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ENTEROBACTERIACEAE IN MOUTH AND CLOACA OF *PODOCNEMIS EXPANSA* AND *P. UNIFILIS* (TESTUDINES: CHELONIA) POPULATIONS OF NATIONAL PARK OF ARAGUAIA PLAINS, BRAZIL

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ABSTRACT

Shigella flexnerii and *Escherichia* coli were the most frequent Gram-negative bacteria found in the mouth cavity and cloacae of the turtles *Podocnemis expansa* and *P. unifilis* on beaches in the National Park of Araguaia, Brazil. Reptiles are known as *Salmonella* carriers, despite rarely isolated in these turtles.

Key words: *Salmonella; Shigella;* Enterobacteriaceae; Turtles; Bananal Island

The association of reptiles with human pathogens, especially *Salmonella,* has been largely documented (3, 8). Mermin *et al*. (11) suggest that reptile and amphibian exposure is associated with ~74,000 *Salmonella* yearly infections in the United States. Turtles have been involved as vectors of salmonellae in captivity as well as in the wild (8, 16). Moreover, other human enteric pathogens have also been isolated in turtles (18, 19).

Studies regarding the microbiota of Brazilian reptiles in the wild are rare, but Serafini *et al*. (21) showed that the Pantanal alligator (*Caiman crocodilus yacare*) and the "jacaré-tinga" (*Caiman crocodilus crocodilus*) carry *Aeromonas* sp., *Acinetobacter* spp., *Citrobacter freundii, Escherichia coli* and *Pseudomonas* sp. Abalem de Sá and Solari (1) found *Salmonella* spp. in 39.1% of Brazilian and imported pet reptiles, including chelonians. Ferronato *et al*. (7) isolated *E. coli, Klebsiella pneumoniae, Enterobacter aglomerans, C. freundii* and *Bacillus* sp. in oral samples of *Phrynops geoffroanus* turtles.

Our work aims at detecting Enterobacteriaceae in the mouth cavity and the cloacae of nesting *P. expansa* and *P. unifilis*, which are extensively used as food as well as for manufacturing utilitarian handicrafts by local populations dwelling along the Amazon and Araguaia/Javaés river basins (17). This causes pressure toward extinction, and also raises the issue concerning health risk to those human populations in contact with these reptiles.

This study is located within the National Park region of the Araguaia Plains (Fig. 1). Field collections were carried out in August and November 2005 under research license 081/04 - IBAMA/RAN. Eighteen female specimens of *P. expansa* were caught on the beaches during oviposition, and 30 males and females of *P. unifilis* captured in the river water while feeding and submitted to cloacal and mouth cavity swabbing.

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This research project has been approved by the Research Ethics Committee of the Federal University of Tocantins (*Universidade Federal do Tocantins*). The swabs were incubated in tubes containing Muller-Kauffmann tetrationate broth (Merck KGaA, Darmstadt, Germany) for one to four days at 35-37°C and taken to the laboratory where they were inoculated on Petri dishes containing EMB Agar (Merck KGaA, Darmstadt, Germany), McConckey Agar (Merck KGaA, Darmstadt, Germany) and SS Agar (Merck KGaA, Darmstadt, Germany) and then incubated for 24 h at 37°C. Typical colonies were purified and identified by Gram staining, TSI (Merck KGaA, Darmstadt, Germany) screening and API 20E™ (BioMerieux, Jacarepaguá, Rio de Janeiro, Brazil) kit tests. Only Gram-negative bacilli and cocobacilli were identified by API20E. APIWEB™ (BioMerieux, Jacarepaguá, Rio de Janeiro, Brazil) was used for identification. Results are expressed as the frequency of occurrence of each bacterial species found within each sampled individual, and two or more isolates from a single individual sample were considered as a single isolate. *Salmonella* serotyping was kindly carried out by LACEN-TO lab, according to standard procedures for human isolates.

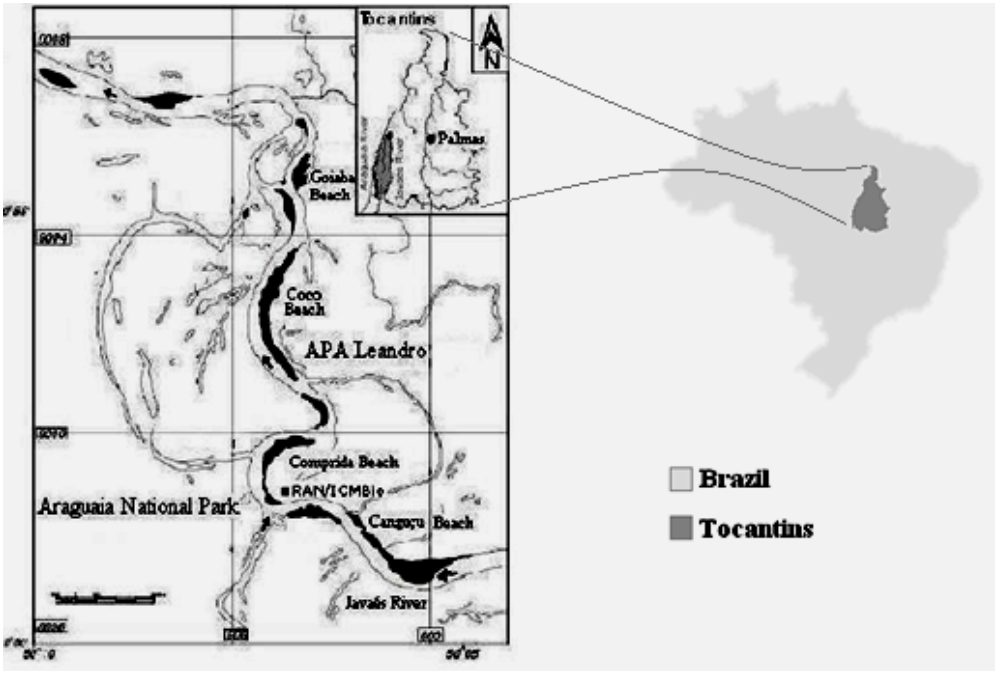

Figure 1. Location map of the study area at the National Park of Araguaia Plains with the nesting beaches of Canguçu, Comprida, Coco and Goiaba and the Javaés River. At the upper right side, the location of the National Park of Araguaia Plains in the Tocantins State, that is shown in the map of Brazil, at the right.

