Tuberculosis in sea lions and fur seals from the south-western Atlantic coast

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Summary: Diverse pathological conditions causing the strandings and/or
deaths of several species of sea lions and seals on the northern coast of the
province of Buenos Aires are being studied.

Tuberculosis was diagnosed in six cases of strandings, involving two otariid
seal species (one Otaria flavescens and five Arctocephalus australis), between

Necropsies were performed on all six cases. Granulomatous lesions were
observed in the prescapular and hepatic lymph nodes. Lesions were also seen
in the lungs, pleura, liver, spleen and peritoneum.

Bacteriological isolation was attempted from all the samples. The isolates
were identified as belonging to the Mycobacterium tuberculosis complex. Some
showed characteristics consistent with M. bovis, whereas others demonstrated
properties of M. tuberculosis.

Genomic deoxyribonucleic acid (DNA) from these strains was analysed by
restriction fragment length polymorphism (RFLP), using IS6110, a genetic
marker found only in the Mycobacterium tuberculosis complex. Using the
IS6110 probe, similar fingerprints were obtained, suggesting a common source
of infection. However, the pattern of DNA differed from DNA patterns of
M. bovis isolated from humans and cattle in Argentina, which generally
contain a unique 1.9 kbp band.

These results suggest that mycobacteria isolated from wild seals form a
different grouping inside the M. tuberculosis complex. This is the first time that
tuberculosis has been detected in wild seals from the south-western Atlantic
coast.

KEYWORDS: Deoxyribonucleic acid pattern – Marine mammals –
Pinnipeds – Restriction fragment length polymorphism – Sea lions – Seals –
Tuberculosis.

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INTRODUCTION

Studies on tuberculosis have been directed principally towards terrestrial mammals, in particular humans and domestic animals because of the pathogenicity of Mycobacterium tuberculosis complex in these species.

Information about tuberculosis in wild species is more recent, and is related principally to terrestrial animals. There are very few reports of this disease in marine mammals (33). The first accounts concerned animals in zoos (3, 15), but those accounts focused specifically on the diagnosis of the disease and not on mycobacterial identification or histopathological lesions.

More recently, cases of tuberculosis have been detected in an Australian sea lion (Neophoca cinerea) and in a New Zealand fur seal (Arctocephalus forsteri), held in captivity at a marine park in Australia (16). These strains were identified as belonging to the M. tuberculosis complex but had unique characteristics (10, 11).

In this article, the authors report six cases of tuberculosis in wild otariid seals (one in Otaria flavescens and five in Arctocephalus australis), found stranded on the coast near the Samborombón Bay (province of Buenos Aires, Argentina) or caught for medical treatment at the Rehabilitation Centre of the Fundación Mundo Marino.

Molecular serotyping techniques were used to differentiate the mycobacterial isolates. The IS6110 probe is an excellent tool to investigate the epidemiology of tuberculosis, but has not yet been extensively used in cases concerning wild animals. IS6110 is an insertion sequence located many times at different points in the chromosome. By using this probe, one can distinguish between many subtypes of M. tuberculosis complex (19).

General characteristics of the species studied

These marine mammals belong to the suborder Pinnipedia, comprising three families: walruses (Odobenidae), seals and elephant seals (Phocidae) and sea lions and fur seals (Otariidae) (23).

Sea lions include five species, as follows:
- New Zealand sea lions (Phocartos hookeri)
- southern sea lions (O. flavescens)
- Steller sea lions (Eumetopias jubatus)
- Australian sea lions (N. cinerea)
- Californian sea lions (Zalophus californianus). There are thought to be three subspecies of this sea lion.

The species involved in this report is O. flavescens (= O. byronia), commonly known as the southern sea lion or, in Spanish, 'lobo marino de un pelo' (26).

This species is found along the coast of South America, from Brazil to Peru. It principally inhabits coastal areas, and has a diet based mainly on demersal coastal fish.

