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Molecular study on human tuberculosis in three geographically distinct and time delineated populations from ancient Egypt

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SUMMARY

We describe the molecular identification of human tuberculosis (TB) from vertebral bone tissue samples from three different populations of ancient Egypt. The specimens were obtained from the predynastic to early dynastic necropolis of Abydos (7 individuals, *c.* 3500–2650 B.C.), from a Middle Kingdom to Second Intermediate Period tomb of the necropolis of Thebes-West (37, *c.* 2100–1550 B.C.) and from five further Theban tombs used in the New Kingdom and the Late Period (39, *c.* 1450–500 B.C.). A total of 18 cases tested positive for the presence of ancient DNA (aDNA) of the *M. tuberculosis* complex. Out of the 9 cases with typical macromorphological signs of tuberculous spondylitis, 6 were positive for mycobacterial aDNA (66·7%). Of 24 cases with non-specific pathological alterations, 5 provided a positive result (20·8%). In 50 cases of normally appearing vertebral bones 7 tested positive (14·0%). There were only minor differences in the frequencies between the three populations. These data strongly support the notion that tuberculosis was present and prevalent in ancient Egypt since very early periods of this civilization. The unexpectedly high rate of mycobacterial aDNA in normal bone samples is presumably due to a pre- to perimortal systemic spread of the bacteria and indicates a generalized infection by *M. tuberculosis*.

INTRODUCTION

The molecular detection of ancient bacterial DNA provides a novel approach to study the presence of infectious diseases in past populations. This also offers a potential new insight into the molecular evolution and the spread of those microbes. The later aspect is of particular significance for the reconstruction of the epidemiological aspects of any such disease, which provides useful information on the role of those infectious diseases during the history of mankind.

There are several studies that have clearly identified a whole variety of microbes in ancient populations, such as the *M. tuberculosis* complex [1–9], *M. leprae* [10, 11], *Plasmodium falciparum* [12–14], *Trypanosoma cruzi* [15], *Yersinia pestis* [16] and *E. coli* [17, 18]. Most of these studies were performed on a case report basis or on only small series of selected samples. The study of larger series of samples is laborious and has only been performed to a limited extent [8, 9, 19].

Most of the molecular research has been focused on the *Mycobacterium tuberculosis* complex. These bacteria are assumed to be better preserved than others due their acid-resistant lipid-rich cell wall. Previous studies have indicated that tuberculosis was present in ancient Egypt. The extent of mycobacterial infection and possible differences in its spread during the 3000-year history of the ancient Egyptian civilization, however, remains unclear at present.

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This study describes the occurrence and frequency of mycobacterial infection in different populations of ancient Egypt by the detection of *M. tuberculosis* complex by molecular methods. We extended our previous studies on the molecular identification of tuberculous mycobacteria to three distinct sample sets originating from two main necropolis of ancient Egypt, covering three time periods which have been defined by the archaeological context. Although our present study deals with only 83 individuals – and is far from the number necessary to establish any epidemiological studies on the frequency of tuberculosis – we provide some initial data on the frequency and spread of the disease. These data suggest:

(i) tuberculosis was present throughout ancient Egypt and over the archaeologically defined 3000 years of its existence,
(ii) the frequency of tuberculosis infection may have been surprisingly high in those populations and
(iii) a rough estimate of the frequency of mycobacterial infection in our study populations indicates no major differences between spatially and temporally different ancient Egyptian populations.

On the grounds of this study one may speculate on factors that may have influenced the presence and spread of this disease in ancient Egypt. Taking archaeological information into account, our data provide initial insights into the potential role of tuberculosis in periods of high and low prosperity and wealth in that ancient population.

MATERIALS AND METHODS

Study populations

Our molecular analysis was performed on osseous material that was obtained from vertebral bodies of 83 individuals from three different cemeteries from Upper Egypt. Most of the material came from skeletonized mummies, which had been damaged by grave robberies so that the skeletal elements were identifiable. All three groups were clearly separated from each other within the archaeological context.

Population 1

The first study group covered a small series of skeletons uncovered in the necropolis of Abydos, Upper Egypt, which was one of the first royal necropolis of ancient Egypt during the predynastic *c.* 3500–3050 B.C.) to early dynastic period (*c.* 3050–2650 B.C.). In this necropolis, the tomb complexes of the first kings of ancient Egypt are located. During these studies, we investigated the remains of 250 skeletonized mummies by anthropological analysis. All had been buried during the predynastic to early dynastic period (see above). Out of these, seven cases in a good state of preservation were selected for further molecular investigation. One presented with vertebral alterations strongly suggestive of tuberculosis, two revealed slight but unspecific vertebral pitting of the osseous surface and four were completely unremarkable. The two cases with non-specific vertebral alterations and two of the four normal appearing vertebras had been already examined previously [9].