Thirteen species of Gram-negative bacteria were isolated from *P. expansa* and *P. unifilis. Chromobacterium violaceum* and *P. aeruginosa* along with two species of *Citrobacter* and one species of *Salmonella* were the most frequent bacteria from the mouth samples of 18 nesting *P. expansa* (Table 1). Five of the samples were negative for growth of enterobacteria and eight isolates could not be identified by the employed methods. *Acinetobacter calcoaceticus* and *C. violaceum* were the most

frequent species in the mouth of *P unifilis*, and one *Aeromonas* and two *Citrobacter* species, *E. coli, E. cloacae* and *Salmonella* Choleraesuis subsp. *arizonensis* were isolated at low frequencies along with four unidentified ones. All samples were positive for the presence of enterobacteria. *Shigella flexnerii* and *E. coli,* which comprised 28 out of 32 isolates from cloacal samples of *P. expansa*. Cloacal samples of *P. unifilis* resulted in the isolation of *S. flexnerii* (nine isolates), *E. coli* (six isolates) and *K. pneumoniae* subsp. *pneumoniae* (six isolates) and one isolate each of *C. youngae, H. alvei* and *S. ficaria.* Seven isolates from cloacae of *P. unifilis* and four from *P. expansa* showed a similar and unknown profile (profile 1 in Table 1) in API20E™.

Table 1. Frequency of occurrence of bacterial species in mouth cavity and cloacae of *P. expansa* and *P. unifilis* adults in four beaches of the Javaés River border of National Park of Araguaia.

Species	*P. expansa* (n[a] = 18)		*P. unifilis* (n = 30)	
	Mouth	Cloaca	Mouth	Cloaca
Aeromonas salmonicida subsp. *salmonicida*			1	
Acinetobacter calcoaceticus			4	
Chromobacterium violaceum	4		4	
Citrobacter freundii	1		1	
Citrobacter youngae	1		1	1
Escherichia coli		8	1	6
Enterobacter cloacae			1	
Hafnia alvei				1
Klebsiella pneumoniae subsp. *pneumoniae*				6
Pseudomonas aeruginosa	2		2	1
Salmonella Cholerasuis subsp. *arizonae*	1		1	
Serratia ficaria				1
Shigella flexnerii		20		9
Non-identified profile 1		4		7
Non-identified profile 2 to 3	8			
Non-identified profiles 4 to 8			12	
Total	17	32	28	32

[a] Number of samples.

The isolation of *Shigella* and *Klebsiella* associated with both turtle species may very well indicate health risk to humans consuming their meat. *Shigella* has rarely been reported as associated with turtles, however solely by Mahmoud *et al.* (10) who isolated *Shigella* spp. from the *Chelonya midas* oviductal fluid and by Dickinson *et al.* (6) from tortoises. David *et al.* (5) proposed that polluted water was the source of *Shigella* in the Nile tilapia in a Kenyan lake, but the Javaés river water presents as highly pristine, according to Morais *et al*. (13). Santoro *et al*. (18) verified that *K. pneumoniae* was the most common microbe identified and the *Enterobacteriaceae* family was the largest Gram-negative group of bacteria in 70 nesting green turtles (*Chelonia mydas*) from Tortuguero National Park, Costa Rica. Boede and Hernández (2) found *Klebsiella* spp. implicated in enteritis and dermatitis in the turtle *Pseudemis scripta*.

Similar to this study, *E. coli* was prevalent in the cloaca and feces of the estuarine diamondback terrapin (*Malaclemys terrapin*) (9) and *Phrynops geoffroanus* (7). Oros *et al*. (14) associated *E. coli* with lesions in *Caretta caretta*, and Raidal *et al.* (15) to juvenile mortality of *Chelonia midas*. Santoro *et al*. (19) found *Aeromonas* spp. and *C. freundii* but not *S. flexnerii,*

E. coli or *C. violaceum* as frequent bacteria in *Lepidochelys olivacea*, a chelonian from Costa Rica.

Salmonella was not obtained from cloaca of the chelonians and only two isolates were obtained from mouth samples. *Salmonella* Choleraesuis subsp. *arizonae* was also isolated from *P. unifilis* eggs in the same area (13). It might be possible that the failure to detect *Salmonella* in cloacal samples was due to a methodological bias. Harwood *et al*. (9) argues that the method of cloacal swabs for sampling presents a great deal of variability due to individual recent activities. In this study, recent oviposition by an individual turtle was not unlikely since the majority of *P. expansa* individuals were females captured on nesting beaches. Also, wild turtles are believed to shed *Salmonella* at lower rates than captive turtles because they either lack exposure to stressors that increase shedding rates or because they are not natural carriers of the bacterium (16).

We conclude that Enterobacteriaceae are part of the normal microbiota of mouth and cloacae of *Podocnemis expansa* and *P. unifilis* in the pristine area of Araguaia National Park and surrounding Plains, since sand and water are not contaminated as shown by Morais *et al.* (13). In regard to the fact that the cold-blooded turtles shed coliform bacteria, including *E. coli*, in their cloacae may have public health significance.

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