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AEROBIC BACTERIAL MICROFLORA OF BROAD-SNOUTED CAIMAN (*CAIMAN LATIROSTRIS*) ORAL CAVITY AND CLOACA, ORIGINATING FROM PARQUE ZOOLÓGICO ARRUDA CÂMARA, PARAÍBA, BRAZIL

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ABSTRACT

The objective of this study was to isolate and identify the aerobic bacterial microflora from the oral cavity mucosa and cloaca's samples, collected from Broad-snouted caiman (*Caiman latirostris*), born and bred in captivity at Parque Zoológico Arruda Câmara, João Pessoa, Paraíba, Brazil. The most common bacteria were *Staphylococcus sp.* (14.74%), *Corynebacterium sp.* (13.68%), *Escherichia coli* (13.68%) and *Shigella sp.* (11.58%), and the less common were *Citrobacter sp.* (1.05%), *Klebsiella pneumoniae* (1.05%) and *Salmonella sp*. (1.05%).This emphasizes the importance of these microorganisms' participation in infectious processes (sepsis) and injuries caused by crocodilians.

Key words: *Caiman latirostris*, cloaca, crocodilians, microbiology, oral cavity

INTRODUCTION

The crocodilians are reptiles with semi-aquatic habits, generally large-sized and belonging to the Order Crocodylia, which counts with one Family and 03 Sub-families. There are divergences between some authors in relation to the number of species, since it varies from 22 to 28 (1,8,15).

In Brazil, there are six crocodilian species, all belonging to the Sub-family Alligatorinae (8): Spectacled caiman (*Caiman crocodilus*), Broad-snouted caiman (*C. latirostris*), Yacare caiman (*C. yacare*), Black caiman (*Melanosuchus niger*), Cuvier's dwarf caiman (*Paleosuchus palpebrosus*) and Schneider's dwarf caiman (*P. trigonatus*).

With the regulation of commercial raising of wild animals from the Brazilian fauna (2) and the normatization of Brazilian crocodilian skin's commercialization (3), a huge number of people became interested in practicing commercial activities involving these animals. According to data from Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA, 67 commercial establishments dealing with alligators are currently registered in Brazil (12).

The Broad-snouted caiman (*Caiman latirostris*), a median-sized crocodilian which can achieve 2.5m, is described by Bassetti (1) as the most watched species in captivity nowadays. This is a species threatened of extinction, most due to its habitat degradation, resulting from agriculture drainage and river pollution, and predatory hunt to supply the meat and skin trafficking (1,19)

This caiman species is classified as *In Low Risk / Less Concern (LR/LC)* in the Red List of the International Union for Conservation of Nature and Natural Resources - IUCN and integrates the Appendix 1 of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) (9).

Bassetti (1) supports the fact that raising Broad-snouted caiman in captivity, focusing on its economical potential, is

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extremely interesting, being considered as a benefic strategy on the management for this species conservation.

In captivity, most of the caiman's skin and mucosa injuries results from an aggressive behavior. Some studies indicate that an aggressive social behavior can be responsible for up to 5% of the mortality cases and 100% of the continuity solutions of a colony (18). Accidents with the staff involved on caimans' farms or the population living near its habitat can also occur. In the Amazonian region, these reptiles were responsible for 71 accidents with humans, registered at IBAMA between January 1990 and September 2001 (14).

The knowledge of the microflora present in caimans is extremely important to establish an adequate therapeutic conduct in cases of accident involving these animals and humans. In face of these facts, the present study was conducted to isolate and identify the bacteria from oral cavity and cloaca of captive Broad-snouted caimans from Parque Zoológico Arruda Câmara, João Pessoa, Paraíba, Brazil.

MATERIAL AND METHODS

The samples were obtained from 21 Broad-snouted caiman pups, with approximately 16 months of age, being born in captivity at Parque Zoológico Arruda Câmara, located in João Pessoa, Paraíba, Brazil. The sexing were not performed due to the difficulties inherent to the animals' size. The animals were kept in an area with 5 m^2, named "nursery", containing a tank and a sandy solarium. The tank water was by the municipal supplying company, being changes by the zoo workers daily. The pups were fed daily with bovine tissue obtained from a slaughterhouse certificated by federal inspection. All the individuals were clinically examined and none presented any clinical signs of illness.

