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## TUBERCULOSIS IN A CAPTIVE COLONY OF PINNIPEDS

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**ABSTRACT:** Tuberculosis was diagnosed in 10 of 16 otariid seals upon post mortem examination. The species involved were New Zealand fur seals (*Arctocephalus forsteri*), Australian sea lions (*Neophoca cinerea*) and an Australian fur seal (*Arctocephalus pusillus doriferus*). Five seals died, four as a direct result of mycobacterial infection. One seal died of unrelated disease. The remaining 10 animals were subsequently tuberculin tested and then killed and necropsied. Tuberculous lesions were seen in five. Gross pathological changes were most commonly seen in the respiratory system. However, a generalized infection, a case with lesions confined to the liver and draining lymph nodes, and a case with tuberculous meningitis also were seen. Histological lesions were characterized by spindle cell proliferation and necrosis without mineralization or giant cell formation. The mycobacteria isolated was identified as belonging to the *Mycobacterium tuberculosis* complex but it appeared to be unique. Intradermal tuberculin testing showed promise as a diagnostic aid; however, the results were not statistically significant. Circumstances suggest that the initial infection was present when the seals were captured from the wild.

**Key words:** Tuberculosis, *Mycobacterium tuberculosis* complex, otariid seals, intradermal tuberculin test, captive study.

### INTRODUCTION

Although mycobacteria have been isolated from many different animals, there are few reports of infections in marine mammals. Tuberculosis has been reported previously in seals but few details were recorded (Blair, 1913; Ehlers, 1965). Atypical mycobacterial infections also have been reported in seals; a generalized infection due to *Mycobacterium smegmatis* (Gutter et al., 1987), and cutaneous infection due to *Mycobacterium fortuitum* (Lewis, 1987). In other marine mammals, cutaneous and pulmonary infections due to *Mycobacterium chelonii* have been described in an Amazon manatee (Boever et al., 1976) and *Mycobacterium marinum* has been isolated from lesions in the lungs and testes of another specimen of this species (Morales et al., 1985). Skin lesions associated with acid fast organisms were recorded in a stranded bottlenosed dolphin (Viale, 1981) but the organism was not isolated in culture. This paper details a

number of cases of tuberculosis in a population of seals in a marine park. The pathology of the disease is examined, the results of intradermal tuberculin testing are presented and some consequences of this episode are briefly discussed.

### MATERIALS AND METHODS

The seal colony was established at an open-air marine park 60 km north of Perth, Western Australia (31°33'S, 115°41'E). Six New Zealand fur seals (*Arctocephalus forsteri*) and three Australian sea lions (*Neophoca cinerea*) were collected from the Archipelago of Recherche in southern Western Australia (34°39'S, 122°27'E) in March 1981. Two additional New Zealand fur seals were collected from the same location between September and October 1983. One Australian fur seal (*Arctocephalus pusillus doriferus*) was imported from another Australian marine park in New South Wales in December 1983 and a hand-raised Australian sea lion was imported from a veterinarian in South Australia in March 1984. Three sea lions were collected as strandings not far from the marine park in December 1984, December 1985, and October 1985 respectively (Table 1).

The general demeanour, dietary intake and body weight of each seal was regularly monitored. Abnormalities in any of these parameters prompted veterinary attention.

Five seals died before May 1986 and were necropsied routinely. Selected tissues were submitted for histological examination. Tissues were

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TABLE 1. Tuberculin testing and post mortem findings in a captive colony of pinnipeds.

