Tuberculosis is an evolving disease: Paleoepidemiological and historical evidence

A thesis submitted by

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Abstract

Tuberculosis is a systemic infection responsible for approximately 20 to 25% of all deaths in Europe during the 18th century. The disease is spread by close contact with infected humans or animals (e.g. aerosol droplets generated through coughing, drinking infected milk).

Even when persons become infected with tuberculosis, they may not show signs or symptoms. In fact, only approximately 10% of infected individuals will develop active disease. This is related to the levels of immunity of the patient; only when immunity has been lowered sufficiently will signs and symptoms develop. Active disease rarely leads to skeletal lesions (3-5% of active cases) but can occur if the bacterium enters the bloodstream. These lesions usually affect vertebrae, but also the hip and knee.

At present, tuberculosis is re-emerging after a long decline and is developing resistance to drugs. The World Health Organization estimates that approximately one third of the world's population is infected. There have been reports of strains with resistance to multiple drugs (MDR-TB) as well as extensive drug resistance (XDR-TB).

This thesis seeks to show that tuberculosis is not just a disease, but a balance and co-evolution between host and pathogen. Previous literature has shown that when immunity is high enough, tuberculosis can have very low mortality rates. Pharmacotherapies (antibiotics plus isoniazid and PAS) are not necessary for this and conservative measures can be used instead. In order to accomplish this, this research involved several parts; each focussing on a separate time scale.

The first analysis was over a long time period (7250 BCE to 1899) and involved the meta-analysis of all reported paleopathological cases of tuberculosis in the literature

(N=531). Results showed frequency of skeletal lesions significantly decreased over time ($P<0.05$) and the distribution of skeletal lesions changed during this same period ($P<0.01$).

The second analysis involved a much shorter time period (1840-1935) of the second epidemiological transition in Switzerland. This research examined the effect of specific factors in the decline of tuberculosis during the 19th and 20th centuries. This showed the impact of improved living conditions and general health on tuberculosis mortality.

The third analysis further investigates the effects of good immunity on skeletal lesions. The Galler Collection contained skeletal remains and medical records of individuals with tuberculosis. This investigation showed how healthcare can result in "healing" of tuberculous skeletal lesions. This research also serves as a guide for paleopathological analyses.

Finally, logistic modelling of tuberculosis mortality in six countries showed reasons for the decline in certain areas. This investigation allowed the understanding of effectiveness of strategies in controlling tuberculosis. Public health measures and sanitation were found to be useful, but antibiotic use resulted in the fastest rate of decline. Milk quality control was important where bovine tuberculosis was at high levels.

The information gathered from these four investigations allows more efficient and cost effective treatment measures to be proposed for areas of the world where tuberculosis is re-emerging. These treatment strategies will also aid in the fight against strains of MDR and XDR tuberculosis.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Kara Holloway and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Kara Holloway Date

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Author Contributions

Manuscripts included in this thesis:

1. Holloway KL, Henneberg RJ, De Barros Lopes M, and Henneberg M. 2011. Evolution of human tuberculosis: A systematic review and meta-analysis of paleopathological evidence. HOMO 62(6):402-458.

2. Holloway KL, Henneberg RJ, De Barros Lopes M, Staub K, Link K, Rühli F, and Henneberg M. 2011. Secular Trends in Tuberculosis during the Second Epidemiological Transition: A Swiss Perspective (accepted in Advances in Anthropology, 17th February 2013, to be published in Vol.3 No.2 (May 2013)).

3. Holloway KL, Link K, Rühli F, and Henneberg M. 2012. Skeletal Lesions in Human Tuberculosis may sometimes heal: An aid to palaeopathological diagnoses (submitted to PLOS ONE, 26th February 2013).

4. Holloway KL, Staub K, Rühli F, and Henneberg M. 2012. Non-pharmacological treatment of tuberculosis may be successful as indicated by long-term epidemiological trends in the past (submitted to the International Journal of Epidemiology, 22nd February 2013).

Contributions for Manuscript 1: Evolution of human tuberculosis: A systematic review and meta-analysis of paleopathological evidence:

Kara Holloway wrote the first draft of the manuscript. Maciej Henneberg, Renata Henneberg and Miguel de Barros Lopes then helped to edit the draft into a final version.

Renata Henneberg provided the initial idea that was developed and conceptualised Maciej Henneberg and Kara Holloway.

Data for this research were collected by Kara Holloway. Maciej Henneberg assisted in providing contacts with additional data.

The analysis of data was completed by Kara Holloway with guidance from Maciej Henneberg, particularly in statistical tasks. The section titled: "Economic-cultural chronology" was devised by Maciej Henneberg.

Interpretations were carried out by Kara Holloway, Maciej Henneberg, Renata Henneberg and Miguel de Barros Lopes.

Contributions for Manuscript 2: Secular Trends in Tuberculosis during the Second Epidemiological Transition: A Swiss Perspective:

The first drafts of the manuscript were prepared by Kara Holloway. Maciej Henneberg, Renata Henneberg, Miguel de Barros Lopes, Kaspar Staub, Karl Link and Frank Rühli all provided comments on the draft. Molly Zuckerman edited the drafts to create the final manuscript.

The original idea for this manuscript was devised by Maciej Henneberg and Kara Holloway, using the guidelines set up by Molly Zuckerman for the Postdoctoral Fellows Conference (South Carolina Institute of Archaeology and Anthropology).

Kaspar Staub provided information on Swiss history for this manuscript.

Data collection was completed by Kara Holloway. Kaspar Staub assisted with collection of data from historical records. Karl Link aided data collection from the Galler skeletal Collection by conducting database searches and providing translations for the medical texts written in German.

Analysis of the data was completed by Kara Holloway, with guidance from Maciej Henneberg.

Interpretations were devised by Kara Holloway and Maciej Henneberg.

Contributions for Manuscript 3: Skeletal Lesions in Human Tuberculosis may sometimes heal: An aid to palaeopathological diagnoses:

Initial drafts of this manuscript were prepared by Kara Holloway. Karl Link and Frank Rühli provided comments and Maciej Henneberg made corrections.

The conceptualisation for this manuscript was completed by Kara Holloway. A trend in the data was noticed after a request from Karl Link for an abstract to send to the 9th International Congress of the German Society for Anthropology. Karl Link and Maciej Henneberg aided in the finalisation of this idea.

Data collection was completed by Kara Holloway. Karl Link provided translations and database searches, the same as for manuscript 2.

Analysis of data was performed by Kara Holloway. Maciej Henneberg provided some guidance and input into this process.

Kara Holloway provided interpretations for this data. Maciej Henneberg added comments.

Contributions for Manuscript 4: Non-pharmacological treatment of tuberculosis may be successful as indicated by long-term epidemiological trends in the past:

Preparation of the initial draft was completed by Kara Holloway. Maciej Henneberg wrote the paragraph titled: “Definition of a disease as a balance between infection and immunity” and made minor corrections. Kaspar Staub and Frank Rühli provided comments for the draft.

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Authorisation by all Coauthors:

Renata Henneberg _____

Karl Link _____

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Holloway KL, Link K, Rühli F, and Henneberg M. 2012. Surviving tuberculosis: healing of skeletal lesions during the recovery from active disease. American Association of Physical Anthropologists. Portland, Oregon: American Journal of Physical Anthropology.Suppl 54, p 165.

Holloway KL, Link K, Rühli F, and Henneberg M. 2011. Skeletal Lesions of Human Tuberculosis: Changes from the Past into the Antibiotic Era. 9th International Congress of the German Society for Anthropology.

Holloway KL, Henneberg RJ, De Barros Lopes M, Staub K, Link K, Rühli F, and Henneberg M. 2011. Tuberculosis in the Second Epidemiological transition in Switzerland. Postdoctoral Fellows Conference, South Carolina Institute of Archaeology and Anthropology. Columbia, South Carolina, United States.

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1. Evolution of human tuberculosis: A systematic review and meta-analysis of paleopathological evidence

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Context

Active tuberculosis (TB) can lead to the development of skeletal lesions. This is rare and occurs only in three to five percent of cases (Ortner 2003; Steinbock 1976). The most common skeletal lesions for TB are destruction of the anterior regions of thoracic and lumbar vertebral bodies. Neural arches and posterior spinal elements are usually unaffected. This can potentially lead to fusion of vertebrae and angular kyphosis. TB can also cause lesions on the articular surfaces of joints, particularly the hip and knee (Steinbock 1976).

The aims of this research were to investigate two hypotheses: 1) the change in frequency of tuberculous skeletal lesions over time and 2) changes in the distribution of lesions on the skeleton through time. This allowed the investigation of the coevolution of host (humans) and pathogen (*Mycobacterium tuberculosis*) over a long time period. The only method possible for this research was to collect all paleopathological cases of TB, covering as many date ranges and geographical regions possible.

There are a large number of reported paleopathological cases in the literature, however there was not a complete summary available for these cases. There are publications authored by Pálfi et al. (1999), Roberts et al. (2009) and Roberts and Buikstra (2003) bringing together large numbers of cases. However, these publications do not cover the full geographic or date ranges. Thus, this research aimed to provide such a summary and in the process show any trends in skeletal lesion manifestation through time. This research showed there was a change in frequency and distribution of skeletal lesions through time.

There were some difficulties in obtaining full text of some older references. This problem was addressed by a typical approach; contacting authors (listed stated in the acknowledgements section of this manuscript) and asking for full text of their articles.

This research is ongoing; more cases can always be added and new analyses can be performed based on the data collected. Many of the variables included in original Excel file have not been fully investigated.

Abstract

Tuberculosis is a re-emerging disease and is a major problem in both developing and developed countries today. An estimated one third of the world's population is infected and almost two million people die from the disease each year. Bone lesions occur in 3-5% of active tuberculosis cases and can be used to diagnose the disease in ancient skeletal remains. A meta-analysis was conducted on 531 palaeopathological tuberculosis cases from 221 sites (7250 BCE to 1899) on all continents for the purpose of testing two hypotheses; 1) the frequency of bone lesions does not change through time and 2) the distribution of lesions throughout the skeleton does not change over time. The frequency of bone lesions was found to significantly decrease over time ($P<0.05$). The distribution of bone lesions was found to change from mainly spinal in earlier time periods to include more cases in other regions of the skeleton (long bones, joints, hands, feet) in later time periods. This difference in distribution was evaluated using a Chi-squared test and found to be significant ($P<0.01$). These findings are an important addition to the current knowledge on the evolution of the disease and the *Mycobacterium tuberculosis*.

Introduction

Many studies have investigated tuberculosis (TB), as it is a major cause of mortality worldwide. The World Health Organization (2010) reports approximately one third of the world's population are infected and nearly 2 million die from the disease each year. The causative agent of TB is primarily *Mycobacterium tuberculosis*, a member of the family *Mycobacteriaceae*, which includes soil dwelling organisms and commensals (Al-Sarie, et al., 1996). *Mycobacterium tuberculosis* itself is labeled as a pathogen, rather than a commensal, but many individuals live with the bacteria without experiencing signs or symptoms of disease. In fact, it only causes active disease in approximately 10% of individuals, frequently in times of lowered immunity (World Health Organization, 2010).

In cases of active TB, three to five percent of individuals develop bone lesions and these can occur in any region of the body (Steinbock, 1976, Ortner, 2003). TB is usually diagnosed in skeletal remains through spinal lesions; specifically osteolytic lesions on the anterior regions of thoracic and lumbar vertebrae. Vertebral bodies are destroyed, potentially leading to angular kyphosis and fusion of vertebrae. Typically only one to four vertebrae are involved and neural arches as well as posterior elements are spared. Other diseases such as brucellosis, fungal infections, pyogenic osteomyelitis and a variety of neoplastic growths can also produce similar lesions complicating differential diagnoses (Steinbock, 1976). Other skeletal lesions are used in combination to produce "most likely" diagnoses and include lesions on the articular surfaces of the joints and on long or flat bones. The hip and knee are frequently involved; making up 15-30% and 10-20% of all non-spinal cases, respectively (Steinbock, 1976). Endocranial lesions and dactylitis can also help to diagnose TB infection, though are rather less commonly used. Two recent studies of TB in Hungarian, French and Italian samples have focused on bone lesions resulting from the disease (Giacon, 2008, Maczel,

2004). Several observations including vertebral hypervascularisation, rib periostitis, endocranial changes and periosteal new bone formation (particularly of the femur) were identified as potentially useful and may be used in future diagnostic work. Rib lesions can also occur from pulmonary TB, however these are subtle lesions, mainly appearing as regions of bone resorption or periosteal new bone formation (Roberts and Buikstra, 2003). Some studies of rib lesions in archaeological samples and skeletal collections have been conducted to investigate an association with TB (Pfeiffer, 1991, Mays, et al., 2002, Raff, et al., 2006, Santos and Roberts, 2006). However these lesions can be caused by a large number of other conditions and consequently are unreliable on their own for the diagnosis of TB. Finally, calcification of the pleura has been considered almost pathognomonic for TB as no other diseases are commonly diagnosed this way (Donoghue, et al., 1998, Pálfi, et al., 1999a, Lombardi and Caceres, 2000, Molnár and Pálfi, 1994).

Studies of DNA from modern strains of *M. tuberculosis* can yield some insight into the molecular evolution of the bacteria through time (Brosch, et al., 2002), but these do not focus specifically on the ancient organisms or the disease they caused in humans.

A large number of paleopathological studies have reported cases of TB in archaeological remains, but this does not help to give a clear image of the co-evolution of the pathogen and host through time. Rather, single and occasionally multiple cases are described, showing only that TB was present in the population. There have been attempts at bringing together the numerous publications (Roberts and Buikstra, 2003, Pálfi, et al., 1999b, Roberts, et al., 2009), but further analysis is needed in this area, for reasons such as limited geographical coverage of previous publications.

Since descriptions of palaeopathological TB cases and ancient and modern DNA studies do not provide a clear view of the co-evolution of humans and the bacterium through time, we conducted a meta-analysis of all cases available in the literature. Two null hypotheses were tested: 1) the frequency of bone lesions due to TB did not change through time and 2) the distribution of lesions throughout the skeleton did not change over time. While meta-analyses are usually rigorous with selection criteria, our study includes, as a starting point, all cases of palaeopathological cases of tuberculosis found in the literature, as this is the only information available.

Materials and Methods

A literature search was conducted using a number of online databases including Academic OneFile, Academic Search Premier, Anthropology Plus, Google Books, Google Scholar, JSTOR, PubMed, ScienceDirect, Scopus, Web of Science and WileyInterScience. Other references were also consulted such as publications by Steinbock (1976), Ortner (2003), Roberts and Buikstra (2003), Dutour et al. (2003) and Brothwell (1967). Additionally, other authors and colleagues were generous enough to mail publications from numerous sources to aid the search. Duplicate references were removed and some others were excluded because they were not considered relevant for the following reasons:

- article described signs and symptoms in living people, not skeletal signs,
- the cases of TB were non-human,
- the cases of TB were mentioned but no description was given,
- article was a review of cases already included from other sources,
- case descriptions were duplications of previously published work,
- only drug treatment for TB was described but without bone signs,

- other diseases were the primary focus of the article (TB only mentioned as an example).

All languages were included and publications were found in English, French, Spanish, Hungarian, Czech, Japanese and German. All publication dates were considered, though some earlier cases have been re-evaluated and this fact was taken into consideration. There was no restriction on publication dates and the earliest publication came from 1886 and the latest from 2010. All geographical regions and time periods were included (Appendix 1 gives the details).

The literature search is up to date as of the end of March 2011 and was conducted using the following terms: "paleopathology," "skeletal remains" and "tuberculosis" in different combinations. The reference lists of the resulting publications were then used to follow up further references. Cases were included where the most probable differential diagnosis was TB based on skeletal lesions (for spinal as well as extra-spinal lesions, Appendix 1) and/or confirmed with ancient DNA (Appendix 2). Each reference was carefully read and the cases entered into a Microsoft Excel file, allowing manipulation of the data. Field titles included reference, geographical location, grave number, date, lesion observation, age, sex and additional notes. For each gravesite, the total number of skeletons and the number of TB cases were also recorded. For convenience of statistical handling, where dates or ages were specified as a range, a single number representing the midpoint was used. Gravesite and number of individual cases were included if available.

The cases range in date from 7250 BCE to 1899. Estimated dates of gravesites and age of individuals at death were recorded as given in the literature. Very young individuals (<15 years) were excluded from the analysis because the immune system development is completed only during adolescence (Jaspan, et al., 2006) and this

decision thus allowed the comparison of individuals with similar immune status. In addition, juveniles are variably under-represented in the skeletal record (Lewis, 2000) and it is difficult to effectively account for this. Consequently, an extensive analysis of childhood TB in the past is impossible using archaeological methods.

Cases were initially grouped according to geographical region, specifically Northern Europe, New World, Asia and Mediterranean (Fig. 1). Some regions have no reports in the literature detailing lesions in palaeopathological cases of TB, including Australia, though investigations were made into the palaeopathology of Aboriginal Australian sites (Webb, 1995).

Sample heterogeneity was assessed by assigning each palaeopathological case a number and then analysing the odd and even numbers separately. Both samples gave the same results and thus the heterogeneity of samples did not affect our results.

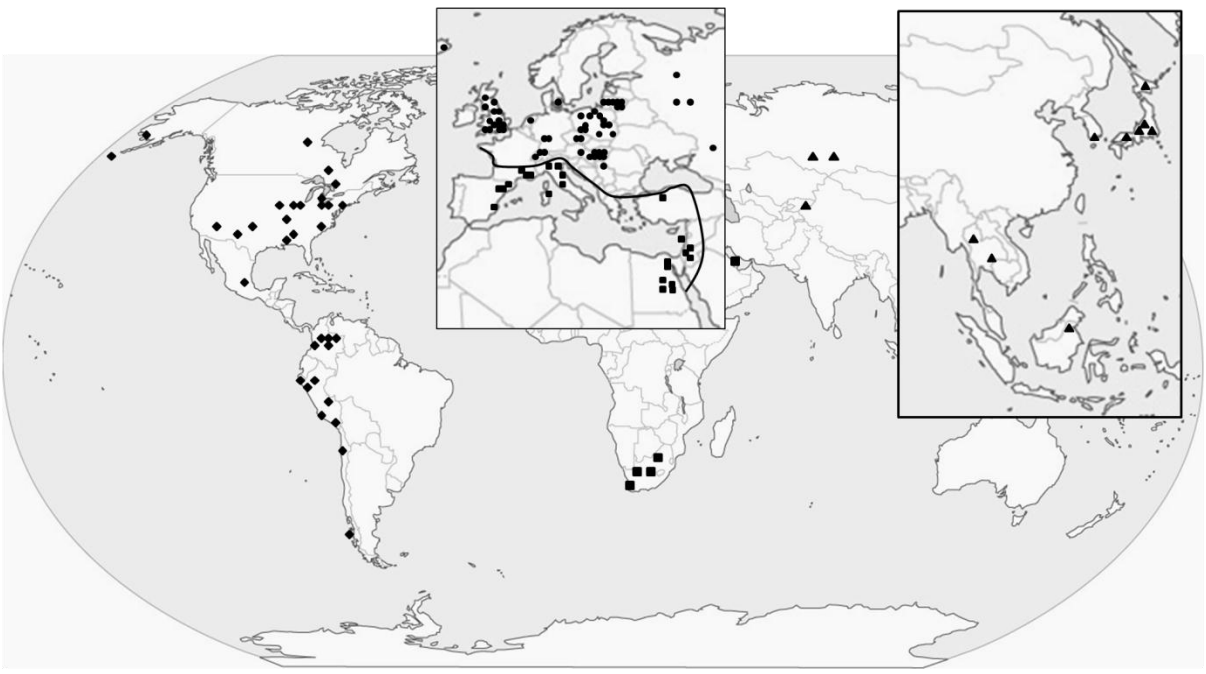

Figure 1: Map showing the location of paleopathological cases of tuberculosis included in the meta-analysis (N=531). Cases were grouped into regions: Northern Europe (circles), New World (diamonds), Asia (triangles) and Mediterranean (squares). The black squares are enlargements of the European and Asian regions.

A German clinical series from Alfer (1892) as reported in Ortner (2003), with 922 adult cases of TB detailing bone involvement was added as a modern comparison. Two other references regarding bone involvement were also consulted, including a group of British patients from a number of hospitals (Cheyne, 1891) and 160 patients from the San Francisco hospital (Rosencrantz, et al., 1941). Lesion location was marked for each individual case, based on reported bone involvement in the literature. Lesions on bones were tabulated as described (e.g. skull, spine, ribs, pelvis, hip sacro-iliac joint, shoulder, elbow and knee). Additionally, lesions of the small bones of phalanges, metacarpals, metatarsals, wrist, ankle, etc. were counted together as "distal limbs" and humerus, ulna, radius, tibia, fibula and femur were classified as "long bones". These groupings were chosen because they represent regions of the body with similar tissue combinations, functions and exposure to environmental factors thus can be expected to have similar reactions to infection and its sequelae.

Frequency of lesions

Frequency of bone lesions for each archaeological or historical site, where sample size was known (N=99), was evaluated by dividing the total number of skeletons with pathological lesions by the total number of skeletons at that site. This was not possible for all gravesites due to absence of sufficient information.

For comparative purposes, we also estimated what frequency rates would be in modern skeletal samples (Fm, N=4, based on data from 1892 (Ortner, 2003), 1909, 1911 and 1958 (Steinbock, 1976)). These were calculated as follows to be comparable with frequencies in skeletal samples:

$$F_m = \frac{M_{TB} * L}{M_t}$$

Where M_{TB} is the total TB mortality rate, L is the percentage of individuals from that population developing bone lesions as reported in Steinbock (1976) and M_t is the total mortality rate for each population. These dates were chosen because the percentage of skeletal involvement in cases of active TB was available. Only one of these is from the post-antibiotic era (streptomycin was introduced in 1946 (Wilson, 2005)), but was not significantly different from the other calculated rates and was thus included. The data used for these calculations are shown in Table 1. Note, that this calculation does not apply to archaeological/historical gravesites.

Table 1: Calculations of tuberculosis lesion frequency (F_m) in modern populations for comparison with paleopathological data. Reference refers to the publication where the percentages of bone involvement in active cases (L) were obtained. Other references were also consulted to obtain the tuberculosis mortality rate (M_{TB}) and total mortality rate (M_t) for each population (Encyclopaedia Britannica, 1911, StasoSphere, 2010, Department of Health and Human Services, 2007, Commonwealth Bureau of Census and Statistics, 1960, Vögele, 1998, United States Bureau of the Census, 1909). Note that the 1909 American Indian data use number of deaths rather than death rates.

Reference	**Geographical location**	**Date**	**Calculation** $\left(F_m = \frac{M_{TB} * L}{M_t}\right)$	**Frequency (F_m, %)**
(Ortner, 2003)	Germany	1892	=(2.41313*0.05)/20.89	0.58
(Steinbock, 1976)	London, England	1911	=(1.477*.063)/15.8	0.59
(Steinbock, 1976)	American Indians	1909	=(824*0.07)/2036	2.83
(Steinbock, 1976)	United States	1958	=(0.8*.052)/9.5	0.44

Regression analysis was performed using linear, power, logarithmic and exponential models. Average frequency of bone lesions was also calculated for each temporal/ecological group (pre-urbanised, early urbanised and early modern, see below) as well as standard error (=standard deviation/√N).

Economic-cultural chronology

To allow analysis of all geographical regions simultaneously, groups were created and defined by cultural and ecological time periods on the basis of cases available. These were termed "pre-urbanised", "early urbanised" and "early modern" and reflect living conditions at the time. The corresponding dates and descriptions for these groups in each region are presented in Table 2. Note, that early modern includes palaeopathological cases of TB up to the end of the 20th century.

Table 2: Descriptions of temporal/ecological groups and corresponding dates for each geographical region. Dates based on information gathered from: Trigger and Washburn (1996a), Trigger and Washburn (1996b), Shively and McCullough (1999), Tsutsui (2009), Keally (2009) and Roberts (1993).

Geographical Region	**Pre-urbanised**	**Early urbanised**	**Early modern**
Description	Hunter-gatherer or small agricultural settlements	Well-developed settlements, some larger cities	Numerous larger cities, beginnings of industrialization
Northern Europe	Before 800 CE	800-1500 CE	After 1500 CE
New World	Before 1500 CE	1500-1700 CE	After 1700 CE
Asia	Before 600 BCE	600 BCE-1600 CE	After 1600 CE
Mediterranean	Before 600 BCE	600 BCE-1600 CE	After 1600 CE

The regions differ in their history of population development. The distinction between Northern Europe and the Mediterranean is based on differences in the ecology and climate created by the Alpine mountain barrier. In the territory of present-day France, this division is somewhat arbitrary, but it separates areas of Mediterranean climate from those of temperate European climate. The way the three periods were defined is of necessity somewhat arbitrary, but the simple fact is that the "early modern" period occurs much later than the "pre-urbanised" in all regions. In Northern Europe the urbanisation spread in the Middle Ages while in the Mediterranean the spread of urbanisation coincided with Greek and Phoenician expansion, though earlier urban centres existed in Egypt and the Eastern Mediterranean. In the New World, with exception of the Mayan, Inca and Aztec empires, there were no large urbanised centres established before Columbus (Trigger and Washburn, 1996a, Trigger and Washburn, 1996b). However, since no cases of TB were found from excavations in these large centres, urbanisation in the New World was considered to have become widespread after European contact. With the scarcity of Asian samples, and a wide variety of Asian cultures, definition of periods is uncertain, here based roughly on changes that occurred in Japan (Shively and McCullough, 1999, Tsutsui, 2009, Keally, 2009). There are numerous specific sources of the literature concerning periodisation of historic developments in various parts of the world. Out of necessity, we had to make some simplifications. The comprehensive description of world history can be found in Roberts (1993).

Lesion distribution

Lesion distribution was tabulated for each geographical region separately, based on spinal involvement only, "other" bone involvement (i.e. extra-spinal) or both (spine

and other). Initially we used time intervals between sub-groups within each geographical region (defined as above and required for comparisons over time) determined by approximately equal numbers of cases. Lesion distribution was then also tabulated for each of the three time periods as defined above (pre-urbanised, early urbanised and early modern) separately for each geographical region. The data were then merged to give only three groups: pre-urbanised, early urbanised and early modern, including cases from all geographical locations for each time period. This merging of data was necessary to maintain statistical robusticity of analysis with the small samples of palaeopathological TB cases. Values from other and both lesions were combined and the distributions of spinal only versus other/both lesions were compared using the Chi-squared test. Contingency tables (2 x 2) contained the number of spinal cases in column one and all other cases (other and both) in column two while rows represented periods. This analysis was performed comparing each of the three time periods with each other one, both for each geographical region separately and for the merged data for all regions. Additionally, the percentage contributions for each skeletal element in all time periods (pre-urbanised, early urbanised and early modern) of the total number of TB cases were calculated, along with standard error (=standard deviation/√N).

Results

Frequency of lesions

A total of 530 paleopathological TB cases from 221 gravesites were summarised, derived from 149 references. The results of the literature review are shown in Table 3. A search of 14 databases yielded a total of 1389 references, 779 of which were removed as duplicates and 514 of which contained no relevant information. An additional 28 references were obtained by following up reference lists in other articles

and 25 were received from Professor Antónia Marcsik, Professor Jane Buikstra, Mi-Ra Kim, Sandra Lösch, Professor Eugen Strouhal, Tamás Hajdu, Tina Christensen, Dunai Józsefné, Sándor Évinger, Associate Professor Ladislava Horáčková and Professor Rimantas Jankauskas.

Frequency of lesions within each site in relation to its date, without any arbitrary divisions into neither time periods nor regions, is shown in Figure 2 and the values decrease towards more recent dates. The minimum value obtained was 0.1% from Zalavár Castle (Hungary), with one case in 744 skeletons. The maximum value was 27.3% from the Tomb of the Shroud (Jerusalem), with three cases in 11 skeletons. The equation of the power function that fitted the data best is y = 0.000d0.323 with an R value of 0.24 (P<0.02). Linear (y=0.00004x + 0.022), logarithmic (y = 0.009ln(x) - 0.038) and exponential (y = 0.012e0.000x) functions were also tested, giving R values of 0.14, 0.20 and 0.20, respectively. Consequently, the power function was chosen as it was the best model for the data.

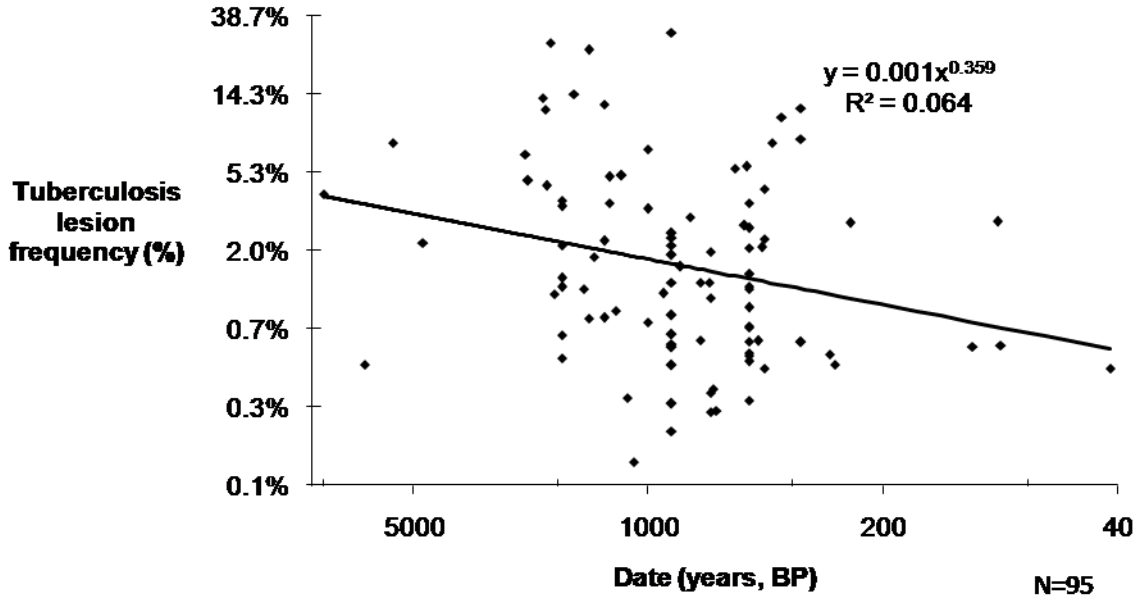

Figure 2: Frequency of tuberculosis bone lesions at each gravesite by date. Note both axes are plotted as natural logarithms.

Table 3: Details of the literature search from numerous databases completed during February 2011.

Database	Search 1	Results	Search 2	Results	Search 3	Results
Academic OneFile	tuberculosis AND palaeopathology in *all fields*	10	tuberculosis AND skeletal remains in *all fields*	7		
Academic Search Premier	tuberculosis AND palaeopathology in *all fields*	86	tuberculosis AND "skeletal remains" in *all fields*	118		
Anthropology Plus	tuberculosis AND palaeopathology in *keywords*	72	tuberculosis AND "skeletal remains" in *keywords*	17		
Google Books	tuberculosis AND palaeopathology	3440	Too many; decided to use this for finding full text of other references instead			
Google Scholar	tuberculosis AND palaeopathology	1660	Too many; decided to use this for finding full text of other references instead			
JSTOR	tuberculosis AND palaeopathology in *all fields*	176	tuberculosis AND "skeletal remains" in *all fields*	289	added "lesions" as a limiter	116
PubMed	tuberculosis AND palaeopathology in *all fields*	79	tuberculosis "skeletal remains" in *all fields*	17		
Science Direct	tuberculosis AND palaeopathology in *all fields*	143	tuberculosis AND "skeletal remains" in *all fields*	177		
Scopus	tuberculosis AND palaeopathology in *all fields*	357	tuberculosis AND palaeopathology in *article title, abstract, keywords*	126	tuberculosis "skeletal remains" in *article title,*	33

					abstract, keywords	
SpringerLink	tuberculosis AND palaeopathology in *full text*	53	tuberculosis AND "skeletal remains" in *full text*	72		
Web of Science	tuberculosis AND palaeopathology in *title*	3	tuberculosis in *abstract* AND palaeopathology in *title*	2		
WileyInter-Science	tuberculosis AND palaeopathology in *all fields*	401	tuberculosis AND "skeletal remains" in *full text*	34	tuberculosis in *abstract* AND "skeletal remains" in *full text*	48
Followed up references		28				
Received from other authors		27				
Total references	**1444**	**Duplicates**	**779**	**Not relevant**	**514**	**Final: 151**

The average frequencies of TB lesions in pre-urbanised, early urbanised and urbanised sites from all geographical regions are shown in Figure 3. Values decreased from 3.2±0.5% to 1.6±0.8% through time. The average frequency of TB lesions decreased significantly ($P<0.05$) between pre-urbanised and early modern time periods. Note that the early modern time period has practically the same absolute dates in all regions and that this period contrasts with the other two significantly.

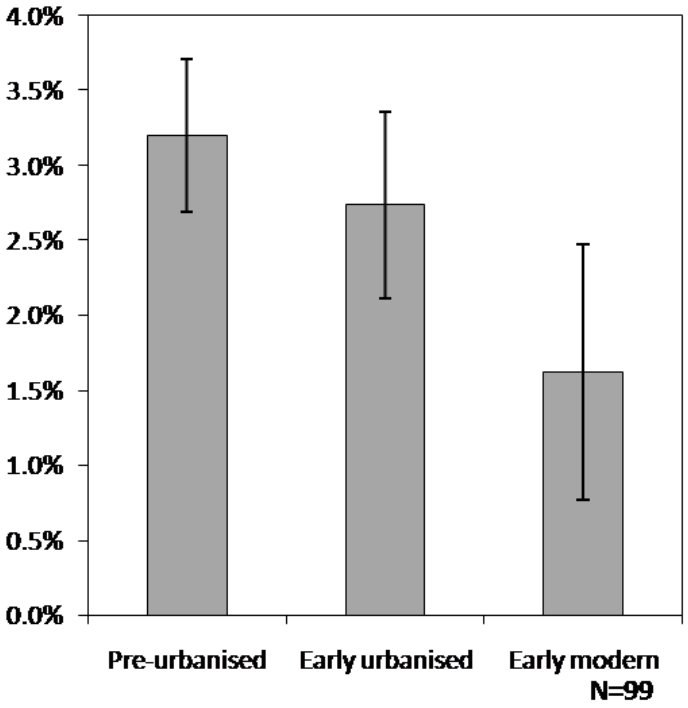

Figure 3: Average frequency of skeletal lesions of tuberculosis calculated for pre-urbanised, early urbanised and early modern samples in all geographical regions. Table 2 shows the dates for the time periods in each geographical region.

Lesion distribution

The distributions of lesions for Northern Europe, the New World, Asia and the Mediterranean with equal numbers of cases within subgroups are given in Figure 4. In Northern Europe (Fig. 4A), the percentage of cases of TB with only spinal involvement

decreases through time from 66.7% (earliest) to 44.4% (latest) with a concomitant increase in other and both lesions. A similar trend is observed for the New World (Fig. 4B), with spinal only TB lesions decreasing from 50.0% to 26.7% through time. In Asia (Fig. 4C), due to a small number of cases (N=25), the data were divided into pre-urbanised and urbanised only. Still, there is a decrease in percentage of spinal only lesions from 70.0% to 60.0% through time. Finally, the Mediterranean data (Fig. 4D) again, show the same trend. Spinal only lesions decrease from 96.0% to 33.3%, with an increase in other and both lesions.

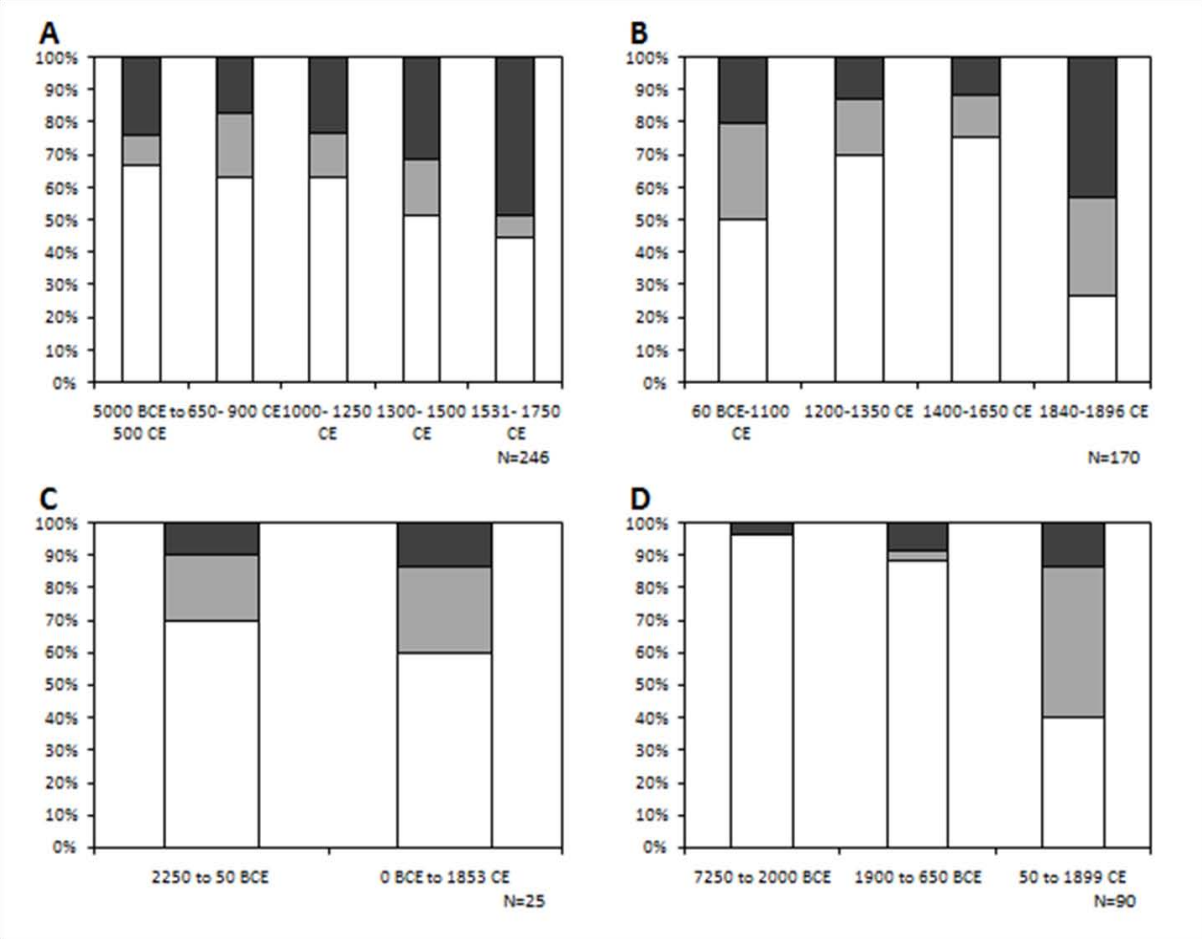

Figure 4: Lesion distribution in percentage of total palaeopathological tuberculosis cases from A) Northern Europe, B) New World, C) Asia and D) Mediterranean, divided by date into groups of approximately equal numbers of cases. Lesions are presented as spine only (unshaded), other skeletal locations (dark shading) and both (light shading).

Lesion distributions for Northern Europe, the New World, Asia and the Mediterranean with time intervals corresponding to pre-urbanised, early urbanised and early modern are presented in Figure 5. The trend of decreasing spinal only involvement over time is also apparent for these data, as observed in Figure 4. In Northern Europe, the percentage of spinal only involvement in cases from pre-urbanised samples is 65.0% and this decreases to 56.0% in early urbanised and 44.4% in early modern samples. This decrease is accompanied by an increase in other and both lesions through time. In the New World, the percentages of spinal only involvement are 62.6% for pre-urbanised, 75.6% for early urbanised and 26.7% in early modern cases. In Asia, spinal only involvement decreases from 66.7% (10 out of 15) in pre-urbanised samples to 60.0% (six out of 10) in urbanised samples. The Mediterranean data also show a similar trend, with pre-urbanised, early urbanised and early modern having 91.4%, 30.0% and 50.0% spinal only involvement, respectively. The reversal of the trend from early urbanised to early modern is likely to be a reflection of the small number of cases for the early modern period (N=8). However, these cases do show a decrease in spinal only lesions compared with the pre-urbanised period, thus continuing the previously observed trend.

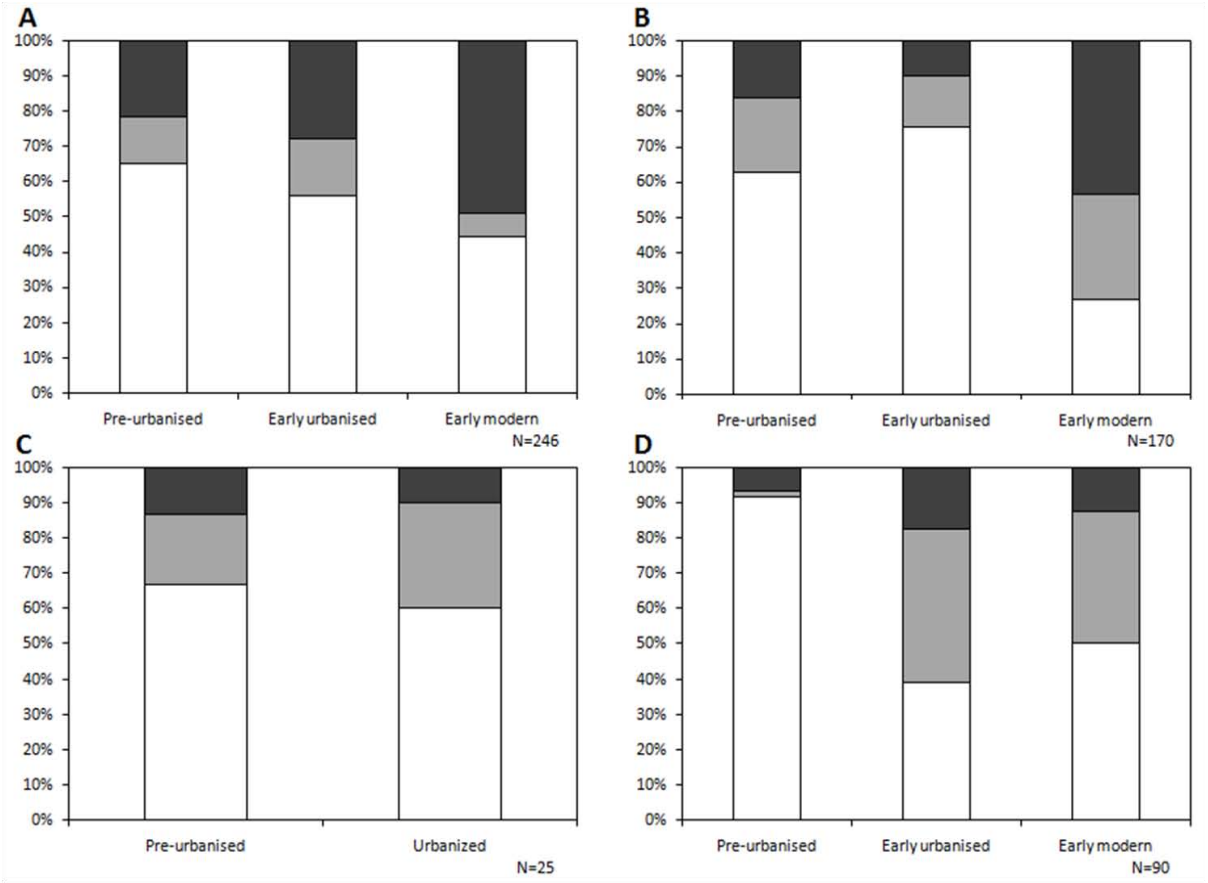

Figure 5: Lesion distribution in percentage of total palaeopathological tuberculosis cases from A) Northern Europe, B) New World, C) Asia and D) Mediterranean, in pre-urbanised, early urbanised and early modern periods. Lesions are presented as spine only (unshaded), other skeletal locations (dark shading) and both (light shading).

