CETACEAN NOCARDIOSIS

Authors: PIER, A. C., TAKAYAMA, A. K., and MIYAHARA, A. Y.

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Nocardial infection is the basis of a number of animal diseases (Pier, 1962, Proc. U.S. Livestock San. Assoc.: 409-416). While the vast majority of reported nonhuman cases of nocardiosis have involved terrestrial mammals, infrequent reports of piscatorial disease involving rainbow trout, golden shiners and neon tetras have been made (Conroy, 1964, Vet. Rec. 76: 676; Snieszko, et al., 1964, J. Bact. 88: 1809-1810). *Nocardia asteroides* has been the primary agent of nocardiosis in lower animals. *Nocardia brasiliensis* and *Nocardia caviae*, while infrequently reported in the veterinary literature, are considered species of medical importance (Pier, 1962, Proc. U.S. Livestock San. Assoc.: 409-416; Gordon, and Mihm, 1962, Annals N.Y. Acad. Sci. 98: 628-636). All three organisms have been isolated from soil, but to the authors' knowledge only *N. asteroides* has been reported as a pathogen of aquatic animals.

It is the purpose of this report to describe three cases of nocardiosis of cetaceans including a pilot whale *(Globiocephala scammoni)*, and two Pacific bottle-nosed dolphins *(Tursiops gillii)*.

Materials and Methods

Animals: All animals were charges of a Hawaiian sea life park. Each had been captured in the wild state and transferred to well managed sea life park facilities where efforts were made to duplicate the essential features of their natural marine environment. They had resided in the park facilities for preiods between 7 weeks and 4 years before signs of illness were noted. Available information pertinent to these cases is listed as follows:

Case 1. Pilot whale *(Globiocephala scammoni)*. A young female 61 inches long was captured off the island of Oahu on May 5, 1965. For approximately 1 month she was force-fed fluids and a mixture of ground fish and squid. By June 12, she was eating voluntarily and eventually consumed 20 pounds of feed daily. Hematologic examinations were conducted on May 16 and May 30. Two days before death, air leakage from the whale's blow hole was noted, but no abnormal odor to the breath was observed. On June 25 the whale died. At postmortem examination, a severe, extensive suppurative pneumonia was present, and disseminated abscesses were observed in the liver. Tissue specimens from the lungs and liver were selected for cultural and histological examination.

Case 2. Dolphin *(Tursiops gillii)*. A mature male Pacific bottle-nosed dolphin, named Eha, captured off the island of Oahu on May 5, 1965, appeared normal and ate well during a 1-week observation period at the sea life park. The animal was transferred to a wire enclosed sea cave at another park where it was kept with another *T. gillii* until January 1966, when it died. At postmortem examination multiple abscesses were noted in the lungs and regional lymph nodes. Tissue specimens were selected for cultural and histological examination.

Case 3. Dolphin *(Tursiops gillii)*. A mature female Pacific bottle-nosed dolphin, named Wela, had been a resident of the sea life park for 4 years and was trained to perform certain maneuvers. On January 11, 1968, the dolphin appeared normal; a blood specimen was taken at the time of a routine injection of iron and liver extract. On January 20, 1968, her trainer noted that she was slow in accomplishing her maneuvers, her appetite was diminished, and there were abrasions at the tip of both the upper and lower rostrum. On the 22nd of January, she swam slowly, lay on the bottom of the pool for long periods, refused to work, and was anorectic; there was inflammation of the oral mucosa and small erosions were present. She was given antibiotics, B - complex vitamins, and liver extracts by injection. The animal ate 18 pounds of fish the following day (Jan. 23) and appeared greatly improved but the rostrum and oral mucosa remained the same. On January 26, she was placed on 2,000 mg tetracycline daily. On January 27, her condition became critical and respirations became erratic. On January 29, she died during convulsions. At postmortem examination two large absecesses were observed in the left lung and multiple smaller nodules of varying sizes were observed in both lungs. There was a fibrinopurulent pleuritis with adhesions on the left side. Sterile swabs were used to obtain specimens for culture; specimens were taken for histological examination*.

