

LEPROSY IN THE 10–13TH CENTURY AD
IN EASTERN HUNGARYZS. CSÓRI¹, H.D. DONOGHUE², A. MARCSIK³

In the examination of the skeletons of past human populations, the various aspects of the pathological changes provide useful information about their living circumstances and may be indicators of their health conditions. The purpose of this study is two-fold: firstly, to review the published cases of the osseous leprosy in Eastern Hungary, and secondly, to present a new case dated to the 10–13th century of a region of present-day Eastern Hungary. In this region 8 cases have already been published from the 10–12th centuries. The new case of osseous leprosy (Hajdúdorog-Szállásfölk) – from the 12–13th centuries – is housed at the J6sa Andr6s Múzeum in Nyíregyháza. The paleopathological investigation was carried out using macroscopic observation and the isolation and analysis of DNA for the detection of *Mycobacterium leprae* was carried out in London. The bone changes were manifested in the skull.

Key words: paleopathology, osseous leprosy, *facies leprosa*, osteoarcheological samples, Hungary, *Mycobacterium leprae* DNA.

INTRODUCTION

The anthropological study of infectious diseases in antiquity is complex and challenging. The interplay of many variables – host resistance, pathogen virulence, cultural practices, ecological settings, malnutrition, crowding – needs to be considered. Accurate interpretation depends on the proper methodology: adequate sample size, accurate age and sex determination, careful recording of the lesion's localization, set in the context of cultural environment so far as it is possible.

The study of specific infectious diseases, including leprosy has been especially emphasized, for these diseases were among the most important selective factors in human populations in antiquity.

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The purpose of this study is two-fold: first to review the published cases of the osseous leprosy in Eastern Hungary from the 10–12th centuries and second to present a new leprosy case dated to the 12–13th century from a region in present-day East-Hungary. In this region 8 cases have already been published dating from the 10–12th centuries (Marcsik *et al.*, 2007). The new case of the osseous leprosy (Hajdúdorog-Szállásföldek) – from the 12–13th centuries – is housed at the Jóna András Múzeum in Nyíregyháza. The paleopathological investigation was carried out using macroscopic observation and the extraction and analysis of ancient DNA for the detection of *Mycobacterium leprae* was carried out in London.

OSSEOUS LEPROSY (PUBLISHED CASES)

Geographical localisation: the region Hajdúság, Nagykunság (Eastern Hungary).

Sites: Sárrétudvari-Hízóföld, Püspökladány-Eperjesvölgy, Ibrány-Esbóhalom, Hajdúdorog-Gyulás (Fig. 1).