Population 2

The second group came from tomb TT 196 of the necropolis of El-Asasif/Thebes-West. Adjacent to the main tomb complex of TT 196, which had been built during the Late Period of ancient Egypt (*c.* 26th dynasty), a further tomb shaft was detected that proved to have been exclusively used during the period from the Middle Kingdom (MK) to Second Intermediate Period (SIP) (*c.* 2100–1550 B.C.). Although both tomb complexes were close together, large amounts of sand and stone debris had sealed the MK shaft so that no further burials had been performed. The archaeological dating was based on the funerary goods found during the excavation, which strongly suggested only MK/SIP material. In addition, the type of mummification was clearly consistent with the embalming procedures used during that time period and differed from later practices [20]. During the anthropological investigation, a total of 183 mummified individuals were identified. From these 37 vertebral samples were selected for the molecular studies. Three samples presented typical macromorphology of tuberculous spondylitis (such as ventral destruction of the vertebral body), 10 further samples revealed non-specific alterations of the vertebral bodies with slight pitting of the osseous surface, while 24 vertebral samples were obtained from completely unremarkable skeletons. Of these samples one case with non-specific morphological alterations and two apparently normal vertebral bodies had been examined in a previous report [9].

Population 3

The third group of samples came from five tombs in the necropolis of Thebes-West that had been built

during the New Kingdom (NK) (*c*. 1550–1070 B.C.) and which had been used during the Third Intermediate Period (TIP) until the Late Period (LP) (*c*. 500 B.C.). These were the tombs TT 84, TT 85 and TT 95 (necropolis of Sheik-Abd-el-Qurna/Thebes-West) which had been constructed *c*. 1450 B.C. and TT 183 which was used since the Ramesside period (*c*. 1250 B.C., necropolis of El-Khokha/Thebes-West). These tombs had been archaeologically investigated by various Egyptologists under the leadership of the German Institute of Archaeology, Cairo, and the Institute of Egyptology of Heidelberg University. Additional material came from two tomb complexes of the nearby necropolis of Dra-Abu-el-Naga/Thebes-West. Most of these tomb complexes had been built during the NK (i.e. since *c*. 1500 B.C.) until the LP. In these tombs, a total of 620 individuals were identified, out of which 39 cases were selected for molecular studies. Five samples showed typical signs of vertebral tuberculosis, in 12 samples non-specific changes were noted in the vertebrae and 22 samples came from apparently normal vertebral bodies. The cases with typical morphological alterations, non-specific changes, and 17 of the 22 unremarkable vertebral bodies, had been examined previously [9].

DNA extraction

To eliminate contamination, all bones were first cleaned with a 0·5% sodium hypochlorite solution and then the outer surface was removed mechanically by sterile instruments. Samples were taken from the inner part of the bones. These were then used to produce a homogeneous bone powder using a mixer mill (Retsch MM200, Haan, Germany); 1 g of the pulverized material was then incubated with 2 ml of 0·5 M EDTA-solution containing proteinase K (0·25 mg/ml) at room temperature for 2 days on a rotatory mixer [21]. Following centrifugation for 15 min at 3000 ***g***, 0·5 ml of the supernatant was removed and 1 ml guanidine isothiocyanate solution and diatomaceous earth [22] were added. After incubation on a rotatory mixer for another 2 h, the diatomaceous earth was pelleted by centrifugation and washed twice with 70% ethanol and once with acetone. The DNA was eluted with 80 μl sterile water. Finally, another washing and concentration step was performed with Microcon-30 filters (Millipore, Bedford, MA). The resulting DNA (*c*. 1 μl fluid) was then diluted with sterile water to a volume of 20 μl. This was done in order to have enough template for repeated PCRs (including subsequent restriction enzyme digestion or sequencing). The final DNA concentration ranged between 20 and 40 ng/μl.

Precautions to avoiding contamination

Precautions were taken to avoid contamination during the extraction procedure and in the PCR reactions. The extraction, PCR and post-PCR analyses were all conducted in separate rooms where no studies of modern mycobacterial or human DNA have been performed. All reagents were purchased as DNAse and RNAse-free molecular biology grade chemicals and autoclaved when appropriate. No positive PCR controls were used. Disposable gloves were worn during all procedures and changed frequently. Sterile aerosol protection tips (Safeseal tips, Biozym, Hess. Oldendorf, Germany) were used to avoid cross-contamination. Two extraction blanks were always performed in the same procedure and additionally a PCR blank was included in each PCR reaction. Finally the specimens were analysed in two different institutions (Munich, Regensburg) using the same analysis procedure in parallel. The results from both sites showed no important differences.

Amplification of mycobacterial DNA

For the specific amplification of mycobacterial DNA we used a primer pair targeting a 123 base pair (bp) segment of the repetitive sequence IS6110 of the *M. tuberculosis* complex, which covers *M. tuberculosis*, *M. bovis*, *M. microti*, *M. africanum* and *M. canettii* [23, 24].

