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MYCOBACTERIUM ABSCESSUS PNEUMONIA IN AN ATLANTIC BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*)

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Abstract

Mycobacterium abscessus pneumonia was diagnosed antemortem in a 23-yr-old male Atlantic bottlenose dolphin (*Tursiops truncatus*). Clinical signs included lethargy, hyporexia, coughing, and bloody respiratory discharge. Diagnostic findings included neutrophilic leukocytosis, anemia, elevated erythrocyte sedimentation rate, and repeated forceful exhaled breath (sputum) cytology, with acute inflammatory cells and acid-fast positive beaded rods. The bacteria were initially identified free in the sputum sample and subsequently were seen within neutrophils. A culture was positive for a rapidly growing, white, colony-forming organism confirmed as *M. abscessus* by polymerase chain reaction and DNA sequencing. Clinical signs initially resolved with multidrug therapy. Concurrent *Pseudomonas aeruginosa* infection complicated clinical management and contributed to terminal decline. The dolphin was euthanized 5 mo after initial diagnosis. Necropsy results demonstrated acid-fast positive bacteria in lung tissue and supported the diagnosis of *M. abscessus* pneumonia. Acid-fast stains and mycobacteria cultures should be considered when evaluating ill dolphins.

Keywords

Bottlenose dolphin; clarithromycin; computed tomography; *Mycobacterium abscessus*; pneumonia; *Tursiops truncatus*

BRIEF COMMUNICATION

This report describes the diagnosis and management of *Mycobacterium abscessus* pneumonia in a 23-yr-old male Atlantic bottlenose dolphin (*Tursiops truncatus*) at the National Aquarium, Baltimore, Maryland. The dolphin was wild caught more than 20 yr ago and had been housed at the National Aquarium for 18 mo. Significant medical history included chronic hepatopathy (Table 1). At the time of presentation, the dolphin was housed in an indoor artificial saltwater facility with eight other bottlenose dolphins.

Initial clinical signs included lethargy and hyporexia. Results of a physical examination demonstrated no significant abnormalities, and the weight was 235 kg. Hematologic and biochemical abnormalities included neutrophilic leukocytosis with a left shift, anemia,

increased erythrocyte sedimentation rate, reduced alkaline phosphatase value, and elevated hepatic transaminases and γ -glutamyl transpeptidase (GGT) values (Table 1). Cytology and culture of fasted gastric fluid, forceful exhaled breath (sputum), and fecal samples were normal. Differential diagnoses included bacterial hepatitis, cholangiohepatitis, and bacterial pneumonia. Symptomatic and supportive treatment with antibiotics, gastroprotectants, and water was initiated (Table 2).

Serial examinations and laboratory work remained abnormal over a 5-wk interval (Table 1). Multiple cytology samples were normal during this period. The dolphin lost weight and condition. Abdominal ultrasound (Sonosite180, SonoSite, Bothell, Washington 98021, USA) demonstrated focal areas of mildly increased hepatic hyperechogenicity. Cholangiohepatitis was the primary differential based on elevated GGT. Medications were modified without improvement in clinical signs or laboratory values (Tables 1, 2). Prednisone (0.25 mg/kg p.o. s.i.d. for 3 days) treatment was initiated for hepatic anti-inflammatory and secondary orexigenic properties. The dosage was reduced over 14 days to 0.04 mg/kg p.o. every 36 hr. Clinical signs resolved within days of starting prednisone but recurred when the administration interval was increased to 48 hr, and, therefore, dosing was continued every 36 hr. Hepatic function support medications were begun (Table 2).

Six wk after initial examination, the animal was presented with acute coughing and weakness and recurrent lethargy and hyporexia. Sputum cytology demonstrated acute inflammation with increased neutrophils. Gastric and fecal cytology samples were normal. Multiple sputum samples over the ensuing days demonstrated continued inflammation and atypical gram-positive, acid-fast positive beaded rod-shaped bacteria. Bacterial morphology was consistent with nontuberculous mycobacteria. Culture on Mitchison agar (7H11) at 37°C resulted in rapidly growing (<48 hr) colonies.