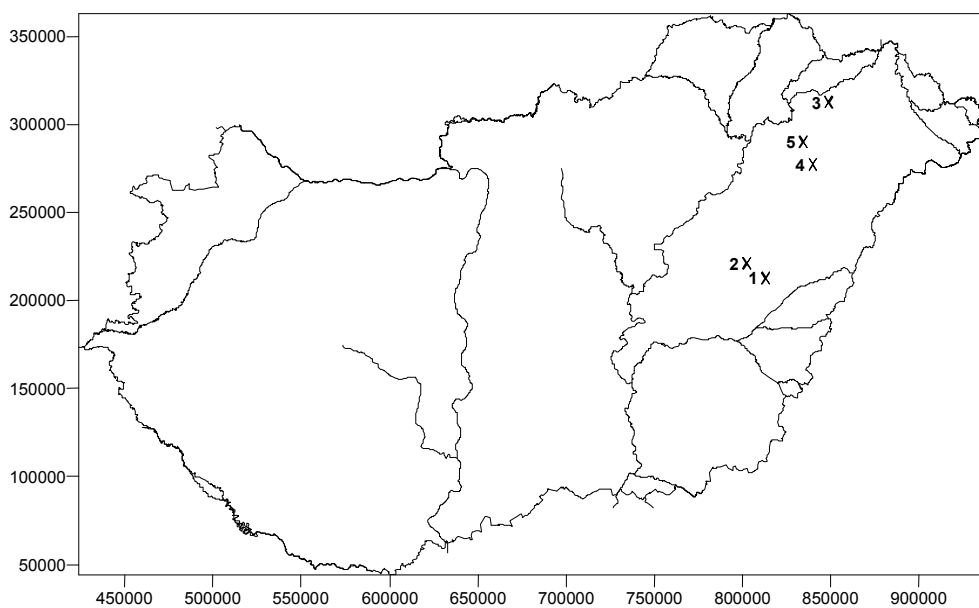


Fig. 1. – Map of Hungary. Sites of skeletons with leprosy: 1. Sárrétudvari- Hízóföld; 2. Püspökladány – Eperjesvölgy; 3. Ibrány – Esbóhalom; 4. Hajdúdorog – Gyulás; 5. Hajdúdorog – Szállásföld.

1. Sárrétudvari-Hízóföld (10th century): there are two cases with leprosy (one female, one male). The female skull is well preserved showing the signs of facies leprosy, the lateral margins of the pyriform aperture are enlarged and remodeled. The changes of the feet are very characteristic. The metatarsals show

marked diaphyseal remodeling with the signs of osteomyelitis and periostitis, and the peg deformity of some phalanges. Periostitis was developed on the tibiae and fibulae. The second case of leprosy is a male showing pathological alteration in the small bones of the feet, with bony ankylosis. The skull shows only periosteal reaction of the hard palate and the nasal region (Pálfi *et al.*, 2002).

The isolation of *Mycobacterium leprae* DNA from both specimens was possible (Haas *et al.*, 2000).

2. Püspökladány-Eperjesvölgy (10–11th centuries): four skulls have maxillary alterations, however, these alterations are slight, early-stage pathological alterations, and not all cases show the specific changes for facies leprosy. The bones of hands and feet are missing (post-mortally) in all of these cases. In the case of 222, in the inferior meatus of the maxilla and in the nasal cavity slight periosteal new bone formation can be observed. In the other skull (503) the border of the nasal aperture is slightly atrophied and there is periosteal apposition on the inner surface of the maxilla and the nasal cavity (Pálfi *et al.*, 2002). These two skulls do not present macroscopic evidence of leprosy, however, the molecular biological study carried out by Donoghue *et al.*, identified *Mycobacterium leprae* (Donoghue *et al.*, 2002, 2005). From specimens 222 and 503 *Mycobacterium tuberculosis* was also identified (Donoghue *et al.*, 2005).

In a further two skulls slight signs of facies leprosa can also be observed (11 and 429), the samples – from the cavum nasale – were positive for *Mycobacterium leprae* DNA (Donoghue *et al.*, 2005).

3. Ibrány-Esbóhalom (10–11th centuries): on one skull (258) a slight alteration of the facies leprosy can be observed. The lateral margins of the pyriform aperture are enlarged and remodelled. The anterior nasal spina has disappeared due to resorption and instead of the spina there is a groove running to the incisors. Porotic processes of the palatine bone, the transverse palatine suture and the hard palate can be noted. The incisive foramen is very large and broad. There is no sign of periostitis on the lower extremity, the bone of the hands and feet are missing postmortem. Molecular analysis was not carried out. This individual is classified as having “possible” leprosy on the basis of those osseous changes (Marcsik, 2003).

4. Hajdúdorog-Gyúlás (10th century): a male skull (56) shows the resorbed anterior nasal spine and the osteolysis and pitting of the interior nasal concha. The anterior border of the nasal bones is also resorbed, the pyriform aperture is less large than normal. No alterations were seen on the extremity (Marcsik *et al.*, 2007). Molecular analysis was carried out (H.D. Donoghue, unpublished observations) and the result was positive for *M. leprae*. This specimen is classified as having leprosy (facies leprosy) on the basis of the osseous changes and molecular data.