The PCR reaction mix contained 10 mM Tris–HCl (pH 8·3), 50 mM KCl, 1·5 mM $MgCl_2$, 200 μM of each deoxynucleotide trisphosphate (Amersham Pharmacia, Uppsala, Sweden), 1 μM of each primer, 0·025 U/μl AmpliTaq Gold (PE Biosystems, Foster City, CA) and 0·5 μl extracted DNA to a final volume of 20 μl. PCR-conditions were as follows: 10 min at 95 °C followed by 45 cycles of 94 °C for 1 min, 66 °C for 1 min and 72 °C for 1 min. After the final cycle another 8 min at 72 °C were added.

Amplification of human DNA

To test whether amplifiable DNA was present in the samples and to ascertain that the PCR was not inhibited, a 202 bp segment of the human β-actin gene was amplified in parallel [25]. The PCR mixture was

prepared as described above. The following amplification protocol was used: 10 min at 95 °C, 45 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, and final extension at 72 °C for 8 min.

As a further amplification control, segments of the amelogenin gene and the SRY gene which are located on the human sex chromosomes were amplified. From the SRY gene, which is solely located on the Y-chromosome, a 93 bp fragment was amplified [26]. The amplification product of the amelogenin gene is a 112 bp fragment from the Y-chromosome and a 106 bp fragment of the X-chromosome [27]. Therefore males will show two PCR products, while females give a single amplification product. The amplification protocol for the amelogenin gene was as follows: 10 min at 95 °C, 45 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 8 min. The SRY PCR was performed with the same conditions, except for an annealing temperature of 58 °C.

Restriction enzyme digestion

In several positive cases, the 123 bp PCR product of IS6110 was digested with *Hae*III [28]. For the digestion 8 μl of the PCR product was incubated with 10 U *Hae*III (Roche Diagnostics, Mannheim, Germany) for 2 h at 37 °C. This resulted in a 94 bp and a 29 bp fragment. These fragments were then identified on agarose gels as described below.

Detection of PCR and digestion products

The PCR products were separated by electrophoresis on a 4% agarose gel and visualized on a UV-screen after staining with ethidium bromide. PCR products of the amelogenin gene amplification were additionally separated on a 15% polyacrylamide gel and visualized by silver staining.

Sequence analysis of PCR products

The nucleotide sequences of the PCR products were determined by direct sequencing: After electrophoresis on a 4% low-melting-point agarose gel, the respective fragment of the PCR reaction was eluted with a purification kit (Freeze'n Squeeze, Bio-Rad, Hercules, CA). With the eluted DNA, cycle sequencing was performed with a dye terminator cycle sequencing kit (PE Biosystems). Automatic sequencing was performed on an ABI PRISM 310 Genetic Analyzer (PE Biosystems).

RESULTS

Morphological findings

The paleopathological investigation of the 83 cases revealed 9 individuals with typical morphological alterations of skeletal tuberculosis (group 1). In a further 24 cases non-specific bone changes were seen providing evidence for a pathological process, but without presenting the typical features of tuberculosis (group 2). Finally, we analysed 50 samples with normal morphology (group 3). The typical morphological characteristics of these three groups are briefly indicated as follows:

Cases typical for tuberculosis (*group 1*)

Our samples contained nine cases presenting typical morphological changes for vertebral tuberculosis. One case came from the predynastic to early dynastic necropolis of Abydos (Fig. 1*a*). It revealed a significant destruction of the ventral part of the vertebral body leading to a typical gibbus seen frequently in osseous tuberculosis. Within the vertebral body fistular defects suggested a chronic inflammatory reaction confirming the presumed diagnosis of tuberculosis spondylitis. Three additional cases with similar vertebral alterations were noted in the series of the MK burials (TT 196, El-Asasif/Thebes-West) again strongly suggesting tuberculosis spondylitis (see Fig. 1*b*). Finally, in five cases of the New Kingdom to Late Period burials from the necropolis of Thebes-West typical signs for tuberculous inflammation of vertebral bodies were noted. Three of these cases presented as mummy torsos with signs of severe spondylitis of the lumbar vertebral bodies and evidence for chronic pleuritis. The combination of the pulmonary and osseous lesions strongly supports the diagnosis of tuberculosis. These three cases have already been described extensively in a previous report [9]. In two additional cases, vertebral fusion and intraosseous lytic destruction with fistular inflammation strongly suggested chronic suppurative inflammation caused by tuberculosis (Fig. 1*c*).

Cases with non-specific skeletal changes (*group 2*)

This group contained a total of 24 samples showing various pathological bone alterations, but without presenting the typical features of tuberculosis (Fig. 1*d*).