	Case number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Species	Af*	Af	Nc	Af	Nc	Apd	Nc	Nc	Af	Af	Af	Af	Nc	Nc	Af	Af
Sex	F	F	F	M	M	F	F	F	M	F	M	F	M	M	M	M
Date acquired	3/81	3/81	3/81	3/81	10/85	12/83	3/81	3/83	3/81	3/81	3/81	10/83	9/83	3/84	12/84	12/85
Origin	AR	AR	AR	AR	St	NSW	AR	AR	AR	AR	AR	AR	AR	SA	St	St
Age died	6	9	5	12	14	7	6	6	6	6	6	4	4	3	2	1
Date died/killed	11/81	5/83	3/85	1/86	3/86	5/86	8/86	8/86	8/86	8/86	8/86	8/86	8/86	8/86	8/86	9/86
Skin test results																
Bovine tuberculin	NA	NA	NA	NA	NA	+	+	+	— <sup>c</sup>	+	+	+	+	—	—	—
Bovine tuberculin (second)	NA	NA	NA	NA	NA	NA	+	+	+	+	+	+	+	—	—	—
Avian tuberculin	NA	NA	NA	NA	NA	NA	+	+	—	+	+	+	+	+	—	—
Lesions/acid fast bacilli																
Lung	+/-	+/+	+/-	+/+	+/+	-/-	+/+	-/-	+/+	+/+	+/+	-/-	-/-	-/-	-/-	-/-
Bronchial lymph node	-/-	+/+	+/+	-/-	+/+	-/-	+/+	-/-	+/+	+/+	-/-	-/-	-/-	-/-	-/-	-/-
Pleura	-/-	+/+	+/+	-/-	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Liver	-/-	+/+	-/-	-/-	+/+	-/-	-/-	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Meninges	-/-	-/-	-/-	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Other	-/-	-/-	-/-	-/-	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Mycobacterial culture	NA	NA	+	+	+	—	+	+	+	—	—	—	—	—	—	—

\* Abbreviations: Af, *Arctocephalus forsteri*; Apd, *Arctocephalus pusillus doriferus*; Nc, *Neophoca cinerea*; AR, Archipelago of Recherche; St, Stranding; NSW, New South Wales; SA, South Australia; NA, Not attempted.

<sup>b</sup> +, positive.

<sup>c</sup> —, negative.

fixed in 10% buffered formol saline before being embedded in paraffin. Five  $\mu$ m sections were cut and stained with haematoxylin and eosin. Selected sections also were stained by the Ziehl-Neelsen technique. Selected tissues were taken for aerobic bacterial cultures on blood and Mackonkey agars, and in some cases (see Table 1), for mycobacterial culture. Routine bacterial cultures and identification were conducted at the Murdoch University Veterinary Hospital (Murdoch University, Murdoch, Western Australia 6150, Australia). Mycobacterial cultures were performed at the Western Australian Department of Agriculture (Baron Hay Court, South Perth, Western Australia 6151, Australia) and isolates were identified by the State Health Laboratory Service of Western Australia (Queen Elizabeth II Medical Center, Nedlands, Perth, Western Australia 6009) and the Commonwealth Scientific and Industrial Research Organization (Animal Health Division, Parkville, Victoria, Australia 3052).

In March 1986, 11 seals were injected intradermally at a shaved site in the dorsal cervical area of the neck with 0.1 ml of 3 mg/ml bovine purified protein derived (PPD) tuberculin (Commonwealth Serum Laboratories, Parkville, Victoria 3052, Australia). The double skin fold thickness of the site was measured with calipers prior to and 72 hr after the injection (Francis et al., 1973; Lesslie and Herbert, 1965). An increase in thickness of 4 mm was regarded as a positive reaction. One of the seals which reacted to the test was anaesthetized with intramuscular ketamine (Ketapex; Apex Laboratories, St. Marys, New South Wales 2760, Australia) and then killed by intracardiac injection of pentobarbitone (Lethabarb; Arnolds of Reading, Boroonia, Victoria 3155, Australia) and necropsied routinely. A comparative tuberculin test was performed 70 days later on the remaining 10 seals in the same manner using 0.1 ml of 1 mg/ml bovine PPD tuberculin and 0.1 ml of 25,000 U/ml avian PPD tuberculin (Commonwealth Serum Laboratories). These animals were subsequently anaesthetized and blood samples taken before being killed and necropsied.

The necropsy performed on the last 10 seals was more detailed than that performed on the other previous seals. Gross lesions and a wide range of lymph nodes were taken for mycobacterial culture. In cases where no gross lesions were seen on initial examination, the lungs were perfused with formalin, frozen and then sliced with a bacon slicer into serial sections approximately 3 mm thick which were individually examined for lesions. Samples for any gross lesions and of lung, liver, kidney, spleen, brain and a range of visceral and peripheral lymph nodes were taken for histological examination

and stained with haematoxylin and eosin. Selected sections also were stained with Ziehl-Neelsen, Martius scarlet blue, Phosphotungstic acid haematoxylin, Giemsa and Gram stains.

