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IDENTIFICATION OF BACTERIA IN BLOOD CULTURES FROM CLINICALLY ILL CAPTIVE ANTILLEAN MANATEES (*TRICHECHUS MANATUS MANATUS*)

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Abstract: Between September 2001 and March 2013, 62 bacterial cultures (37 aerobic and 25 anaerobic) were performed on 37 blood samples from 23 Antillean manatees (*Trichechus manatus manatus*) that were kept in captivity at the Brazilian National Center for Research and Conservation of Aquatic Mammals (CMA) in Pernambuco (CMA-PE) and Alagoas (CMA-AL), Brazil. All of the animals sampled exhibited clinical signs at the time of sampling including abscesses ($n = 8$), debilitation and anorexia ($n = 22$), and profound lethargy-moribundity ($n = 7$). The 4 animals with profound lethargy-moribundity died shortly after sampling of unknown causes. Bacteria were isolated from 15/37 (40.5%) and aerobic blood cultures from 13/23 animals (56.5%). None of the anaerobic cultures were positive. *Aeromonas caviae*, *Aeromonas hydrophila*, *Aeromonas* sp., *Escherichia coli*, *Leclercia adecarboxylata*, *Pantoea agglomerans*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Pseudomonas* sp., *Sphingomonas paucimobilis*, coagulase-negative *Staphylococcus*, and *Staphylococcus epidermidis* were each found in only one animal; *Staphylococcus* spp. was found in two; and *Vibrio fluvialis* in four. Thirteen samples had only one bacteria isolated, one sample had two bacteria, and one sample had three bacteria isolated. Regarding sex, age group, and origin among the manatees examined, 54.5% (6/11) of the females, 58.3% (7/12) of the males, 40% (2/5) of the calves, 66.7% (8/12) of the juveniles, 50% (3/6) of the adults, 55.5% (10/18) at CMA-PE, and 60% (3/5) at CMA-AL were found to be positive for bacterial growth during at least one sampling time. All Antillean manatees were clinically ill. Regarding clinical signs, bacteria were found in 50% (11/22) of blood samples of the animals showing debilitation and anorexia, 1 of 8 (12.5%) of blood samples of the animals showing abscesses, and 3 of 7 (42.9%) of blood samples of the animals showing profound lethargy-moribundity.

Key words: Antillean manatee, aquatic mammal, bacteria, Brazil, Sirenia, *Trichechus manatus manatus*.

INTRODUCTION

The presence of living microorganisms in the blood of mammals has substantial clinical importance. Bacterial cultures of blood samples are important tools for diagnosing bacteremia. From the diagnostic standpoint, a positive blood culture yielding a clinically important microorganism

represents failure of the host's defenses to contain an infection at its primary focus and the presence of bacteremia also is an indicator of disseminated infection.¹⁸ However, despite the clinical importance of blood cultures, few investigations have been conducted on bacteremia in aquatic mammals, including manatees.

The Antillean manatee (*Trichechus manatus manatus*) is a subspecies of the manatee (*Trichechus manatus*) and is classified as a vulnerable species by the International Union for Conservation of Nature (IUCN)⁶ and as "critically endangered" by the Brazilian Action Plan for Aquatic Mammals.⁵ In Brazil, bacteria were isolated of the upper respiratory tract from six Antillean manatees at the Brazilian National Center for Research and Conservation of Aquatic Mammals (CMA) of the Chico Mendes Institute for Biodiversity Conservation (ICMBio), on Ilha de Itamaracá, Pernambuco, and from two Antillean manatees at Barra de Mamanguape, Paraíba.¹⁶

While studies of bacteria isolated at necropsy from the tissues^{4,14,17} or antemortem from the respiratory tract¹⁶ of manatees have been pub-

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lished, no reports of blood culture data could be found in the literature.

The aim of the present study was to investigate aerobic and anaerobic bacteria cultured from clinically ill Antillean manatees kept in captivity in Brazil.

MATERIALS AND METHODS

The captive Antillean manatees in this study were from two units of the CMA, located on Ilha de Itamaracá in the state of Pernambuco (CMA-PE) (7°48'33.40"S; 34°50'16.36"W) and at Porto de Pedras, in the state of Alagoas (CMA-AL) (9°13'05.47"S; 35°19'59.01"W). The CMA has been responsible for rescue, rehabilitation, and release of this species in Brazil for the last 35 years.

At CMA-PE, some of the manatees are brought in from the wild and others are born there. They are housed individually or in groups, in artificial pools for an average of 3 to 4 yr. When they are deemed healthy, they are transferred to a captive environment at CMA-AL for adaptation before release. The animals are kept at the latter location for an average of 3 to 12 mo before being released to the wild.