In these cases, parts of the vertebral column showed either focal destruction of isolated vertebral bodies, pitting of the periosteal surface, anterior and/or

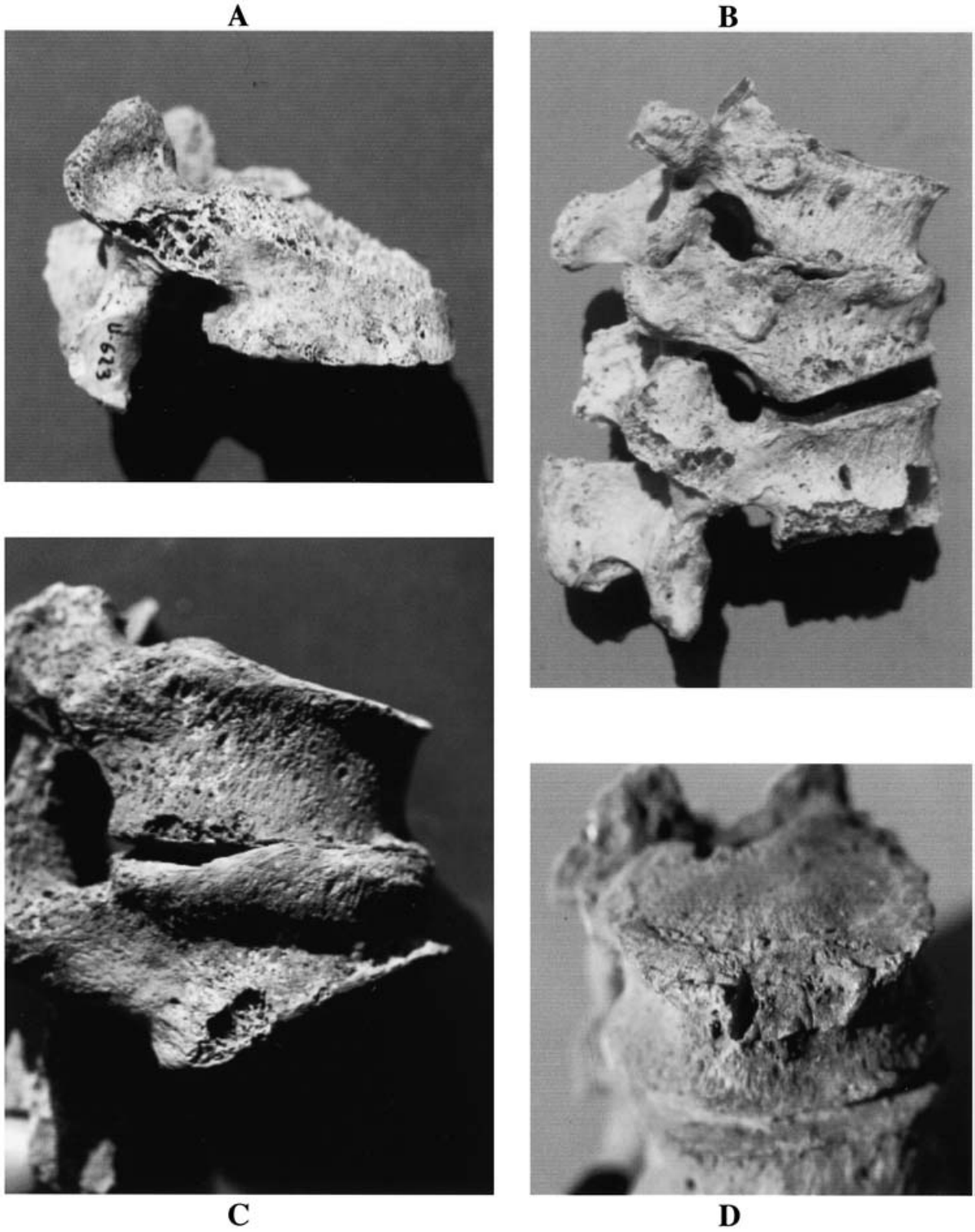

Fig. 1. Vertebral bone samples with either typical or non-specific morphological alterations of skeletal tuberculosis. The cases U-623 (*a*) from Abydos (Predynastic period), DAN93.11-33 (*b*) from the necropolis of Thebes-West (New Kingdom to Late Period) and TT196-2-25 (*c*) from Thebes-West (Middle Kingdom) presented with a typical destruction of the ventral part of the vertebral body leading to a gibbus formation often seen in osseous tuberculosis. The case TT183-29 (NK/LP) showed a focal destruction with periosteal lipping of the ventral edge of the vertebral body suggesting a non-specific inflammatory reaction (*d*).

