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

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S U M M A R Y

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 above-mentioned 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 (δ^{15}N, δ^{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 the group buried there. In addition, recent environmental samples (soil, water, plants) were investigated for Sr isotopic composition in order to establish a local/dietary signal for comparison (details are being published elsewhere [43]).

3. Results

Demography: The age at death distribution in both populations do not match our expectations – neither when we consider the whole samples, nor when we separate by sex. Table 1a and Figure 1a show a very high and similar child mortality rate in both populations: at the hill-top settlement 64.4% of the individuals died before the age of 20; at the valley settlement the subadults account for 57.8%. If we disregard the juveniles from this calculation, differences between the populations increase, and show – again – a considerably higher relative frequency of subadults buried at the fortified hill-top settlement (hill-top: 58.6%; valley: 45.3%), which is statistically significant ($Chi^2 = 3.791$, $df = 1$, $p = 0.05$). Also of interest is the age at death distribution in males and females in the two groups (see Table 1b, Figure 1b): Although interpretation is limited by the small number of adult individuals recorded in the valley population, we can determine that a) the mortality peak of the valley settlers lies in a higher age category than that of the hill-top settlers, and b) within the latter, the relative frequency of young adult males exceeds that of the females. This finding is in contrast to the typical pattern for ancient populations, where the frequency of females surpassed that of males in the young age category (adult). This result seems to corroborate the assumption of a military function of the hill-top construction (A reduced mortality table, the mortality rate and other parameters will be calculated and discussed in detail in the upcoming monograph).

Pathological alterations: Although we have recorded the complete spectrum of traumatic and pathological skeletal alterations (results will be published elsewhere), we selected two groups of conditions for this study that seem to be most relevant in the given context: inflammatory reactions possibly associated with tuberculosis (Figure 2; Table 2a–c; Figure 3a), and symptoms of malnutrition (Table 3a and b; Figure 3b). As reported previously [10,11], there is strong evidence for the presence of tuberculosis infection in the fortified hilltop settlement. Through morphological inspection we observed three cases of spinal tuberculosis and one case of joint tuberculosis. The first was a 12-year-old individual with features of 'classical' Pott's disease, an advanced destruction of the vertebral column resulting in the characteristic angular deformity, leaving the rudimentary, wedge-shaped bodies of four thoracic vertebrae and involving some of the inter-vertebral joints. The second specimen, attributable to a male, is characterised by severe destructions of the lumbar spine, the predominant location of bone tuberculosis infection. Both these specimens show healing as evidenced by gibbus and residual cavitary defects. Interestingly, these two individuals also exhibit a feature often found and discussed in the context of tuberculosis, viz unilateral new bone formation on the visceral surfaces of the ribs. The third individual affected by tuberculosis infection shows a lesser degree of vertebral destruction, thus representing the granulomatous phase with local cavitations in the cancellous bone and perifocal osteoporosis. The fourth case, again a male, presents a pronounced, yet remodelled, destruction of the shoulder joint accompanied by a severe deformation of the articular surface and a substantial shortening of the humerus. This implies an infection through the growth period, because 'foci destroying a growth plate will leave a growth deficit and/or deformity of the involved bone' [44]. Just recently a single very similar case was also discovered among the riverine skeletal population of Gars/Thunau.

Table 1a
Age-at-death distribution of the Early Mediaeval sub-populations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria.

	HS (n = 309)		VS (n = 64)	
	n	%	n	%
Fetus/neonatus	21	6.8	2	3.1
Infans I	126	40.8	21	32.8
Infans II	34	11.0	6	9.4
Juvenile	18	5.8	8	12.5
Adult	45	14.6	7	10.9
Mature	51	16.5	14	21.9
Senile	12	3.9	5	7.8
Unknown adult	2	0.6	1	1.6

Besides this 'direct' evidence, a significant number of indirect – although unspecific – features, such as newly built bone structures at the visceral surface of ribs, representing an inflammatory response (pleurisy), often induced by a late primary infection of tuberculosis, were also identified. Adults as well as subadults from the hill-top settlement exhibit these lesions in a high frequency (14 out of 88 = 15.9% and 20 out of 142 = 14.1%), whereas the riverine settlers lack these alterations almost completely (only 1 subadult). It further became clear that males were more prone to pleurisy than were females (21.3%: 11.4%).

