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MELIOIDOSIS IN A BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) AFTER A HURRICANE IN THE CARIBBEAN ISLANDS

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Abstract: Melioidosis is an emerging infectious disease of humans and animals caused by the bacterium *Burkholderia pseudomallei* and endemic in tropical regions, principally Southeast Asia and northern Australia. In September 2017, after Hurricane Maria impacted the Dolphin Discovery facility in the Federation of St. Kitts and Nevis, a juvenile male bottlenose dolphin (*Tursiops truncatus*) died within 96 hr of presenting with acute anorexia, lethargy, and respiratory distress. Histopathology demonstrated necrohemorrhagic bronchopneumonia, necrotizing hepatitis, splenitis, and lymphadenitis, with intralesional Gram-negative bacilli. *B. pseudomallei* was confirmed by bacteriological culture and DNA sequencing. This case emphasizes the challenges of melioidosis diagnosis, the importance of awareness for both early detection and efficacious treatment, and recognition in tropical regions where it has been either not reported or underreported. To the authors' knowledge, this is the first case of cetacean melioidosis in the Caribbean Islands, an often severe and fatal disease with increasing prevalence on the American continent.

Key words: Bottlenose dolphin, *Burkholderia pseudomallei*, cetacean, marine mammal, melioidosis.

BRIEF COMMUNICATION

Burkholderia pseudomallei is a saprophytic Gram-negative bacillus found in soil and surface water. It is highly pathogenic, classified as a biosafety level 3 and a potential biological terrorism agent, and causes melioidosis. Melioidosis is a serious and often fatal disease of humans and a wide range of animals[12] affecting zoo, wildlife, domestic, and laboratory animals across many taxonomical groups, including aquatic and terrestrial mammals, birds, and reptiles.[14] There is variation in species susceptibilities and clinical manifestations; therefore, many other diseases may resemble melioidosis clinically.[13,14] Melioidosis is endemic mainly to tropical and subtropical climates, particularly northern Australia and Southeast Asia; however, it has been increasingly recognized in the Western Hemisphere, including Central America, South America, and the Caribbean.[4,10,13]

Melioidosis was first reported in marine mammals in 1975 when 24 dolphins died at Ocean Park, an oceanarium in Hong Kong.[6] Most documented cases of melioidosis in marine mammals are from this facility, with a preponderance of infections occurring during the rainy "typhoon" season.[1,5,7–9,11] Acute septicaemic melioidosis was the most common clinical presentation in cetaceans and associated with high mortality.[7]

In September 2017, the Dolphin Discovery facility in St. Kitts, located in the dual island Federation of St. Kitts and Nevis of the Leeward Islands chain of the Lesser Antilles, was affected by Hurricane Maria, a Saffir-Simpson category 5 storm. The facility is an open-water enclosure, surrounded by a breakwater wall and support meshes that allow natural seawater flow. Dolphins are housed in a 3,700 m^2 area with an average depth of 5 m, divided in a main lagoon and two holdings. A group of 11 bottlenose dolphins (*Tursiops truncatus*) was evacuated 500 me by truck to an outdoor saltwater pool during the passing of Hurricane Maria in St. Kitts. This pool, located uphill approximately 13 m above sea level, was selected because it was close by and protected from storm surge. The average evacuation time per dolphin was 15 min. The dolphins showed normal attitude and appetite while evacuated. Four days after the hurricane passed and following repairs, the dolphins were transported back to their original facility. The next day, a 6-yr-old male showed reduced activity and decreased appetite, rapidly progressing to anorexia, lethargy, and respiratory distress and dying within 96 hr. Relevant symptoms, abnormal hematologic and biochemical findings compared with reference values,[2] and treatment are summarized in Table 1. No other dolphins showed clinical signs, and all

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Table 1. Symptoms, relevant and abnormal clinical laboratory results, and treatment for a 6-yr-old bottlenose dolphin (*Tursiops truncatus*) during the 4-day course of acute fulminating melioidosis.

