

Transmission of *Mycobacterium pinnipedii* to humans in a zoo with marine mammals

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SUMMARY

OBJECTIVES: An outbreak of tuberculosis (TB) in sea lions occurred recently in a zoo in the Netherlands. The disease was detected in a captive colony consisting of 29 animals kept in an open air basin with an indoor night house. Approximately 25 animal keepers were in close contact with the animals.

METHODS: The sea lions were investigated using the tuberculin skin test (TST) with avian and bovine purified protein derivative (PPD) and, in case of positivity, necropsied. A survey was conducted among the animal keepers including TSTs with *Mycobacterium tuberculosis* complex PPD tuberculin, a chest X-ray and an interferon-gamma release assay (IGRA).

RESULTS: Necropsy was positive for TB in 13 of the 29

sea lions. Three cases of pulmonary involvement were found. Only one of these was infectious and it was therefore regarded as the source case. The causative mycobacterium was identified as *M. pinnipedii*. Six of the 25 animal keepers were TST-positive; in five of these, infection was confirmed by a positive IGRA.

CONCLUSION: Transmission of *M. pinnipedii* infection from sea lions to humans was established by TST. IGRA results largely agreed with the TST results. Nebulisation when cleaning the sea lions' enclosure was most likely the main cause of transmission to humans.

KEY WORDS: tuberculosis; *Mycobacterium pinnipedii*; transmission; latent tuberculosis infection; interferon-gamma assay

BETWEEN 1986 and 2000, *Mycobacterium tuberculosis* complex organisms were isolated in various marine mammals in South America, the United Kingdom and Australia.^{1–5} In 1994, it was reported for the first time that isolates from seals captured on the coast of Argentina in 1992 had a characteristic insertion sequence (IS) 6110 restriction fragment length polymorphism (RFLP) pattern.⁶ This seal bacillus was later designated *M. pinnipedii* and appeared to have a unique position in the *M. tuberculosis* complex.⁷ In the majority of cases in which *M. pinnipedii* was isolated, pulmonary lesions were found. Widely disseminated infection affecting the liver, spleen, kidneys, pancreas, meninges and bronchial, mesenteric, colonic and duodenal lymph nodes was also described.¹ Cough, however, is often not a prominent feature in diseased animals. In 1993, pulmonary tuberculosis (TB) was described in an Australian seal trainer caused by the same *M. tuberculosis* complex organism that caused TB in seals. He had been working with the seals 2 years earlier.²

In a large zoo in The Netherlands, a sea lion was diagnosed with TB, causing concern that the causative bacterium might have been involved in transmission

to animal keepers. The cultured bacteria were identified as *M. pinnipedii*. As little is known about the transmission of *M. pinnipedii* from animals to humans, we decided to extend our investigations. In the present study, we report the management of the outbreak, including the examination of animal keepers and other zoo personnel for TB infection by tuberculin skin test (TST) and interferon gamma release assay (IGRA).

MATERIAL AND METHODS

Sea lions

At the zoo, 29 Southern sea lions (*Otaria flavescens*) were held in a 750 m² open-air basin. Of these, 28 had been born in captivity and only one was wild (exact origin unknown) and had been obtained from a zoo in Denmark in 2000.

The sea lions were skin tested by simultaneous intradermal injection of avian and bovine purified protein derivative (PPD) (respectively 2500 and 3000 international units [IU]) in a comparative test, for which the results are read by a diagram with a negative, positive and intermediate area for (bovine) TB. The injections were performed under general anaesthesia.

If an animal tested positive, it was euthanised and necropsied. In case of negative test results, TST was repeated after 6 weeks.

All animals that died or were euthanised were necropsied and samples were taken for histological and bacteriological studies (Veterinary Faculty of the University of Utrecht, Central Institute for Animal Disease Control, Lelystad, the Netherlands). Material for bacteriological study from the sea lion with open TB was sampled and tested for susceptibility against the main anti-tuberculosis drugs (National Institute of Public Health and the Environment, RIVM, Bilthoven, the Netherlands).

Other animals

A total of 95 animals kept in proximity to the basin of the sea lions, including moose, bison, waterbucks, wildebeests, impalas, dwarf goats, Nubian goats, squirrel monkeys and baboons, were also skin tested. The moose and bison live closest to the sea lion basin, at a distance of approximately 20–25 m. The squirrel monkeys could freely reach the trees around the basin, but usually lived at a greater distance.