Chi squared tests showed for all geographical regions that a significant difference between the pre-urbanised, early urbanised and early modern samples was present (Table 4).

Table 4: Chi-squared probability values for comparisons of early urbanised and early modern time periods with pre-urbanised samples in each geographical location separately. See Table 2 for the dates of the time periods in each geographical region.

	Northern Europe	**New World**	**Mediterranean**
Pre-urbanised to early urbanised	0.026	0.086	1.8×10^{-19}
Pre-urbanised to early modern	0.004	4.7×10^{-5}	0.00002
Early urbanised to early modern	0.117	0.000	0.53

The combined data from all geographical regions grouped into pre-urbanised, early urbanised and early modern are shown in Figure 6. A clinical series from Germany (1892), detailed in (Ortner, 2003) was included as a comparison to the palaeopathological data. Once again, the trend of decreasing spinal only involvement over time is apparent. The percentage values of spinal only lesions decrease from 70.8% in pre-urbanised samples to 57.7% in early urbanised and 40.0% in early modern. Additionally, the 19th century German clinical series showed a percentage value of 6.1% for spinal only involvement. Other and both lesions become increasingly more common through the three time periods and make up a majority of the cases in the clinical series. The average age of affected individuals at death, as calculated from the literature, is also presented in Figure 6 for each time period, and does not change significantly.

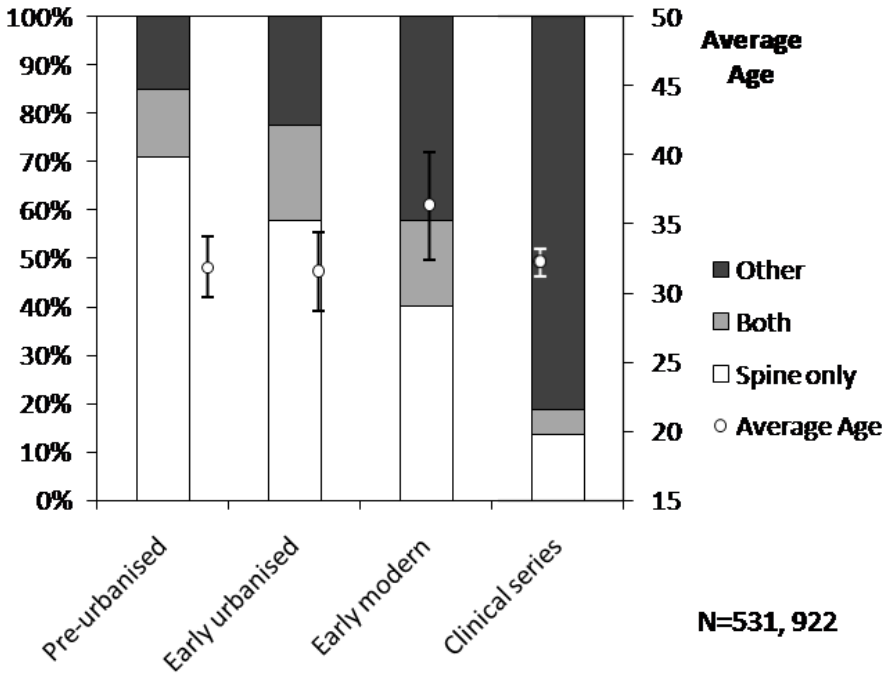

Figure 6: Lesion distribution in percentage of total palaeopathological tuberculosis cases for all geographical regions, grouped by time period (N=531). A clinical skeletal series from a 19th century setting (1892), described in Ortner (2003) is included as a modern comparison for the palaeopathological data (N=922). Calculated average age at death is also presented for each time period on the secondary ordinate.

Chi-squared analysis of the distribution of spinal only versus other/both lesions was performed. Comparisons showed the ratios of spinal only involvement in early urbanised and early modern samples were both significantly different (Chi-squared probability values of 1.5 x 10-4 and 3.3 x 10-9, respectively) from pre-urbanised samples. The early urbanised and early modern samples were also significantly different from each other (Chi-squared probability value of 0.004).

The percentage of total cases of TB for each skeletal element grouped as pre-urbanised, early urbanised and early modern is shown in Table 5. Lesions in the spine, including the thoracic and lumbar vertebrae, decrease significantly through time

(P<0.05). Spinal lesions decreased from 85.0% to 74.2% and 57.6% during the pre-urbanised, early urbanised and early modern time periods, respectively. For thoracic vertebrae, the values were 38.6%, 20.2% and 12.9%, respectively. Values for lumbar vertebrae decreased from 33.5% to 11.3% and 12.9% during the three time periods. Additionally, a significant increase (P<0.05) was observed for lesions of the ribs (28.8%, 10.3% and 34.1%, respectively) as well as long bones (12.9%, 5.2% and 24.7%). An increase was also observed for lesions of the skull, shoulder, elbow and knee, but these were not statistically significant.

Table 5: Percentage of total cases for each lesion type observed in pre-urbanised, early urbanised and early modern time periods. Values are percentage ± 2 x standard error.

Time period	Skull	Spine	Cervical vertebrae	Thoracic vertebrae	Lumbar vertebrae	Sacral vertebrae	Ribs	Pelvis
Pre-urbanised	2.6±2.0%	85.0±1.8%	5.2±2.8%	38.6±5.0%	33.5±5.0%	6.9±3.2%	28.8±5.0%	3.4±2.3%
Early urbanised	1.9±1.8%	74.2±3.0%	3.3±2.4%	20.2±4.9%	11.3±4.1%	2.3±2.1%	10.3±3.9%	3.3±2.4%
Early modern	5.9±5.0%	57.6±7.0%	2.4±3.2%	12.9±6.8%	12.9±6.8%	4.7±4.5%	34.1±8.3%	2.4±3.2%

Hip	Sacro-iliac joint	Long bones	Shoulder	Elbow	Knee	Distal limbs	**Total cases**
6.4±3.1%	2.6±2.0%	12.9±4.1%	4.3±2.6%	0.4±0.9%	1.3±1.5%	6.4±3.1%	**233**
8.9±3.7%	1.9±1.8%	5.2±3.0%	0.9±1.3%	0.9±1.3%	3.3±2.4%	2.8±2.2%	**213**
5.9±5.0%	2.4±3.2%	24.7±8.1%	3.5±3.9%	2.4±3.2%	8.2±5.7%	2.4±3.2%	**85**

Discussion

It is possible that not all cases have been included in our meta-analysis, and hence, our results and interpretation may change if further literature becomes available. However, the inclusion of several additional cases among over 500 would be unlikely to change the results substantially. There are also other aspects of the data that were not

investigated during this analysis. For example, the presence of multiple extra-spinal lesions was not addressed separately (only as other), but this may be an important next step in further investigations. One of the main problems underlying this work is the reliance on other author's interpretations of TB cases in palaeopathological remains. However, over and under-diagnosis was handled during our analyses by carefully considering the descriptions given in the literature and this was assisted by the publication of Roberts and Buikstra (2003), which highlights well known cases of TB and uses uniform diagnostic criteria.

Another important limitation of this work is the differing preservation of samples (Évinger, et al., 2010), however, although taphonomically and diagnostically important, will not affect the time trends we observed. Depending on the taphonomic conditions, various parts of a skeleton may be differentially preserved. The long bones, containing large amounts of compact tissue preserve better than mostly spongy-bone vertebrae (Willey, et al., 1997). This is also true for largely trabecular epiphyses of long bones. However, publications of paleopathological cases do not always contain clear statements regarding the state of preservation of entire skeletons, and thus differential preservation could not be adequately addressed in our analyses. Since preservation of archaeologically recovered skeletons depends on local taphonomic circumstances rather than on antiquity of a burial (Hermann and Hummel, 1994, Nawrocki, 2009), differential preservation should not bias time trends as more recent sites do not always yield more complete skeletons compared with ancient sites. Rather, the effect of incomplete preservation is to reduce the number of observable lesions in some regions of skeletons overall.

Finally, the exclusion of individuals younger than 15 years of age limits the possible interpretations from our results.

As far as we are aware, there are no other publications that studied the distribution of lesions over time and this work can make a useful contribution to the literature. There are however, three works bringing together a large number of palaeopathological cases, one of which is the volume by Roberts and Buikstra (2003) titled: "The Bioarchaeology of Tuberculosis. A Global View on a Reemerging Disease". The second is the publication edited by Pálfi, et al. (1999b), titled "Tuberculosis: Past and Present" and contains a number of important cases as well as some other related topics. The third is titled "Understanding the Impact of Infectious Disease on European Populations: Contributions from the Global History of Health Project" by Roberts and others, presented at the American Association of Physical Anthropology Symposium in 2009. This work discusses a major palaeopathological study of over 10,000 skeletons from Europe and the Mediterranean. Approximately 1% of observed spines showed lesions on thoracic or lumbar vertebrae and a similar number showed rib periostitis. Another important finding from this study is that the frequency of spinal lesions decreased through time (Fig. 6 of the publication). This is the same result as obtained in the current study.

Frequency of lesions

The frequency of lesions may be considered an estimate of the prevalence of skeletal lesions of TB among persons dying. Making such an estimate requires the assumption that the excavated skeletal sample is representative of the living population. This assumption may be incorrect if the sample is somehow selected based on sex, age or disease status. Skeletal samples used in the publications considered here have not been described as being specifically sex, age or otherwise biased. The majority of samples come from common burial arrangements. In terms of age composition, it is

theoretically possible that due to the non-zero natural increase, age composition of a skeletal sample does not reflect age structure of a living population. There are examples of skeletal samples where such a situation occurred (Henneberg and Steyn, 1994). For the majority of skeletal samples, however, it is either impossible to obtain an estimate of natural increase, or their age structures do not have clear indications of biases. Following the rule of parsimony, the stationary population model (stable death and birth rates, zero natural increase) can be applied in those situations as argued by Acsádi and Nemeskéri (1970). Validity of this approach has been theoretically debated (Buikstra, 1997, Buikstra and Konigsberg, 1985, Sattenspiel and Harpending, 1983).

Empirically, however, it turns out that assumption of stationarity holds when comparing age structures of skeletal samples derived from burial grounds and from a disaster. Henneberg and Henneberg (2002) describe a comparison between two excavated cemeteries in Pantanello (Metaponto) and Ponte di Ferro (Paestum) with skeletons representing living population of Pompeii. The remains at Pompeii represent the living population because the skeletons come from people who died in various locations in the city during the volcanic eruption in 79 CE. The comparison showed that the cemetery samples sex and age structures did not differ from that of Pompeii, meaning that typical cemeteries are representative of the living population at that site. An exception is that skeletal samples are clearly biased by underrepresentation of subadult individuals (Lewis, 2000, Henneberg, 1977). Thus, only adult individuals were considered here.

The frequency of bone lesions attributed to TB in historical samples was shown to significantly decrease through time, and although an R value of 0.24 seems low, the fact that a significant trend could be observed from palaeopathological data is surprising. The relationship is detectable and significant despite varying sample size and

quality of reporting of cases that introduce sizeable random errors. There is no reason to assume that such errors, resulting from sample sizes or reporting quality, are correlated with time. These errors depend on preservation of skeletons at various archaeological sites that primarily is a result of taphonomic conditions, not dates and observability of pathological signs. Standard errors of the moment-product correlation coefficients are also high due to the method of calculation, relying on combination of covariance with variances of correlated variables (Snedecor and Cohran, 1980), meaning, that the actual R value in our case would be much larger.

We must be careful here however, due to the osteological paradox, where only those individuals with sufficient health and immunity to survive a chronic condition will develop bone lesions (Wood, et al., 1992). Theoretically, we may not see bone lesions in cases where an individual died quickly from TB, and hence lesions represent individuals with higher general immunity. In the case of TB bone lesion frequency, a decrease over time may also indicate that the number of individuals with sufficient immunity to resist the disease for a long period during their life has decreased. This is unlikely however, since the average age at death was the same through the three time periods (Fig. 6). So, the decrease in frequency of bone lesions would be likely to reflect an increase in immunity or decrease in the virulence of the bacterium. A reason to favour a decrease in the virulence of the bacteria is that general pressures of natural selection have become relaxed through time, meaning, immunity may not be as high now as it was in earlier times (Stephan and Henneberg, 2001). However, we also need to take into account that the cause of death in many individuals with TB bone lesions may not have been TB. Thus, the observed decrease in frequency of bone lesions reflects a reduction in the number of individuals developing these lesions, though the same number may be dying of the disease in all time periods.

The substantial decrease in frequency of TB bone lesions from early urbanised to early modern times may reflect increases in health care and sanitation (Roberts and Buikstra, 2003). It was stated previously that this may have reduced host immunity, but treatment for TB, and indeed other infectious diseases, did not rely mainly on drugs until recent times. The early modern cases here represent populations where antibiotics were not yet available, and the TB treatment regimes were to improve general immunity through sanatoria. Consequently the "cure" relied upon the host immunity, supported by improved sanitation, hygiene, nutrition, rest and reduced stress levels rather than drugs (Roberts and Buikstra, 2003, Dormandy, 1999). Consequently, natural selection was still operating in these periods to select hosts with improved general immunity, supporting the theory of host co-evolution with the bacterium. The decrease in frequency of TB bone lesions in early modern times is also supported by the literature; TB rates between 19th and early 20th century were reported to be decreasing before antibiotics were developed (Smith, 1988).

Lesion distribution

The presence of lesions has changed from mainly in the spine only to other regions throughout the body over the time period investigated, and the distribution of lesions is significantly different between time periods. While many of the reported cases come from Northern Europe, the same trends were also observed in each geographical region separately (New World and Mediterranean), indicating the trend is not limited to Northern Europe. There are differences in the time when the changes in distribution occur, and these correspond well to the introduction of agriculture in each region (i.e. Mediterranean earliest, Northern Europe second, New World latest). Unfortunately, the number of cases from Asia (N=25) was not large enough to allow the formation of any

conclusions, though they did contribute to the entire picture of lesion distribution over time. Additionally, the trend observed is not simply due to differing observational methods, because it is present when periods represented only by archaeological bone samples, pre-urbanised and early urbanised periods, are compared.

A limitation of these data is that the earlier cases of TB described in the literature were diagnosed based on spinal lesions, and other lesions have only come into use in more recent times (Maczel, 2004). However, the average publication dates for cases of each lesion type, that is spinal, other and both, are not significantly different (1991.7±1.9, 1995.6±2.2 and 1992.7±2.4, respectively). This indicates that the date of publication is not influencing the results of the lesion distribution observations.

The clinical series reported by Ortner (2003) has a large number of distal limb cases (Appendix 1), which includes phalanges, metacarpals, etc. and the palaeopathological evidence is disadvantaged in this regard as small bones are not always recovered (Matheson and Brian, 2003). This may be a reason there are fewer cases of lesions outside the spine in the archaeological samples compared with the clinical series. The data retrieved from the two other references (Cheyne, 1891, Rosencrantz, et al., 1941) gave only information regarding the percentages of spinal only and other lesions, but not of "both". For the British cases the values were 23.2% and 76.8%, while for the American cases 33% and 77% were reported for spinal only and other lesions, respectively. The overall frequencies of spinal only versus other lesions in these series are similar to Ortner (2003) in that most of the cases involve lesions outside the spine. Thus, we considered the German clinical series as being representative of the modern circumstances.

Ancient DNA (aDNA) studies have been conducted on Chinese and ancient Egyptian samples from the pre-Dynastic (3000-2500 BCE) to the Late Period (500

BCE) (Zink, et al. (2005), Zink, et al. (2007) and Fusegawa, et al. (2003). These studies have helped to estimate the number of individuals infected with the bacterium in the past. The bone samples from Egypt and China with characteristic, non-specific or no bone lesions associated with TB were examined for the presence of *M. tuberculosis* DNA. The results showed the percentage of bones positive for aDNA in all three groups did not change significantly through time (see Appendix 3). Additionally, data presented at the American Association of Physical Anthropologists Conference in April 2011 (Roberts et. Al.), reported that 30% of samples from Europe and the Mediterranean covering the time period 0 CE to 2000 CE were positive for the presence of *M. tuberculosis* DNA (Appendix 3). This is in agreement with the previous two studies. These results have important implications for the current work because if more individuals were becoming infected with Mycobacteria, then we would expect to see more spinal and non-spinal cases. Since the number infected remains the same, there is no influence of this on the frequency and distribution of lesions observed.

Conclusions

Frequency of bone lesions due to tuberculosis decreased significantly through time. Lesion distribution was shown to change from mainly spinal lesions in early time periods to extra-spinal lesions (or both spinal and extra-spinal) in later time periods. We hope that these observations can lead to further research and interpretations of the co-evolution of host and pathogen through time.

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References

Acsádi, G., Nemeskéri, J., 1970. History of Human Life Span and Mortality. Akademiai Kiado, Budapest.

Al-Sarie, I., Al-Shiyab, A., El-Najjar, M., 1996. Cases of tuberculosis at 'Ain Ghazal, Jordan, Paléorient 22, 123-128.

Brosch, R., Gordon, S.V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., Garnier, T., Gutierrez, C., Hewinson, G., Kremer, K., Parsons, L.M., Pym, A.S., Samper, S., Van Soolingen, D., Cole, S.T., 2002. A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc. Natl Acad. Sci. U. S. A. 99, 3684-3689.

Brothwell, D., Sandison, A.T., 1967. Diseases in Antiquity. Charles C. Thomas, Illinois.

Buikstra, J., Konigsberg, L.W., 1985. Paleodemography: Critiques and controversies. Am. Anthropol. 87, 316-333.

Buikstra, J., 1997. Paleodemography: Context and Promise. In: Paine, R.R. (Ed.), Integrating Archaeological Demography: Multidisciplinary Approaches to Prehistoric Population. Center for Archaeological Investigations, Carbondale, Illinois, pp. 367-380.

Cheyne, W.W., 1891. Lectures on the pathology of tuberculous diseases of bones and joints. Br. Med. J. 1, 896-901.

Commonwealth Bureau of Census and Statistics, 1960. Official year book of the Commonwealth of Australia, Australian Bureau of Statistics, Canberra.

Department of Health and Human Services, 2007. U.S. Annual Death Rates per 1,000 Population, 1900–2005.

Donoghue, H.D., Spigelman, M., Zias, J., Gernaey-Child, A.M., Minnikin, D.E., 1998. Mycobacterium tuberculosis complex DNA in calcified pleura from remains 1400 years old. Letters in Applied Microbiology 27, 265-269.

Dormandy, T., 1999. The White Death: A History of Tuberculosis. The Hambledon Press, London.

Dutour, O., Ardagna, Y., Maczel, M., Signoli, M., 2003. Epidemiology of Infectious Diseases in the Past: Yersin, Koch, and the Skeletons. In: Greenblatt, C., Spigelman, M. (Eds.), Emerging Pathogens: Archaeology, Ecology and Evolution of Infectious Diseases. Oxford University Press Inc., New York, pp. 151-165.

Encyclopaedia Britannica, 1911. Tuberculosis., Accessed November 2010, http://www.1911encyclopedia.org/Tuberculosis

Évinger, S., Bernert, Z., Fóthi, E., Wolff, K., Kővári, I., Marcsik, A., Donoghue, H.D., Kiss, K.K., Hajdu, T., 2011. Paleoepidemiology of skeletal tuberculosis in ancient Transdanubia, Hungary. HOMO 62, 165-183.

Fusegawa, H., Wang, B.H., Sakurai, K., Nagasawa, K., Okauchi, M., Nagakura, K., 2003. Outbreak of tuberculosis in a 2000-year-old Chinese population, Kansenshogaku Zasshi 77, 146-149.

Giacon, M., 2008. Palaeopathology in the Osteological Collection of Anthropological Museum in Padova University: the Cases of Tuberculosis. Ph.D. dissertation, Dipartimento di Scienze Medico Diagnostiche e Terapie Speciali, University of Padova, Padova.

Henneberg, M., 1977. Proportion of dying children in paleodemographical studies: Estimation by guess or by methodical approach? Przegląd Antropologiczny 43, 105-114.

Henneberg, M., Henneberg, R.J., 2002. Reconstructing medical knowledge in ancient Pompeii from the hard evidence of bones and teeth. In: Renn, J., Castagnetti, G. (Eds.), Homo Faber: Studies on Nature, Technology, and Science at the Time of Pompeii, Ministero Per I Beni E Le Attivita Culturali, Soprintendenza Archeologica Di Pompeii, Rome, pp. 169-187.

Henneberg, M., Steyn, M., 1994. A preliminary report on the paleodemography of the K2 and Mapungubwe populations, Hum. Biol. 66, 105-120.

Hermann, B., Hummel, S., (Eds.), 1994. Ancient DNA: Recovery and Analysis of Genetic Material from Paleontological, Archaeological, Museum, Medical and Forensic Specimens. Springer-Verlag, New York.

Jaspan, H.B., Lawn, S.D., Safrit, J.T., Bekker, L.-G., 2006. The maturing immune system: implications for development and testing HIV-1 vaccines for children and adolescents. [Editorial], AIDS 20, 483-494.

Keally, C.T., 2009. Historic Archaeological Periods in Japan, Accessed 20th December 2010, http://www.t-net.ne.jp/~keally/hist.html (This is a web-page)

Lewis, M., 2000. Non-adult palaeopathology: current status and future potential. In: Cox, M., Mays, S. (Eds.), Human Osteology in Archaeology and Forensic Science. Cambridge University Press, London.

Lombardi, G.P., Caceres, U.G., 2000. Multisystemic tuberculosis in a pre-Columbian Peruvian mummy: Four diagnostic levels and a paleoepidemiological hypothesis. Chungara (Arica) 32, 55-60.

Maczel, M., 2004. On the Traces of Tuberculosis: Diagnostic Criteria of Tuberculous Affection of the Human Skeleton and the Application in Hungarian and French Anthropological Series, Department of Anthropology, University of La Mediterranee & University of Szeged, Marseille, France & Szeged, Hungary.

Matheson, C., Brian, D., 2003. The molecular taphonomy of biological molecules and biomarkers of disease. In: Greenblatt, C., Spigelman, M. (Eds.), Emerging Pathogens: Archaeology, Ecology and Evolution of Infectious Disease. Oxford University Press Inc., New York, pp. 127-142.

Mays, S., Fysh, E., Taylor, G.M., 2002. Investigation of the link between visceral surface rib lesions and tuberculosis in a Medieval skeletal series from England using ancient DNA. Am. J. Phys. Anthropol. 119, 27-36.

Molnár, E., Pálfi, G., 1994. Probable cases of skeletal infections in the 17th century anthropological series from Bácsalmás (Hungary). Acta Biologica Szegediensis 40, 117-132.

Nawrocki, S.P., 2009. Forensic taphonomy. In: Blau, S., Ubelaker, D.H. (Eds.), Handbook of Forensic Anthropology and Archaeology. Left Coast Press, Inc., California, pp. 284-294.

Ortner, D.J., 2003. Identification of Pathological Conditions in Human Skeletal Remains, second ed. Elsevier, USA.

Pálfi, G., Ardagna, Y., Molnár, E., Dutour, O., Panuel, M., Haas, C.J., Zink, A., Nerlich, A., 1999a. Coexistence of tuberculosis and ankylosing spondylitis in a 7-8th century specimen evidenced by molecular biology. In: Pálfi, G., Dutour, O., Deák, J., Hutás, I. (Eds.), Tuberculosis: Past and Present, Golden Book Publishers and Tuberculosis Foundation, Budapest/Szeged, pp. 403-409.

Pálfi, G., Dutour, O., Deák, J., Hutás, I., 1999b. Tuberculosis: Past and Present. Golden Book Publishers and Tuberculosis Foundation, Budapest/Szeged.

Pfeiffer, S., 1991. Rib lesions and New World tuberculosis. Int. J. Osteoarchaeol. 1, 191-198.

Raff, J., Cook, D.C., Kaestle, F., 2006. Tuberculosis in the New World: A study of ribs from the Schild Mississippian population, West-Central Illinois. Memorias do Instituto Oswaldo Cruz 101, 25-27.

Roberts, J., 1993. History of the World. Oxford University Press, New York.

Roberts, C.A., Buikstra, J., 2003. The Bioarchaeology of Tuberculosis: A Global View on a Reemerging Disease. University Press of Florida, Florida.

Roberts, C., Betzinger, T.K., Steckel, R.H., Larsen, C.S., Walker, P.L., Blondiaux, J., Grupe, G., Jankauskas, R., Maat, G., McGlynn, G., Papathanasiou, A., Teschler-Nicola, M., Wittwer-Backofen, U., Agnew, A., Assis, S., Bereczki, Z., Bertrand,

B., Boulter, S., Bourbou, C., Boylston, A., Brickley, M., Bürli, L., Cooper, C., Coppa, A., Coughlan, J., Drozd, A., During, E., Eng, J., Engel, F., Fox, S., Furtado, M., Gerhards, G., Haebler, K., Harkins, K., Holck, P., Holst, M., Hotz, G., Justus, H., Kaminska, K., Kjellström, A., Knüsel, C.J., Kozlowski, T., Lagia, A., Lopes, C., Manolis, S., Marcsik, A., Marques, C., Moenke, C., Moutafi, I., Niel, C., Novak, S.A., Novotny, F., Peck, J., Potiekhina, I., Rega, B., Richman, R., Rijpma, F., Rose, J., Ruiz, J., Sannen, P., Sciulli, P., Soficaru, A., Spannagl, M., Storm, R., Subirà, M.E., Swales, D., Tritsaroli, V., Tyler, E., Ulrich-Bochsler, S., Vatteoni, S., Villena-Mota, N., Wiggins, R., Williams, L.L., 2009. Understanding the Impact of Infectious Disease on European Populations: Contributions from the Global History of Health Project. Paper presented at the American Association of Physical Anthropology Symposium 2009, dates of the meeting Chicago, USA., American Journal of Physical Anthropology Supplement, pp. 222-223

Rosencrantz, E., Piscitelli, A., Bost, F.C., 1941. An analytical study of bone and joint lesions in relation to chronic pulmonary tuberculosis. J Bone Joint Surg. Am. 23, 628-638.

Santos, A.L., Roberts, C.A., 2006. Anatomy of a serial killer: Differential diagnosis of tuberculosis based on rib lesions of adult individuals from the Coimbra Identified Skeletal Collection, Portugal. Am. J. Phys. Anthropol. 130, 38-49.

Sattenspiel, L., Harpending, H.C., 1983. Stable populations and skeletal age. American Antiquity 48, 489-498.

Shively, D.H., McCullough, W.H., 1999. The Cambridge History of Japan: Heian Japan. Cambridge University Press, Cambridge.

Smith, F.B., 1988. The Retreat of Tuberculosis 1850-1950. Croom Helm Ltd., New York.

Snedecor, G.W., Cohran, W.G., 1980. Statistical Methods, seventh ed. Iowa State University Press, Ames, IO.

StasoSphere, 2010. Effect Of Sanitary Improvements On Death-Rate., Accessed November 2010, http://chestofbooks.com/architecture/House-Construction/Effect-Of-Sanitary-Improvements-On-Death-Rate.html

Steinbock, R.T., 1976. Paleopathological diagnosis and interpretation: Bone diseases in ancient human populations, Charles C Thomas, Springfield, Illinois, USA.

Stephan, C.N., Henneberg, M., 2001. Medicine may be reducing the human capacity to survive, Medical Hypotheses 57, 633-637.

Trigger, B.G., Washburn, W.E., 1996a. The Cambridge History of the Native Peoples of the Americas: North America, Volume 1, Cambridge University Press, Cambridge.

Trigger, B.G., Washburn, W.E., 1996b. The Cambridge History of the Native Peoples of the Americas: South America, vol. 3. Cambridge University Press, Cambridge.

Tsutsui, W.M., 2009. A Companion to Japanese History, Wiley Blackwell, Chichester, UK.

United States Bureau of the Census, 1909. Mortality statistics, Government Printing Office, Washington.

Vögele, J., 1998. Urban Mortality Change in England and Germany, 1870-1913. Liverpool University Press, Liverpool.

Webb, S., 1995. Palaeopathology of Aboriginal Australians. Health and Disease across a Hunter-Gatherer Continent. Cambridge University Press, Cambridge.

Willey, P., Galloway, A., Snyder, L., 1997. Bone mineral density and survival of elements and element portions in the bones of the Crow Creek massacre victims. Am. J. Phys. Anthropol. 104, 513-528.

Wilson, L.G., 2005. Commentary: Medicine, population, and tuberculosis. Int. J. Epidemiol. 34, 521-524.

Wood, J.W., Milner, G.R., Harpending, H.C., Weiss, K.M., Cohen, M.N., Eisenberg, L.E., Hutchinson, D.L., Jankauskas, R., Česnys, G., Katzenberg, M.A., Lukacs, J.R., McGrath, J.W., Roth, E.A., Ubelaker, D.H., Wilkinson, R.G., 1992. The osteological paradox: Problems of inferring prehistoric health from skeletal samples [and Comments and Reply], Curr. Anthropol. 33, 343-370.

World Health Organization, 2010. WHO | Tuberculosis. http://www.who.int/topics/tuberculosis/en/

Zink, A.R., Grabner, W., Nerlich, A.G., 2005. Molecular identification of human tuberculosis in recent and historic bone tissue samples: The role of molecular techniques for the study of historic tuberculosis. Am. J. Phys. Anthropol. 126, 32-47.

Zink, A.R., Molnár, E., Motamedi, N., Pálfi, G., Marcsik, A., Nerlich, A.G., 2007. Molecular history of tuberculosis from ancient mummies and skeletons, Int. J. Osteoarchaeol. 17, 380-391.

2. Secular Trends in Tuberculosis during the Second Epidemiological Transition: A Swiss Perspective

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Context

The second epidemiologic transition is defined by Omran (1983) as "the age of receding pandemics." In this transition, mortality decreases as life expectancy increases, resulting in population growth. The major causes of death also change. Prior to the transition, infectious diseases are responsible for the majority of deaths. Following the transition, degenerative diseases such as cancer and heart disease become more prominent.

The Postdoctoral Fellows Conference, South Carolina Institute of Archaeology and Anthropology conference (2011), focused on the second epidemiological transition, provided an opportunity to explore the effects of the second epidemiological transition on the mortality rate of tuberculosis (TB). This involved an investigation of Swiss TB mortality data for Canton Zürich and the city of Zürich. The research focussed on the factors responsible for decline in TB before pharmacotherapies were introduced; specifically how an increase in general immunity affected the mortality rate. Specific factors could be examined, including population density, urban vs rural setting, sanitation and Public Health Laws. This investigation helped to provide an indication of how many people had sufficient immunity to prevent deadly, active TB and consequently, the effectiveness of conservative measures (e.g. sanitation, diet) on mortality rates.

As far as we are aware, there are no specific publications on the second epidemiological transition in Switzerland. There are many publications for other countries such as England and Wales (Nathanson 2007), Japan (Johnston 1995), France (Preston and Walle 1978), United States (Condran and Cheney 1982; Doege 1965), Brazil (Antunes and Waldman 1999), The Netherlands (Wolleswinkel-van den Bosch et al. 1997) and Australia (Lewis et al. 1998). Switzerland poses an interesting research

topic because of some unique characteristics including (but not limited to) neutrality during wars, mountainous terrain, positioning in Europe, lack of natural resources, multiple national languages and governmental system (Steinberg 1996).

This research led to the first study of the second epidemiological transition in Switzerland (specifically for Canton Zürich and Zürich city). This manuscript also highlights the importance of the Galler skeletal Collection and will hopefully encourage more research using the specimens stored within. This research showed the effects of public health and sanitation on the decline of TB (as well as other infections) in Switzerland during this time, indicating that conservative measures are effective in reducing mortality from the disease.

This research could be continued to a more in-depth study of specific areas in Switzerland, since the country has a mix of urban and rural areas. The data presented here included Canton Zürich as well as Zürich city. There was a noticeable difference between the city and the canton. It would be interesting to explore this further. There are also geographical differences between areas around Switzerland; some have mountainous terrain, others do not. Presently, this research has already led to the development of the final manuscript in this thesis (logistic graphing).

Abstract

The second epidemiologic transition is defined as "the age of receding pandemics", wherein mortality declines, life expectancy increases, and population growth occurs. The major causes of death also shifted from predominantly acute infectious diseases to degenerative and "man-made" diseases (Omran, 1983). The aim of this study was to determine the timing of the transition in Zürich (Switzerland) and to investigate patterns of tuberculosis mortality during this period. This is one of the first studies to specifically investigate the timing of the second transition in Zürich, Switzerland. The data sources for this study were Swiss records of mortality from the Staatsarchiv (Canton Archives), Stadtarchiv (City Archives) and a published volume of State Statistics (Historische Statistik der Schweiz). The changes in mortality through time were addressed for all causes of death in the city of Zürich for the years 1893 to 1933 that is, the time including the second epidemiological transition. After 1933 the structure of the mortality data collection changed as the responsibility was transferred away from the canton archives. Mortality from tuberculosis was then examined in greater detail and compared with changes in living standards as well as population density occurring at the time.

Introduction

An Overview of Tuberculosis

Tuberculosis is an ancient disease and has been one of the biggest causes of death among societies throughout history (Kaufmann & Britton, 2008). Tuberculosis occurs in individuals infected with a bacterium called Mycobacterium tuberculosis and who cannot control the organism effectively due to a lowered immunity (North & Jung, 2004). Tuberculosis is usually transmitted by inhalation of aerosols generated primarily by coughing (Cole, Eisenach, McMurray, & Jacobs Jr, 2005). However, ingestion of contaminated meat and dairy products is a route of infection for the related bacterium, Mycobacterium bovis, which predominantly infects cattle but can cause infection in humans (Waddington, 2004; Wilbur, Farnbach, Knudson, & Buikstra, 2008).

Mycobacterium tuberculosis does not always cause disease; in fact only approximately 10% of infected individuals suffer pathological signs and symptoms (Wilbur et al., 2008). A patient can even be asymptomatic and thus appear to be "cured" for long periods of time, yet may show signs and symptoms later in life. Since M. tuberculosis spreads mostly through the respiratory tract, the common pathological signs and symptoms include coughing, difficulty breathing, bloody sputum, weakness, lethargy, loss of appetite and weight, night sweats, pallor and chest pain (Dormandy, 1999; Wilbur et al., 2009). Tuberculosis can affect any part of the body and with time, the bacteria can disseminate from soft tissues of the lungs to the other parts of the body, including the bones (Wilbur et al., 2008).

In Europe, tuberculosis was likely responsible for 20% to 25% of all deaths during the 1500s (Stead, 2001). There are difficulties with this estimate, however, because relevant historical records can be absent, or incomplete and ambiguous on cause of death in the pre-modern era. For example, in many countries, physicians were

hesitant to diagnose tuberculosis because of the social implications for the patient and instead recorded the cause of death as some other unspecified pulmonary disease (Johnston, 1995). The term "tuberculosis" also did not become widely used until the 1830s (Wilbur et al., 2008). A number of other names were given to pulmonary conditions presenting tuberculosis-like signs and symptoms including "consumption", "phthisis" and "the white plague", while diseases of other organs caused by M. tuberculosis were referred to as tabes mesenterica and "scrofula" or "King's evil" (Herzog, 1998; Miller & Thompson, 1992; S. Newsom, 2006; Smith, 1988). In addition, disease diagnosis prior to the 19th century did not have a high degree of certainty, and new systems of disease classification were introduced in the early 20th century (Dormandy, 1999; Rucker & Kearny, 1913). Improving accuracy in diagnostics and shifts in disease classification can cause problems in interpretation of the mortality and morbidity of tuberculosis through time as disease categories associated with tuberculosis can also include several other conditions. For example, "phthisis" may also include other pulmonary diseases with symptoms similar to pulmonary tuberculosis. However, the disease now known as tuberculosis has been reported in some ancient literature such as a Babylonian text written by the monarch Hammurabi between 1948 and 1905 BCE (Herzog, 1998; Kaufmann & Britton, 2008; S. Newsom, 2006; Steinberg, 1996). We investigated patterns in tuberculosis during the 19th and 20th centuries, many years after it was first described in these ancient writings. Thus, while some diagnoses are likely to be incorrect, the majority will not be. We considered the terms "tuberculosis", "consumption", "phthisis", "scrofula" and "King's Evil" as evidence of tuberculosis since these were the most commonly used terms during the time period under investigation (Burke, 2011).

Switzerland – Public Health, Demography and Economy

General Overview of Switzerland

Switzerland is a central European country comprised of 26 regions called "cantons". These cantons differ in size, population, language, geographical features, and extent of urbanization. The borders of Switzerland are irregular because they represent negotiations and agreements in the past (Steinberg, 1996). For example, the Canton of Basel was split into two half-cantons (Basel-Stadt and Basel-Land) after a disagreement between urban and rural areas in 1830 (Bouvier, Craig, Gossman, & Schorske, 1994; Steinberg, 1996). The Swiss government has been stable since 1848 (Butler, Pender, & Charnley, 2000; Steinberg, 1996), but each canton has its own constitution, executive, legislative and judiciary, laws and practices, flag, and coat of arms as well as a separate Parliament (Steinberg, 1996). Multiple religions are accepted in Switzerland and in the past the population was approximately equally divided between Protestant and Catholic. The country has been neutral since 1815, but still maintains an army (Remak, 1993). The country's economy, while highly specific (banks, watches, cheese, and chocolates), has been very successful. This success has been partly due to dependence on foreign workers, which reduces labor costs. These workers came from a variety of countries including France, Poland, Germany and Italy (Bouvier et al., 1994).

A Short History of Switzerland

At the end of the 1700s, Switzerland had no central government, no common treasury, troops, currency, judiciary or mark of sovereignty despite the long history of Confederation dating back to the 13th century (Bouvier et al., 1994; Remak, 1993). At the time, each canton was governed separately, though there was a larger Parliament for the country, which consisted of representatives from each canton. However, in 1798

major changes occurred when Napoleon invaded Switzerland from France (Remak, 1993). Following the invasion, Napoleon converted Switzerland into the Helvetic Republic, which united and centralized the cantons. Napoleon also granted freedom of speech, movement and religion to the country, and introduced a common currency, law code, and postage system (Remak, 1993). However, due to dissatisfaction with some of these changes among the populace, especially the loss of traditional rights of the individual cantons, Napoleon altered the Helvetic Republic with "The Act of Remediation" in 1803, which did not return the cantons to Swiss control but did reintroduce some traditional rights. Napoleon lost control of Switzerland in 1814 and in 1815 a Federal Treaty was signed. This reverted the country to pre-Napoleonic policies and provided regulations for political relationships between the cantons (Bouvier et al., 1994; Remak, 1993; Steinberg, 1996). France was also required to pay compensation for damages incurred during Napoleon's military campaigns, which supported Switzerland's economic development. The Treaty also introduced the concept of neutrality. While the Swiss citizenry was supportive of this, they did not support the Treaty's centralization of the country (Remak, 1993).

In 1831, the 1815 Treaty was effectively rejected (Steinberg, 1996). Religious schisms also arose, driven in part by issues of economic inequality: more urban, prosperous cantons were gradually converting to Protestantism while the poorer and rural cantons remained Catholic (Remak, 1993). This religious division had progressed slowly throughout the 1830's as Liberalism became increasingly popular in Switzerland and other neighboring European countries. This led to religion-based alliances between the Catholic cantons, and widespread unrest, with some unsuccessful attempts made by protestant Radical groups to overthrow the government in 1844 and 1845. In 1847, a Civil War erupted when seven Catholic cantons tried to establish a separate alliance, though it ended in less than a month with few casualties (Butler et al., 2000; Remak,

1993; Steinberg, 1996). After the war, unification between the cantons became favored, precluding the revolutionary uprisings, which plagued much of Europe, and a new constitution was drafted in 1848, which remains in use today. This new Constitution resolved the longstanding conflict between national centralization and traditional canton-based freedoms (Steinberg, 1996), by forbidding alliances between the cantons. It created a "bottom-up" system that allowed citizens to vote on all decisions by the government. Thus power rests with the people rather than a central authoritative group (Bouvier et al., 1994).

Industrialization and the Economy of Switzerland

Switzerland was one of the earliest industrialized countries in Europe, on a par with the United Kingdom, the United States, and France (Butler et al., 2000; Steinberg, 1996). By 1780, the entire region of eastern Switzerland was largely industrialized; producing exports such as milk products, silk textiles, cotton and mechanical parts (e.g. watches) (Steinberg, 1996). From 1880 to 1950 only the UK had a higher gross national product per head. However, several features distinguished industrialization in Switzerland from that experienced by other early industrializers: a slow rate of urbanization, high levels of economic specialization (e.g. high quality mechanical parts and textiles, see below), slow spread of railways, a strong dependence upon foreign labor, limited geochemical natural resources, such as coal, a high rate of international investment, a geographical concentration of economic activities in micro-units, and a very high level of industrialization (Butler et al., 2000; Steinberg, 1996). In fact, by 1850, Switzerland ranked fourth in the level of industrialization behind the United Kingdom, Belgium and the United States (Siegenthaler, 1972). Railway systems developed quickly in the mid 19th century in other early industrializers, but lagged in

Switzerland, even in comparison to other aspects of economic growth. This was due to difficulty with the mountainous terrain, the absence of coal (industry and development was fueled largely with hydropower) the small size of Swiss cities, and lack of a strong, central government. Mountainous terrain was also an overall obstacle to economic development in Switzerland: 25.5% of the land is unproductive (e.g. High Alps), 30.3% is "forested", and only 38.3% is available for agriculture (Steinberg, 1996). Only 5.8% is considered surface area suitable for habitation and infrastructure. In a small country, this amounts to a very small area.

