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Salmonellosis in an Antillean manatee (*Trichechus manatus manatus*) calf: a fatal case

Jociery Einhardt Vergara-Parente¹, José Júlio Costa Sidrim²,
Maria Fátima da Silva Teixeira¹, Milton César C. Marcondes³ and
Marcos Fábio Gadelha Rocha¹

¹School of Veterinary, Post-Graduation Program in Veterinary Science, State University of Ceará, Fortaleza-CE, Brazil, Avenida Dedé Brasil, 1700, Itaperi, Fortaleza, CE, 60740-000, Brazil

²Department of Pathology and Legal Medicine, School of Medicine, Medical Mycology Specialized Center, Federal University of Ceará, Fortaleza-CE, Brazil, Rua Monsenhor Furtado, sn, Bairro Rodolfo Teófilo, Fortaleza, CE, 60340-350, Brazil

³Aquatic Mammals Center/IBAMA, Estrada do Forte Orange, sn, Itamaracá, PE, 53900-000, Brazil

Abstract

A calf Antillean manatee (*Trichechus manatus manatus*) stranded on a beach along the Ceará coast of Brazil, was rescued and transferred to a Rehabilitation Center. This animal died 25 days later without apparent clinical symptoms. The necropsy showed an accumulation of fibrinous exudates deposited in the mesentery, on the intestinal surface and on the walls of the abdominal cavity. The mesentery was hyperemic, with areas of great congestion. Macroscopic alterations of the digestive tract were not observed. It was noted that certain regions of the liver showed altered consistency. The histological analysis showed necrotic alterations in the stomach mucosal and the caecum. The liver exhibited a discreet interstitial edema. Furthermore, samples of the liver and caecum were submitted for primary cultures in selective media. Following this procedure, a rapid serum agglutination assay was performed together with standard biochemical tests, which confirmed the presence of *Salmonella panama*. Therefore, it was concluded that the cause of death of the young *Trichechus manatus manatus* was an acute salmonellosis, caused by *Salmonella Panama*.

Key words: manatee, *Trichechus manatus manatus*, *Salmonella panama*, strand, death, mortality.

Introduction

The Antillean manatee (*Trichechus manatus manatus*), an herbivorous marine mammal, is distributed throughout the Atlantic Ocean stretching from Mexico to Brazil, where it inhabits the north and northeast coasts, the State of Ceará (Northeast

being the area of largest frequency in Brazil (Lima, 1997).

This species is classified as 'vulnerable' (VU A1cd, C2a) by the International Union of Conservation (Hilton-Taylor, 2000) and as 'critical danger of extinction' by the Plan of Action for the Marine Mammals of Brazil (IBAMA, 2001).

According to Elliot *et al.* (1981), the knowledge of diseases in sirenians is thus very limited, particularly when compared to other marine mammals. Although White & Francis-Floyd (1990) reported that the Antillean manatee is a marine mammal that is relatively resistant to diseases. Picanço *et al.* (1998) concluded that the first six months in the life of a manatee calf is critical for its survival in captivity.

In marine mammals, bacterial diseases are the main cause of mortality (Dunn *et al.*, 2001). Among the main agents that cause these diseases is *Salmonella* spp., which has been identified worldwide in various species of marine mammal (Gilmartin *et al.*, 1979; Stroud & Roelke, 1980; Howard *et al.*, 1983; Banish & Gilmartin, 1992; Baker *et al.*, 1995; Higgins, 2000).

The main objective of this study was to report a fatal case of Salmonellosis in a young Antillean manatee calf (*Trichechus manatus manatus*) in northeast Brazil.

Materials and Methods

A young female manatee, weighing 31 kg, was stranded on Canoa Quebrada Beach (4°33.5'S; 37°47'W) along the coast of Ceará, Brazil, on 29 March 2001. This animal was transferred to the Rescue and Rehabilitation Unit of the Aquatic Mammals Center/IBAMA, situated on Itamaracá