Molecular typing

Spoligotyping was performed as described previously.⁸

Humans

Standard TST of the animal keepers working with the sea lions and a number of zoo personnel working in their immediate vicinity was carried out by injecting 0.1 ml PPD-tuberculin 2 tuberculin units (TU) RT 23 intradermally. After 3 days, the infiltrate was measured by trained technicians. An infiltration >10 mm was considered positive;^{9,10} none of the individuals tested had had a previous bacille Calmette-Guérin (BCG) vaccination or positive TST. In case of a negative result, the test was repeated after 2 months. In this second test, the TST was also considered positive when the result of the first test (<10 mm) increased by ≥10 mm. An increase of <10 mm was considered to be due to error inherent to the test or to boosting.

Individuals who were not skin tested due to previous BCG vaccination or an episode of TB were examined by X-ray for active TB. In individuals in whom the TST was ≥5 mm, an IGRA, the QuantiFERON®-TB Gold test, was carried out.

Informed consent was obtained from all individuals.

RESULTS

Sea lions

A 5-year-old female sea lion born in a zoo in France died in September 2006 after a period of about 10 months of lethargy, anorexia and weight loss. At one point, anal loss of blood was observed in this animal. She never coughed markedly. Necropsy showed mul-

iple tubercles with central necrosis, caseation and epithelioid macrophages in the kidneys, spleen, liver, lungs and lymph nodes and an ulcerative tracheitis. The lungs showed granulomatous infiltration with necrosis and calcification and eruption towards the bronchi. Acid-fast bacilli (AFB) were observed; the polymerase chain reaction (PCR) was positive for *M. tuberculosis* complex, and spoligotyping yielded patterns characteristic of *M. pinnipedii*.

It was decided to examine the whole colony of sea lions. TST was performed on 24 animals and was found positive in 11 and indeterminate in five. Two animals that were to be transported to another zoo were preventively euthanised; two other animals died during general anaesthesia before they were skin tested. At necropsy, no signs of TB were found in these animals. It was expected that skin tests would be found to be positive when the remaining eight animals were tested again, and that this might continue until no animals were left alive. It was therefore decided to euthanise the remaining eight animals without further skin testing.

Necropsy was positive for TB in 13 animals, including the index case, and negative in 16. In most of the animals, positive bronchial, mediastinal and mesenteric lymph nodes were found. Pulmonary involvement was found in two cases, neither of which was yet in a state of infectiousness. Only the index case was found to be infectious. Apart from the index case, a positive Ziehl-Neelsen (ZN) could be obtained from samples taken from bronchial lymph nodes in two animals and a positive spoligotyping for *M. pinnipedii* was obtained from one of these.

None of the animals in the vicinity of the sea lions' water basin had a TST suggestive of infection with *M. tuberculosis* complex.

Transmission to humans

A group of 25 animal keepers had been in close contact with the sea lions. All were native Dutch and only one had any previous risk of TB infection. They worked in shifts both in the open air and in the night house. To clean the night house, which was poorly ventilated, high pressure fresh water was used. This procedure took 1 to 2 h daily. The animal keepers did not wear masks during this work.

Skin testing was started 1 week after TB was detected, in September 2006, in 24 of the 25 animal keepers who had been in close contact with the sea lions. The tests were repeated after 2 months.

In this first group of 24 keepers, six (25%) were positive for TB infection (see Table). None had symptoms of active TB and chest X-rays revealed no abnormalities. All except one of these six keepers had regularly used the high pressure hose. Those who were negative had never or hardly ever used it, except for one of the sea lion keepers, who was the only other person to regularly use this device.

Subject 6 had a positive TST but a negative IGRA.

Table Results TST and IGRA

Subject	TST 1	TST 2	TST result	IGRA	IGRA result	Final result
6	9	19	+	0.04	-	+
8	19		+	2.64	+	+
12	8	12	-	1.05	+	+
18	11	9	-			-
43	18		+	13.0	+	+
51	13		+	0.48	+	+
59	0	12	+	0.78	+	+

TST = tuberculin skin test; IGRA = interferon gamma release assay.