	Day 1	Day 2	Day 3	Day 4
Symptoms	Low activity Decreased appetite	Lethargy Anorexia	Lethargy Anorexia Respiratory distress	Lethargy Anorexia Respiratory distress
Clinical analysis[a]				
WBC (10^3/μl, 5–9)	5.35	5.75	2.3	4.6
Neutrophil (mature) (10^3/μl, 3.23–4.85)	4.066	4.485	1.518	3.082
Neutrophil band (%)	0	0	6	8
ESR (at 60 min, 4–17)	13	25	32	24
ALT (U/L, 28–60)	144	110	151	198
AST (U/L, 190–300)	428	507	798	1,296
GGT (U/L, 30–50)	57	84	88	113
LDH (U/L, 350–500)	2,184	2,369	10,319	>10,000
ALP (U/L, 300–1,300)	313	232	36	33
Antibiotic therapy[b]				
Ofloxacin	600 mg PO BID	600 mg PO BID	—	—
Amikacin			1.7 g IM SID	1.7 g IM SID
Ceftriaxone			2.3 g IM SID	2.3 g IM SID
Metronidazole				800 mg PO BID
Other treatments[b]				
Hydration	1.5 L PO BID	1.5 L PO BID	1.5 L PO BID	2 L PO TID
Diazepam	15 mg PO SID	15 mg PO SID	—	—
Dexamethasone			20 mg IM SID	10 mg IM SID
Doxapram			100 mg IM SID	—
Tramadol		100 mg IM SID	100 mg IM TID	100 mg IM BID

[a] Units followed by reference interval.[2] WBC = white blood cell count; ESR = erythrocyte sedimentation rate; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = γ glutamyltransferase; LDH = lactate dehydrogenase; ALP = alkaline phosphatase.

[b] Doses were calculated for 115 kg estimated body weight. PO = orally; BID = twice a day; IM = intramuscular; SID = once a day; TID = three times a day. Dashes indicate drug was discontinued.

remained apparently healthy for the months following the hurricane.

At necropsy, numerous off-white to yellow nodules, 1–5 mm in diameter and containing thick, similarly colored exudate, were disseminated throughout the lungs, liver, spleen, and lymph nodes (Fig. 1). These were grossly interpreted as abscesses. Several foci <1.5 cm of subpleural hemorrhage were also observed in both lungs. The pericardium and peritoneal cavity contained 10 and 20 ml, respectively, of yellowish, slightly turbid thin fluid.

Histology demonstrated lesions consistent with acute septicemia, including severe acute multifocal necrohemorrhagic bronchopneumonia and acute necrotizing hepatitis, splenitis, and lymphadenitis, with intralesional Gram-negative bacilli (Fig. 2). In the lung, alveoli were filled with hemorrhage, neutrophils, necrotic cell debris, and few lymphocytes and macrophages. Severely affected areas showed microvascular thrombosis. Multifocal necrosis predominated in other organs, consisting of eosinophilic cytoplasmic debris and remnants of pyknotic and karyorrhectic nuclei, admixed with fibrin and variable but generally few neutrophils. Bacilli were long, slender rods and most readily identified in foci of necrosis, present extracellularly in loose tangles forming colonies or within the cytoplasm of degenerate neutrophils.

Pericardial and peritoneal fluid, liver, lung, tracheal, and bronchial swabs were plated semiquantitatively to blood, chocolate, phenylethyl alcohol, and MacConkey agar and incubated at 30°C. Blood agar yielded heavy growth of smooth creamy-white colonies within 24 hr, progressing to large mucoid colonies within 72 hr. Small light pink colonies also grew on MacConkey agar within 24 hr and progressed to heavy growth of large pink colonies by 72 hr. Gram stain showed short Gram-negative rods with bipolar staining. The bacteria were motile, and biochemical testing showed the isolate to be indole negative and oxidase positive. Sensitire™ Gram-negative GNID ID Plate

