

NOTE

Bacteria associated with stranded cetaceans from the northeast USA and southwest Florida Gulf coastsJohn D. Buck¹, Neal A. Overstrom², Geoffrey W. Patton³, Howard F. Anderson³, Jay F. Gorzelany³¹ Department of Marine Sciences and Marine Research Laboratory, The University of Connecticut, Noank, Connecticut 06340, USA² Mystic Marinelife Aquarium, Sea Research Foundation, Inc., Mystic, Connecticut 06355, USA³ Mote Marine Laboratory, 1600 Thompson Parkway, Sarasota, Florida 34236, USA

ABSTRACT: A total of 64 stranded cetaceans from the northeast USA and southeast Florida Gulf coastlines were sampled for bacteria during the period 1984 to 1990. Thirty-six individuals were dead when examined and 27 were alive but died shortly after stranding; one was released. Cultures were recovered from a variety of external and internal surfaces. Species of *Vibrio* were isolated from all Florida strandings; *V. alginolyticus*, *V. parahaemolyticus*, and *V. damsela* represented 35% of the total number of isolates (382). *Vibrios* were recovered from 10 individuals from the northeast; the 3 species above accounted for 17% of the total (139) and were most common from strandings between May and September. Other bacteria which represented $\geq 5\%$ of the total number of isolates in one or both areas included *Edwardsiella tarda*, *Morganella/Proteus/Providencia* spp., *Pseudomonas putrefaciens*, and other pseudomonads. Some geographical differences were noted.

The causes of individual cetacean strandings remain poorly understood despite the popular and scientific interests which the incidents attract. Microbial disease is a possible explanation and some generalized information is available (Hall et al. 1971, Cordes 1982, Simpson & Cornell 1983, Buck 1984, Baird et al. 1988, Martineau et al. 1988). In many cases, stranded carcasses have been too badly decomposed for adequate microbiological examination to establish one or more microbes as the etiological agent of death. The ultimate death of hundreds of stranded Atlantic bottlenose dolphins *Tursiops truncatus* along the eastern USA coast in 1987 may have involved several factors, including massive infection by opportunistic bacteria, particularly *Vibrio* spp., following immune suppression (Brody 1989, Smith 1990). The exact cause of death has not been shown unequivocally and hypotheses vary (Brody 1989, Geraci 1989, Smith 1990). However, it is recognized that species of *Vibrio* are extremely aggressive and dangerous to humans (Janda et al. 1988, West 1989). *Vibrio* species have been reported to cause

death and illness in dolphins (Tangredi & Medway 1980, Schroeder et al. 1985, Fujioka et al. 1988, Palmer et al. 1989) and are found in apparently healthy marine mammals (Buck & Spotte 1986).

We sampled a large number of stranded cetaceans and present herein results of microbiological studies for individuals found in 2 different geographical areas. The data in checklist form may be valuable in assessing previous observations on stranding deaths and examination of future occurrences.

Materials and methods. A total of 64 cetaceans were sampled during the period 1984 to 1990 in 2 study areas; coastlines between Portland, Maine, and Atlantic City, New Jersey (29 individuals) and between Tampa and Ft. Myers, Florida (35 individuals). Thirty-six individuals were dead when examined and 27 were alive but died shortly after stranding; one was released. Several were maintained for a few hours in aquariums before subsequent death.

Samples for bacteriological analysis were collected using Culturette II swabs (Marion Scientific Co., St. Louis, Missouri) from a variety of external and internal sites, depending on the condition of the cetacean studied and whether or not a necropsy was performed. Most swabs from the northeast region were processed within 6 h; some samples were overnight air-shipped, including all but 2 samples from Florida which were processed immediately upon receipt.