The total population of O. flavescens is estimated to be approximately 275,000 individuals and, of these, about 175,000 are thought to inhabit the Argentine coast, in some 70 rookeries, both insular and continental (Fig. 1). The population distributed along the Uruguayan coast is estimated to be about 35,000 (6, 25).
Fur seals include nine species, as follows:
- northern fur seal (Callorhinus ursinus)
- Antarctic fur seal (A. gazella)
- South American fur seal (A. australis)
- Galapagos fur seal (A. galapagoensis)
- New Zealand fur seal (A. forsteri)
- Subantarctic fur seal (A. tropicalis)
- Guadalupe fur seal (A. townsendi)
- Juan Fernández fur seal (A. philippi)
- South African/Australian fur seal (A. pusillus).
The second species of wild seal which was found to have tuberculosis is *A. australis*, commonly known as the South American fur seal or 'lobo marino de dos pelos'. This species is distributed along the Atlantic and Pacific coasts of South America, from Brazil to Peru, although most of the population is concentrated in the rookeries of the Uruguayan islands (Fig. 2).

**FIG. 2**

**Distribution of rookeries of *Arctocephalus australis* in the south-western Atlantic**

The total population of *A. australis* is estimated to be approximately 500,000 animals (250,000 of which inhabit the island colonies of Uruguay). The population on the coasts of Argentina and Chile has been greatly reduced as a consequence of intensive human exploitation. The population along the coast of Peru is slightly larger than in Argentina and Chile (13, 32).
A. australis has fewer coastal habits than O. flavescens, with a pelagic stage during the non-breeding season. This seal feeds on both demersal coastal fish and pelagic fish. Squid is also an important item in the natural diet.

In the Samborombón Bay area (Argentina), where the stranded specimens were found (Fig. 3), there are no rookeries of either O. flavescens or A. australis. The specimen of O. flavescens found on the beach may have come from a colony in Uruguay or Argentina. The A. australis specimens probably came from the Uruguayan rookeries, as reports indicate that these seals frequently migrate towards Buenos Aires province during the pelagic stage (2). The possibility that the stranded A. australis seals came from Argentine stocks is unlikely, as the rookeries are located along the Patagonian coast, very far from Samborombón Bay, and the population consists of no more than a few thousand individuals.

Most of the individuals of both species which were found stranded on the Samborombón Bay coast were physiologically depressed, with a higher percentage of diverse illnesses than is generally seen in animals found in natural colonies.

**Fig. 3**

Northern coast of Buenos Aires province – stranding zone
MATERIALS AND METHODS

Case studies

Case 1

An adult female *A. australis* (W/N) was found in Cariló, Buenos Aires province, Argentina, on 27 February 1989. The initial (and final) weight of this seal was 40 kg. The specimen showed dyspnoea, anorexia and weakness and was in poor physical condition.

**Medical treatment**

Cloramphenicol (10 mg/kg/12 h) and colistin metasulfonate (7.50 international units [IU] per kg every 12 h) were administered by intramuscular injection. The animal was fed 500 g of minced fish through a tube. A total of 500 ml of multivitamin solution and calcium gluconate were added to the diet.

The animal died 48 hours after arriving at the centre.

Case 2

An adult male *A. australis* (M-17-92) was found in San Clemente del Tuyú, Buenos Aires province, on 22 June 1992. Initial weight: 60.5 kg. Final weight: 78.5 kg. This specimen arrived in very poor physical condition, but recovered significant weight at the Rehabilitation Centre.

**Medical treatment**

The animal was treated with Enrofloxacin (5 mg/kg/24 h) and a multivitamin supplement and force-fed with 3 kg of fish. This ration was slowly increased to 7 kg by day 15 after arrival at the centre.

The animal died 28 days after arriving at the centre.

Case 3

An adult female *A. australis* (M-31-92) was found in Las Toninas, Buenos Aires province, on 18 August 1992. Initial weight: 27.5 kg. Final weight: 26.0 kg. This specimen was in very poor physical condition, with variable appetite. It drank fresh water. Defecation was normal but the animal was in a permanent ‘decubitus’ position and had a cut on the left side of the body.

**Medical treatment**

Enrofloxacin (5 mg/kg/24 h) was administered. A vitamin supplement was given with the fish diet. The injury on the left side was treated in the usual way.

The seal was force-fed with an initial ration of 500 g of fish, increased by day 7 to 2.0 kg. The animal died 24 hours later.

Case 4

An adult female *A. australis* (M-38-92) was found in Mar de Ajó, Buenos Aires province, on 24 September 1992. Initial weight: 27.5 kg. Final weight: 24.0 kg. The animal was very weak, in a permanent ‘decubitus’ position. Faecal material was black in colour.
Medical treatment

Ampicilin (22 mg/kg/8 h) was administered. Multivitamins and a 5% glucose solution were administered intravenously. An electrolyte solution was given by tube. The animal was initially force-fed with small quantities of fish. The amount was increased to 2 kg of fish after nine days.

