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Clostridium perfringens as the Cause of Death of a Captive Atlantic Bottlenosed Dolphin (*Tursiops truncatus*)[1]

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ABSTRACT: A previously healthy captive female bottlenosed dolphin (*Tursiops truncatus*) died suddenly. At necropsy, *Clostridium perfringens* was isolated from dorsal muscle, blood, left heart ventricle, thoracic fluid, and abdominal fluid. An identical strain was recovered from pool water. A male dolphin in the same pool had inflicted several "rake" marks on the dorsal surface of the female. Water-borne bacteria probably entered these lesions which served as the focus for anaerobe penetration and spread.

Key words: Clostridium perfringens, Atlantic bottlenosed dolphin, *Tursiops truncatus*, anaerobic bacterial infection, case history.

Although culture for anaerobic bacteria is recommended when evaluating the cause of disease or death in marine mammals (Howard et al., 1983), the literature is generally vague on the involvement of anaerobes. The drowning of a captive killer whale (*Orcinus orca*) was precipitated by *Clostridium perfringens* hepatoxic encephalopathy (Griffin and Goldsberry, 1968; Klontz, 1970). A blackleg-like disease in the iliopsoas muscle group of one bottlenosed dolphin (*Tursiops truncatus*) resulted in isolation of a *Clostridium* species (Sweeney and Ridgway, 1975) which had not been reported previously in these animals (Wood, 1973). Three cases of clostridial myositis in a killer whale, an Atlantic bottlenosed dolphin, and a California sea lion (*Zalophus californianus*) have been noted (Greenwood and Taylor, 1978). All occurred at the site of intramuscular injections administered by lay persons, and all involved *C. perfringens* except in the killer whale from which an unspecified *Clostridium* sp. was observed in muscle smears. *Clostridium chauvoei* and *C. novyi* were found in various tissues of marine mammals (Medway, 1980), but no other details were given. A captive manatee (*Trichechus manatus*) in Florida died of a clostridial infection termed "fat neck" (Caldwell and Caldwell, 1985). This study describes the isolation of *C. perfringens* and other bacteria at necropsy from a previously healthy Atlantic bottlenosed dolphin that had been at Mystic Marinelife Aquarium (Mystic, Connecticut) for eight years after captivity in Florida. The water system has been described previously (Dunn et al., 1982).

The animal was a 19-yr-old female; length 270 cm and weight 205 kg. It had been housed with two to four other bottlenosed dolphins and one to three belukha whales (*Delphinapterus leucas*) and was eating normally 24 hr prior to death. There were multiple lacerations over the right eye, at the right commissure of the mouth, on the dorsal aspect of the dorsal fin, on the shank, and to the left of the blowhole above the left eye. On incision, gas was released dorsal to insertion of the right pectoral fin, lateral to the scapula. There were copious amounts of gas present in the muscles, liver, and under the capsules of the kidney. An area approximately 20 × 8 cm of the vascular network and the underlying musculature in the right thoracic cavity was necrotic. Swabs (Culturette II, Marion Scientific Corp., Kansas City, Missouri 64114, USA) were used to obtain

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TABLE 1. Bacteria recovered at necropsy from an Atlantic bottlenosed dolphin.

Anus	*Edwardsiella tarda*
	Proteus mirabilis
	Providencia sp.
	Staphylococcus epidermidis
	Staphylococcus hyicus
	Streptococcus faecalis
Blowhole	*Staphylococcus aureus* (coagulase +)
	P. mirabilis
Mouth fluids	*P. mirabilis*
	S. aureus (coagulase +)
	Vibrio alginolyticus
Lungs	*P. mirabilis*
	S. aureus (coagulase +)
	V. alginolyticus
Dorsal muscle	*Clostridium perfringens*
	Morganella morganii
	P. mirabilis
	S. aureus (coagulase +)
	S. hyicus
	V. alginolyticus
Blood	*C. perfringens*
Heart ventricle (left)	*C. perfringens*
Thoracic fluid	*C. perfringens*
Abdominal fluid	*C. perfringens*

TABLE 2. Biochemical characterization of dolphin muscle and pool water isolates of *Clostridium perfringens*.