The following media were used for bacterial isolation: blood agar (Columbia agar base + 5% sheep blood), MacConkey agar, mannitol salt agar and thiosulfate-citrate-bile-sucrose (TCBS) agar. All media were from Difco Laboratories (Detroit, Michigan) or Becton Dickinson Microbiology Systems (Cockeysville, Maryland). The top section of each plate was swabbed directly. Streaking of the plate was com-

Table 1. Bacteria isolated from stranded cetaceans

| Bacteria recovered | Cetacean species and location where isolated ^{a,b} | Site of isolation ^c |
|---|---|--|
| <i>Achromobacter</i> / <i>Acinetobacter</i> / <i>Alcaligenes</i> /CDC IV E/ <i>Moraxella</i> / <i>Pasteurella</i> / <i>Pseudomonas</i> sp. (AAACMPP) | D. l. (CT) G. m. (MA) L. al. (CT) T. t. (FL, NJ) | bh, sl bh bh, lu, v a, bh, hb, lu, o, sl, sp |
| <i>Achromobacter</i> / <i>Acinetobacter</i> / <i>Alcaligenes</i> /CDC IV E sp. | Z. c. (FL) T. t. (FL) | sl gs |
| <i>Achromobacter</i> / <i>Pseudomonas</i> sp. | G. m. (MA) S. c. (NY) | bh bh |
| <i>Achromobacter</i> sp. | T. t. (FL) | o |
| <i>Acinetobacter</i> / <i>Flavobacterium</i> / <i>Pseudomonas</i> sp. | T. t. (FL) | gs |
| <i>Acinetobacter</i> / <i>Pseudomonas</i> sp. | D. l. (CT) G. m. (MA) P. m. (FL) | bh bh bh |
| <i>Acinetobacter calcoaceticus</i> var. <i>anitratum</i> | T. t. (FL) T. t. (FL) | a, hb, o, sl, sp a, gs, o |
| <i>Acinetobacter calcoaceticus</i> var. <i>lwoffii</i> | T. t. (FL) | o |
| <i>Acinetobacter</i> sp. | G. m. (MA) | bh |
| <i>Aeromonas hydrophila</i> | G. m. (MA) L. al. (CT) T. t. (FL, NJ) | a bh a, bh, gs, m, o |
| <i>Alcaligenes</i> sp. | T. t. (FL) | bh |
| <i>Chromobacterium</i> sp. | T. t. (FL) | gs |
| <i>Citrobacter freundii</i> | G. m. (MA) L. a. (CT) S. c. (RI) T. t. (FL) | a a a a, bh, gs |
| <i>Citrobacter</i> sp. | L. al. (CT) S. c. (RI) T. t. (FL) | bh bh gs |
| <i>Corynebacterium</i> sp. | L. al. (CT) T. t. (FL) | bh gs, sl |
| <i>Edwardsiella tarda</i> | D. l. (CT) G. m. (CT, MA) L. a. (MA, RI) L. al. (CT) T. t. (FL) | bh a, bh a, ac, bh, h a, v a, gs, lu, o |
| <i>Enterobacter</i> / <i>Klebsiella</i> sp. | T. t. (FL) | lu |
| <i>Enterobacter</i> / <i>Serratia</i> sp. | L. a. (MA) | a |
| <i>Enterobacter aerogenes</i> | T. t. (FL) | a, o |
| <i>Enterobacter agglomerans</i> | L. a. (MA) T. t. (FL) | bh o, sl |
| <i>Enterobacter cloacae</i> | D. l. (CT) T. t. (FL) Z. c. (FL) | sl bh, gs a |
| <i>Escherichia coli</i> | D. d. (NY) D. l. (CT) G. m. (MA) L. a. (MA) T. t. (FL, NY) | a, bh sl a, bh a, bh, h a, bh, gs, lu, o, sl |
| <i>Escherichia hermannii</i> | T. t. (FL) | gs |
| <i>Flavobacterium</i> / <i>Cytophaga</i> sp. | T. t. (FL) | gs |
| <i>Flavobacterium</i> / <i>Pseudomonas</i> sp. | G. m. (MA) T. t. (NJ) | bh sl |
| <i>Flavobacterium meningosepticum</i> | P. p. (NY) T. t. (FL) | bh gs |