The animal died ten days after arriving at the centre.

Case 5

An adult male O. flavescens (M-47-92) was found in Mar de Ajó, Buenos Aires province, on 20 November 1992. Initial and final weight: 180 kg. The specimen was in a very poor physical state, with an injury to the front left flipper. The animal was in a permanent ‘decubitus’ position, but displayed very aggressive behaviour.

Medical treatment

Enrofloxacin (5 mg/kg/24 h) was administered in the diet. On the fifth day, the sea lion was force-fed with 2 kg of fish, an amount which was slowly increased to reach a total of 7.5 kg. The diet was supplemented with a multivitamin solution.

The animal died twelve days after arriving at the centre. Respiratory distress was observed before death.

Case 6

A young male A. australis (M-11-91) was found in Las Toninas, Buenos Aires province, on 15 March 1991. Initial weight: 19 kg. Final weight: 25 kg. The animal was in good physical condition.

Medical treatment

The seal was treated with a multivitamin solution by tube, and force-fed with 300 g of fish. This amount was slowly increased to 1.5 kg by day 10 after arrival at the centre.

The animal was progressively rehabilitated, and housed in the Rehabilitation Centre for experimental studies. This seal died one year later, because of a traumatic accident.

Necropsies

Necropsies were conducted on the above cases, according to standard techniques for small carnivore animals (4, 22).

The carcasses were opened using the ventral midline to observe all the internal organs. Samples were obtained from selected organs for pathological and histopathological examination.

Histology

Samples were taken from the liver, spleen, lungs, lymph nodes and kidney, fixed in 10% formalin and processed and stained with haematoxylin and eosin, according to standard procedures.
Bacteriology

Samples obtained from necropsies were processed by the Petroff decontamination method and inoculated on Löwenstein-Jensen and Stonebrink media (27). Culture isolates were identified by biochemical, drug susceptibility and biological tests, according to standard procedures (5, 20) (Table I).

**Table I**

**Necropsy samples from five seals and one sea lion from which Mycobacterium species were isolated**

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample No.</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arctocephalus australis</td>
<td>1203</td>
<td>Right lung</td>
</tr>
<tr>
<td>(W/N)</td>
<td>1204</td>
<td>Pleura</td>
</tr>
<tr>
<td></td>
<td>1205</td>
<td>Abdominal and thoracic exudate</td>
</tr>
<tr>
<td>A. australis</td>
<td>1849</td>
<td>Hepatic lymph node</td>
</tr>
<tr>
<td>(M-17-92)</td>
<td>1850</td>
<td>Lung</td>
</tr>
<tr>
<td>A. australis</td>
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<td>1856</td>
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<td>1857</td>
<td>Lymph node</td>
</tr>
<tr>
<td></td>
<td>1858</td>
<td>Lymph node</td>
</tr>
<tr>
<td>A. australis</td>
<td>1862</td>
<td>Lymph node</td>
</tr>
<tr>
<td>(M-38-92)</td>
<td>1863</td>
<td>Lung</td>
</tr>
<tr>
<td></td>
<td>1864</td>
<td>Liver</td>
</tr>
<tr>
<td>Otaria flavescens</td>
<td>1866</td>
<td>Lung</td>
</tr>
<tr>
<td>(M-47-92)</td>
<td>1867</td>
<td>Lymph node</td>
</tr>
<tr>
<td>A. australis</td>
<td>1868</td>
<td>Peritoneum</td>
</tr>
<tr>
<td>(M-11-91)</td>
<td>1869</td>
<td>Lymph node</td>
</tr>
</tbody>
</table>

**Deoxyribonucleic acid techniques**

Isolates were grown on standard mycobacterial media (Stonebrink) and checked for purity by microscopic examination after Ziehl-Neelsen staining. Bacterial cells were harvested and deoxyribonucleic acid (DNA) extractions were performed as described previously (30, 31).

Genomic DNA from five wild seal isolates was analysed by the restriction fragment length polymorphism (RFLP) technique, using the IS6110 probe (1, 7, 8, 9, 12, 14).