In complement to industrialization, Switzerland also maintained a vigorous agricultural economy. This economy was focused on specialized milk products, such as cheese and chocolate, which, together with tourism, watch making, and textile production, provided consistent income for rural areas throughout the 19th century. While the country maintained a high rate of importation for cereals, exports, particularly of luxury items, formed a critical component of the economy; only Belgium had a higher export rate. Switzerland's overwhelming economic focus on export and luxury items, like watches and embroidery, was due to the nation's dearth of raw materials, and high transport costs (Butler et al., 2000; Siegenthaler, 1972; Steinberg, 1996). Little economic centralization occurred; Swiss industrialists and canton governments were not supportive of large scale production and industrialization or the construction of factories (Steinberg, 1996). Instead, machines were installed in worker's home, allowing a continuation of cottage industry and piece production (Bouvier et al., 1994; Butler et al., 2000). This practice resulted in a much lower urban population density than was found in 19th and 20th century Britain or Germany (Steinberg, 1996). While exportation was expensive, Swiss goods remained competitive because of low labor costs: wages were 15% lower and hours 15% longer when compared with Germany (Steinberg, 1996). This advantage did not overcome all of Switzerland's disadvantages, however,

translating into a heavy emphasis on economic specialization and high levels of skilled labor, high product quality, and involvement in international trade, open markets, and optimization of trade conditions (Butler et al., 2000).

Switzerland employed child labor throughout the 19th century, but also maintained a system of compulsory education. Children participated in industrial activities within the home, and thus were also able to attend school. Education of children between the ages of 6 and 16, both boys and girls, became compulsory in the 16th century (Bouvier et al., 1994; Steinberg, 1996). Economic assistance was provided to parents who could not afford schooling for their children (Steinberg, 1996). Much of Switzerland's economic success has been attributed to this emphasis on high quality, accessible education even in the face of rapid economic growth (Butler et al., 2000).

Living Conditions in Switzerland

Switzerland maintained a uniformly high standard of living in rural and urban areas during industrialization in comparison to other early industrializers. This was largely due to the use of white coal (hydropower) brought into the city from rural areas (rather than black) which produced less pollution, and also to low urban population density (Schoch, Staub, & Pfister, 2011; Steinberg, 1996). For instance, in 1900, only 6% of Swiss citizens lived in towns with a population of over 10,000. By 1910 this had only increased to 25%. In comparison, Great Britain had reached 25% by the 1840s. Zürich had a population of 28,000 during the late 1800s, though in-migration and incorporation of surrounding regions into Zürich due to economic difficulties in 1893 pushed this number up to 100,000 (Steinberg, 1996).

Economic hardships also affected Switzerland in the 19th century. Despite intensive agriculture, there were periodic food shortages in the 1810s, particularly in

urban areas and this was associated with the internal conflict and fall of Napoleon's Empire in 1814 (Bouvier et al., 1994; Steinberg, 1996). Later, in 1845, Switzerland also experienced an epidemic of potato blight, which precipitated widespread poverty, increased levels of child labor, and famines during the 1850s (Butler et al., 2000). However, dietary and economic conditions improved during the 1880's, with increased consumption of animal protein as well as cheap grain (Schoch et al., 2011). With this influx of grain from cheap imports, local farmers decided to focus on milk products instead. Around this time (1870 to 1912), the public healthcare system was established and public sanitation (such as the introduction of sewerage systems, waste removal and public health education), immunization as well as housing/working conditions were further improved (Schoch et al., 2011). The public health system may have addressed factors such as water pollution, poor quality foods in the marketplace, disposal of animal waste from slaughterhouses, cemeteries, problems with leaky sewers and septic tanks, street cleaning, housing with inadequate ventilation or sunlight and harmful chemicals or working conditions in industry (Bourdelais, 2006). Major investments in the urban water supply and sewerage systems were completed during the last third of the 19th century in Switzerland (Condrau & Tanner, 2000). These methods were more effective in urban areas than in rural areas; likely because the former have a higher population density and these measures could be more effectively implemented in cities than in smaller, country towns. However, economic difficulties associated with a rise in the price of wheat and flour products due to poor harvests during 1880-1888 caused many farmers to become unemployed and forced many people to migrate from rural areas to cities in search of a new occupation (Graber, 1926; Schoch et al., 2011). However, while bread prices were rising substantially in other countries (especially in Europe) the Swiss Government established an association called the "Wheat Administration" on the 9th of January 1915 (Graber, 1926). The task of this association

was to moderate the prices of wheat products in Switzerland, to control imports of these products into the country, maintain reserve stocks of cereals and to encourage the cultivation of home-grown wheat. Since this administration was in control of purchasing wheat, when prices increased during the 1880's, it became difficult to afford Swiss-grown wheat. The Wheat Administration was very successful during WWI and consequently, bread prices in Switzerland were tolerable for the consumer, unlike in other countries around the world.

The conflicts and economic disturbances that ravaged much of Europe through the 19th and 20th centuries had a moderate effect upon Switzerland's economy and living standards. Switzerland did not participate in WWI but did experience minor economic declines in part because the country maintained a standing army during the period. Numerous refugees from Russia, France and Germany were also admitted during the 1910s, resulting in additional economic declines because these individuals required housing and subsistence, thus reducing the amount of land and food available (Steinberg, 1996). Even during WWI, the standard of living in Switzerland did not decline, largely because of governmental attempts to control and prevent malnutrition; government subsidies ensured that milk products were highly accessible and a school lunch policy was instituted (Schoch et al., 2011). In addition, the Government also provided sufficient wheat for bread products through direct cost to the country during WWI (Graber, 1926). During the 1930s, Swiss manufacturing (watches and textiles) was severely affected by the Great Depression and in some areas, entire industries collapsed. Changes in currency exchange rates also severely affected Swiss profits and in north-eastern Switzerland, many towns relying upon single industries, became economically vulnerable (Steinberg, 1996).

The City of Zürich – An Overview of Sanitation and Hygiene

Zürich is a very wealthy city located in northeastern Switzerland, within the canton of Zürich. Economic growth associated with industrialization began in approximately 1827, and became focused on cotton textiles around 1857. Industrialization affected the city in several ways including an increase in population density, decrease in the level of sanitation and beginning of social stratification. However, these changes occurred to a lesser extent in Switzerland compared to other countries (Steinberg, 1996). The sanitation and hygiene situation in Zürich in the early 1800s was similar to the rest of Europe; in 1837, a British tourist published an article in a Swiss magazine describing the amount of filth in the streets of Zürich. He stated: "when it rained the streets turned into lagoons covered in half a foot of mud" (Lemann, 2008). Due to a lack of sewage systems in Zürich, human and animal waste and refuse drained into small alleyways between houses and then into the River Limmat, and water-borne diseases such as cholera (at the time the records did not state the type of cholera) frequently raged through the city. To correct this, major sewage reforms were instigated between 1866 and 1870, resulting in the regular removal of household refuse, introduction of simple sewerage systems for waste and rainwater, and a system of waste management based on composting and deposition in rural areas. This strategy seems to have been effective; in 1883, Zürich hosted a national exhibition that presented the city as one of the cleanest and healthiest in Europe. Furthermore, in the 1890s, Swiss authorities proposed the adoption of a mode of waste disposal currently popular in Britain—incineration—and in 1899, the citizens of Zürich allocated 1 million Swiss francs to construction of an incinerator in Zürich. This was to help control the city's waste in the future, since the current management would be unable to cope with an increasing population. This facility, finished in 1904, was the first in Switzerland and the fourth in mainland Europe. Products of this facility did not impact the health of

individuals living in Zürich because it was built some distance from the city. Other effective, though smaller scale public health reforms included the use of sealed containers with sliding lids for storing unwanted household refuse. These were designed for the purpose in the early 1900s, quickly made mandatory for all households in Zürich, and eventually mass-produced throughout the country until plastic rubbish bags made them obsolete in the 1970s (Lemann, 2008).

Switzerland's Role in the Treatment of Tuberculosis

Switzerland played a substantial role in the 19th century declines in mortality from tuberculosis. The country's great number of sanatoria distributed through its alpine regions—and its neutrality, which facilitated migration to these centers—long encouraged individuals infected with tuberculosis to establish short or long term residence there. The main reason for the migrations were the reputation of Switzerland's sanatoria in aiding those with tuberculosis; many with the disease believed if they could travel to these establishments that they would be cured. These establishments were dedicated to the care of patient with active tuberculosis, and were built throughout the 1800s and 1900s to offer a 'cure' for tuberculosis (Rucker & Kearny, 1913). Contemporary medical thinking about active disease held that the "open-air" climate found in the high mountainous areas of Switzerland was an effective cure for the disease (Warren, 2006). The sanatoria also encouraged rest, including sitting in the sun, and 'satisfactory' exercise (Dormandy, 1999; Rucker & Kearny, 1913), and provided clean, hygienic environments for patients, as well as a substantial nutritional regime: up to seven meals a day, consisting largely of dairy products and cod-liver oil (Roberts & Buikstra, 2003; Warren, 2006). All of these treatment methods related to the bolstering of disease resistance among patients through aiding the immune system with proper

nutrition and this allowed many to recover from active tuberculosis. Rucker & Kearny (1913) describe the success rates of sanatorium treatment of at least four weeks for tuberculosis patients from 1905 to 1911. For those with early stage tuberculosis, more than 95% improved, 3.2% were unimproved and only 0.1% died in the sanatoria. Of cases with more advanced tuberculosis, 85% were improved, 12% were unimproved and only 1.0% died in the sanatoria. Very advanced disease resulted in 62% improved, 34% unimproved and only 3% deaths in the sanatoria. During this period, in the 19th and early 20th centuries, estimates of the frequency of tuberculosis in Switzerland are rough, as only mortality from the disease was recorded. However, in 1928, a law (Anfrage des Stadtrates betr. Erlass von Vorschriften über die Wohnungsinspektion) was passed that required the reporting of all cases of active disease. Importantly, this law also required treatment of all recorded individuals, not just those who could afford a stay in a sanatorium (Gesetzgebung: Zürich, 1928).

However, tuberculosis has re-emerged in many other countries around the globe in the past few decades, following the development of drug resistance and the HIV/AIDS epidemic (Corbett et al., 2003; World Health Organization, 2012). Currently, the WHO estimates that one-third of the world's population is infected and two million die from tuberculosis each year. Thus, it is important to study this ancient disease in order to help us combat the current problem of re-emerging and drug resistant tuberculosis.

Materials and Methods

Data Sources

Historical Records and State Statistics

Data employed in this study include vital records on mortality from both the Stadtarchiv ("city-archive" (Stadt Zürich, 2012)) and Staatsarchiv ("canton-archive" (Canton of Zürich, 2012)) in the city of Zürich. The Stadtarchiv held records for causes of death in Zürich city from 1893 (likely because this was when the original city and the surrounding regions were combined) to 1933, which included mortality attributed to tuberculosis. There were many records in this archive, and we examined:

- Statistik der Infektionskrankheiten (Statistics of Infectious Diseases)
- Tuberkulose (Tuberculosis): two volumes; 1912-1932 and 1932 to 1935
- Tuberkulose Sterbefaelle (Tuberculosis mortality): five volumes; 1903-1905, 1905-1915, 1915-1920, 1920-1934 and 1929-1936

The Staatsarchiv held information detailing the introduction of Anfrage des Stadtrates betr. Erlass von Vorschriften über die Wohnungsinspektion (the Law regarding the compulsory recording and treatment of tuberculosis). Data were also collected from a volume of primary historical statistics for Switzerland (State Statistics) titled "Historische Statistik der Schweiz" (Ritzmann-Blickenstorfer, 1996). Both the Staatsarchiv and State Statistics yielded information on mortality attributed to tuberculosis for thirteen cantons (i.e. Zürich, Berne, Lucerne, Uri, Schwyz, Obwalden, Nidwalden, Glarus, Zug, Fribourg, Solothurn, Basel-Stadt and Basel-Land) for 1876 to 1935. From the book of State Statistics, the relevant table was "D43. Todesfälle infolge Lungentuberkulose nach Kantonen 1876-1935" (Mortality due to pulmonary tuberculosis by Canton).

The State Statistics also provided data on population sizes for Zürich city (through censuses) and a number of cantons (i.e. Zürich, Berne, Lucerne, Uri, Schwyz, Obwalden, Nidwalden, Glarus, Zug, Fribourg, Solothurn, Basel-Stadt and Basel-Land) for the years 1888, 1900, 1910 and 1930. This was used to calculate the population density for those years with available data: 1888, 1900, 1910 and 1930 (Gwillim Law, 2009). Each canton was assigned an arbitrary category of population density: low (<40 people per km2), medium (40-80), high (81-100) or very high (>101). These reflect relative predominance of rural and urban styles of living. Cantons with low population density were Uri (19.0 people per km2) and Obwalden (34.0). Medium density included Nidwalden (49.7), Glarus (49.3), Schwyz (62.2) and Fribourg (79.4). High density cantons were Berne (101.7), Lucerne (107.0) and Zug (115.6). Finally, very high density cantons included Solothurn (141.5), Basel-Land (144.4), Zürich (273.2) and Basel-Stadt (3221.6). Since population density increased through time in all cantons, this designation was attributed based on the population density estimated for each at the beginning of observations in 1888. Due to political and economic stability, population densities in studied areas would remain comparatively steady in comparison to each other thus without changing the nature of low-density and high-density areas.

The state statistics also provided average newborn life expectancies for 1878 to 1991. The available data represent an average for the whole of Switzerland (Zürich canton and city specific data were not available) and were used to give an indication of living conditions for 1840 to 1933.

Data Analysis

Causes of death were grouped into four categories according to type of disease. These include infectious, “organ” (i.e. diseases of organ systems such as the renal and

reproductive systems), degenerative, and "other" (e.g. accidents, poisoning). While we are aware that some of the diseases placed in the group "organ" may be the result of infectious processes, we could not be sure and consequently, we simply chose to remove them from our general overview in order to reduce biases. Diagnoses were used as reported and no attempt was made to re-interpret them. Although some terms changed around 1900 (e.g. "phthisis" to "tuberkulose" (Rucker & Kearny, 1913)), an attempt was made to keep the causes of death consistent over the time period by determining which diseases had changed in nomenclature and their corresponding new names. The percentage contributions of each of these four disease groups were calculated by dividing the total deaths from all diseases in that group by the total deaths from all groups.

Data from literature investigating or describing the second epidemiological transition in other countries were consulted to provide a comparison for the Swiss data. Tuberculosis mortality data were available for England and Wales (1860-1960), Japan (1925-1964), Chile (1915-1965), Sri Lanka (1939-1967), The Netherlands (1875-1992), Australia (1907-1990) and cities in the United States (Philadelphia (1870-1930), New York (1866-1965)). The countries used for comparisons were chosen due to the availability of data and because they represent a diverse set of conditions and degrees of urbanization. These comparisons are thus useful to determine the effects of different living conditions and social factors on tuberculosis mortality through time. Graphs of percentage mortality from infectious, organ, degenerative, and other diseases were produced from the data presented in publications describing tuberculosis mortality in the other countries stated above (Carter et al., 2011; Condran & Cheney, 1982; Lewis, Taylor, & Powles, 1998; Omran, 1983, 2005; Wolleswinkel-van den Bosch, Looman, Van Poppel, & Mackenbach, 1997). In some cases this was difficult, especially where the causes of death were grouped or not clearly described. In some cases, graphs from

publications were not clear and these were reproduced in a different format to allow an estimation of the timing of the second epidemiological transition.

Following established methods, the percentage of tuberculosis mortality was calculated by dividing tuberculosis mortality by total all-cause mortality. This was plotted for both Canton Zürich and the city of Zürich in order to observe trends and patterns over time, but also for comparison of the two. Tuberculosis mortality, expressed as a rate per 100,000 living individuals, was plotted against the calculated population densities for several Swiss cantons (Zürich, Lucerne, Uri, Schwyz and Berne) in order to determine any correlation between the two.

Results

Part One: The Timing of the Second Epidemiological Transition in Zürich City

The total number of recorded deaths for the causes in each disease group (i.e. infectious, organ, degenerative, and other) was expressed as a percentage of the total mortality for 1893 to 1933. This was plotted along with newborn life expectancy and is shown in Figure 1. The second epidemiological transition, defined by Omran (1983) as the time when degenerative diseases become more important than epidemic infectious diseases in terms of mortality, is clearly shown in Figure 1. This transition takes place in 1911, according to the intersection point of linear trend lines for degenerative and infectious diseases. The rate of increase of degenerative diseases was equal to the rate of decrease of infectious diseases; 0.0079±0.0007 and 0.0078±0.0007 per year, respectively. This transition is reflected in the increase in life expectancy; as individuals began living longer, they became more likely to develop degenerative diseases. The newborn life expectancy of males increased from 60.5 years in 1895 to 66.5 years in

1935. For females, the values increased from 62.2 years to 69.6 years over the same time period.

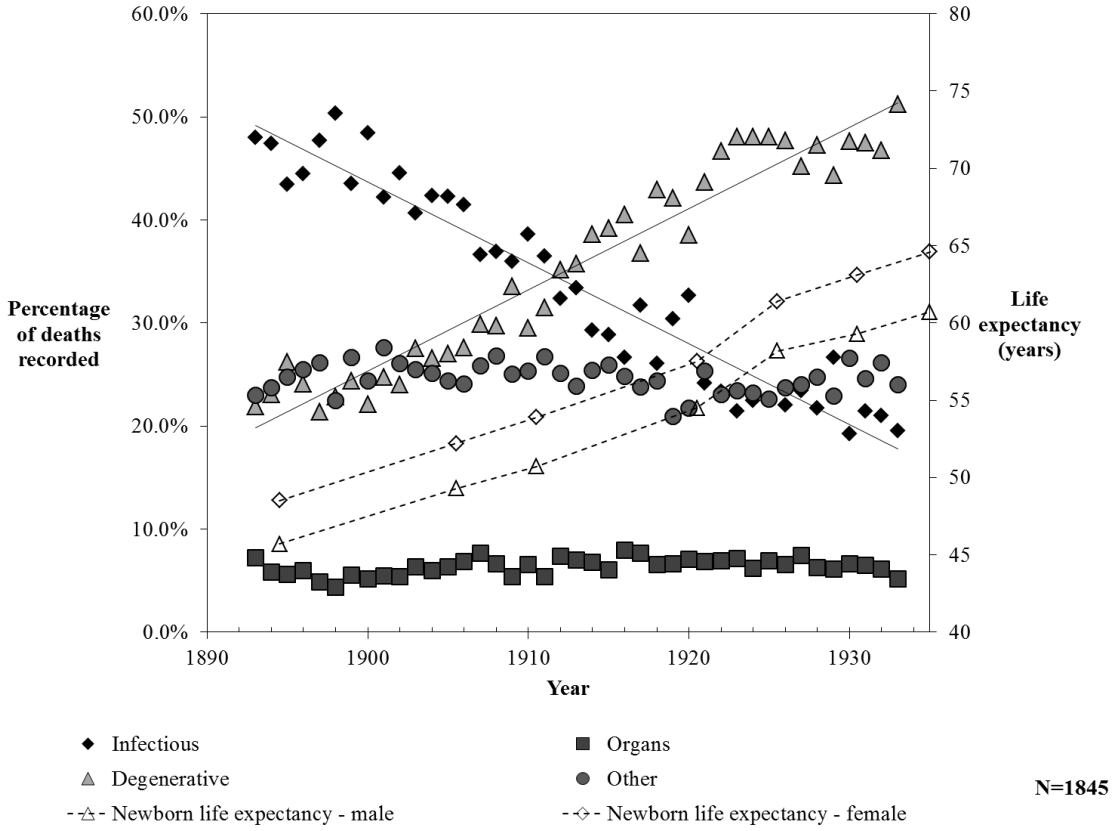

Figure 1: Percentage mortality in the city of Zürich for infectious, organ, degenerative and other diseases, between 1893 and 1933. Newborn life expectancies for males and females are also plotted.

Part Two: Trends in Tuberculosis Mortality during the Second Epidemiological Transition in Zürich City and Zürich Canton

Mortality from tuberculosis, expressed here as a percentage of total mortality, was calculated for the canton of Zürich for 1840 to 1933 (Stadt Zürich 2012) and Zürich city for a shorter period, 1893 to 1933 (Ritzmann-Blickenstorfer, 1996). The data are

plotted in Figure 2, along with annotations of important historical dates for Switzerland, healthcare and tuberculosis treatment.

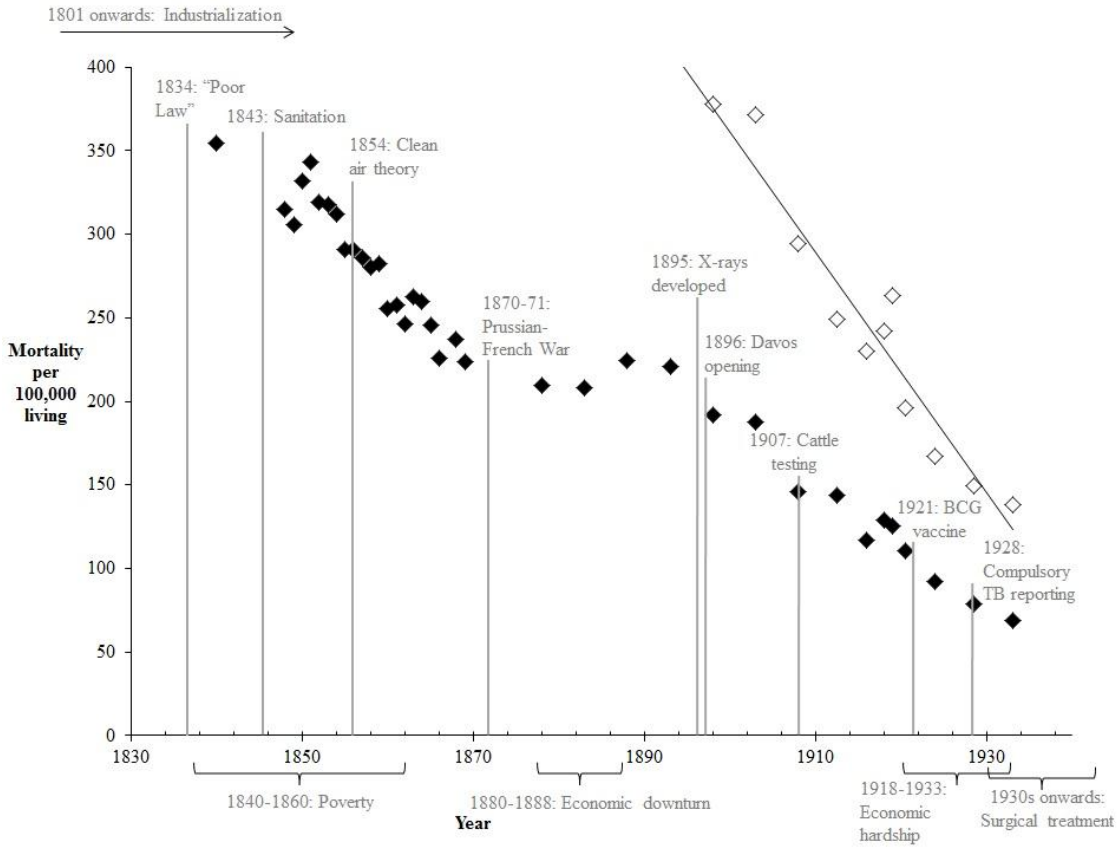

Figure 2: Percentage of total mortality from tuberculosis in Canton Zürich (1840-1933) compared with Zürich city (1893-1933). Historical events are also shown.

In the city of Zürich, the percentage of total mortality from tuberculosis decreased between 1893 and 1933. In the canton of Zürich there was a peak in tuberculosis mortality around 1890. This could be a result of economic difficulties involving the wheat market (as mentioned above) as well as migration, resulting in lower nutritional value of the Swiss diet through rationing (Graber, 1926). The data for the city differed from the cantonal values in that the initial percentage was higher, likely due to the higher population density of the city (average 1485 people per km2 between 1893 and 1933). Towards 1933, the percentage of total mortality from tuberculosis in

the city began to approach the value for the entire canton. Finally, the decrease in tuberculosis mortality is more dramatic in the city than in the entire canton. This could reflect changes in living conditions as well as improved access to medical treatment and medical advances in the city, which would be greater in a higher population density area. In more crowded areas, before effective public health measures, there was a higher level of infectious diseases due to poor sanitation (waste products were left to decompose in the streets) as well as higher transmission rates due to population density. With the introduction of public health education and sanitation, these issues can be quickly and efficiently resolved. There is another interesting observation from Figure 2; the percentage of mortality from tuberculosis was decreasing even before medical advances related to tuberculosis care. This is potentially due to the improvements in living conditions and public health care in Switzerland rather than specific tuberculosis treatments. Additionally, tuberculosis mortality was also in decline in the city of Zürich before the second epidemiological transition occurred there, around 1911 (see Figure 1).

Part Three: Patterns of Tuberculosis in Other Swiss Cantons during the Second Epidemiological Transition and the Effect of Population Density

Population density (people/km2) was plotted against the tuberculosis mortality rate per 100,000 living individuals (Figure 3) to determine whether correlations existed between these two variables in different cantons during the several years for which data were available: 1888, 1900, 1910, and 1930.

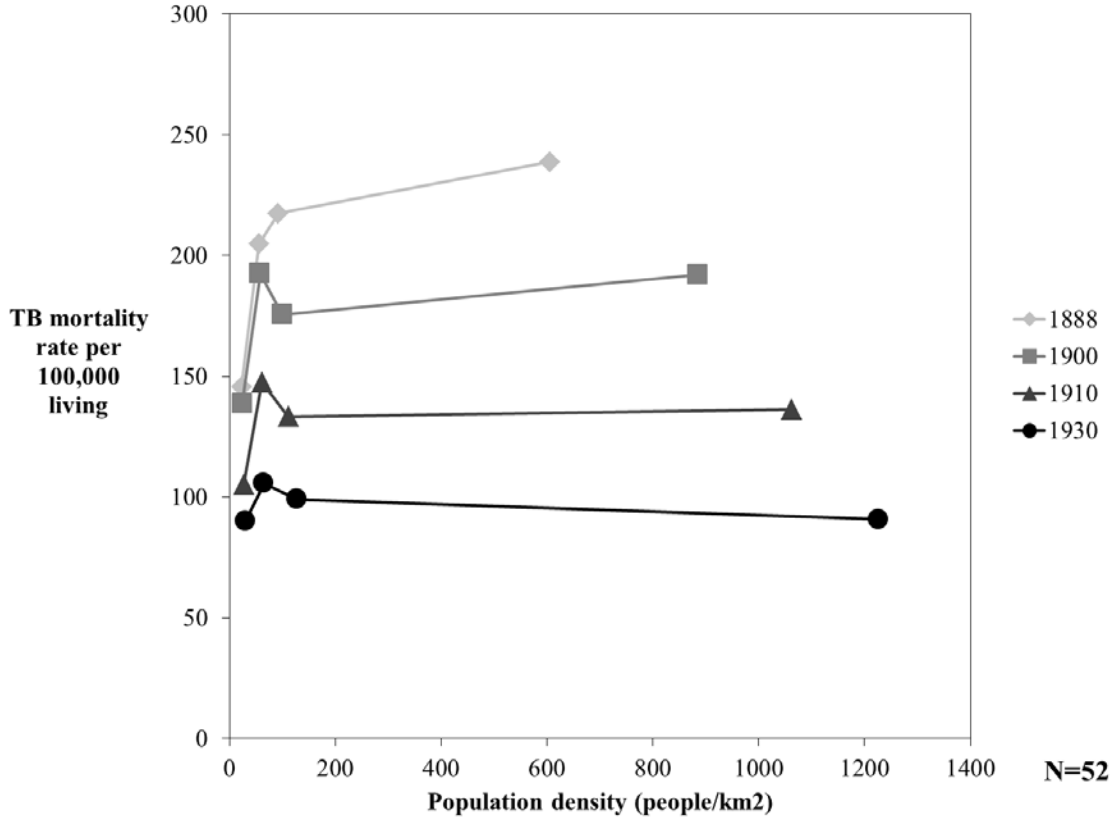

Figure 3: Tuberculosis mortality rate per 100,000 living people compared with population density (logarithmic scale) in various Swiss cantons (1888-1930). Cantons include: Zürich, Berne, Lucerne, Uri, Schwyz, Obwalden, Nidwalden, Glarus, Zug, Fribourg, Solothurn, Basel-Stadt and Basel-Land.

A positive relationship existed between population density and tuberculosis mortality in the earlier years, namely 1888 and 1900 (Figure 3). The mortality rate decreased through time, despite an increase in population density. The decrease in tuberculosis mortality is more dramatic in cantons with a higher population density than those with a lower population density. In those with lower density, the tuberculosis mortality rate in 1888 was 146 (per 100,000 living) but decreased to 90 by 1930. In cantons of very high population density, the tuberculosis mortality rate was 239 (per 100,000 living) in 1888 and decreased to 91 by 1930. Note that in 1930 the correlation between tuberculosis mortality and population density disappeared and both high and

low density areas had the same tuberculosis mortality rates (approximately 90 per 100,000).

Discussion

Part One: The Timing of the Second Epidemiological Transition in Zürich City

When compared with data from other countries that industrialized in the 19th and early 20th centuries, and the cities within them, evidence here suggests that Switzerland and Zürich underwent the second transition early. For instance, in the earliest industrializers, the transition occurred during the 1920s in Great Britain (Omran, 2005), and before 1930 in major urban areas in the U.S., namely Philadelphia and New York (Condran & Cheney, 1982; Omran, 1983). In the Netherlands, for example, which experienced intensive industrialization in the 1860s and 1870s, cause of death and all-cause mortality data, covering the period from 1875 to 1992, suggest that the transition occurred there only in the 1930s (Wolleswinkel-van den Bosch et al., 1997). Other, later industrializing countries, including Nauru, Japan, Mexico, and Chile exhibited patterns of an epidemiological transition later, in 1955, 1951, 1957, and 1974, respectively (Carter et al., 2011; Omran, 1983, 2005). Evidence from Australia, for example, which industrialized mainly throughout the 20th century, seems to suggest that the transition occurred there in the 1920s; infectious disease mortality declined in the 1940s, stabilizing by 1950, and degenerative diseases, specifically cardiovascular disease and cancer, increased from 1920 on, only plateauing in 1940. Overall, the epidemiological transition in all of these other countries occurred later than in Switzerland.

The decrease in infectious disease mortality observed so early is attributable to several possible causes. Refrigeration, which would have reduced morbidity and mortality from gastrointestinal disease, is one possible cause. Refrigeration was under

development in the early 1800s and was in widespread use in 1884 in the United States (Krasner-Khait, 2011); it likely became widespread in Switzerland at approximately the same time. The mortality rate from gastrointestinal diseases, many of which may be caused by an infectious agent (though not always), in Zürich city decreased substantially from 17% of all deaths in 1898 to 2% in 1914. This pattern suggests that the decline may be linked with improvements in food technologies, such as refrigeration. The decline would have substantially lowered mortality among children, the age group most affected by this type of disease, thus allowing more individuals within the population to reach adulthood. This theory is supported by the increase in life expectancy among newborns through time as shown in Figure 1.

Improvements in general sanitation also likely played a role in precipitating Switzerland's early transition. For instance, sewage control, which was introduced between 1866 and 1870 in Zürich, and the increased accessibility of potable running water in Zürich and other cities in the same time period, would have facilitated the epidemiological transition in Switzerland by further reducing mortality from water borne and many gastrointestinal diseases. Similar structural changes were occurring in both England and Germany, but at a slightly later date. For instance, in England, a Public Health Act in 1875 required appropriate waterworks to be present and functional, while in Germany, all larger cities (i.e. more than 25,000 people) had efficient sewerage systems by the year 1900 (Vögele, 1998). These changes, lagging slightly behind those in Switzerland, are reflected in the decline of infectious diseases through time in these nations. For tuberculosis, these improvements in living conditions helped to increase the general immunity of the population and consequently decreased the number of people who developed active disease.

Importantly, Switzerland was a stable country during the time periods investigated, and unlike many other early and late industrializing countries, did not take part in any international wars in the mid-19th and early 20th centuries (and into the present day). Data from Switzerland also, unlike these other countries, show little evidence of poorer living conditions in highly populated cities compared with rural areas. In contrast, Great Britain, for example, experienced extensive damage to their urban, economic, and public health infrastructures during WWI and WWII. In WWII in particular, living conditions in urban areas dramatically deteriorated due to damage to buildings and homes, leading to overcrowding as well as rationing of food. This did not occur in Switzerland; living conditions remained stable throughout the wars. In Switzerland, many of the penalties associated with urban factories were avoided because individuals could work at home and were not subject to the poor conditions of living and working in large cities. This kept many native Swiss in their hometowns and encouraged some immigrants to move to these areas; thus helping to prevent cities becoming overcrowded. These factors, combined, seem to have contributed to a buffering of the epidemiological costs associated with industrialization and urbanization in Switzerland, and a comparatively earlier transition to a reduced regime of infectious disease and greater longevity among its citizens.

Part Two: Trends in Tuberculosis Mortality during the Second Epidemiological Transition in Zürich City as well as the Entire Canton of Zürich

The 19th century witnessed several major shifts in mortality from tuberculosis in Zürich. Between 1840 and 1870, the percentage of total mortality from tuberculosis in the Canton of Zürich decreased substantially. This coincided with changes in health policies, including sanitary reforms and enactment of the "New Poor Law" (the "Old

Poor Law" was introduced in the 16th century), which required the segregation of wealthy and poor individuals and encouraged those with the ability to work to find employment and support themselves (Wilson, 2005). The Newer Poor Law aimed at providing housing, clothing, food and education (for children) to the poor in return for several hours of labor per day in a workhouse (The National Archives, 2012). Separating these poorer individuals from those of higher socioeconomic status helped to reduce tuberculosis among the latter because they had less contact and opportunity for transmission of the disease with poor people with lower immunity due to a lower nutritional status. However, after the Prussian-French War (1870-1871), the percentage mortality due to tuberculosis increased, which may be attributed to the influx of a large number of both French troops and refugees, who were likely poorly nourished and stressed, into the canton near the end of the war. This trend persisted until approximately 1910, when the percentage decreased again in both the city and Canton of Zürich. This decline coincided with a number of important developments in tuberculosis prevention and control in Switzerland, including the discovery of X-rays in 1895 (these were later used as a method of diagnosing active pulmonary infection) (Herzog, 1998), and the initiation of testing cattle to control bovine tuberculosis around 1907 in many European countries (S. W. B. Newsom, 2006). These innovations were later followed by the first use of the Bacille Calmette Guerin (BCG) vaccine in 1921, which was the first—and only—vaccine to provide protection from tuberculosis, particularly in children (Herzog, 1998). Lastly, the 1928 Swiss law which required reporting and treatment of cases of tuberculosis (Gesetzgebung: Zürich, 1928), and the invention of surgical treatment for pulmonary tuberculosis in the 1930s (Herzog, 1998) likely contributed to the steady early 20th century decline in the disease in Switzerland and Zürich specifically. Interestingly, the years associated with the beginning of

industrialization and economic hardship in Zürich (19th century), and thus potentially reduced overall health were not associated with increased mortality from tuberculosis.

The fact that tuberculosis mortality decreased before medical interventions in Zürich suggests that medical treatment and advances on their own may not be major causes of declines in mortality from tuberculosis in a given population. This finding is congruent with other scholarship on the second epidemiological transition, such as Omran (2005), which suggests that ecobiological changes, such as aspects of the environment, and the biology of hosts and pathogens, and socioeconomic, political and cultural changes, such as standards of living, hygiene and nutrition, exerted far more influence in precipitating the second transition than did medical intervention, such as surgery, drugs and vaccination. Likewise, our results suggest that in Switzerland, the second transition occurred before the implementation of major medical advances, such as chemical therapy/antibiotics and in the case of tuberculosis, before use of BCG vaccine. It seems that this early occurrence of the epidemiological transition is a result specific for Switzerland's combination of sanitation, local health care arrangements and living conditions. These latter ones included lack of overcrowded extensive urban agglomerations, working at home or in small establishments, relatively good food supply, and stable social relationships. This observation has important implications in the current global situation because there are a number of low-income countries struggling with the problem of multi-drug resistant bacteria (World Health Organization, 2012). Our findings suggest that clinical endeavors and public health initiatives in these contexts should emphasize improving overall health, general nutrition, and living standards, as much—if not more so—developing and providing new drugs and treatment regimens.

Tuberculosis, however, does defy easy categorization into the shifting patterns of mortality characteristic to the second transition. This is because it is an infectious condition, but also a chronic disease which, like degenerative diseases, often does not manifest symptoms for many years. As such, the picture of a decline in infectious disease mortality in Zürich and of tuberculosis specifically in the early 20th century is not straightforward; instead the data suggest a combination of infectious and non-infectious causes producing mortality through a chronic and long-lasting process. Tuberculosis does have an infectious origin, but active disease is a result of an individual's inability to control the disease. Mortality from the disease gives us information about a combination of the levels of transmission, nutritional status and level of public health, but it is very difficult to split these factors during corresponding interpretations. However, this information is useful for providing a general overview of the population's ability to resist other infectious agents and as this increases, the second epidemiological transition is observed.

Part Three: Patterns of Tuberculosis in Other Swiss Cantons during the Second Epidemiological Transition and the Effect of Population Density

The results presented here suggest that tuberculosis mortality rates declined more rapidly in high population density cantons than in low-density cantons (from 1910 onwards), which may be due to progressive improvements in sanitation and changing strategies for handling tuberculosis patients which may be more effective in more crowded areas (as mentioned previously). However, high density areas also showed higher initial rates of tuberculosis mortality (during the years 1888 and 1900 specifically) likely due to poorer living conditions and increased human contact in these urban areas in comparison to low density rural areas.

These results further suggest that in 19th and 20th century Switzerland, living conditions exerted a substantial impact on mortality rates from tuberculosis, while medical interventions exerted less of an effect. Sanitation methods may have become effective in improving living conditions in high-density areas to make them comparable with lower density areas. The effect of good sanitation may result in a reduction of transmission of diseases because the population's immune systems are bombarded by infectious agents to a lesser extent than in the case of poor sanitation. With a lower level of infectious agents in the environment, accompanied by an increase in nutrition, the immune system of an individual will have a higher chance of controlling diseases. Additionally, outbreaks of specific diseases (e.g. cholera) will be less likely when there are fewer opportunities for the pathogens to be transmitted from one person to another. In the case of lower density areas, there are already fewer opportunities for the spread of pathogens. This has implications for developing nations, where a similar situation is currently present (poor living conditions, overcrowding and poor nutrition). Regions such as sub-Saharan Africa, South-East Asia and some countries in South America currently are considered high burden areas in terms of tuberculosis (World Health Organization, 2012). Consequently, improving the standard of living through sanitation (improving waste management and especially public health education) and reviewing and modifying how tuberculosis patients are handled could possibly be used to obtain the same effect as observed for Switzerland. Currently, many individuals are given antibiotic therapy for tuberculosis, but often do not finish the course of treatment (Tiemersma, van der Werf, Borgdorff, Williams, & Nagelkerke, 2011). This can be for a number of reasons including inability to obtain or afford the drugs, non-compliance (due to side effects) and lack of time to visit the clinic. However, there are obvious differences between modern developing nations and 19th and 20th century Switzerland including economical and geographical considerations. Thus it is impossible to predict

the outcomes of improved sanitation, vaccination, and immunotherapy, but it should still be considered important to use all methods available to improve human health and combat a disease that has been a major cause of death for many years. In particular, sanitation and public health education have been very effective in reducing the mortality due to tuberculosis in Switzerland. Part of the gradual decline in tuberculosis mortality may be due to the country's attitude towards the disease. Many highly reputable sanatoria were built in Switzerland and people migrated from around the world to spend time in these establishments. Consequently, the Swiss population would have known about the disease and hygienic measures that aid in its treatment (Rucker & Kearny, 1913). Vaccination and drug therapy were introduced many years after the initial decline in tuberculosis, indicating that these are not necessary for the decline in tuberculosis mortality, though they do assist where implemented.

Limitations of the Study

There are several possible interpretive issues and limitations involved in analyses examining mortality and morbidity from tuberculosis in the second epidemiological transition. For instance, historical records, which are relied upon here, can be incomplete and biased through uneven distribution of ages or socioeconomic status (Doege, 1965; Rieder, Zwahlen, & Zimmermann, 1998). There may also be issues with the disease nomenclature, wherein certain diseases may be identified by several different cognates (e.g., phthisis) (Puranen, 1999). These issues may also lead to an inability to determine the actual cause of a disease in specific terms (i.e. the single disease which caused death) as well as whether an infectious agent was involved. In some cases, the nomenclature does not give us the opportunity to determine the cause of a disease as infectious or other. For this reason, we created the "organ" group to help

remove this bias that would prevent us from giving an informative overview of the epidemiological transition in Switzerland. However, since the records employed here cover a time period after the modern nomenclature ("tuberculosis") had come into widespread use (1901 in Switzerland (Rucker & Kearny, 1913)), this issue likely did not substantially affect the results presented here. Difficulties with determining population size, and thus density, also present a possible limitation (see Antunes & Waldman, 1999), as both the population size and the geographical size of urban areas in Switzerland increased with urbanization throughout the mid to late 19th and 20th centuries. While the city of Zürich expanded substantially in 1893, the records employed begin at this time and thus it is unlikely to have affected the results presented here. Only data from the entire Canton of Zürich predate 1893 but no major increases in population size occurred across the whole canton before this time.

Another limitation is that much of the data employed here are derived from Zürich city only, and thus do not necessarily represent larger patterns within other cantons or Switzerland as a whole. This is however, also an advantage in that the death records are likely to be more complete as compared with an entire canton since the entire canton includes rural areas which may be overlooked during a census. Additionally, comparison of the results presented here with other literature regarding the second epidemiological transition showed that the trends observed in Switzerland are similar to what occurred in other countries, meaning our results can be extended to a wider area of Europe.