During the samples collection the animals were physically restrained and a sterile swab was inserted on the cloaca and oral cavity (palate and tongue) of each individual. The swabs were kept chilled in an isothermal recipient and transferred to the Laboratório de Doenças Infecto-contagiosas of Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, where they were processed.

The material from the swab was planted on plates containing the culture medium agar with 8% sheep blood and Levine agar, being incubated in an aerobic environment at 37ºC for 72h. After the incubation period, the bacteria colonies were submitted to Gram staining and identified according to their morphologic characteristics and biochemical reactions (Triple sugar iron (TSI), Citrate, Lysine decarboxylase, Urea hydrolysis, Metil Red, Voges Proskauer, Motility and Indole production) (13).

RESULTS AND DISCUSSION

From the 42 samples (oral cavity and cloaca) obtained, an aerobic bacteria growth was observed in 93.24% (40/42). The absence of growth was verified in 4.76% (2/42) of the samples, which can be explained by the low concentration of microorganisms from the samples obtained with the swabs. Most of the bacteria species isolated belong to the Family Enterobacteriaceae (73.33%), being the rest (26,67%) identified as Gram+ microorganisms (Table 1). The high level of enterobacteria isolation may suggest fecal contamination of the tank water, since the animals spend most of their time underwater.

In a similar study, Ramos *et al*. (18) identified oral cavity bacteria from 19 Broad-snouted caiman, isolating Enterobacteriaceae in 17 animals (94.7%).

According to Cupul-Magaña *et al*. (4), studies on the crocodilian flora are scarce and the few existent only focus two species: the American alligator (*Alligator mississippiensis*) and the Broad-snouted caiman (*Caiman latirostris*). These same authors, working with free-ranging American crocodile (*Crocodylus acutus*), isolated *Aeromonoas hydrophila*, *Arizona* sp., *Citrobacter diversus*, *C. freundii*, *Enterococcus sp*., *Eschecrichia coli*, *Klebsiella pneumoniae*, *Neisseria sp*., *Pseudomonas sp* and *Strepetococcus viridans* from the animal's oral cavities. Such findings corroborate with the present work, which also found *E. coli* and *K. pneumoniae* in Broad-snouted caiman's oral mucosa. According to these authors, these animals are particularly pathogenic for humans and a single scar due to a bite can facilitate infection and cause sepsis. Generally crocodilians present a certain resistance to microbial infections. Blood chemistry studies on American alligators verified that serum from these animals presented anti-bacterial properties (16). However, Novak e Seigel (17) demonstrated sepsis cases in American crocodiles (*Alligator mississippiensis*), caused by six species from the Enterobacteriaceae Family (*Citrobacter freundii, Enterobacter agglomerans, Proteus* sp., *Morganella morganii, Serratia marcescens,* and *Klebsiella oxytoca*).

Microorganisms associations were present in 31 (73.81%) samples. From the 11 (26.19%) which did not presented any association, three were originating from oral cavity - *Staphylococcus sp.*, *Escherichia coli* and *Klebisiella pneumonae* - and eight from the cloaca - *Escherichia coli*, three times; *Enterobacter aerogenes*, twice; *Corynebacteirum sp.*, *Micrococcus sp.* and *Shigella sp.*, only one time each.

The most prevailing bacteria were *Staphylococcus sp*. (isolated 14 times), *Corynebacterium sp.* and *Escherichia coli* (13 times each) and *Shigella sp.* (isolated 11 times), being the less ones *Citrobacter sp.*, *Klebsiella pneumoniae* and *Salmonella sp.* (isolated once each). Researchers from Oswaldo Cruz Foundation in Manaus, Brazil, during a study with Brazilian crocodilian species which frequently attack fishermen in the Amazonian Region, identified seven species of bacteria from oral cavity, being the most prevalent *Pseudomonas cloacae*, *Escherichia coli* e *Enterobacter cloacae*. This last one was also isolated from fishermen' injuries made by these crocodilians

Table 1. Aerobic bacteria isolated from Broad-snouted caiman (*Caiman latirostris*) kept in captivity at Parque Zoológico Arruda Câmara, João Pessoa, Paraíba, Brazil.