Restriction enzyme digests of mycobacterial DNA were prepared by using approximately 1 µg of chromosomal DNA and 5 µ of Pvu II for 3 h at 37°C. The digested fragments were separated by electrophoresis through 0.8% agarose gels in tris-borate ethylene diamine tetra-acetate (TBE) at 20 volts overnight. The DNA was
transferred to a positively charged nylon membrane by using a vacuum transfer apparatus. The DNA was fixed to the nylon membrane on the ultraviolet (UV) transilluminator for 10 minutes. The membrane was prehybridised for 2 h at 65°C in a hybridisation solution containing 1% sodium dodecyl sulphate (SDS); 2.5 × SSC (IsomM NaCl, IsmM sodium citrate), 0.01% sodium pyrophosphate and 0.1% polyanetholesulfonic acid. After prehybridisation, the DNA probe was added and the membrane incubated overnight at 65°C. The 245 base pair (bp) polymerase chain reaction (PCR) fragment IS6110, labelled with P\(^{32}\) by a random priming method, was used as a probe to detect Pvu II restriction fragments containing the right arm of IS6110 (17, 18). After hybridisation, the membrane was washed twice, for five minutes each time, at room temperature with 1 × sodium salt nitrate (SSC)/0.1% SDS, and twice for 15 minutes at 65°C with 0.1 × SSC/0.1% SDS. Membranes were exposed to X-Omat film at -70°C for various lengths of time.

**RESULTS**

**Strandings**

The number of stranded animals found in the Samborombón area during the present study was considered normal, compared to records of previous years. During 1992, there was a total of 52 strandings of wild seals (*O. flavescens*, *A. australis* and *A. tropicalis*). Of these strandings, 19 cases (36.54%) were successfully rehabilitated, and subsequently released into the wild. The remaining 33 cases (63.46%) died from various causes, including tuberculosis in four instances (12.12%), during 1992.

As a general rule, the stranded specimens were emaciated and physiologically depressed.

**Pathological observations**

Necropsies were performed for pathological examination in all six cases of tuberculosis. The most important macroscopic lesions observed (Fig. 4) are as follows:

**Case 1**

*Arctocephalus australis* (27/02/89)

The lungs contained numerous, round, yellow caseous lesions, 30 to 40 mm in diameter.

In the abdominal cavity, similar nodular yellow caseous lesions were found on the peritoneum and in the liver.

Thoracic and abdominal lymph nodes were enlarged and caseous in appearance.

**Case 2**

*Arctocephalus australis* (M-17-92)

The trachea was congested, with an overlying purulent membrane. In the thorax, there was a copious amount of a liquid, yellowish exudate. The pleural lining was thickened and covered by an abundant fibrinous layer.
The hepatic and thoracic lymph nodes were enlarged and firm with caseous and calcified foci.

**Case 3**

*Arctocephalus australis* (M-31-92)

Purulent exudate was found adhering to the trachea and mucosa and the pleural surface of the lungs. Extensive and irregularly shaped yellow friable foci, 40-50 mm in diameter, were present in the caudo-dorsal aspects of the caudal lung lobes.

In the liver parenchyma, there were small, irregular, yellow friable caseous areas, approximately 30-40 mm in diameter. Similar lesions were observed in the spleen.

The thoracic and abdominal lymph nodes were enlarged and, on cut surfaces, revealed calcified and caseous foci.

**Case 4**

*Arctocephalus australis* (M-39-92)

In the trachea, a purulent exudate was present. The left pulmonary lobes were consolidated and contained numerous, scattered firm yellow foci, approximately 4 mm in diameter. Similar large foci were observed in the right lung lobes.

The lymph nodes, particularly those of the axillary, thoracic and abdominal regions, were enlarged, and caseous and calcified areas were evident on cut surfaces.
Case 5

*Otaria flavescens* (M-47-92)

Numerous small, firm, yellow caseous foci, approximately 5 mm in diameter, were observed scattered throughout the lung lobes.

The prescapular, cervical, mediastinal, retropharyngeal and thoracic lymph nodes were enlarged, with caseous foci on cut sections.

Case 6

*Arctocephalus australis* (M-11-91)

There was evidence of trauma to the skull and brain.

The thoracic and abdominal lymph nodes were enlarged and, when cut, revealed calcified and caseous foci.

