# Fatal *Citrobacter* Coelomitis in a Juvenile Green Turtle (*Chelonia mydas*): A Case Report

**Daphne W. Goldberg[1], Camila T. Cegoni[1], Daniel Wagner Rogério[1], Juçara Wanderlinde[1], Eron Paes e Lima[2], Renato Luiz Silveira[3], Hassan Jerdy[4] & Eulógio Carlos Queiróz de Carvalho[4]**

*[1]Fundação Pró-Tamar, Caixa Postal 5098, Trindade, Florianópolis, SC, CEP: 88040-970, Brazil (E-mail: daphne@tamar.org.br); [2]Centro Nacional de Proteção e Pesquisa das Tartarugas Marinhas (Projeto TAMAR), Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO), Rua Professor Ademir Francisco 140 , Barra da Lagoa SC, CEP: 88061-160, Brazil; 3Universidade Federal Fluminense, Rua Hernani Mello 101, Centro, Niterói, RJ, CEP:24210-130, Brazil; [4]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego 2000, Parque Califórnia, Campos dos Goytacazes, RJ, CEP: 28013-602, Brazil*

Gram-negative bacteria are the most common bacterial pathogens among sea turtles, which is not a surprising fact, because gram-negative bacteria are common isolates in healthy reptiles (Alfaro et al., 2006). This report describes the post mortem lesions in a juvenile green turtle (*Chelonia mydas*) that died during rehabilitation due to a severe coelomitis.

On 12 November 2014 a juvenile green sea turtle (*Chelonia mydas*) was rescued by Projeto Tamar (Brazilian sea turtle conservation program) after stranding on Jurerê beach, Florianópolis, Santa Catarina State, Brazil. On admission, the animal measured 60 cm curved carapace length, 52.5 cm curved carapace width, and weighed 14.9 kg. The turtle exhibited signs of cachexia, dehydration, lethargy, anemia (packed cell volume or PCV = 12%), positive buoyancy and it was covered with leeches and barnacles. The initial treatment consisted of ceftazidime (20 mg/kg IV), clindamycin (5 mg/kg IV), an injectable vitamin supplement and intravenous fluids.

Death occurred three days after initial supportive care and a complete necropsy, following a standardized protocol, was performed on the turtle, revealing a severe generalized coelomitis, with multiple cystic structures and bulky caseous masses of different sizes, throughout the serous tissues of the coelomic cavity (Figs. 1, 2, 3) and the following organs: liver, heart, lungs, stomach, large and small intestines, ovaries, oviduct and urinary bladder. On cut surfaces, the cystic structures had a typical onion appearance, with a fibrous layer lining it and multiple concentric laminated layers in its interior (Fig. 4).

Tissue samples were collected and fixed in 10% neutral formalin solution and sent to the Laboratory of Animal Pathology, Universidade Estadual do Norte Fluminense - UENF (Darcy Ribeiro State University of Northern Rio de Janeiro. Swabs were taken from the contents of the cysts/masses and sent to Citovet©, a private laboratory, in Florianópolis. In order to avoid contamination, the sampled structures and surrounding areas were cleaned with gauze and sterile saline and an incision was made over them with a #24 sterile blade, cutting through the fibrous layer into the cavity. Immediately after sampling, the swabs were placed in Stuart transport medium and kept under refrigeration.

Tissue samples were cleaved and packaged in disposable plastic tissue cassettes. Infiltration and blocking were performed in paraffin and leaf material was sliced into 5-µm-thick sections using a rotary microtome. Sections were stained with hematoxylin and eosin (HxE) and mounted on the slide for subsequent histopathologic examination by light microscopy.

