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## Tuberculosis in a Wild Australian Fur Seal (*Arctocephalus pusillus doriferus*) from Tasmania

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**ABSTRACT:** Tuberculosis was found in a wild, mature male Australian fur seal (*Arctocephalus pusillus doriferus*) at Hobart, Tasmania on 8 March 1992. We observed fibrogranulomatous and pyogranulomatous lesions in the lung, pleura, lymph nodes and spleen. The SDS/PAGE profile of this Tasmanian isolate was similar to other seal strains; however, differences were detected using pTBN12 and insertion sequence IS6110 probes.

**Key words:** Australian fur seal, *Arctocephalus pusillus doriferus*, tuberculosis.

Australian fur seals (*Arctocephalus pusillus doriferus*) are found in Bass Strait, and at rocky and exposed places on the coasts of Tasmania and in Victoria, and southern New South Wales of Australia (King, 1983). The Tasmanian population of Australian fur seals has been estimated to be between 6,500 and 30,000 animals (Pearse, 1979; R. Kirkwood, unpubl.). Although not often encountered by the public, these seals commonly are seen around fish farms located in southern Tasmania, from which they are captured and relocated to other areas in Tasmania. Occasionally they haul out on beaches located in populated areas where they are of great interest to the public. We report the finding of tuberculosis in a wild, mature, male Australian fur seal at Hobart, Tasmania.

The seal had been seen swimming around and intermittently hauling out in the area of the Hobart docks (42°53'S, 147°19'E) for approximately 2 wk during late February and early March 1992. It was watched by officers from the Department of Parks, Wildlife and Heritage and a perimeter fence about 2 to 3 m from the seal was erected at its haul out site to minimize interference by the public.

On 3 March 1992 the animal was bright and alert and appeared to be in average body condition for a male Australian fur seal at that time of year, weighing an estimated 230 kg. There was a penetrating wound to the left distal aspect of the neck which was clean and dry though there was inspiratory dyspnea. Based on examination of its feces, it appeared that the seal had been feeding; however, the animal appeared to have difficulty swimming.

Five days later, on 8 March 1992, the animal's condition seemed to have deteriorated. It appeared weak, lethargic, and no longer exhibited interest in its surroundings. There was thick, tenacious green pus discharging from the penetrating wound on the neck and profound inspiratory dyspnea with gasping mouth breathing. The animal was considered to be suffering excessively and the long term prognosis for full recovery was considered minimal. The animal was euthanized using a 7.62 caliber rifle shot to the head.

At necropsy the animal was relatively thin for an animal at that time of year. The neck wound appeared consistent with a penetrating injury or gun shot wound, but neither a bullet nor evidence of penetration of the thoracic cavity or other structures could be found.

Blood and pus occurred in the upper esophagus. Subcapsular ecchymotic hemorrhages were present on the visceral surfaces of the kidneys. A superficial well-circumscribed, yellow-white, multilobulate mass approximately 10 × 15 cm was associated with the penetrating wound of the neck. Ecchymotic hemorrhages and bruising were present in the left axilla. The

spleen was pale and flaccid; the pericardium was opaque, white, and diffusely thickened. The left submandibular, thoracic and abdominal lymph nodes were swollen and edematous but there was no gross evidence of abscessation. The trachea and bronchi contained blood and thick, creamy, yellow pus. There was bilateral pyothorax which contained about 1.5 l of thin, watery, mucopurulent effusion. The parietal pleura and mediastinum were opaque and diffusely thickened and the right apical lung lobe appeared collapsed. Firm, nodular lesions with a central core of pus varying in size from that of a pin head to confluent, consolidated areas approximately  $10 \times 6 \times 4$  cm were visible in both caudal lung lobes. They were rubbery to cut. The larger nodules contained inspissated pus and the smaller nodules liquid pus. Grossly the lesions were similar to those described by Forshaw and Phelps (1991) from tuberculous seals. An eroded area was present on the right, costal lung surface where a nodule had ruptured into the thoracic cavity.

Samples were taken for histopathological and bacteriological examination and submitted to the Mt. Pleasant Laboratories, Launceston, Tasmania. After necropsy the carcass was buried immediately.

Samples of liver, spleen, bronchial lymph node, lung, portions of the mass associated with the penetrating wound and a sample of the pleural effusion were preserved in 10% formalin, embedded in paraffin, sectioned at  $5 \mu\text{m}$ , and stained with hematoxylin and eosin. Selected paraffin embedded tissues also were stained by the Ziehl-Neelsen method for acid fast bacilli (Corner, 1993). Lung and pleural fluid were submitted for bacteriology. Duplicate smears were taken from each sample and streaked onto 5% sheep blood, MacConkey and Edwards Agars (Oxoid, West Heidelberg, Victoria, Australia), and incubated aerobically; the blood agar was incubated with 8%  $\text{CO}_2$ , at  $37^\circ\text{C}$  for 2 days. Mycobacterial culture (Corner, 1993) also was carried out on each sample.

