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The Early Mediaeval manorial estate of Gars/Thunau, Lower Austria: An enclave of endemic tuberculosis?

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SUMMARY

In recent decades, an increasing number of studies have aimed to shed light on the origin and spread of tuberculosis in past human populations. Here we present the results of a systematic palaeodemographic and palaeopathological survey of the Early Mediaeval population of Gars/Thunau (Lower Austria), which – at this stage – includes 373 individuals recovered at two archaeological sub-sites: a fortified settlement (including a necropolis) at the top of a hill – probably reserved for social and military elites; and a large riverine settlement at the foot of the hill, a so-called 'suburbium', where burials and an area of 'industrial' character were discovered. We recorded a great number of pathological alterations and a variety of 'classical' features of tuberculosis, such as vertebral destructions (Pott's disease) and joint destructions, and other pathological (unspecific) features probably linked with *Mycobacterium tuberculosis* infection (e.g. new bone formation at the inner surface of the ribs, endocranial alterations in the form of 'pits', and new bone formation at the cranial base). We hypothesize that the two contemporaneous (~900–1000 AD) populations of Gars/Thunau differed not only in their social affiliation/condition, but also in the type and frequencies of their population-density-related infectious diseases (in particular tuberculosis). Moreover, we investigated the molecular genetic evidence of the causative organism in a few selected immatures exhibiting pathological changes at the inner wall of the cranium and discuss these findings in regard to the macroscopic features observed. Finally, we analysed carbon and nitrogen stable isotopes of both populations and strontium isotope ratios of the hill-top inhabitants in order to reconstruct certain aspects of diet and mobility to test our hypothesis concerning the specific social and/or military character of the site.

1. Introduction

Thunau am Kamp in north-eastern Austria, approximately 25 km north of the river Danube, represents an archaeologically well-documented Early Mediaeval fortified central settlement [1–3]. The site saw repeated prehistoric occupation, but settlement activity intensified in the late phase of the Early Middle Ages, between the ninth and tenth century AD [4]. The importance of the settlement can be explained by its location on a trade route on the periphery of the former East-Franconian Empire, linking the East Franks in the south and the Moravians and Bohemians in the north [5]. On the basis of structural features and finds indicating the presence of a social elite, it is assumed that the site may have played an important military role.

Large scale excavations by the Department of Prehistoric and Historical Archaeology of the University of Vienna began in the mid 1960s and remain ongoing [6]. The site comprises two sub-areas, a

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fact that is of particular importance in the particular epidemiological context under investigation here. These are: a) a fortified settlement on a hill; and b) a settlement at the foot of the hill, in the valley of the river Kamp. Obenaus [7,8] and Szameit [9] have stated that the settlement forms and human populations of the two sub-areas underwent a parallel growth and development from the Neolithic period onwards, notably during the Bronze Age; however, they observed differences between the two sub-areas of the site during the Early Middle Ages, particularly in regard to spatial structures, the relative quality of finds, and in the arrangement and concentration of the graves. For example, the hill-top settlement included a manorial farm with fortified ramparts, as well as a cemetery with densely packed graves, which was probably reserved for social and military elites. In contrast, the riverine area was characterised by an unfortified rural settlement, where extensive archaeological evidence for large-scale craft production (such as tannery and flax- or metal-processing) and agricultural activities has been documented. The archaeologists identified this obviously less densely populated area as an 'industrial zone', presumably inhabited by craftspeople, farmers and their families, who probably supplied the occupants of the manor with necessary goods. Thus, although the settlements belong to the same period, the archaeological finds suggest that their inhabitants probably experienced different living conditions.

In the course of an anthropological pilot study of human skeletal remains unearthed at the Gars/Thunau hill-top settlement, we identified a noticeably high frequency of features related to infectious diseases, in particular tuberculosis [10,11]. At first glance, the skeletal remains recovered at the open riverine settlement complex in the valley of the river Kamp showed fewer pathological manifestations and thus we hypothesize that the inhabitants of the two settlements – despite their close proximity – were subject to different levels of stress, whether demographic, nutritional, activity- or hygiene-related, and that tuberculosis may have been a particular burden in the enclosed and tightly confined hill-top settlement, being well known for its population-density dependent transmission.