Between September 2001 and March 2013, 62 bacterial cultures (37 aerobic and 25 anaerobic) were performed on 37 blood samples from 23 Antillean manatees. Samples were collected once for 15 animals, twice for 4 animals, 3 times for 3 animals, and 5 times for 1 animal. For animals with multiple samples, at least 3 mo passed between each sample collection and each sample collection occurred during a separate and distinct period of illness. Among these animals, 17 presented clinical signs such as debilitation and anorexia, 4 presented abscesses, and the remaining 4 had profound lethargy-moribundity. During the study, two Antillean manatees changed their clinical signs. One animal presented debilitation and anorexia and later presented abscesses, and the other presented debilitation and anorexia and later presented profound lethargy-moribundity. Samples were collected from 11 females and 12 males; 5 calves (1–30 days old), 12 juveniles (31 days to 2 yr old) and 6 adults (>2 yr old); and 18 were at CMA-PE and 5 at CMA-AL.

To obtain the blood samples, the manatees were contained in slings that had been constructed for handling this species and were kept on mattresses soaked with water. The animals' skin was kept constantly moistened so that it would not dry out. The sample collection had been authorized through a license for scientific purposes issued

by Sistema de Autorização e Informação em Biodiversidade (Authorization and Information System in Biodiversity) (number 20685).

The blood samples were obtained from the brachial plexus between the radius and ulna, using disposable syringes. Masks and gloves were used and, before sample collection, asepsis was performed on the animal's flipper, comprising triple cleansing alternating between iodinated alcohol and 70% alcohol. The liquid was then wiped away using sterile gauze. A volume of 5 ml of blood from each animal was placed in each of two sterile glass flasks: one containing the blood culturing media BacT/ALERT® PF (bioMérieux Inc., Durham, North Carolina 27712, USA) and BacT/ALERT FA (bioMérieux Inc.) for aerobic growth, and one containing BacT/ALERT FN (bioMérieux Inc.) for anaerobic growth. The flasks were kept in insulated boxes without refrigeration and were taken within 6 hr to the clinical analysis laboratory of Real Hospital Português in Recife, Pernambuco State, Brazil.

All the samples were analyzed using colorimetric detection, through the BacT/ALERT (bioMérieux Inc.) automated blood culturing equipment.⁹

Samples identified with positive growth in the flasks were subcultured on both blood agar and MacConkey agar. In addition, slides were produced for Gram staining. After the plates had been seeded, they were incubated at 35°C under an aerobiosis regimen for 24 h. The species was identified by means of the VITEK 2® Compact equipment (bioMérieux S.A., Marcy l'Etoile 69280, France).⁹

RESULTS

In total, 62 blood cultures were performed, among which 37 were done using aerobic growth media (Tables 1, 2) and 25 with anaerobic growth media. Bacteria were isolated from 15/37 (40.5%) of the aerobic cultures from 13/23 (56.5%) of the animals. All of the anaerobic blood cultures produced negative results.

Bacteria isolated once from a single animal were *Aeromonas caviae*, *Aeromonas hydrophila*, *Aeromonas* sp., *Escherichia coli*, *Leclercia adecarboxylata*, *Pantoea agglomerans*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Pseudomonas* sp., *Sphingomonas paucimobilis*, coagulase-negative *Staphylococcus*, and *Staphylococcus epidermidis*. *Staphylococcus* spp. was found in two animals and *Vibrio fluvialis* in four.

Both sexes (54.5% [6/11] of females and 58.3% [7/12] of males) had animals with positive results

Table 1. Results from aerobic blood cultures on 37 samples of Antillean manatees (*Trichechus manatus manatus*), according to etiological agent, number of positive animals, percentage, sex, age group, and origin.^a

Etiological agent	No. positive samples	% (n = 37)	Sex	Age group	Origin
<i>Aeromonas caviae</i>	1	2.7	Male	Juvenile	CMA-AL
<i>Aeromonas hydrophila</i>	1	2.7	Male	Juvenile	CMA-AL
<i>Aeromonas</i> sp.	1	2.7	Male	Calf	CMA-PE
<i>Escherichia coli</i>	1	2.7	Female	Juvenile	CMA-PE
<i>Leclercia adecarboxulata</i>	1	2.7	Male	Adult	CMA-PE
<i>Pantoea agglomerans</i>	1	2.7	Male	Calf	CMA-PE
<i>Pseudomonas aeruginosa</i>	1	2.7	Female	Juvenile	CMA-PE
<i>Pseudomonas stutzeri</i>	1	2.7	Male	Adult	CMA-PE
<i>Pseudomonas</i> sp.	1	2.7	Male	Adult	CMA-PE
<i>Sphingomonas paucimobilis</i>	1	2.7	Female	Adult	CMA-PE
coagulase-negative <i>Staphylococcus</i>	1	2.7	Male	Juvenile	CMA-PE
<i>Staphylococcus epidermidis</i>	1	2.7	Female	Juvenile	CMA-PE
<i>Staphylococcus</i> spp.	2	5.4	1 female, 1 male	Calves	CMA-PE
<i>Vibrio fluvialis</i>	4	8.1	1 female, 3 males	Juveniles	CMA-AL