Comparison with the Literature

It is possible to compare evidence from Switzerland on tuberculosis mortality with that of numerous other nations for the period after 1870 because of the increased

availability and completeness of data for this this period. Comparisons made between Switzerland and England or France, for instance, are useful because similar events (e.g. sanitation, improvements in public health) were occurring around the same time period at the same rate in these countries and therefore, any differences between them can be interpreted as being the result of differences in the country, such as culture and extent of urbanization and development. A comparison of Switzerland and Japan is also useful because the latter underwent urbanization later (at the end of the 19th century and early 20th century) and at a more rapid rate; thus the differences between the two countries can be used to give an indication of the impact of urbanization on tuberculosis mortality. In Switzerland, in 1901, tuberculosis mortality was 273 per 100,000 population for the entire country. Two countries, namely Hungary and France exhibited tuberculosis mortality rates above 300 per 100,000 in 1900 (Figure 4) (Johnston, 1995). Italy, Netherlands, Spain, United States, Denmark, England and Japan exhibited values closer to those of Switzerland, ranging between 160 and 199 per 100,000 (Gubéran, 1980; Johnston, 1995). Based on these comparisons, Switzerland has a higher tuberculosis mortality rate than many other countries in Europe (as well as the US). The reason for this could be a higher accuracy of historical records in Switzerland compared with other countries due to compulsory reporting of cases of tuberculosis. Another possibility is that Swiss cities did not grow in size until late in the 19th century. This is certainly true for Zürich; in 1893, the city expanded substantially and this would have had a major impact on the levels of overcrowding as immigrants had a larger area to migrate into. For cities such as London and Paris, their areas were defined earlier in the 19th century and thus public health and sanitation efforts were introduced into a well-known area.

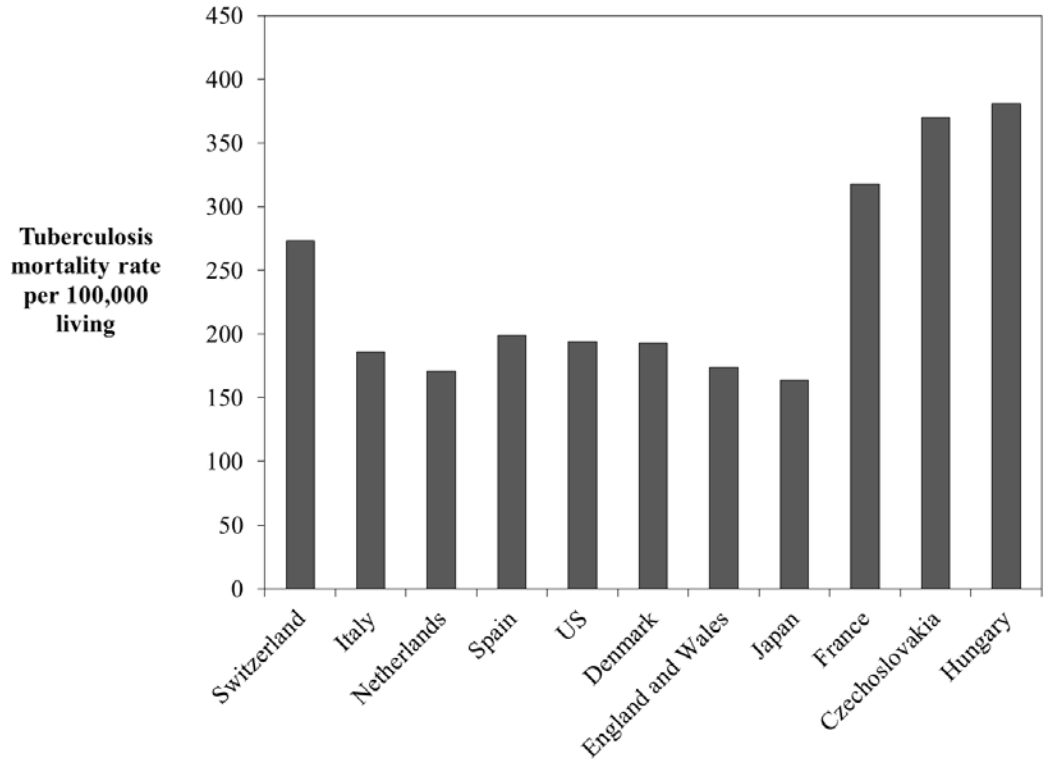

Figure 4: Tuberculosis mortality rates per 100,000 living population for Switzerland and several other countries in the year 1900.

Of these countries, mortality data for tuberculosis are also available for several major cities (London, Paris and Tokyo). In 1900, tuberculosis mortality in Zürich city was 422 per 100,000. In Tokyo, it was 480 per 100,000 in 1900 (Johnston, 1995), and in London the tuberculosis mortality rate was estimated at 180 per 100,000 in 1891-1900 (Nathanson, 2007). Paris tuberculosis death rates were 173 per 100,000 (Preston & Walle, 1978). This comparison is useful because it shows that Zürich and Tokyo had similar, very high rates of tuberculosis mortality. London and Paris also had similar rates of mortality, but much lower than those of Zürich and Tokyo. Both Zürich and Tokyo had poor sanitation and hygiene around 1900, but Switzerland improved this situation at a faster rate than in Japan. This is because they both adopted different

measures to help control tuberculosis. Switzerland initiated public health systems as well as sanitation and Japan used much cheaper methods of facilities (for the care of patients with tuberculosis) and public education (Johnston, 1995). The reason that both London and Paris have lower rates of tuberculosis mortality than either Zürich or Tokyo may be due to the fact the former had introduced Public Health Acts long before the latter did. England and Wales first introduced Public Health Acts in the 1840's (Szreter, 1988) and France in the 1850's (Preston & Walle, 1978). Thus, by 1900, both of these cities had improved living conditions when compared to Zürich and Tokyo, which did not introduce Public Health Acts until later, although Zürich introduced sewerage reforms earlier than Germany and England and Wales.

Over the 19th and 20th centuries, several major shifts were observed in tuberculosis mortality among developing nations undergoing the second transition. In most countries, tuberculosis mortality decreased through time in parallel with improvements in living conditions and sanitation. This is true for the U.S., where tuberculosis mortality declined in two stages: first during the first few years of the 20th century, and second between 1944 and 1950 (Doege, 1965). These two periods were associated with two different events; the introduction of sanitation and then later, specific drug therapy. Switzerland shows a decline equivalent to that in the U.S. in the early 1900s, likely due to the same reasons of improving living conditions and public health campaigns. Data for the 1940's in Switzerland Gubéran (1980) reveal another similar trend to the US. During WWII, tuberculosis mortality rates remained stable, but later when the war had ended and streptomycin was introduced, the rate declined substantially. Declines in tuberculosis mortality have also been documented for Great Britain starting from the 1830's due to the introduction of a large number of Public Health Acts (Szreter, 1988). In contrast, data suggest that Japan experienced high mortality from tuberculosis starting around 1895 and 1900, and extending to after

WWII (Johnston, 1995). Antibiotics and other medical interventions (e.g. surgery, other chemical agents) were introduced after the war and this was responsible for almost all of the decline in tuberculosis mortality in Japan. Rather than shifts in the disease nomenclature and diagnostic accuracy for tuberculosis, declines in tuberculosis mortality have also been described in low-income countries undergoing industrialization and the second transition later during the 20th century, such as Brazil, which witnessed major declines in mortality rates in São Paulo between 1945 and 1985 (Antunes & Waldman, 1999). However, in Brazil and other low-income nations, the transition was facilitated by medical interventions, such as antibiotics, as well as by improvements in living conditions and public health (Omran, 2005).

Changes in the age distribution of tuberculosis mortality have also been discussed for high-income nations undergoing industrialization and the second transition in the 19th and 20th centuries. For instance, Preston and Walle (1978) showed that in urban areas of France, such as Marseilles, Paris, and Lyon, tuberculosis mortality was highest in young adults (20 to 29 years) but changed to older age groups (30 to 39 years) through the 19th century (1816-1882). Doege (1965) showed that for the U.S. this trend was also observed from 1900 to 1960 and not simply attributable due to an increase in life expectancy, but rather due to a change in the manifestation of tuberculosis across age groups. Tuberculosis was becoming a disease of the elderly and remained latent in younger individuals before reactivation later in life. Additionally, the average age of fatal tuberculosis increased in the U.S. from 34.4 years in 1900 to 58.1 years in 1960 (Doege, 1965). While age information is not available for Zürich, life expectancy did increase in Switzerland over the time period 1893 to 1933 (Figure 1) while tuberculosis mortality decreased. Additionally, one study by Gubéran (1980) for Switzerland as a whole showed that older age groups were more commonly affected by tuberculosis through time for the studied period: 1875 to 1935. This may indicate that

living conditions and health of children and young adults improved such that older individuals were far more affected by the disease due to lowered immunity in later life (Omran, 2005). This theory supports the other data presented here, suggesting that tuberculosis mortality decreases with improvement in living conditions.

Some studies have also found sex-based differences in tuberculosis mortality for some high-income industrializing countries undergoing the second transition. Data on sex in relation to tuberculosis mortality in Switzerland are largely unavailable, but Gubéran (1980) has demonstrated that for the country as a whole, females aged 15 to 29 years had a higher mortality rate than did their male peers between 1900 and 1960. Similar patterns have been found in the US and Japan (Doege, 1965; Johnston, 1995).

Several reasons for the decrease in tuberculosis mortality through time during the 19th and 20th centuries in many countries have been suggested. For instance, Miller and Thompson (1992) describe some criteria that assisted in the control and prevention of tuberculosis in Newcastle, Britain around the year 1907. These included compulsory notification of tuberculosis, sanatoria, building of hospitals and dispensaries, prevention of infection of the lungs and abdominal tract, education, and establishment of a national health authority. Other authors agree with these suggestions and describe them in relation to their own work on different countries. Preston and Walle (1978) reported that urban areas in France had poorer living conditions than the rest of the country as a whole. They additionally suggested that water supply, public health, and hygiene all must be controlled for a decrease in tuberculosis mortality to occur. This did occur in France during the late 19th and early 20th centuries. They highlighted that medicine, including vaccination, surgery and drug therapy, was not important in the decline of tuberculosis in France. This was also true for the U.S. (Doege 1965) during the initial decline in the early 1900s. However, antibiotics were responsible for the secondary

decline between 1944 and 1950. In São Paulo (Antunes and Waldman 1999), the situation was slightly different, with tuberculosis mortality rates remaining high until after WWII. Reasons for the decline there included, besides the most important medication, preventative and therapeutic measures (such as public education, antibiotics, surgical interventions and a general increase in immunity through improvements in nutrition and living conditions), increased provision of health services (e.g. more clinics), and social changes (e.g. how the population interacted with one another, avoiding contact with others when they were contagious). However, recently the tuberculosis mortality rate has begun to increase again at an exponential rate, most likely alongside the growing HIV/AIDS problem in Brazil.

The situation in Japan (Johnston, 1995) is similar to what occurred in Brazil (Antunes & Waldman, 1999). Antibiotics to combat tuberculosis were not available until after 1950 but after that date were an important factor in the decline. Before this, the Japanese used facilities (such as sanatoria and specialist hospitals) and health education in order to combat the disease. While these measures are less expensive than social and economical changes, they are also less effective (Johnston, 1995). In Japan, transmission of the disease was mostly due to female industry workers returning to their homes in rural locations from urban areas and from migrating soldiers. Both of these groups were exposed to poor living conditions and thus were more likely to develop active tuberculosis. Finally, Szreter (1988) presents a series of arguments demonstrating that both improved nutrition and various public health measures were responsible for the historical decline in Great Britain. Szreter (1988) also mentions the numerous Public Health Acts in England and Wales that would have impacted living conditions. They also highlighted how nutrition is important, but so are a number of other factors including overcrowding, lack of sunlight, ventilation and occupational hazards (e.g. dust

and smoke), all of which were common problems in the initial stages of industrialization.

In Switzerland, many of these factors did play some role in the decline of tuberculosis through time. Living conditions improved substantially during the second half of the 1800's, following the first sewerage reform in 1866. Public Health services were excellent (e.g. many hospitals, well-educated medical personnel) in Switzerland and contributed to the overall high quality of life in Swiss cities, which experienced few health disadvantages as observed in other countries. Industrialization did not affect Switzerland as negatively as other countries, due to some unique characteristics of the economy, such as the small number of factories and production centered within the home, and the slow growth of urban centers.

Conclusion

From these descriptions, it is clear that prior to chemical therapy and antibiotics, a series of factors were important in the decline of tuberculosis mortality through time. No single factor could be pointed out, but rather a combination of improved living conditions as well as public health changes was ultimately responsible for the decline in Switzerland. This is also true for other countries, but Switzerland is unique in that it was neutral during the years when conflict was common and has had a stable government since 1848. This allowed the second epidemiological transition to occur quickly and earlier than in other countries. This knowledge could be implemented in the high burden countries at present and consequently reduce mortality from tuberculosis in these areas, just as it occurred in Switzerland and other countries, many years ago. Many of these countries are currently undergoing the third epidemiological transition, characterized by an increase in the drug resistance of the tuberculosis bacterium. This limits the

usefulness of medicine as a method of therapy. Instead, consideration of the ideas and practices employed by the Swiss and later, in other areas in Europe, may present another viable option for treatment, prevention, and ultimately reducing the global burden of tuberculosis.

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References Cited

Antunes, José Leopoldo Ferreira, & Waldman, Eliseu Alves. (1999). Tuberculosis in the twentieth century: time-series mortality in São Paulo, Brazil, 1900-97. Cad. Saúde Pública, Rio de Janeiro, 15(3), 463-476.

Bourdelais, Patrice. (2006). Epidemics Laid Low: A History of What Happened in Rich Countries. Baltimore: The Johns Hopkins University Press.

Bouvier, Nicolas, Craig, Gordon A., Gossman, Lionel, & Schorske, Carl E. (1994). Geneva, Zurich, Basel: History, Culture and National Identity. New Jersey: Princeton University Press.

Burke, Stacie D. A. (2011). Tuberculosis: Past and Present. Reviews in Anthropology, 40(1), 27-52.

Butler, Michael, Pender, Malcolm, & Charnley, Joy (Eds.). (2000). The Making of Modern Switzerland, 1848-1998. London, UK: Macmillan Press Ltd.

Canton of Zürich. (2012). Staatsarchiv Retrieved 7 September 2012, from http://www.staatsarchiv.zh.ch/internet/justiz_inneres/sta/de/home.html

Carter, K., Soakai, T. S., Taylor, R., Gadabu, I., Rao, C., Thoma, K., & Lopez, A. D. (2011). Mortality trends and the epidemiological transition in Nauru. Asia Pacific Journal of Public Health, 23(1), 10-23.

Cole, S. T., Eisenach, Kathleen Davis, McMurray, David N., & Jacobs Jr, W. R. (Eds.). (2005). Tuberculosis and the Tubercle Bacillus. Washington DC, USA: ASM Press.

Condran, Gretchen A., & Cheney, Rose A. (1982). Mortality Trends in Philadelphia: Age- and Cause-Specific Death Rates 1870-1930. Demography, 19(1), 97-123.

Condrau, Flurin, & Tanner, Jakob. (2000). Working-class experiences, cholera and public health reform in nineteenth-century Switzerland. In Sally Sheard & Helen Power (Eds.), Body and City: Histories of Urban Public Health (pp. 109-122). England: Ashgate Publishing Ltd.

Corbett, E. L., Watt, C. J., Walker, N., Maher, D., Williams, B. G., Raviglione, M. C., & Dye, C. (2003). The growing burden of tuberculosis: Global trends and interactions with the HIV epidemic. Archives of Internal Medicine, 163(9), 1009-1021.

Doege, Theodore C. (1965). Tuberculosis Mortality in the United States, 1900 to 1960. Journal of the American Medical Association, 192(12), 1045-1048.

Dormandy, Thomas. (1999). The White Death: A History of Tuberculosis. London: The Hambledon Press.

Gesetzgebung: Zürich. (1928). Anfrage des Stadtrates betr. Erlass von Vorschriften über die Wohnungsinspektion. Zürich, Switzerland.

Graber, Paul. (1926). Concerning the Wheat Monopoly in Switzerland (Vol. 3). Geneva, Switzerland: French University Press.

Gubéran, E. (1980). Tendances de la mortalité en Suisse: 2. Maladies infectieuses 1876-1977. Schweizerische Medizinische Wochenschrift, 110(15), 574-588.

Gwillim Law. (2009). Switzerland Cantons Retrieved Accessed: February 2011, from http://www.statoids.com/uch.html

Herzog, H. (1998). History of tuberculosis. Respiration, 65(1), 5-15.

Johnston, William. (1995). The Modern Epidemic: A History of Tuberculosis in Japan. Cambridge (Massachusetts): Harvard University Press.

Kaufmann, S. H. E. , & Britton, W. J. (Eds.). (2008). Handbook of Tuberculosis: Immunology and Cell Biology. Weinheim, Germany: Wiley-VCH.

Krasner-Khait, B. (2011). History Magazine – The Impact of Refrigeration Retrieved Accessed 21st January 2011, from http://www.history-magazine.com/refrig.html

Lemann, Martin F. (2008). Waste Management. Bern, Switzerland: Peter Lang.

Lewis, M., Taylor, R., & Powles, J. (1998). The Australian mortality decline: all-cause mortality 1788-1990. Australian and New Zealand journal of public health, 22(1), 27-36.

Miller, F. J., & Thompson, M. D. (1992). Decline and fall of the tubercle bacillus: the Newcastle story 1882-1988. Archives of Disease in Childhood, 67(2), 251-255.

Nathanson, Constance A. (2007). Disease Prevention As Social Change: The State, Society, and Public Health in the United States, France, Great Britain, and Canada. New York: Russell Sage Foundation.

Newsom, S. W. B. (2006). The history of infection control: Tuberculosis: Part two - Finding the cause and trying to eliminate it. British Journal of Infection Control, 7(6), 8-11.

Newsom, Swb. (2006). The history of infection control: Tuberculosis, part one: Defining a disease and its social consequences. British Journal of Infection Control, 7, 14-17.

North, R. J., & Jung, Y. J. (2004). Immunity to tuberculosis. Annual Review of Immunology, 22, 599-623.

Omran, Abdel R. (1983). The Epidemiologic Transition Theory. A Preliminary Update. Journal of Tropical Pediatrics, 29(6), 305-316. doi: 10.1093/tropej/29.6.305

Omran, Abdel R. (2005). The Epidemiologic Transition: A Theory of the Epidemiology of Population Change. Milbank Quarterly, 83(4), 731-757. doi: 10.1111/j.1468-0009.2005.00398.x

Preston, Samuel H., & Walle, Etienne van de. (1978). French Mortality in the Nineteenth Century. Population studies, 32(2), 275-297.

Puranen, Bi. (1999). Tuberculosis and the Decline of Mortality in Sweden. In Edited by:, R. Schofield, D. Reher & A. Bideau (Eds.), The Decline of Mortality in Europe (pp. 97-117). New York: Oxford University Press.

Remak, Joachim. (1993). A Very Civil War: The Swiss Sonderbund War of 1847. Colorado, USA: Westview Press, Inc.

Rieder, H. L., Zwahlen, M., & Zimmermann, H. (1998). Mortality from respiratory tuberculosis in Switzerland. Sozial- und Präventivmedizin/Social and Preventive Medicine 43(3), 162-166.

Ritzmann-Blickenstorfer, Heiner. (1996). Historische Statistik der Schweiz [Historical Statistics of Switzerland]. Zürich, Switzerland: Chronos Verlag.

Roberts, C. A., & Buikstra, J. (2003). The Bioarchaeology of Tuberculosis: A Global View on a Reemerging Disease. Florida: University Press of Florida.

Rucker, W. C., & Kearny, R. A. (1913). Tuberculosis in Switzerland: Results of the Campaign against the Disease. Public Health Reports (1896-1970), 28(52), 2815-2829.

Schoch, Tobias, Staub, Kaspar, & Pfister, Christian. (2011). Social inequality and the biological standard of living: an anthropometric analysis of Swiss conscription data, 1875-1950. Economics and Human Biology, [in print].

Siegenthaler, Jürg K. (1972). A Scale Analysis of Nineteenth-Century Industrialization. Explorations in Economic History, 10(1), 75-107.

Smith, F.B. (1988). The Retreat of Tuberculosis 1850-1950. New York: Croom Helm Ltd.

Stadt Zürich. (2012). Stadtarchiv - Stadt Zürich Retrieved 7 September 2012, from http://www.stadt-zuerich.ch/content/prd/de/index/stadtarchiv.html

Stead, W. W. (2001). Variation in vulnerability to tuberculosis in America today: Random, or legacies of different ancestral epidemics? International Journal of Tuberculosis and Lung Disease, 5(9), 807-814.

Steinberg, Jonathan. (1996). Why Switzerland? (Second ed.). Cambridge, UK: Cambridge University Press.

Szreter, Simon. (1988). The Importance of Social Intervention in Britain's Mortality Decline c. 1850-1914: a Re-interpretation of the Role of Public Health. The Society for the Social History of Medicine, 1(1), 1-38.

The National Archives. (2012). 1834 Poor Law Retrieved 7 September

Tiemersma, Edine W., van der Werf, Marieke J., Borgdorff, Martien W., Williams, Brian G., & Nagelkerke, Nico J. D. (2011). Natural History of Tuberculosis: Duration and Fatality of Untreated Pulmonary Tuberculosis in HIV Negative Patients: A Systematic Review. PloS ONE, 6(4).

Vögele, Jörg. (1998). Urban mortality change in England and Germany, 1870-1913. Liverpool: Liverpool University Press.

Waddington, K. (2004). To Stamp Out "So Terrible a Malady": Bovine Tuberculosis and Tuberculin Testing in Britain, 1890-1939. Medical History, 48(1), 29-48.

Warren, P. (2006). The evolution of the sanatorium: the first half-century, 1854-1904. Canadian Bulletin of Medical History, 23(2), 457-476.

Wilbur, A. K., Bouwman, A. S., Stone, A. C., Roberts, C. A., Pfister, L. A., Buikstra, J. E., & Brown, T. A. (2009). Deficiencies and challenges in the study of ancient tuberculosis DNA. Journal of Archaeological Science, 36(9), 1990-1997.

Wilbur, A. K., Farnbach, A. W., Knudson, K. J., & Buikstra, J. E. (2008). Diet, tuberculosis, and the paleopathological record. Current Anthropology, 49(6), 963-991.

Wilson, L. G. (2005). Commentary: Medicine, population, and tuberculosis. International Journal of Epidemiology, 34(3), 521-524.

Wolleswinkel-van den Bosch, J. H., Looman, C. W., Van Poppel, F. W., & Mackenbach, J. P. (1997). Cause-specific mortality trends in The Netherlands, 1875-1992: a formal analysis of the epidemiologic transition. International Journal of Epidemiology, 26(4), 772-781.

World Health Organization. (2012). WHO | Tuberculosis, from http://www.who.int/topics/tuberculosis/en/

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3. Skeletal Lesions in Human Tuberculosis May Sometimes Heal: An Aid to Palaeopathological Diagnoses

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Context

As mentioned in the context for manuscript 1, *"Evolution of human tuberculosis: A systematic review and meta-analysis of paleopathological evidence,"* skeletal lesions can develop as the result of active tuberculosis. These can be used to produce differential diagnoses in archeological specimens (Ortner 2003; Steinbock 1976). However, other diseases can produce similar skeletal lesions such as brucellosis, fungal infections, pyogenic osteomyelitis and neoplastic growths. This complicates the diagnosis. Often, when skeletal remains are observed with only destructive lesions, TB becomes a possible diagnosis. However, once bone deposition is observed, TB is often removed as a possibility.

As described previously, TB can be active or latent. When a patient's immunity decreases, active disease may develop (World Health Organization 2012). TB can change from active to latent several times during a person's life.

I was asked by Dr Karl Link to prepare an abstract for the 9th International Congress of the German Society for Anthropology and this resulted in an investigation of Galler Collection skeletal evidence. The initial aim of this manuscript was simply to investigate any differences in skeletal lesion manifestation in association with the introduction of antibiotics in Switzerland. However, an interesting trend was observed and focus was directed to showing how antibiotics and improved patient immunity affected the manifestations of skeletal TB. The results later showed the effect of antibiotics on the healing of skeletal lesions due to TB and resulted in a useful guide for palaeopathology.

These investigations serve mainly as a guide to paleopathologists, for assisting with the diagnosis of atypical cases of skeletal TB. However, this paper also explores the effect of high immunity on the manifestations of skeletal TB. Long term and short

term effects were already investigated previously, but these did not include pharmacotherapies including antibiotics, as a factor in the decline in mortality due to TB. This research then became the next logical step.

The results of this study showed skeletal lesions can heal and appear macroscopically different when this has occurred. Towards the aims of this thesis, the results of this study showed the effects of high levels of immunity through healthcare as well as antibiotics on skeletal lesions resulting from TB. Medical records also gave an indication of the health of the patient during treatment.

Due to the nature of the collection, there is a lot of unexplored information in the medical records which could be later investigated to provide future research opportunities. Additionally, some of the Galler Collection is not yet fully documented and thus more information may be available when this task is completed.

Abstract

In three to five percent of active cases of tuberculosis, skeletal lesions develop. Typically, these occur on the vertebrae and are destructive in nature. In this paper, we examined cases of skeletal tuberculosis from a skeletal collection (Galler Collection) with focus on the manifestation of bony changes due to tuberculosis in various body regions in association with antibiotic introduction. This skeletal collection was created in 1925-1977 by a pathologist at the University Hospital in Zürich, Ernst Galler. It includes the remains of 2426 individuals with documented clinical histories as well as autopsies. It contained 29 cases of skeletal tuberculosis lesions. We observed natural healing of vertebral lesions through several processes including fusion of vertebrae, bone deposition and fusion of posterior elements. In these cases, we observed a higher frequency and proportion of bone deposition and fusion of posterior vertebral elements where pharmacological agents were used. There were also four cases of artificial healing through surgically induced posterior spinal fusion. With the introduction of pharmaceutical treatments, the number of individuals with multiple tuberculous foci decreased from 80% to 25% when compared to individuals who did not receive any drug therapy. Investigation of comorbidities showed that pneumonia, pleuritis and being underweight were consistently present, even with pharmaceutical treatment. Our results have applications in paleopathological diagnoses where healing and consequent bone deposition may complicate differential diagnoses.

Key words: Paleopathology; Galler Collection; Switzerland

Introduction

In palaeopathology, diagnosis of tuberculosis is rare (review in (Holloway et al., 2011)) because skeletal lesions occur in no more than 5% of all active cases while these lesions are not always pathognomonic. Therefore it is important to present well documented cases of skeletal TB involvement to improve the possibility of palaeopathological diagnosis.

Tuberculosis (TB) is primarily a pulmonary disease that can affect individuals of all ages, but occurs mostly among individuals with lowered immune function (Santos and Roberts, 2001; Eley and Beatty, 2009). The most common signs and symptoms of pulmonary TB include localized damage to the lungs resulting in respiratory distress (coughing, difficult breathing, bloody sputum and chest pain), as well as a general "wasting" that may include fatigue, weight loss and pallor (Dormandy, 1999; Wilbur et al., 2008). The clinical manifestation of TB varies depending on the immune status of an individual after becoming infected by the causative agent, *Mycobacterium tuberculosis* (Kaplan, 1959). Signs and symptoms can range from an active, debilitating illness in those with low immunity to a chronic, sub-clinical, latent infection in those with sufficient immunity to control the bacterium (Abdelwahab et al., 1997; Cole et al., 2005).

In three to five percent of cases of active TB, osteolytic skeletal lesions develop; these occur mainly on the vertebrae (Lafond, 1958; Steinbock, 1976). The typical bone lesion for TB is destruction of the anterior region of vertebral bodies with subsequent collapse of the spine (Steinbock, 1976; Ortner, 2003). However, posterior regions are affected in some cases (Abdelwahab et al., 1997). Usually only up to four vertebrae are affected in this process and fusion of partly destroyed vertebrae is commonly observed in the later stages of the disease. Other diseases that can produce similar spinal lesions

include brucellosis, fungal infections, pyogenic osteomyelitis, vertebral fractures and neoplasms (Morse, 1967; Steinbock, 1976; Évinger et al., 2011). A careful differential diagnosis can eliminate other diseases as potential causative agents. Bone lesions caused by TB can occur in other locations on the skeleton, but occur most frequently at major joints such as the hip and knee. These sites account for 15% to 30% and 10% to 20% of non-spinal cases of skeletal TB, respectively (Steinbock, 1976; Roberts and Buikstra, 2003). The typical lesion of these sites is destruction of the articular surfaces of the joint (Steinbock, 1976; Ortner, 2003). No new bone formation is expected; except where the disease has been inactive for a long time (Kaplan, 1959). In general, paleopathological diagnoses based on skeletal lesions do not include TB when bone deposition has occurred as this is not considered characteristic of the disease (Ortner, 2003). There is, however, a possibility of bone deposition occurring in TB cases. This needs to be investigated on bone specimens coming from patients with well-diagnosed TB.

Before the 1940's and 50's the only treatments available for TB were surgical interventions as well as a general improvement in an individual's immunity through rest, good nutrition and hygiene (Kaplan, 1959; Herzog, 1998; Roberts and Buikstra, 2003). These treatments were provided at sanatoria, which increased in popularity in most countries from around the year 1854 (Warren, 2006).

Besides the fairly common rib resection procedure, surgical intervention included posterior spinal fusion which was considered necessary when extensive vertebral destruction had occurred. This procedure was developed in the United States by Dr Fred H. Albee in 1909 (Albee, 1935). In his original article, Albee highlights the importance of immobilizing the intervertebral joints in order to allow ankylosis and consequently healing of the spine to occur. However, because the vertebral joints are

always moving due to breathing and other normal activities, the use of braces and casts does not provide sufficient immobilization of the spine. Albee's original procedure involved using a bone graft from the individual's own tibia, which has been shown to help initiate bone deposition and ankylosis of the spinal joints. It was noted that not only did the grafts internally immobilize the spine, but also provided mechanical support, especially where the spine had collapsed due to severe destruction of vertebral bodies. The bone taken from the tibia also had vascularising ability; healing of the spine through formation of new blood vessels and return of blood flow to areas of the vertebrae severely damaged by TB were both observed.

In 1943, the first antibiotic to specifically combat TB, streptomycin, was produced, but did not become widely available until 1946 (Wilson, 2005; Newsom, 2006). In 1952, a second pharmacological agent called isoniazid (INH) was implemented into TB treatment regimes (Tiemersma et al., 2011). Para-aminosalicylic acid (PAS) was available earlier than this but was not as effective as INH. Unfortunately, these pharmacological treatments were unable to cure all individuals with TB but were certainly able to prolong the life of a person diagnosed with the disease. With the combination of immune based therapy and pharmacological intervention, mortality from TB declined from the 1800's until recently, when the development of drug resistance, the beginning of the HIV epidemic and a shift away from government funded medical support reversed the trend (Corbett et al., 2003; Centre for Disease Control and Prevention, 2011; World Health Organization, 2012).

In our previous work, we have shown using historical records from both the Stadtarchiv ("city-archive") and Staatsarchiv ("canton-archive") in the city of Zürich (Switzerland) that TB was common there during the 19th and early 20th centuries (1893 to 1933). Data were obtained for both the city of Zürich and the entire Canton of Zürich

(Switzerland comprises a number of smaller regions known as "cantons"). A comparison of TB mortality rates for The Netherlands, Denmark, Belgium and England and Wales was conducted, showing that at the beginning of the 20th century.

TB mortality rates were between 150 and 200 per 100,000 living for all countries as well as the Canton of Zürich. However, in the city of Zürich, the mortality rate was higher; at 400 per 100,000. Many countries including Switzerland, show two peaks of TB mortality through time, the first occurring just before 1920 and the other between 1940 and 1950. These correspond to the first and second World Wars as well as the influenza outbreak in 1918 (Spanish flu) (Doege, 1965; Puranen, 1999). During these periods, immunity of populations decreased because of food shortages, hard working conditions, overcrowding and military service (Szreter, 1988). Despite these peaks in mortality, a general trend of decreasing mortality from TB continued through time in Europe and North America.

In this study, our aim was to describe the skeletal manifestation of TB in the well-documented pathology collection covering the period from the early 20th century to 1970s (Galler collection, Switzerland) in order to obtain more information about the processes of skeletal lesion formation and healing through time. This period covers the time where no effective pharmacological treatments for TB were available as well as the time of their introduction (late 1940s) and eventual widespread use. We investigated the number of regions of the body affected by the disease as well as co-morbidities in addition to TB.

Materials and Methods

Skeletal Collection

The skeletal collection used in this study is the Galler collection; initiated in the early 1900's by Ernst Galler and now stored at the Natural History Museum in Basel (Galler 1) and at the University of Zürich (Galler 2). The aim of Ernst Galler was to collect examples of skeletal lesions useful in studies of pathologies affecting bones. It contains the skeletal remains of 597 individuals (Galler 1) and 1829 individuals (Galler 2) from the Zürich Canton who died throughout the 20th century. These individuals were from all backgrounds including, among others, construction workers, health workers and housewives. Medical records, autopsy reports, medical photographs and X-rays are available for many individuals in the collection and it is thus a useful sample for investigating the development of bone lesions with background knowledge of the soft tissue pathology. A summary of Galler 1 can be found in Rühli, et al. (2003), stating that most specimens have original autopsy reports available, in addition to information on age, sex, origin and profession. The partially handwritten reports are approximately four pages in length each with detailed macroscopic and microscopic descriptions as well as a summary of clinical history, autopsy findings and medical treatment history. Descriptions of soft tissue pathology are given, however, for this study we were more interested in skeletal pathologies which were also described. There are avilable photographs of skeletal lesions taken at the time the specimens were deposited. Thus these photographs are of the quality obtainable with earlier photographic techniques, yet they demonstrate sufficently well skeletal lesions. There is no publication describing Galler Collection 2 at this time. It comes from somewhat later time period, has better quality autopsy reports and the specimens were available for direct study and digital photography.

Medical records and autopsy reports for individuals from the Galler Collection were consulted in order to compare changes in TB manifestation during 1925-1977 in Switzerland. For this study, we searched the Galler Collection database for individuals who had been diagnosed with any type of TB (pulmonary, skeletal, major joints, lymph nodes, etc.) during their life. The cause of death did not have to be TB. We found 69 individuals who met this criterion, however, only 29 of these had bone lesions possibly associated with TB. The remainder (40 individuals) were kept in the collection because they were good examples of other bone pathologies, although the individual may have had TB during their lifetime that did not result in skeletal lesions. All were autopsied during the 20th century, but only one (Autopsy Number: 2289, Autopsy Year: 1968) died of TB. We investigated the possibility of other diseases causing the observed bone lesions and firstly consulted the medical records for any evidence of other causes. We reviewed the medical records to determine any evidence of TB at the site of a lesion. Where significant evidence was documented, we considered the lesion to be tuberculous in origin. Due to time and financial restrictions, radiography was not performed, but may be done in future studies. Differential diagnoses were also attempted based solely on skeletal lesions observed and several other possible diseases considered with focus on compression fractures, Paget's disease, osteomyelitis and neoplasms as these were considered the most probable other causes of spinal lesions in this sample (age range was 16 to 98 years). Septic arthritis was considered as a potential alternative diagnosis for cases involving joints such as the hip or knee. The criteria we used for macroscopic diagnosis of spinal TB were based on guidelines described by Ortner (2003) and Steinbock (1976). These criteria included (at least two):

- Destructive lesions on the anterior region of the vertebral body; posterior regions of vertebrae remained unaffected
- Usually 2-4 adjacent vertebrae were affected

- Presence of kyphosis and/or scoliosis
- The lower thoracic and upper lumbar vertebrae were affected (instead of the cervical or sacral regions)
- Intervertebral disc destruction

We did not include minimal bone deposition as a criterion in this study because our aim was to show that bone deposition does indeed occur in TB. Since TB was common in Switzerland at this time, these cases likely represent common manifestations rather than unusual cases.

Individuals were grouped into three time periods based on the availability of pharmacological intervention in Switzerland, starting with streptomycin in 1946 (Wilson, 2005). The time periods are defined in this study as: before 1946 (first period), 1946-1950 (second period) and after 1950 (third period. The third period defines the time period where pharmacological agents (including antibiotics) became widely used to treat individuals. The second time period is used to ensure separation of the periods when antibiotics were not regularly used and when they were available to most individuals. Individual records used did not contain information about pharmacological treatment, but it can be safely assumed that medical practitioners in Switzerland would not deny an individual with TB treatment that was routinely available at the time. Indeed this is true; a Law was passed in 1928 in Switzerland, making it mandatory for cases of TB to be reported and treated (Gesetzgebung: Zürich, 1928). Since all of the individuals in our study were autopsied after 1928, it can be expected that all were recorded and treated in some way.

Osteological lesions of the spine were examined and the level of "healing" was recorded. Healing is defined here as a cessation of osteolytic processes and evidence of healing processes such as ossification of ligaments and fusion of destroyed bones. We

considered both spinal and non-spinal cases of TB in the analysis of skeletal lesion healing. Since this sample included a large number of spinal cases, we identified four types of lesions (Figure 1); no healing (A), fusion of vertebral bodies (B), bone deposition (C) and fusion of posterior elements (e.g. spinous processes) (D). These categories were created in order to separate different processes that occur during lesion healing in TB. Fusion of the anterior regions of vertebral bodies (B) is quite common in TB and is thus considered separately from bone deposition (C) and fusion of posterior elements (D); that do not occur very often in TB. (C) refers to bone deposited on any part of the vertebra, that does not result in fusion. It is uncommon for bone deposition to occur in TB (Ortner, 2003) except through direct fusion of vertebrae. The fusion of posterior elements is rare and not recorded in the literature and thus we considered it separately from normal vertebral fusion and bone deposition, which are far more likely to occur. We considered fusion of posterior elements a sign of well healed TB, as this could not occur unless the disease had become inactive so that destructive processes usually occurring during active disease have stopped. We used these categories also in the cases of non-spinal TB. For example, in a case of hip TB, there can be no healing (Figure 2A), fusion of the joint (2B) and bone deposition (2C) as the lesion heals.

Information was obtained from medical records, autopsy reports and skeletal samples. In all cases there was agreement between the written reports and skeletal samples, but occasionally the records described lesions on bones of the skeleton that were not available for examination. Diagnoses were made by several individuals; firstly the original medical staff to treat these patients, secondly Dr. Karl Link through searching the database for the Galler Collection (this found any cases of TB) and finally through macroscopic investigations completed by Kara Holloway and Maciej Henneberg. What follows are case descriptions each illustrated by at least one photograph. Out of necessity, these photographs are of differing quality, depending on

when they were taken (some in the early 20th century). We also had to curtail the number of photographs due to space limitations. Thus, case descriptions should be treated as primary source of information while photographs only illustrate some major points.

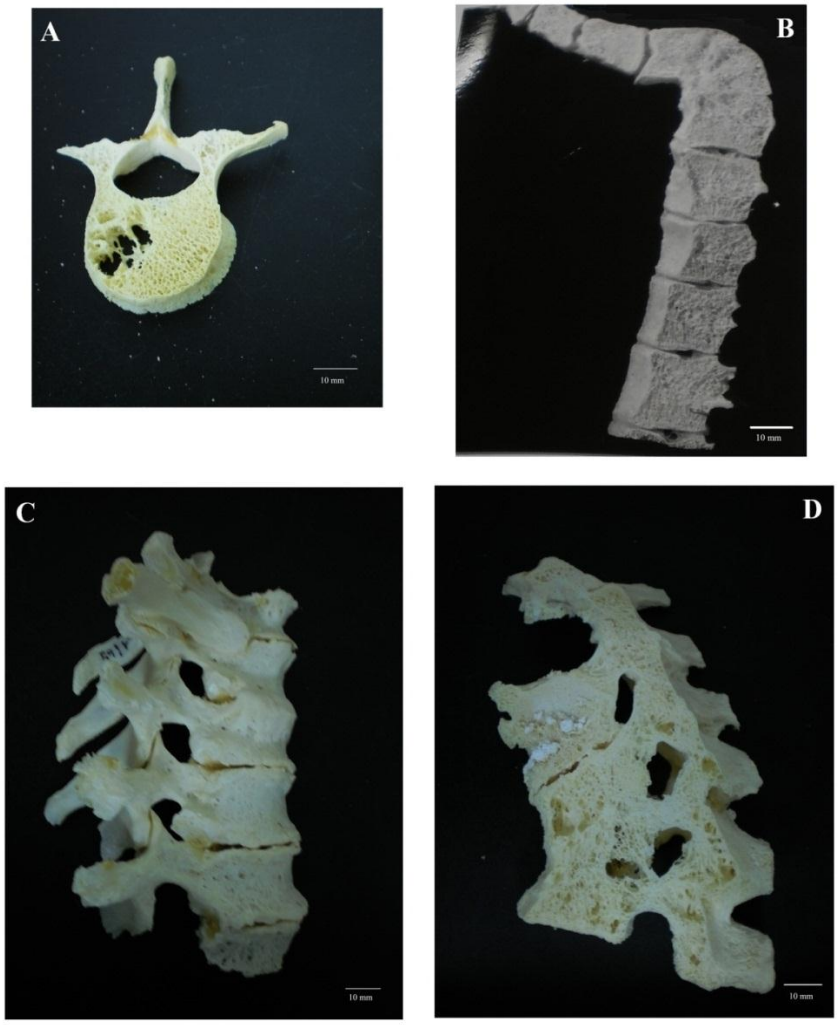

Figure 1: Examples of categories of spinal lesions due to tuberculosis used in this study. All cases presented here are from the Galler Collection and are fully described in Appendix 4. A) Autopsy Number: 1645, Autopsy Year: 1957, No evidence of "healing." B) Autopsy Number: 411, Autopsy Year: 1955, fusion of vertebrae has occurred. C) Autopsy Number: 2461, Autopsy Year: 1969, bone has been deposited on the anterior bodies of affected vertebrae. D) Autopsy Number: 785, Autopsy Year: 1963, posterior elements (as well as vertebral bodies) have fused. Scale bar represents 10 mm

Four individuals had received a posterior spinal fusion operation as a treatment for spinal TB. One was from the first period, one from the second and two from the third.

The number of organs affected by TB in individuals from the Galler Collection was also recorded by using information from medical records. Cases were divided into two groups: those with TB of a single region in the body and those with TB of more than one region. Co-morbidities were also recorded to provide further background information. Average ages at death were calculated along with standard error (=standard deviation/√N). Table 1 shows an overview of the sample and the analysis groups.

Table 1: Overview of the cases of tuberculosis from the Galler Collection (Switzerland).

