

# Tuberculosis in Pacific Green Sea Turtles, *Chelonia mydas*<sup>1,2</sup>

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## ABSTRACT

Six cases of tuberculosis were found in captive Pacific green sea turtles, *Chelonia mydas*. The diagnoses were based on detection of gross lesions in lungs and livers, positive acid-fast bacilli in impression smears, characteristic lesions on microscopic examinations of tissues, and the isolation of the bacilli. The bacilli were identified as *Mycobacterium avium*, Serotype 8, and represent the first instance of *M. avium* in cold-blooded species. Tuberculosis has not previously been reported in the green sea turtle.

The occurrence of tuberculosis in cold-blooded animals is not unusual. Nigrelli and Vogel (1963) listed 186 species of fishes, amphibians, and reptiles in which tuberculosis has been reported. Although tuberculosis in cold-blooded species has been found mainly in captive animals (Griffith 1930), cases of tuberculosis in feral populations have also been reported (Nigrelli 1953). Friedmann (1903) described two cases in loggerhead turtles, *Chelone corticata*, which died in a Berlin aquarium with tubercle formation and typical giant cells. Tubercle bacilli were obtained from tuberculous terrapins which died (Wilson 1925).

This report describes a spontaneous outbreak of tuberculosis in captive Pacific green sea turtles, *Chelonia mydas*, caused by tubercle bacilli of the *Mycobacterium avium* complex.

## METHODS

One hundred-twenty hatchling Pacific green sea turtles from the French Frigate Shoals, 800 km west-northwest of Hawaii, were brought to the University of Hawaii for studies. These islands are bird and wildlife sanctuaries and uninhabited except for a small U.S. Coast Guard station. Three to five turtles were kept

in 60–80 liter tanks of filtered seawater which was changed weekly. The water was obtained from a 26-meter coral filtration well. Turtles were fed twice daily by being placed in dish-pans containing experimental diets, consisting of cereal grains and frozen squid and fish, suspended in water.

Turtles that died were immediately refrigerated and examined within 24 hours or were frozen until postmortem examinations could be performed. When gross lesions suggestive of tuberculosis were found, impression smears of the lesions were made and stained by the Ziehl-Neelsen method. Specimens for histologic examination were fixed in 10% formalin and the prepared tissue sections were stained with hematoxylin and eosin.

Some specimens of lung were forwarded to the National Animal Disease Center in Ames, Iowa for isolation and identification of the mycobacterium.

Chickens housed in a room adjacent to the turtles were intradermally injected with 0.1 ml of avian tuberculin in the wattles and observed for 24–72 hours. Postmortem examinations were performed on chickens that died and on apparently normal chickens.

## RESULTS

Of the original 120 turtles, 22 died during the first 5 months of captivity. Compaction of feces in the large intestines was determined to be the cause of death in some, but the cause of death was not determined in a large number of cases. No evidence of tuberculosis was found in these turtles.

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TABLE 1.—Summary of the results of histopathologic examinations of tissues of turtles and microscopic examination of acid-fast stained impression smears.

Animal number	Impression smear acid-fast stain	Granulomatous lesion			
		Lung	Liver	Kidney	Spleen
73-40	+ (lung)	3+ <sup>a</sup>	1+	1+	-
73-73	-	± <sup>b</sup>	-	-	-
73-74	N.D. <sup>c</sup>	±	-	-	-
73-85	N.D.	3+	1+	2+	-
73-95	+ (lung) + (kidney)	3+	±	3+	-
73-113	N.D.	1+	-	-	-
73-114	N.D.	N.D.	1+	-	-
73-143	+ (lung)	3+	2+	1+	1+
73-158	N.D.	±	-	-	-

<sup>a</sup> Severity of lesions: 3+ = severe; 2+ = moderate; 1+ = few lesions or only giant cells seen; - a negative.

<sup>b</sup> ±: granuloma, not typical of tuberculosis.

<sup>c</sup> N.D.: not done.

Nine turtles died from the 6th through the 7th month of captivity. All cases of tuberculosis described in this report were found in this latter group of 9 turtles. Grossly visible lesions in the lungs consisted of yellowish-white nodules ranging in size from 0.5 to 2 mm in diameter. The nodules were also seen in the liver and kidneys of some turtles. Table 1 represents a summary of the results of microscopic examinations of impression smears stained for acid-fast organisms and of histologic sections of lung, liver, kidney, and spleen.

Acid-fast organisms were observed in impression smears of lungs from turtle 73-40, 73-95, and 73-143, and in the kidney smear of turtle 73-95.

Specimens of lung from turtles 73-40 and 73-95 were sent to the National Animal Disease Center for culture and identification. Bacilli were isolated from turtle 73-40 and were identified as *Mycobacterium avium*, Serotype 8. Organisms were not isolated from turtle 73-95.