Cultural methods: Routine medical microbiological procedures were used to isolate and identify the more common bacterial pathogens; these included streaking aseptically ground tissue suspensions and swab specimens over a variety of solid mediums including nutrient agar and blood agar and incubating aerobically at 37 C. After 48 to 72 hours of incubation, rough, raised adherent colonies were observed; these were comprised of Gram-positive rods and branching filaments resembling Nocardia. Representative colonies were selected, isolated and sent to the National Animal Disease Laboratory, Ames, Iowa, for definitive cultural identification. Nocardia were identified by criteria previously described (Gordon, and Mihm, 1962, Annals N.Y. Acad. Sci. 98: 628-636; Pier, 1969, Mycology, Chapter 18 in: *Textbook of Veterinary Clinical Pathology*. Medway, Prier, and Wilkinson, The Williams and Wilkins Co., Baltimore, Md.). Differential cultural reactions were observed at weekly intervals for 3 weeks.

Histologic methods: Tissue specimens selected for histopathology were fixed in 10% formalin and embedded in paraffin. Sequential tissue sections were selected; one was stained with hematoxylin and eosin (H and E) and the other by the Gram-Weigert method.

Results

Nocardia were isolated from one or more preparations from each of the three cases. The pilot whale, Case 1, was infected by *N. asteroides;* the Pacific bottle-nosed dolphin, Case 2, was infected by *N. brasiliensis* and the dolphin in Case 3 was infected by *N. caviae*. Reactions leading to the identification of these isolants are summarized and presented in Table 1. In addition to *N. asteroides,* which was recovered from the whale's lung, *Staphylococcus aureus* was isolated from the liver and *Proteus sp.* was isolated from some preparations of the whale's lung. In addition to *N. brasiliensis,* which was recovered from both the lung and lymph nodes of Case 2, *S. aureus* was isolated from the lymph node and *Staphylococcus epidermidis* was isolated from the lung.

Two hemograms were obtained from the whale (Case 1) before the onset of clinical disease; the results of these were as follows:

	May 16	May 30
RBC x $10^6/mm^3$	4.46	3.80
WBC x $10^3/^3$	7.10	12.60
Differential: (% of cells)		
Heterophil	78	81
Lymphocyte	1	12
Eosinophil	8	7
Monocyte	3	0
Hemoglobin (gm/100 ml)	16.5	—

It appeared possible that the modest elevation in leukocyte count on May 30 might have heralded the onset of pulmonary infection.

Histologic features of these processes were typical of nocardiosis in other animal species (Pier, 1962, Proc. U.S. Livestock San. Assoc.: 409-416). Granulomatous reaction with suppuration were the essential features of the tissue response. The whale's lung (Case 1) was involved by an extensive suppurative pneumonia which involved individual

*Preliminary cultural and histopathological processing was accomplished by Pathology Associates Medical Laboratories, Honolulu, Hawaii.

TABLE 1. *Cultural features of cetacean nocardial isolants.*

Source	Morphology & staining: Branching	Fragmentation	Gram stain reaction	Acid fastness (Kinyoun's)	Aerial mycelium	Growth: Aerobic	Survive 60°C, 4 hr.	Acid production: Arabinose	Dextrose	Mannose	Sorbitol	Decomposition: Casein	Tyrosine	Xanthine	Identification
Case 1: Whale *(G. scammoni)*	+	+	+	+	+	+	+	—	—	—	—	—	—	—	*N. asteroides*
Case 2: Dolphin *(T. gillii)*	+	+	+	+	+	+	—	—	+	+	—	+	+	—	*N. brasiliensis*
Case 3: Dolphin *(T. gillii)*	+	+	+	+	+	+	+	—	+	—	—	—	—	+	*N. caviac*

alveolae, lobules and adjacent bronchioles (Fig. 1). The alveolar septa were prominent and infiltrated by macrophages. Proliferative changes and histiocytic infiltrations were observed but encapsulation was minimal. Giant cells were not seen. Numerous alveolae were packed with heterophils and some alveolae contained acellular aggregates of tissue debris which enveloped microcolonies of nocardial filaments (Figs. 2 and 3). The liver had a greater proliferative response to the nocardial infection and more evidence of encapsulation than did the lungs. Accumulations of fibroblasts and histiocytes were present at the periphery of suppurative areas in the liver and nocardial filaments were loosely distributed near the periphery.