Analysis conducted	Number of individuals	Average Age (years)	Sex (M/F/?)
Full Collection	69	62±2	27/34/8
First time period (before 1946)	5	39±10	3/2/0
Second time period (1946-1950)	8	57±5	3/4/1
Third time period (After 1950)	51	65±3	21/28/2
Non-spinal TB	3	30±7	3/0/0
Analysis of bone lesion healing	25	60±4	11/14/0
Number of tuberculous foci	61	63±2	24/34/3
Co-morbidities	As above	As above	As above

Results

Cases of spinal tuberculosis

A total of 29 (42%) out of 69 individuals with medically diagnosed TB had observable skeletal lesions. This high frequency of skeletal involvement is unsurprising because the cases were specifically chosen for the presence of exemplary skeletal

lesions. Of the spinal lesions observed, three (10%) were from the first time period, four (14%) from second time period and nineteen (66%) from third time period. Three additional non-spinal cases were discovered for the first (2 cases, 7%) and second (2 cases, 3%) time periods during the original search.

This first part of this study focused on spinal lesions as they are the most commonly recognized diagnostic lesion for TB. Ten representative cases are outlined below that show an array of pathological bone changes observed in the Galler Collection. All additional cases can be found in Appendix 4. The second part of the study describes the three cases of TB affecting a region of the skeleton other than the spine.

First time period (before 1946)

Description: Figure 2 shows the fourth thoracic to the six lumbar vertebrae of a 35 year old male. Spondylitis has affected multiple sites on the spine, including the anterior side of the eighth to twelfth thoracic vertebrae. The individual had back pain in 1937 (two years before death) and the spondylitis was diagnosed at this time. The anterior regions of thoracic vertebral bodies nine to twelve have bone erosion and thoracic vertebra twelve is almost completely destroyed. Lumbar vertebrae one and two are also affected by the disease process. Thoracic vertebra twelve and lumbar vertebra one have collapsed, causing an angulation of the spine. This has also occurred around the eighth and ninth thoracic vertebrae. The vertebral discs beginning at the level of thoracic vertebra eleven and ending at the first sacral vertebra are all destroyed. There is a small, circular lesion observable on the anterior surface of lumbar vertebra one (visible in Figure 2). It measures approximately 2 mm x 4 mm. There is also a small

amount of bone deposition on the anterior surface of the first sacral vertebra (visible in Figure 2). There is no evidence of healing of the spinal lesions.

In differential diagnosis, compression fractures, Paget's disease, osteomyelitis and neoplasms were considered, however, there were no mentions of these diseases in the medical records for this individual. This case shows anterior destruction of a single vertebra, which is consistent with TB. There is a mild kyphosis and the posterior elements are unaffected. In addition to the diagnosis of spondylitis TB, we considered this to be a case of TB.

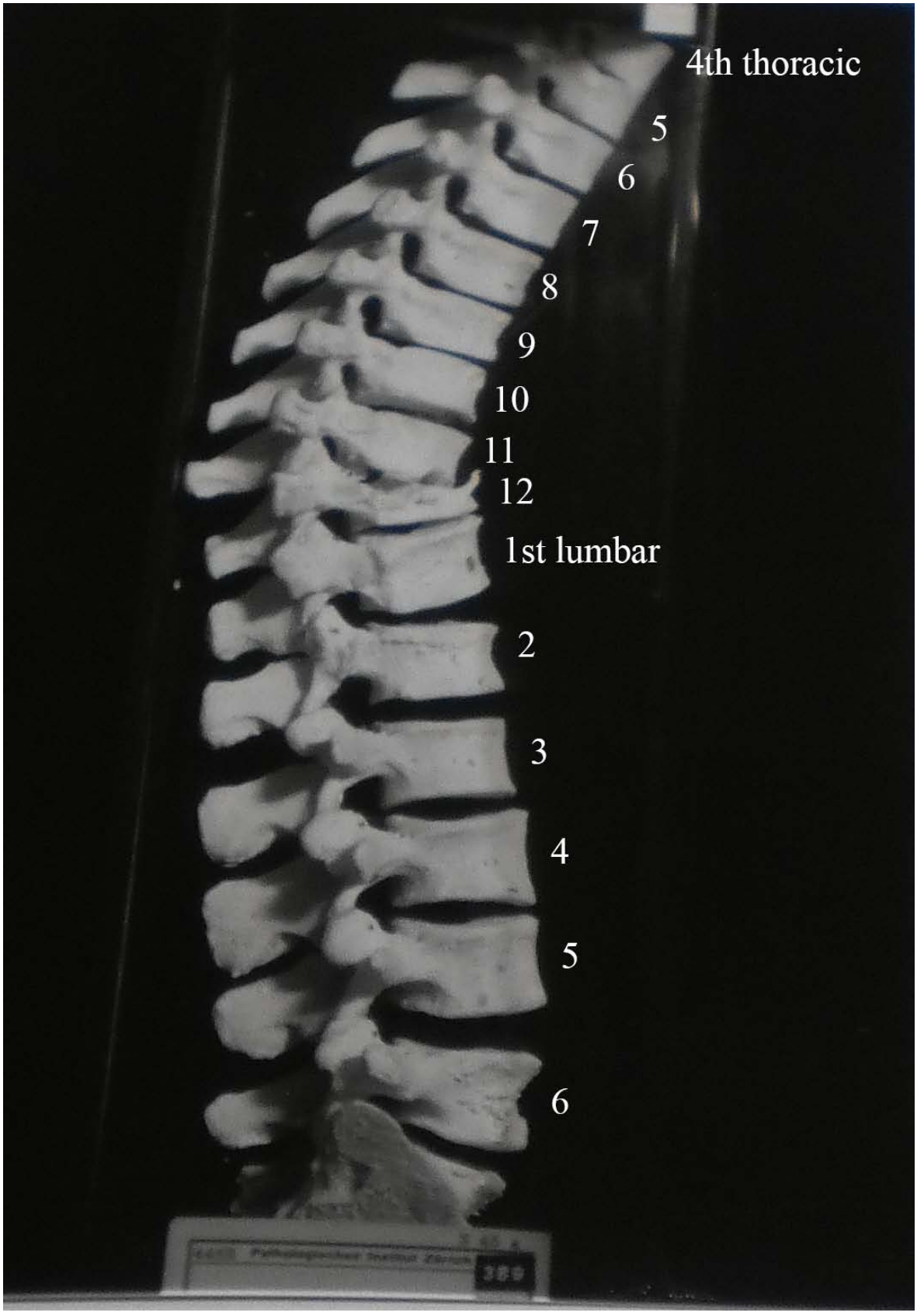

Figure 2: Autopsy Number: 751, Autopsy Year: 1939, Age: 35, Sex: Male.

Description: Figure 3 shows first thoracic to sixth lumbar vertebrae of a 78 year old female. She had spondylitis of the eleventh and twelfth thoracic as well as the first lumbar vertebrae. An abscess is present on the left side of the first lumbar vertebra. There is also healed spondylitis of thoracic vertebrae six through nine, involving fusion and kyphosis. The fusion is extensive and involves the entire vertebral disc surface of all vertebrae. There is an abscess on thoracic vertebra eleven, measuring approximately 4.5 mm x 7 mm. The disc between thoracic vertebrae eight and nine has been destroyed. Other than thoracic vertebrae six to nine, eleven, twelve and lumbar one, all other vertebrae appear normal. There is reduction of intervertebral disc space between the fourth and fifth lumbar vertebrae as well as between the fifth and sixth lumbar vertebrae. This individual also had her right leg and a finger amputated due to TB. Healing has occurred in this case through fusion of vertebrae.

Differential diagnosis included compression fractures, Paget's disease, osteomyelitis and neoplasms, but these were not mentioned in the medical diagnoses. Additionally, in this case, more than four vertebrae were affected, but in two different regions of the spine. This was a result of two separate events. Considered separately, only four and three vertebrae are affected in these two separate instances. The skeletal lesions are destructive and occur on the anterior regions of vertebral bodies. In the upper thoracic region, the vertebral destruction has led to kyphosis. Posterior regions of the vertebrae were unaffected. Thus we considered this a case of TB.

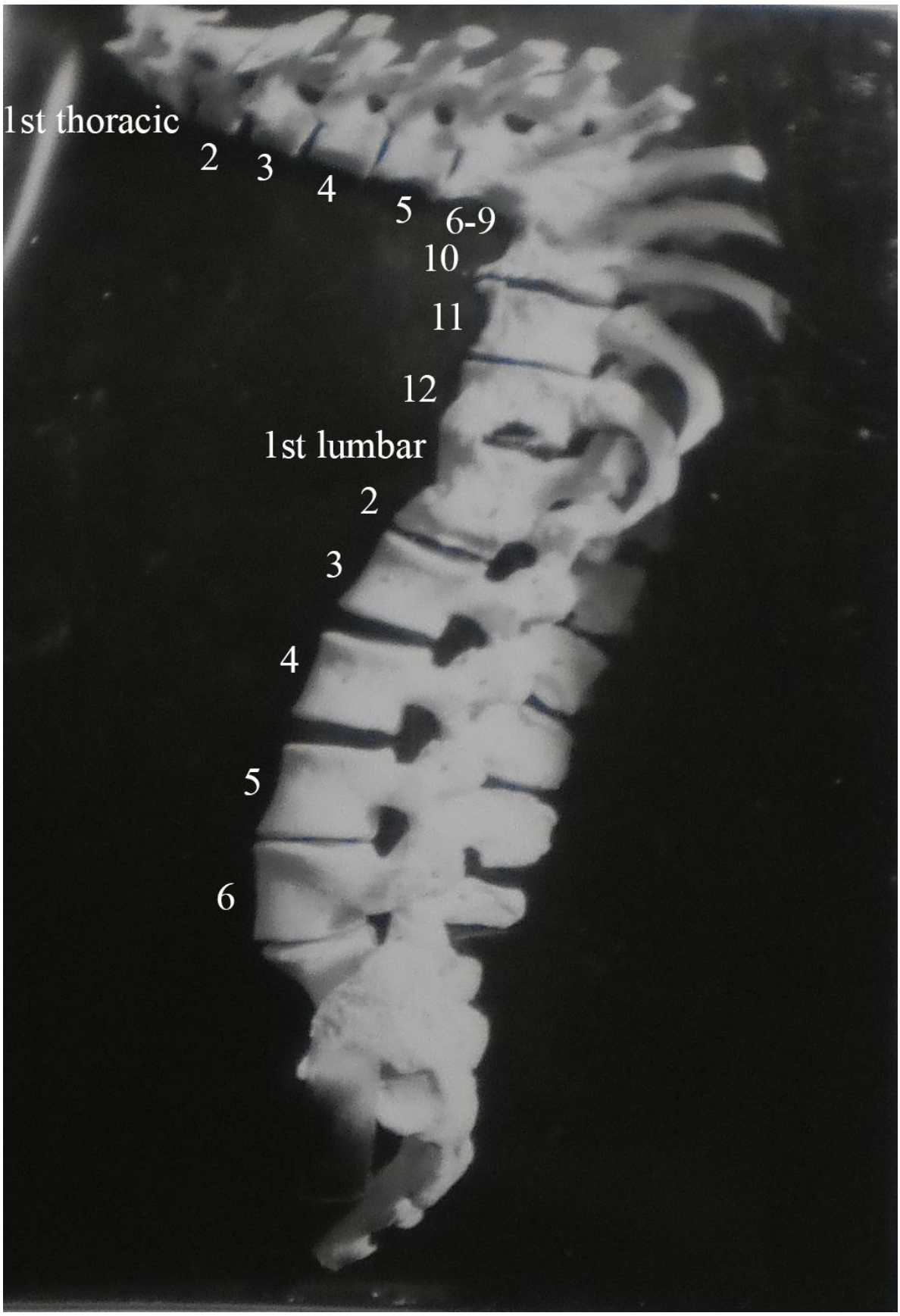

Figure 3: Autopsy Number: 793, Autopsy Year: 1936, Age: 78, Sex: Female.

Description: Figure 4 shows lower thoracic and upper lumbar vertebrae of a 65 year old female. We were unable to determine which thoracic vertebrae were involved here. Lytic lesions are present on the anterior of lower thoracic and upper lumbar vertebral bodies and have led to collapse of the spine. Healing has occurred through spontaneous fusion of vertebrae and posterior spinal elements near the site of collapse.

There was no indication of other diseases likely to have possibly caused the skeletal lesions (compression fractures, Paget's disease, osteomyelitis and neoplasms) in the medical records. Pulmonary TB was described and the destruction of the anterior region of the body of a single thoracic vertebra with resulting kyphosis indicates TB as the most likely cause of the lesions.

Figure 4: Autopsy Number: 732, Autopsy Year: 1949, Age: 65, Sex: Female.

Description: Figure 5 shows the fifth thoracic to the second lumbar vertebrae of a 52 year old female. Both thoracic vertebra seven and lumbar vertebra one were affected by TB spondylitis. The seventh thoracic vertebra has been completely destroyed, leading to collapse of the spine. The collapse of this vertebra resulted in a 90 degree angulation of the spine. Thoracic vertebrae six through twelve as well as lumbar vertebra one were all affected by the disease process. The spondylitis at the first lumbar vertebra has manifested as a deposition of bone on the superior surface of lumbar vertebra two which has caused destruction of the adjacent bone on the inferior surface of lumbar vertebra one. The bony protrusion is "pointed" and measures approximately 7 mm x 2 mm. Healing has occurred by fusion of some posterior spinal elements. Interestingly, there is very little fusion between vertebral bodies, except between thoracic vertebrae eleven and twelve.

There was limited information regarding this individual in the medical records. It specifically details the presence of TB spondylitis of thoracic vertebra seven and lumbar vertebra one. Based on the specific destruction of the seventh thoracic vertebral body and resulting kyphosis, this diagnosis would be accurate.

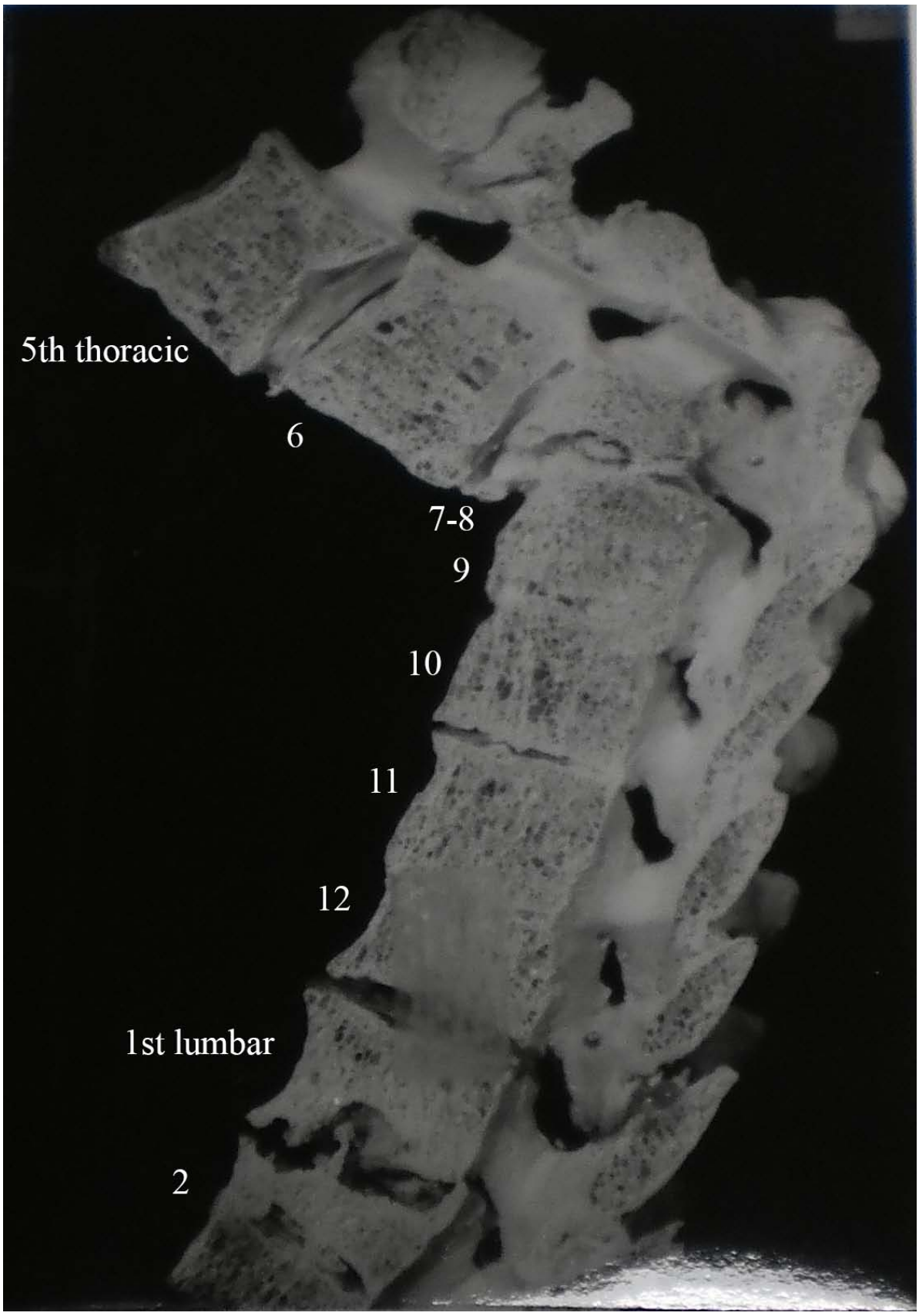

Figure 5: Autopsy Number: 901, Autopsy Year: 1948, Age: 52, Sex: Female.

Third time period (After 1950)

Description: Figure 6 shows the eleventh thoracic to fifth lumbar vertebrae of a 67 year old female. Lumbar vertebrae two to five have fused together through extensive bone deposition between vertebral bodies. There is evidence of lipping on the anterior edges of these vertebral bodies. Major lipping as a result of bone deposition is also present on the inferior edges of thoracic vertebra twelve and lumbar vertebra one. The TB spondylitis of lumbar vertebrae three to five occurred between 1933 and 1939; 20 years before death. This individual also had TB of the right knee and this became involved in 1949; 7 years before death. At autopsy, it was discovered that the knee was ankylosed and unable to be moved.

There was no mention of other diseases such as compression fractures, Paget's disease, osteomyelitis or neoplasms that might have been the cause of the skeletal lesions observed. The medical records describe the presence of TB spondylitis of the lower lumbar vertebrae as well as the knee. In this case, there is no kyphosis or collapse of the spine. There is an extensive amount of fusion, however this does not include posterior spinal elements. Due to the involvement of a single knee joint (TB is usually unilateral), it is likely that the spinal lesions are also caused by TB.

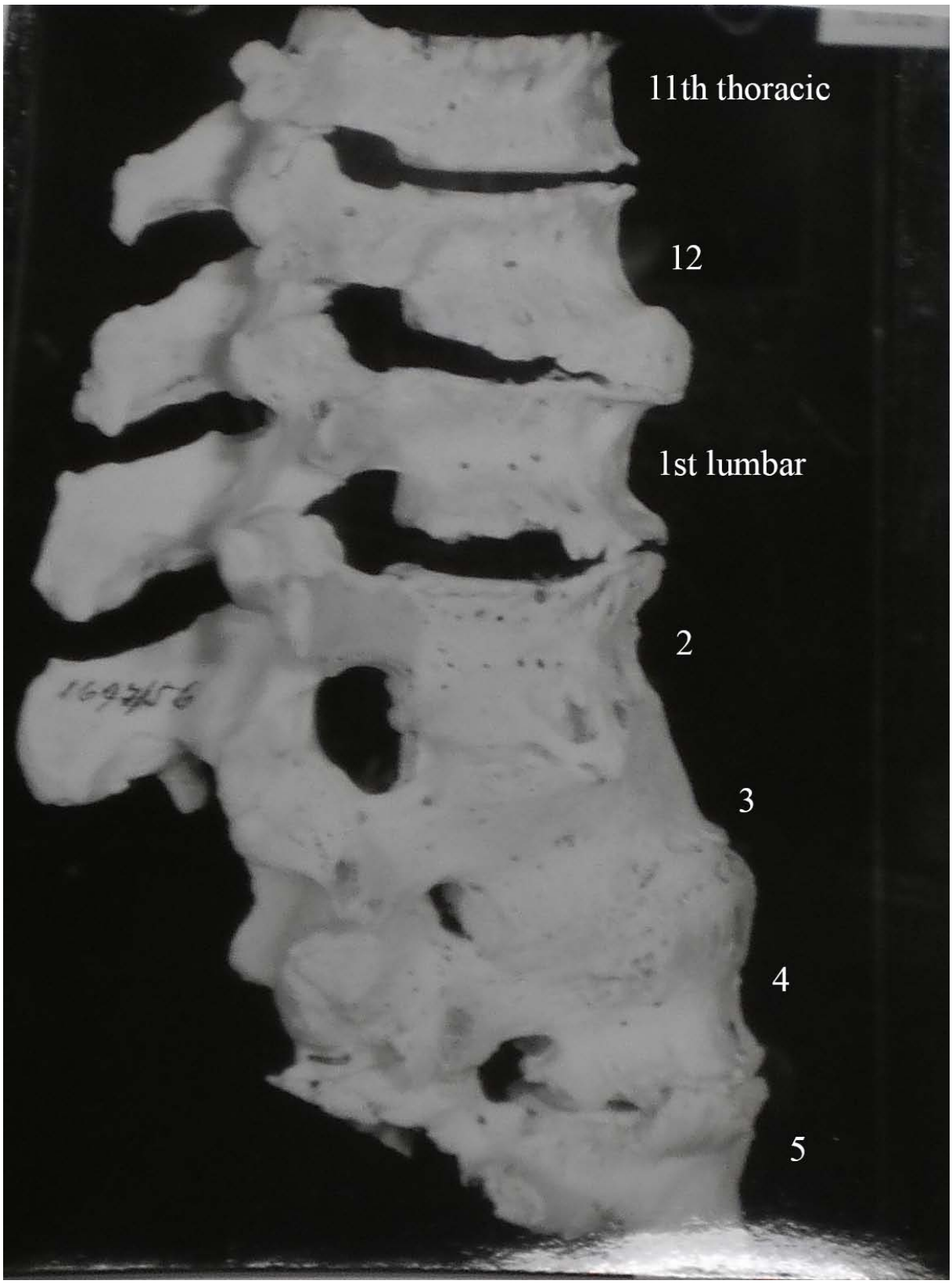

Figure 6: Autopsy Number: 1697, Autopsy Year: 1956, Age: 67, Sex: Female.

Description: Figure 7 shows lower thoracic vertebrae of a 52 year old female. We were unable to determine which vertebrae are involved in this case. One of the vertebral bodies has been almost completely destroyed and the inferior surface has fused to the superior surface of the adjacent vertebra. There has been a large amount of bone deposition on the anterior edge of the vertebra below that which had collapsed; effectively creating a bridging structure. This extends across two vertebrae, skipping the one which had collapsed. Healing in this case is by bone deposition.

There was mention of neoplasms in the liver and iliac lymph nodes in this individual. There are details regarding the surgery attempted. The medical records also describe TB spondylitis of the thoracic vertebrae. One of the vertebrae has been almost completely destroyed, with the lytic lesion starting in the anterior region of the vertebral body. This has resulted in collapse of the spine. Posterior elements were unaffected. These are characteristics of TB rather than metastases and thus we considered TB the most likely cause of these lesions.

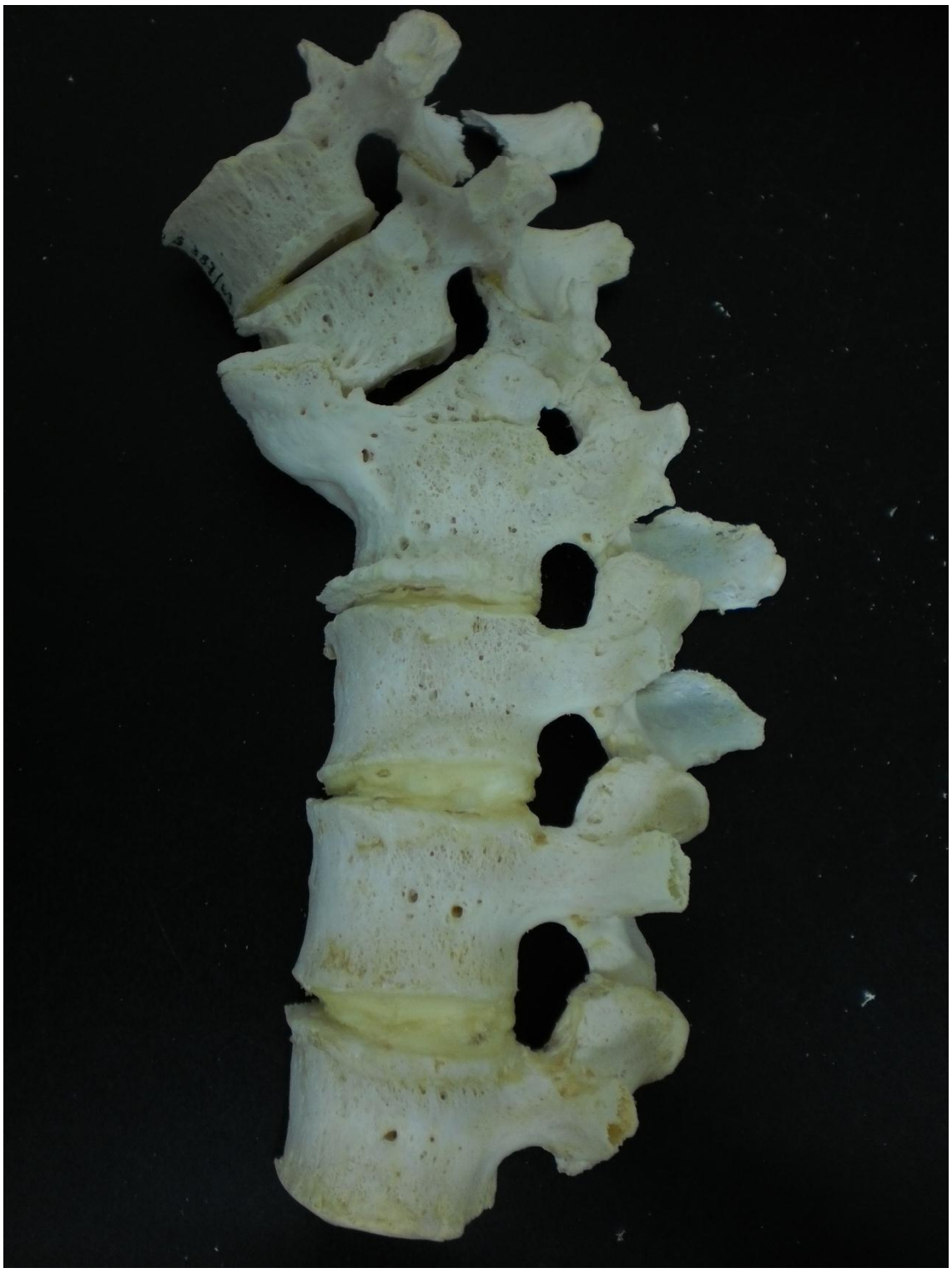

Figure 7: Autopsy Number: 387, Autopsy Year: 1963, Age: 52, Sex: Female.

Cases of spinal tuberculosis treated with surgically induced posterior spinal fusion

Four individuals from the Galler Collection were treated by this method. These are summarized below.

First time period (Before 1946)

Description: Figure 8 shows the fifth thoracic to fifth lumbar vertebrae of a 30 year old female. She had received a spinal operation for tuberculosis spondylitis, resulting in destruction of the anterior region of thoracic vertebrae ten to twelve. The spine has collapsed and spinal angulation had occurred. The lesions are completely destructive, however, a small mass of bone has been deposited on the left side of lumbar vertebrae one and two. This bone deposit measures approximately 22 mm x 11 mm. No healing of the vertebral collapse is evident.

Since the autopsy of this case was carried out in 1928, there is limited information in the medical record. Spondylitis of the thoracic vertebrae due to TB was described. There was no mention of compression fractures, Paget's disease, osteomyelitis or neoplasms. However, based on the lesions observed, it is likely that TB is the cause. There is extensive destruction of the anterior regions of several thoracic vertebral bodies, leading to a collapse of the spine. The posterior elements were unaffected by the disease process, however, there was artificial fusion of the spine through surgical intervention.

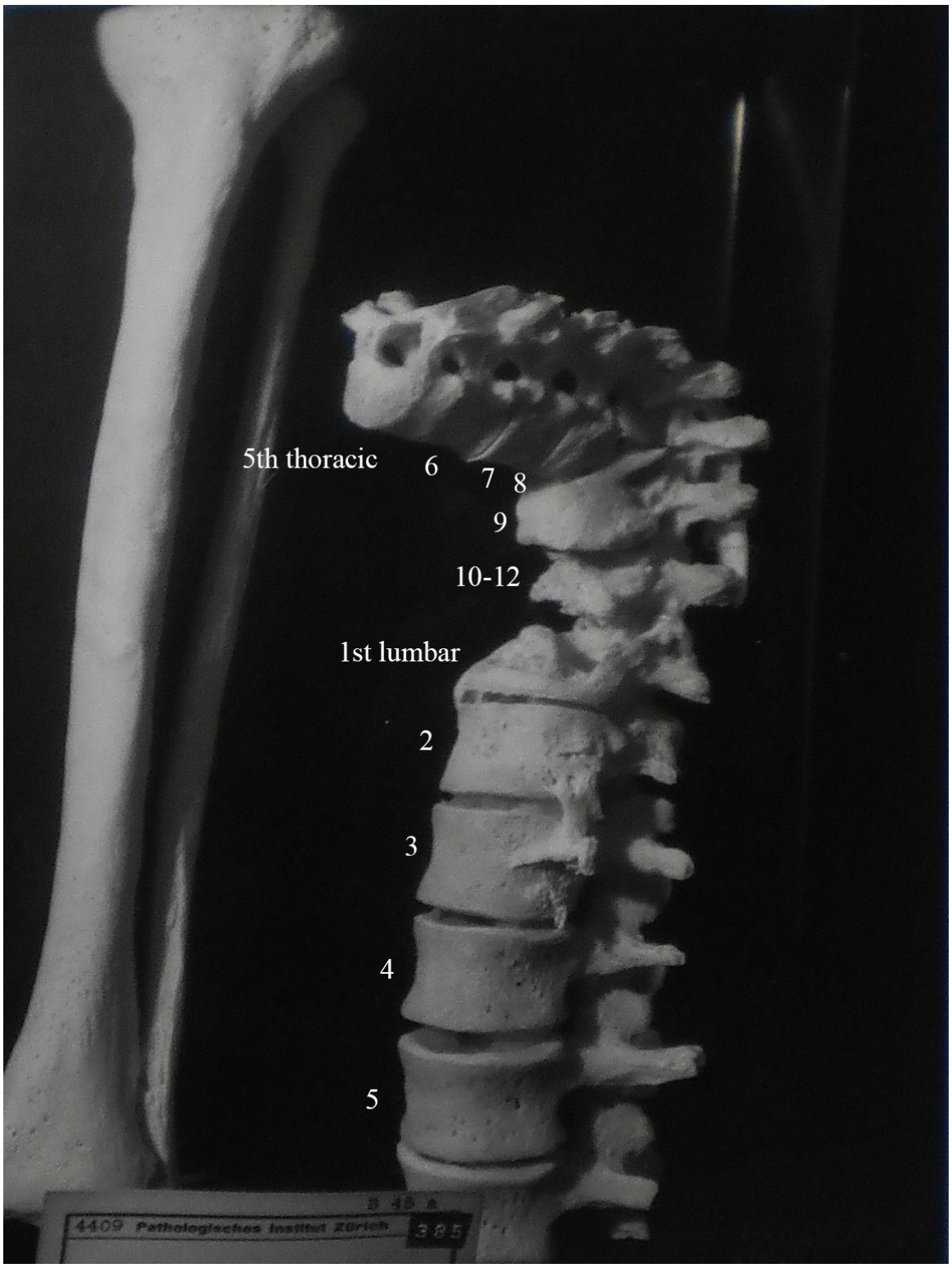

Figure 8 (part one): Autopsy Number: 7, Autopsy Year: 1928, Age: 30, Sex: Female.

Figure 8 (part two): Autopsy Number: 7, Autopsy Year: 1928, Age: 30, Sex: Female.

Second time period (1946-1950)

Description: Figure 9 shows the eighth thoracic to third lumbar vertebrae of an individual with unknown age and sex. Extensive fusion on all edges (anterior, lateral and posterior) of thoracic vertebra nine to lumbar one has occurred. There is slight spinal angulation around thoracic vertebra eleven. This individual was treated with a spinal operation, 24 years before death and it was successful in that further spinal destruction has been avoided in this case. This operation was performed due to spondylitis of thoracic vertebrae eleven and twelve as well as lumbar vertebrae one to three. Healing has occurred spontaneously by bone block formation between vertebral bodies (fusion of vertebrae). Although bone deposition has occurred around posterior elements, upon examination, it was found these were not fused together. In this case, it is interesting to note that the level of bone deposition and vertebral fusion obscures any remaining destructive lesions, indicating this is a well healed case and the disease has been inactive for some time.

There is no mention in the medical records regarding compression fractures, Paget's disease, osteomyelitis or neoplasms, but there is a description of TB spondylitis. This case is more difficult to diagnose because of the extensive amount of bone deposition, atypical for TB. There is presence of a mild kyphosis and this is a case where TB was inactive for many years. Thus we can only diagnose this case as TB using information obtained from medical records, suggesting the possibility that these lesions may be the result of a condition other than TB. However, since this case involved artificial fusion of posterior spinal elements, it was not included in other analyses and therefore the inability to give a confident diagnosis does not affect our results.

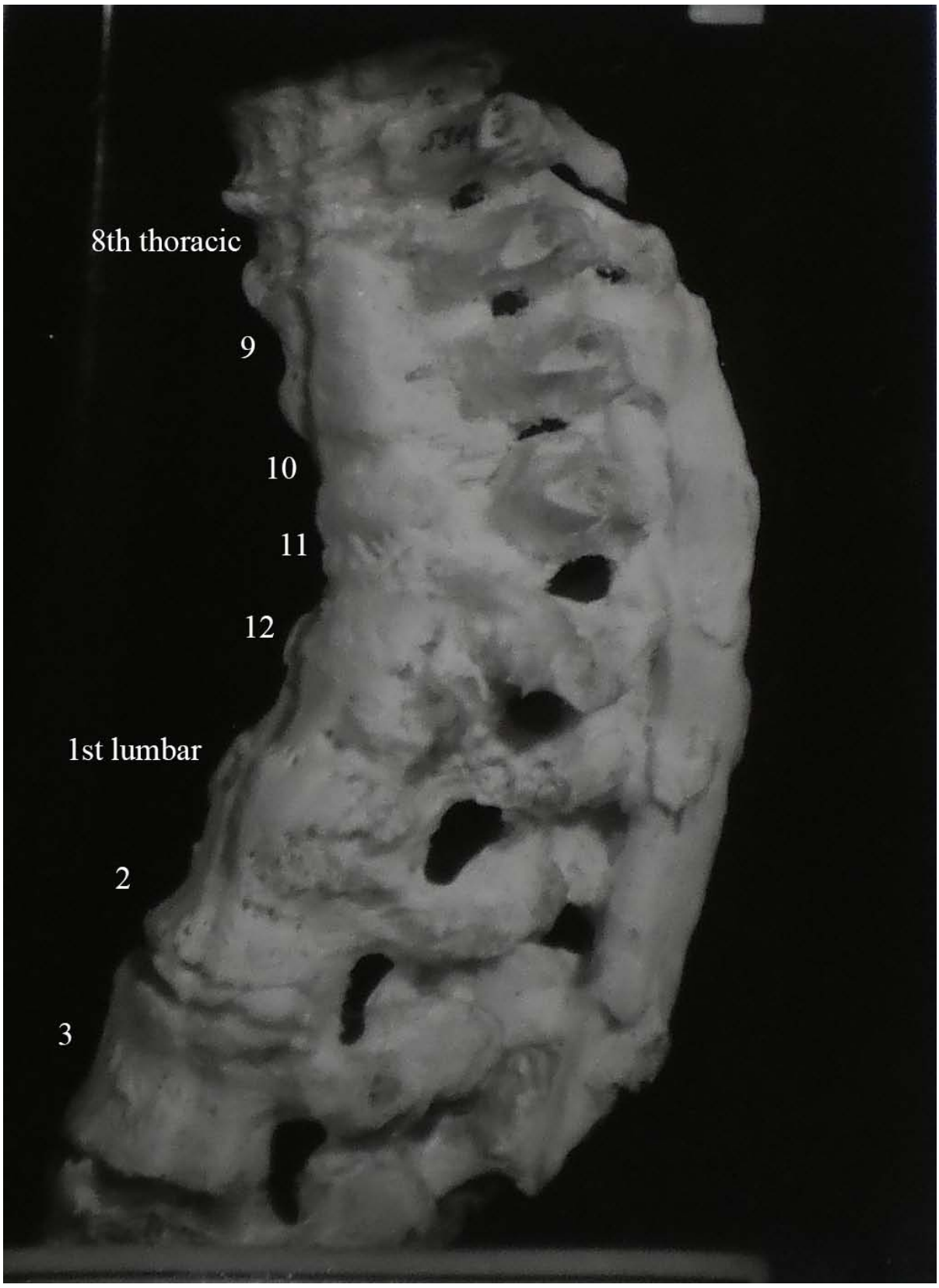

Figure 9: Autopsy Number: 262, Autopsy Year: 1948, Age: No information, Sex: No information.

Third time period (After 1950)

Description: Figure 10 shows lumbar vertebrae four and five as well as the sacrum of a 50 year old female (anterior view). Minor destructive lesions are evident on the fourth and fifth lumbar vertebrae as the result of tuberculosis spondylitis starting 39 years before death, but this diagnosis was not made until 1925 (28 years before death). The individual was treated with a spinal operation and in this case, it has assisted in preventing massive destruction of the vertebrae. Both the diagnosis of spondylitis and the spinal operation occurred 39 years before death. Healing has occurred through a large amount of vertebral fusion including posterior elements. Lumbar vertebrae two to four are fused, four and five are fused into a square block and there is evidence of vertebral fusion of thoracic vertebra twelve to lumbar vertebra three. In addition to bony changes of the vertebrae, there was also extensive destruction of the sacrum.

The medical records describe spondylitis TB for this individual. No mention is made of compression fractures, Paget's disease, osteomyelitis or neoplasms. The lesions observed also indicate TB rather than another causative agent. There were lytic lesions on lumbar vertebrae and fusion of these vertebrae has resulted.

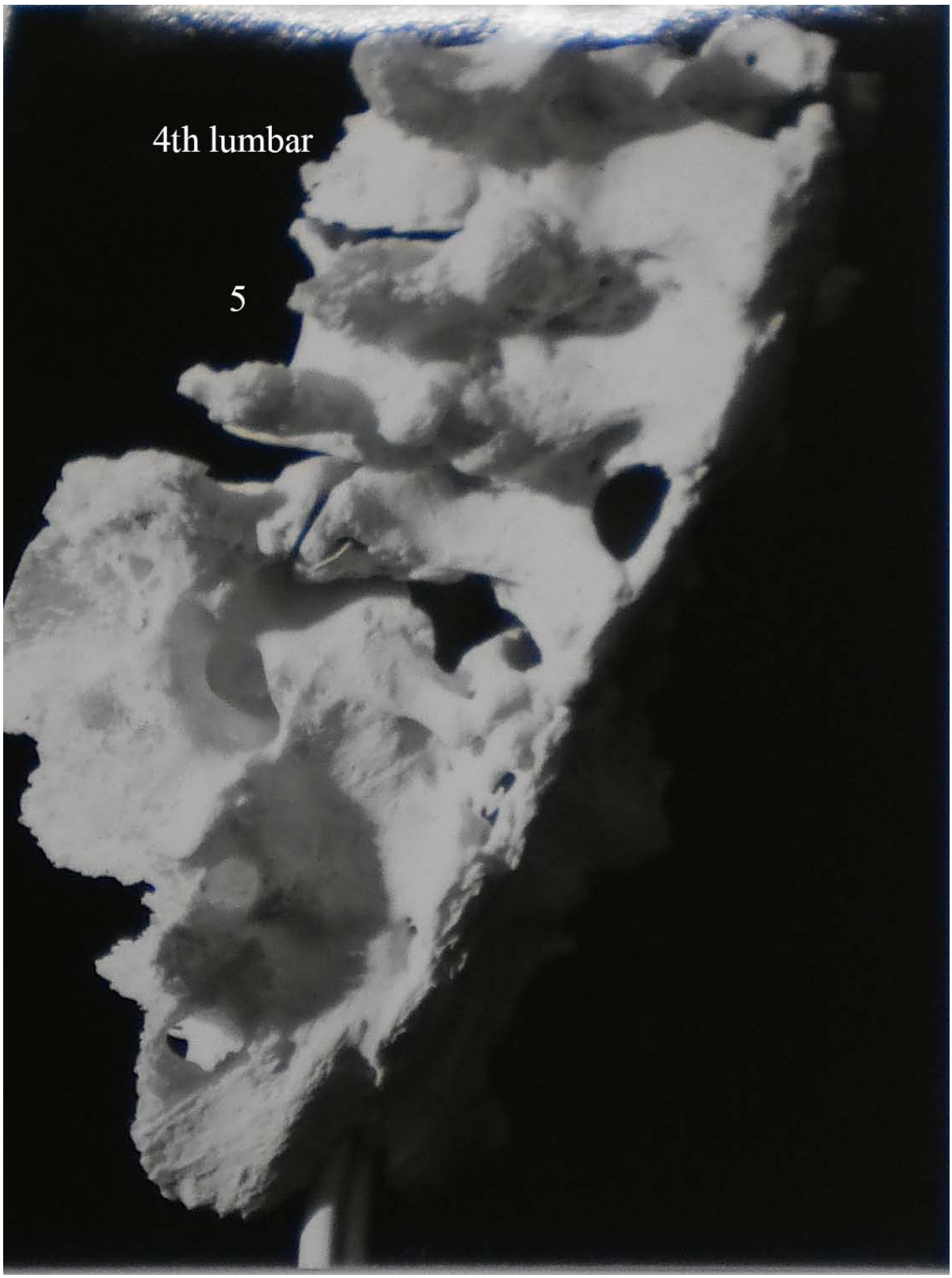

Figure 10: Autopsy Number: 779, Autopsy Year: 1953, Age: 50, Sex: Female.

