

OCCURRENCE OF *CRYPTOSPORIDIUM* SPP. IN ANTILLEAN MANATEES (*TRICHECHUS MANATUS*) AND AMAZONIAN MANATEES (*TRICHECHUS INUNGUIS*) FROM BRAZIL

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Abstract: Infections by *Cryptosporidium* spp. in aquatic mammals is a major concern due to the possibility of the waterborne transmission of oocysts. The aim of the present study was to report the occurrence of *Cryptosporidium* spp. in Antillean manatees (*Trichechus manatus*) and Amazonian manatees (*Trichechus inunguis*) from Brazil. Fecal samples were collected and processed using Kinyoun's method. Positive samples were also submitted to the direct immunofluorescence test. The results revealed the presence of *Cryptosporidium* spp. oocysts in 12.5% (17/136) of the material obtained from the Antillean manatees and in 4.3% (05/115) of the samples from the Amazonian manatees. *Cryptosporidium* spp. infection was more prevalent in captive animals than in free-ranging specimens.

Key words: Coccidian, diagnosis, *Trichechus manatus*, *Trichechus inunguis*, sirenians, zoonosis.

INTRODUCTION

Among the aquatic mammals with critically compromised populations, the Antillean manatee (*Trichechus manatus manatus*) and the Amazonian manatee (*Trichechus inunguis*) are classified as vulnerable by the World Conservation Union.¹⁴ In Brazil, the Antillean manatee occurs in coastal marine habitats and river estuaries with the present distribution discontinuous along the northern and northeastern coast and the Amazonian manatees are distributed within the river systems of the Amazonian basin. According to the Brazilian Action Plan for Aquatic Mammals, marine species are "in critical danger of extinction" while the freshwater species are considered "vulnerable."¹³ These status levels for aquatic species are the result of various human-related causes that represent direct risks to these species including habitat degradation and loss, deforestation, pollution, predatory hunting,²¹ entanglement in fishing gear, and the stranding of orphaned calves.^{19,22,27}

Although the manatee is considered to be resistant to many diseases,³⁵ it is also susceptible to several agents with zoonotic potential.³ *Cryptosporidium* spp. have been diagnosed in dugongs (*Dugong dugon*)¹¹ and Antillean manatees.¹ Clinical findings such as weight loss, diarrhea, abdominal discomfort, and lethargy have been reported, along with the death of a dugong naturally infected by this coccidian.²⁴

According to Fayer et al.,⁴ contaminated water and food are the main transmission routes. Considering the predisposing factors such as the wide variety of food consumed by these animals in captivity, the clinical findings observed in infected individuals, the public health relevance of this disease, the dissemination and resistance of the causative agent to adverse environmental conditions, and the limited information on coccidian infection in Antillean and Amazonian manatees, it was judged necessary to conduct a study on these species. Hence, the aim of the present study was to determine the prevalence of *Cryptosporidium* spp. in Antillean and Amazonian manatees in Brazil.

MATERIALS AND METHODS

Sample collection

Samples were obtained from 34 Antillean manatees ranging in age from calves to adults. Twenty-eight of these animals were kept in pools in the state of Pernambuco, Brazil; two were maintained in an enclosure in an estuarine area in the state of Paraíba, Brazil; and four were rehabilitated-and-released specimens monitored through a satellite tracking system between the states of Sergipe and Rio Grande do Norte (northeastern Brazil). Four fecal samples were

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Table 1. Absolute and relative frequency of infection by *Cryptosporidium* spp. in Antillean and Amazonian manatees.

Origin	Antillean manatees		Amazonian manatees		Antillean manatees		Amazonian manatees	
	Animals	Samples	Animals	Samples	AF ^a	% RF ^b	AF	% RF
Pools	28	112	2	2	13	76.4	— ^c	—
Captivity in natural setting	2	8	0	0	2	11.8	0	0
Free ranging	4	16	10	10	2	11.8	2	40
Floating samples	0	0	—	103	0	0	3	60
Total	34	136	12	115	17	100	5	100

^a Absolute frequency.

^b Relative frequency.

^c — = unidentified.

collected from each specimen every 2 mo during the handling procedures.

Samples from Amazonian manatees were collected from 12 individuals ranging in age from calves to adults. Two of these individuals were kept in pools in the state of Pará (northern Brazil) and 10 were free-ranging individuals. Moreover, 103 fecal samples found floating free on the surface of Amanã Lake (state of Amazonas, northern Brazil) were collected during a field trip. Due to the difficulty in gaining access to these specimens, only one sample was obtained from each individual.

Prior to the handling procedures, all manatees were submitted to a clinical evaluation in which their health status, as well as the color, odor, and consistency of their feces, was recorded. The samples were placed in a mixture containing alcohol (ethyl alcohol 95% PA, Revitec Produtos para Laboratórios, São Paulo, SP, 04261-080, Brazil), formalin (formaldehyde PA, Revitec Produtos para Laboratórios), and acetic acid (glacial acetic acid PA, Revitec Produtos para Laboratórios) using a method described by Ueno and Gonçalves.³⁴ Parasites were identified, labeled, and sent to the Parasitic Diseases of Domesticated Animals Laboratory of the Universidade Federal Rural de Pernambuco, Brazil for confirmation.