The swabs were cultivated in BHI broth, MacConkey agar and Blood agar plates, which were incubated under aerobic conditions at 29 °C for 24 hours. After this time, the broths were seeded again in Blood agar and MacConkey agar plates. The plates were observed after 24, 48 and 72 h. Imprints of isolated bacteria were Gram-stained and morphologically characterized. The following tests were performed on all isolated strains: Gram-staining, motility, catalase and oxidase activity. Two species of bacteria were isolated from the swabs: *Citrobacter freundii* and *C. amalonaticus.*

The histological analysis revealed an inflammatory response characterized by an eosinophilic border containing large numbers of heterophils in different organs and its vessels, including liver, heart, lungs, stomach, large and small intestines, ovaries, oviduct

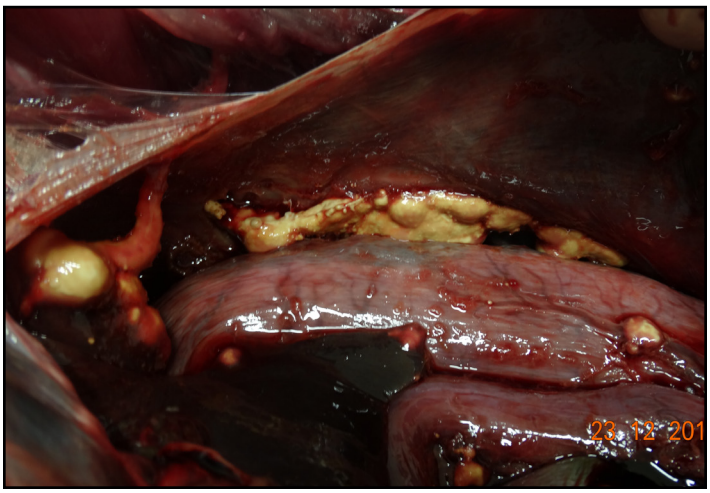

**Figure 1.** Multiple caseous yellow masses throughout the coelomic cavity of a juvenile green turtle.

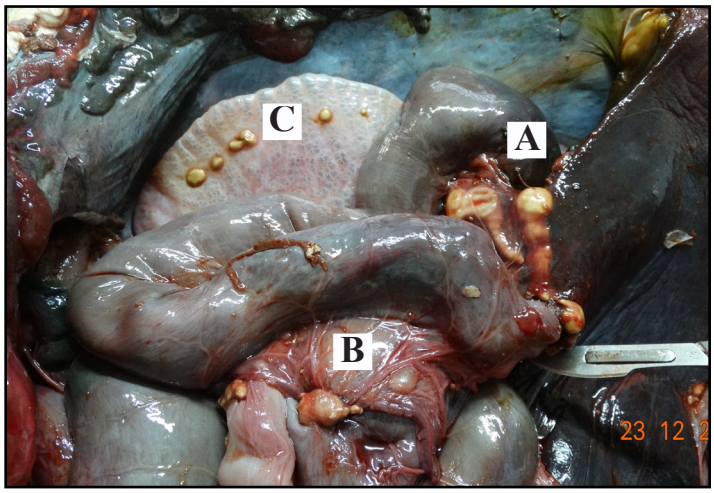


**Figure 2.** Multiple caseous yellow masses on the serosa of the liver (A), intestines (B) and lungs (C).

and urinary bladder (Figs. 5, 6, 7). The cystic structures had a fibrous layer lining it and multiple concentric laminated layers in its interior. On the other hand, the caseous masses (abscesses) also had a fibrous layer lining them, but a solid material of cheeselike consistency in the interior. Bacterial colonies were seen as thin basophilic granules within the abscesses and cysts.

*Citrobacter* species are straight, facultative anaerobic, Gram-negative bacilli and are typically motile by means of flagellae. They are commonly found in water, soil, food, and the intestinal tracts of animals and humans. Orós *et al*. (2005) associated the presence of *Citrobacter* sp. and other bacteria with exudative bronchopneumonia, granulomatous pneumonia, necrotizing granulomatous hepatitis, granulomatous nephritis and renal abscesses in sea turtles from the Canary Islands. Raidal *et al*. (1998) related bacteremia caused by *Citrobacter* sp., *Salmonella* sp., *Moraxella* sp. and *Escherichia coli* with vascular flukes in free-ranging green turtles from Western Australia.