Other features discussed that favour identification with tuberculosis infection are endocranial features [27,29,45,46]. Table 2a–c and Figure 3a summarize our findings regarding alterations of proliferative character and small, often conflating, grooves and pits on the internal layer of the cranial vault and cranial base. We observed structural changes on the inner layer of the cranium for the anterior, the middle and the posterior region. What becomes clear in this simplified graph is that proliferative changes occur more often in subadult individuals than in adults. Many of these peculiar reactions are located at the base; especially in the region of the pars orbitalis of the frontal bone and the sphenoid bone around the foramen spinosum and ovale (see Figure 2c and d). Table 2c shows that 62.3% of the hill-top immatures exhibit proliferative changes at the cranial vault (the valley group: 21.7%), 51.2% show alterations exclusively at the cranial base (the valley group: 40.0%) and 39.8% exhibit a combination of these features (the valley group: 20%). Thus, we assume, as suggested earlier, that these structures, in particular those at the cranial base, are probably indicative of tuberculosis infection of immatures.

On the other hand, small pits (foveolae), often located on the margins of the anterior cranial fossa and in the middle cranial fossa (sometimes reaching the squama of the temporal bone) are much more frequent in adults. As asymmetry was not observed, Table 2a and b presents the frequencies recorded at the right side of the skull. Again, males are more often affected. Interestingly, there are only a few skeletons recovered at the riverine site on which small pits, interpreted by Templin and Schultz [25,26] as impressions of calcified tubercles, could be found.

Molecular genetic analysis: To verify our presumption that these features may indicate tuberculosis infection, we recently carried out a molecular genetic analysis of 4 individuals (see above). In PCR experiments with all combinations of primers, 3 out of the 4 individuals yielded positive results with the primer pair TbC and TbD (GT25030/male individual, selected example with progressive joint destruction probably caused by tuberculosis; GT25246/2–3-year-old immature with alterations at the cranial base; GT25054/4–5-year-old child with severe alterations, including destructive and proliferative changes to the cranial vault), that is, sequences with high sequence similarity to *M. tuberculosis* in a BLAST search in GenBank (http://www.ncbi.nlm.nih.gov). There were two different types of sequences: one obtained from the bone samples of all three