Table 1. *aDNA results in the three different populations*

Location/ morphology	β-actin	Amelo-genin	SRY gene	*M. tuber-culosis* (IS 6110)	*M. tuber-culosis* and human DNA
Abydos (Pre-/Early Dynastic) total	3/7	2/7	0/7	2/7	2/7
Typical Tb	1/1	0/1	0/1	1/1	1/1
Non-specific	1/2	1/2	0/2	1/2	1/2
Normal	1/4	1/4	0/4	0/4	0/4
Thebes (MK) Total	18/37	12/37	9/37	9/37	6/37
Typical Tb	2/3	1/3	1/3	2/3	2/3
Non-specific	6/10	2/10	1/10	2/10	1/10
Normal	10/24	9/24	7/24	5/24	3/24
Thebes (NK/LP) Total	21/39	13/39	3/39	7/39	6/39
Typical Tb	5/5	3/5	1/5	3/5	3/5
Non-specific	7/12	4/12	1/12	2/12	2/12
Normal	9/22	6/22	1/22	2/22	1/22
Total	42/83	27/83	12/83	18/83	14/83

All data indicate the number of positive cases of all samples analysed in the group/population.

posterior fusion of vertebrae or spondylophytic lipping with partial fusion of vertebral bodies. These features were more suggestive for spondylosis or DISH than for tuberculosis. Several cases showed additional periosteal bone reaction of the ribs or the clavicle suggesting an inflammatory reaction.

Specimens without morphological evidence for tuberculosis (*group 3*)

Besides samples with evidence for inflammatory bone reaction, we analysed morphologically unremarkable bone samples originating from the same burial places as groups 1 and 2, but coming from individuals clearly different from the other groups. This material comprised samples from 49 individuals, mostly of the lumbar ($n=38$) and the lower thoracic vertebrae ($n=11$). These samples were clearly unique and different from those indicative of TB or with the non-specific changes described above.

Frequencies of morphological findings in the different populations

The numbers of samples classified into the three morphological groups were within a comparable range between the three time periods. Out of the 9 cases from the predynastic to early dynastic necropolis of Abydos 1 was in group 1, 2 in group 2 and 4 in group 3. The MK tomb of TT 196 (El-Asasif/Thebes-West) produced 3 cases with typical vertebral tuberculosis (group 1), 10 cases with non-specific alterations (group 2) and 24 samples of group 3, i.e. having normal morphology. The NK to LP material from Thebes-West (population 3) contained 5 cases with vertebral tuberculosis (group 1), in 12 cases we noted alterations of group 2 (non-specific changes) and 22 appeared normal (group 3) (Table 1).

Molecular findings

Amplification of human β*-actin DNA*

In order to exclude the presence of PCR inhibitors and to verify the existence of amplifiable DNA, we first analysed all extracted samples for a 202 bp fragment of the human β-actin gene. In 42 of the 83 cases (50·6%) human β-actin DNA could be isolated and amplified (Fig. 2). Although there were more β-actin positive cases in the 'younger' samples NK–LP: 53·8%) *vs.* the 'older' material (MK 48·6%, predynastic – early dynastic 42·8%) these differences were not statistically significantly different.

Amplification of amelogenin and SRY-DNA

As a further test for amplifiable aDNA, we identified the molecular gender of the individuals by testing all samples for both the presence of the amelogenin gene and the SRY gene. This test provided a positive result

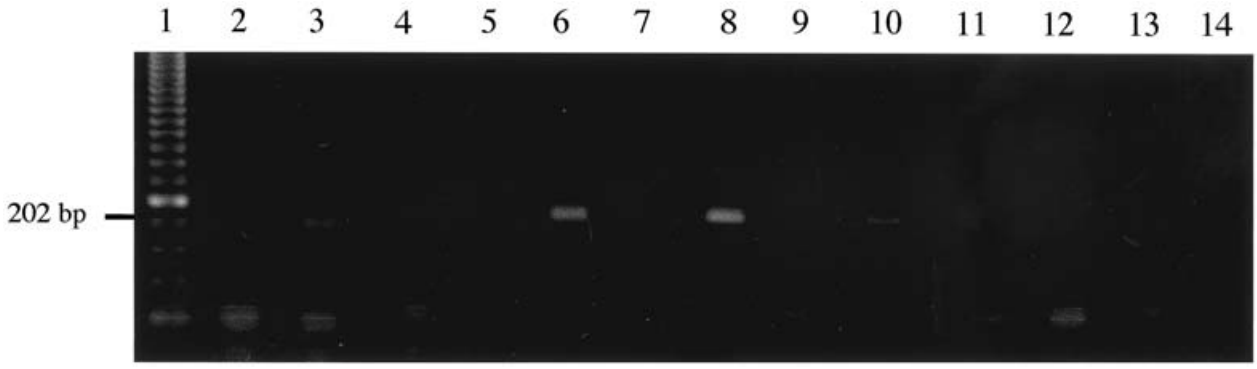

Fig. 2. Amplification of the human β-actin gene in the non-specific samples from the tomb complex TT196, Thebes-West (MK, see Table 1). Lane 1, 50-bp standard ladder; 2–11, amplification products of the samples; 12–13, extraction blank controls; 14, PCR blank control.