Bacteria seem to play an important role in sea turtle diseases, both as primary pathogens and as opportunistic agents, infecting an injured or immunocompromised host (Alfaro 2008). Bacterial diseases are quite common in reptiles, with most infections caused by gram-negative bacteria, such as *Citrobacter* sp. (Alfaro 2008). However, the simple presence of bacteria in the bloodstream does not necessarily cause any symptoms. But if they start to proliferate and become persistent, particularly in hosts with a weakened immune system, bacteremia can lead to septicemia (Leekha *et al*. 2011), which is defined as a serious bloodstream infection that becomes systemic (Cooper 1983).

Because microbiological results do not become available for a week or two, an initial empirical therapy is often required, being frequently guided by the clinical signs of the patient. According to Novak & Seigel (1986), using broad-spectrum antimicrobial agents as initial empiric therapy is preferable to waiting for the test results. Data from medical literature show that delays in antimicrobial therapy lead to a significantly increased risk of mortality (Houck *et al.* 2004; Kumar *et al.* 2006). Especially critical for septic shock, the risk of dying increases by approximately 10% in humans, for every hour of delay in receiving antibiotics (Kumar *et al*. 2006).


**Figure 3.** Macro view of a cystic structure, with a fibrous layer lining it.

Once microbiology results are available and can identify the etiologic pathogen and its antimicrobial susceptibility, every attempt should be made to narrow the antibiotic spectrum. This is critically important to avoid toxicity, to reduce costs and to prevent bacterial resistance (Leekha *et al*. 2011). Although antibiotics are critical to fight bacteria, the successful treatment of infection diseases also requires appropriate husbandry, nutrition and the correction of predisposing factors (*e.g.*, stress, immunosupression, primary infections and others).

Our results indicate that the turtle died from acute coelomitis associated with *Citrobacter freundii* and *C. amalonaticus*. No evidence of clinically relevant infection by any other pathogens was found in the present case. Unfortunately, the source of infection remains unknown. Although we cannot positively determine whether these bacteria were primary pathogenic agents or secondary invaders, it seems likely that they are capable of producing disease in sea turtles.

***Acknowledgements.*** Projeto TAMAR, a conservation program of the Brazilian Ministry of the Environment, is affiliated with