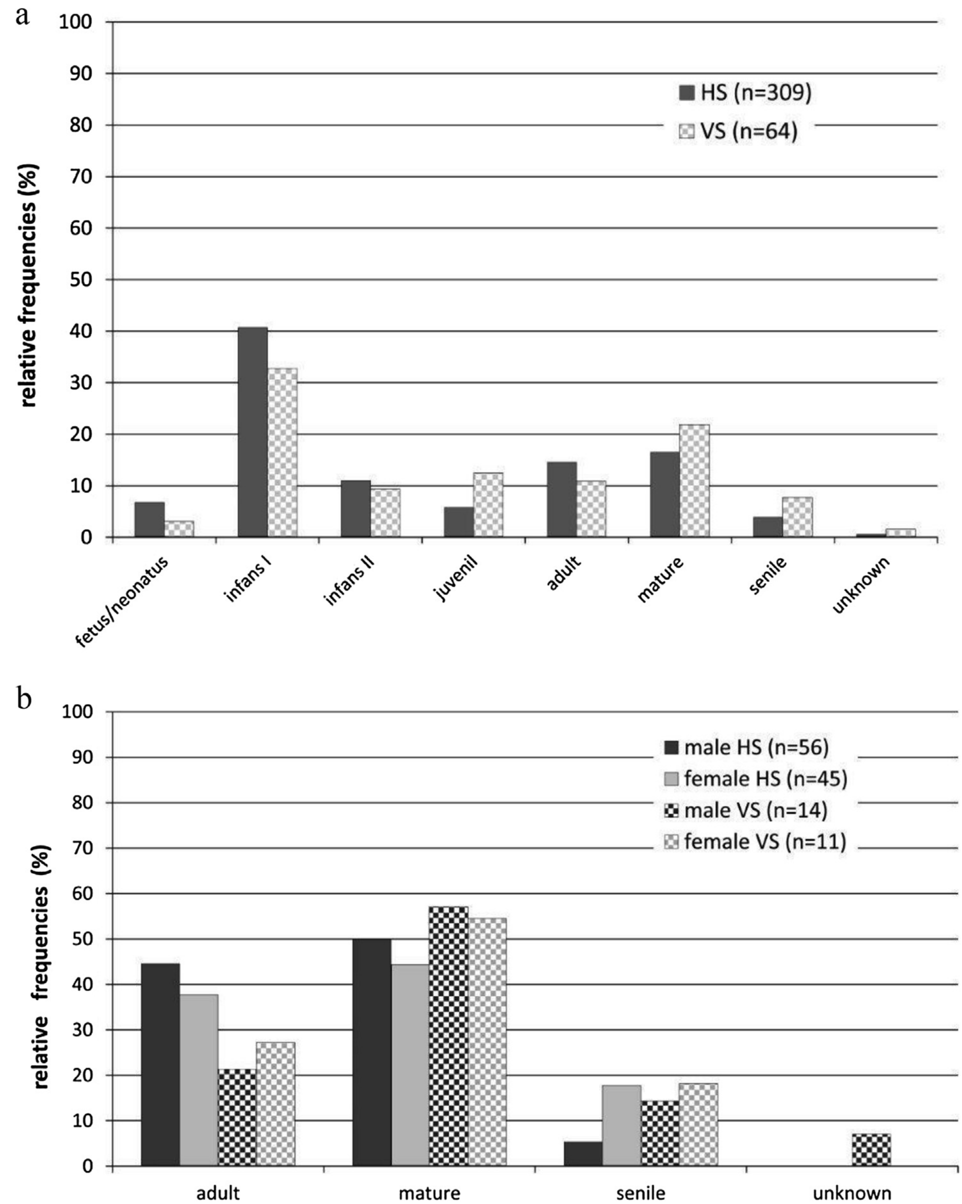

Figure 1. a) Age-at-death distribution (relative frequencies) of the Early Mediaeval sub-populations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria. b) Age-at-death distribution (relative frequencies) of males and females of the Early Mediaeval sub-populations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria.

individuals, and another obtained in addition from the tooth sample of GT25030, the individual showing severe destruction at the shoulder joint. This is obviously due to the fact that these primers bind to a repetitive region and therefore have various binding sites within the genome of *M. tuberculosis*. Data from this preliminary study will be presented elsewhere in detail.

Table 1b
Age-at-death distribution of males (m) and females (f) of the Early Mediaeval sub-populations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria.

	HS/m (n = 56)		HS/f (n = 45)		VS/m (n = 14)		VS/f (n = 11)	
	n	%	n	%	n	%	n	%
Adult	25	44.6	17	37.8	3	21.4	3	27.3
Mature	28	50.0	20	44.4	8	57.1	6	54.5
Senile	3	5.4	8	17.8	2	14.3	2	18.2
Unknown	0	0.0	0	0.0	1	7.1	0	0.0

Unspecific stress features: Tuberculosis infection is spread by airborne transmission and, thus, influenced by population density. But it also more often affects immunocompromised individuals. Consequently, our study also captured the frequencies of unspecific stress features (Table 3a and b, Figure 3b). It is obvious that subadults exhibit higher frequencies of cribra than do adults in both populations, but the hill-top inhabitants were generally affected more often than the riverine settlers. Subadults of the fortified settlement show also a higher frequency of proliferative changes at the mandibular ramus and the maxillary alveolar rim. In contrast, porotic hyperostosis and periosteal reactions were found more often in adult individuals and, again, the hill-top settlers were more frequently concerned. Males and females exhibit conspicuous differences in their frequencies of unspecific features: males are more often affected by porotic hyperostosis and periostitis (further detailed studies are ongoing).