Table 1 (continued)

| Bacteria recovered | Cetacean species and location where isolated ^{a,b} | Site of isolation ^c |
|---|---|--------------------------------|
| <i>Flavobacterium</i> sp. | L. a. (MA) | bh |
| | S. c. (RI) | a |
| | T. t. (FL) | bh, gs |
| Fluorescent <i>Pseudomonas</i> sp. | D. d. (NY) | it |
| | G. m. (MA) | bh |
| | L. a. (MA) | bh |
| | P. m. (FL) | bh |
| | S. c. (NY) | a |
| | T. t. (FL, NJ) | a, bh, gs, sl |
| <i>Klebsiella oxytoca</i> | T. t. (FL) | lu, sl |
| <i>Klebsiella pneumoniae</i> | G. m. (MA) | a |
| | T. t. (FL) | bh |
| <i>Klebsiella</i> sp. | Z. c. (FL) | sl |
| <i>Micrococcus</i> sp. | G. m. (MA) | a, bh |
| | S. c. (RI) | a |
| | T. t. (FL) | a, gs, hb, sl |
| <i>Moellera wisconsensis</i> | P. p. (CT) | bh |
| <i>Morganella morgani</i> | T. t. (FL) | a, bh, hb, gs, lu, o |
| <i>Pasteurella multocida</i> | D. l. (CT) | bh |
| | T. t. (FL) | a |
| <i>Plesiomonas shigelloides</i> | T. t. (FL) | a, gs |
| <i>Proteus/Providencia</i> sp. | T. t. (FL) | bh, o, sl |
| <i>Proteus mirabilis</i> | T. t. (FL) | a, bh, gs, o |
| <i>Proteus penneri</i> | T. t. (FL) | a |
| <i>Proteus vulgaris</i> | G. m. (ME) | a, bh |
| | T. t. (NY) | a, bh, gs, o, sl |
| <i>Proteus</i> sp. | G. m. (ME) | a |
| | T. t. (FL) | bh |
| <i>Providencia rettgeri</i> | T. t. (FL) | a, bh, o |
| <i>Providencia stuartii</i> | G. m. (ME) | a |
| | T. t. (FL) | o |
| <i>Pseudomonas aeruginosa</i> | T. t. (FL) | a, gs, lu |
| <i>Pseudomonas maltophilia</i> | G. m. (MA) | bh |
| | L. a. (MA) | bh |
| | T. t. (FL) | sp |
| <i>Pseudomonas putrefaciens</i> | D. l. (CT) | bh |
| | G. m. (ME) | a |
| | L. a. (RI) | o |
| | L. al. (CT) | y |
| | T. t. (FL, NJ) | a, bh, gs, hb, lu, o, sl, u |
| <i>Pseudomonas</i> sp. | B. e. (FL) | sl |
| | D. l. (CT) | sl |
| | G. m. (MA) | a, bh |
| | P. m. (FL) | bh |
| | P. p. (CT) | y |
| | T. t. (FL) | a, bh, gs, o, sl |
| <i>Serratia liquefaciens</i> | T. t. (FL) | bh, lu |
| <i>Serratia rubidaea</i> | T. t. (FL) | lu |
| <i>Serratia</i> sp. | T. t. (FL) | a, sl |
| <i>Staphylococcus capitatus</i> | T. t. (FL) | gs |
| <i>Staphylococcus cohnii</i> | S. c. (RI) | a |
| | T. t. (FL) | bh |
| <i>Staphylococcus epidermidis</i> | B. e. (FL) | bh |
| | L. a. (MA) | a, bh |
| | L. al. (CT) | v |
| | S. c. (RI) | a |
| | T. t. (FL) | bh |
| <i>Staphylococcus hominis/saprophyticus</i> | L. a. (MA) | a, bh |

Table 1 (continued)