Histopathological findings

Significant lesions were observed in the lungs and lymph nodes (4, 22). The lungs contained numerous granulomatous lesions, characterised by a central zone of caseous necrosis and mineralisation, and an intermediate zone of large epithelioid cells with abundant eosinophilic cytoplasm and a central nucleus. No giant cells were observed in this zone. The peripheral zone was composed of an inner layer of highly vascularised granulation tissue and an outer layer of mature collagenous tissue. Lymphocytic cells were infiltrating the peripheral zone.

Similar granulomatous lesions were evident in the lymph nodes and no normal lymphoid nodules could be discerned.

Bacteriology

Acid-fast rods were observed microscopically, after Ziehl-Neelsen staining, in all samples studied. The results are shown in Table II.

The slow growth of these strains on Stonebrink media at 37°C should be noted. In primary isolation, strains needed four to eight weeks to grow (29). A few strains were positive for niacin production. The isolated mycobacteria were susceptible to isoniazid, streptomycin, rifampicin, ethambutol and thiophene-2-carboxylic acid hydrazide, and relatively resistant to p-aminosalicylic acid. The experimental inoculation of isolated strains into guinea-pigs produced significant and generalised lesions, and the intradermal tuberculin test was strongly positive in guinea-pigs to *M. bovis* purified protein derivative (PPD), 50 IU (Table III).

Restriction fragment length polymorphism analysis

The isolates came from four animals. (It was not possible to obtain DNA from the mycobacteria isolated from two of the specimens.) All four isolates gave patterns with considerable similarities. But some polymorphisms were observed in three of the patterns. Whereas three bands (1.70, 1.90 and 4.00 kilobase [kb]) were shared by all four strains, a further two bands (1.95 and 5.55 kb) were observed in three strains. Finally, one strain (isolate 1849) showed six bands, incorporating a unique 5.50 kb band.
TABLE II
Useful characteristics to differentiate mycobacteria species

<table>
<thead>
<tr>
<th>Tested property</th>
<th>Cases and sample no.</th>
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<td>At 37°C</td>
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<td>Niacin accumulates</td>
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</table>

-: growth not present
+: growth present
ND: growth not detected

Results of differential tests used for characterising species of *Mycobacteria*. Results obtained were consistent with *M. bovis* for isolates from cases 1, 3, 4 and 5, while isolates from case 2 showed some properties characteristic of *M. tuberculosis*. Properties of isolates from case 6 were difficult to interpret.
TABLE III

Strains of the Mycobacterium tuberculosis complex isolated from sea lions and seals

<table>
<thead>
<tr>
<th>Cases and sample no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>Tested property</td>
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<td>1849</td>
<td>1855</td>
<td>1862</td>
<td>1866</td>
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<td>1856</td>
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<td>1857</td>
<td>1864</td>
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<td></td>
<td></td>
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<td>1858</td>
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</table>

Biological assays

<table>
<thead>
<tr>
<th>Experimental inoculation of guinea-pigs</th>
<th>PPD Bov.</th>
<th>10 mm</th>
<th>PPD Bov.</th>
<th>26 mm</th>
<th>PPD Bov.</th>
<th>16 mm</th>
<th>PPD Bov.</th>
<th>22 mm</th>
<th>PPD Bov.</th>
<th>13 mm</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intradermal tuberculin tests</td>
<td>PPD Av.</td>
<td>7 mm</td>
<td>PPD Av.</td>
<td>12 mm</td>
<td>PPD Av.</td>
<td>10 mm</td>
<td>PPD Av.</td>
<td>10 mm</td>
<td>PPD Av.</td>
<td>6 mm</td>
<td>ND</td>
</tr>
<tr>
<td>Necropsy results of guinea-pigs</td>
<td>Generalised lesions</td>
<td>Lesions in liver, hind legs, lymph nodes enlarged, size of spleen increased (approx. 10%)</td>
<td>Haemorrhagic caseum liver, spleen and lung with lesions, mediastinal nodes with caseum</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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</tbody>
</table>

PPD Bov.: purified protein derivative bovine
ND: not detected
PPD Av.: purified protein derivative avian

Characteristic virulence for guinea-pigs was found for isolates from cases 1, 2 and 3

After digestion by BclII, five bands of 22, 18, 6, 4 and 2.7 kb were observed, except for the 1856 isolate in which the 6 and 2.7 bands were lacking (data not shown).