Bacteria	Samples / Body location																																														
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17		18		19		20		21	
	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C
Arizona sp (4 / 4.21%)*				X										X										X				X																		
Citrobacter sp (1 / 1.05%)																																														X	
Enterobacter aerogenes (10 / 10.53%)				X	X	X					X				X	X	X			X									X								X									
Enterobacter cloacae (3 / 3.16%)				X												X																						X								
Enterobacter sp (2 / 2.11%)		X					X																																							
Escherichia coli (13 / 13.16%)	X		X					X		X	X	X							X					X					X	X	X		X											X	
Klebsiella pneumoniae (1 / 1.05%)																																				X										
Providencia alcalifaciens (3 / 3.16%)	X		X												X																															
Salmonella sp (1 / 1.05%)																									X																					
Shigella sonnei (6 / 6.32%)	X		X										X		X						X		X																								
Shigella sp (11 / 11.58%)												X	X		X		X						X						X	X				X	X			X		X						
Bacillus sp (4 / 4.21%)											X													X				X										X								
Corynebacterium sp (13 / 13.68%)		X		X				X			X					X	X				X	X			X	X	X								X					X					
Micrococcus sp (9 / 9.47%)		X										X		X				X					X		X	X				X					X										
Staphylococcus sp (14 / 14.74%)								X			X			X	X	X						X	X				X	X			X		X	X					X			X				X	

O = Oral cavity; C = Cloaca; * (n) = Number of time the species was isolated; ■ = Samples with only one isolated species; ■ = Samples without bacterial growth after 72 hours.

(14). In this work, the *Enterobacter cloacae* was isolated in 14.29% (3/21) of the animals. These findings indicate the importance of knowing the bacterial composition of the Brazilian crocodilian's oral mucosa. A successful treatment of the injuries caused by these animals - in captive specimens of the same colony, humans or even other species of wild and domestic animals - depends on the knowledge of the flora present in the oral cavity and the results of the antimicrobial sensibility test of the microorganisms isolated.

Although several of the isolated microorganisms are not pathogenic in natural conditions, they can become a problem in periods of high stress levels. The crocodilians are very sensitive to stress and, under such condition, the bacteria present in the intestinal tract can break the mucosa barrier and lead to a septic shock (11). Novack and Seigel (17) identified several microorganisms species in samples collected from captive American crocodiles (*Alligator mississippiensis*) which died of sepsis, being the most frequent *Citrobacter freundii*, *Enterobacter agglomerans, Proteus sp., Morganella morganii*, *Serratia marcescens* and *Klebsiella oxytoca*. The authors suggest that such bacteria were involved in the septic process which leads the animals to death. From these six species, three (*Citrobacter sp., Enterobacter sp.* e *Klebsiella sp*.) were found in the samples collected from the Parque Zoológico Arruda Câmara animals'.

The presence of *Salmonella sp.* in the reptiles' digestive tract has been reported extensively (5,6,7). All the serological variant of this bacteria are able to produce illness on the digestive tract and sepsis on humans and animals, and the infection can be related to the high levels of mortality (10). The identification of *Salmonella sp.* in the sample number 13 (from the cloaca of an animal on the present study) reinforce even more how important management is, in this case, particularly involving the Broad-snouted caiman.

In conclusion, there is a vast variety of aerobic bacteria isolated from the oral cavity and cloaca of captive Broad-snouted caiman from Zoológico Arruda Câmara, João Pessoa, Paraíba, Brazil. These bacteria can eventually be involved in pathological processes on this caiman species and other ones, including humans.

RESUMO

Microflora bacteriana aeróbica da cavidade oral e cloaca de jacaré-de-papo-amarelo (*Caiman latirostris*) procedentes do Zoológico de João Pessoa, PB, Brasil

O presente estudo teve como objetivo isolar e identificar a microflora bacteriana aeróbica presente na mucosa da cavidade oral e da cloaca de exemplares de jacarés-de-papo-amarelo (*Caiman latirostris*) nascidos e criados em cativeiro no Parque Zoológico Arruda Câmara, localizado na cidade de João Pessoa - PB. As bactérias mais freqüentes foram *Staphylococcus sp.* (14,74%), *Corynebacterium sp.*(13,68%), *Escherichia coli* (13,68%) e *Shigella sp.* (11,58%), e as menos prevalentes foram *Citrobacter sp.*(1,05%), *Klebsiella pneumoniae* (1,05%) e *Salmonella sp*. (1,05%). Ressalta-se a importância da participação desses microrganismos em processos infecciosos (septicemias) e em feridas provocadas por crocodilianos.

Palavras-chaves: *Caiman latirostris*, cloaca, crocodilianos, microbiologia, cavidade oral

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