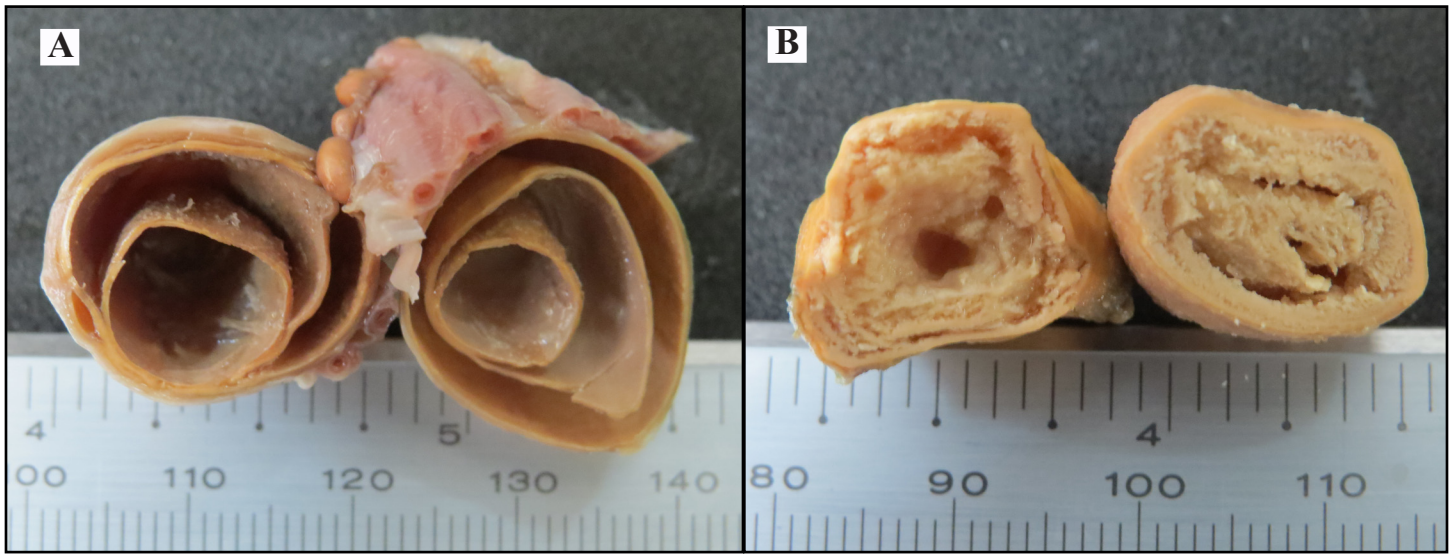


**Figure 4.** Gross appearance of a cut-open cyst (A) and of a cut-open caseous abscess (B).

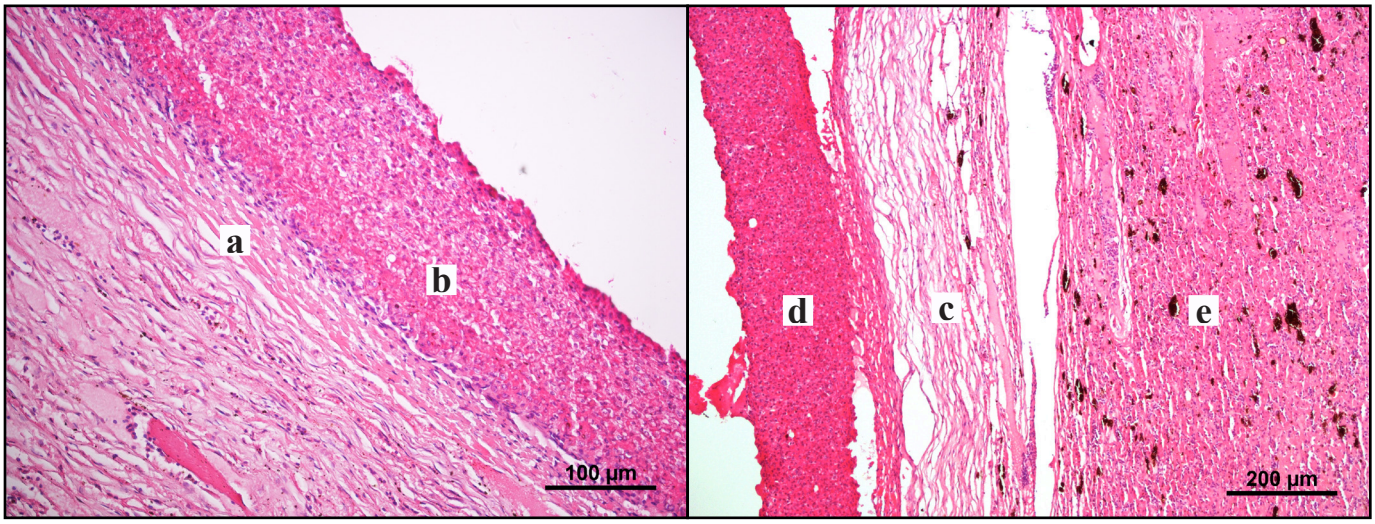


**Figure 5.** Left panel - Diffuse thickening of the coelomic membrane (a), which is overlaid by a granular eosinophilic material (caseous structure) (b). Right panel - Liver serous membrane (c) covered with caseous material (d). On the right, normal hepatic parenchyma with melanomacrophages (e), HxE.

