systems. Either, *Aeromonas* and *Plesiomonas* may cause systemic or local lesions on skin and fins of marine mammals, especially when these animals were submitted to stress conditions. Furthermore, immunocompromised persons are more susceptible to infections caused by these pathogens (8, 13).

The Vibrionaceae family is considered an important group of autochthonous pathogens inhabitant of marine environment and spreading worldwide. The pathogenic species of *Vibrio* are usually found in low incidence when compared with the more abundant saprophytes bacteria. However, their presence in aquatic environment may represent a risk of transmission of diseases to animals. Furthermore, these pathogenic bacteria were considered a potential hazard to humans, aquatic organism and marine animals due the possibility on causing severe diseases as cholera and others gastrointestinal diseases to human and vibriosis to fishes (4, 16)

Vibrio cholerae, *V. parahaemolyticus* and *V. vulnificus* had been recognized as potential pathogens to humans and considered as major causative agents of illness associated with foodborne and environment diseases. The gastrointestinal disease caused by these pathogens are more dangerous to immunocompromised, *diabetes mellitus* and others chronical diseases patients. Some species as *Vibrio mimicus*, *Vibrio fluvialis*, and *Vibrio anguillarum* have been associated with human and marine animals' diseases, leading mortality of fishes, mussels and crustaceans. Otherwise, these species may cause superficial infection at localized lesions in humans and animals after exposure to marine environment. This epidemiological aspect is relevant especially to food chain and aquaculture professionals (16).

The occurrence of gastrointestinal illness is linked to virulence factors, including adhesiveness and invasiveness, and lesional factors such as exoenzymes and enterotoxins, including those with potent haemolytic activity. Several species of *Vibrio* produces haemolysins, proteases and citotoxins which increase their pathogenic potential. The invasion ability and the resultant tissue damage suggest the role of toxins, enzymatic compounds, or the action of substances with citolytic and proteolytic activities (4, 5, 9,16).

In Brazil, several studies appoint the occurrence of these pathogens isolated from aquatic ecosystem, food origin (fish, oysters, mussels and crabs) and coetaneous lesions in humans caused by fisheries. These studies are important to Public Health due the association of some of these microorganisms on foodborne disease and extraintestinal infections (9, 15, 17).

The aim of this study was to evaluate the occurrence of genus *Vibrio*, *Aeromonas* and *Plesiomonas shigelloides* from superficial lesions (wounds) and other sites (e.g. blowhole, anus, genital slit, and mouth) of marine mammals beached or accidentally captured by fishing net in Rio de Janeiro and Rio Grande do Sul, Brazil.

MATERIALS AND METHODS

In the present investigation we evaluated a total of 198 swabs (Cary-Blair transport medium) of the following marine mammals species: whales samples – southern right whale (*Eubalaena australis*), bryde's whale (*Balaenoptera edeni*), humpback whale (*Megaptera novaeangliae*), minke whale (*Balaenoptera acutorostrata*) and True's beaked whale (*Mesoplodon mirus*), dolphins samples- franciscana (*Pontoporia blainvillei*), marine tucuxi dolphin (*Sotalia guianensis*), atlantic spotted dolphin (*Stenella frontalis*), rough-toothed dolphin (*Steno bredanensis*), common dolphin (*Delphinus* sp.) and a pinniped: South American sea lion (*Otaria byronia*).

From 2003 to 2004, the swabs from blowhole, anus, genital slit, mouth, nostrils umbilicus, eyes and open wounds of marine mammals were collected by DEENSP (Departamento de Endemias da Escola Nacional de Saúde Pública Sérgio Arouca – FIOCRUZ), GEMARS (Grupo de Estudos de Mamíferos Aquáticos) and CECLIMAR/UFRGS (Centro de Estudos Costeiros Limnológicos e Marinhos da Universidade Federal do Rio Grande do Sul) institutes where a group of specialists developed a biological and geographical data and health treatment of animals beached in coastal zones of Brazil. The samples were sent to Laboratório de Referência Nacional de Cólera e outras Enteroinfecções Bacterianas (LRNCEB), Instituto Oswaldo Cruz (IOC), Fundação Oswaldo Cruz (FIOCRUZ) in order to evaluate the occurrence of pathogens concerning to Aeromonadaceae family and *Plesiomonas shigelloides*.