Dietary reconstruction: The differences observed between the hill-top and the riverine population concerning the frequencies of

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Figure 2. a–d) Endocranial features: a) Gars/Thunau grave no. 119, adult, confluent pits at the squama of the temporal bone; b) Gars/Thunau grave no. 100, subadult, newly build bone structures (proliferative changes) at the cranial vault; c) Gars/Thunau grave no. 184, subadult, newly build bone structures at the cranial base; d) Gars/Thunau grave no. 197, subadult, newly build bone structures at the cranial base.

pathological alterations and unspecific stress lesions led us to a further approach, the investigation of the δ^{15}N and δ^{13}C values. The δ^{15}N values of the humans (8.3‰–11.8‰) indicate an omnivorous diet, consisting of variable proportions of plant and animal resources. With two exceptions [33], the δ^{13}C values range from −19.8‰ to −17.4‰ and imply a terrestrial, mainly C_3-based diet. The δ^{13}C and δ^{15}N values data set of the human remains was investigated for differences between population groups (hill-top and valley population, males and females) by multivariate analysis of variance and ANOVA. There was no statistically significant difference between the hill-top and valley populations observable. Although the data from the investigated males from both locations seem to show a tendency for higher δ^{13}C and δ^{15}N values compared to the females, the differences were not statistically significant.

Provenience: A further aspect of this multidisciplinary study dealt with the question of provenience, the identification of non-locals and locals within the hill-top sample. The strontium isotope ratio approach was used to provide supporting information for the interpretation of the ascertained mortality pattern. Based on

Table 2a
Endocranial alterations of subadults and adults of the Early Mediaeval subpopulations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria (prol = proliferative structures, pits = impressions, CV = cranial vault, CB = cranial base, A = anterior, M = middle, P = posterior).

Endocranial location/type of alteration	HS adult (n = 111)		HS subadult (n = 198)		VS adult (n = 27)		VS subadult (n = 37)	
	n/N	%	n/N	%	n/N	%	n/N	%
CV/prol	26/90	29.0	100/171	58.4	1/20	5	8/28	28.6
CBA/prol	3/55	5.6	17/79	21.5	0/15	0	2/18	11.1
CBA/pits	16/55	29.0	12/92	13.0	0/15	0	0/18	0
CBA/prol + pits	3/55	5.5	1/92	1.1	0/15	0	0/18	0
CBM/prol	4/63	6.4	37/100	33.6	0/16	0	6/19	31.6
CBM/pits	20/63	31.7	8/110	7.3	2/16	12.5	1/19	5.3
CBM/prol + pits	2/63	3.2	3/110	2.7	0/16	0	0/19	0
CBP/prol	5/65	7.7	34/99	34.3	0/15	0	6/17	35.3
CBP/pits	21/65	32.2	6/99	6.1	0/15	0	1/17	5.9
CBP/prol + pits	2/65	3.1	2/99	2.0	0/14	0	1/17	5.9

Table 2b
Endocranial alterations of males (m) and females (f) of the Early Mediaeval subpopulations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria (prol = proliferative structures, pits = impressions, CV = cranial vault, CB = cranial base, A = anterior, M = middle, P = posterior).

Endocranial location/type of alteration	HS/m (n = 56)		HS/f (n = 45)		VS/m (n = 14)		VS/f (n = 11)	
	n/N	%	n/N	%	n/N	%	n/N	%
CV/prol	15/49	30.6	9/34	26.5	0/10	0	1/8	12.5
CBA/prol	1/28	3.6	2/23	8.7	0/8	0	0/5	0
CBA/pits	11/28	39.3	5/23	21.7	0/8	0	0/5	0
CBM/prol	2/33	6.1	1/24	4.2	0/8	0	0/5	0
CBM/pits	13/33	39.4	6/24	25.0	2/9	22.2	0/5	0
CBP/prol	4/37	10.8	0/25	0	0/8	0	0/5	0
CBP/pits	16/37	43.2	4/25	16.0	0/8	0	0/5	0

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Table 2c
Endocranial proliferative (prol) alterations at the cranial vault (CV) and the cranial base (CB) in subadults of the Early Mediaeval sub-populations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria.

