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Coccidioidomycosis in a Bottlenose Dolphin

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ABSTRACT: A stranded bottlenose dolphin (*Tursiops truncatus gilli*) succumbed to a pulmonary infection of *Coccidioides immitis*. The dolphin initially presented with mild inspiratory dyspnea that rapidly worsened over 48 hr to include buoyancy abnormalities and finally death. At necropsy, caseous nodules were observed throughout the lungs and perihilar lymph nodes. On histological examination of tissues, double walled organisms containing endospores characteristic of *C. immitis* were observed in lung, perihilar lymph nodes, and brain. Pyogranulomatous infiltrates were observed in the lung and perihilar lymph nodes only. A DNA Gen-Probe test performed on a purified isolate confirmed infection by *C. immitis*. Serum was positive for antibodies to *C. immitis* at a titer of 1:128 and was negative for all known marine morbilliviruses. Although there have been reports of *C. immitis* infections in free ranging marine wildlife, including California sea lions (*Zalophus californianus*) and sea otters (*Enhydra lutris*), this is the first reported case of coccidioidomycosis in a cetacean.

Key words: Bottlenose dolphin, case report, *Coccidioides immitis*, coccidioidomycosis, fungal disease, pulmonary disease, mycosis, *Tursiops truncatus gilli*.

An adult female bottlenose dolphin (*Tursiops truncatus gilli*) was rescued from a beach in La Jolla, California (117°15'W/32°51'N) and taken to Sea World of California's beached animal quarantine facility, on 3 June 1995 (San Diego, California, USA). On presentation, the dolphin appeared very thin, weighed 167 kg (normal weight might be expected to be 250 kg), and displayed mild inspiratory dyspnea. A blood sample was drawn, culture swabs of the blowhole and anus were smeared on blood and Sabouraud's agar (Remel, Lenexa, Kansas, USA) for microbiology, and the dolphin was then placed into a 9 × 9 × 1.2 m deep pool.

The dolphin initially appeared alert, swam normally, and after 3 hr began to eat

voluntarily and swim strongly in a counterclockwise fashion. At nearly the same time her inspiratory dyspnea worsened and she began to list to the right when resting. A CBC, serum fibrinogen, and serum chemistries were performed which provided evidence of moderate inflammation including neutrophilia, left shift, lymphopenia, eosinopenia, elevated erythrocyte sedimentation rate, hyperfibrinogenemia, and low serum iron (Table 1).

Based on the severe respiratory clinical signs and the abnormal hematologic findings, a tentative diagnosis of bacterial pneumonia was made and trimethoprim sulfadiazine (Smith Kline Beecham, West Chester, Pennsylvania, USA) was administered orally at 15mg/kg daily. Approximately 12 hr after administration of the first antibiotic dose, the dolphin stopped eating, started listing while swimming and appeared to have difficulty remaining buoyant. Despite an apparent stabilization of her respiratory clinical signs and swimming pattern during the next day, she died the following evening.

On post-mortem examination, the dolphin was emaciated with an average blubber thickness of 0.75–1 cm (normal = 3–4 cm). The lungs contained diffuse miliary nodules, measuring 0.3–10 cm throughout both lobes, and single large firm caseous nodules, measuring 8 to 9 cm in diameter, in both the left and right caudoventral regions. Both perihilar lymph nodes were firm and enlarged, containing caseous material. In addition, there was a 7 cm diameter ulcer in the body of the first stomach compartment along with a few *Anisakis* spp. nematode larvae, kelp, and four, 5 by 3 cm latex rubber strips.

Representative tissue samples were collected and fixed in 10% neutral buffered

TABLE 1. Selected hematological and biochemical values from a free-ranging bottlenose dolphin (*Tursiops truncatus gilli*) with coccidioidomycosis.

| Parameter | Units | Value | Normal ^a Mean (SD) |
|--------------------------------|----------------------|-------|----------------------------------|
| White blood cells (WBC) | X10 ³ /μl | 8,200 | 6,400 (1,100) |
| Band neutrophils | X10 ³ /μl | 246 | 0 |
| Segmented neutrophils | X10 ³ /μl | 7,216 | 4,288 (408) |
| Lymphocytes | X10 ³ /μl | 492 | 1,152 (320) |
| Monocytes | X10 ³ /μl | 164 | 128 (64) |
| Eosinophils | X10 ³ /μl | 82 | 768 (320) |
| Erythrocyte Sedimentation Rate | mm in 60 min | 25 | 0 ^b |
| Fibrinogen | mg/dl | 1,058 | <250 ^b |
| Serum iron | μg/dl | 78 | 132 (27) |