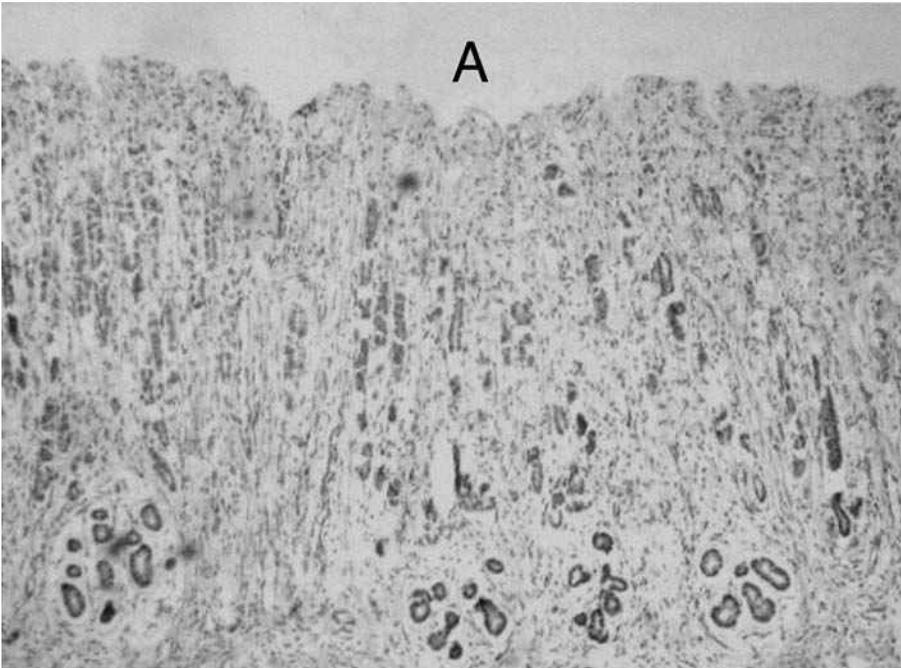


Figure 1. Stomach. (A) Necrotic alterations in the stomach mucosa of a calf Antillean manatee. H&E $\times 40$.

Island ($7^{\circ}44'52''S$; $34^{\circ}49'32''W$), in Pernambuco Brazil. It remained in isolation in a pool, each day receiving, four nursing bottles of a mix of powdered milk without lactose as described in Vergara *et al.* (2000). This animal (S0112/114) increased 3 kg in weight during the period of captivity.

The calf was kept in a circular fiberglass pool, with capacity of 5.15 m^3 which was filled with saline water of 18 ppm, filtered and chlorinated, and refilled twice a day. The physiochemical parameters of the water were evaluated daily and fecal coliforms were quantified twice on 5 and 19 April.

On 22 April, 25 days after the rescue, the animal died without any previous signs. The necropsy was carried-out according international protocol for Manatees (Bonde *et al.*, 1983). Tissue fragments were collected and preserved in a 10% formaldehyde solution, for histological analysis. The tissues were stained with hematoxilin and eosin (HE) and examined by a pathologist from the Adolfo Lutz Laboratory in Recife, Pernambuco, Brazil.

During the necropsy, specific tissue samples were chosen for microbiological analysis. Samples from liver, caecum, and peritoneal exudate were collected using sterile swabs (Culturette®) for the bacteriological procedures. The samples were cultured in different enriched media (blood agar, MacConkey agar, and chocolate agar) and incubated micro-aerophilic, at 37°C , for 24 h. In addition, rapid

serum agglutination and standard biochemical tests were performed.

Results

The necropsy showed an accumulation of fibrinous exudates deposited in the mesentery, on the intestinal surface, and on the walls of the abdominal cavity. The mesentery was shown to be hyperemic, with areas of accentuated congestion. The stomach was replete with food in the digestive process, without macroscopic alterations. There were no noteworthy alterations found in the intestines. The left lobe of the liver showed altered consistency, breaking-up when cut. On the posterior edge of the right lobe, although with an apparently normal consistency, the coloration was dark-red, differing from other parts of the organ that was bright red colour. In the respiratory tract, trachea was congested however the lungs were found to be unaltered macroscopically. The urinary bladder displayed areas of congestion.

From the histological analysis, it was observed by pathologist from the Adolfo Lutz Laboratory that the mucosa of the stomach exhibited necrotic alterations (Fig. 1), ectasia, and vascular congestion in the vessels of the internal and external muscular layers. In the caecum, were observed autolytic alterations in the mucosal membrane (Fig. 2). The