During wk 7, the dolphin developed bloody respiratory discharge, and severe weakness. Endoscopy of the upper respiratory tract demonstrated frank blood, blood clots, and foamy hemorrhagic fluid in the nasal passages. Acid-fast positive bacteria were present in respiratory secretions (including within neutrophils), gastric, and fecal samples. Prothrombin time (17.5 sec) was comparable with previous values (12.2–17.2 sec; $n = 14$) and inconsistent with coagulopathy as the etiology for bleeding. A presumptive diagnosis of pulmonary mycobacteriosis was made based on clinical signs, foamy hemorrhagic fluid, and diagnostic testing. The organism was identified as *M. abscessus* at the University of Texas Mycobacteria–Nocardia Laboratory via polymerase chain reaction (PCR) and DNA sequencing.

Multidrug antibiotic therapy was instituted with amikacin (2,300–2,800 mg i.m. s.i.d.) and azithromycin (1,500 mg p.o. s.i.d. for 5 doses, 1,000 mg p.o. s.i.d.). Imipenem (1,000 mg i.m. b.i.d.) was initially administered for 4 days. Based on sensitivity results, azithromycin was changed to clarithromycin (750–1,750 mg p.o. b.i.d.) at approximately 2 wk. Nebulization with amikacin 4 ml, acetylcysteine 2 ml, and 0.9% NaCl 3 ml driven by oxygen was conducted for 5 min s.i.d. or b.i.d.

From 8–21 wk after presentation, clinical signs resolved, and cytology samples normalized except for scant but persistent acid-fast positive bacteria in sputum samples. Although hematologic and biochemical values remained largely unchanged, the dolphin was behaviorally normal 12 wk after presentation and regained weight (Table 1). The dolphin's condition acutely declined with severe lethargy, weakness, anorexia, coughing, and tachypnea 22 wk after presentation. Bloodwork demonstrated increased neutrophilia (Table 1). Gastric fluid was purulent, characterized by severe neutrophilic inflammation. Other cytology samples were normal. Culture of gastric fluid produced repeatable heavy, pure

growth of *Pseudomonas aeruginosa*. Differential diagnoses included secondary pseudomonas infection; recrudescence of mycobacterial infection; and gastritis, gastric abscess, or gastric ulceration.

Wk 23–29 after presentation, the dolphin's clinical condition continued to decline. Blood-work and gastric fluid cytology samples remained abnormal. Gastric endoscopy revealed minor (<1 cm, superficial) esophageal ulceration but was otherwise normal. Pulmonary interstitial pattern consistent with pneumonia was radiographically evident. Thoracic ultrasound demonstrated severe, generalized lung tissue consolidation. Thoracic computed tomography demonstrated cavitating pulmonary lesions and significantly increased, generalized parenchymal thickening. Based on clinical decline and severity of underlying disease, the dolphin was euthanized (Table 3).

Gross necropsy revealed multifocal to coalescing chronic bronchopneumonia with multiple cavitating lesions, fibrinous pleuritis, and severe multifocal atelectasis with more than 70% of the pulmonary tissue abnormal. The liver was congested. Microscopic examination of lung revealed diffuse pyogranulomatous bronchopneumonia without discrete granuloma formation. Rare acid-fast positive bacterial rods were found within pulmonary macrophages on impression smears and tissue sections. The liver had moderate chronic-active periportal hepatitis with bridging fibrosis and biliary hyperplasia. Hemosiderin deposits were present in the liver and kidney. Culture results included *P. aeruginosa* isolation from bronchial fluid, lung abscess, gastric fluid, and fecal samples. Bronchial fluid was positive for *M. abscessus* based on both culture and PCR with DNA sequencing.

