

## **DIRECT SENSITIVE DETECTION OF TUBERCULOSIS AND LEPROSY IN ANCIENT AND MODERN INFECTIONS**

Oona Y.C. Lee<sup>1</sup>, Athina Papaemmanouil<sup>1</sup>, Israel Hershkovitz<sup>2</sup>, Helen D. Donoghue<sup>3</sup>, Angela M. Gernaey<sup>4</sup>, Ehud Galili<sup>5</sup>, Vered Eshed<sup>2</sup>, Charles L. Greenblatt<sup>6</sup>, Eshetu Lemma<sup>6†</sup>, Gila Kahila Bar-Gal<sup>7</sup>, Mark Spigelman<sup>3,6</sup>, David E. Minnikin<sup>1</sup>, Gurdyal S. Besra<sup>1</sup>

<sup>1</sup>School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K.

<sup>2</sup>Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel 69978

<sup>3</sup>Centre for infectious Diseases and International Health, University College London, London W1T 4JF, U.K.

<sup>4</sup>Biosciences Research Institute, University of Salford, Salford, M5 4WT, U.K.

<sup>5</sup>Marine Archaeology Branch, Israel Antiquities Authority, Jerusalem 91004, Israel.

<sup>6</sup>Kuvin Center for the Study of Infectious and Tropical Diseases, Hebrew University-Hadassah Medical School, Jerusalem 41120, Israel.

<sup>7</sup>Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, 76100, Israel.

<sup>†</sup>Present address: Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia.

Tuberculosis is a debilitating disease from past to now, it still kills 2 million people annually worldwide. Tuberculosis in humans and cattle is caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis*, respectively; human leprosy is due to *Mycobacterium leprae*. Ancient tuberculosis and leprosy infection can be detected by DNA amplification and mycolic acid lipid biomarkers. Mycolic acids (MA) are essential components of mycobacterial cell envelopes, with characteristic long carbon chains (C<sub>70-90</sub>) that not present in uninfected mammals, so it can be used for direct detection of tuberculosis. Previous fluorescent derivatisation for HPLC analysis was not quantitative and the derivatives were labile; an alternative robust protocol is reported here.

Liberated MA were converted to pentafluorobenzyl (PFB) esters, followed by reaction with pyrenebutyric acid (PBA) for fluorescence high performance liquid chromatography (HPLC). The procedure was applied to ancient skeletons or organs suspected to have been infected with tuberculosis and leprosy, and biopsies from cattle with bovine tuberculosis. Characteristic profiles of alpha-, methoxy- and ketomycolates were found in *M. tuberculosis* and *M. bovis*, but *M. leprae* lacked of methoxymycolates, as expected.

The ancient tuberculosis of celebrated Granville mummy in the British Museum was confirmed. Leprosy was confirmed in some Hungarian skeletons, including some cases of co-infection with tuberculosis. The facile detection of bovine tuberculosis in cattle offers a rapid positive diagnosis where infection from badgers is suspected.