Tissue changes in Case 2 (dolphin) resembled those in the liver of Case 1 (whale). There was a loosely distributed network of *N. brasiliensis* hyphae at the periphery of encapsulated lesions at the juncture of the suppurative and proliferative zones (Fig. 4); neither microcolonies nor tissue granules containing branched filaments were seen. In other areas, particularly in the lymph node, there were well-defined tissue granules complete with peripheral clubs of mineralized material (Fig. 5); these appeared to be botryomycotic granules and they uniformly contained Gram-positive cocci, often in tetrad configurations. Both *N. brasiliensis* and *S. aureus* were isolated from lymph nodes of this animal.) Nocardial filaments were not observed in the area of these granules but were observed in small numbers in other areas of the same section and in large numbers in other tissue specimens. Conversely, recognizable staphylccoccal aggregates were not seen in areas where *N. brasiliensis* filaments were present; this absence was considered a less definitive marker, however, because of the ease with which a few staphylococci could be overlooked in such a specimen.

Lung tissue specimens taken at postmortem examination from Case 3 showed changes similar to those seen in the other two animals. A purulent bronchopneumonia with multiple abscesses and a fibrinous pleuritis were the essential features of this involvement. The hemogram obtained 9 days before clinical signs of disease in this dolphin was as follows:

Hematocrit	40.0
WBC x $10^3/mm^3$	13.6
Differential: (% of cells)	
Heterophil	77
Lymphocyte	19
Eosinophil	1
Monocyte	3
Hemoglobin (gm/100 ml)	13.8

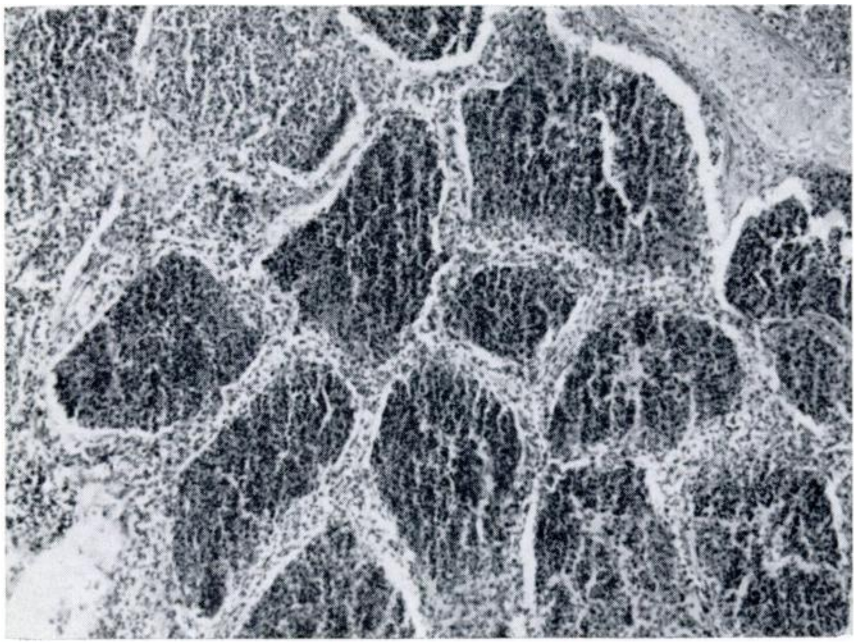

FIGURE 1. *Suppurative bronchopneumonia in the pilot whale, Case 1. The alveolar septae were prominent and infiltrated by histiocytes. Encapsulation and giant cell formation were not observed. H and E x 120.*