Endocranial location	HS subadult (n = 181)		VS subadult (n = 29)	
	n/N	%	n/N	%
CV/prol	96/154	62.3	5/23	21.7
CB/prol	63/123	51.2	8/20	40.0
CV + CB/prol	43/108	39.8	3/15	20.0

the enamel samples of 56 adult individuals we identified a remarkable strontium isotope ratios variability (min = 0.70863; max = 0.71499; x = 0.71140, SD = 0.00128). Interestingly – and this is an extraordinary finding – 83–89% of the humans and >50% of the investigated historical animals show a non-local signal [47] (we abstain from detailed comparative interpretation, as the riverine population is undergoing study).

4. Discussion

One of the most interesting findings of this study concerns the mortality pattern, in particular the high, and similar, child mortality rate in the two populations (64.4% and 57.8%, respectively), and the higher mortality rate of young males in the fortified hill-top. If we consider the frequencies of pathological alterations and the high rate of infectious diseases detected in the remains of the hill-top-subadults (especially the high rate of endocranial indices of meningitis/meningoencepahlitis), we would assume a strong relation to either bad hygienic conditions and/or cramped living conditions.

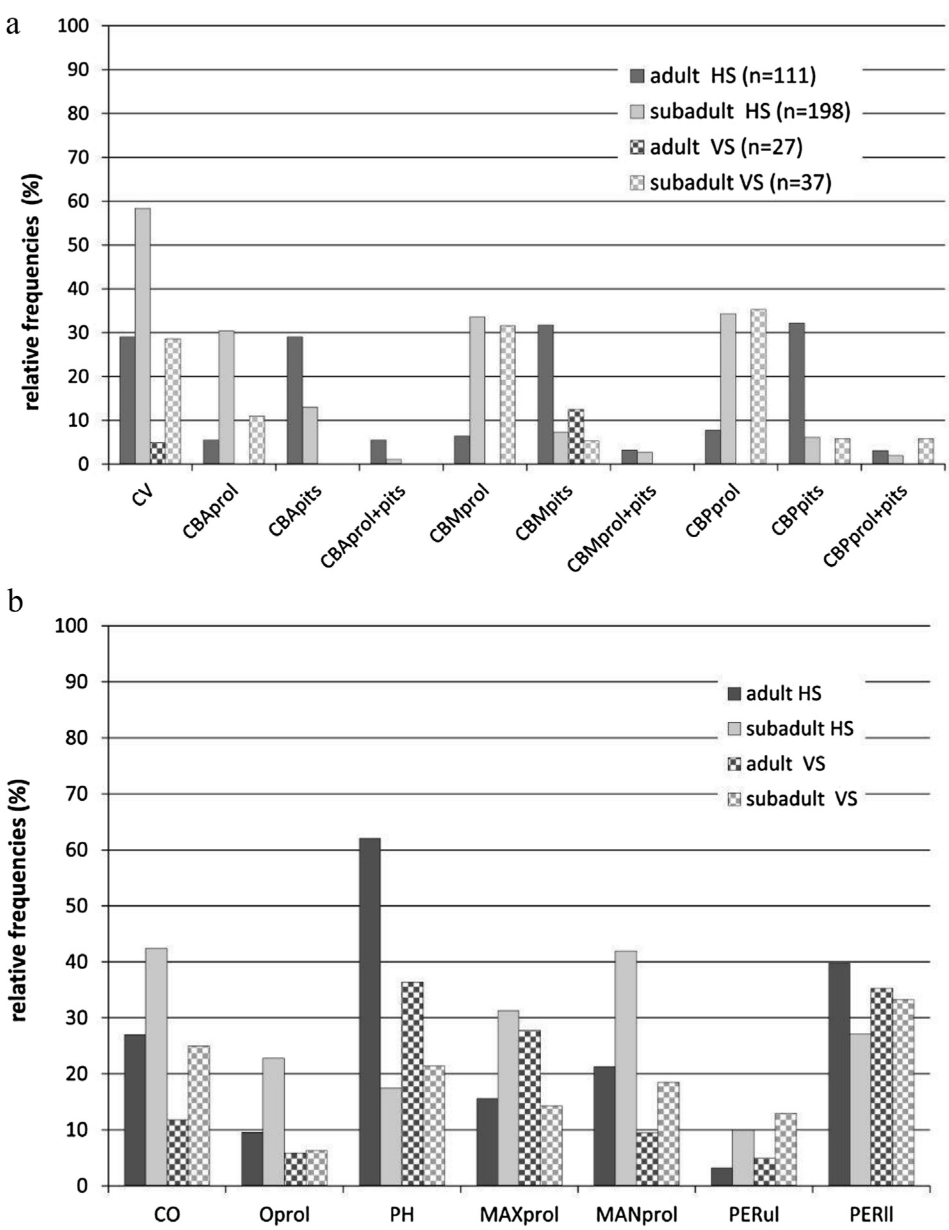

Figure 3. a) Endocranial alterations (relative frequencies) of subadults and adults of the Early Mediaeval sub-populations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria (prol = proliferative structures, pits = impressions, CV = cranial vault, CB = cranial base, A = anterior, M = middle, P = posterior). b) Unspecific stress features (relative frequencies) of subadults and adults of the Early Mediaeval sub-populations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria (prol = proliferative structures, ul = upper limbs, ll = lower limbs, CO = cribra orbitalia, O = orbital walls, PH = porotic hyperostosis, MAX = maxilla, MAN = mandible, PER = periostitis).

Table 3a
Unspecific stress features of subadults and adults of the Early Mediaeval subpopulations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria (prol = proliferative structures, ul = upper limbs, ll = lower limbs, CO = cribra orbitalia, O = orbital walls, PH = porotic hyperostosis, MAX = maxilla, MAN = mandible, PER = periostitis).