## RESULTS

### Clinical and necropsy findings

In the first instance, the diagnosis of tuberculosis was made retrospectively. This animal died of post partum haemorrhage and at necropsy a single pulmonary granuloma was noted as an incidental finding.

Clinical signs referable to tuberculosis were observed in four seals which subsequently died (Table 1, cases 2 to 5). They varied from a short period of anorexia with or without dysphagia to one case (3) in which intermittent anorexia and loss of weight occurred over 14 mo. Coughing was not a prominent feature. Thoracic and abdominal radiographs revealed no abnormalities. Pulmonary lesions were found on necropsy of all four seals.

Two cases (2, 3) exhibited a marked pleural reaction with generalized thickening of both parietal and visceral surfaces and large volumes of serosanguineous fluid in the pleural cavity. Multiple nodular proliferations, 0.5 to 4 cm in diameter, projected from the diaphragmatic and pericardial pleural surfaces in one case (3, Fig. 2). When sectioned, these nodules had a firm, fibrous external coat and a paler core composed of softer, more friable material. Generally, the pleura was shiny, smooth, and pale yellow. It was up to 1 cm thick on the visceral surface. In this case there was minimal gross involvement of the lung itself whereas there was marked consolidation, collapse and focal granuloma formation within the lung in the other case with generalized pleuritis (2).

A widely disseminated infection was present in one case (5). Multiple 2 to 4 mm pale yellow, soft nodules occurred throughout the lungs which were generally collapsed with focal areas of emphysema involving the anteroventral borders and the dorsal aspects of the diaphragmatic lobes. Similar lesions were present



FIGURE 1. Lesions in the subpleural parenchyma of the lung of a New Zealand fur seal. These were the only lesions detected in this animal (case 9).

in the liver, spleen and kidney, and in the bronchial, mesenteric, colonic and pancreatoduodenal lymph nodes.

In one case (4) in which only a small lung lesion was seen grossly, the cause of death was not clear on gross examination. Grossly, the brain appeared normal.

Lesions were seen in five of the 11 seals that were tuberculin tested. Lung lesions were the most common finding and varied from a single  $5 \times 3 \times 2$  cm firm, uniformly pale grey nodule deep within the parenchyma to multiple 2 to 5 mm pale firm amorphous nodules in the subpleural parenchyma which were either isolated or arranged in small rosette formations (Fig. 1). In two cases (10, 11), lesions were found only on examination of serial sections of the lung.

The bronchial lymph nodes were enlarged in most cases. They were firm to cut and pale with indistinct cortico-medullary borders. In one case (9), the bron-