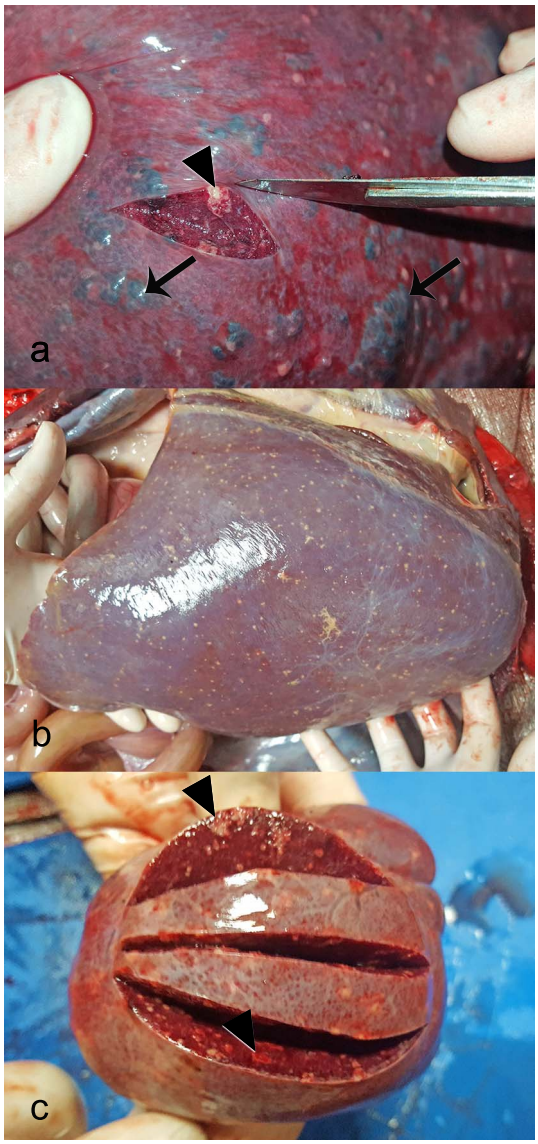

Figure 1. Gross lesions of a bottlenose dolphin (*Tursiops truncatus*) that died from melioidosis. A. Lungs show numerous pale yellow round foci representing abscesses (arrowhead) as well as foci of subpleural hemorrhage (arrows). B. The capsular surface of the liver bears similar pale yellow foci representing foci of necrosis. C. The cut surface of the spleen shows similar pale yellow foci representing foci of necrosis (arrowheads).

(ThermoFisher Scientific, Waltham, MA 02451-02454, USA) failed to identify the isolate. The isolate was sent to Eurofins Genomics LLC in the United States for 16s rRNA polymerase chain reaction and sequencing, and results were consistent with *B. mallei/pseudomallei*. The final

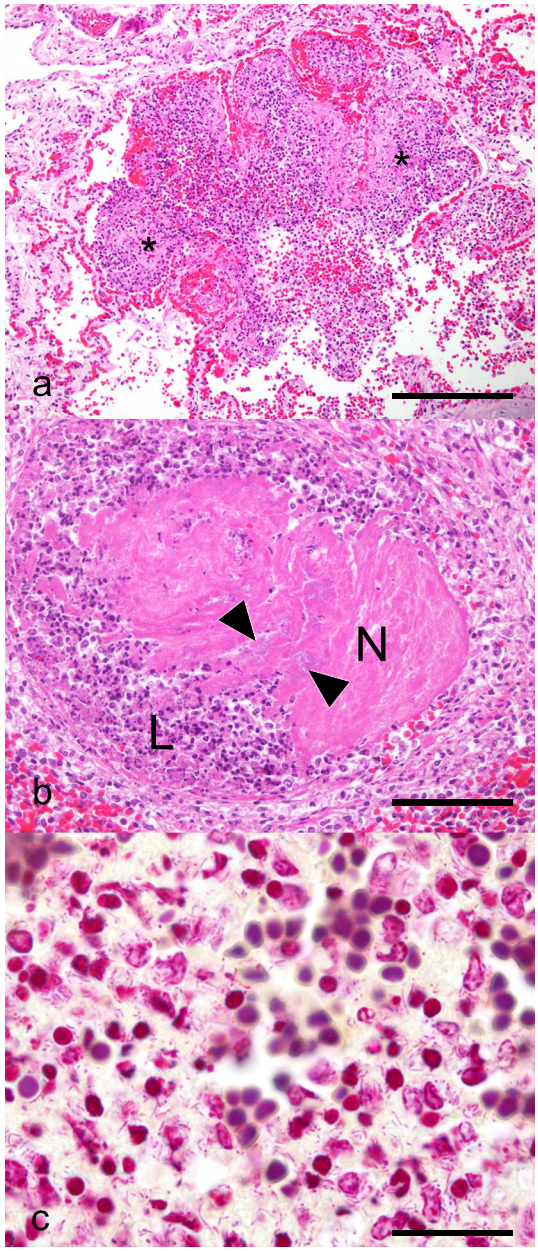

Figure 2. Microscopic lesions of a bottlenose dolphin (*Tursiops truncatus*) that died from melioidosis. A. Necrosuppurative and hemorrhagic bronchopneumonia showing alveoli filled with erythrocytes, leukocytes, and necrotic debris (asterisks), H&E, bar = 100 μm; B. Necrotizing splenitis characterized by areas of necrosis (N) variably infiltrated by neutrophils (L) and intralesional bacteria colonies (arrowheads), H&E, bar = 50 μm. C. Long slender rods within the cytoplasm of degenerate neutrophils in an area of pneumonia, Brown Hopps Gram stain, bar = 20 μm.

identification of *B. pseudomallei* rather than *B. mallei* was based on colony morphology, growth on MacConkey agar, bipolar Gram staining, the presence of motility, and oxidase positivity. Sensitivity studies showed that *B. pseudomallei* isolate was resistant to two of the antibiotics administered, amikacin and metronidazole, whereas sensitivity was not assessed for ofloxacin and ceftriaxone. The isolate was sensitive to ceftazidime, imipenem, meropenem, and amoxicillin/clavulanic acid.