Tuberculosis is a chronic granulomatous infectious disease caused by *Mycobacterium tuberculosis*, which may befall the skeletal system. The clinical presentation depends on the individual's response to the presence of the pathogen [12]. The infection starts usually as a direct infection ofthe lung by inhalation of microorganisms, or – rarely – through the alimentary tract, or via skin, or connatally. The prevalence of this disease in ancient human skeletal remains is mainly evidenced by osteomyelitic changes to the spine (Pott's disease [13]) or joints [14,15], which usually appear as post-primary, late manifestations of tuberculous reinfection. Skeletal tuberculosis is macroscopically and radiologically identifiable and well documented in ancient human skeletal remains [16–20]. Although there is documentary evidence that tuberculosis was a common problem in archaic populations, in particular in the Middle Ages [21], osteopathological findings are rare, since only 3–5% of persons with tuberculous infection develop skeletal or joint destructions. In recent decades, an increasing number of palaeoepidemiological studies have also recorded other (unspecific) manifestations of this disease, probably linked with *M. tuberculosis* infection, for instance inflammatory, osteolytic and osteoproliferative lesions at the visceral surface of the ribs caused by pleurisy [22–24], or structural changes on the internal cranial layer in the form of small foveas and crypts [25,26] or new bone formations and inflammations at the endocranial base, which can probably be related to *Meningitis tuberculosa* [10,11,27,28,29]. They are caused by an inflammatory meningeal reaction, which leads to fibrous exudates around basal cisterns and the brain stem, and variable destructions of the internal skull base [30]. These specific and unspecific skeletal features, as well as other unspecific stress symptoms implying malnutrition, will be used to test the abovementioned hypothesis of different living conditions within the fortified and open riverine Early Mediaeval settlements of Gars/Thunau. We will also discuss the findings in relation to the results of dietary (stable carbon and nitrogen isotope ratios) and provenience (strontium isotope ratio) studies. Moreover, we performed preliminary PCR experiments to detect *M. tuberculosis* in selected bone and tooth samples to shed light on the possible relation between pathomorphological features and the causative pathogenic agent.

2. Material and methods

The sample comprises a total of 373 individuals, 309 of whom were recovered at the hill-top settlement (the 'Schanze'), and 64 at the riverine settlement. To test the assumption of probable different living conditions at the two sites, or whether the inhabitants of the two separate areas represent different social strata, we used three empirical approaches: the morphological approach, the PCR approach, and the isotope approach.

Sex and age-at-death estimation and the investigation of pathological alterations (we recorded inflammatory reactions possibly associated with tuberculosis and symptoms attributed to malnutrition, such as cribra orbitalia, porotic hyperostosis and periosteal lesions) were conducted using the conventional methods of macromorphology [31-37], reflected-light microscopy and radiology [38].

For the (pilot) molecular genetic analysis of the pathogenic agent (*M. tuberculosis*), four individuals (GT25030 = grave no.75, GT25246 = grave no.1980/1, GT25054 = grave no.94, GT25256 = grave no.1985/2) were selected, from whom tooth as well as bone samples were analysed. They all are from the hill-top settlement: GT25030 is a male with joint TB (=tuberculosis) (also investigated by Helen Donough); GT25054 is a 4 to 5-year-old child exhibiting severe destructions in the form of newly built bone structures and hypervascularisation at the inner skull vault; GT25246 and GT25256 are taken from two subadults, ca. 3 and 4 years old respectively, showing newly built bone structures around the nerve channels at the cranial base. All samples were pulverized with a Retsch MM400 grinding mill, using wolfram carbide grinding jars and balls. DNA was extracted with the *Genial* All-tissue DNA-Kit according to the manufacturer's instructions for DNA extraction from bone and teeth. Final elution of DNA solution was in 30 μl. Primers TbA, TbB, TbC, and TbD (targeted to the insertion sequence IS6110 [39]) were used as described in Bachmann et al. [40]. In addition, two overlapping primers designed in the present study were used: TbE (5′-CCCGCCGATCTCGTCCAGCGCCGCTTCGG-3′) and TbF (5′- GCCCAGGATCCTGCGAGCGTAGGCG-3′). PCR was performed with QiagenTopTaq Polymerase in 25 μl 0.5 μM of each primer, 0.2 mM of each dNTP (Roche, Mannheim, Germany) and with 1 μl DNA solution. Thermal cycling conditions: 94 °C for 2 min; 45 cycles of 94 °C for 30 s, 60–72 °C for 30 s, and 72 °C for 30 s; final extension at 72 °C for 10 min. PCR products were extracted from agarose gels with the QIAquick Gel ExtractionKit (Qiagen, Inc.) and cloned using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA). Sequencing of the clones (both directions) was performed at LGC Genomics.

The isotope approach included carbon and nitrogen stable isotope ($\delta^{15}N$, $\delta^{13}C$) analysis of collagen extracts of 41 human bones, 27 faunal bones and 2 human teeth from both sites, the hill-top and the riverine settlement, in order to identify a purported social stratification as reflected in the subsistence (for further details see Rumpelmayr [41,42]) and strontium isotope ratio ($^{87}Sr/^{86}Sr$) analysis of 76 human first or second molars and 10 (non modern) animal teeth recovered from within the hill-top area (the riverine inhabitants remain under study) to shed light on the provenance of