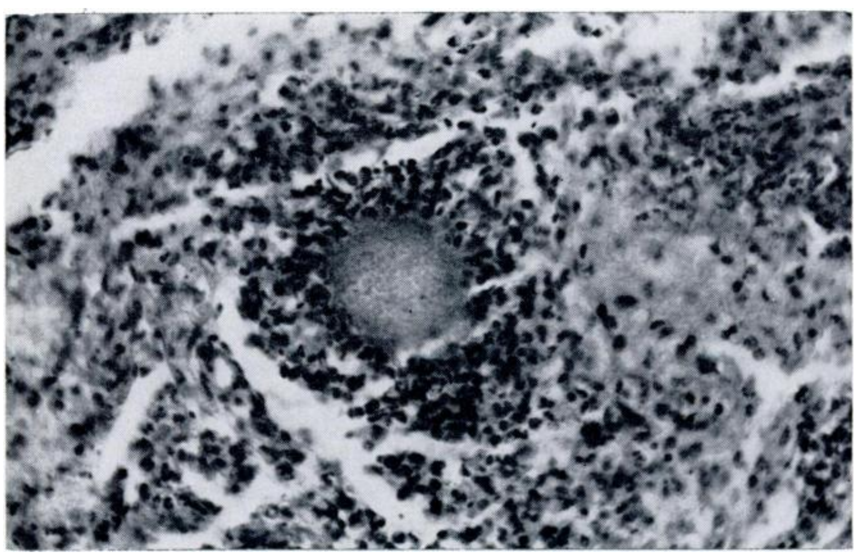

FIGURE 2. *Acellular aggregate of necrotic debris in the center of a granulomatous focus in the whale lung, Case 1. Both heterophils and histiocytes were abundant. H and E x 250.*

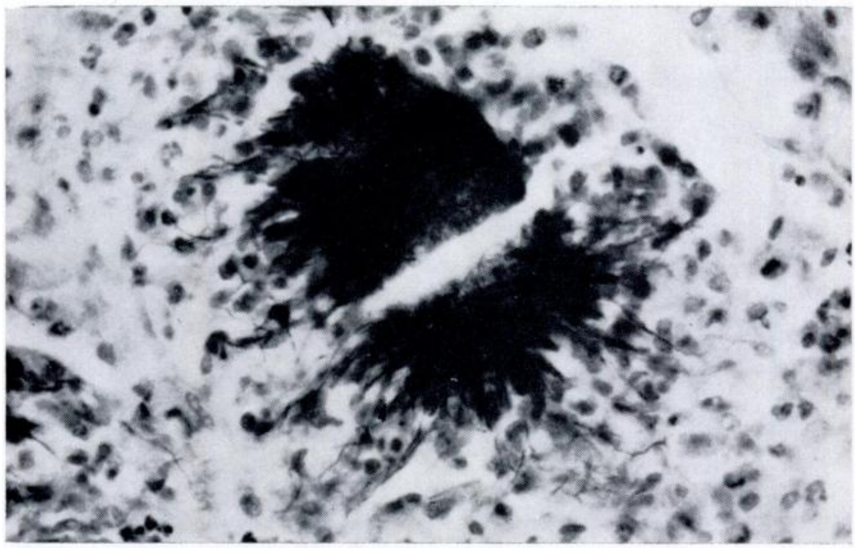

FIGURE 3. *Microcolony of* Nocardia asteroides *at the center of the same aggregate shown in Figure 2. Gram-Weigert x 500.*