^aNo. positive samples = total number of positive samples of Antillean manatees for the type of etiological agent; % = percentage; CMA-AL = Brazilian National Center for Research and Conservation of Aquatic Mammals, located in Alagoas; CMA-PE = Brazilian National Center for Research and Conservation of Aquatic Mammals, located in Pernambuco.

from at least one sample. Similarly, all age groups (40% [2/5] of the calves, 66.7% [8/12] of the juveniles, and 50% [3/6] of the adults) and animals from both facilities (55.5% [10/18] at CMA-PE and 60% [3/5] at CMA-AL) had animals with positive results.

All animals in the study were clinically ill. Of the 37 samples collected from 13 animals when they were ill, 50% of them (11/22) were positive of Antillean manatees with debilitation and anorex-

ia, 12.5% of them (1/8) were positive of Antillean manatees with abscesses, and 42.9% of them (3/7) were positive of Antillean manatees with profound lethargy-moribundity.

DISCUSSION

This study provides the first report on isolation of bacteria from aerobic blood cultures from Antillean manatees. However, no direct compar-

Table 2. Results of percentage from aerobic blood cultures on 37 samples of Antillean manatees (*Trichechus manatus manatus*), according to identification of the animal, sex, age group, origin, and etiological agent.^a

Animal ID	No. positive samples	No. samples collected	%	Sex	Age group	Origin	Etiological agent
2	1	2	50	Male	Juvenile	CMA-PE	coagulase-negative <i>Staphylococcus</i>
6	1	5	20	Female	Juvenile	CMA-PE	<i>Pseudomonas aeruginosa</i>
9	2	3	66.7	Male	Adult	CMA-PE	<i>Leclercia adecarboxulata</i> , <i>Pseudomonas</i> sp.
10	1	3	33.3	Male	Juvenile	CMA-AL	<i>Vibrio fluvialis</i>
11	1	3	33.3	Female	Juvenile	CMA-PE	<i>Escherichia coli</i>
12	1	1	100	Female	Juvenile	CMA-PE	<i>Staphylococcus epidermidis</i>
13	1	1	100	Male	Adult	CMA-PE	<i>Pseudomonas stutzeri</i>
15	1	1	100	Male	Juvenile	CMA-AL	<i>Vibrio fluvialis</i> , <i>Aeromonas hydrophila</i> , <i>Aeromonas caviae</i>
16	1	1	100	Female	Juvenile	CMA-AL	<i>Vibrio fluvialis</i>
17	1	1	100	Male	Juvenile	CMA-AL	<i>Vibrio fluvialis</i>
18	1	1	100	Female	Calf	CMA-PE	<i>Staphylococcus</i> spp.
19	2	2	100	Male	Calf	CMA-PE	<i>Pantoea agglomerans</i> , <i>Aeromonas</i> sp., <i>Staphylococcus</i> spp.
23	1	1	100	Female	Adult	CMA-PE	<i>Sphingomonas paucimobilis</i>

^aNo. positive samples = total number of positive samples of Antillean manatees; No. samples collected = total number of samples collected of Antillean manatees; % = percentage; CMA-PE = Brazilian National Center for Research and Conservation of Aquatic Mammals, located in Pernambuco, Brazil; CMA-AL = Brazilian National Center for Research and Conservation of Aquatic Mammals, located in Alagoas, Brazil.

isons of these results could be made because no studies with descriptions of bacteria from blood cultures on marine mammals, either in captivity or free-living, were found. Therefore, comparisons were made between the bacteria found in these captive Antillean manatees in Brazil and isolates from other hosts that had been obtained through other culturing media and different biological materials.

In the 11 of 22 samples from 17 Antillean manatees that presented debilitation and anorexia, the following were found to be present: coagulase-negative *Staphylococcus*, *Staphylococcus epidermidis*, *Staphylococcus* spp., *L. adecarboxylata*, *P. stutzeri*, *Pseudomonas* spp., *V. fluvialis*, *A. hydrophila*, *A. caviae*, and *Sphingomonas paucimobilis*.