| Bacteria recovered | Cetacean species and location where isolated ^{a,b} | Site of isolation ^c |
|-----------------------------------|---|-------------------------------------|
| <i>Staphylococcus warneri</i> | G. m. (MA) | bh |
| | T. t. (FL) | sp |
| <i>Staphylococcus xylosus</i> | T. t. (FL) | gs, o |
| <i>Staphylococcus sp.</i> | G. m. (MA) | bh |
| | T. t. (FL) | bh, hb |
| <i>Streptococcus dysgalactiae</i> | T. t. (FL) | lu |
| <i>Streptococcus equinus</i> | T. t. (FL) | bh |
| <i>Streptococcus faecalis</i> | T. t. (FL) | a, li, lu, sp |
| <i>Streptococcus mitis</i> | T. t. (FL) | bh |
| <i>Streptococcus sp.</i> | G. m. (MA, ME) | a |
| | T. t. (FL) | a, bh, gs, hb, m, o, sl |
| <i>Vibrio alginolyticus</i> | D. d. (NY) | a |
| | G. m. (MA, ME) | a, bh |
| | L. a. (MA, RI) | a, bh, o |
| | L. al. (CT) | a, bh, ps |
| | P. m. (FL) | bh |
| | S. c. (RI) | bh |
| | T. t. (FL, NJ) | a, bh, b1, gs, hb, li, lu, m, o, sl |
| <i>Vibrio damsela</i> | B. e. (FL) | sl |
| | D. d. (NY) | a, bh, v |
| | G. m. (MA) | bh |
| | L. a. (MA) | a, v |
| | P. p. (NY) | a |
| | T. t. (FL) | a, bh, gs, lu, o |
| <i>Vibrio fluvialis</i> | L. al. (CT) | lu |
| | P. m. (FL) | bh |
| | T. t. (FL) | a, bh, m, u |
| <i>Vibrio mimicus</i> | T. t. (FL) | gs |
| <i>Vibrio parahaemolyticus</i> | L. al. (CT) | bh |
| | T. t. (FL, NJ) | a, bh, gs, lu, o, sl |
| <i>Yersinia enterocolitica</i> | L. a. (MA) | bh |
| | T. t. (FL) | bh |

^a B. e. = *Balaenoptera edeni* (Bryde whale). D. d. = *Delphinus delphis* (saddleback dolphin), D. l. = *Delphinapterus leucas* (beluga whale), G. m. = *Globicephala melaena* (longfin pilot whale), L. a. = *Lagenorhynchus acutus* (Atlantic whiteside dolphin), L. al. = *Lagenorhynchus albirostris* (white-beak dolphin), P. m. = *Physeter macrocephalus* (sperm whale), P. p. = *Phocoena phocoena* (harbor porpoise), S. c. = *Stenella coeruleoalba* (striped dolphin), T. t. = *Tursiops truncatus* (Atlantic bottlenose dolphin), Z. c. = *Ziphius cavirostris* (goosebeak whale)

^b CT = Connecticut, FL = Florida, MA = Massachusetts, ME = Maine, NJ = New Jersey, NY = New York

^c a = anus, ac = abdominal cavity, bh = blowhole, bl = bladder, gs = genital slit, h = heart, hb = heart blood, it = intestinal tumor, li = liver, lu = lung, m = mammary gland, o = oral cavity (esophagus, larynx, mouth, throat, tracheae), ps = pericardial sac, sl = skin lesion, sp = spleen, u = umbilicus, v = vagina

pleted with a sterile wire loop to obtain isolated colonies. The tips of swabs were broken off in tubes of Tryptic Soy Broth (Difco). All cultures were incubated at 37°C for 18 to 24 h. Colonies from all media were transferred to Tryptic Soy agar slants; isolates from TCBS plates were also maintained on slants of Difco Marine Agar 2216.

The Gram (Buck 1982) and cytochrome oxidase (Pathotec strips; Organon Teknika, Durham, North Carolina) reactions were recorded for all isolates. Gram-negative bacteria were identified by the API 20E system (Analytab Products, Plainview, New York); for typical *Vibrio* isolates from TCBS sugar, 20% artificial

seawater was used as diluent (MacDonell et al. 1982) in test strips. Additional tests for vibrios included susceptibility to the vibriostatic agent 0/129, growth at various NaCl concentrations, swarming reaction, and gas production in modified MOF medium (Lemos et al. 1985). Gram-positive cocci were identified using STAPH Trac or Rapid STREP systems (both Analytab Products) plus morphology and pigment characterization.

Results and discussion. Table 1 is a composite list of bacteria isolated from all cetaceans sampled. Species of *Vibrio* were recovered from all Florida strandings (total of 35) which occurred in all months except December. *Vibrio alginolyticus* was the most frequent isolate (28

individuals) followed by *V. parahaemolyticus* (19), *V. damsela* (15), *V. fluvialis* (8), *V. vulnificus* (1), and *V. mimicus* (1). *Vibrio* occurrence is temperature-dependent with greatest numbers in seawater associated with higher water temperature (Janda et al. 1988). Consequently, it was not considered unusual that vibrios were recovered from Florida cetaceans where Gulf of Mexico waters would be warm enough year-round to support these bacteria.