However, recent TB infection was considered highly probable due to intensive contact with the sea lions. In subject 12, TST was considered negative but a positive IGRA suggested TB infection. Subject 18 was classified in the first group due to his work. Because he appeared to have had many foreign contacts with pinnipeds in the past and only relatively little contact with the sea lions in our zoo, it was concluded that the slight reaction to the TST, which did not increase in the second test, was probably due to an older infection caused either by *M. tuberculosis* complex or non-tuberculous mycobacteria (NTM). In concordance with the conclusion of no recent infection, no IGRA was performed.

A second group of 38 zoo personnel consisted of keepers of the surrounding animals, technicians, trainees and visitor guides who visited the night house in the evenings when the sea lions were inside. These individuals had much less or no direct contact with the sea lions, and were therefore considered to have a much lower risk of infection. All were native Dutch and none had any previous risk of TB infection. It was only when a number of positive TSTs were found in the first round of investigation, that it was decided to add this group to the study.

In this second group, 27 of the 38 persons were skin tested. There were no positive TSTs: all but one had no intradermal reaction at all, and the only reaction that was seen was only 3 mm induration.

Of the remaining individuals in both groups, five were not skin tested due to previous BCG vaccination or an episode of TB, and they were examined by X-ray for active TB. Another seven individuals in the second group were not recruited into the study at all. The chest X-rays of the five individuals who were not skin tested showed no signs of active TB.

Bacteriology

M. pinnipedii was found to have normal susceptibility to the main anti-tuberculosis drugs isoniazid, rifampicin, ethambutol, streptomycin and pyrazinamide.

DISCUSSION

M. pinnipedii infections have been reported in various marine mammals. One case report described a

seal trainer who developed TB following contact with seals suffering from TB.² In the present study, we describe an outbreak of TB in marine mammals in a large zoo in the Netherlands. Investigation of the zoo personnel showed that six subjects were infected, which was confirmed both by TST and by IGRA.

The TST-positive subjects were treated with isoniazid for 6 months. Prophylactic treatment was initiated because it had been reported that humans may develop TB from *M. pinnipedii*. This can be expected because the bacillus belongs to the *M. tuberculosis* complex and seems to be more closely related to *M. tuberculosis* than to *M. bovis* in terms of evolution. It is, however, unknown if the risk of humans developing active disease due to this bacillus is the same as for *M. tuberculosis*.

In addition to the TST, an IGRA was carried out and proved helpful in diagnosing TB. IGRA was added because positive skin reactions to tuberculin PPD caused by NTM infection were expected in this group of zoo personnel who are in close contact with many different species. IGRA can differentiate between such infections and infection with *M. tuberculosis* complex. In this small group, no additional clinical information was obtained when an IGRA test was combined with the classical TST. As expected, a positive IGRA test was observed in subjects infected with *M. pinnipedii*. The positive IGRA only strengthens this diagnosis, because it makes infection with NTM improbable.

To prevent the problem we encountered with boosting vs. recent infection, the use of a two-step TST could be considered. The use of an IGRA test remains necessary to differentiate between infections with *M. tuberculosis* complex and NTM.

This study demonstrates that sea lions transmitted the infection to their keepers. On analysing the type of work performed by each subject, it became obvious that cleaning the night house with a high pressure water hose was the greatest risk factor for infection. The night house was soiled by urine and faeces contaminated by TB bacilli caused by TB in the kidneys and liver. It is therefore likely that nebulisation when cleaning the night house was the most likely cause.

The water in the basin may also have been contaminated, and may have been the mode of transmission to the other animals. At the same time, infection by coughing in frequently coughing animals is certainly not excluded. In humans, however, no evidence for infection by coughing was found.

The negative screening results in the second group of zoo personnel and of the surrounding animals provided sufficient evidence for the general population to suggest that the risk of infection caused by visiting the zoo was minimal. Measures were taken to protect the keepers, such as wearing masks during high pressure cleaning, and protocols were established to prevent future outbreaks—the new population of sea lions is

now skin tested annually. Local newspapers were used to regularly inform the public.