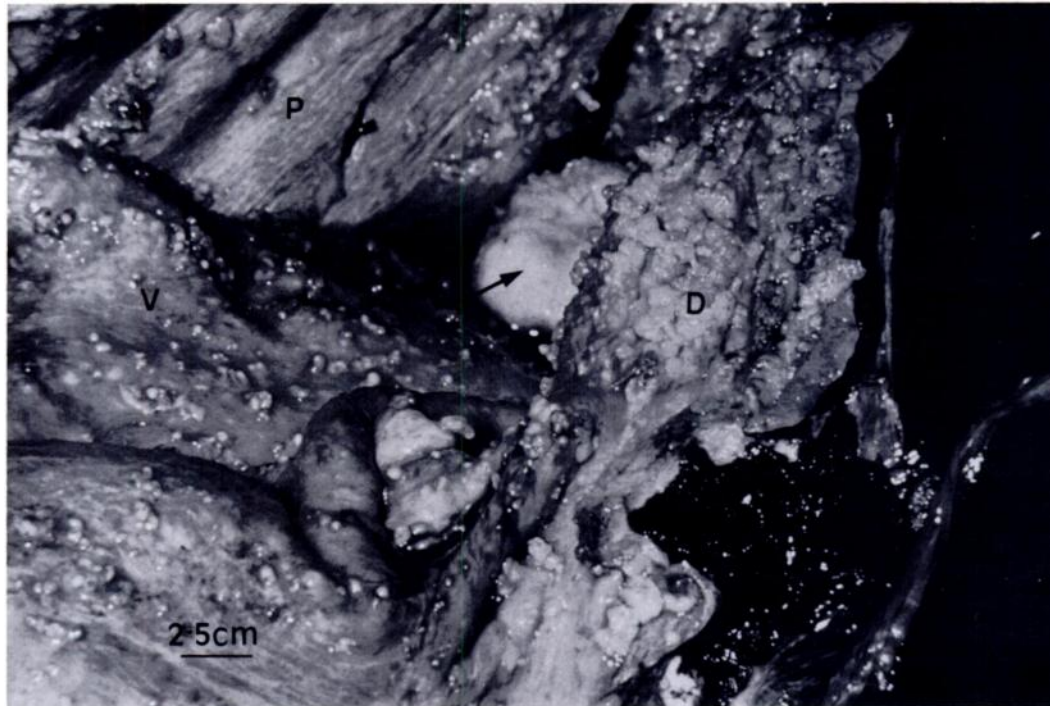


FIGURE 2. Generalized granulomatous pleuritis in an Australian sea lion with tuberculosis. The large nodule (arrow) was found floating in the pleural cavity (case 3). V: visceral pleura. P: parietal pleura. D: diaphragmatic pleura. L: liver.



chial lymph node was grossly enlarged and the centre occupied by a 5 cm diameter caseous pale mass, speckled with pinpoint white foci.

In one case (8), lesions were confined to the liver and hepatic lymph node. The liver lesions were 2 to 3 cm diameter focal; they were sometimes confluent, raised and pale nodules, the larger of which had depressed centres. The hepatic node was enlarged and it was similar to the bronchial nodes seen in the majority of the other cases.

#### Histopathology

Histological examination of lesions generally revealed a consistent pattern of well orientated spindle cell proliferation. Typically, the cells had a single, large, oval vesicular nucleus and sparse eosinophilic cytoplasm. An outer rim of lymphocytes and occasional plasma cells were seen in most lesions although the intensity of the infiltrate varied. Polymorphonuclear leukocyte infiltration, a feature of case 5, was minimal in other cases. Central necrosis was a feature of many lesions but mineralization was not prominent.

The lesions in the lymph nodes consisted of almost pure spindle cell infiltration ramifying through nodal sinusoids (Fig. 3). Occasional foci of necrosis were present, usually with a fine microscopic pattern of mineralization. The lymph nodes were markedly hyperplastic. In some cases, histological lesions were seen in the lymph nodes when gross lesions were not.

The pleural reaction seen in the earlier cases (2 and 3) was remarkable for the degree of fibrosis present. There was intense fibroplasia emanating from the pleural surface with marked lymphocytic infiltration along the deep border. Occasional neutrophils were scattered throughout the reaction. The superficial layers were necrotic, remaining in situ as an intensely eosinophilic coagulum within which a few pyknotic nuclei were seen (Fig. 4).

In the case in which the cause of death was not obvious on gross inspection (4) there



FIGURE 3. Affected lymph node with predominantly spindle cell infiltration through the sinusoids in an Australian sea lion with tuberculosis (case 7). C, capsule. L, loose connective tissue. V, blood vessel.

was a severe meningeal reaction presenting as intense hyperemia and fluid exudation, with focal areas of coagulative necrosis throughout the meninges. The foci of necrosis were rimmed by large epithelioid mononuclear cells with abundant pink staining cytoplasm and large oval nuclei. Granulocytes infiltrated into the areas of necrosis and prominent lymphocyte accumulations were seen around larger vessels. A mild neutrophilic arteritis also was present in individual vessels but this was not a prominent feature.

Acid fast bacilli were seen in lesions from the four animals that died as a result of mycobacterial infection and in two which underwent tuberculin testing.

