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Detection of *Mycobacterium tuberculosis* complex ancient DNA in human skeletal remains

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The aim of this research was to detect the presence of the causative agent of tuberculosis (TB), the *Mycobacterium tuberculosis* complex (MTBC) in ancient Hungarian human remains, in order to obtain a better understanding of the prevalence and pathology of this devastating disease throughout different historical time periods.

We have analyzed skeletal remains dating from the Neolithic to the Late Medieval periods (including Bronze Age, Avar Age and Árpádian Age samples). All the skeletal series selected for investigation contained cases showing macromorphological skeletal alterations indicative of TB. Both classical/advanced stage bone lesions and atypical/early-stage symptoms were searched for. The bone material has been screened for the presence of MTBC aDNA both in morphologically positive and negative cases. The members of the MTBC contain a multicopy repetitive insertion sequence element called *IS6110*. The presence of TB aDNA was assessed by applying a PCR-based assay targeting the MTBC *IS6110* region. To increase the sensitivity of the assay, a nested PCR strategy was applied. Initially, conventional PCR was performed using primers *IS6110*F and *IS6110*R to generate a 123 base pair product. The examination was carried out in a special pre-PCR area with a stringent environment for the studies of aDNA including the use of protecting clothing, UV-light exposure of the equipment and bleach sterilization of the surfaces. The aDNA work has been carried out in the aDNA laboratory of the EURAC Institute for Mummies and the Iceman in Bolzano and the Archaeogenetics Laboratory of the Institute of Archaeology, Research Centre for the Humanities, Hungarian Academy of Sciences.

Our preliminary biomolecular results indicate high prevalence of TB infection in the Late Medieval population of Bácsalmás-Óalmás (Pósa et al. 2013). Further analyses were carried out in this particular series, however, has drawn attention to the complementarity of biomolecular and macromorphological investigations; in addition to double-positive cases, morphologically negative/PCR positive and morphologically positive/PCR negative cases have also came to light in the course of the examinations (Pósa et al. 2015a). As for the sampling strategies, remnants of MTBC aDNA were found in skeletons from both sexes and all age groups. Corresponding to the scientific literature, we have sampled compact bone for DNA extraction in each case. However, we have also made several successful attempts to follow protocols of sampling teeth for MTBC aDNA extraction (Faerman et al. 1999; Nguyen-Hieu et al. 2011; Pósa et al. 2012). We also carried out a paleomicrobial study of the Alsónyék Neolithic series. Relatively high prevalence of TB infection was proved in the grave group no. 13 (Pósa et al. 2015b). The PCR analyses of some of the included series are still in progress, similarly to the ongoing spoligotyping and sequencing of the previously extracted MTBC aDNA remains possibly providing more detailed information on the different MTBC pathogens.

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