Description: Figure 11 shows thoracic vertebrae of a 36 year old female. We were unable to identify which thoracic vertebrae these are. A small, circular lesion is present on the thoracic vertebra at the bottom of Figure 11. It measures approximately 4 mm x 3 mm. A spinal operation was performed eight years before death. Two vertebrae have fused spontaneously along the entire surface area of their vertebral bodies. Additional stabilization of the spine is provided by the surgical intervention.

Medical records describe spondylitis TB, but not compression fractures, Paget's disease, osteomyelitis or neoplasms. Only two thoracic vertebrae are involved, with destruction of the vertebral bodies, leading to a collapse of the spine. The two affected vertebrae have fused together. These are all characteristics of TB and thus we diagnosed this as a case of TB.

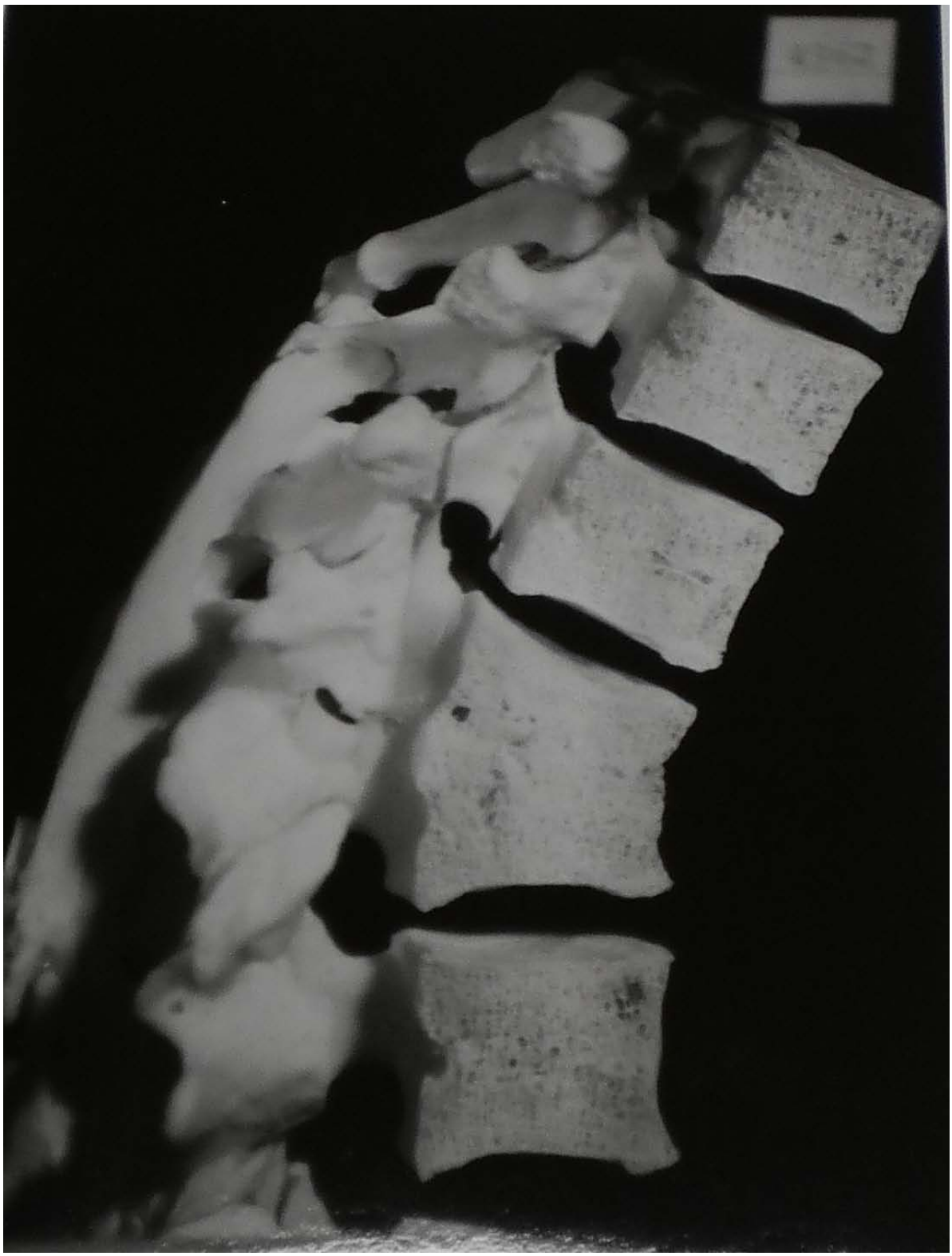

Figure 11: Autopsy Number: 5717, Autopsy Year: 1964, Age: 36, Sex: Female.

Cases of skeletal tuberculosis not involving the spine

Description: Figure 12 shows the right femur and acetabulum of a 16 year old male. There is extensive destruction of the articular surfaces of the hip joint. Although the individual had TB (beginning with meningeal) since 1924 (4 years old), bone destruction in the hip did not begin until shortly after he sustained an injury during 1929. Three years later, he returned to hospital. No evidence of healing is present.

We considered the possibility that these lesions could be the result of septic arthritis. Usually in septic arthritis, the bone destruction is limited and ankylosis very common (Williams and Snortland-Coles, 1986; Aufderheide and Rodriguez-Martin, 1998). This case shows extensive destruction of both articular surfaces of the hip and no evidence of ankylosis. This individual was also very young and TB of the hip usually begins at an early age. Additionally, this case was from the first time period (before 1946) where antibiotics were unavailable. If the cause of these lesions were septic arthritis, it is likely this individual would have died before bone lesions could have developed (McLain, 1991). This case was thus considered to be the result of TB.

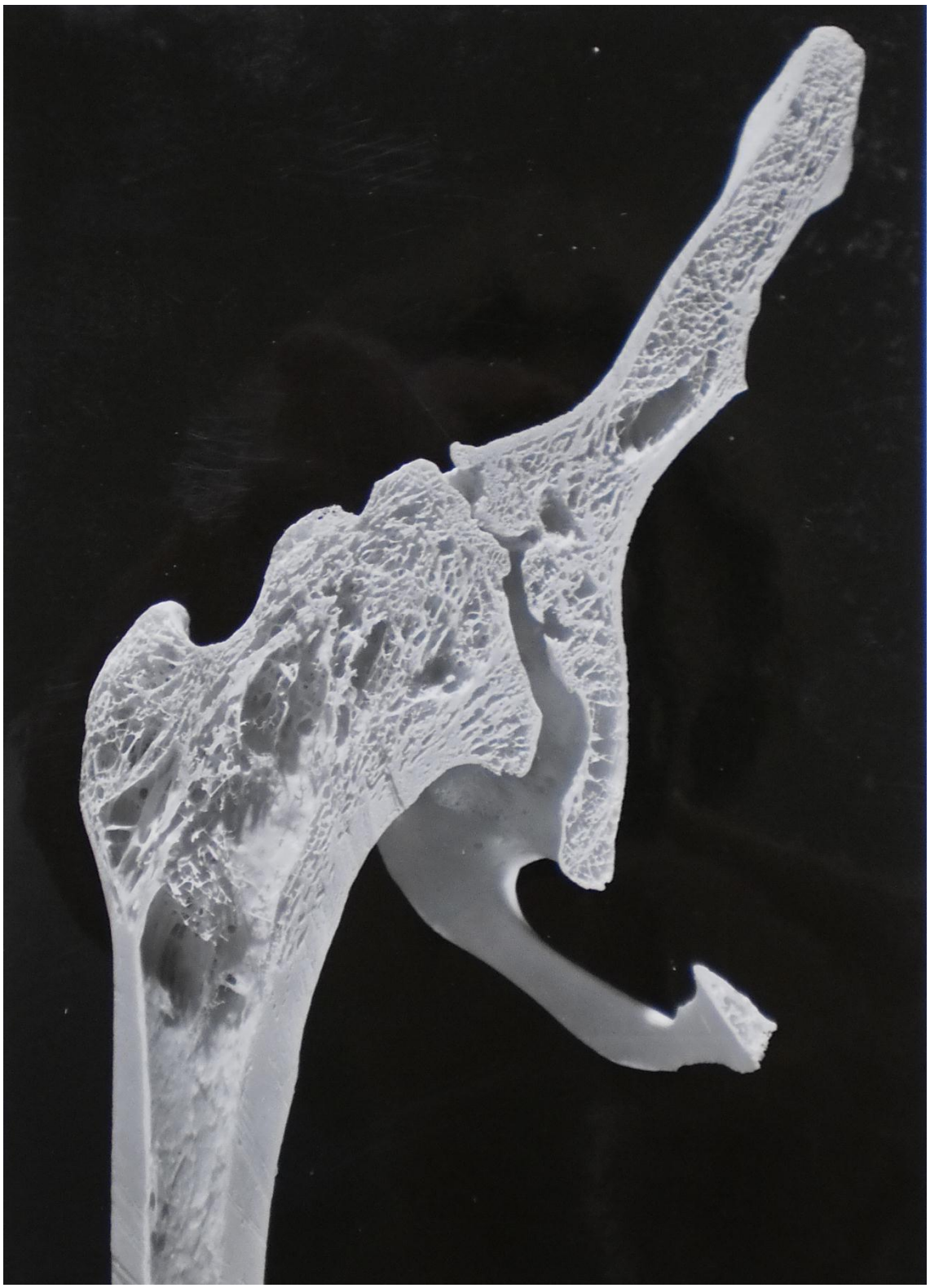

Figure 12: Autopsy Number: 325, Autopsy Year: 1936, Age: 16, Sex: Male.

Description: Figure 13 shows the foot bones and distal tibia and fibula of a 36 year old male. The individual also had skeletal TB of the metacarpal, elbow and left temporal bone since he was a child. These lesions, however, were not preserved in the collection. The final results of TB processes on the foot were healed by fusion of the fibula and tibia. This fusion had been going on for 20 years before death, indicating it was well healed and that the disease had been inactive for some time. The individual also received a rib resection and the right elbow is ankylosed to the point where it has become immobile.

This individual suffered from severe sepsis around the time of death. Due to this, the ankylosis of the elbow may be a result of septic arthritis rather than TB. However, for this study, we are more concerned with the bony changes in the foot because there is documented evidence of tuberculous diseases of this region. The medical records describe a well-healed example of foot TB and that other bones in the body were affected. Although the cause of death in this case would likely have been due to sepsis, TB occurred earlier in this person's life and the bone lesions are well healed at death. This indicates that sepsis was not the cause of these lesions, or healing would not have been observed.

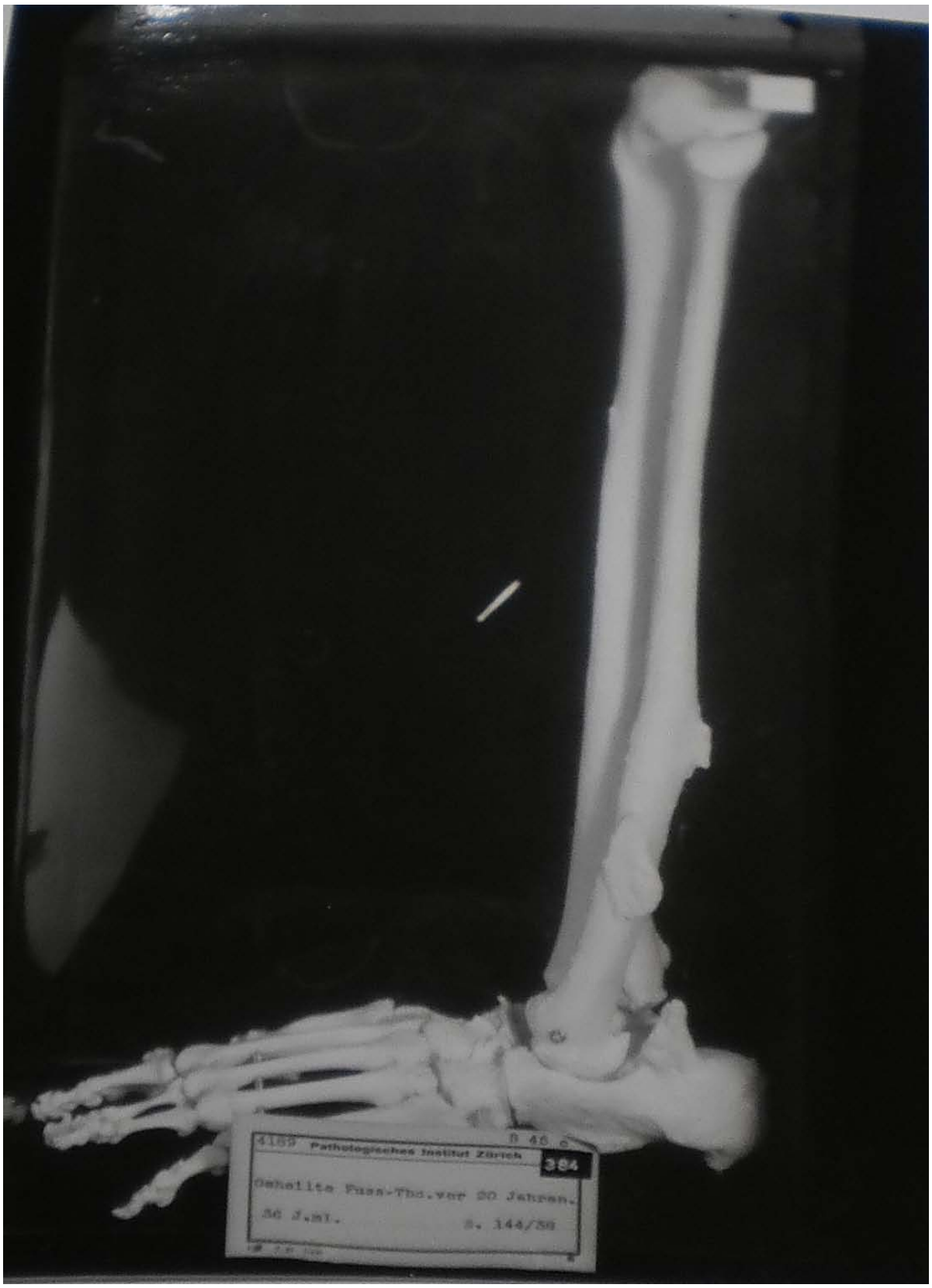

Figure 13: Autopsy Number: 144, Autopsy Year: 1938, Age: 36, Sex: Male.

Description: Figure 14 shows the right femur and acetabulum of a 37 year old male. There is a high level of destruction of articular surfaces of the femur and acetabulum due to TB infection. The femoral head is almost completely destroyed. The joint surface is mostly destroyed, however the joint is still mobile.

The diagnosis recorded in the medical records was one of two possibilities; TB or heart failure. Doctors were unable to decide. Although the cause of death could have been TB in this case, we are concerned with the etiology of the skeletal lesions of the hip. The medical records do clearly describe damage to the hip after infection with TB. The extensive destruction of the bone on the articular surfaces of the joint without ankylosis indicates TB is more probable than septic arthritis.

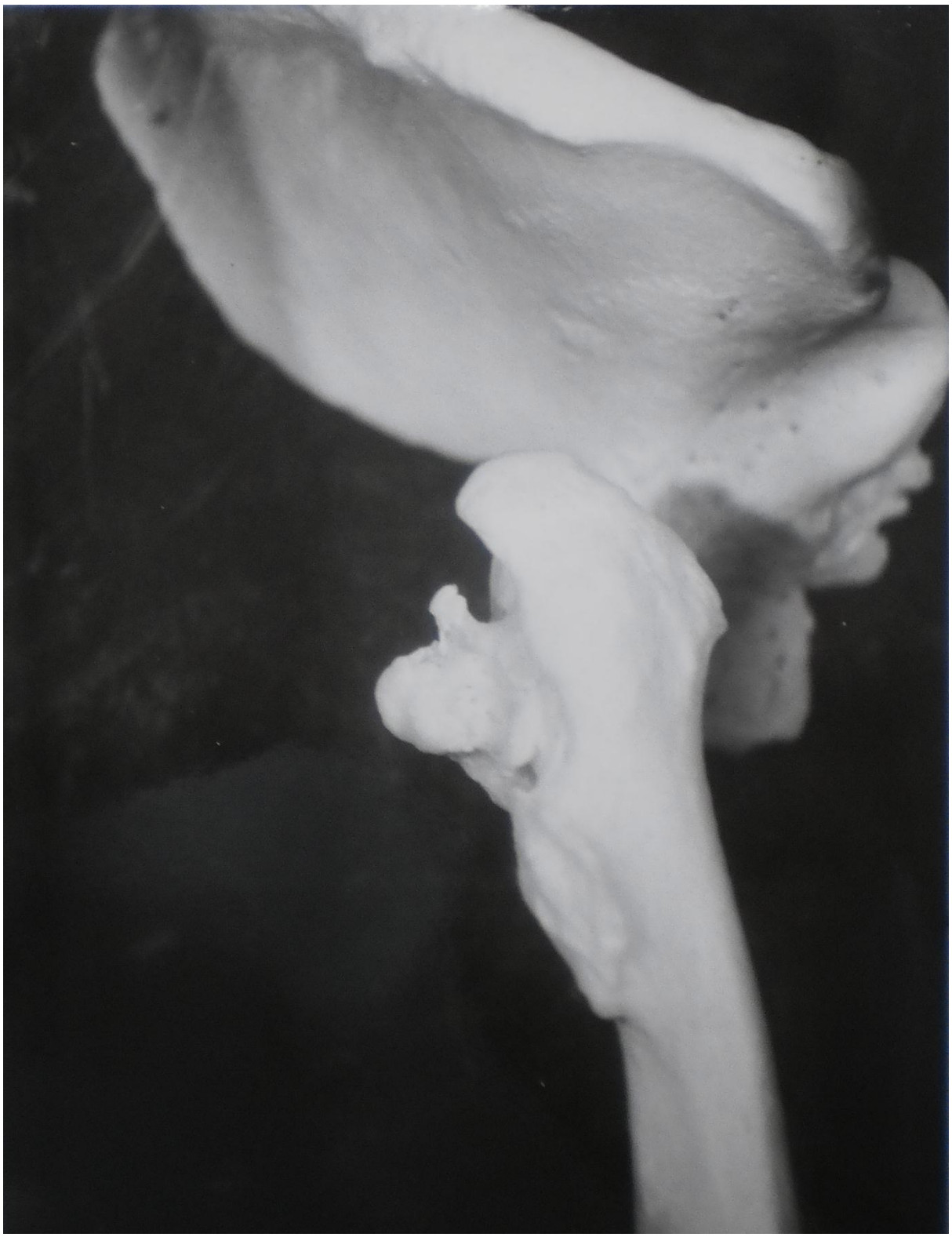

Figure 14: Autopsy Number: 304, Autopsy Year: 1947, Age: 37, Sex: Male.

Analysis of bone lesion healing

There were two categories of lesion healing in the Galler Collection cases; the first followed targeted surgery for bone lesion repair and the second involved natural healing. The latter involved interventions not directly aimed at healing spinal lesions, but rather aiding the individual to combat the disease. The cases of artificial healing by posterior spinal fusion have been presented (N=4), so we now focus upon the cases of natural healing (N=25). The presence and extent of natural bone lesion healing in individuals from the Galler Collection are presented in Figure 15.

These results show a trend through time correlating with availability of pharmacological agents. Lesion healing in the first time period either did not occur or was achieved through fusion of vertebrae (which commonly occurs in skeletal TB). There were two cases where no healing of spinal lesions occurred and one with healing. However, in the second time period, where pharmacological agents (including antibiotics) were first being used to treat individuals, there are cases of healing involving fusion of posterior spinal elements. There was one case of no healing and three where healing had occurred. Further use and implementation of chemotherapeutic regimes resulted in all categories of bone lesion healing considered in this study, being present in samples from the third time period, including vertebral fusion, bone deposition, and fusion of posterior elements. In this time period, there was one case of unhealed skeletal lesions and 16 with healing.

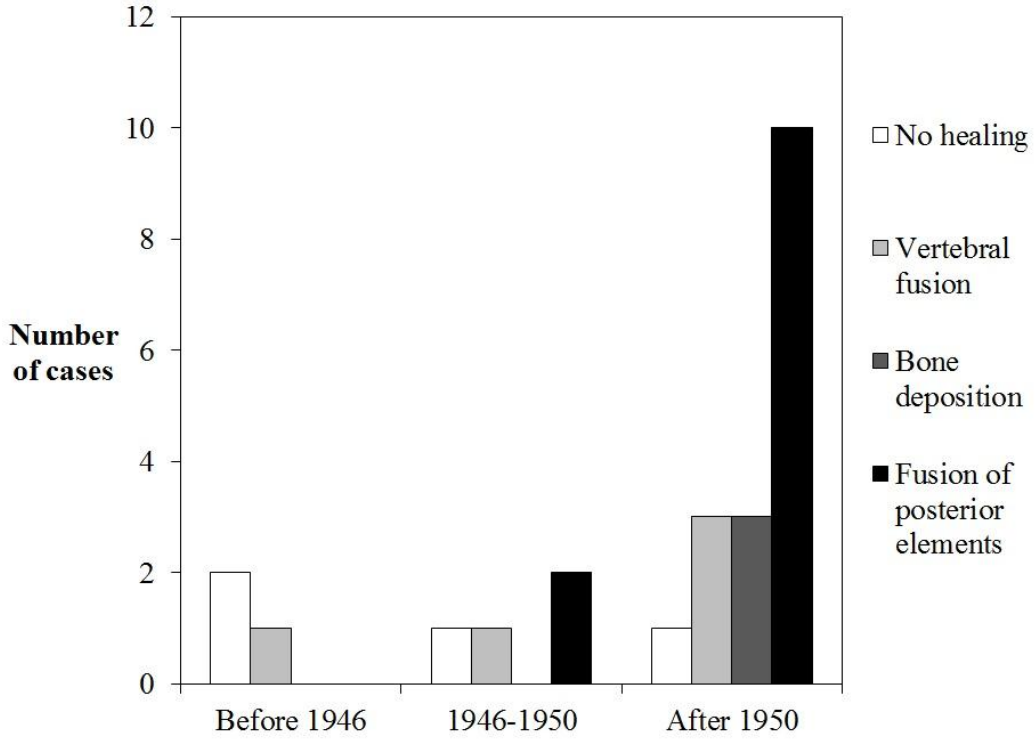

Figure 15: Number of cases from the Galler Collection for each category of healing of bone lesions due to tuberculosis through time (N=25). Cases that received surgery (N=4) were excluded.

Cases of TB affecting skeletal elements other than the spine were infrequent; there were only three cases in 69 individuals diagnosed with the disease. Two of these cases involved the hip and the last involved the foot. The cases affecting the hip joint show destruction of the articular surfaces; typical for TB. The individual with TB of the foot however, shows atypical lesions. The disease process is well healed and an extensive amount of fusion of bones has taken place.

The process of healing through vertebral fusion occurred throughout all time periods. There was no difference in appearance of lesions healed in this way when comparing examples throughout all time periods. Our results also show that different types of healing occur in later time periods (i.e. after the introduction of antibiotics), indicating that antibiotics did not affect the usual mechanism of healing (vertebral

fusion), but did impact healing in general. A higher proportion of individuals in later time periods show healing than those in earlier time periods.

Soft Tissue Involvement of TB

The number of sites affected by TB was recorded for each individual in the Galler Collection as either one (single) or more than one (multiple). The analysis involved comparison of single/multiple foci through time. For some individuals (N=8), we were unable to determine a year of death and these cases were consequently excluded from this analysis.

The results (Figure 16) show that in the first time period, most individuals had multiple tuberculous foci. However, after the introduction of pharmacological intervention, the proportion of individuals with multiple foci decreased substantially. The third time period showed an essentially identical distribution to the second time period. The average age at death increased significantly through time.

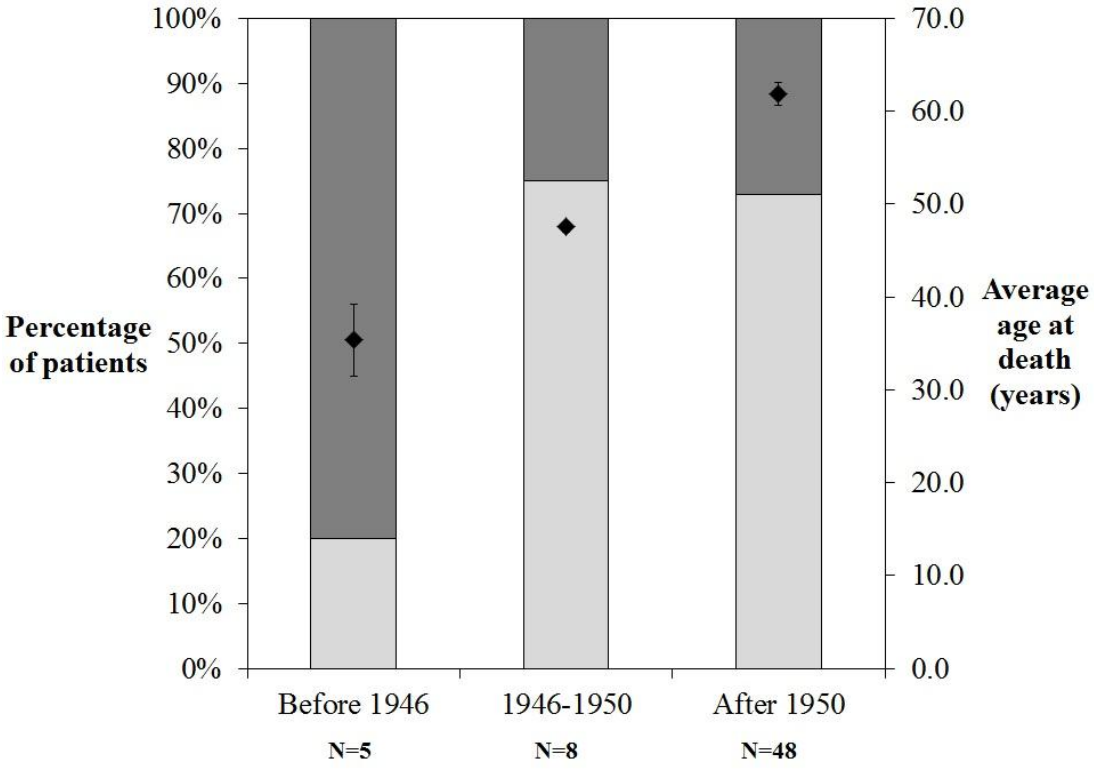

Figure 16: Percentage distribution through time of individuals from the Galler Collection with one region (single) and more than one region (multiple) of the body affected by tuberculosis. Average age of death (filled diamonds) is also plotted on the secondary (right) ordinate. Light and dark shading correspond to single and multiple foci, respectively.

It was possible to determine which regions of the body were affected by TB for every individual in the Galler Collection (N=69), including those without observable skeletal lesions, using medical records (Table 2). Many individuals had TB in multiple tissues and bones of the body.

Table 2: Number of cases of tuberculosis affecting various soft tissues (and skeleton) of individuals in the Galler Collection (N=69). Note that some individuals had tuberculosis of multiple tissues and bones.

Tissue/bone	Number of cases	Percentage of total cases
Lung	36	52%
Spine	29	42%
Lymph nodes or vessels	9	13%
Urogenital	8	12%
Liver	7	10%
Other skeletal lesions (not already covered elsewhere)	7	10%
Spleen	5	7%
Suprarenal (adrenal)	5	7%
Hip	5	7%
Miliary	4	6%
Meningeal	3	4%
Elbow	2	3%
Knee	2	3%
Ear	1	1%
Gastrointestinal tract	1	1%
Aorta	1	1%
Psoas muscle	1	1%
Hand	1	1%
Tibia	1	1%
Feet	1	1%

Unsurprisingly, the most common site of TB was the lungs, closely followed by the spine. The lymph nodes/vessels, urogenital system, liver, skeleton, spleen, suprarenal glands and hip were affected in several cases (5-9 individuals each). Miliary (widespread) and meningeal TB affected fewer individuals; 4 and 3 cases respectively. Sites infrequently affected (1-2 cases each) included the elbow, knee, ear,

gastrointestinal tract, aorta, psoas muscle, hand, tibia and foot. It was considered that there may be a link between regions of the body affected and skeletal lesions and this analysis will form a part of future studies of the Galler Collection.

Through time, the regions of the body affected by TB do not significantly change in frequency of infection and the lungs, spine, lymph nodes, etc. remain the main sites of pathology, regardless of the time period. There is a difference in the less frequently affected sites; all of the cases of TB affecting the meninges, elbow, aorta, psoas muscle, hand, tibia and foot occurred during the first time period.

A summary of the cases where multiple regions of the body were affected by TB is given in Table 3 and there are cases from each time period. The age range was 16 to 93 years, meaning that individuals of all ages were affected by TB in multiple tissues and bones of the body. The specific tissues affected varied between individuals and time periods but in all cases, the lungs were affected initially, though at the time of death lung pathology may not have been obvious and therefore not recorded.

Table 3: Summary of individuals with diagnosed tuberculosis of multiple regions of the body from the Galler Collection (N=16).

Autopsy Number	Autopsy Year	Age (years)	Sex	Time Period	Soft tissues affected by tuberculosis
Data not available Patient ID: 404	Data not available	Data not available	Data not available	N/A	Skeleton (excluding spine), lung, urogenital, spleen and liver
793	1936	78	Female	Before 1946	Miliary: Lungs, urogenital, spleen, liver, suprarenal, aorta and hand
325	1936	16	Male	Before 1946	Meninges, hip, spleen and lungs
144	1938	36	Male	Before 1946	Skeleton (excluding spine), elbow, feet, tibia and meninges
751	1939	35	Male	Before 1946	Meninges, spine, lung, urogenital, psoas muscle,
304	1947	37	Male	1946-1950	Lung, lymph vessels and hip
779	1953	50	Female	After 1950	Spine, lungs, lymph nodes, suprarenals, liver and urogenital
1445	1954	88	Female	After 1950	Lung, lymph nodes, hip and knee
411	1955	34	Male	After 1950	Lung, lymph node, meninges, spine and urogenital
60	1956	69	Male	After 1950	Miliary: Spine, skeleton, lymph nodes, liver, spleen, suprarenal, genitourinary system and hip
1697	1956	67	Female	After 1950	Spine and knee
572	1957	93	Male	After 1950	Lungs, tracheal lymph node
487	1958	80	Male	After 1950	Lung and gastrointestinal system
749	1960	64	Male	After 1950	Skeleton (excluding spine), lung and spine
2420	1965	37	Male	After 1950	Spine and lungs
932	1966	72	Male	After 1950	Miliary: Lymph nodes, spleen and liver

Co-morbidities

Many of the individuals in the Galler Collection had a number of other conditions in addition to TB. Table 4 shows the co-morbidities for all individuals with a year of death (N=61) in each time period.

Table 4: Co-morbidities by time period associated with introduction and use of pharmacological interventions. Values reflect the percentage of total individuals in that time period with a specific disease or condition. *Highlighted are causes related to TB that consistently appear in all time periods.*

First period (before 1946)	%	Second period (1946-1950)	%	Third period (after 1950)	%
Fatty liver	60.0	**Pleuritis**	**25.0**	Heart pathology	48.2
Pleuritis	**40.0**	None	25.0	Arterio/atherosclerosis	39.3
Pneumonia	**40.0**	Heart pathology	12.5	Osteoporosis	37.5
Edema	40.0	Arterio/atherosclerosis	12.5	Edema	35.7
Peritonitis	40.0	Osteoporosis	12.5	**Pneumonia**	**21.4**
Tonsilitis	40.0	**Pneumonia**	**12.5**	Emphysema	19.6
None	20.0	**Underweight**	**12.5**	Bronchitis	19.6
Underweight	**20.0**	Metastases	12.5	**Underweight**	**17.9**
Emphysema	20.0	Fatty liver	12.5	**Pleuritis**	**16.1**
Sepsis	20.0	Congenital hip luxation	12.5	Metastases	10.7
Addison's disease	20.0	Blood disease	12.5	Cystitis	10.7
		Aortic aneurism	12.5	Uremia	10.7
				Senile marasmus	10.7

Across all time periods, there are several co-morbidities that were common among all groups. These include pleurisy, pneumonia and being underweight. Additionally, the effect of antibiotics can be observed between groups. In the first time period, infectious diseases are included in the co-morbidities list. However, in the second and third time periods, the co-morbidities become chronic and "degenerative" in nature, reflecting a shift away from infectious diseases due to the introduction of pharmacological agents. Several chronic co-morbidities from the second and third time periods include heart pathologies, osteoporosis and metastases (cancer).

Discussion

This study showed that "healing" of spinal lesions due to TB can be achieved through the processes of vertebral fusion, bone deposition and fusion of posterior elements. We also show one example of healed foot TB where bones have become extensively fused together. Although medical records may be inaccurate due to difficulty in diagnosis, we also considered skeletal evidence in each case. We did encounter difficulties with incomplete skeletons in the collection. However, in some cases, the medical records did provide additional information regarding bones that were not present. Through a combination of skeletal evidence and medical records, we were able to confidently suggest that all individuals presented here except for three (of 29) had skeletal lesions as a result of TB. Consequently, the results presented here can be of use to palaeopathologists when considering skeletal samples with bone lesions suggestive of TB, but with some atypical characteristics.

The small sample size (N=25) is a major limitation of this study, but can be addressed by reviewing published literature regarding similar findings in the past. Several studies have previously described cases of diagnosed spinal TB from the pre-

antibiotic era (Cofield, 1922; Perlman and Freiberg, 1943; Lafond, 1958)). In two of these studies (Cofield, 1922; Perlman and Freiberg, 1943), none of the individuals received pharmacological treatment but bone deposition did still occur (Table 5). Individuals were aged between 18 and 39 years. Each case was diagnosed as TB using biopsy, smear tests and inoculation of guinea pigs. The ancestry of each individual was noted in Table 5 because the living standards in the United States at the time the studies were conducted (early 20th century) were different for those with European compared with African-American ancestry (Lambert, 2006). It has also been noted that African-Americans as well as American Indians appear to have a higher risk of developing active TB than Europeans (Stead, 2001). Despite these differences, bone deposition, defined in these studies as "bony bridging," was noted in several individuals of different ancestry. An estimate of the frequency of "bony bridging" (recorded from the Cincinnati General Hospital, Ohio) in the early 20th century was 10% in all spinal cases of TB (Cofield, 1922). This value is the same as that reported by (Steinbock, 1976) for 1922. In another study by (Kaplan, 1959), it was reported that the use of antibiotics increased the percentage of cases healing through bony fusion to 50%. In another separate study, a similar observation was found from 44% (no antibiotics) to 55% (antibiotics). "Superior healing" has also been described in cases treated with streptomycin compared to those who were left unhealed (Public Health Service, 1952; Lafond, 1958). Finally, spinal fusion was found to be 69% in a series of cases treated with streptomycin, while fusion was 31% in a comparable sample that was untreated (Falk, 1958).

Table 5: Summary of cases of spinal tuberculosis (TB) from the pre-antibiotic literature for comparison with this study. Note that "L1" refers to lumbar vertebra number one and "L2" to the second lumbar vertebra, etc.

Reference	Country	Ancestry	Age (years)	Sex	Date of admission	Date of release or death	Disease focus	Bone lesions	Treatment
(Perlman and Freiberg 1943)	United States	European	32	Male	May 6, 1940	December 12, 1940 (death)	Spinal TB affecting lumbar vertebrae	Disc space between L2 and L3 is narrowed, a calcified deposit bridges this space	Bed rest at the hospital
(Perlman and Freiberg 1943)	United States	African American	18	Male	April 9, 1937	June 1940 (released)	Pulmonary, lumbar spine, psoas muscle and right hand	Destruction of L5, dense bony bridging between L3/L4 and L4/L5, sacroiliac involvement	Not specified
(Perlman and Freiberg 1943)	United States	African American	32	Male	August 8, 1937	February 13, 1938, readmitted on April 17, 1940	Pulmonary, lumbar spine, lymph nodes, both psoas muscles	Gibbus of L4/L5, destruction of L4/L5, bony bridging between L3/L4	Not specified
(Perlman and Freiberg 1943)	United States	European	25	Male	January 9, 1941	Not specified	Lower lumbar, left hip, psoas muscle, lymph node	Bony bridging between L1/L2	Not specified
(Perlman and Freiberg 1943)	United States	European	39	Male	July 14, 1939	Not specified	Left hip, lumbar spine, psoas muscle, lymph nodes	Intervertebral disc between L2/L3 destroyed, bony bridging between L2/L3, L3/L4 and L4/L5	Surgery on left hip
(Cofield 1922)	Italy	European	24	Male	November 1917	Not specified	Lumbar spine, psoas muscle	Bony bridging between L2/L3	

Our suggestion for the reason these types of bony changes occurred in individuals from the Galler Collection is due to elimination or complete control of the bacterium through a combination of an individual's immune system and treatment by pharmacological intervention (including antibiotics). Although this may not result in complete removal of the bacterium from an individual's body, it may be sufficiently controlled by granulomatous tissue to render it "neutralized." (Kaplan, 1959) observed that destructive processes can be stopped in as little as 6 to 9 months with antibiotics. After cessation of those processes involving the bone, other changes can occur. In the cases where a longer time period has passed since the disease was active, bone may be deposited to provide strength to a spinal column weakened by bone destruction. We found this to be true in at least three cases, where the disease had been arrested and bone deposition had occurred over a long time span. Bone deposition and fusion has been found to take a long time; usually several years (Bradford and Cotton, 1900). However, it has been reported that bone solidification, fusion and ankylosis is an outcome for TB affecting the skeleton (Bradford and Cotton, 1900; Ortner, 2003). (Kaplan, 1959) has reported the ankylosis of cases of TB of the hip and knee that were only treated using immobilization techniques. The same authors have also reported a large proportion of lumbar vertebrae (66%) lesions progressing to ankylosis and bony fusion. Additionally, fusion of posterior spinal elements such as the spinous process can also provide physical support in an area around a collapsed vertebra. (Kaplan, 1959) also made this observation in patients who were recommended for posterior spinal fusion. Upon surgery, it was discovered that in some, spontaneous fusion of the vertebrae at the apex of the kyphosis has occurred. Some individuals (from all time periods) from our study as well as other studies from the literature received spinal operations. While conservative therapy such as rest, hygiene and improved nutrition can produce satisfactory results in the treatment of TB, this takes a considerable amount of time (Ito

et al., 1934). Surgery can offer a faster and more effective means of treatment (Orell, 1951). The specific surgery we observed has been very popular and successful in the past. (Ito et al., 1934) report on five cases treated with posterior spinal fusion for correction of kyphosis and prevention of further deformity in the spine. All patients survived this operation and it was successful in preventing further deformity.

Healing of TB lesions occurs through the processes of fibrosis, organization of abscesses, resorption of sequestra, osteogenesis and occasionally bony fusion between vertebrae (Perlman and Freiberg, 1943). In Pott's initial report of TB spinal lesions, he described calcification of intervertebral ligaments and production of new bone but most clinicians consider these elements not diagnostic of the disease (Cofield, 1922; Perlman and Freiberg, 1943). Spinal fusion is most likely to occur when kyphosis has caused adjacent vertebrae to come in contact with one another (Bradford and Cotton, 1900). Bone can react in only a limited number of ways to pathological processes and is heavily influenced by a number of factors including the presence of other infectious agents or pathology, the immunity of an individual, genetics of the pathogen and location of the lesion (Cofield, 1922). (Kaplan, 1959) also suggests that there may be a difference in healing when certain antibiotics are used. Work on renal TB showed that when streptomycin and PAS were used, healing occurred by fibrosis. When streptomycin and isoniazid were used, lesions healed with minimal scarring. These problems however, do not affect our results because we collected data from historical records, medical and autopsy reports in addition to skeletal samples. The medical reports may be considered to be accurate because TB was well described by the 20th century in Europe and a number of routine diagnostic techniques (e.g. culture, guinea pig inoculation, smear tests) were available at this time (Dye and Williams, 2010).

A comparison of descriptions published regarding palaeopathological cases of TB with our cases was made. We included only several examples from the newer literature because these are more likely to have very extensive descriptions which will allow the best comparison. We chose recent articles from (Suzuki et al., 2008; Murphy et al., 2009; Klaus et al., 2010; Évinger et al., 2011; Köhler et al., 2012) as these represent some newer cases from various geographical regions (Hungary, Peru, Siberia and Korea). The cases from Peru described by (Klaus et al., 2010) all showed typical destructive lesions for TB, with no new bone formation. (Suzuki et al., 2008) and (Köhler et al., 2012) both report on spines with extensive amounts of vertebral fusion from Korea and Hungary, respectively. However, they note that no new bone was formed, despite the large amount of fusion. In (Murphy et al., 2009), many of the cases from Siberia show destructive lesions, but in one individual (XXXI.77, Figure 3 of publication), some bone deposition is present on the edges of two vertebral bodies. Finally, (Évinger et al., 2011) report on several cases from Hungary and one of these (Grave No.: 39/02 (9th century), Inventory no.: 2008.4.118., Figure 8) had lesions very similar to one of our cases (Autopsy Number: 732, Autopsy Year: 1949, Figure 4). Several separate foci with vertebral fusion were present, along with bone deposition on a lumbar vertebra. While some of our cases do show a lack of bone formation, many show bone deposition, similar to the last example from Evinger et al. (2011) above. Therefore, our results may be useful for applications in paleopathological studies. Where some cases display bone deposition atypical for TB (spinal or otherwise) that would automatically exclude the disease from differential diagnoses, these observations may assist to identify unusual cases. Our results may also help to initiate further investigations such as ancient DNA (Donoghue et al., 2009; Murphy et al., 2009; Klaus et al., 2010; Évinger et al., 2011) or lipid analysis (Hershkovitz et al., 2008; Redman et al., 2009; Donoghue et al., 2010; Mark et al., 2011) of skeletal remains that would

otherwise not be considered as potentially useful samples for TB studies. This may be useful since these methods are expensive and time consuming.

Another factor to consider in this study is the way in which the individuals were divided into time periods. TB is a chronic disease with both active and latent periods (Cole et al., 2005). Older individuals may have developed TB during the first time period, but died only in the third time period, complicating the results. However, if the individual survived until antibiotic treatment was available, then they would experience healing after control of the bacterium, same as another individual who developed skeletal lesions of TB during the third time period would. Additionally, when the Swiss Law (Gesetzgebung: Zürich, 1928) was introduced, it required the compulsory reporting and treatment of TB cases. As such, when antibiotics were introduced, it can be expected that in a wealthy country such as Switzerland with excellent healthcare, individuals with TB would be treated with these pharmaceutical agents. A study in Minnesota, United States during 1946-1948 reported the rates of streptomycin usage (Falk, 1958). In 1946, only 5% of patients received streptomycin. In 1947 and 1948, the rates were 52% and 43%, respectively. Since streptomycin was only introduced in 1946 (Wilson, 2005), this is a rapid adoption of its use into general practice. Therefore we can consider that *at least* half of the individuals in our collection who died after 1946 were treated with antibiotics. This allows a generalization of the effects of streptomycin on skeletal lesions.