The samples were enriched at Alkaline Peptone Water (APW) containing 1% sodium chloride (NaCl) and APW plus 3% NaCl (37°C/18-24 hours). After, the samples were streaked onto *Pseudomonas-Aeromonas* Selective Agar Base (GSP Agar), Tiossulfate Citrate Bile Sucrose (TCBS Agar) and the oxidase positive colonies were submitted to biochemical tests including resistance to O/129 vibriostatic agent (2, 4 diamino-6, 7 diisoprylpteridine), ONPG production (α nitrophenyl-β-D galactopyranoside), VP (Voges-Proskauer), lysine/ornithine descarboxylase and arginine dihydrolase (1, 6).

RESULTS AND DISCUSSION

After the evaluation of the total results we observed a high frequency of microorganisms isolated from franciscana (59%) that can be directly associated to number of swabs evaluated and animals captured or beached in Rio Grande do Sul (Table 1). Although members of the genus *Aeromonas* and *Plesiomonas shigelloides* are well known as pathogens of fish and amphibian species and are recognized as important causes of diseases in immunocompromised individuals, in the present investigation, there was not reported the presence of these bacteria from humpback, mink and bryde's whales.

On the other hand, the results to the others marine mammals investigated appoint the isolation of one hundred and fourteen (114) bacteria strains distributed in 10 *Aeromonas* species and *Plesiomonas shigelloides*. The main pathogens isolated were *Aeromonas veronii* biogroup *veronii* (19.3%), *A. caviae* (12.2%), *A. hydrophila* (9.6%), *A. veronii* biogroup *sobria* (8.8%), *Aeromonas* sp. (15.7%) and *Plesiomonas shigelloides* (7%). In this study, other *Aeromonas* species were isolated in low rates such as *Aeromonas jandaei* (5.3%), *A. sobria* (5.3%), *A. schubertii* (4.4%), *A. media* (4.4%), *A. eucrenophila* (4.4%) and *A. trota* (3.5%).

However, among the diverse sites of isolation in all of them there was observed an increased distribution of microorganisms from genital slit and anus with the predominance of *Aeromonas veronii* biogroup *veronii*. There was possible to note that *Aeromonas hydrophila* was most isolated from blowhole, anus and umbillicus (South American sea lion), *Plesiomonas shigelloides* was isolated from genital slit, blowhole, anus, mouth and right eye (franciscana) and *Aeromonas sobria* was isolated from an open wound from True's beaked whale. Concerning the widespread distribution of the bacteria isolated from different sites of

marine mammals, additional studies should be developed to increase the knowledge about their virulence in cases of opportunistic infections especially caused after stress conditions.

In the present investigation among the whales species (southern right whale and True's beaked whale) we observed the isolation of *Aeromonas veronii* biogroup *veronii*, *A. hydrophila* and *A. sobria* that represents important pathogens due the probability of causing primary or secondary septicemia and wound infections in animals and humans. The same *Aeromonas* species were isolated from an outbreak of diarrhea in Pernambuco and also from a human wound after aquatic exposure. These results are similar to others researches developed around the world (5, 9, 14, 18).

About the dolphin samples, we observed even though in low frequency the isolation of *Aeromonas eucrenophila*, *A. veronii* biogroup *sobria* and *A. jandaei*, whereas the franciscana samples showed *Aeromonas veronii* biogroup *veronii* and *Aeromonas* sp. Other studies revealed enterobacteria species (*Escherichia coli*) and gram positive bacteria isolated from wild sea lion (10). In this study, the samples from South American sea lion represented by a captive animal without state health for adaptation to wild life appoint *Aeromonas veronii* biogroup *sobria*, *Aeromonas* sp., *A. caviae*, *A. jandaei*, *A. hydrophila*, *A. media* and *Plesiomonas shigelloides* as the main pathogens isolated.