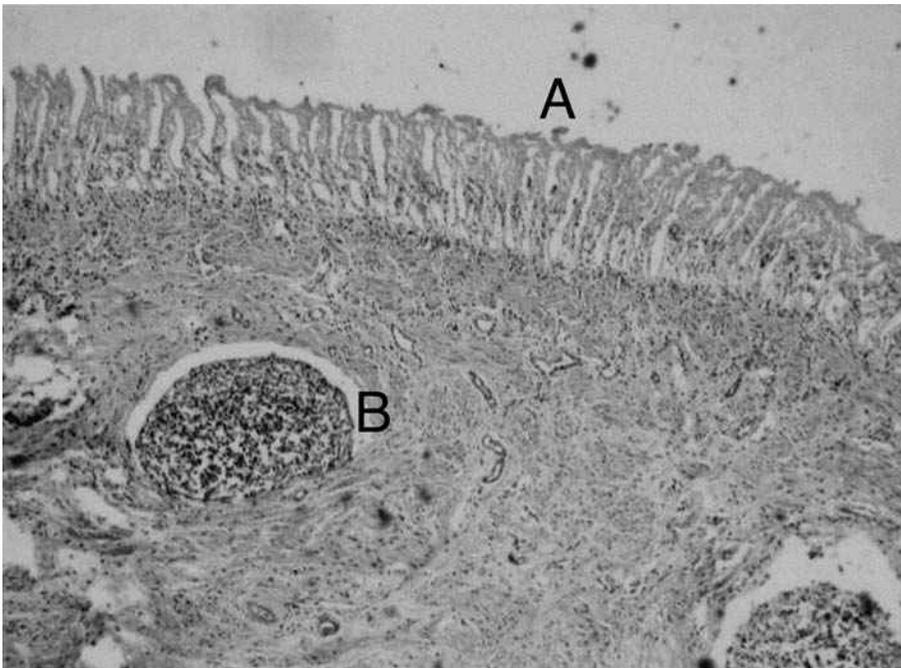


Figure 2. Caecum. (A) Necrotic alterations in the mucosa of a calf Antillean manatee. (B) Densification of a lymphoid follicle. H&E \times 40.

liver had only a discreet interstitial edema. The lung exhibited light capillary hyperaemia, ectasia, and congestion of the interstitial vessels. The kidneys showed moderate interstitial capillary hyperaemia in the medullar region. In the urinary bladder, an exudative inflammatory process was observed, severe and intense, characterized by diffusely distributed polymorphonuclear cells.

The presence of *Salmonella* spp. in the liver and caecum was detected by specific biochemical tests (triple-sugar-iron, Simmon citrate, arginine, ornithine, phenylalanine deaminase, MILi, Christensen urea, and Voges-Proskauer); as well as through the rapid serum agglutination assays (polyvalent and group E, both from Probac[®]). Subsequently, this microorganism was identified as *Salmonella panama*, at the Adolfo Lutz Institute, SP, Brazil, according to the methodology described by Ewing (1986) and following the Kaufmann & White model, outlined by Popoff & Le Minor in 1972 (Popoff & Le Minor, 1997).

Discussion

There are little researches done on behalf of the order Sirenia, in particular the Antillean manatee, mainly when compared to other marine mammals. This is a situation that intensifies with every

passing moment, due to the restricted size of the population, to the irregular distribution of the subspecies, and to the constant number of stranded calves present in Brazil.

The diseases caused by pathogenic bacteria, like Salmonellosis, are common causes of mortality in marine mammals. According to Howard *et al.* (1983), *Salmonella* was the most frequently isolated bacterium in deaths from septicemia in a study carried-out in cetaceans and pinnipeds.

The *Salmonella* genus has been of great importance for public health, when one considers its zoonotic character and its ample distribution in nature (Almeida *et al.*, 2000). Nowadays, more than 2435 serotypes of *Salmonella* are known (Popoff *et al.*, 1997) and only 80 or 90 are common in cases of infection in humans and animals. Salmonellosis generally is related to contaminated food and water intake. However it also can be transmitted through direct contact with animals and humans (Van Krunigen, 1998; Bopp *et al.*, 1999).

Salmonella has been noted worldwide, in diverse species of marine mammals, whether apparently sick or healthy, in captivity or in the wild (Gilmartin *et al.*, 1979; Stroud & Roelke, 1980; Banish & Gilmartin, 1992; Baker *et al.*, 1995). Cases of the *Salmonella* genus have occurred in cetaceans (Howard *et al.*, 1983) and principally in pinnipeds, in which the following species were isolated:

S. enteritidis, *S. newport*, *S. oranienburg*, *S. heidelberg*, *S. sieburg*, *S. minnesota* (Gilmartin et al., 1979; Stroud & Roelke, 1980; Banish & Gilmartin, 1992). In sirenians, there are records of *S. lohbruegge*, in a dugong (*Dugong dugon*) (Campbell & Ladds, 1981; Elliot et al., 1981) and *S. heidelberg* in two Florida manatees (Campbell & Ladds, 1981). There were no records for the subspecies (*Trichechus manatus manatus*) that exist in Brazil.

Animals, which are apparently healthy, but are hosts of *Salmonella*, may spread Salmonellosis as non-symptomatic carriers. These animals generally are adults, which have already recovered from an infection or which have been in contact with small quantities of the microorganism. These non-symptomatic carriers continue to eliminate *Salmonella* through faeces for months or even years (Corrêa & Corrêa, 1982; Campos, 2000).