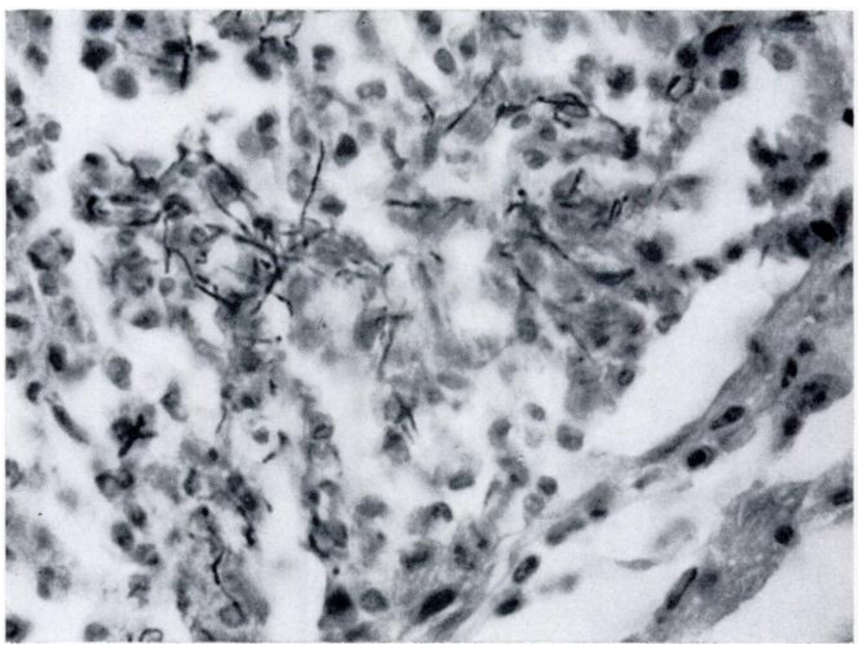

FIGURE 4. *Beaded, branched filamentous hyphae of* Nocardia brasiliensis *were present at the periphery of a granulomatous abscess in the Pacific bottle-nosed dolphin, Case 2. Gram-Weigert x 900.*

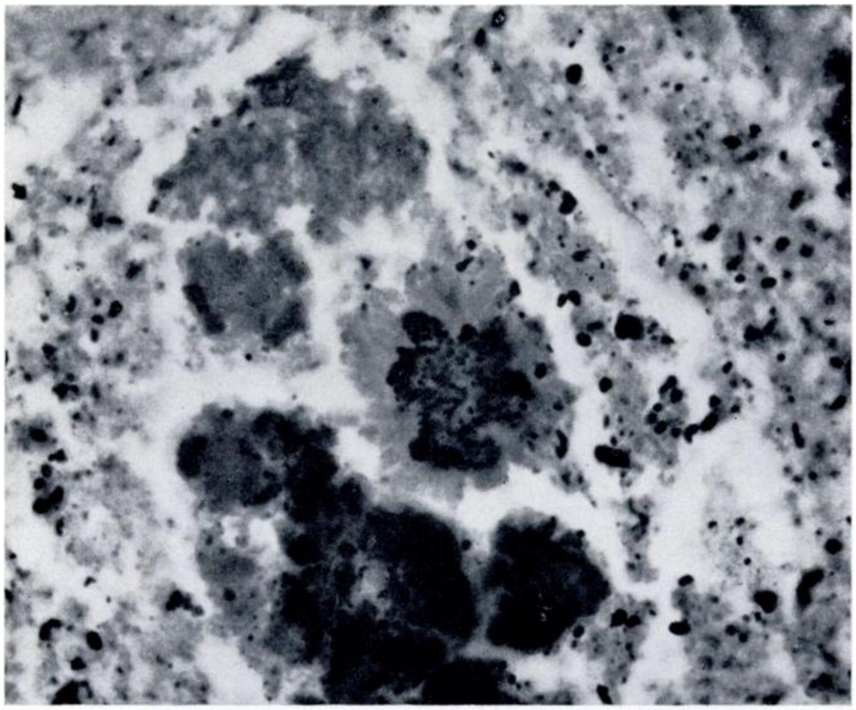

FIGURE 5. *Botryomycotic tissue granules in a necrotic focus from the dolphin, Case 2. Gram-positive cocci in tetrad formations were seen in these granules; filamentous forms were not. Gram-Weigert x 500.*

Discussion

Nocardia, particularly *N. asteroides*, may be either primary pathogens (Pier, 1962, Proc. U.S. Livestock San. Assoc.: 409-416) or opportunists. (Cross and Binford, 1962, Lab. Investig. 11: 1103-1109). Cockrill (1960, Brit. Vet. J. 116: 133-144, 175-190) in his study on the pathology of cetaceans, observed that the normal environment of whales precluded exposure to most known bacterial pathogens. However, recent studies of actinomycetes in oceanic sediments by Weland (1969, Nature 223: 858) imply that Nocardia as well as other Actinomycetes may be normal constituents of the oceanic flora.