In Brazil, a study developed in two hospitals at Rio Grande do Sul appoints to the isolation of *Aeromonas* species from humans with the prevalence in lactants and children. The main pathogens isolated on this investigation were *Aeromonas hydrophila*, *A. caviae* and *A. veronii* biogroup *sobria* and these bacteria were isolated from marine mammals in our investigation (7). A similar study developed in three municipal hospitals at Rio de Janeiro city from newborns in Intensive Unit Care showed *Aeromonas caviae* and *A. media* associated to multiresistance antimicrobial profiles as the main pathogens isolated (15). According to some authors, *Aeromonas trota*, *A. eucrenophila*, *A. caviae* and *A. jandaei* were isolated from a fish experimental station and these bacteria were isolated from marine mammals (4).

The real role of *Aeromonas* and *Plesiomonas shigelloides* as pathogens on diarrheal disease agents are unclear but these microorganisms were epidemiologically associated to acute diarrhea in human and others diseases in animals. Our study has revealed significant results from diverse sites of marine mammals and the different species of bacteria characterized from marine mammals can be evaluated on distinct aspects: as causative agents of pathogenic diseases or opportunistic condition when these animals were on stress situations, beached or captured accidentally by fishing net. These pathogens were important to public health due the capability of cause disease in humans and animals so their monitoring is considered a relevant tool to prevent diseases (4, 7, 15, 16).

These microorganisms can be considered as cosmopolitan due their occurrence in soil, untreated and chlorinated water, human and animal infections. Therefore, several studies report that multiple infections by two or more *Aeromonas* are rare (11) Otherwise in this investigation we observed the simultaneous occurrence of species isolated from the marine mammals and these results should not be ignored.

Concerning the results to Vibrionaceae family we observed the distribution of one hundred and eight (108) strains which *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus* and *V. fluvialis* were the most prevalent species isolated. The whales samples showed the predominance of *V. alginolyticus* (26%) and *V. vulnificus* (17,4%). There were observed the isolation of *V. parahaemolyticus* and *V. fluvialis* considered relevant pathogens to Public Health.

In the present investigation, *Vibrio algynolyticus* was the major specie isolated from the whale's species analyzed showing the relevance to aquatic environment. Furthermore, the high incidence of this microorganism appoint to the risks for human health (fisheries, swimmers and seafood consumers). This microorganism has been associated with

gastrointestinal and extra-intestinal infections as otitis and coetaneous lesions in humans and animals. Moreover, it has been involved in epizootic outbreaks in aquaculture systems causing mortality and economic losses. These results were corroborated by others researches that describes the cosmopolitan distribution of this bacteria. These studies were conducted in Italy, France and Brazil and showed that *V. alginolyticus* was the most prevalent bacteria isolated from different sources as mussels, marine water, diverse fishes species (sardines, anchovies), followed by *V. parahaemolyticus* (9,16).

The Brazilian coastal regions are considered as the highest biodiversity area due the presence of diverse marine mammals' species, fishes, crustaceans, mollusks, algae as well as numerous invertebrates and microorganisms that coexist in harmony, in spite of the intrinsic characteristics of each group or specie. On the other hand, El Niño and La Niña, two climate phenomenons contributes to increase the major impacts on marine and coastal ecosystems as fisheries, global climate changes, degradation of habitat, invasions of exotic species and contamination or pollution of aquatic ecosystem (12).

Concerning these bacteria were recognized as emergent pathogens, we would like to emphasize the importance of this investigation which appoints the aquatic environment as a possible route of transmission for marine mammals. Studies suggests that bioaccumulation of many compounds can be absorbed by membranes and mucosa's animals causing various injuries to them and contributing to situation of stress and reduction of resistance to infections. Whereas, situations as intense traffic of ships or boats, and other coastal activities as recreational and tourism cause the loss of habitat and represent negative anthropogenic influence. Activities of exploitation of oil and other industries products can affect the natural bed of marine mammals causing alteration or negative influence to the migratory routes of whales and others animals (19).

Moreover these studies appoint to the relevance of epidemiological tools and microbiological monitoring of aquatic ecosystem. This research can increase the knowledge about these microorganisms, their habitat and relationship of them with marine mammals and also the consequences to human health. Furthermore, these data reinforce the need to implement environment protection programs that may be a real possibility to preserve animals especially endangered cetaceans species.

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