Salmonella panama is a serotype that causes gastroenteritis in patients of all ages, with severe clinical manifestations, including septicemia (Olive et al., 1995). This agent has already been isolated in humans, octopus, seawater, sewage, and eggs, among others. The epidemiology of infections caused by *S. panama* is not well known (Soto et al., 2001).

In normal adult animals, Salmonellosis can cause enterocolitis, which in general disappears within a week. However, in young animals, systemic Salmonellosis are more prevalent, especially with regard to neonate animals. Stress can be an important cause to start the clinical illness, and the predetermining factors include, transportation, pathological alteration, irregular or inadequate feeding, crowded unsanitary conditions, and environmental extremes (Stroud & Roelke, 1980; Corrêa & Corrêa, 1992; Campos, 2000).

Failure in the transference of passive immunity is an important primary predetermining factor in neonatal septicemia caused by gram-negative bacteria, such as *Salmonella* spp (Corrêa & Corrêa, 1992). In this way, newborn calves that fail to receive maternal antibodies could succumb to bacterial or viral infections of the respiratory and digestive tracts (Fraser, 1991). Thus, if a manatee calf becomes separated from its mother immediately after birth, not having ingested the adequate colostrum, it will be an immunodeficient calf, which strengthens the thesis of Picanço et al. (1998), which affirm that the first six months of a manatee orphan's life are the most critical for its survival.

The alterations found in the microscopy of the stomach and caecum of calf from this study suggest severe infection caused by a virulent *Salmonella*, such as *S. panama*, which occur through the action of cytotoxins which destroy the epithelial cells of the intestine (Corrêa & Corrêa 1992; Olive et al., 1995).

Although the diffuse fibrinous exudate present in the abdominal cavity did not analyzed microscopically, this has been attributed to proinflammatory mediators that induce vascular alterations, increasing vascular permeability, establishing an analogy to that mentioned for the respiratory system by López (1998). As a result a substantial extravasation of liquids and proteins (fibrin) occurs. The fibrin accumulates at the peritoneal surfaces, eventually going on to form yellow plates (Sigal & Yacov, 1993).

The pathogenicity of the salmonellae vary according to the serotype, age, and health of the host (Campos, 2000). In the case considered here, the serotype, *S. panama*, caused gastroenteritis, with severe clinical manifestations including septicemia (Olive et al., 1995). The host was young; according Campos (2000) at this age Salmonellae may be manifested in a very serious form, frequently as a septicemia. Stress is considered by Corrêa & Corrêa (1992) as one of the major causes of the beginning of the clinical illness. The potential stressors in this case include maternal separation, stranding, and transport.

The septicemic form of Salmonellosis occurs following the penetration of the agent in the blood vessels, initially affecting the liver and subsequently the other organs such as lungs, spleen and kidneys (Corrêa & Corrêa, 1992). This was confirmed in this study, where, although without observing macroscopic lesions and with only small characteristic microscopic alterations, *Salmonella* was isolated, in the caecum, as well as in the liver. In septicemic cases, the most common scenario is sudden death from shock, due to the septicemia itself or to the large concentration of *Salmonella* endotoxins.

As regards to the source of infection, the following possibilities need to be considered: (1) The calf was contaminated by its mother, a possible unapparent carrier, before being stranded, or even in the marine environment. According to a study carried-out by Soto et al. (2001), where *S. panama* was isolated from seawater; (2) Contamination occurred in captivity in pools supplied with seawater or water from a cistern, or (3) The composition of the alimentary formula (which included eggs), was contaminated. According to Van Kruiningen (1998), the disease is transmissible between animals and humans and can be transmitted through fingers, flies, and utensils. In addition, death caused by Salmonellosis as a result of alimentation has already been recorded in two Florida manatees (*Trichechus manatus latirostris*) (Forrester, 1992).

Buck (1980) recommended that all animals newly introduced into a captive environment are subjected to microbiological examination, including culture for potentially pathogenic yeasts. Furthermore,

all animals and pool waters should be monitored periodically for the presence of these organisms. Another important factor to be considered is the need for the collection of material for bacteriological research during necropsies, ensuring a more exact evaluation of the agents involved in the mortality.

Extra caution in the care of animals in rehabilitation should be taken, aiming to prevent the presence of *Salmonellae* in the area. The zoonotic potential of *Salmonella* must also be considered, calling for special attention towards the people who handle or care for the animals. Research should be conducted for the diagnosis of *Salmonella* in captive marine mammals and people involved in the handling.

Finally, based on the macroscopic, microscopic, and bacteriological alterations, it is concluded that the causa mortis of this *Trichechus manatus manatus* calf was an acute case of Salmonellosis, caused by *Salmonella panama*.

Acknowledgments

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