Sample processing

The fecal samples were processed using the sedimentation method with formaldehyde-ether (formaldehyde PA and ethyl ether PA, both from Revitec Produtos para Laboratórios) and Kinyoun's acid-fast stain² (carbol fuchsin 620513, Interlab Distribuidora de Produtos Científicos, São Paulo, SP, 04330-130, Brazil) for the identification of *Cryptosporidium* spp. oocysts. Positive samples were also submitted to a direct immuno-

fluorescence test (Meridian Bioscience Diagnostics, Merifluor[®] *Cryptosporidium*/*Giardia*, Meridian Bioscience, Inc., Cincinnati, Ohio 45244, USA).³³

RESULTS

Cryptosporidium spp. oocysts were found in 25% (34/136) of the Antillean manatees, corresponding to 50% (17/34) of the total number of samples collected from this species. Among the samples from the Amazonian manatees, 4.3% (5/115) tested positive for the parasitic agent, 1.7% (2/115) having originated from captured individuals and 2.6% (3/115) from feces found on the surface of the water. Thus, the frequency of the coccidian was higher in the marine species (Table 1).

Among the Antillean manatees evaluated and diagnosed as positive for parasitic infection, 76.47% (13/17) exhibited clinical findings compatible with this coccidian such as diarrhea, abdominal discomfort, increased respiratory intervals, lethargy, and weight loss. However, not all these signs were always present in the same individual manatee. In Amazonian manatees with a positive diagnosis, it was not possible to evaluate these signs due to the difficulty locating the individuals, as most were free-ranging animals.

DISCUSSION

The results of the present study confirm the occurrence of *Cryptosporidium* spp. in Antillean and Amazonian manatees, with the marine species infected more intensively. Among the several transmission routes of this parasite, contamination of coastal waters by oocysts was incriminated as one of the possible ways that favor the waterborne transmission of the pathogen.^{5,8,10,15,17,20,32}

According to Robertson et al.,³⁰ *Cryptosporidium* spp. oocysts exhibit considerable resistance in

adverse environmental conditions, including those found in coastal^{13,7} and estuarine⁶ environments. These areas constitute the habitat of the Antillean manatee,^{18,26} which may have contributed toward the infection observed in the animals kept in captivity within an estuarine area and in the free-ranging individual.

The smaller number of infected samples from Amazonian manatees may be related to lesser contamination of the water, as observed by Queiroz,²⁹ a finding that indicates the integrity and protection of the Amanã Sustainable Development Reserve in the state of Amazonas. However, the sanitary problems in local communities, the release of feces into river habitats by boats, and the release of animal waste into lakes and black-water forest streams are important sources of contamination and may, therefore, favor the dissemination of *Cryptosporidium* spp. oocysts.

Besides these environmental factors, another important point that may have influenced the infection intensity of the Amazonian manatees was the impossibility of obtaining a larger fecal sample from the same individuals in different periods, as performed with the Antillean manatees in the present study. It should also be borne in mind that, on certain occasions, oocyst numbers in feces may be small due to the animal recovering from infection,⁴ thereby hampering the detection of the parasite.

Based on the results and on comparison of the two species, a higher number of positive samples were observed in captive manatees. This finding suggests that, beside the possibility of waterborne transmission, other factors may be associated with the dissemination of the *Cryptosporidium* including the wide variety of available food items (seagrass, seaweed, carrots, beets, lettuce, and occasionally fruit and water hyacinth), contact between animals and handlers, and the manipulation of food items by people who deal directly with these animals.^{12,16,23,25,31} The constant interaction among captive animals and the typical behavior of consuming feces observed in manatees⁹ may also exert an influence over infection rate.²⁸

The confirmation of clinical signs only occurred with the marine species. Among the 17 animals that tested positive for *Cryptosporidium* spp., 76.47% (13/17) exhibited diarrhea, abdominal discomfort, and increased respiratory intervals. In the absence of a detailed clinical investigation for the determination of the occurrence of other pathogens, the clinical manifestations cannot be attributed the coccidian alone, although it is likely that this parasite contributed to the symptoms.

Animals that tested positive, but did not exhibit any clinical manifestations, corresponded to 23.53% (4/17) of the marine species and 100% (2/2) of the freshwater species. The occurrence of asymptomatic reservoirs implies a certain resistance to the infection. However, these individuals continue to eliminate oocysts into the environment, which underscores their epidemiologic importance to the dissemination of cryptosporidiosis.

Considering the findings described herein and the possibility of the transmission of *Cryptosporidium* spp. oocysts, it is necessary that institutions dedicated to the rescue and rehabilitation of stranded marine mammals reevaluate their handling routines in order to help prevent the spread of this parasitic agent. Moreover, the best way to minimize infection in free-ranging populations is to stimulate public policies for basic sanitation and foster the conservation and maintenance of aquatic habitats.

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