^a Asper et al., 1990^b T. H. Reidarson and J. McBain (unpublished data).

formalin. These included heart, lung, pulmonary and mesenteric lymph nodes, liver, all four chambers of the stomach, small intestine, kidney, ovary, urinary bladder, skeletal muscle, adrenal gland, skin, and brain. Culturette swabs of the large pulmonary nodules and perihilar lymph nodes were taken and placed on blood and Sabouraud's dextrose agar (Remel, Lenexa, Kansas, USA)

On histological examination of tissues, thick double walled organisms containing endospores characteristic of *Coccidioides immitis* were observed in lung, perihilar lymph nodes, and brain with pyogranulomatous infiltrates observed in the lung and perihilar lymph nodes only. The significance of the lack of inflammatory infiltrate surrounding the spherules in the brain is uncertain. The only other significant lesions were diffuse severe chronic periportal hepatitis, cirrhosis of unknown origin, and gastric ulceration.

A DNA Gen-Probe test (Accuprobe, San Diego, California, USA) performed on a purified isolate from Sabouraud's dextrose agar, by the Texas Fungus Testing Laboratory (University of Texas Health Science Center, San Antonio, Texas, USA) confirmed an infection with *Coccidioides immitis*. A serum sample tested by quantitative immunodiffusion for antibodies to *C. immitis*, performed at the University of California Coccidioidomycosis Serology

Laboratory (Davis, California, USA), revealed a titer of 1:128. In humans and animals, a titer of this magnitude is strong evidence for disseminated coccidioidomycosis (Pappagianis and Zimmer, 1990). A serum also tested negative for antibodies to all known marine morbilliviruses, at the USDA Foreign Disease Diagnostic Laboratory (Plum Island, New York, USA).

Cytology and culture of the blowhole swabs from blood and Sabouraud's agar yielded no evidence of a fungus. Two swabs taken 24 hr apart showed occasional polymorphonuclear leukocytes and epithelial cells with mixed gram positive and negative bacteria, including *Vibrio* sp., alpha hemolytic *Streptococcus* sp., and a *Corynebacterium* sp. More invasive techniques such as bronchoalveolar lavage or percutaneous biopsies are necessary for detecting deep seated fungal infections (Reidarson et al., 1998).

Coccidioides immitis is a dimorphic fungus that grows as a mold in the soil. Arthroconidia form within the mold hyphae and are dispersed into the environment by the wind. Humans and animals are infected almost exclusively by the respiratory route. Arthroconidia lodge in the lungs and develop into spherules that undergo repeated divisions resulting in thousands of endospores. Sixty percent of infections in humans are asymptomatic with spontaneous resolution, while 40% produce

pneumonia with a 5 to 10% dissemination rate. Certain risk factors enhance the occurrence of dissemination, including immune compromise, immune suppressive drugs, and late term pregnancy, however dissemination can also occur in normal healthy individuals (Kirkland and Fierer, 1996).

Coccidioides immitis is endemic to Arizona, California, Nevada, New Mexico, Texas and Utah, as well as parts of Mexico and South America (Pappagianis, 1988). It is capable of blowing great distances in wind, and can survive in saline soil and sea water (Dzawachiszwilli et al., 1964). Since bottlenose dolphins are known to venture close to the California shore, it is believed that an offshore wind may have carried the arthroconidia to this individual from one of several endemic areas of California where there have been numerous reports of *C. immitis* infections in domestic animals, as well as captive and free ranging wildlife. Coccidioidomycosis has previously been described in free ranging marine mammal species, including California sea lions (*Zalophus californianus*) and sea otters (*Enhydra lutris*) (Fauquier et al., 1996; Cornell et al., 1979; Reidarson et al., 1998; Pappagianis, 1988; Thomas et al., 1995). While these animals spend most of their lives in the water, infection of this bottlenose dolphin is the first reported case of coccidioidomycosis in a free-ranging purely aquatic marine wildlife species. This provides another example of the occurrence of coccidioidomycosis in the marine environment, in marked contrast to the usual arid or semi-arid endemic zones.

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