DISCUSSION

The first bacteriologically confirmed case of tuberculosis in wild seals was reported by Cousins et al., in three specimens of otariid seals (N. cinerea and A. forsteri) which were found dead on Australian beaches (12). It is worth mentioning that this was to be expected, because information about tuberculosis in captive pinnipeds has existed since 1913 (3, 15, 16, 24, 28).

Forshaw and Phelps reported the presence of an organism, biochemically identified as M. bovis, which was considered to be the cause of tuberculosis in a colony of captive Australian sea lions (N. cinerea) and New Zealand fur seals (A. forsteri) at a marine park near Perth, in Western Australia (16). This agrees with the previous finding of the same disease in wild pinnipeds of the same species and in the same geographical areas.
This article is the second report of tuberculosis in wild seals, and the first for species of the Atlantic ocean. The findings affect two species of otariid seals typical of South America (O. flavescens and A. australis).

In addition to the importance of the finding itself, it is interesting to note that this disease has affected marine mammals other than the Australian species, in a totally unrelated area, without bio-geographical connections.

This fact gives rise to interesting questions about the origin of mycobacteria in outbreaks of tuberculosis in marine mammals. It would, however, be very difficult to propose a hypothesis, before further research has been carried out.

In the absence of such research, it should be supposed that this disease could also appear in other geographical areas, and in other species. These aspects should be investigated in other regions of the world.

It must be remembered that in both reports, from Australia and from Argentina, these cases of tuberculosis affected wild animals that were away from their natural rookeries. The cases reported from Australia were of stranded animals, found dead on shore. The cases reported in Argentina were of stranded animals which were alive but in very weak general physical condition. As of the present time, the prevalence of tuberculosis in natural rookeries in Australia and Argentina is unknown.

In cases where similar diseases exist in terrestrial and marine environments, it is challenging to investigate the possible relationship between the causative agents.

In regard to these aspects, Cousins et al. speculate about the source of origin of infection in Australian wild seals (11, 12). It was thought that the cases of infected animals in the Perth marine park may not have been due to infection by humans or cattle after capture (12).

In relation to tuberculosis in wild seals, Cousins et al. question the possibility that the animals may have been infected by cattle. If this were so, they assume, the seals must have been infected before 1978, as this was the year in which the last case of tuberculosis in cattle was recorded in Australia. From the point of view of the authors, this argument seems inconsistent because the viability of these bacteria is very high, even under unfavourable conditions. Moreover, there is no sanitary control on wild seals that could guarantee the absence of tuberculosis in wild populations. Furthermore, one must remember that tuberculosis is a disease of chronic evolution, and short periods of time (less than twenty years) do not provide sufficient argument against the possibilities of transmission.

As for South American species of wild seals, one may not discard the possibility of close contact with cattle at some time, since the colonisation of South America in the 16th century, and the first introduction of cattle, but in reality this possibility is remote. The rookeries of A. australis and O. flavescens in Uruguay are insular, in areas where cattle do not prosper, and the colonies in Argentina are found in the Patagonia region, where sheep are the main domestic animal. Tuberculosis has never been reported among sheep in Argentina.

Cousins et al. also speculate about the possible transmission of tuberculosis from humans, but consider it unlikely that the infection could have been spread to these animals in days when sealing was a major industry (12). This comment is based on the fact that humans were in close contact principally with dead seals, for their pelts. Cousins et al. also discuss the remote possibility that a seal could have eaten the corpse of a human tuberculosis sufferer who was buried at sea, as old seals, which are not
active enough to capture prey, may scavenge for food. This might explain the fact that the mycobacteria isolated from seals resemble *M. tuberculosis* rather than *M. bovis* (12, 21). However, there are no bibliographic references to such scavenging in seals and the authors have never observed this behaviour in South American species. The possibility that a seal may eat a human corpse is most unlikely.

Finally, Cousins *et al.* discuss the more feasible possibility that tuberculosis has been endemic in wild seals for hundreds or thousands of years, just as *M. tuberculosis* and *M. bovis* have been present in humans and cattle, respectively (12). The authors would also accept this speculation. However, if this were so, Cousins *et al.* note that one might expect to find more genetic diversity in the strains isolated from wild seals, and more cases of tuberculosis in wild seals from different parts of the world.

Very few reports of tuberculosis in wild seals are available from other countries. Perhaps this is due to the fact that specialists in tuberculosis rarely receive samples from marine mammals.