MOLECULAR EXAMINATION OF HAJDÚDOROG-GYÚLÁS (10TH CENTURY, GRAVE 56)

DNA extraction

The recommended protocols of ancient DNA (aDNA) work (O'Rourke *et al.*, 2000) were followed, with separate rooms for different stages of the process. Approximately 67 mg of the palatal sample was crushed in a sterile pestle and mortar, transferred into a 9 ml tube of NucliSens[®] (bioMérieux) lysis buffer containing 5 mol⁻¹ guanidium thiocyanate solution and incubated at 56°C for 24 hours. Negative extraction controls were processed in parallel with the test sample. The 9 ml tube was boiled for 1 hour, vortex-mixed and allowed to cool. To complete the disruption of the bone scrapings and any pathogenic microorganisms, the sample was then snap-frozen in liquid nitrogen and thawed in a 65°C water bath. This was repeated twice. The sample was spun at 5000g for 15 mins at 5°C and the supernatant carefully removed into a clean, sterile tube. DNA was captured by mixing with 50 µl silica suspension (NucliSens[®]) and centrifugation. The silica pellet was washed once with wash buffer (NucliSens[®]), twice with 70% (v/v) ethanol (-20°C) and once with acetone (-20°C). After drying in a heating block, DNA was eluted from the silica using 60 µl elution buffer (NucliSens[®]), aliquoted and used immediately or stored at -20°C.

DNA amplification and detection

M. leprae DNA was detected by targeting a specific region of the repetitive element RLEP (37 copies/cell). A two-tube nested PCR was used which give an outer product of 129 bp and a nested PCR product of 99 bp (Donoghue *et al.*, 2001). A hot-start *Taq* polymerase was used to minimise non-specific primer and template binding. Negative DNA extraction and PCR controls were processed alongside the test sample. Detection of PCR product was by staining with ethidium bromide and visualising under ultraviolet light. The result was positive using nested PCR (Fig. 2).

Therefore, the specimen – on the basis of morphologic appearance – suffered from leprosy and this diagnosis is supported by the molecular analysis.

NEW CASE OF OSSEOUS LEPROSY

5. site: Hajdúdorog-Szállásföldek (Fig. 1)

At Hajdúdorog-Szállásföldek 1245 skeletons were discovered in 2004. Based on the archaeological finds they are dated to the 12–13th centuries (1141–1235) (Fodor, 2006). During the paleopathological analysis one skull showed serious alterations on its facial bones.



Fig. 2. – Gel of nested PCR for *Mycobacterium leprae* RLEP
 Lanes (left to right): 1–2, other positive archaeological samples; lane 3–8: other negative archaeological samples; lane 4: Hajdúdorog-Gyúlás 56 (positive); lanes 9–10: negative controls; lane 11: molecular markers.

Skeleton No. 53 (obj. 560, str. 775)

Sex: male (64–70 years)

This skeleton is well preserved, however, some bones - mainly the bones of hands and feet – are missing postmortally (there are only five phalanges and one metacarpal bone). The age at death and the sex were determined as described by Acsádi-Nemeskéri (1970), Éry *et al.*, (1963), Harsányi-Nemeskéri (1964) (obliteration=5, facies of symphysis pubis=4, trajectorial system of humerus=3, and of femur=4). Because of the older age at death some bones show porotic lesions (iliac fossa, medial surface of the ribs, patellas). Mild osteoarthritis can be noted in the semilunar notch.

The alterations on the facial bones are the following. The margins of the pyriform aperture are rounded and the alveolar maxillary exhibits destructive pathological remodeling. There is a bilaterally symmetrical resorption with sharply defined lateral margins, a mild surface pitting and progressive resorption of the anterior nasal spine (with initial loss of cortical bone and exposure of cancellous bone) and of the prosthion (Fig. 3). The alterations of the nasal surface of the palatine process of the maxilla appear as gross patterns: porosity, pitting with remodelling subperiosteal new bone. The incisive foramina are larger than normal (Fig. 4a). On the lateral side of the nasal cavity and of the maxillary sinus there are smaller exostoses and pitting (Fig. 4b). Pitting and perforations can be seen also on the oral surface of the maxillary palatine process and the palatine bone is total resorbed (Fig. 5). These changes relate to the rhinomaxillary syndrome (facies leprosa).



Fig. 3. – Hajdúdorog-Szállásföldek, 12–13th century, objectum 560.
Facies leprosa – rounded margins of the pyriform aperture due to absorption.



Fig. 4a. – Pitting and porosity on the nasal surface of the palatine process due to inflammation.