Mycobacterium abscessus is a rapidly growing, environmental mycobacterium commonly found in soil and water. Molecular testing such as PCR with DNA sequencing is necessary to differentiate *M. abscessus* from closely related organisms, for example, *Mycobacterium chelonae*.⁵ In humans, *M. abscessus* pulmonary infection is associated with chronic pulmonary disease, such as cystic fibrosis, although infection in patients who are immunocompetent and without preexisting disease has also been reported.^{5,10} *M. abscessus* does not appear to be transmitted horizontally.⁵ Infections in humans are managed with long-term antibiotic therapy and surgical resection of infected tissue when appropriate.^{5,7} Treatment goals for pulmonary infection include symptomatic improvement, radiographic regression of disease, and reduction in positive sputum cultures, but full resolution is rare.⁵

Environmental exposure to nontuberculous mycobacteria is expected in cetaceans and measurable titers to *Mycobacterium marinum*, *Mycobacterium fortuitum*, and *M. chelonae* have been reported in wild bottlenose dolphins.¹ Disease associated with *M. marinum*, *M. chelonae*, *Mycobacterium mageritense*, and *M. abscessus* has been reported in cetaceans.^{2,3,9,11} *Mycobacterium abscessus* was reported in a beluga whale (*Delphinapterus leucas*), with mammary gland and cutaneous infection diagnosed antemortem.³ Treatment with amikacin, enrofloxacin, and clarithromycin did not resolve infection, and the whale was euthanized.³ In this case, antibiotic administration did not fully resolve infection based on histology, but clinical condition and sputum cytology improved. Respiratory mycobacteria shedding was evaluated by serial sputum cytology but repeated cultures would be more consistent with monitoring in humans.⁵ Postmortem pulmonary mycobacteria culture was negative despite positive histology, likely due to low organism numbers. *Pseudomonas* overgrowth can contribute to clinical decline in human cases and was identified in this case.⁶

Blood serum levels for clarithromycin and the 14-hydroxy active metabolite were evaluated for a 3.6 mg/kg dose at the National Jewish Research Center. Clarithromycin levels at 3.25 hr and 7 hr were 9.75 µg/ml and 2.50 µg/ml, respectively. The 14-hydroxy active metabolite levels at 3.25 hr and 7 hr were 2.86 µg/ml and 2.50 µg/ml, respectively. These levels were

considered consistent with those used in humans for managing infection. (Carroll, Perl, pers. comm.)

Due to pulmonary disease, nebulization was used during management. Nebulizing efficacy is not documented in cetaceans, but the procedure was tolerated during manual restraint treatments and ultimately trained as a voluntary behavior. The device in this case was designed and fabricated in-house but a nebulizer (Dolphin Nebulizer, Taylors Pharmacy, Winter Park, Florida 32789, USA) for cetaceans is now available.

Initial presenting signs may have been due to *M. abscessus* infection, but this cannot be confirmed. Earlier thoracic diagnostic imaging would have provided improved pulmonary evaluation. Acid-fast stain and mycobacteria cultures were performed after identification of a gram-positive beaded rod on sputum cytology during wk 6 after presentation. It is possible that bacteria were shed earlier and not identified. The dolphin had chronic liver disease, and hemosiderin deposits were supportive of iron storage disease.⁸ Chronic prednisone administration was used at a dosage not typically associated with immunosuppression. Chronic dexamethasone administration at 0.016–0.096 mg/kg s.i.d. in combination with altrogenest was speculated to contribute to cutaneous *M. chelonae* infection in a dolphin.¹¹ Immunosuppression from chronic liver disease or drug administration cannot be excluded, but *M. abscessus* pulmonary infection may develop in humans who were immunocompetent.⁵ It is currently unknown how immune status or pulmonary condition relates to *M. abscessus* infections in bottlenose dolphins.

Repeated positive acid-fast cytology or *Mycobacterium* culture on diagnostic samples should be considered abnormal in bottlenose dolphins in closed, artificial saltwater systems.⁴ As in this case, nontuberculous mycobacteria infection may have scant numbers of bacteria on histology, and organisms may only be appreciated with the appropriate staining technique.^{2,11} The lack of severe granulomatous inflammation should not preclude consideration of mycobacterial infection. Nontuberculous mycobacteriosis should be considered in animals with chronic leukocytosis or respiratory disease. Appropriate staining and culture techniques are required to identify these organisms.

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Table 1

Summary of select hematologic and biochemical values in a bottlenose dolphin (*T. truncatus*) with *M. abscessus* pneumonia.