Location and type of alteration	HS adult (n = 111)		HS subadult (n = 198)		VS adult (n = 27)		VS subadult (n = 37)	
	n/N	%	n/N	%	n/N	%	n/N	%
CO	20/74	27.0	39/92	42.4	2/17	11.8	4/16	25.0
O/prol	7/73	9.6	21/92	22.8	1/17	5.9	1/16	6.3
PH	59/95	62.1	30/171	17.5	8/22	36.4	6/28	21.4
MAX/prol	12/77	15.6	31/99	31.3	5/18	27.8	3/21	14.3
MAN/prol	19/89	21.3	55/131	42.0	2/21	9.5	5/27	18.5
PERul	3/95	3.2	12/121	9.9	1/20	5.0	3/23	13.0
PERll	37/93	39.8	38/140	27.1	6/17	35.3	8/24	33.3

But these findings may also reflect a general European trend during the transition between the Early Middle Ages and the High Middle Ages. Whereas continued de-urbanisation due to political instability accounts for a low but stable population level in the Early Middle Ages, towards the end of this period, in the tenth century, when levels of raiding fell, the population increased due to improved economic conditions and new cultural and agricultural technologies [48]. A ‘Climate Optimum’ also boosted population growth [49]' [50]. Increasing population size in past communities is – as with the first dramatic demographic changes in prehistory, stimulated by sedentarisation in the Neolithic – known to be accompanied by a high subadult mortality rate. The assumption that our findings do not necessarily reflect inadequate living conditions is further strengthened by the fact that the calculated mean age-at-death of male individuals in the fortified hill-top area reached ca. 39 years, and at the riverine settlement ca. 44 years, which is higher than the mean age-at-death estimate as generated from adult skeletal populations recovered from late antique and Early Mediaeval cemeteries dating to the period 300 AD and 800 AD in Austria [51]' [52]. Despite this increased mean value of age-at-death based on adults, the life expectancy of the inhabitants of Gars/Thunau was very low. But independently of these considerations, we assume that the higher mortality rate of young males identified in the cemetery at the fortified settlement (which also exceeded that of the females at the site) could indeed support the archaeological arguments in favour of a military function of the construction at the border of the Frankish Empire between ~900 and 1000 AD. Two cases of executed males (individuals exhibiting features of decapitation) seem to corroborate this interpretation.