Analysis of the number of tuberculous foci after the introduction and use of pharmacological agents to treat Swiss individuals during the time period 1946-1977 showed that an individual's prognosis was improved when these agents were used in treatments. (Kaplan, 1959) has also reported a decrease of multiple foci after streptomycin was introduced. The reduction was 50%; from 10% to 5%. While these

values do not agree with our findings this can be explained by differences in the samples. Individuals in the Galler Collection are a small sample of adults with exemplary skeletal lesions, while the sample described by Kaplan et al. (1959) is a generalization of the South African population and includes all ages, whether they have skeletal manifestations or not. Another study (Public Health Service, 1952) reported a positive general effect on the immunity of patients. They report that although streptomycin was unable to reach the site of a lesion directly, the benefits to the immune system allowed a patient to combat the bacterium more effectively. This resulted in cessation of the destructive processes. The results for the regions of the body affected by TB in individuals from the Galler Collection agree with previous reports of tuberculous involvement of soft tissues and bone. TB is primarily a pulmonary disease and it is expected that the most frequent site of infection would be the lungs, which is supported here. Other soft tissues involved in the immune response are also frequently affected in individuals from the Galler Collection, such as the liver and spleen. Skeletal TB makes up 10.1% of the cases and these involve the spine, elbow, hip, knee, tibia and feet. Several of these sites are commonly reported as major sites of tuberculous involvement in active disease (hip and knee, specifically). Additionally, the gastrointestinal tract is affected in only a single case, suggesting that the primary mode of transmission is through inhaled aerosolized droplets generated by coughing although abdominal TB can be transmitted from cattle by ingestion of contaminated milk and meat products (Waddington, 2004). Additionally it was observed that the main sites of tuberculous involvement (lungs, spine, lymph nodes, etc.) remained consistent through time. However, there were additional cases of TB of infrequently affected sites (meningeal, elbow, aorta, psoas muscle, hand, foot and tibia) during the first time period. The reason for this change in disease manifestation may be due to the implementation of pharmacological treatment during later time periods. Without drug

therapy, an individual's immune system was not supported by medical means and consequently the disease was able to spread to areas of the body that are infrequently involved in TB. A summary of the cases with TB of multiple regions of the body showed no trends associated with age or time period. Individuals from all time periods were affected, showing that the implementation of pharmacological therapy regimes did not completely prevent an individual from developing TB in multiple tissues and bones of the body.

The co-morbidities observed to be common to all three time periods (underweight, pneumonia and pleurisy) were expected. TB often causes a loss of appetite leading to weight loss and consequently underweight individuals (Orell, 1951; Eley and Beatty, 2009). Pneumonia and pleurisy are both conditions affecting lung tissues and this may reduce an individual's immunity to pulmonary TB. The increase in chronic, degenerative conditions through time indicates that antibiotics were an effective treatment for infectious diseases and that individuals were living long enough with TB to develop these diseases. This is also reflected in an increase in the average age at death through time. Despite these additional co-morbidities, most patients can be expected to recover after some time. This is due to antibiotics as well as other treatments such as rest as well as improved nutrition and hygiene (Kaplan, 1959).

Conclusions

This study showed that "healing" of osteolytic TB lesions in both the spine and other regions of the skeleton occurred during the 20th century in Switzerland. This may occur through fusion of anterior and posterior parts of vertebrae, bone deposition and was aided by surgical intervention (e.g. posterior spinal fusion). Although bone lesion healing occurred naturally prior to the introduction of pharmacological interventions,

our results suggest an increase in the frequency and effectiveness of healing after the introduction of pharmacological agents. This information may be used to aid differential diagnosis in unusual paleopathological cases of TB.

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References Cited

Abdelwahab I.F., Camins M.B., Hermann G., and Klein M. (1997) Vertebral arch or posterior spinal tuberculosis. Skeletal Radiology, 26: 737-740.

Albee F.H. (1935) Spinal Tuberculosis: Climatic and Operative Treatment. American Journal of Surgery, 30(1): 60-65.

Aufderheide A.C., and Rodriguez-Martin C. (1998) The Cambridge Encyclopedia of Human Paleopathology. Cambridge University Press, Cambridge, United Kingdom.

Bradford E.H., and Cotton F.J. (1900) Treatment of Pott's Disease After the Development of the Deformity. Boston Medical and Surgical Journal, 142(12): 277-283.

Centre for Disease Control and Prevention. (2011) Chapter 1 – Overview of Tuberculosis Epidemiology in the United States. In: Centre for Disease Control and Prevention, (eds.). Core Curriculum on Tuberculosis: What the Clinician Should Know. National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Atlanta, Georgia p.

Cofield R.B. (1922) Bony Bridging in Tuberculosis of the Spine. Journal of the American Medical Association, 79(17): 332-341.

Cole S.T., Eisenach K.D., McMurray D.N., and Jacobs Jr W.R. (2005) Tuberculosis and the Tubercle Bacillus. In: Cole S.T., Eisenach K.D., McMurray D.N., and Jacobs Jr W.R., '(eds.), Book Tuberculosis and the Tubercle Bacillus. ASM Press, Washington DC, USA p.

Corbett E.L., Watt C.J., Walker N., Maher D., Williams B.G., Raviglione M.C., and Dye C. (2003) The growing burden of tuberculosis: Global trends and interactions with the HIV epidemic. Archives of Internal Medicine, 163(9): 1009-1021.

Doege T.C. (1965) Tuberculosis Mortality in the United States, 1900 to 1960. Journal of the American Medical Association, 192(12): 1045-1048.

Donoghue H., Pap I., Szikossy I., and Spigelman M. (2009) Detection and characterisation of Mycobacterium tuberculosis DNA in 18th-Century Hungarians with pulmonary and extra-pulmonary tuberculosis. Programme and Abstracts 1st Bolzano Mummy Congress, Mummies and Life Sciences. EURAC – Institute for Mummies and the Iceman, Bolzano, Italy. p 24.

Donoghue H.D., Lee O.Y.C., Minnikin D.E., Besra G.S., Taylor J.H., and Spigelman M. (2010) Tuberculosis in Dr Granville's mummy: A molecular re-examination

of the earliest known Egyptian mummy to be scientifically examined and given a medical diagnosis. Proceedings of the Royal Society B: Biological Sciences, 277(1678): 51-56.

Dormandy T. (1999) The White Death: A History of Tuberculosis. The Hambledon Press, London.

Dye C., and Williams B.G. (2010) The population dynamics and control of tuberculosis. Science, 328(5980): 856-861.

Eley BS, Beatty DW (2009) The basic immunology of tuberculosis. In: Schaaf S, Zumla A, eds. Tuberculosis: a comprehensive clinical reference. Philadelphia, PA: Saunders, 75-86.

Évinger S., Bernert Z., Fóthi E., Wolff K., Kővári I., Marcsik A., Donoghue H.D., O'Grady J., Kiss K.K., and Hajdu T. (2011) New skeletal tuberculosis cases in past populations from Western Hungary (Transdanubia). Journal of Comparative Human Biology, 62 (3):165-183.

Falk A. (1958) A Follow-up Study of the Initial Group of Cases of Skeletal Tuberculosis Treated with Streptomycin, 1946-1948. The Journal of Bone and Joint Surgery, 40-A(5): 1161-1168.

Gesetzgebung: Zürich. (1928) Anfrage des Stadtrates betr. Erlass von Vorschriften über die Wohnungsinspektion. In: Zürich S., editor. Zürich, Switzerland.

Hershkovitz I., Donoghue H.D., Minnikin D.E., Besra G.S., Lee O.Y.C., Gernaey A.M., Galili E., Eshed V., Greenblatt C.L., Lemma E. et al. . (2008) Detection and molecular characterization of 9000-year-old Mycobacterium tuberculosis from a neolithic settlement in the Eastern mediterranean. PloS ONE, 3(10).

Herzog H. (1998) History of tuberculosis. Respiration, 65(1): 5-15.

Holloway K.L., Henneberg R.J., De Barros Lopes M., and Henneberg M. (2011) Evolution of human tuberculosis: A systematic review and meta-analysis of paleopathological evidence. Journal of Comparative Human Biology, 62(6): 402-458.

Ito H., Tsuchiya J., and Asami G. (1934) A New Radical Operation for Pott's Disease. The Journal of Bone and Joint Surgery, 16(3): 499-515.

Kaplan C.J. (1959) Conservative Therapy in Skeletal Tuberculosis: An Appraisal Based on Experience in South Africa. Tubercle, 40: 355-368.

Klaus H.D., Wilbur A.K., Temple D.H., Buikstra J.E., Stone A.C., Fernandez M., Wester C., and Tam M.E. (2010) Tuberculosis on the north coast of Peru: skeletal and molecular paleopathology of late pre-Hispanic and postcontact mycobacterial disease. Journal of Archaeological Science, 37(10): 2587-2597.

Köhler K., Pálfi G., Molnár E., Zalai-Gaál I., Osztás A., Bánffy E., Kirinó K., Kiss K.K., and Mende B.G. (2012) A Late Neolithic Case of Pott's Disease from Hungary. International Journal of Osteoarchaeology, doi: 10.1002/oa.2254.

Lafond E.M. (1958) An Analysis of Adult Skeletal Tuberculosis. The Journal of Bone and Joint Surgery, 40-A(2): 346-364.

Lambert P.M. (2006) Infectious disease among enslaved African Americans at Eaton's Estate, Warren County, North Carolina, ca. 1830-1850. Memórias do Instituto Oswaldo Cruz, 10(1): 107-117.

Mark L., Gulyas-Fekete G., Marcsik A., Molnár E., and Pálfi G. (2011) Analysis of ancient mycolic acids by MALDI TOF MS: response to "Essentials in the use

mycolic acid biomarkers for tuberculosis detection" by Minnikin et al., 2010. Journal of Archaeological Science, 38: 1111-1118.

McLain R.F. (1991) The Nature of Joint Disease in Early Man. The Iowa Orthopaedic Journal, 11: 94-100.

Morse D. (1967) Chapter 19: Tuberculosis. In: Brothwell D., and Sandison A.T., (eds.). Diseases in Antiquity. Charles C. Thomas, Illinois p 249-271.

Murphy E.M., Chistov Y.K., Hopkins R., Rutland P., and Taylor G.M. (2009) Tuberculosis among Iron Age individuals from Tyva, South Siberia: palaeopathological and biomolecular findings. Journal of Archaeological Science, 36(9): 2029-2038.

Newsom S.W.B. (2006) The history of infection control: Tuberculosis: Part two - Finding the cause and trying to eliminate it. British Journal of Infection Control, 7(6): 8-11.

Orell S. (1951) Chemotherapy and Surgical Treatment in Bone and Joint Tuberculosis. Acta Orthopaedica Scandinavica, 21(3): 190-203.

Ortner D.J. (2003) Identification of pathological conditions in human skeletal remains. Elsevier, USA.

Perlman R., and Freiberg J.A. (1943) The Bridging of the Vertebral Bodies in Tuberculosis of the Spine. The Journal of Bone and Joint Surgery, 25(2): 340-350.

Public Health Service. (1952) Evaluation of Streptomycin Therapy in a Controlled Series of Ninety Cases of Skeletal Tuberculosis. The Journal of Bone and Joint Surgery, 34-A(2): 288-298.

Puranen B. (1999) Tuberculosis and the Decline of Mortality in Sweden. In: Edited by:, Schofield R., Reher D., and Bideau A., (eds.). The Decline of Mortality in Europe. Oxford University Press, New York p 97-117.

Redman J.E., Shaw M.J., Mallet A.I., Santos A.L., Roberts C.A., Gernaey A.M., and Minnikin D.E. (2009) Mycocerosic acid biomarkers for the diagnosis of tuberculosis in the Coimbra Skeletal Collection. Tuberculosis, 89(4): 267-277.

Roberts C.A., and Buikstra J. (2003) The Bioarchaeology of Tuberculosis: A Global View on a Reemerging Disease. University Press of Florida, Florida.

Rühli F.J., Hotz G., and Böni T. (2003) Brief communication: The Galler Collection: A little-known historic Swiss bone pathology reference series. American Journal of Physical Anthropology, 121(1): 15-18.

Santos A.L., and Roberts C.A. (2001) A picture of tuberculosis in young Portuguese people in the early 20th century: A multidisciplinary study of the skeletal and historical evidence. American Journal of Physical Anthropology, 115(1): 38-49.

Stead W.W. (2001) Variation in vulnerability to tuberculosis in America today: Random, or legacies of different ancestral epidemics? International Journal of Tuberculosis and Lung Disease, 5(9): 807-814.

Steinbock R.T. (1976) Paleopathological diagnosis and interpretation: Bone diseases in ancient human populations. Charles C Thomas, Springfield, Illinois, USA.

Suzuki T., Fujita H., and Jong G.C. (2008) Brief communication: New evidence of tuberculosis from prehistoric Korea - Population movement and early evidence of tuberculosis in far East Asia. American Journal of Physical Anthropology, 136(3): 357-360.

Szreter S. (1988) The Importance of Social Intervention in Britain's Mortality Decline c. 1850-1914: a Re-interpretation of the Role of Public Health. The Society for the Social History of Medicine, 1(1): 1-38.

Tiemersma E.W., van der Werf M.J., Borgdorff M.W., Williams B.G., and Nagelkerke N.J.D. (2011) Natural History of Tuberculosis: Duration and Fatality of Untreated Pulmonary Tuberculosis in HIV Negative Patients: A Systematic Review. PloS ONE, 6(4).

Waddington K. (2004) To Stamp Out "So Terrible a Malady": Bovine Tuberculosis and Tuberculin Testing in Britain, 1890-1939. Medical History, 48(1): 29-48.

Warren P. (2006) The evolution of the sanatorium: the first half-century, 1854-1904. Canadian Bulletin of Medical History, 23(2): 457-476.

Wilbur A.K., Farnbach A.W., Knudson K.J., and Buikstra J.E. (2008) Diet, tuberculosis, and the paleopathological record. Current Anthropology, 49(6): 963-991.

Williams J.A., and Snortland-Coles S. (1986) Pre-Contact Tuberculosis in a Plains Woodland Mortuary. Plains Anthropologist, 114: 249-252.

Wilson L.G. (2005) Commentary: Medicine, population, and tuberculosis. International Journal of Epidemiology, 34(3): 521-524.

World Health Organization. (2012) World Health Organization Tuberculosis burden estimates.

4. Non-pharmacological treatment of tuberculosis may be successful as indicated by long-term epidemiological trends in the past

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Abstract

This study investigated the trends in tuberculosis mortality through time in Switzerland. Information on the decline in mortality may be useful in third world countries where tuberculosis is now re-emerging. Swiss data were collected from historical records and comparative data were obtained from the literature for England and Wales, New York, Japan, Brazil and Sierra Leone. Logistic curves were fitted to examine the rate of decline before introduction of pharmacotherapies and show that the decline would have continued without the introduction of chemical therapies, including antibiotics. In Switzerland, England and Wales and New York, the decline had occurred long before the introduction of specific anti-tuberculosis agents. In Brazil and Japan, chemical therapy was co-incident with the decline in tuberculosis mortality rates. Overall, we suggest that the effective control of tuberculosis can be achieved through a combination of chemical interventions, conservative therapy (rest, good nutrition, ventilation, etc.) as well as public health interventions addressing hygiene, nutrition, reducing exposure to infections and educating the population about tuberculosis.

Keywords: Tuberculosis, Epidemiology, Streptomycin, 20th century, Mortality

Introduction

Definition of a disease as a balance between infection and immunity

There is a multitude of definitions of "a disease" because like with most common words, the usage is varied. It is nearly impossible to reach an agreement on one formal definition of a disease. The most general statement would be that disease is a detrimental deviation from structural or functional norm (anatomical, physiological or psychological norm in case of living organisms). What a "norm" is remains equally debatable. One of the most common categories of diseases are infectious diseases. In the classic Koch's definition, these are diseases caused by a parasitic organism, usually a small one like a bacterium or a virus entering a body and altering its normal functions (Kaufmann 2003). A human body is a microcosm of flora and fauna kept at balance by interactions of the vertebrae organism with a multitude of its microorganismic inhabitants. The immune system plays the decisive role in controlling how those inhabitants interact with the host organism. Tuberculosis is a good example of the alteration between infestation by a particular category of bacteria, *Mycobacterium* and immunity. Most humans are infected by *Mycobacteriae*, but do not develop any pathological signs or symptoms (Cole et al. 2005). Some humans, with lower immunity, will suffer consequences of uncontrolled expansion of mycobacterial flora that produces a disease invading various tissues of the body and, if immunity does not improve or the parasite growth is not artificially checked by chemotherapy, may die (Cole et al. 2005; Roberts and Buikstra 2003).

Killing germs versus improving immunity: what can eradicate tuberculosis ?

Tuberculosis (TB) is an ancient disease and caused the deaths of approximately 25% of the European adult population during the 1700's and early 1800's (Stead 2001). At present, the World Health Organization (WHO) estimates that approximately one third of the world's population are infected with the causative organism, *Mycobacterium tuberculosis* (World Health Organization 2012*)*. However, only approximately 10% of all individuals infected, most commonly those with lowered immunity, will ever develop active disease (Wilbur et al. 2008). Signs and symptoms of TB are usually associated with the respiratory system and include coughing, difficulty breathing, bloody sputum, weakness, lethargy, loss of appetite and weight, night sweats, pallor and chest pain. Transmission of the bacterium occurs through generation of aerosolized droplets containing bacteria; usually through excessive coughing.

Antibiotics and other chemical therapies were introduced in the 1940's and 1950's, which allowed the "cure" of TB by killing the pathogenic organism in the patient's body. However, their use caused the development of drug-resistant bacteria through the process of natural selection (Herzog 1998). This is a major problem in many countries worldwide. In some of those countries the majority of infections is now with the multi-drug resistant strains (e.g. Peru (Manjourides et al. 2012)). Some strains of the TB bacillus have been found to be multiple-drug resistant (MDR), meaning they are resistant to the two first-line chemical agents (isoniazid and rifampin) (Wang et al. 2008). Some strains have even been observed to be extensively-drug resistant (XDR) and are almost untreatable.

Before chemical therapies including antibiotics appeared, the typical treatment for TB involved increasing a patient's immunity through improved nutrition, hygiene and rest (conservative therapies) (Kaplan 1959). All of these measures were used at

sanatoria (Rucker and Kearny 1913) and helped to cause and maintain a decline in mortality due to TB throughout the 19th century, long before antibiotics were introduced. The main factors in this decline are important because they can help to devise new treatment methods for patients with MDR or XDR that are untreatable with antibiotics, in the same way that antibiotics were unavailable throughout the 19th and early 20th century.

Materials and Methods

Information about tuberculosis mortality was collected from numerous sources for this study. We collected information for Canton Zürich from both the Stadtarchiv ("city-archive" (Stadt Zürich 2012)) and Staatsarchiv ("canton-archive" (Kanton Zürich 2012)) in the city of Zürich, Switzerland. The Stadtarchiv held records for causes of death in Zürich city from 1893 to 1933, which included mortality attributed to tuberculosis. There were many records in this archive, but we focused on:

- Tuberkulose (Tuberculosis): two volumes; 1912-1932 and 1932 to 1935
- Tuberkulose Sterbefaelle (Tuberculosis mortality): five volumes; 1903-1905, 1905-1915, 1915-1920, 1920-1934 and 1929-1936

Data were also collected from a volume of primary historical statistics for Switzerland (State Statistics) titled "Historische Statistik der Schweiz" (Ritzmann-Blickenstorfer 1996).

Data for the entire country (Switzerland) were also collected from these sources as well as from the World Health Organization (WHO) for the years 1867 to 2005.

Much of the information was obtained from previously published literature regarding England and Wales (Blower et al. 1995) New York (United States) (Drolet

and Lowell 1952) Japan (Johnston 1995), Brazil (Antunes and Waldman 1999) and Sierra Leone (World Health Organization 2012). Data from publications regarding mortality for TB in England and Wales as well as New York (United States) were collected and entered into Microsoft Excel for direct comparison with the data collected in this study (from Canton Zürich as well as the whole of Switzerland). England and Wales and New York (United States) were considered similar in terms of TB mortality trends (all Westernized, similar history regarding anti-TB health measures). Data from Japan, Brazil and Sierra Leone were collected for comparison as these countries had different history of TB prevention and control (as well as lacking Westernization for many years).

Important historical dates for events in the prevention and control of TB were added for further interpretations of the mortality data. These events included (among others) the introduction of Public Health and Sanitation Acts, compulsory reporting of TB cases and introduction of streptomycin.

We chose the logistic curve as the basic description of change in the relationship between disease prevalence and population characteristics. This curve describes the alteration of an occurrence of a particular feature in a population after the introduction of a change in the relationship of this feature to the population. The curve has a characteristic shape resulting from the fact that a change initially has small effects, then its effects increase as the change spreads and when it exhausts the prevalence of a feature, it comes to an asymptotic end. Logistic models were fitted to the data for Switzerland (whole country), England and Wales, and New York in the form:

$$M = f_0 + \frac{f_z - f_0}{1 + 10^{(t-c)*(-w)}}$$

Where:

M is the mortality rate observed at a given year (t)

t is the year

f_0 is the lower asymptote (the lowest mortality rate observed)

f_z is the upper asymptote (highest mortality rate observed)

c is the inflection point, where the rate of decline in mortality is the highest

w is the rate of decline at the inflection point (i.e. the highest rate)

The values of the parameters f_0, f_z, w and c were initially estimated by observing the scatter of data against time and the best fit was obtained by their iterative alterations (bootstrapping) using the least squares method. The year (t) and mortality (M) were the independent and dependent variables, respectively and thus did not need estimating.

These models were extrapolated to show the expected trend in TB mortality without the introduction of antibiotics in order to investigate the efficiency of conservative therapies (rest, good nutrition, ventilation, etc.) as well as public health improvements (addressing hygiene, nutrition and reducing exposure to infections as well as educating the population about TB) on the disease.

Results

Historical events in the decline of tuberculosis

THE CITY OF ZÜRICH AND CANTON ZÜRICH (SWITZERLAND)

The TB mortality rate for Canton Zürich (Switzerland) was above 350 per 100,000 during the 1840's (Figure 1). The mortality rate declined from that time onwards, reaching 224 by 1869. The lack of information for the early 1870's is likely due to the Prussian-French War during 1870 and 1871. Although Switzerland did not participate in this war, it took in a large number of refugees and the instability at the time would have affected external and international actions such as trade. From 1878 to 1888, the TB mortality rate increased slightly from 210 to 224 per 100000. There was a major economic crisis period in the 1880's and many people emigrated from Switzerland (Schoch et al. 2011). From 1893, the TB mortality rate dropped steadily until the end of the data shown here (1933). This decline was associated with the development of X-rays for diagnosis (1895), the opening of the first sanatorium (in Davos, 1896), introduction of the BCG vaccine (1921) and finally compulsory TB reporting and treatment introduced in 1928 (Gesetzgebung: Zürich 1928)).

For the city of Zürich, the records start only from 1893. This is easily explained because in 1893, the city expanded substantially in terms of area and population (Steinberg 1996). Thus the statistics collected are for a completely differently defined "City of Zürich" than in years preceding 1893. The initial TB mortality rate was higher in the city than in the entire canton. In fact, the rate in the city for 1893 was 180 per 100,000 higher than in the canton for this same year. Despite the initial rate being higher, the decline in mortality in the city was faster than in the canton. From 1893 to 1933, the mortality rate declined from 401 to 138 per 100,000. There was a reversal

from the decline in 1918, when the Spanish flu (influenza) epidemic caused a brief increase in mortality from TB.

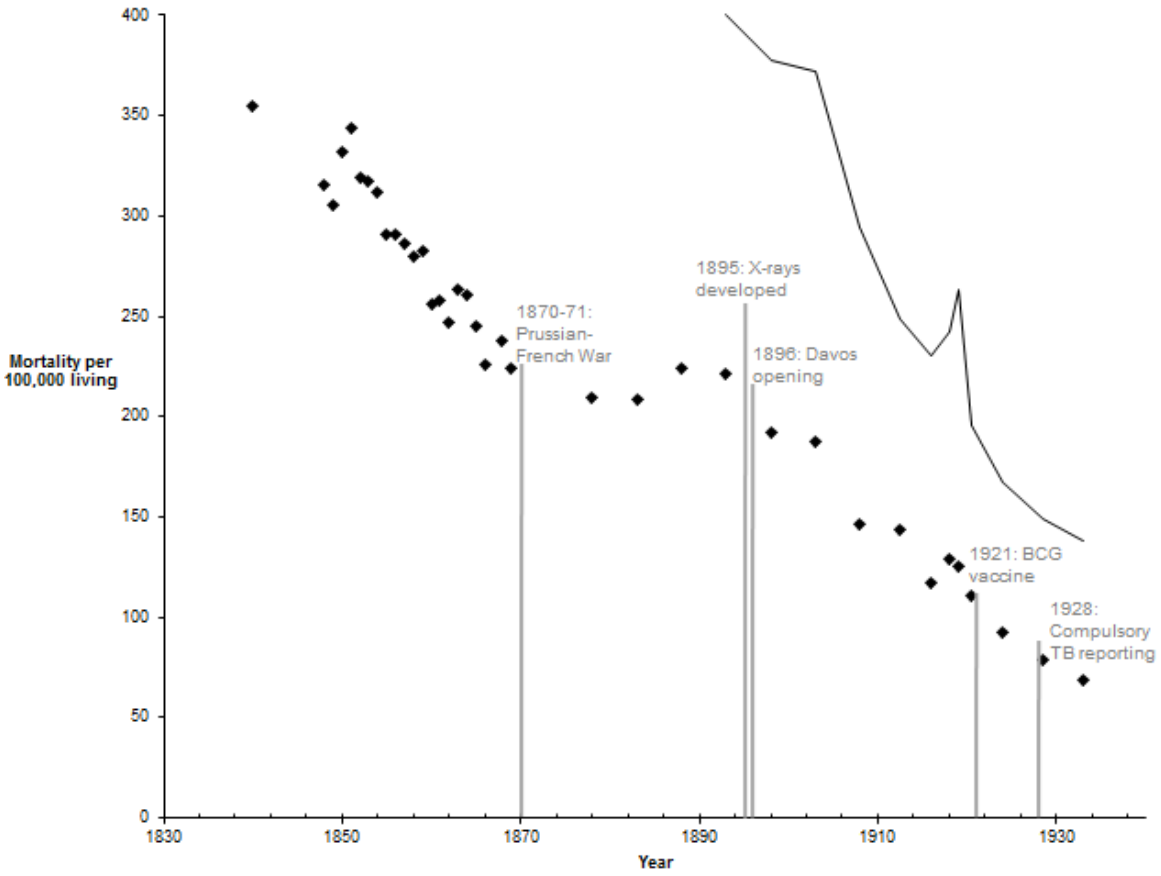

Figure 1: Tuberculosis mortality rate per 100000 population for Canton Zürich (diamonds) and the City of Zürich (line) in Switzerland. Data for Canton Zürich cover 1840 to 1943. The City of Zürich data cover 1893 to 1933. Historical dates associated with tuberculosis prevention and control are included.

ENGLAND AND WALES

In England and Wales, similar to Switzerland, TB mortality rates exceeded 350 (per 100,000) prior to the 1840's (Figure 2). By 1846, the rate had fallen to around 300 per 100,000. Mortality continued to decline steadily, reaching about 50 per 100,000 in 1940s, prior to the introduction of pharmacotherapies. Since then it declined further

until approximately 1960, when it had reached 5 per 100000. After this, the mortality rate was close to zero and could not decline much further. During the period of steady decline, a large number of events aimed at the control and prevention of TB occurred.

A number of these events included the introduction of laws including the "Poor Law," Sanitary and Public Health Acts (1846, 1848, 1866 and 1870's), Housing Act (1890) and a Public Health Act specific for TB (1921) (Szreter 1988; Wilson 2005). These Acts helped to prevent the spread of disease between groups in the population with different levels of risk for TB, addressed hygiene, living conditions, exposure risk (for bovine TB) and nutrition. In 1836, the registration of death became compulsory and this resulted in an improvement in reporting of causes of death (Szreter 1988). A notification of Infectious Diseases Act was established in 1889, requiring the reporting of all cases of infectious diseases. This allowed many of the previously unknown cases of TB to be located and dealt with more appropriately. Surgical interventions for TB were introduced in 1910, when the first pneumothorax (lung collapse) operation was performed. Compulsory reporting of TB did not come into effect until 1913 and as such, before this, many cases may have gone unreported. The final step in the control of TB in England and Wales was the introduction of the specific anti-TB antibiotic, streptomycin. After this time, mortality from the disease had declined substantially and the disease was no longer a major cause of death among the population.

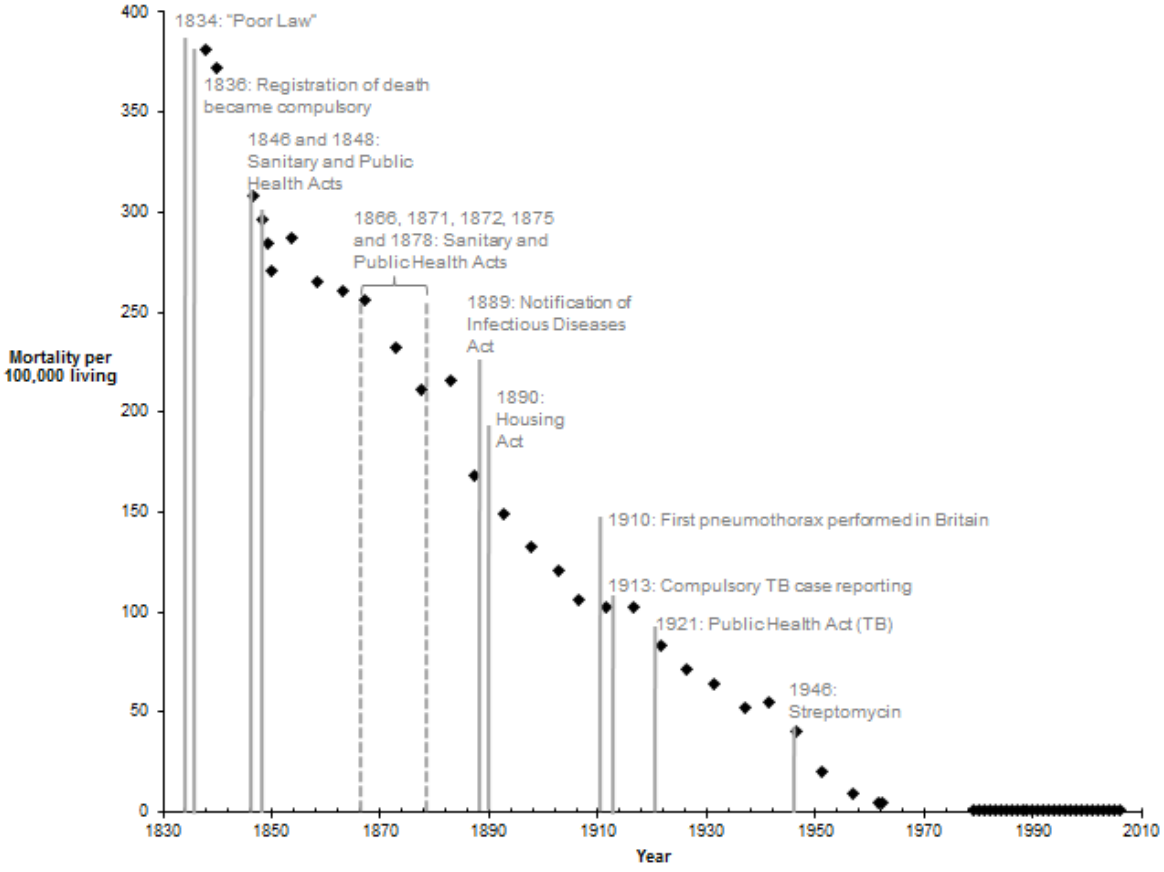

Figure 2: Tuberculosis mortality rate per 100000 population for England and Wales between 1838 and 2006. Historical dates associated with tuberculosis prevention and control are included.

NEW YORK (UNITED STATES OF AMERICA)

The TB mortality rate in New York was above 350 per 100,000 until about 1887 and did not drop below 300 until after 1890 (Figure 3). During the years 1918 to 1924, the mortality rate due to TB declined from around 200 to 100 per 100,000. After this time, the mortality rate declined slower at a relatively constant rate until 1950 when pharmacotherapies became established.

As in England and Wales, a number of laws were introduced to help control TB. One of these included an improved reporting of cases, free examination of sputum and

home visits for consumptives (1894) (Drolet and Lowell 1952). Other modifications to public health were made early in the 20th century including separating TB from the rest of the population (1901), Housing Law (1901), home visits by nurses (1939), X-rays for school teachers (1942) and government funded treatment (1947). Compulsory reporting of TB was introduced in 1897. The anti-TB drug, streptomycin was introduced in 1947. This allowed effective treatment of TB but did not significantly impact the rate of mortality decline. The last measure to be implemented was the BCG vaccine in 1949. By this time, the mortality due to TB was low and this final measure had little impact on the rate.

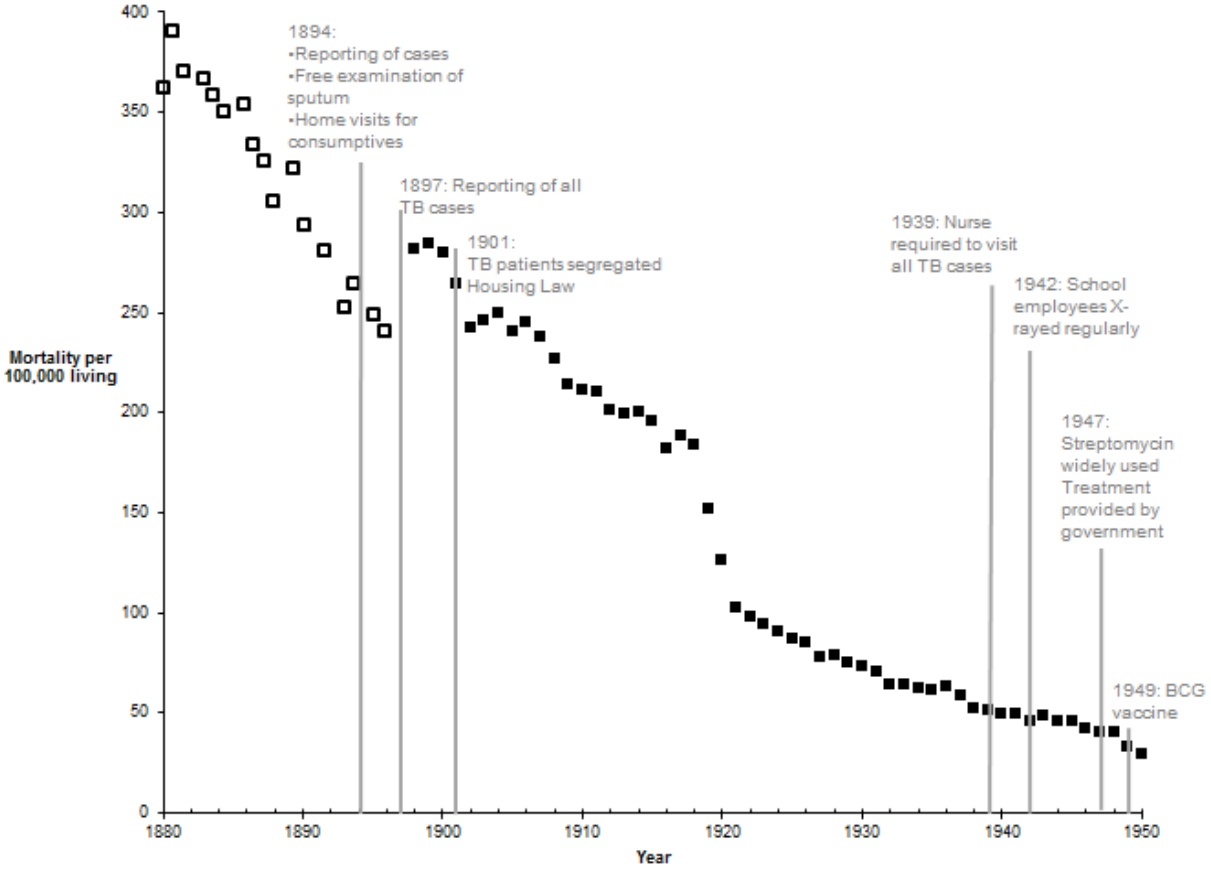

Figure 3: Tuberculosis mortality rate per 100000 population for New York (United States) between 1880 and 1950. Historical dates associated with tuberculosis prevention and control are included.

JAPAN

In contrast to Switzerland, England & Wales and New York, the mortality rate in Japan was below 100 per 100,000 between 1886 and 1888 (Figure 4). The rate increased steadily until 1910, when it was 231 per 100,000. The mortality rate fluctuated between 1911 and 1918, but was increasing overall. This increase in mortality rate occurred during the initial heavy industrialization of the country (Johnston 1995). TB mortality would have increased partly as a result of poor living conditions in "on site" accommodation for factory workers. In 1918, workers were allowed to commute to their workplace rather than living on site and this gradually resulted in a decrease in mortality due to TB (Johnston 1995). In 1918, the mortality rate was 251 and this decreased to 194 per 100,000 by 1924. In 1926, a Factory Law was introduced, protecting workers as well as women and children in the workplace. However, from 1933, the recorded mortality rate began to increase. This is a combination of compulsory reporting of cases introduced in 1931 as well as the Great Depression and war-time effects on economy. It is worth noting, however, that mortality rates during the early 20th century remained well below 300 per 100,000, roughly comparable to what these rates were at the same period in Europe and New York. Towards the end of World War II, TB had become a major problem and some further measures were introduced to help combat the disease. In 1941, reporting and treatment of TB were made compulsory in young males. The BCG vaccine was introduced in 1943. After World War II, cases of TB were reported more accurately and medicine was able to help fully control the disease. From 1946 to 1985, the mortality rate decreased from 185 to 4.5 per 100,000. Most of this decline is associated with the introduction of streptomycin in 1948, followed by the other anti-TB drugs, para-aminosalicylic acid and isoniazid in the early 1950's. Japan is different in that the pattern of mortality does not begin at a high level. Mortality increased and was then controlled by public health

measures, before medication finally reduced the disease to a level where it was no longer a major cause of death. Mortality rates also never reached the same level as in Switzerland, England and Wales, and New York. It is difficult to say to what extent the post-1950 decline of mortality was impacted by non-pharmacological measures.

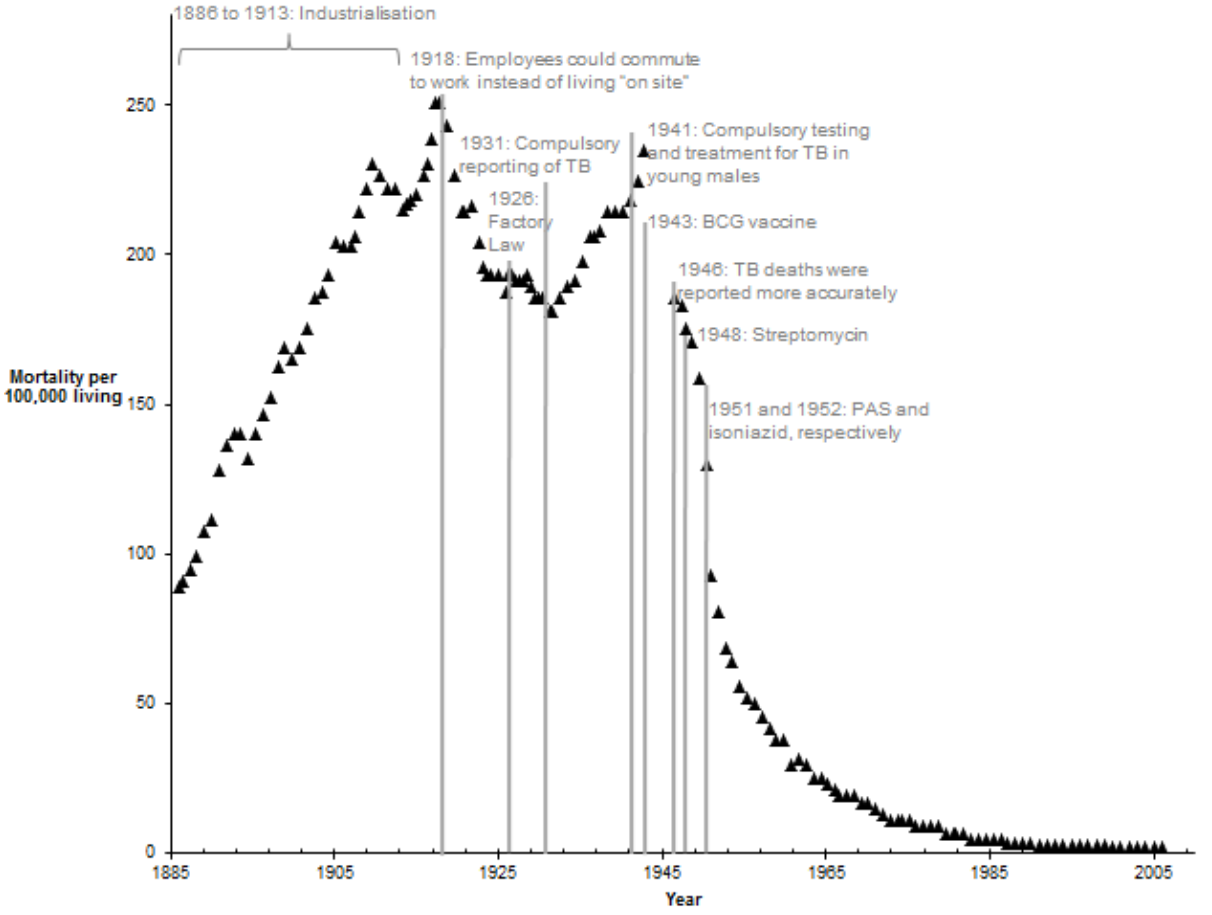

Figure 4: Tuberculosis mortality rate per 100000 population for Japan between 1886 and 2006. Historical dates associated with tuberculosis prevention and control are included.