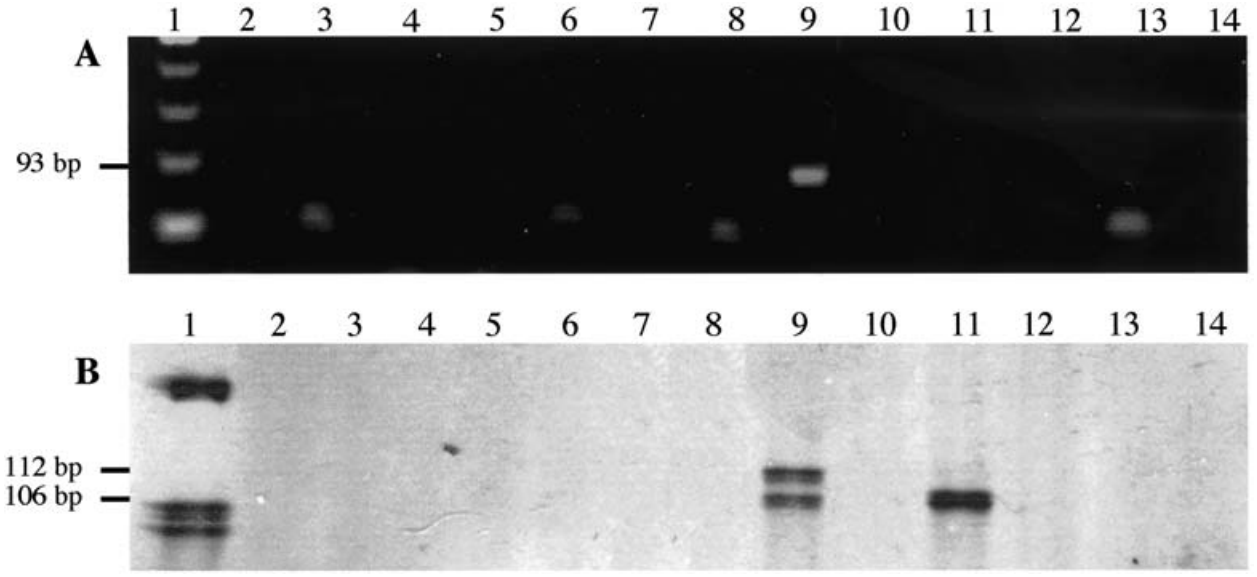

Fig. 3. Molecular sex identification of the ancient vertebral bone samples from the tomb complex TT196, Thebes-West (MK, see Table 1). (*a*) Amplification of the SRY gene. Lane 1, 50-bp standard ladder; 2–11, amplification products of the samples; 12–13, extraction blank controls; 14, PCR blank control. (*b*) Amplification of the amelogenin gene. Lane 1, 100-bp standard ladder; 2–11, amplification products of the samples; 12–13, extraction blank controls; 14, PCR blank control.

in 12 cases for SRY (Fig. 3*a*) and in 27 of the 83 cases (32·5%) for amelogenin (Fig. 3*b*). Since the SRY gene is only expressed in male individuals, this does not rule out amplifiable aDNA in the negative cases. The amelogenin and SRY gene expression was successful in eight cases that were β-actin negative.

The overall rate of samples with amplifiable aDNA was 50 of 83 (60·2%). Twenty-three cases were only positive for β-actin, 8 cases only for amelogenin and 19 cases positive for both (Tables 1, 2).

Amplification and identification of mycobacterial DNA

For the investigation of mycobacterial aDNA, we applied a primer set specifically recognizing the insertion sequence IS6110 [23] indicating the presence of mycobacteria from the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. microti*, *M. africanum* and *M. canettii*) causing tuberculosis. Of all 83 cases analysed, 18 were positive for mycobacterial DNA of the *M. tuberculosis* complex (Fig. 4*a*). Of these samples 14 had already been found positive for the presence of human aDNA while 4 specimens were positive for MTB only. All blank controls were negative.

As a further control to confirm these data, the specificity of the PCR was confirmed by digestion with the restriction enzyme *Hae*III [28]. This reaction revealed the expected 94 bp and 29 bp fragments as shown previously. We performed direct sequencing of

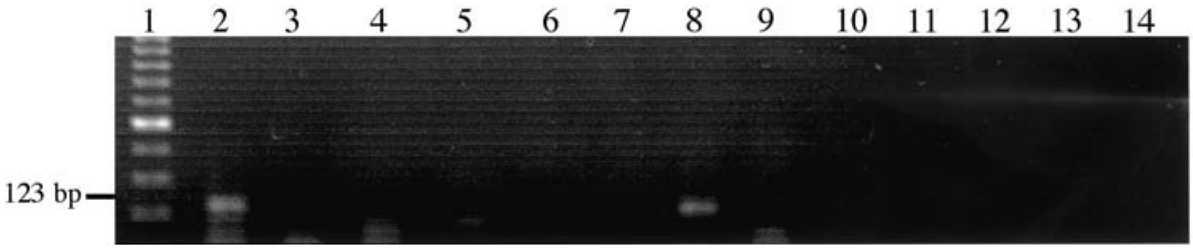

Fig. 4. PCR products for *M. tuberculosis* complex DNA (IS6110) in the ancient Egyptian samples from the tomb complex TT196, Thebes-West (MK, see Table 1). (*a*) Lane 1, 50-bp standard ladder; 2–11, amplification products of the samples; 12–13, extraction blank controls; 14, PCR blank control.