Of most interest in the given context was the observation of a high frequency of infectious diseases, especially of TB infection, among the population of the fortified rampart. Besides a particular genetic disposition, a number of factors make people more susceptible to TB infection. For instance, TB is closely linked to overcrowding and malnutrition. At the hill-top site, a high number of features implying extrapulmonary TB infections were recorded. These occur more commonly in immunosuppressed individuals and young children, and affect, for example, bones and joints, the central nervous system (in tuberculous meningitis), and the pleura (in tuberculous pleurisy). Tuberculosis of bones and joints usually appears as a post-primary late manifestation of tuberculous reinfection after the primary tuberculosis has healed. It is most often diagnosed by specific macroscopic features, for example malformations of the vertebrae that can lead to hunchback deformity with gibbus and abscess formation. It is well known that only 3–5% of persons infected by tuberculosis develop bone tuberculosis (40% attacking the vertebral column, 25% the hip joint, 20% the knee joint). This implies that the probability of identifying such cases of tuberculosis is very low – and is further decreased by the often inadequate preservation status of the skeletons. Recent epidemiological statistics may show that 6–10% of adults and 26–35% of infected immatures develop meningitis. A well-progressed meningitis is the consequence of a hematogenuous spread of the primary complex (= early tuberculous distribution) or of a tuberculosis of an organ (e.g. the lung). According to Sandritter and Thomas [53], a tuberculosis meningitis inflammation is mainly localized in the basal cistern, typically including the inflammation of brain nerves during the early stage (see also Bostroem [54]). We identified four cases of bone and joint tuberculosis in the hill-top skeletal assemblage and one among the riverine settlers. If we postulate tuberculosis as having been endemic, especially in the hill-top site, we should be able to identify a minimum of approximately 30% of the individuals with features of meningitis (primarily at the cranial base). Surprisingly, we identified a much higher frequency. More than the half of the subadult population at the fortified rampart, namely 62.3%, and 21.7% of the riverine immatures show features at the internal cranial vault caused by meningitis/meningoencephalitis (in general) – features which may have been caused by several bacterial and fungal infections. But inspection of the cranial base showed that 51.2% of the hill-top subadults and 40% of the riverine subadults have developed alterations in the form of proliferative changes, small areas of newly built bone structures around the foramen, which is the predilection area of tuberculous meningitis. Interestingly, the adults are less affected by proliferative changes (29% show an affected internal layer of the vault and only 6.4% an affected middle cranial base, see Table 2a and Figure 3a) than are the subadults. Instead, the internal structural alterations appear as small impressions (‘pits’). Templin and Schultz [26] discussed these features as being caused by the impression of calcified tubercles. Hence they may represent a later stage of tuberculosis infection. Another extra-pulmonary location of TB infection is the pleura. Even if exudative pleurisy, induced by a late primary infection of tuberculosis, may be caused by several other diseases (rheumatic, cancerous) or by trauma, evidence from recent epidemiological examinations suggests that 25% of pleural effusions are of tuberculous genesis, increasing to 50% for persons less than 30 years of age. Surprisingly, the frequency of this feature within the two adult groups (hill-top: ca. 16%; riverine: no cases) falls below our expectation and is thus not able to support our assumption of endemic TB in these communities.

The study also attempted to address the PCR identification of the causative organism. The *M. tuberculosis* complex includes four other TB-causing mycobacteria: *M. bovis*, *M. africanum*, *M. canetti* and *M. microti*. The preliminary experiments provided further evidence of *M. tuberculosis* in three of the four selected samples that were characterized by their specific, TB-related features (1 joint TB; 3 subadults featuring endocranial alterations at the base and the

Table 3b
Unspecific stress features of males and females of the Early Mediaeval subpopulations (hill-top site = HS, valley site = VS) of Gars/Thunau, Lower Austria (prol = proliferative structures, ul = upper limbs, ll = lower limbs, CO = cribra orbitalia, O = orbital walls, PH = porotic hyperostosis, MAX = maxilla, MAN = mandible, PER = periostitis).