The source of infection was suspected to be contamination of seawater with soil or exposure through dispersal and possible aerosolization of *B. pseudomallei* during hurricane winds and heavy rain. The facility's main lagoon was virtually divided into three areas, and 100-ml samples of seawater, muddy soil from the surrounding area, and seafloor sand were collected in each area. These nine samples were cultured using similar methods, but the organism was not isolated, and the specific source of the bacterium remained undetermined.

To the authors' knowledge, this is the first report of melioidosis in a cetacean in the Caribbean Islands and the first report of melioidosis in the Federation of St. Kitts and Nevis in any species.[13] Melioidosis is an emerging disease, increasingly present in the Americas and likely endemic in the Caribbean, Mexico, and Central and South America.[10,13] Although cases of human melioidosis have been reported during the rainy season in the Caribbean islands, the public health concern has not been fully recognized in the region, and the disease may be more common than currently appreciated.[10,13] There is only a single report of melioidosis in animals of the Caribbean islands, involving sheep, goats, and pigs in Aruba from the 1950s.[13,15] Cetacean melioidosis has rarely been observed in the Western Hemisphere. Two Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) were diagnosed with melioidosis in a dolphinarium on the west coast of Mexico. One dolphin died, but the other survived after being administered appropriate antimicrobial therapy following the isolation of the microorganism (Ibarra, pers. comm.). Marine mammals seem to be especially susceptible to *B. pseudomallei* infection[7] and could be important sentinel species for human health risk, particularly in regions where melioidosis is not well documented. This occurred in Hong Kong, where melioidosis was diagnosed in humans only after it was first recognized in dolphins.[7]

Diagnosis of melioidosis may be challenging due to its protean clinical manifestations and the lack of rapid reliable diagnostic tests, particularly in areas where the disease occurs sporadically.[13] Isolation of *B. pseudomallei* from clinical samples using Ashdown's selective agar is the "gold standard" for diagnosis of melioidosis, but it may take 3–7 days.[7,13] The organism also grows on standard laboratory media, such as MacConkey, sheep blood, and chocolate agar, but it may be confused with closely related bacteria in laboratories that have limited experience with *B. pseudomallei*.[13] As seen in this case, *B. pseudomallei* is predictably resistant to many broad-spectrum antibiotics.[3] Treatment of septicaemic melioidosis consists of two phases: an intensive phase that involves intravenous antimicrobial therapy for 2 wk, followed by long-term oral treatment or eradication therapy for 20 wk, necessary to prevent relapse. Ceftazidime or carbapenems are the antibiotics of choice for treating melioidosis during the acute phase and amoxicillin/clavulanic acid for eradication therapy in both human and cetacean infections.[3,7]

In the present case, the clinical presentation and pathological features of the disease were consistent with septicemia, the acute fulminating form of melioidosis, similar to previously described cases of cetacean melioidosis.[5,7,11] Treatment for septicemia was initiated soon after presentation, but melioidosis was not a primary differential and was diagnosed only postmortem. Therefore, the elected antimicrobial therapy was not effective. In addition, steroids were administered, which are considered a risk factor for melioidosis in humans and may have potentiated disease progression. Pyrexia seems to be a nonspecific but consistent early indicator of melioidosis,[5,7,11] and although body temperature was not measured in this case, it may be a useful tool for monitoring marine mammals after inclement weather where melioidosis is of concern.

It is crucial to include melioidosis as a differential diagnosis for dolphins showing an acute onset of anorexia, pyrexia, and lethargy, especially in tropical areas and after an extreme weather event. Cetacean melioidosis is a rapidly fatal disease, and appropriate antimicrobial therapy may need to be initiated while pursuing a definitive diagnosis.

This case underscores the importance of melioidosis as a potential emerging infectious disease in tropical areas of the American continent. It is important that the medical and veterinary communities are aware of its presence in this region because of the severity of disease resulting in high

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2019-0050R1

mortality rates, challenges in diagnosis and treatment, the range of susceptible species, and its public health significance.

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