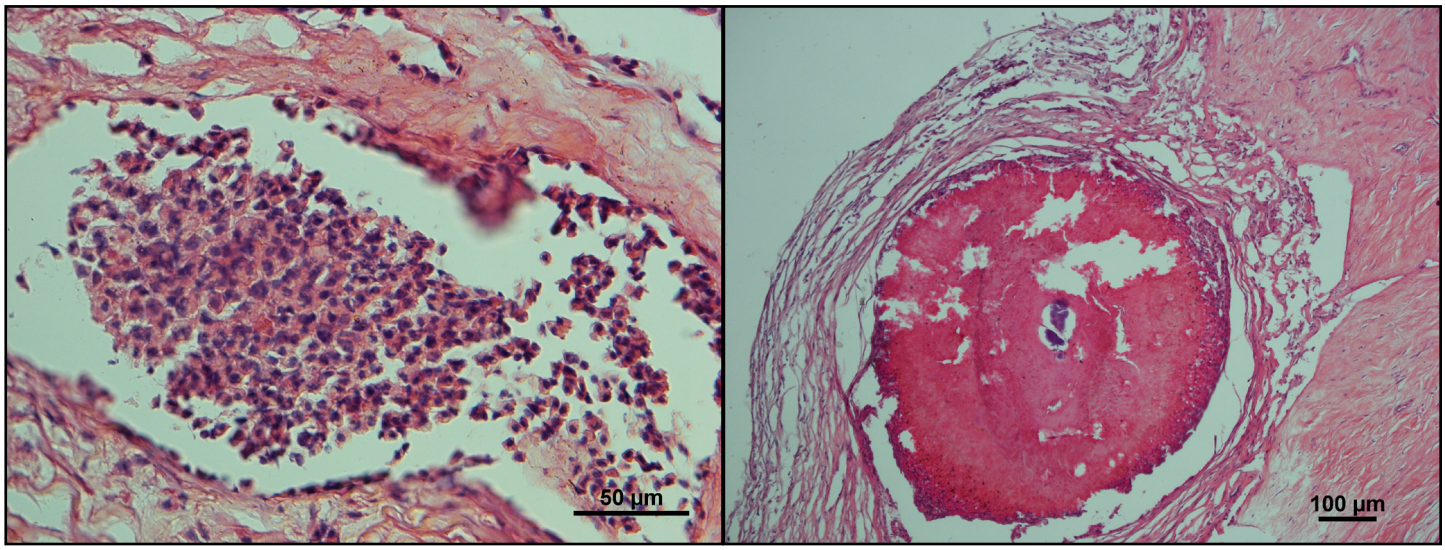


**Figure 6.** Left panel - Sea turtle lung. Pulmonary blood vessel with heterophilia. Right panel - Intestinal serosa. Caseous mass with a central basophilic substance (arrow), consistent with bacterial colony, HxE.

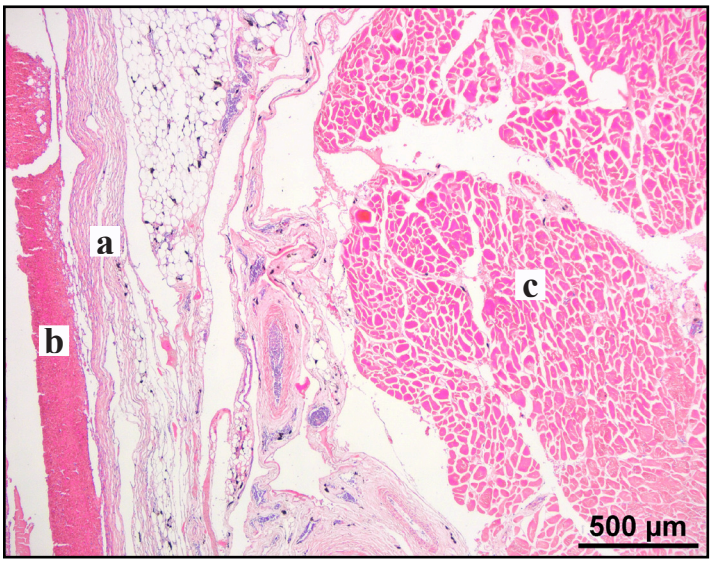


**Figure 7 (left).** Diffuse thickening of the pericardial membrane (a), which is overlaid by a granular eosinophilic material (caseous structure) (b). On the right, normal cardiac (striated) muscle (c), HxE.