Based on histopathological examination of the tissues, we observed fibrogranulomatous and pyogranulomatous lesions in the lung, pleura, lymph nodes, and spleen. Sub-acute to chronic proliferative pleuritis was evident. Fibroplasia was evident in the bronchiolar lymph node and spleen as were foci of necrosis and old abscessation. Ziehl-Neelsen-stained sections had red filamentous acid-fast organisms occurring either as single bacilli or in small aggregates. The organisms were associated with necrotic debris at the center of the pyogranulomas and within macrophages at the periphery of these lesions. These organisms were most prevalent in the pleuropneumonic lesions. Pleural fluid and lung cultures had large numbers of mixed bacterial flora by Gram-stain; however, cultures for *Mycobacterium* spp were overgrown with a contaminant and no *Mycobacterium* spp were isolated. Because of the contamination problems encountered and the importance of determining whether the causative organism belonged to the pathogenic *Mycobacterium tuberculosis* complex, the remaining specimens of pleural fluid and lung were forwarded to the Tuberculosis Laboratory at the Department of Agriculture, Western Australia for testing by polymerase chain reaction (PCR). The deoxyribonucleic acid (DNA) was extracted from pleural fluid and lung tissue and tested using a multiplex PCR as described by Cousins et al. (1992). Specimens received in Western Australia were cultured using the procedures of Corner (1993) except that they first were decontaminated with 2% NaOH because of the likelihood of bacterial contamination. In preliminary results, using direct PCR on pleural fluid, we detected DNA bands consistent with that of the *M. tuberculosis* complex. An acid-fast organism was isolated from the pleural fluid after 2 wk incubation. No growth was detected from the lung tissue after 8 wk of incubation. The acid-fast organism isolated was negative when tested for MPB70 antigen using the immunoperoxidase test (Corner et al., 1988); however, using con-

ventional biochemical tests and drug sensitivity tests (Kent and Kubica, 1985), we identified the strain as *M. bovis*. Based on the multiplex PCR performed on the isolate, we identified it as consistent with a *M. tuberculosis* complex organism.

The SDS/PAGE protein analysis and DNA fingerprinting was performed on this isolate using the methods of Cousins et al. (1993). The resulting patterns were compared with those from the *M. tuberculosis* complex strains isolated from six captive seals at a marine park near Perth, Western Australia (Cousins et al., 1990; Forshaw and Phelps, 1991), a seal trainer who had worked at the affected marine park from 1983 to 1985 (Thompson et al., 1993), and two wild pinnipeds found on the coast of Western Australia at Albany and Bremer Bay (Cousins et al., 1993). Extracted genomic DNA was digested with the restriction endonucleases *Alu* I, *Taq* I, *Bst* EII, *Bcl* I and *Pvu* II (Cousins et al., 1993) and evaluated with the repetitive element pTBN12 (Ross et al., 1992) and the insertion element IS6110 (Thierry et al., 1990).

The SDS/PAGE profile of the isolate from the Australian fur seal from Tasmania was similar to the other seal isolates. Based on a Western blotting test (Cousins et al., 1990), we failed to detect the MPB70 protein, which is present in *M. bovis* as a 22 kiloDaltons (kDa) antigen. The DNA fingerprints of the *Alu* I digests evaluated with pTBN12 produced the same pattern as found in other seal isolates. When *Taq* I digests were evaluated with pTBN12, all seal isolates had the same pattern with the exception of a single band difference in the isolate from the Tasmanian seal. Single band differences were detected in the *Bst* EII and *Bcl* I profiles using the pTBN12 probe. When the insertion sequence IS6110 was used as probe, we also detected differences between the Tasmanian isolate and previous isolates. The profiles of the seal tuberculosis isolates all were more similar to each other than to other isolates belonging to the *M. tuberculosis* complex. The Tasmanian seal isolate had bands in

common with the other seal isolates using IS6110 but differences were detected in each of the *Bst* EII, *Pvu* II and *Bcl* I digests. Because fewer bands are detected using the IS6110 probe, the differences were easier to see than when using pTBN12 as probe. The Tasmanian seal isolate appeared to belong to the unique cluster of seal isolates identified by Cousins et al. (1993); these, in turn, are different from other members of the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*). Isolates from this cluster have now been detected in three different species of wild seals which inhabit southern Australian coastal waters.

This is the first reported case of tuberculosis in an Australian fur seal and the first confirmed case of tuberculosis due to the *M. tuberculosis* complex in a seal outside of Western Australia. The disease also was found in a mature animal, with lesions consistent with the chronic progressive disease as reported in other species. The clinical, gross and histopathological findings were consistent with those reported from captive Australian sea lions (*Neophoca cinerea*) and New Zealand fur seals (*Arctocephalus forsteri*) with tuberculosis in Western Australia (Forshaw and Phelps, 1991). Tuberculosis recently has been reported in two adult male Australian sea lions and one adult male New Zealand fur seal found on beaches on the south coast of Western Australia (Cousins et al., 1993). Although the prevalence of tuberculosis in wild, free-ranging seals is unknown, the risk of humans or domestic stock becoming infected with tuberculosis probably is low because of the usually rare and/or brief duration of their contact with wild seals. Appropriate precautions should, however, be taken when handling seals or seal carcasses because of the zoonotic nature of the disease and marine park staff, seal researchers, other scientists and the public should be aware of the possibility of tuberculosis in pinnipeds.

Studies of prevalence and incidence of the disease may be of value in areas where

tuberculosis has been diagnosed in pinnipeds and humans are in contact with the animals. In wild animals, an enzyme-linked immunosorbent assay (ELISA) test (Cousins, 1987) on serum and PCR on nasal sputum may be the best tests as animals need to be captured and restrained on two occasions, 72 hr apart, to perform the comparative intradermal tuberculin test; results of this latter test can be misleading due to cross reaction with atypical mycobacteria. More practical, readily applicable tests are required for routine screening of wild animals for this disease.

Until studies into prevalence and incidence of tuberculosis in wild pinnipeds are undertaken, the disease should be suspected in cases where solitary sick animals, whether in a colony or stranded away from colonies, have clinical signs of respiratory embarrassment consistent with tuberculosis (such as coughing or breathing difficulties). In these cases, standard safety precautions should be taken to avoid infection when handling animals and ELISA, PCR or comparative intradermal tuberculin tests performed. A positive result may be sufficient evidence to warrant euthanasia.

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