It appeared impossible to obtain positive AFB results by necropsy of all of the animals. Although the necropsy results of 11 of the 13 animals were histologically concordant with the diagnosis of TB caused by *M. pinnipedii*, bacteria were not found by microscopy. This may be because we were dealing with relatively recent infections with a very small bacterial load. This is probably also the reason for the negative spoligotyping in one of the ZN-positive cases.

In the year before the TB outbreak, two animals died unexpectedly in an interval of about 6 months. Necropsy only showed a large amount of pleural fluid, but no signs of TB. Most other animals in the zoo are skin tested on a regular basis, but this is not the case in marine mammals because of the high death rate after general anaesthesia due to ventilatory insufficiency. Moreover, in the period before the outbreak there had been no signs of TB in this colony of sea lions. It is nevertheless advised that careful screening of animals of wild origin is necessary to avoid new outbreaks of TB in a zoo.

After this outbreak, other zoos in Europe were contacted to share our experience, as we believe that other marine mammals might also be infected with *M. pinnipedii*. In the French zoo from which the source case originated, TB was indeed detected among the sea lions. Results from other zoos are yet unknown. We also advised other zoos to test their keepers for TB infection if TB is suspected among animals.

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RÉSUMÉ

OBJECTIFS : On a observé récemment une mini-épidémie de tuberculose (TB) chez les otaries dans un zoo des Pays-Bas. La maladie a été détectée dans une colonie captive comportant 29 animaux qui étaient gardés dans un bassin en plein air, avec logement nocturne à l'intérieur. Environ 25 gardiens de ces animaux avaient été en contact étroit avec les otaries.

MÉTHODES : On a fait une recherche chez les otaries par test cutané (TST) à la dérivé protéique purifié (PPD) aviaire et bovine et on les a autopsiées en cas de positivité. Parmi les gardiens des animaux, on a mené une enquête comportant des TST à la tuberculine PPD pour le complexe *Mycobacterium tuberculosis*, un cliché thoracique et un test d'interféron gamma (IGRA).

RÉSULTATS : L'autopsie a démontré une TB chez 13 des

29 otaries, parmi lesquelles on a trouvé trois cas d'atteinte pulmonaire. Une d'entre elles seulement a été contagieuse et a été considérée pour cette raison comme le cas index. La mycobactérie causale a été identifiée comme *M. pinnipedii*. Au total, les TST ont été positifs chez six des gardiens. Chez cinq de ces derniers, l'infection a été confirmée par un test positif d'IGRA.

CONCLUSION : Les TST ont démontré la possibilité de transmission de l'infection par *M. pinnipedii* à partir d'otaries vers des sujets humains. Les résultats des tests sérologiques ont été largement concordants avec ceux des TST. L'aérolisation liée au nettoyage des locaux des otaries a été le plus probablement la cause principale de transmission vers les sujets humains.

RESUMEN

OBJETIVOS : Recientemente ocurrió un brote epidémico de tuberculosis (TB) en los leones de mar de un zoológico en los Países Bajos. La enfermedad se detectó en una colonia de 29 animales cautivos, que permanecían en un estanque al aire libre con un abrigo nocturno cubierto.

Cerca de 25 cuidadores estuvieron en contacto cercano con los animales.

MÉTODOS : Se estudiaron los leones de mar mediante pruebas cutáneas (TST) con una proteína pura derivada (PPD) de origen aviar y en caso de resultado positivo se

practicó una autopsia. Los cuidadores de las otarias se evaluaron mediante la TST con la PPD del complejo *Mycobacterium tuberculosis*, la radiografía de tórax y una prueba de liberación de interferón gama (IGRA).

RESULTADOS : La autopsia fue positiva para TB en 13 de 29 otarias y se demostró la localización pulmonar en tres de ellas. Solo una de las otarias era infecciosa y se consideró como el caso inicial. Se identificó como micobacteria causante al *M. pinnipedii*. En total, 6 de 25 cui-

dadores de las otarias presentaron TST positivas ; en cinco se confirmó la infección mediante el resultado positivo de la IGRA.

CONCLUSIÓN : Mediante la TST quedó demostrada la transmisión de la infección por *M. pinnipedii* de las otarias al hombre. Los resultados de IGRA concordaron ampliamente con los resultados de la TST. La principal causa de la transmisión a los hombres fue muy probablemente la limpieza de los locales mediante nebulización.