ICMBio (Chico Mendes Institute for Biodiversity Conservation) and is comanaged by Fundação Pró-TAMAR. Data collection was authorized by ICMBio, through special license number 14122, issued by Biodiversity Authorization and Information System (SISBIO)

ALFARO, A. 2008. Synopsis of infections in sea turtles caused by virus, bacteria and parasites: an ecological review. In: A.F. Rees, M.G. Frick, A. Panagopoulou & K. Williams (Comps). Proceedings of the 27th Annual Symposium on Sea Turtle Biology and Conservation. NOAA Tech Memo NMFS-SEFC-569, p. 5.

COOPER, J.E. 1983. Diseases of the Reptilia. Volume 1 and 2. Academic Press. New York. 615pp.

HOUCK, P. M., D.W. BRATZLER, W. NSA, A. MA, & J.G. BARTLETT. 2004. Timing of antibiotic administration and outcomes for Medicare patients hospitalized with community-acquired pneumonia. Archives of Internal Medicine 164: 637-644.

KUMAR, A., D. ROBERTS, K.E. WOOD, B. LIGHT, J.E. PARRILLO, S. SHARMA, R. SUPPES, D. FEINSTEIN, S. ZANOTTI, L. TAIBERG, D. GURKA, A. KUMAR & M. CHEANG. 2006. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Critical Care Medicine 34: 1589-1596.

LEEKHA, S., C. TERRELL & R. EDSON. 2011. General principles of antimicrobial therapy. Mayo Clinic Proceedings 86: 156-167.

NOVAK, S.S. & R.A. SIEGEL. 1986. Gram-negative septicemia in American alligators (*Alligator mississippiensis*). Journal of Wildlife Disease 22: 484-487.

ORÓS, J., A. TORRENT, P. CALABUIG & S. DÉNIZ. 2005. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998-2001). Diseases of Aquatic Organisms 63: 13-24.

PINERA-PASQUINO, L. 2006. Patterns of antibiotic resistance in bacteria isolated from marine turtles. Master Thesis.

RAIDAL, S.R., M. O'HARA, R.P. HOBBS & R.I.T. PRINCE. 1998. Gram-negative bacterial infections and cardiovascular parasitism in green sea turtles (*Chelonia mydas*). Australian Veterinary Journal 76: 415-417.

# LETTER TO THE EDITORS

## Exertional "Rigor Mortis" Preceding Death in Sea Turtles - A Research Opportunity

The paper by Phillips *et al.* (2015) on exertional myopathy in a juvenile green turtle (*Chelonia mydas*) reminded me of my observations of stiffening of propulsive lateral muscles in live channel catfish (*Ictalurus punctatus*) experimentally subjected to strenuous exercise (Caillouet 1967). At the time, I likened this "stiffening" to physiological contracture and rigor mortis, and suggested that it might contribute to death (Caillouet 1967).

The time lapse between death and development of rigor mortis in humans can be used to estimate time of death, but Chakravarthy (2010) described a case of "rigor mortis" in a live patient.

If pre-death muscle stiffening occurs in sea turtles subjected to forced submergence or cold-stunning, this phenomenon may be worthy of further research as a possible contributor to delayed mortality (Caillouet 2012).

CAILLOUET, C.W., JR. 1967. Hyperactivity, blood lactic acid and mortality in channel catfish. Iowa State University Agriculture and Home Economics Experiment Station Research Bulletin 551: 898-915.

CAILLOUET, C.W. JR. 2012. Does delayed mortality occur in sea turtles that aspirate seawater into their lungs during forced submergence or cold stunning? Marine Turtle Newsletter 135: 1-4.

CHAKRAVARTHY, M. 2010. Rigor mortis in a live patient. The American Journal of Forensic Medicine and Pathology 31: 87-88.

PHILLIPS, B.E., S.A. CANNIZZO, M.H. GODFREY, B.A. STACY & C.A. HARMS. 2015. Exertional myopathy in a juvenile green sea turtle (*Chelonia mydas*) entangled in a large mesh gillnet. Case Reports in Veterinary Medicine 2015: Article ID 604320.

**Charles W. Caillouet, Jr.,** *119 Victoria Drive West, Montgomery, Texas 77356 USA (E-mail: waxmanjr@aol.com)*