Fig. 4b. – Pitting and smaller exostoses on the lateral side of the nasal cavity and of the maxillary sinus.

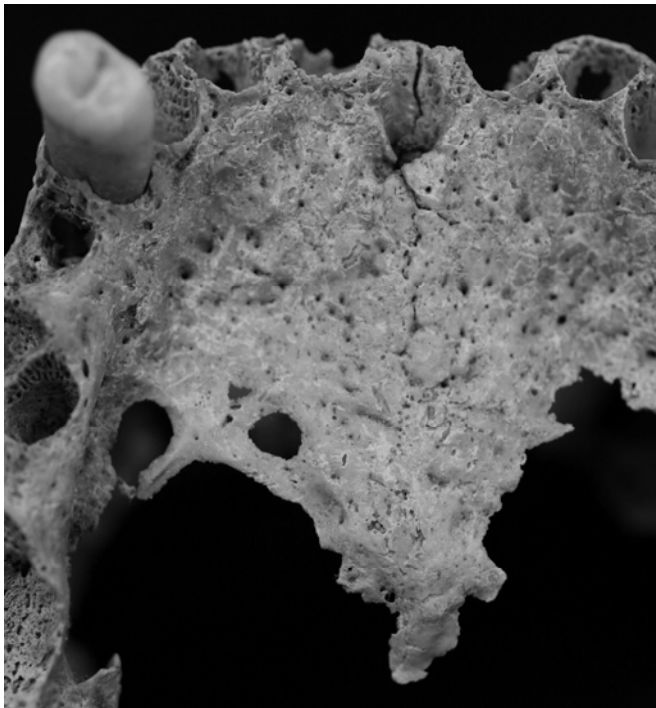
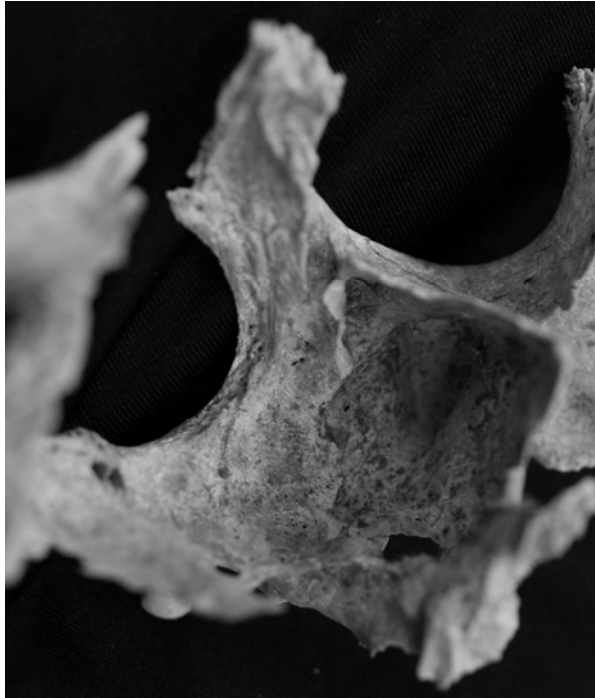


Fig. 5. – Perforations on the palatine process, resorbed palatine bone.

No changes are seen on the infracranial bones, apart from the slight signs of periostitis on the tibiae, fibulae and femora.

SUMMARY

On the basis of paleopathological observations, leprosy appeared in the 10th century AD causing typical osseous morphological alterations. However, it was not widespread, and has been found only at a few archeological sites on the region of the north-eastern part of the Great Plain in Hungary (Marcsik *et al.*, 2007). In southern parts of the country, leprosy has also been found from the 11th and the early 12th centuries (Marcsik, 1998). In later centuries and the Hungarian Medieval Period, based on the appearance of skeletal material, bony manifestations of leprosy are observed less frequently. This is not too surprising as it has been suggested that the leprous patients were isolated from the non-infected population relatively early in Medieval Hungary (Pálfi *et al.*, 2002). Although the skeletons from Hajdúdorog-Szállásföldek dated to the 12–13th centuries, it is possible that the specimen with leprosy is from the early 12th century.

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