Value	Pre-illness			Wk 1-12			Wk 13-21			Wk 22-29			Normal ^a Range
	Average	n	Range	Average	n	Range	Average	n	Range	Average	n	Range	
White blood cell/ μ l	8,188	8	6,050-11,550	25,396	19	13,475-35,585	28,435	3	25,300-31,570	49,518	9	25,080-78,430	5,000-9,000
Neutrophil/ μ l (mature)	5,396	8	4,477-8,432	17,952	19	11,723-24,523	22,821	3	19,228-26,203	30,200	9	11,888-54,901	3,230-4,850
Neutrophil/ μ l (band)	378	8	0-1,304	2,833	19	248-9,964	2,044	3	947-4,048	13,326	9	752-29,564	0
Lymphocyte/ μ l	969	8	666-1,130	1,022	19	340-1,848	832	3	631-1,012	1,219	9	509-2413	840-1,660
Hematocrit (%)	41	8	39.5-45	37.5	19	33-40	35	3	34.5-36	38	9	35-41	38-44
Erythrocyte sedimentation rate (mm/hr)	2.3	7	1-8	58	19	27-86	27	3	10-45	40	3	35-50	4-7
Total serum iron (μ g/dl)	368	7	234-491	375	19	193-591	309	2	236-381	228	6	69-372	120-340
Alkaline phosphatase (U/L)	625	6	573-651	208	18	120-317	204	3	191-228	125	6	99-180	300-1,300
Alanine transaminase (U/L)	88	7	71-102	112	18	74-191	66	3	63-69	65	6	51-79	28-60
Aspartate transaminase (U/L)	868	6	665-1,060	789	18	621-1,198	440	3	424-462	443	6	343-526	190-300
glutanyl transpeptidase (U/L)	309	6	261-354	954	18	562-1,343	315	3	298-334	250	6	186-280	30-50

^aBossart, G. D., T. H. Reidarson, L. A. Dierauf, and D. A. Duffield. 2001. Clinical Pathology. In: Dierauf, L. A., and M. D. Frances (eds.). CRC Handbook of Marine Mammal Medicine: Health, Disease, and Rehabilitation. CRC Press, Boca Raton, Florida. P. 393.

Table 2

Summary of medications administered to a bottlenose dolphin (*T. truncatus*) with *M. abscessus* pneumonia (wk 1–6).

Medication	Dose and route	Frequency ^a	Wk in which administered
Enrofloxacin	1,156 mg p.o.	b.i.d.	1, 2
Metronidazole	1,625 mg p.o.	b.i.d.	1–3
Ranitidine	750 mg p.o.	s.i.d. or b.i.d.	1–6
Sucralfate	2,000 mg p.o.	b.i.d.	1
Water or 0.9% NaCl	1–2 L p.o.	p.r.n.	1–6
Amikacin	3,290 mg i.m.	s.i.d.	1–3
Ampicillin	2,350 mg i.m.	s.i.d.	1
Amoxicillin	2,500 mg p.o.	b.i.d.	1–4
Ciprofloxacin	1,125 mg p.o.	b.i.d.	2–6
Nystatin	750,000 IU p.o.	t.i.d.	2–6
Clavulanate-amoxicillin	2,250 mg p.o.	b.i.d.	4–6
Cefixime	400 mg p.o.	b.i.d.	6
Clindamycin	300 mg p.o.	b.id.	6
Milk thistle	300 mg p.o.	s.i.d. or b.i.d.	6
Artichoke extract	325 mg p.o.	b.i.d.	6

^a Actual administration may have varied due to inconsistent eating.

Table 3

Euthanasia protocol in a bottlenose dolphin (*T. truncatus*) with *M. abscessus* pneumonia.

Medication	Dose and route	Dosage (mg/kg)
Midazolam	30 mg i.m.	0.15
Butorphanol	20 mg i.m.	0.1
Pentobarbital sodium with phenytoin sodium ^a	19,500 mg and 2,500 mg i.v., respectively	95 and 12, respectively

^a Administered 15 min after midazolam and butorphanol, the dolphin was deeply sedated and had no visible reaction to injection.