Typical tuberculous lesions (Fig. 1) were seen in the tissues of six turtles (73-40, 73-85, 73-95, 73-113, 73-114, and 73-143). These lesions could be described as focal granulomas characterized by central necrosis, sometimes with calcification, and infiltrated by macrophages, lymphocytes, and Langhan's-type multinucleated giant cells. The lesions were surrounded by fibroblastic elements.

Histopathologic lesions were graded as

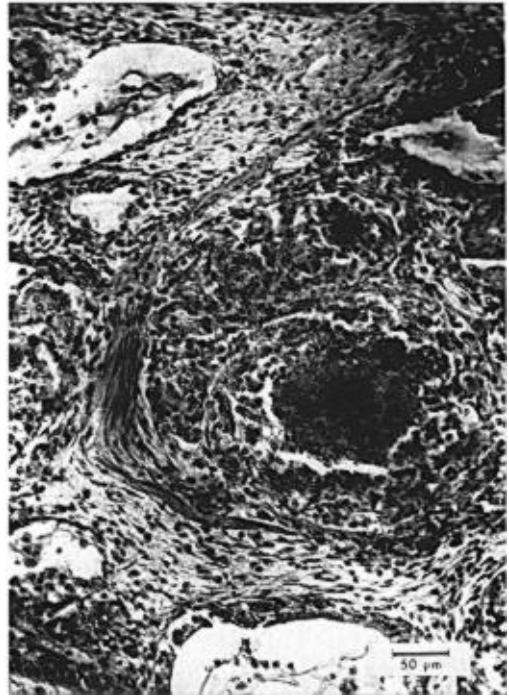


FIGURE 1.—Tuberculous lesion in the lungs of turtle No. 73-143. Hematoxylin-eosin stain.

severe (3+), moderate (2+) or mild (1+). Mild lesions consisted of only a few granulomas or sometimes only an aggregate of a few giant cells (Table 1). Lesions in lung, liver, and kidney were seen in four turtles. Additionally, one of these had splenic lesions. Microscopic lesions were seen in tissues of two other turtles that were not detected grossly.

Lesions not typical of tubercular granulomata were seen in four turtles (±) (Table 1). A granulomatous lesion in the lung of one turtle contained mycelial elements suggestive of a fungal infection. Necrosis with many neutrophils and bacteria-like bodies were seen in the lungs of two turtles and the liver of another. With the exception of the one with the liver necrosis which had characteristic tuberculous lesions in other organs, these turtles were not considered to have tuberculosis.

Evidence of tuberculosis was not found in gross and microscopic examinations of the other 22 turtles which died in the first 5 months of captivity. Acid-fast stained impres-

sion smears were made of only eight of these 22 turtles and acid-fast organisms were not found.

Postmortem examinations of mature chickens housed in a room adjacent to the turtles did not show evidence of tuberculosis. All skin tests with avian tuberculin were negative in chickens examined 24–72 hours after inoculation of the antigen.

#### DISCUSSION

As far as can be determined, bacilli of the *M. avium* complex have not heretofore been isolated and identified in cold-blooded species. Furthermore, these are the first known cases of tuberculosis in the green sea turtle, *Chelonia mydas*. The six cases of tuberculosis reported here occurred during a 2-month period, which represents a high attack rate. The severity of the disease and the high attack rate may suggest a relative susceptibility of turtles to this bacillus.

The severity and consistency of the pneumonic lesions may indicate the greater likelihood that infection was by airborne transmission rather than by ingestion of the bacilli.

The source of the infection in turtles was not determined and the more likely possibilities considered were:

1. Contact with tuberculous animals and poultry. *M. avium* Serotype 8 is usually associated with poultry, cattle, and swine (Thoen et al. 1972). There were no known direct contacts of the turtles with these animals; however, some laboratory personnel had contact with these species and the turtles. Tuberculosis was not detected in chickens housed in an adjacent room.

2. The feed may have contained infective bacilli. However, there was no evidence of lesions in the digestive tract to support this possibility.

3. Human beings in contact with the turtles may have been a source of the bacteria. Tuberculosis caused by bacilli of the *M. avium* complex is rare in human beings.

4. The turtles could have become infected at the hatching site, either directly from the dam or indirectly from the wild seabirds that share the same environment in the French Frigate Shoals.

The occurrence of this serious disease in turtles points out the need for care and vigilance in raising captive species. If these animals were being raised for commercial purposes, diseases could result in slowed growth, mortalities, and be of public health significance in contact or consumption by human beings.

Animals raised in captivity and being released to replenish diminishing stocks of endangered species should be free of disease. Epizootics initiated by infected animals released in nature could counter the well-meaning intentions and efforts of conservationists.

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