



Animal-to-human transmission of *Mycobacterium pinnipedii*

To the Editor:

Mycobacterium pinnipedii, the known causative agent of tuberculosis (TB) in marine mammals, was only recognised as a member of the *Mycobacterium tuberculosis* complex in 2003 [1] and is believed to cause TB in several species, including nonmarine mammals [2, 3] and even humans [4]. The assumption of zoonotic transmission has been strongly reinforced by a disruptive study published in 2014 by a team of archaeologists from Tübingen, Germany [5]. Based on archaeological and genomic investigations on millennial human skeletons, the authors implicated sea mammals infected with *M. pinnipedii* as a source of New World human TB. Considering that this phenomenon pre-dates the human migrations to South America by several centuries, they refuted the previous scientific hypothesis of TB driven by human contact [6]. The authors suggested as the most plausible hypothesis that human TB in South America was initiated by animal-to-human transmission of *M. pinnipedii*, probably due to hunting of sea lions. However, although the zoonotic potential has been assumed, previous attempts to unequivocally demonstrate such hypothesis have failed and the isolation of a *M. pinnipedii* human strain has never been described. In fact, although in 1993 a case of a putative zoonotic transmission was described in a marine park in Western Australia, the strains were identified as *M. bovis*, and no robust molecular tools were available for fine-tuned species identification [7]. More recently, a report of a TB outbreak in a sea lion population in a zoo in the Netherlands assumed zoonotic transmission to the animal keepers [4]. However, there were no cases of human disease and only serological tests (tuberculin skin test and interferon- γ release assay) were used. Although the animal-to-human transmission was probable, considering that these tests lack *M. pinnipedii*-specific antigens, the identified latent TB cases can be attributed to any of the members of the *M. tuberculosis* complex.

In this regard, we aim to report the first confirmed animal-to-human transmission of *M. pinnipedii*.

In February 2019, one sea-lion keeper from a Portuguese zoo was admitted to hospital with clinical symptoms of TB, namely cough for >2 weeks, fever, night sweats and loss of weight. The subject was immunocompetent, but a heavy smoker and did not seem to be healthy. TB diagnosis was confirmed after laboratory isolation and susceptibility testing of a *M. tuberculosis* complex strain using the standard methodologies (performed using liquid media isolation with BACTEC MGIT 960 automated system (BD, Franklin Lakes, NJ, USA)) at the National Reference Laboratory for Tuberculosis of the Portuguese National Institute of Health (NIH). As no *in vitro* resistance was detected, the patient was subjected to a successful treatment for 6 months using the standard first-line scheme (2 months isoniazid, rifampin, pyrazinamide, ethambutol + 4 months isoniazid, rifampin).

Public health screening for active or latent TB in close contacts (family, friends and co-workers) was initiated. No other active human cases were found, but one case of TB in a sea lion (*Zalophus californianus*) from the same zoo had been diagnosed in December 2018 (figure 1). This animal underwent a long-term treatment for lameness and after a sudden considerable drop in weight, loss of mobility and onset of productive cough. After a period in isolation, it was considered to be a terminal case and the decision to humanely euthanise the animal was taken. *Post-mortem* samples were collected from both the respiratory tract and bone lesions for bacteriological analysis. Isolated strains from the two sites were grown using the standard methodologies, as for human samples, and positively identified as *M. pinnipedii* using an in-house multiplex PCR targeting regions RD9 and RD2^{seal} [8] at the Portuguese National Institute for Agrarian and Veterinary Research.

This information was shared among the enrolled institutions and, considering the successful isolation of strains from both animal and human origin potentially underlying the first confirmed case of zoonotic transmission of *M. pinnipedii*, we proceeded with whole-genome sequencing (WGS) analysis at the NIH.



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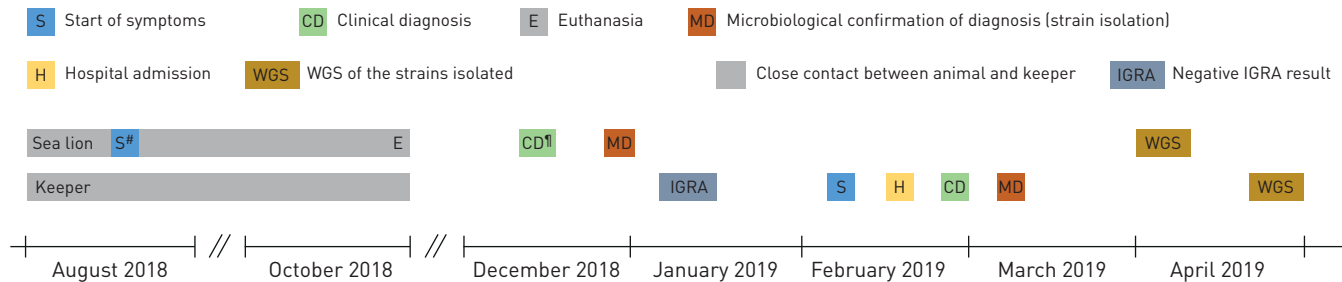


FIGURE 1 Timeline of transmission of *Mycobacterium pinnipedii* from the sea lion to the animal keeper. WGS: whole-genome sequencing; IGRA: interferon-γ release assay. #: respiratory symptoms; †: post-mortem.

The DNA from the strains isolated from the human and animal respiratory samples was subjected to Nextera XT Library Preparation (Illumina, San Diego, CA, USA) and paired-end sequencing (2×250 or 2×150 bp) on Illumina MiSeq equipment. The analysis of the resulting WGS data (mean depth of coverage of 50× and 60× for the human and animal strains, respectively) confirmed the identification of *M. pinnipedii* species for both strains as well as their genetic relatedness. The single nucleotide variants analysis, performed using the *M. tuberculosis* H37Rv genome as reference (NC_000962.3), revealed that the two strains only differed in one position (a single nucleotide polymorphism) among the ~4 Mb analysed (accession numbers ERR4143897 and ERR4143898 from Bioproject PRJEB29446).

Although this constitutes the first confirmed report of animal-to-human transmission of *M. pinnipedii*, it is not possible to speculate about the frequency of these events. In fact, it is only possible to unequivocally demonstrate such unexpected transmission chains due to the rampant technological advances in WGS-based approaches in the past decade. These data illustrate a typical scenario of a new occupational disease and future studies will be needed in order to draw conclusions about the potential transmission of *M. pinnipedii*, particularly due to the famous “sea lion kisses” given to children in zoo shows worldwide.

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