Coagulase-negative *Staphylococcus* is an organism that has been associated with true bacteremia, but these bacteria form part of the microbiota of the skin and it is difficult to know whether they are clinically significant. For this reason, the puncture site should ideally be prepared, as was done at the time of sample collection in the present study.

Staphylococcus epidermidis is coagulase-negative, which is an indication that this microorganism has low virulence. Nonetheless, some lineages have been found to be capable of causing diseases. Since the infections generated by this bacterium occur both as commensal infections of the skin and as environmental contaminants, they may have either endogenous or exogenous origin. Many of the infections are opportunistic and are related to trauma or immunosuppression, or are concurrent with parasitic or fungal infections, allergic conditions, or endocrine or metabolic disorders.¹³ *Staphylococcus* spp. may produce a variety of virulence and toxicity factors, frequently related to pyogenic infections. The significance of some of these factors remains unclear.⁹

The clinical importance of *L. adecarboxylata* is uncertain. It has previously been isolated from samples of blood, saliva, urine, feces, and wounds in both humans and healthy domestic pigs.⁹

Species of *Pseudomonas* have also commonly been isolated in aquatic environments and have been seen to cause bacterial diseases in marine mammals such as pinnipeds and cetaceans. Mixed infections involving *Pseudomonas* spp. and *E. coli* have been reported in manatees that presented with omphalitis with generalized peritonitis.¹⁷ Pinnipeds and cetaceans may have deep skin ulcers that cause damage to the dermis and lead the animals to vocalize. These animals may also develop septicemia due to infection by these

bacteria.¹⁰ These findings suggest that opportunistic infection by *Pseudomonas* spp. may also occur in manatees.

Species of *Vibrio* have been found in marine environments and have frequently been isolated from aquatic mammals. Several species have been identified as normal inhabitants of small cetaceans.³ However, they have also been correlated with primary or opportunistic disease, including chronic active dermatitis and necrotizing hepatitis, and possibly with acute bronchopneumonia in one case.¹⁵

Aeromonas spp. is present especially in aquatic environments and has previously been isolated from clinically healthy marine mammals.^{7,11} These microorganisms are capable of producing opportunistic disease, such as gastroenteritis, septicemia, respiratory tract infection, skin infection, and soft-tissue infection in fish, reptiles, and mammals.⁸ In a study on dugongs (*Dugong dugon*), *Aeromonas* spp. was identified as a potential pathogenic agent for pneumonia. Such findings may also be observed incidentally. *Aeromonas* spp. has frequently been isolated in cases of mixed infections, with reports of infections in both manatees and dugongs.¹²

Sphingomonas paucimobilis is present in the ground, water, and sediments. It has previously been isolated from blood, urine, wounds, and the vagina and uterine neck in humans, and from hospital environments.⁹ Its clinical significance in manatees is unclear.

In the only one of eight samples from four Antillean manatees that presented abscesses, presence of *Pseudomonas aeruginosa* was observed. This bacterium is responsible for bronchopneumonia, dermatitis, osteomyelitis, and septicemia in bottlenose dolphins (*Tursiops truncatus*) and has also been identified in other aquatic mammals held in captivity.^{1,2}

The bacteria found in three of seven blood samples of the four Antillean manatees that presented profound lethargy-moribundity were *Escherichia coli*, *Staphylococcus* spp., *Pantoea agglomerans*, and *Aeromonas* spp. *Escherichia coli* is one of the species most commonly isolated in clinical laboratories,⁹ and its presence has been correlated with infections affecting practically all tissues and organic systems. It is one of the microorganisms most commonly involved in sepsis due to gram-negative bacteria and shock induced by endotoxins in humans.⁹ In Antillean manatees held in captivity in Brazil, it has been observed that this bacterium is also present in the water in the animals' enclosure.¹⁶

Pantoea agglomerans has been isolated from plant and seed surfaces, from water, and from humans in wounds, blood, urine, and internal organs. It forms part of the family Enterobacteriaceae: these are gram-negative bacteria that produce a variety of toxins and virulence factors. This bacterium has been correlated with injuries involving plants.⁹

This paper presented the first description of isolates from blood cultures from captive Antillean manatees in Brazil and identified several species of bacteria. Although the clinical significance of many of these isolates is difficult to determine, it is important for them to be documented, so that a database of these species can be created. These bacterial species might be normal occurrences in aquatic mammals, or might be pathogenic or opportunistic. Further blood culture studies conducted on healthy animals might help interpret the results of this study. Moreover, further studies on prevalence and clinical correlations need to be conducted.

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