It is worth noting the possibility of tuberculosis being transmitted to humans (12). The body of findings, from both captive and wild seals, indicates that it is necessary to set strict biosafety norms in two areas of activity related to humans. First, trainers in oceanaria who have close contact with seal species should follow safety recommendations provided by the animal curator and other professionals. Secondly, those who work closely with these animals in rookeries must also consider at least minimal safety guidelines.

To characterise the mycobacteria present in wild seals, the authors used diverse techniques (i.e. biochemical tests and fingerprints) to confirm that the isolated organism was a member of the *M. tuberculosis* complex. This organism displayed a great similarity to *M. bovis* in some cases, and to *M. tuberculosis* in others, according to the technique used, but with its own characteristics.

Cousins *et al.*, using restriction enzyme analysis (REA), Western blots, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/Page) and RFLP for the first time, concluded that the isolated strains from seals were different from all the other members of the *M. tuberculosis* complex, suggesting that such strains could belong to a genetically different group within the complex, as has been found for *M. africanum* (12).

The authors have also used RFLP techniques for the first time on the strains isolated from animals on the south-western Atlantic coasts, and the results are consistent with those of Cousins *et al.* (12). These strains do belong to the *M. tuberculosis* complex, but clearly differ from *M. bovis* isolated from cattle and other terrestrial mammals, both domestic and wild.

Although the overall band pattern is similar, differences can be detected in the position and number of the different bands. This indicates diversity between these strains, suggesting that tuberculosis is endemic in wild seals. These results are somewhat different from those of Australian researchers who found only two patterns. The authors found three different patterns. In regard to the identity of these strains and those found in Australia, the restriction enzyme pattern seems to be different, but this assumption should perhaps be confirmed or discarded, based on concurring migration profiles.

*M. tuberculosis* isolated from local patients has multiple bands (data not shown). These patterns are different from those of *M. bovis* isolated from Argentinian cattle,
which generally contain a unique 1.9 kb band and, on occasion, two bands (as does *M. bovis* Bacillus Calmette-Guérin (BCG) used for humans in Argentina). They are also different from the patterns of *M. tuberculosis* H37Rv (Fig. 5).

**FIG. 5**

Restriction fragment length polymorphism of mycobacteria from wild seals and reference strains

Deoxyribonucleic acid was digested by Pvu II, developed on an 0.8% agarose gel, transferred to nylon membrane and hybridised with a $^{32}$P labelled IS6110 probe. Lane 1: *M. tuberculosis* H37Rv. Lanes 2, 3, 4, 5 and 6: wild seal strains 1849, 1856, 1862, 1864 and 1868, respectively. Lanes 7, 8 and 9: *M. bovis* bovine isolates. Lane 10: *M. bovis* AN5. Lane 11: Bacillus Calmette-Guérin Pasteur. Lanes 12, 13 and 14: *M. bovis* human isolates.
**CONCLUSIONS**

The patterns obtained in Southern blots of mycobacteria isolated from wild seals show that:

1. These mycobacteria belong to the *M. tuberculosis* complex
2. These mycobacteria differ from *M. bovis* isolated from cattle and from BCG, in that they have more copies of the IS6110 band: Wild seal-specific mycobacterial DNA showed hybridisation to a probe which is considered specific for *M. tuberculosis* and *M. africanum* (A. Cataldi et al., unpublished findings)
3. Such mycobacteria differ from *M. tuberculosis* because mycobacteria isolated from wild seals have fewer copies of IS6110, and because Löwenstein-Jensen medium with glycerin does not support the growth of such mycobacteria. Moreover, some biochemical characteristics are not consistent with those of *M. tuberculosis*
4. The type of polymorphism found within the group suggests that tuberculosis in wild seals may not be recent. This conclusion arises from the fact that IS6110 moves very slowly (17, 18).

Future studies will be aimed at defining the relationship which exists between the mycobacteria isolated from sea lions and fur seals and *M. bovis*, which is present in terrestrial mammals. Sea lions and fur seals would be reservoirs for strains of this organism. Such reservoirs would have to be taken into account in programmes for the final control and eradication of animal tuberculosis.