#### Tuberculin testing

Results of the intradermal tuberculin testing are summarized in Table 1. The



FIGURE 4. Diaphragm showing marked fibrocytic response in an Australian sea lion with tuberculosis (case 3). Superficial layers (S) are necrotic. D, diaphragmatic muscle.

reaction to avian PPD tuberculin was less pronounced than the reaction to bovine PPD tuberculin in all but one case and one seal (14) which did not respond to bovine PPD tuberculin, reacted to avian PPD tuberculin. Statistical analysis of the correlation between the presence of lesions and the results of the intradermal tuberculin testing using Fishers Exact Test revealed that they were not significantly related.

#### Bacteriology

Mycobacteria were isolated from lesions of six of the 14 seals whose tissues were cultured for mycobacteria. In the two cases (10, 11) in which lesions were seen but cultures were negative, lesions were only found upon fine slicing of the lung and the actual lesions were not cultured. Although originally identified as *Mycobacterium*

*bovis*, genetic analyses revealed significant differences to *M. bovis*. Final classification placed the isolate within the *M. tuberculosis* complex; however, it may be a unique species (Cousins, 1990).

#### DISCUSSION

The nature and the distribution of the lesions of tuberculosis vary according to the species of animal affected, the species and strain of mycobacteria involved, the immunity of the host, the route of infection and probably other ill-defined variables (Jubb et al., 1985).

*Mycobacterium tuberculosis* infections in humans and primates and *M. bovis* infections in ruminants, pigs and nonhuman primates usually result in the formation of distinctive granulomas with central areas of necrosis, epithelioid cells, and Langerhans type giant cells (Luke, 1958; Thoen and Himes, 1980; Cordes et al., 1981; Jones and Hunt, 1983; Jubb et al., 1985). Mineralization is a marked feature of the lesions in cattle (Jubb et al., 1985). Although multifocal granulomas with central necrosis were consistent, mineralization and giant cell formation were not prominent features of the disease in seals.

Tuberculosis due to *M. bovis* infections in horses is a chronic proliferative disease producing lesions grossly resembling a sarcoma and caseation is not a feature (Innes, 1949; Luke, 1958). The pleural reactions seen in two seals grossly resembled sarcomas and, histologically, the fibrocytic proliferation also had features in common with a sarcoma. This is similar also to the reaction seen in dogs (Jubb et al., 1985). There were no details recorded in a reported outbreak of tuberculosis in hooded seals (*Cystophora cristata*) (Blair, 1913) or in a case in a California sealion (*Zalophus californianus*) (Ehlers, 1965).

The majority of seals developed pulmonary lesions; therefore, inhalation was probably the major route of infection. However, in one case only liver and hepatic node lesions were seen, so alimentary

infection also was presumed to have occurred.

The lack of specific clinical signs and the failure of radiographic examinations to reveal lesions, even in advanced cases, suggests that their use is limited in the diagnosis of tuberculosis in pinnipeds. The results of tuberculin skin testing were not statistically significant. However, the apparently high degree of sensitivity among this small number is encouraging and suggests that this technique may be a useful test in seals. The low degree of correlation may be due to the low numbers of animals tested. An enzyme-linked immunosorbent assay also appears to have merit (Cousins, 1987).

The first two cases in which tuberculosis was diagnosed occurred after the colony was established by collection of wild seals and before any additional seals were imported. These cases were not confirmed by culture and in the first case the diagnosis was made retrospectively solely on the basis of histopathological changes. Acid fast organisms were not seen within lesions. However, the pathology conformed with that seen in the ensuing cases. Because all seals of which culture results were available were infected with the same strain of organism and since tuberculosis is not commonly recognized in seals, it was assumed that the infection spread within the confines of the park. Given that no cases of tuberculosis were identified in any of the park staff or any other animals kept on the premises and the fact that the seals previously had little contact with the public at the time of the first case being diagnosed, it is possible that at least one of the seals was infected at the time of capture. This has ramifications for all colonies of captive seals because until the efficacy of diagnostic procedures are determined, it seems prudent to at least tuberculin test all seals being added to existing collections including wild caught animals. This is particularly important because of the zoonotic potential of tuberculosis and the difficulty of treatment and control.

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