Location and type of alteration	HS/m (n = 56)		HS/f (n = 45)		VS/m (n = 14)		VS/f (n = 11)	
	n/N	%	n/N	%	n/N	%	n/N	%
CO	12/39	30.8	7/31	22.6	0/10	0	2/6	33.3
O/prol	5/39	12.8	1/31	3.2	1/10	10.0	0/6	0
PH	40/52	76.9	17/37	45.9	7/11	63.6	1/9	11.1
MAX/prol	8/43	18.6	4/31	12.9	3/9	33.3	2/7	28.6
MAN/prol	10/51	19.6	9/33	27.3	0/10	0	2/9	22.2
PERul	1/49	2.0	11/33	5.1	1/10	10.0	0/8	0
PERll	19/47	40.4	16/39	41.0	4/7	57.1	1/9	11.1

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vault). Despite these positive results we faced two major problems in relation to the study design. Using repetitive genomic regions is suboptimal, especially when using old samples, where the quality of DNA is expected to be bad. Only very small fragments (~100 bp) could be obtained from the available material. For future studies it will be essential to establish PCR-based markers that allow amplification of various single copy sequences of *M. tuberculosis* and related species. It is crucial to use a combination of short gene sections with optimally designed primers that amplify with high success rate and provide unambiguous information on species and even strains.

In the given context, unspecific stress features played a further important role. Cribra orbitalia and porotic hyperostosis are frequently linked with both acquired and inherited hemolytic and megaloblastic anemias, but they can also originate from vitamin deficiencies or infectious diseases. Given their prevalence and etiology, they are a key indicator for assessing past quality of life and are often associated with substandard living conditions. Most of the features investigated appear more frequently in the skeletal remains recovered at the hill-top cemetery. This applies also to the Vitamin-C-deficiency features of osseous plaques at the mandibular ramus. In contrast, the periosteal lesions, irregular new bone formations and elevations at the limb bones do not show differences between the two sub-site populations. Many events may lead to periosteal reactions (e.g. trauma, metabolic or neoplastic conditions, circulatory impairment or infectious processes), but they can also result from hemorrhagic diseases such as scurvy. Although these changes are not pathognomic, they suggest a health-disruptive process often as an outcome of environmental constraints. Hence, although we have grounds for arguing that there was an inadequate supply of vitamins, the δ^{13}C values and δ^{15}N values showed no difference neither between the sites, nor between the males and females at those sites. The populations of both settlements consumed C_3 plants (such as wheat, rye or oats); only two individuals show substantially higher δ^{13}C values (-16.3‰ and -15.8‰), which can be explained either by ingestion of a significant proportion of C_4 plants, most likely millet, or a marine component in their diet (which is rather unlikely, given Thunau's central European location). The nitrogen isotopic signatures of these two individuals lie within the same range as the other individuals and indicate the consumption of terrestrial protein. In conclusion, neither reflected the δ^{13}C and δ^{15}N values a difference between the two populations (which we believed to be linked to social status), nor did they reflect differences in diet between males and females of these populations [33].

Finally, we return to the important issue of identifying the provenience of the inhabitants of the hill-top area (valley settlement investigation is still ongoing) using strontium isotope ratios. The fact that we identified 83–89% as non-locals supports the hypothesis that the ‘central settlement’ character of the site may be related to a particular military function, with frequently changing occupation forces on this periphery of the Frankish Empire. Furthermore, due to its strategically favourable location in a border-zone between east and west, Gars/Thunau was probably linked with an increasing mercantilism and commercialism and assumed a key role as an interregional market. This development may also have promoted an increase in population size, requiring provisioning with an increased number of non-local animals. This population phenomenon, along with high mobility (and poor hygienic conditions), was an important factor in the increased transmission of infectious disease, as our documentation and analysis of skeletal alterations caused by TB in the fortified hill-top site demonstrates.

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