Table 2. *Percentage of aDNA/IS6110-positive cases in the three morphological groups. Group 1 contains cases with typical morphological alterations of skeletal tuberculosis, group 2 cases with non-specific alterations and group 3 samples with normal morphology*

Group	*n*	aDNA-positive cases* (%)	IS 6110-positive cases (%)
1	9	88·9	66·7
2	24	58·3	20·8
3	50	54·0	14·0
Total	83	60·2	21·7

* Number of samples positive for the β-actin and/or amelogenin/SRY gene.

amplified DNA of the IS6110 insertion sequence in several cases. This reaction provided a mycobacterial sequence confirming the presence of *M. tuberculosis*.

Frequencies of mycobacterial aDNA in the different populations and the various groups of morphological changes

We detected aDNA of the *M. tuberculosis* complex in all three populations. Since we had selected a percentage of these cases with a suggestive morphology for osseous tuberculosis, the resulting values per population did not provide adequate data on the disease frequency. A comparison of the disease frequencies between the three morphological groups provided more applicable data (Tables 1, 2).

In the morphological group suggestive of tuberculosis (group 1) most cases contained specific mycobacterial aDNA. The single case from Abydos, 2 out of 3 MK cases (El-Asasif/Thebes-West) and 3 out of 5 cases of later burials (Thebes-West, various places) tested positive. In total, about two thirds of these cases could be identified as positive at the molecular level (66·7%).

The frequency of *M. tuberculosis* in those cases with non-specific alterations (group 2) was significantly lower. We found 5/24 (20·8%) of all cases to have been infected by mycobacteria. The frequency of mycobacterial infection was not significantly different between the three populations (MK 20%, NK–LP 16·7%). One of the two respective cases from the oldest population (predynastic to early dynastic) was tested positive, the second was negative.

Most surprisingly, group 3, normally appearing bones, produced 7 positive samples with molecularly proven mycobacterial infection from 50 cases (14%). These were 5/24 cases (20·8%) of the TT196 samples (MK) and the 2/22 cases (9·1%) from the NK–LP material. None of the four Abydos cases contained mycobacterial aDNA.

DISCUSSION

Several previous studies have reported on the molecular analysis of human tuberculosis in various historic populations [1–9]. These analyses provided clear evidence that mycobacterial infections can be identified in material dating back several thousands of years. This seems to be supported by a high stability of mycobacterial DNA in ancient specimens. Our findings, that in 4 of 18 MTB positive samples no human DNA could be amplified, strongly support the presumption that the preservation of MTB DNA is better than that of human DNA. In this regard, the investigation of mycobacteria in ancient material seems to be warranted for several reasons:

(i) mycobacterial species are better preserved than other bacteria due to their lipid-rich cell wall [29]; this enhances the chances of detecting enough intact mycobacterial aDNA fragments necessary for a positive analysis;

(ii) tuberculosis is identifiable by the amplification of specific mycobacterial gene sequences that are present as multicopy genes, increasing the chance to be detected even in poorly preserved biomaterial;

(iii) tuberculosis is usually a chronic infectious disease that affects bone tissue which is the most easily available material in palaeopathology;
(iv) the disease is assumed to have had a major impact on life expectancy and the development of a population.

This is the first study not only to describe the presence of the *M. tuberculosis* complex from different time periods and populations, but also to provide an estimation of disease frequencies from different historic populations. We are fully aware that this initial study bears major limitations and that should be taken into consideration: (i) we were able to analyse only a smaller number of individuals although this is the largest group of ancient Egyptian samples analysed so far; (ii) we investigated only bone material that came from selected vertebral bodies – this strongly reduces the database, since bone tissue is only affected (on macromorphological grounds) to a very limited extent. In present day, it can be assumed that 0·3–2·5% of all tubercular infections spread to the bone [30, 31]. In addition, the microbes often affect only selected vertebral bodies so that any selection process may result in overlooking affected individuals.