Reaction	Pool water	Muscle
Indole	−	−
n-acetyl glucosaminidase	+	+
α-glucosidase	+	+
α-arabinosidase	−	−
β-glucosidase	+	+
α-fucosidase	+	+
Phosphatase	+	+
α-galactosidase	+	+
β-galactosidase	+	+
Indoxyl acetate	+	+
Arginine dihydrolase	−	−
Leucine amino peptidase	−	−
Proline amino peptidase	−	−
Tryosine amino peptidase	−	−
Arginine amino peptidase	+	+
Alanine amino peptidase	−	−
Histidine amino peptidase	−	−
Phenylalanine amino peptidase	−	−
Glycine amino peptidase	−	−
Pyroglutamic acid arylamidase	+	+

swabs at necropsy and were processed in the laboratory within 2 hr. Swabs were used to inoculate directly the upper section of petri dishes containing blood agar (tryptic soy agar plus 5% sheep blood), MacConkey agar, mannitol salt agar, and TCBS agar (all from Difco Laboratories, Detroit, Michigan 48232, USA). A sterile wire loop was used to spread the inoculum and subsequently secure isolated colonies. Plates were incubated aerobically at 35 C in Gas Pak jars (BBL Microbiology Systems, Cockeysville, Maryland 21030, USA). Representatives of all colony types were picked and maintained on tryptic soy agar slants or fluid thioglycollate broth (Difco Laboratories) for anaerobes. The latter were confirmed as obligate anaerobes by streaking on blood agar and incubating in air at 35 C, but there was no bacterial growth after 2 days. Anaerobic growth on blood agar showed colonies surrounded by a double zone of hemolysis which is characteristic of *Clostridium perfringens* (Smith and Dowell, 1980; Finegold and Martin, 1982). Cells were nonmotile, Gram positive straight rods; no spores were observed. Gram negative bacteria were identified using API 20 E strips (Analytab Products, Plainview, New York 11803, USA). STAPH-IDENT and STAPHase III systems (Analytab) were used for identification of *Staphylococcus* spp. Table 1 lists all bacteria recovered at necropsy.

The large number of skin lacerations ("rake" marks) were probably caused by interaction with an aggressive male dolphin housed in the same pool. It is assumed that *C. perfringens* entered the animal through these external wounds and localized in the dorsal musculature. Oral transmission was possible, but evidence of lung or other internal organ damage or infection caused by *Clostridium* spp. or any other organism such as *Staphylococcus* spp. was not found at necropsy. The dolphin had not received an intramuscular injec-

TABLE 3. Antibiotic susceptibility of muscle and pool water isolates of *Clostridium perfringens*.

Antibiotic	MIC[a] (μg/ml)	
	Pool water	Muscle
Amikacin	>64.0	>64.0
Ampicillin	<1.0	<1.0
Aztreonam	>128.0	>128.0
Cefazolin	<1.0	<1.0
Cefoxitin	<1.0	<1.0
Ceftriaxone	<1.0	<1.0
Cefuroxime	<1.0	<1.0
Cephalothin	1.0	2.0
Chloramphenicol	<2.0	<2.0
Clindamycin	<0.1	<0.1
Erythromycin	2.0	2.0
Gentamicin	>32.0	>32.0
Imipenum	<0.3	<0.3
Mezlocillin	<2.0	<2.0
Netilmycin	>64.0	>64.0
Oxacillin	<0.3	<0.3
Penicillin	<0.1	<0.3
Rifampin	<4.0	<4.0
Tetracycline	<1.0	<1.0
Ticarcillin	<2.0	<2.0
Timentin	<2.0	<2.0
Tobramycin	>16.0	>16.0
Vancomycin	<1.0	<1.0

[a] Minimum inhibitory concentration.

tion for approximately 1 yr, and this potential source of the organism was also rejected.

Clostridium perfringens was found in the feces of a California sea lion and other bottlenosed dolphins sharing the same water as the animal that died and in the pool water. The organism was cultured also from an anal swab from a newly-introduced bottlenosed dolphin shipped from Mississippi. Because *C. perfringens* occurs in nature in the spore form and is not killed effectively by chlorination (Cabelli, 1977; Olivieri, 1982), it would be expected to be found in the aquarium environment.

Clostridium perfringens isolates from dorsal muscle and pool water were subjected to a variety of biochemical tests. Results are presented in Table 2. Response of these two isolates to antimicrobial agents was tested also, and antibiograms are listed in Table 3. Because these two strains were identical, the likelihood that the disease was transmitted by pool water was confirmed.

We believe that the death of the dolphin reported here was caused by the accidental exogenous introduction of spores of *C. perfringens* into the musculature through the skin breaks and subsequent vegetative cell development under anaerobic conditions and exotoxin production. *Clostridium perfringens* infections spread rapidly because of the destructive effects of the toxin. The dolphin showed several bruises in the musculature which may have contributed to bacterial invasion.

This incident represents additional documentation of the (probably) common presence of pathogenic bacteria in aquarium waters. It is important to monitor for these organisms and to recognize their potential for animal (and possibly) human disease. Immunization against *C. perfringens* toxins should be routine (Greenwood and Taylor, 1978). Appropriate management of unimmunized captive marine mammals with cutaneous lesions should include isolation.

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