In northeast USA waters, 29 individuals were sampled that stranded in all months except February and October. *Vibrio* spp. were recovered from 11 of these; 6 of the 8 that stranded between May and September were positive for *Vibrio* spp., whereas these organisms were only cultured from 5 of the 21 that stranded between October and April. These observations are consistent with the temperature relationships noted above, although 2 isolations of *V. alginolyticus* were from individuals that stranded in November and *V. damsela* was found in 2 strandings which occurred in November and December. Some species of *Vibrio* (e.g. *V. cholerae*) adhere to the surfaces of live copepods (Huq et al. 1984) and the survival and distribution of these bacteria may be affected by absorption to larger animals as well.

Table 2 considers, albeit arbitrarily, only those bacterial species representing more than 5% of the total number of isolations from each geographical area. In the northeast, 4 organisms comprised approximately the same representation; 10 to 12% (*Edwardsiella tarda*, *Escherichia coli*, various *Pseudomonas* species other than *P. putrefaciens*, *Vibrio alginolyticus*). Conversely, *V. alginolyticus* isolations were 19% of the Florida total (about twice that seen in northeast samples) and the other bacteria (or group) were 6% or less each. Two other species of *Vibrio* (*V. parahaemolyticus* and *V. damsela*) were collectively 6% of the northeast isolates and 16% of those from Florida. Thus, the 3

Vibrio species in Table 2 were 16% of the total number of isolates from the northeast and 35% of the Florida total. The latter considered a larger number of isolates from the oral cavity and genital slit which could offer more surfaces for adsorption of bacteria. The data do indicate that vibrios are common in recently dead or debilitated cetaceans as well as in healthy specimens (Buck & Spotte 1986) and colonize a wide variety of external and internal surfaces and organs. Several individuals from the Florida strandings showed the occurrence of several *Vibrio* spp. in internal organs which suggested rapid colonization by these bacteria. It should be noted that bacteria, other than vibrios, listed in Table 2 have also been found in stranded cetaceans in widely different geographical areas (e.g. Coles et al. 1978, Martineau et al. 1988, Walsh et al. 1988) and should be considered as opportunistic pathogens.

Unfortunately, necropsies could not be performed in all strandings reported here so that a more valid assessment of bacterial involvement could not be obtained. However, it is apparent from our observations that vibrios are common (often the most frequent isolate) in and on stranded cetaceans. This is probably not unusual because of the ubiquity of various *Vibrio* spp. in seawater but given the pathogenic potential of these organisms to humans (Janda et al. 1988, West 1989) it is clear that vibrios should be included in microbiological examination of any cetacean, whether it be wild, captive, or stranded. In addition, care should be exercised by all personnel handling marine animals because several species of *Vibrio* can produce severe bacteremia via cuts and punctures.

With respect to recent mass dolphin deaths, assessment of dinoflagellate toxin levels and bacteriological studies on live populations and at necropsy in future strandings may clarify ambiguities on the cause of mortality. The data provided here will be useful for comparative purposes.

Table 2. Bacteria representing $\geq 5\%$ of the total number of isolates from each stranding area

| Isolate | Northeast USA | | | Florida Gulf Coast | | |
|--|--------------------------------|------------------------------|------------|---------------------------------|------------------------------|------------|
| | No. of individuals (n = 29) | No. of isolates (n = 139) | % of total | No. of individuals (n = 133) | No. of isolates (n = 382) | % of total |
| <i>Edwardsiella tarda</i> | 12 | 16 | 12 % | 8 | 10 | 3 % |
| <i>Escherichia coli</i> | 11 | 16 | 12 % | 14 | 21 | 6 % |
| <i>Morganella/Proteus/Providencia</i> spp. | 2 | 5 | 4 % | 19 | 38 | 10 % |
| <i>Pseudomonas putrefaciens</i> | 5 | 7 | 5 % | 21 | 34 | 9 % |
| Other <i>Pseudomonas</i> spp. | 11 | 13 | 10 % | 12 | 21 | 6 % |
| <i>Vibrio alginolyticus</i> | 8 | 14 | 10 % | 28 | 74 | 19 % |
| <i>V. damsela</i> | 3 | 6 | 4 % | 15 | 29 | 8 % |
| <i>V. parahaemolyticus</i> | 2 | 3 | 2 % | 19 | 30 | 8 % |

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