When the different types of bone morphology were examined, the detection frequency of mycobacterial aDNA was significantly different, as expected from previous studies [8, 9]. Of the nine cases with macromorphological evidence for osseous tuberculosis two-thirds were positive for the TB complex (88% of these total cases contained aDNA). The group with non-specific morphological alterations contained 24 samples of which 5 were TB-positive (20·8%). The re-evaluation of our earlier results within the scope of this study did not reveal any differences in the molecular analyses. Thereby, all previously MTB positive cases could be confirmed. Again these rates are well within the expected values from previous studies [8, 9]. As shown by our previous study on Egyptian MTB infection [9], it is interesting that the group with normal bone morphology also contained several cases with specific evidence for mycobacterial infection. This supports the findings of Faerman et al. [5], who first described this phenomenon. This rate was 14·0% of those samples tested, suggesting a high rate of infections in the overall population. This high frequency of DNA positive cases without morphological alterations is difficult to interpret. It remains uncertain whether those people suffered from manifest tuberculosis or were infected during an outbreak of the disease. Knowing the pathobiology of mycobacterial infection, the latter option seems to be very unlikely. We think that those positive cases represent a systemic spread of the bacteria immediately prior to death that led to the deposition of *M. tuberculosis* DNA in the bone tissue. However, it remains unclear whether the dissemination of the tubercle bacilli occurred close to the death of an individual suffering from tuberculosis or resulted from an earlier non-reactive spread or a more common chronic miliary tuberculosis. A recent study on present-day cases with evidence for pulmonary tuberculosis suggests that even localized pulmonary TB may result in a (haematogeneous or lymphatic) spread to the adjacent vertebral bodies of the lower thoracic or lumbar spine. In a recent autopsy series, MTB DNA was detected by PCR in vertebral bone samples from those cases shown TB positive with a concomitant pulmonary or lymph node infection; none of the numerous controls tested MTB positive (Zink et al., unpublished results). One may thereby assume that those individuals suffered from organ tuberculosis with bacteraemia.

It is assumed that the lack of early diagnosis and chemotherapeutic intervention may have led to a higher rate of osseous tuberculosis in ancient times. Although the population analysed in this study is quite small and contains a disproportionate number of cases with pathological spinal abnormalities, we conclude that the rate of tuberculosis may have been considerably higher in those ancient Egyptian populations investigated, at least significantly higher than previously suggested by other authors [32].

It has been suggested that genetic factors may play a role in susceptibility to tuberculosis [33]. Family relationships and living in close proximity could have, therefore, increased the transmission within the investigated populations leading to a higher incidence of tuberculosis compared to the general ancient Egypt population. Indeed, the tomb complexes were originally built as burial places for the family members of high officials. However, the numerous shafts and chambers of the tomb complexes were extensively used for later burials. Moreover, the burial sites were heavily disturbed by grave robberies (MK and NK/LP) or former archaeological excavations (Abydos) allowing no further kinship reconstruction of the occupants.

As a further observation, the rates of mycobacterial infection were not significantly different between the three populations (in particular when the different sizes of the populations are taken into account). This

hold true when the overall data are examined: In the Abydos population 28% of cases were positive for the *M. tuberculosis* complex. The proportion of MTB MK and the NK/LP populations of Thebes-West ranged at 24% and 18%. It has to be taken into consideration that any selection of pathologically altered samples may clearly bias the results. Thus, the rate of mycobacterial aDNA in normal bone samples differs from the three groups: there was no case in the Abydos group, 5/24 cases in the Theban MK material, and 2/22 in the later Theban population. The low numbers of samples investigated make clear that those frequencies are very similar.

We conclude that there were no major differences in the disease frequencies, with or without morphological bone alteration, over a time period of approx. 2500 years. This of course holds true for the higher social classes which are represented in our material (at least for the pre-/early dynastic and NK/LP material). This also means that the disease was not only present in the different populations, but affected comparable numbers of individuals. Although our database is too small to draw any statistical conclusions, this is the first evidence for an extensive presence of tuberculosis in various ancient Egyptian populations. In addition, this indicates that the different time periods with political and economic differences did not influence the percentage of mycobacterial infection.

Recently, it has been shown that a further typing of the different members of the *M. tuberculosis* complex can be successfully applied to mycobacteria found in ancient human remains by spoligotyping [34, 35]. This technique seems to be suitable for investigating evolutionary aspects of human tuberculosis and may help to clarify the origin and transmission of the disease in humans of various timer periods and populations. In a current study we were able to characterize a few of the ancient Egypt MTB samples by spoligotyping (Zink et al., unpublished results). The preliminary findings provided a *M. tuberculosis* or *M. africanum* specific signature, but no *M. bovis* specific pattern was found. This ongoing study supports the reliability of our data and will probably allow us to compare these findings on a population level. Thereby, in the future we may be able to draw conclusions on the evolutionary pathway and spread of mycobacteria.

We have described a relatively low rate of life expectancy in the Theban population [36], which is confirmed by the Theban MK population (Graefe et al., unpublished results) and the Abydos material [37]. This reduced life expectancy may have come from a fairly considerable level of chronic infection by various pathogenic organisms, such as tuberculosis and parasitosis. Both conditions have been previously documented to be present in ancient Egypt [38, 39]. Although this study provides evidence that tuberculosis may have contributed considerably to this poor life expectancy, we feel that further studies on other ancient Egyptian populations are necessary to substantiate this notion.

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