**ACKNOWLEDGEMENTS**

The authors would like to express gratitude to the owners and personnel of Mundo Marino Oceanarium for their support and cooperation, and to Dr I.N. de Kantor (Pan-American Institute for Food Protection and Zoonoses/Pan American Health Organisation/World Health Organisation) (INPPAZ/PAHO/WHO) for her important advice. Dr V. Quse and L.D. Rodríguez kindly helped during the preparation of the manuscript.

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**Résumé :** Les auteurs présentent plusieurs cas, actuellement à l'étude, d'une affection causant la mort et/ou l'échouement de diverses espèces d'otaries et de phoques sur les plages de la côte nord de la Province de Buenos Aires.

La tuberculose a été diagnostiquée chez six pinnipèdes échoués sur la plage entre mars 1989 et décembre 1992 ; il s'agissait respectivement d'une otarie (Otaria flavescens) et de cinq phoques (Arctocephalus australis).

Lors des autopsies, des lésions granulomateuses ont été observées dans les ganglions lymphatiques précapsulaires et hépatiques. Des lésions ont par ailleurs été constatées dans les poumons, la plèvre, le foie, la rate et le péritoine.
Des prêlevements effectués à partir de ces six animaux ont été soumis à une recherche bactériologique. Les isolats obtenus ont été identifiés comme appartenant au complexe de Mycobacterium tuberculosis. Certaines souches présentaient des caractéristiques comparables à celles de M. bovis, tandis que d’autres présentaient les propriétés de M. tuberculosis.

L’acide désoxyribonucléique (ADN) génomique de ces souches a été analysé à l’aide de la cartographie RFLP (qui met en évidence le polymorphisme de taille des fragments de restriction) en utilisant le marqueur génétique IS6110, lequel n’est observable que dans le complexe de M. tuberculosis. A l’aide d’une sonde IS6110, des empreintes similaires ont été obtenues, ce qui suggère une source commune d’infection. Cependant, la structure de l’ADN différait de celles observées dans l’ADN de M. bovis isolé à partir d’êtres humains et de bovins en Argentine, qui présentaient généralement une bande unique de 1,9 kbp.

Ces résultats suggèrent que les mycobactéries isolées chez les pinnipèdes sauvages constituent un groupe différent au sein du complexe de M. tuberculosis. C’est la première fois que la tuberculose est identifiée chez des pinnipèdes sauvages des côtes du sud-ouest de l’Atlantique.


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TUBERCULOSIS EN LEONES Y LOBOS MARINOS EN EL OCÉANO ATLÁNTICO SUD-OCIDENTAL. – A. Bernardelli, R. Bastida, J. Loureiro, H. Michelis, M.I. Romano, A. Cataldi y E. Costa.

Resumen: Los autores presentan varios casos debidos a una patología que produce varamieneto y/o la muerte de diversas especies de leones y lobos marinos en la costa norte de la Provincia de Buenos Aires.

Casos de tuberculosis fueron diagnosticados en seis ejemplares de dos especies de otaridos (uno en Otaria flavescens y cinco en Arctocephalus australis) varados en la playa entre marzo de 1989 y diciembre de 1992.

Fue realizada la necropsia en todos los casos. Lesiones granulomatosas se observaron en los linfonódulos pre-escapular y hepáticos. Asimismo se comprobaron lesiones en pulmón, pleura, hígado, bazo y peritoneo.

El aislamiento bacteriológico fue realizado en todas las muestras. Los aislamientos fueron identificados como pertenecientes al complejo Mycobacterium tuberculosis. En la realización de las pruebas algunas de las cepas mostraron características compatibles con M. bovis, mientras que otras exhibían las propiedades de M. tuberculosis.

El estudio genómico del ácido desoxirribonucleico (ADN) de las cepas fue analizado mediante cartografía RFLP (restriction fragment length polymorphism) usando la técnica que emplea el marcador genético IS6110 encontrado solamente en el complejo M. tuberculosis. Usando la sonda IS6110 fueron obtenidas huellas dactilares (fingerprints) similares. Ello sugiere una fuente común de infección. Sin embargo, las estructuras del ADN fueron diferentes de aquellas de M. bovis, aisladas de humanos y ganado de la Argentina, las cuales contienen principalmente una única banda a 1,9 kbp.
Estos resultados señalan que las micobacterias aisladas de lobos y leones marinos forman un grupo diferente dentro del complejo M. tuberculosis. Ésta es la primera vez que la tuberculosis es identificada en pinnípedos salvajes de las costas del Atlántico sud-occidental.


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