It is not clear whether nocardiosis occurred in these cetacean species because of their incarceration or whether their confinement merely permitted the observations to be made. Two other cases of cetacean infection by Nocardia or Nocardia - like organisms have been called to our attention. Geraci (1969, Personal communication: J. R. Geraci, Dept. Zool., Univ. Guelph, Guelph, Ontario) observed in 1965 a culturally confirmed case of *N. asteroides* infection in a young, captive, female harbor porpoise *(Phocaena phocaena);* this case was attended by pneumonia, pleuritis and abscesses of the lymph nodes and kidney. Cowan (1968, 2nd Conf. on Diseases of Aquatic Mammals, Boca Raton, Florida) cited the morphologic recognition of an organism of "the Actinomyces-Nocardia group" in histopathologic sections of a mass taken from the lung of a wild pygmy sperm whale; cultural confirmation of the latter case was not obtained.

Of the four culturally confirmed cases of cetacean nocardiosis (three reported here and Geraci's observation), all were in captive animals. Our Pacific bottle-nosed dolphin, Case 2, however, had resided for 6 months before illness in a sea cave pen that had free exchange with ocean water and thus was in as nearly a wild environment as captivity would permit.

It appeared possible that the environment of cetaceans in the aquatic park was different from that of free-living individuals, and these differences may have influenced the water temperature as well as the bacterial population of the tank; either or both of these factors might lower the resistance of animals to infection.

The fact that the three animals we report here became infected with Nocardia was unusual as judged by the absence of reported cases of cetacean nocardiosis. The observation that in our series of three cases, three different species of medically important Nocardia were represented seemed statistically fortuitous. Since all three species of Nocardia are known to have a free-living state in soil, it is possible that soil contamination of the tank water may have contributed to the infections. Finally, the observation of microcolonies of *N. asteroides* enveloped by dense aggregates of tissue debris; of diffuse accumulations of *N. brasiliensis* filaments in an obviously systemic process; and of botryomycotic granules in adjoining tissues but not cohabiting with demonstrable *N. brasiliensis* infection, reawakened old anxieties about the value of tissue granules as presumptive diagnostic aids. According to the old rule-of-thumb, largely derived from medical experience in man, *N. brasiliensis* rarely causes systemic nocardiosis and usually produces tissue granules. Obviously, all tissue granules are not of nocardial origin, and differences in host, organ and duration of infection can shape the course of the infection as well as the tissue response to it.

In summary: three cases of cetacean nocardiosis are reported involving two species of marine mammals and three species of Nocardia. A pilot whale *(G. scammoni)* was infected by *N. asteroides;* one Pacific bottle-nosed dolphin *(T. gillii)* was infected by *N. brasiliensis;* and another Pacific bottle-nosed dolphin was infected by *N. caviae*. All three animals were residents of a sea life park and all had respiratory involvement. A concurrent botryomycotic process, attributed to *S. aureus*, was seen in the dolphin (Case 2) infected with *N. brasiliensis*.

Acknowledgement

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A. C. PIER,* A. K. TAKAYAMA,** and A. Y. MIYAHARA†

**Leader, Bacterial and Mycotic Diseases Investigations, National Animal Disease Laboratory, USDA, ARS, Ames, Iowa.*

***Veterinary practitioner at Aina Haina Pet Hospital, 5140 Kalanianaole Highway, Honolulu, Hawaii.*

†Formerly veterinary pathologist at State of Hawaii Department of Agriculture, Honolulu, Hawaii; present address: Extension Service, College of Tropical Agriculture, University of Hawaii, Honolulu, Hawaii.

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