BRAZIL AND SIERRA LEONE

In these two countries, public health interventions occurred very late in comparison with Switzerland, England and Wales and United States. Thus they serve as

a useful comparison showing that anti-TB drug therapy is indeed effective at controlling TB mortality (Figure 5).

In Brazil, at the end of the 19th century the mortality rate was just below 200 per 100,000, a moderately high rate compared to Europe and New York in the same period. The decline since then was slow and unsteady, but brought the mortality to less than 100 per 100,000 population in mid-1940 just prior to the introduction of pharmacotherapies. When medication was introduced, the mortality rate declined substantially at a rapid rate until it reached a level low enough to remove TB as a major cause of mortality in Brazil (Antunes and Waldman 1999). As in the case of Japan it is impossible to separate effects of non-pharmacological measures.

In Sierra Leone, there are data for only 1990 to 2010, but it is clear the TB mortality rate was, and still is, increasing at an alarming rate. In this African country, TB control is complicated by poor public health, lack of hygiene, HIV and probably increasing number of drug-resistant TB strains. In this case, although chemical therapies are available, they are ineffective. A strategy to reverse this trend can be developed but it must take into account a number of approaches.

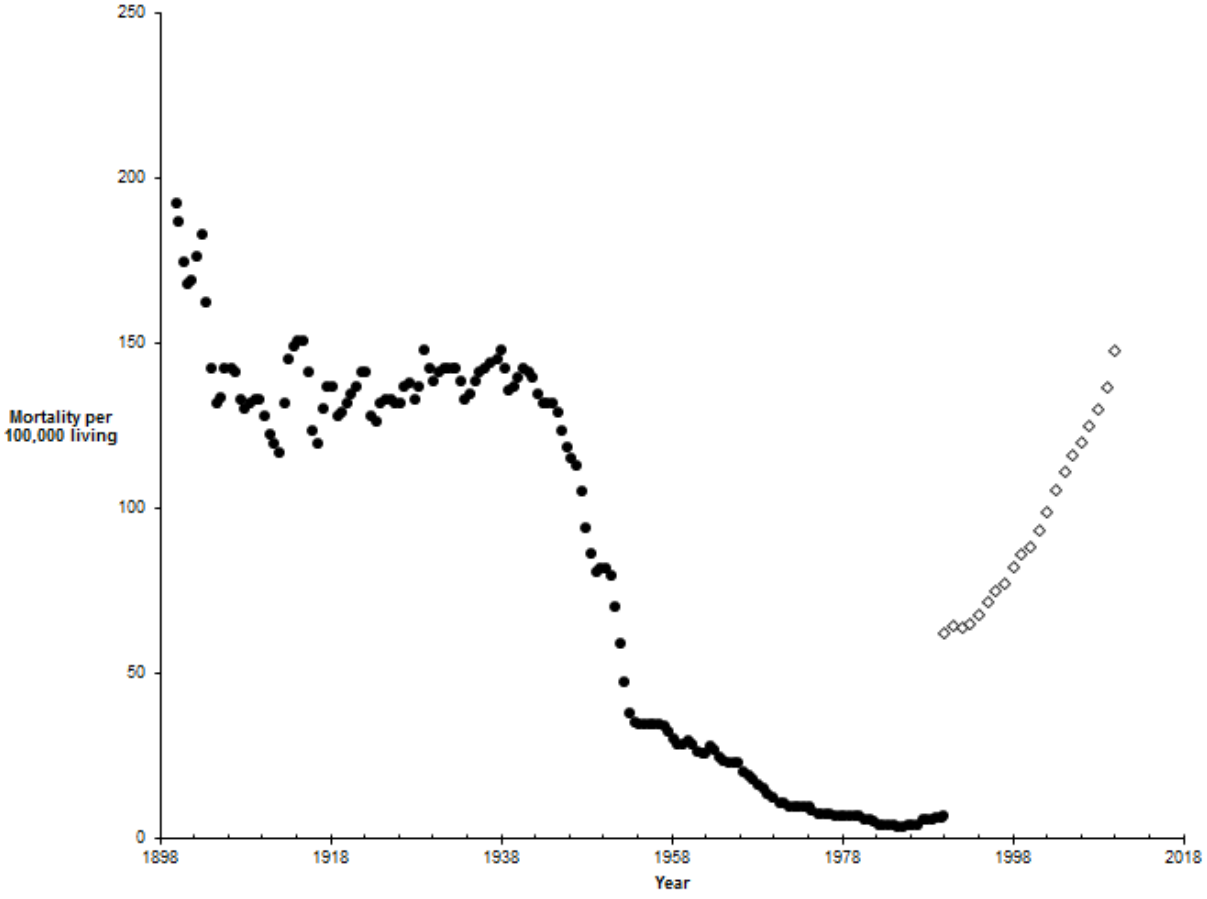

Figure 5: Tuberculosis mortality rate per 100000 population for Brazil (1900 to 1990) and Sierra Leone (1990 to 2010).

Logistic fitting

Logistic curves were fitted to the data for Switzerland (whole country), England and Wales, and the United States. Two (and in one case, three) separate equations were required to fully describe the data. Time periods best fitted by separate curves may have corresponded to a specific event during the decline of TB; the introduction of medication. Streptomycin implementation was observed to cause an increase in the rate (w) of decline compared with earlier time periods. In many countries during the first few years of streptomycin use, the datum points show a significant decrease and it was

very difficult to include these data when estimating parameters of a single curve, so they were excluded from the estimations.

SWITZERLAND

The mortality rate for Switzerland is shown in Figure 6. We chose not to model the data for Canton Zürich or Zürich city because the date range ended before the introduction of antibiotics and this method was intended to compare the rate of decline before and after the introduction of these therapies. The two time periods fitted with separate logistic curves were: (i) 1867 to 1941 and (ii) 1946 to 2005. The equations that best fit the data for these two time periods are:

(i) $M = \frac{305}{1+10^{(t-1925)(-1*-0.034)}}$ and

(ii) $M = \frac{450}{1+10^{(t-1927)(-1*-0.042)}}$

The R squared value for this double-logistic fit was 0.993, indicating a good fit. From the logistic models, the rate of decline can be observed to increase after 1946; when antibiotics were introduced. However, this is only a 24% increase in rate indicating that TB was well controlled before the implementation of antibiotics. The inflection point (*c*; where the rate of decline is highest) does not differ much between the two separate models as if they were parts of the same process. This could indicate again, that antibiotics did not have a significant impact on TB mortality in Switzerland, but rather supported the decline already occurring.

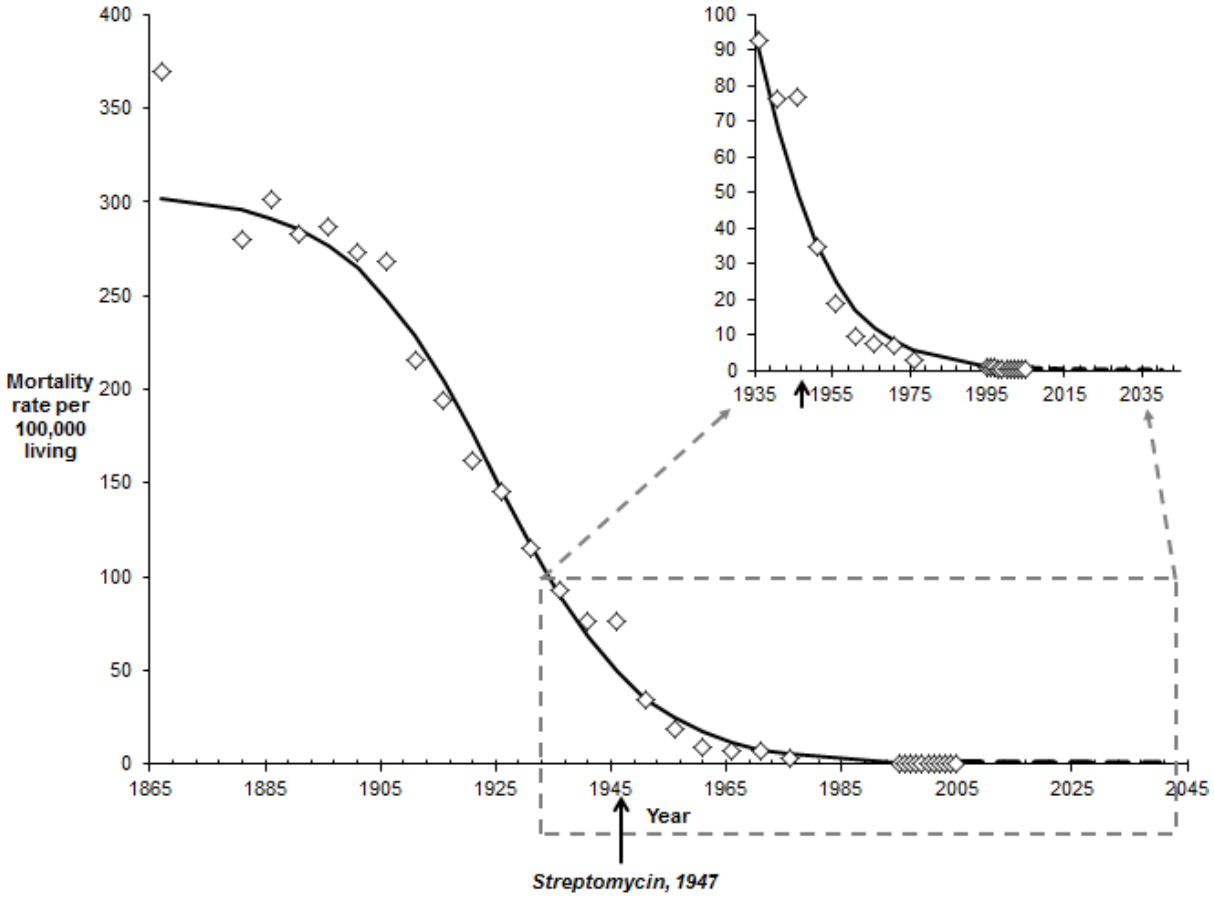

Figure 6: Tuberculosis mortality rate per 100000 population for Switzerland for 1867 to 2005. A logistic curve with R^2=0.993 has been fitted to the data. The logistic fit has been extrapolated. The inset shows a closer view of the time when streptomycin was first introduced. Symbols correspond to the real data, solid line is the logistic fit and dashed line is an extrapolation of the logistic fit using only the first equation (pre-pharmacotherapy).

ENGLAND AND WALES

For England and Wales, fitting of data required three separate models: (i) 1838 to 1878, (ii) 1883 to 1937 and (iii) 1941 to 2006 (Figure 7). This indicates there were three time periods where different factors were active in reducing the mortality from TB. The equations for these three different time periods are:

(i) $$M = \frac{250}{1+10^{(t-1840)(-1*-0.050)}}$$

(ii) $$M = \frac{405}{1+10^{(t-1880)(-1*-0.020)}}$$

(iii) $$M = \frac{304.3}{1+10^{(t-1929)(-1*-0.055)}}$$

These three models were combined in order to fully explain the data and the R squared value for this fit is 0.995, indicating the very close fit. When comparing the three different equations, corresponding to different time periods, the rates (*w*) begin at 0.050, decrease to 0.020 and then increase again to 0.055. Thus it can be observed that the initial decline of TB during time period (i) (1838 to 1878) was relatively fast, nearly as fast at following the introduction of antibiotics. After 1878, up to 1941, the decline slowed, but still a decrease in mortality occurred. During the final time period, 1941 to 2006, the decline increased in rate again, to slightly more than it was in the first time period. It is likely that the initial decline was due to the factors addressed by public health interventions (nutrition, hygiene, living conditions, etc.), while the final decline was influenced by antibiotics. The inflection point (*c*, date at the highest rate of decline) increases through the three different time periods; from 1840 to 1880 to 1929 in time periods (i), (ii) and (iii), respectively. The different values of *c* indicate that three separate processes are occurring.

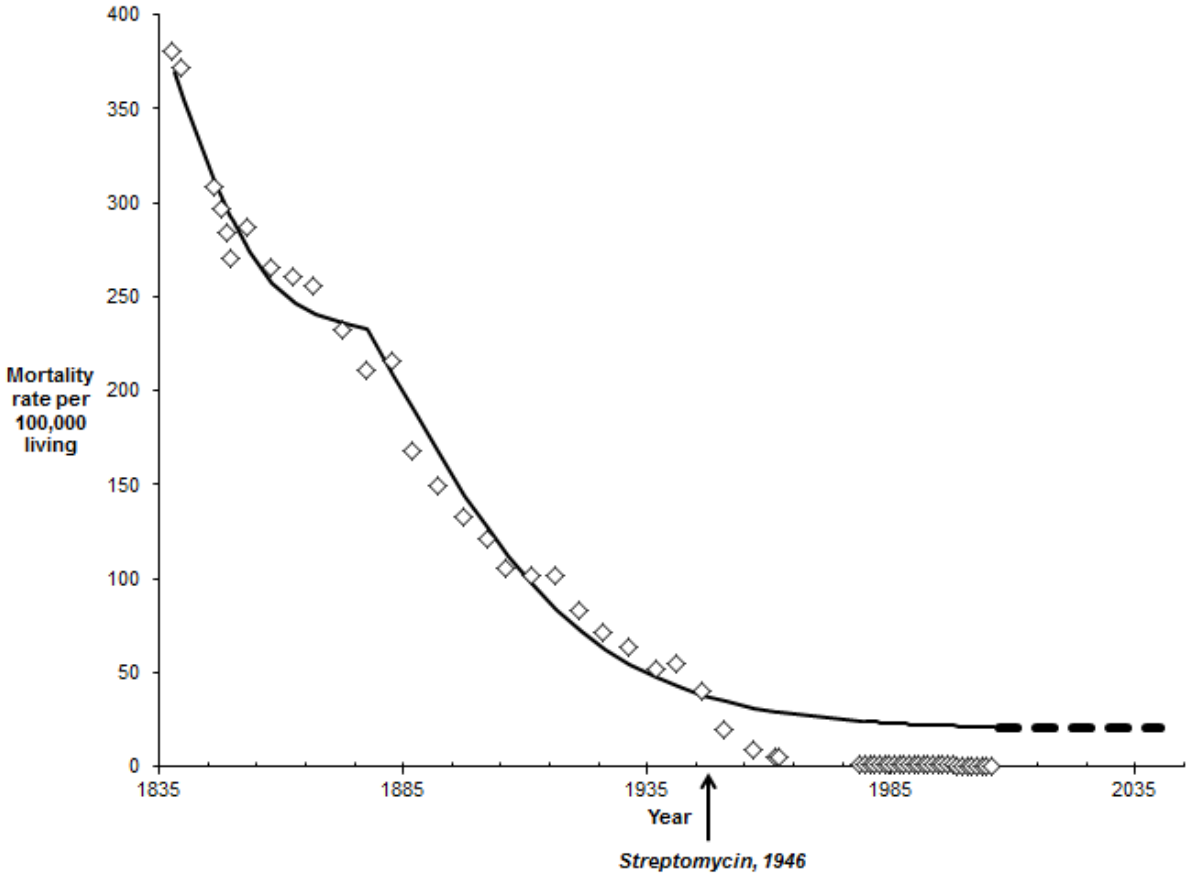

Figure 7: Tuberculosis mortality rate per 100000 living for England and Wales for 1878 to 1950. A set of three logistic curves with R^2=0.995 has been fitted to the data. The logistic fit has been extrapolated. Symbols correspond to the real data, solid line is the logistic fit and dashed line is an extrapolation of the logistic fit using the second logistic curve (pre-pharmacotherapy).

THE UNITED STATES OF AMERICA (NEW YORK)

For New York, fitting required to distinguish two periods with separate logistic models (Figure 8). These two time periods were: (i) 1878 to 1917 and (ii) 1921 to 1948. The time span corresponding to the third period in England and Wales (1940s to 2006) was not studied. The equations fitted to these data were:

(i) $M = \frac{230}{1+10^{(t-1892)(-1*-0.050)}}$ and

(ii) $M = \frac{297}{1+10^{(t-1904)(-1*-0.025)}}$

These two equations gave a combined R squared value of 0.971, indicating a reasonably good fit, but some data were not well represented by the model. This discrepancy is likely due to changes in the reporting system, where some cases of TB went unreported and after a certain year, compulsory reporting of TB cases was introduced. An attempt was made to solve this issue by artificially increasing the mortality rate values before 1898 (Figure 9). A logistic model was fitted to the modified data, with equation:

(iii) $M = \frac{370}{1+10^{(t-1892)(-1*-0.052)}}$

The other data (after 1920) retained the same equation as before ((ii) above).

If we compare the equations (i) and (ii) we can observe that the rate (*w*) decreases by half between the earlier and later time periods (0.05 to 0.025), indicating that the rate of decline was faster during the time period 1878 to 1917 than during 1921 to 1948. This is interesting because the latter time period included, at its very end, the introduction of antibiotics. This indicates that antibiotics had little impact on the already rapid and largely completed decline of TB mortality. The same decrease is observed when comparing (i) and (iii) (modified data). (i) and (iii) also have the same value for *c* (inflection point, where the decline is most rapid). However, between the two time periods, the value of *c* is different. In the earlier time period, the rate of decline is highest at 1892 and in the later time period during 1904. This difference in the value of *c* indicates that at least two separate processes are affecting the decline of TB during the entire time period reported (1878 to 1897). There were only a few differences between the modified and unmodified data for New York. The rate is slightly higher in the

modified data and the upper asymptote (highest mortality rate) was higher for the modified data compared to the unmodified data. This is expected, since the modification was to artificially increase values to account for the underreporting of TB cases before 1898.

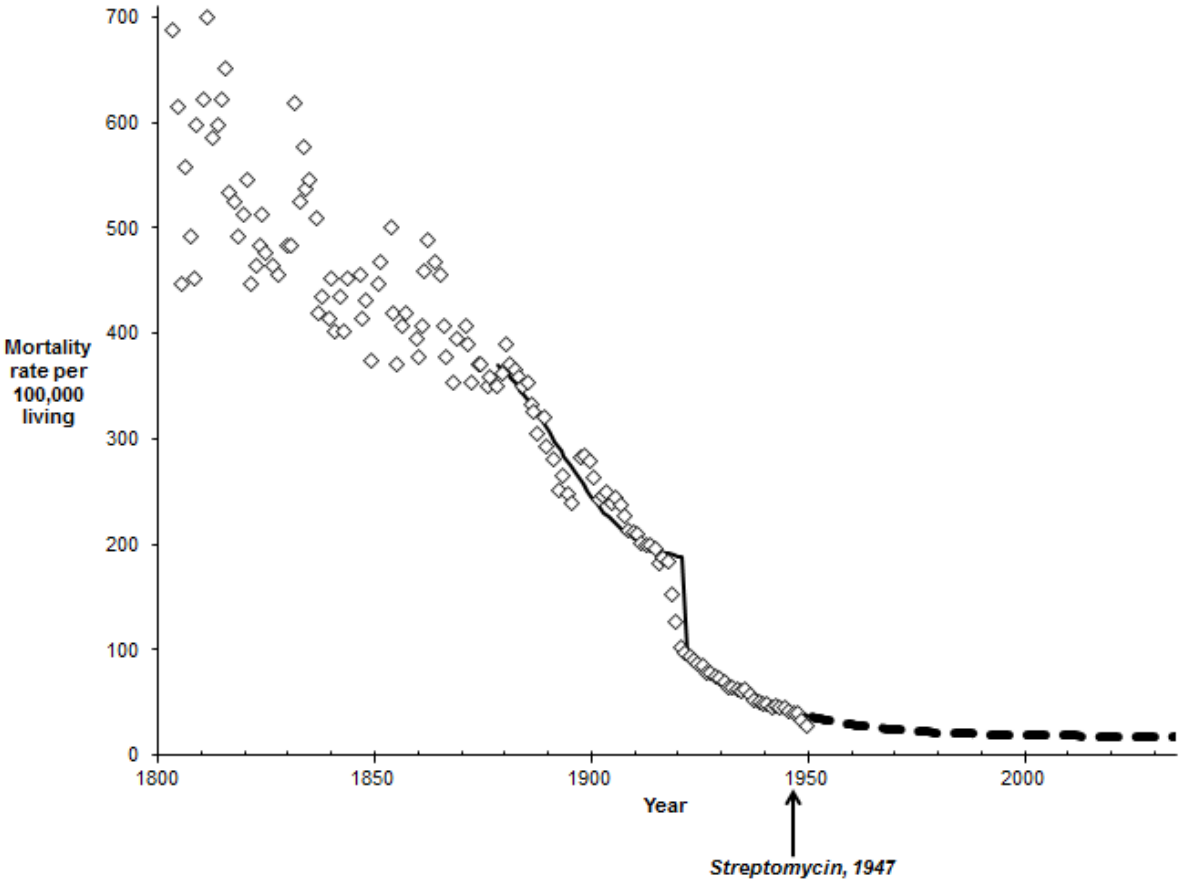

Figure 8: Tuberculosis mortality rate per 100000 living for New York (United States) for 1878 to 1950. A logistic curve with R^2=0.971 has been fitted to the data. The logistic fit to pre-pharmacotherapy data has been extrapolated. Symbols correspond to the real data, solid line is the logistic fit and dashed line is an extrapolation of the logistic fit.

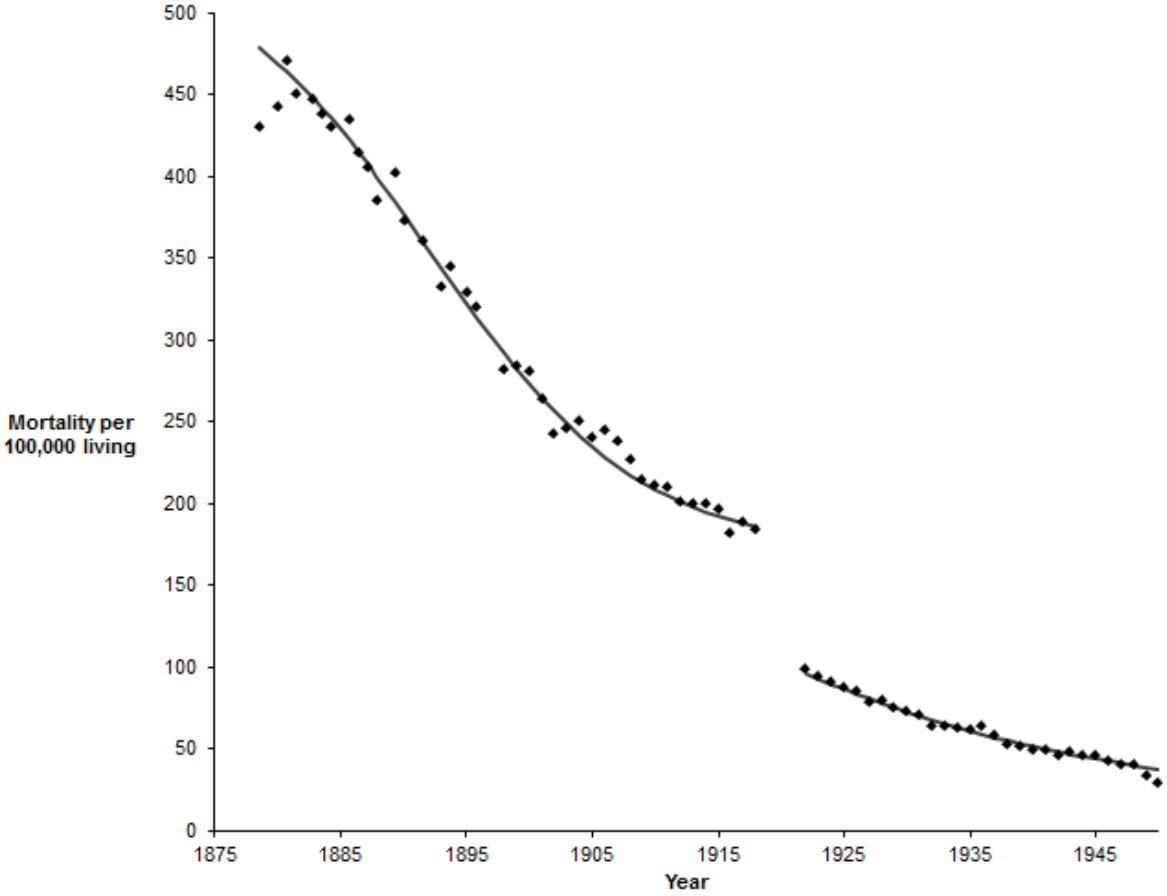

Figure 9: Tuberculosis mortality rate per 100000 living for New York (United States) for 1878 to 1950. The data for 1878 to 1897 have been modified from Figure 8 due to underreporting before the introduction of compulsory recording of TB cases in 1898. A logistic curve with an R squared value of 0.996 has been fitted to the data.

EXTRAPOLATION OF LOGISTIC MODELS

The models fitted to the data for Switzerland, England and Wales and the United States (New York) were extrapolated to show the expected trend of TB mortality if antibiotics had not been introduced. For Switzerland, this was achieved by extrapolating equation (i) to the year 2040. For England and Wales, equation (ii) was used to model the years from 1941 to 2040 while equation (i) was retained to model the earlier period

1838 to 1878. New York data were extrapolated using equation (ii) to model data beyond 1948.

In Switzerland (Figure 6), streptomycin was introduced during 1947. The rate of decline for TB after the introduction of this therapy was 0.042 (equation (ii), above) but with extrapolation, the rate is only 0.034 (equation (i), above). This corresponds to a 24% increase in rate of decline through the use of antibiotics. Both the model data and the real data reach a similar mortality rate of less than 1 per 100,000 by the end of the real data (2005). At the time that streptomycin was introduced, mortality from TB had declined from 370 per 100,000 in 1867 to 77 in 1946. This is a decline of 71%. The logistic model predicts a rate of 0.58, while the real data is 0.28. Overall, in Switzerland, the introduction of streptomycin only served to slightly improve the rate of mortality decline.

In England and Wales (Figure 7), streptomycin was available in 1946. At this time, the rate of decline with this therapy was 0.055 (equation (iii) above). The rate of decline modelled by the logistic fit (extrapolation) is 0.020 (equation (ii) above). This is an increase of 175% in the rate of mortality decline. Thus, antibiotics did substantially improve the rate of decline. Interestingly, the modelled data and the logistic data do not reach the same levels by 2006 (when the real data end). The modelled data reached 21 per 100,000 living while the real data declined to 0.70. This difference suggests that there was a decline already occurring but that the factors responsible were not sufficient to fully control the disease. However, when streptomycin was introduced the mortality rate had already declined by 90%. The rate decreased from 381 per 100,000 in 1838 to 40 in 1946.

As in Switzerland, streptomycin was available in 1947 in New York (Figure 8). We chose to use the raw data (i.e. not Figure 9) for the extrapolation because the logistic fit chosen was for 1922 to 1950. The logistic fit of the data before this was not important for the extrapolation (i.e. the section that was modified in Figure 9). The rate of decline after this time was 0.025 (equation (ii) above). The data did not significantly alter with the introduction of antibiotics, so there was no need to modify the logistic fit. However, in 1950, the real and modelled data do differ slightly in terms of actual rate. The modelled data predicted a rate of 38 per 100,000 in 1950, while the real data show a rate of 29. When streptomycin was introduced in 1947, the mortality rate had already declined from 350 per 100,000 in 1878 (687 in 1803) to 42 in 1946. This is a decline of 88%. This suggests that although streptomycin did not make a significant impact on the mortality rate during its introduction, it still was aiding the decline already occurring.

Discussion

The decline of tuberculosis

In Switzerland, England and Wales and the United States (New York), the decline of mortality due to TB was continuous and steady throughout a period of more than 100 years. Medical intervention in the form of the pharmacotherapies including the antibiotic, streptomycin was not responsible for the majority of the decline. Mortality rates in Switzerland, England and Wales and the United States (New York) had declined by 71%, 90% and 88%, respectively before the introduction of streptomycin. There was no specific event that was responsible for a majority of the decline; instead it was a combination of many smaller changes in public health and disease control.

In Japan, mortality did not decline from an originally high level as was observed in Switzerland, England and Wales, and in the United States (New York). Instead, the

mortality increased from an originally low level, which would be associated with an increasing level of industrialization at this time. During the 1930's, the Great Depression affected living conditions of the Japanese, resulting in an increase in TB mortality. This occurred just before World War II, which resulted in a continuation of the same trend. It was not until after the war, when anti-TB drugs were introduced, that TB in Japan was reduced to low mortality rates and controlled. Thus in Japan, the decline in TB mortality was largely due to the implementation of drug therapy for the disease. It could be postulated that the improvements in sanitation and living conditions in post-war Japan may have lowered TB mortality rates even without the introduction of pharmacotherapies, but it is impossible to separate the effects of these measures from those of chemical intervention.

In Brazil, drug therapy was the major factor in the decline of TB mortality, though the data show a slow decline approximately halving mortality rates between the end of the 19th century and the time pharmacotherapies became available. Then when drug therapy was introduced, mortality declined quickly within several years. The shape of this mortality decline is not observed for Switzerland, England and Wales, or, New York (United States) indicating that the major factors of the decline in TB mortality were different from medical intervention in those countries. Sierra Leone shows the data for a country where TB is becoming a major health problem. This trend would likely have been observed for Switzerland, England and Wales and the United States in earlier years not captured by the sources of information we used.

Logistic fitting

Logistic curves were fitted to the mortality data for Switzerland, England and Wales and the United States (New York) and all had an R squared values above 0.97,

indicating a good fit. This also indicates that the decline of TB mortality followed a logistic pattern. One single logistic equation was not satisfactory for a good fit and consequently multiple equations were needed. This indicated that multiple processes of decline in TB mortality were operating during different years. In the years at the beginning of our data, it may be expected that implementation of public health reforms addressing hygiene, nutrition, living conditions, etc. were the reason for the mortality decline. The mortality decline associated with drug treatment was in some cases substantially faster than for conservative methods and public health however, both had clear effects on decline in mortality rates. Extrapolation of the models to exclude the influence of antibiotics, showed that the mortality decline would have continued into the 21st century, even without the introduction of pharmacotherapies. However, the mortality rates declined quicker and reached lower values with antibiotic therapy.

Limitations of the study

One limitation of this study is the reliance on historical records. However, this study includes data from multiple countries over a number of years, all of which show the same trend over time. These data also include years when TB notification was compulsory and the records can be considered reasonably accurate during these time periods.

One other limitation with this study is the change in cause of death nomenclature through time. The disease has been recorded under many names including "tuberculosis," "phthisis," and "consumption." Non-pulmonary TB has been known by multiple names including "scrofula" and "King's Evil." However, the nomenclature was defined by the ICD codes for causes of death in 1900, which was used by all Western as well as some Eastern countries (including Japan). Thus this limitation is only a concern

before this year while much of the decline in TB mortality occurred during the early years of the 20th century.

Another problem with this study is the difficulty in differentiating the factors responsible for the mortality decline. Each factor cannot be distinctly assessed for its role in the mortality decline because the data provide only an overview of the effects of all factors operating at that time.

This type of investigation has been completed previously by several authors (Fairchild and Oppenheimer 1998; McKeown 1976; Szreter 1988). McKeown (1976) produced one of the original works on this topic. In his book, McKeown describes the growth in population size through history, attributing the increase in to a reduction in mortality, rather than an improvement in fertility. He then goes on to describe the possible causes of the mortality decline, focusing on the major causes of death during the 19th century; infectious diseases. He focuses mainly on airborne infectious diseases because they were a large part of the decline. Several factors were considered, including a decrease in virulence of microorganisms, medical interventions (immunization and therapy), levels of exposure and nutrition. All of these factors were considered in turn, with the final conclusion that nutrition was the main reason for the decline in mortality (due to infectious airborne diseases). For TB, the authors suggest the reason for the mortality decline was a reduction in exposure because the disease was less prevalent; brought about by an improvement in nutrition. The work done by Griffith (1926) was also highlighted, giving reasons why the interpretation of medical interventions as the main reason for mortality decline is incorrect.

A later investigation into the same area was conducted by Szreter (1988). The author appraised the earlier work by McKeown and highlighted various problems. He stated that the interpretation was done via elimination and that the nutrition hypothesis

was not as carefully investigated as the other factors were. Nutrition also did not account for decline of some diseases such as smallpox that declined for other reasons (in this case, immunization). Additionally, food and waterborne diseases were a substantial part of the mortality decline. These were significantly affected by hygiene and living conditions and less affected by nutritional status. McKeown also grouped "public health" as a broad factor and did not consider many of the smaller parts. The work also treated all airborne diseases together, but mortality trends for separate groups were different. Overall, Szreter suggests that only TB would really support the nutrition hypothesis proposed by McKeown. However, despite this, there are certainly other factors affecting the decline of TB including addressing overcrowding, lack of sunlight, ventilation, occupational hazards (dust/smoke) through public health interventions. Additionally, urban and rural populations had a different exposure and risk of TB. Szreter instead, suggests a different reason for the mortality decline, associated with public health. The author lists the number of public health acts introduced in Britain addressing a variety of factors including hygiene, living conditions, ventilation and food quality. The author also notes that McKeown's theory about a larger quantity of food resulting in better nutrition, may not be true if food quality is poor. In fact, it may spread disease faster than if a smaller amount of food was available.

Another later study (Fairchild, 1998) reviews both McKeown's and Szreter's work, highlighting that although each has their own interpretations, neither has a sufficient amount of evidence to support their conclusions. There is a need for multifactorial models that include housing, ventilation, hygiene, working conditions, nutrition, other infections, but it is impossible to test for each one of these while keeping the others constant. The author continues on with a description of sanatoria and how segregation and consequent lowering of transmission of the disease has been the only consistently effective measure for controlling TB. The authors finally conclude that

"social medicine" was responsible for the decline in TB and this included public health, awareness, environmental conditions, housing, food and working hours. Overall, these previous works show the difficulty in determining the exact reasons for the decline in TB mortality through time. It is likely that a combination of factors related to public health, nutrition, segregation of the infected and hygiene were responsible for the decline in mortality. We have shown here that pharmacological interventions were not responsible for the majority of the decline in Switzerland, England and Wales and the United States (New York). Instead, conservative and public health measures implemented before the introduction of streptomycin were effective in reducing the mortality from TB. This is even despite the obvious problems with compliance and efficiency of the introduction of various public health measures. Hygiene/sanitation and availability of proper healthcare was not uniformly distributed in the 19th and early 20th centuries. This is shown by data from (Drolet and Lowell 1952), describing the differences in mortality rates for different areas of New York between 1900 and 1950. This is also true for many other cities in this time period. Clearly however, even partly ineffective implementation of conservative measures and public health can help to control TB and this can be used in situations where TB is at high levels currently. Conservative methods and public health will be also effective at controlling drug resistant TB, as pharmacotherapeutics are not the focus of treatment.

However, we also included data from Japan and Brazil, which show the rapid reduction in TB mortality due most likely to pharmacological interventions. This shows that drug therapy as well as conservative measures and public health can be used to control TB. Overall, as suggested by McKeown (1976), as long as the prevalence of TB is decreasing through any means, a reduction in prevalence results in a lower level of exposure and transmission, which, in the case of airborne infections, is the most important part of controlling the disease. The only concern with using drug therapies as

a major part of a disease control program is the development of drug resistance. This may be exacerbated by patient non-compliance. Unlike public health measures and conservative methods, drug therapy needs to be fully and efficiently implemented to be effective in controlling infections.

Drug resistant TB has recently surfaced as a result of poor drug therapy practices including patient noncompliance (usually resulting in incomplete treatment). Non-compliance is a major issue in the treatment of TB because the regime lasts for several months, providing a long time frame for drug resistant bacteria to develop. Multiple drug resistant (MDR) and extensively drug resistant (XDR) strains of the tubercle bacillus have become difficult for physicians to treat with the current pharmaceuticals. This study suggests another method; improving an individual's general health and immunity to allow them to control the bacterium themselves. Yet a different method has been attempted in the recent past involving the use of surgical procedures (Jacobaeus 1923; Wang et al. 2008). The aim of these procedures was to remove a section of infected lung tissue, which reduced the bacterial load. This allowed the immune system of a patient to more effectively combat the fewer remaining bacilli and in many cases, was successful.

Conclusion

The decline of TB mortality during the 19th and 20th centuries in Switzerland, England and Wales and the United States was largely due to a combination of conservative measures (rest, good nutrition, ventilation, etc.) and public health (addressing hygiene, nutrition, reducing exposure to infections and education about TB). The introduction of anti-TB drug therapy played a small role in the mortality decline in these countries. Drug therapies can be useful to reduce mortality due to TB,

but must be implemented efficiently in order to prevent resistance. With regard to drug-resistant forms of TB it should be recommended to use the time-honoured and proven methods of increasing general immunity and limiting probabilities of infection through specific and general public health measures rather than to find expensive and short-lived chemical therapies aimed at killing germs that have already entered bodies of patients. These conservative measures and public health have been shown to be effective, even when not implemented at full efficiency. Improving economies and public health policies of TB infested countries is a life-saving imperative.

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Declaration of Interest

The authors wish to express no conflicts of interest.

References

Antunes JLF, and Waldman EA. 1999. Tuberculosis in the twentieth century: time-series mortality in São Paulo, Brazil, 1900-97. Cad Saúde Pública, Rio de Janeiro 15(3):463-476.

Blower SM, McLean AR, Porco TC, Small PM, Hopewell PC, Sanchez MA, and Moss AR. 1995. The intrinsic transmission dynamics of tuberculosis epidemics. Nature Medicine 1(8):815-821.

Cole ST, Eisenach KD, McMurray DN, and Jacobs Jr WR, editors. 2005. Tuberculosis and the Tubercle Bacillus. Washington DC, USA: ASM Press.

Drolet GJ, and Lowell AM. 1952. A Half Century's Progress Against Tuberculosis in New York City: 1900-1950. New York: New York Tuberculosis and Health Association.

Fairchild AL, and Oppenheimer GM. 1998. Public health nihilism vs pragmatism: history, politics, and the control of tuberculosis. Am J Public Health 88(7):1105-1117.

Gesetzgebung: Zürich. 1928. Anfrage des Stadtrates betr. Erlass von Vorschriften über die Wohnungsinspektion. In: Zürich S, editor. Zürich, Switzerland.

Herzog H. 1998. History of tuberculosis. Respiration 65(1):5-15.

Jacobaeus HC. 1923. The Cauterization of Adhesions in Artificial Pneumothorax: Treatment of Pulmonary Tuberculosis under Thoracoscopic Control. Proceedings of the Royal Society of Medicine 16:45-62.

Johnston W. 1995. The Modern Epidemic: A History of Tuberculosis in Japan. Cambridge (Massachusetts): Harvard University Press.

Kanton Zürich. 2012. Staatsarchiv.

Kaplan CJ. 1959. Conservative Therapy in Skeletal Tuberculosis: An Appraisal Based on Experience in South Africa. Tubercle 40:355-368.

Kaufmann SHE. 2003. A short history of Robert Koch's fight against tuberculosis: Those who do not remember the past are condemned to repeat it. Tuberculosis 83(1-3):86-90.

Manjourides J, Lin H-H, Shin S, Jeffery C, Contreras C, Cruz JS, Jave O, Yagui M, Asencios L, Pagano M et al. . 2012. Identifying multidrug resistant tuberculosis transmission hotspots using routinely collected data. Tuberculosis 92:273-279.

McKeown T. 1976. The Modern Rise of Population. London: Edward Arnold.

Ritzmann-Blickenstorfer H. 1996. Historische Statistik der Schweiz [Historical Statistics of Switzerland]. Zürich, Switzerland: Chronos Verlag.

Roberts CA, and Buikstra J. 2003. The Bioarchaeology of Tuberculosis: A Global View on a Reemerging Disease. Florida: University Press of Florida.

Rucker WC, and Kearny RA. 1913. Tuberculosis in Switzerland: Results of the Campaign against the Disease. Public Health Reports (1896-1970) 28(52):2815-2829.

Schoch T, Staub K, and Pfister C. 2011. Social inequality and the biological standard of living: an anthropometric analysis of Swiss conscription data, 1875-1950. Economics and Human Biology [in print].

Stadt Zürich. 2012. Stadtarchiv - Stadt Zürich.

Stead WW. 2001. Variation in vulnerability to tuberculosis in America today: Random, or legacies of different ancestral epidemics? International Journal of Tuberculosis and Lung Disease 5(9):807-814.

Steinberg J. 1996. Why Switzerland? Cambridge, UK: Cambridge University Press.

Szreter S. 1988. The Importance of Social Intervention in Britain's Mortality Decline c. 1850-1914: a Re-interpretation of the Role of Public Health. The Society for the Social History of Medicine 1(1):1-38.

Wang H, Lin H, and Jiang G. 2008. Pulmonary Resection in the Treatment of Multidrug-Resistant Tuberculosis: A Retrospective Study of 56 Cases. The Annals of Thoracic Surgery 86:1640-1645.

Wilbur AK, Farnbach AW, Knudson KJ, and Buikstra JE. 2008. Diet, tuberculosis, and the paleopathological record. Current Anthropology 49(6):963-991.

Wilson LG. 2005. Commentary: Medicine, population, and tuberculosis. Int J Epidemiol 34(3):521-524.

World Health Organization. 2012. World Health Organization Tuberculosis burden estimates.

Appendix 1: For manuscript 1: "Evolution of human tuberculosis: A systematic review and meta-analysis of paleopathological evidence."

Summary of paleopathological cases of tuberculosis.

Reference	Geographic Location	ID/grave No.	Date	Date for analysis	Skull	Spine	Ribs	Pelvis	Hip	Sacro-iliac joint	Long Bones	Shoulder	Elbow	Knee	Distal Limbs	Age	Age for analysis	Sex	Total cases	Sam. size	aDNA/ notes
Northern and Central Europe																					
(Roberts and Buikstra, 2003)	Zlota, Poland		5000 BCE	-5000		1												M	1	218	
(Steinbock, 1976)	Heidelberg, Germany		5000 BCE	-5000		1												M			
(Roberts and Buikstra, 2003)	Manych, Russia		1000 BCE	-1000		1												M			
(Mays and Taylor, 2003)	Tarrant Hinton, England	7	400-230 BCE	-315		1										30-40	35.0	M	1	15	*
(Roberts and Buikstra, 2003)	Igrzyczna, Poland		450-100 BCE	-275										1					1	21	
as above	Alsted, Denmark		500-1 BCE	-250		1															
(Jankauskas, 1998)	Marvelė, Lithuania	917	100-200	150		1	1		1						1	30-35	32.5	F			*
(Anderson, 2001)	Towcester, England	SK22	100-300	200		1										30-40	35.0	M	1	27	
(Stirland and Waldron, 1990)	Alington Ave., England		100-300	200		1										20-30	25.0	M	2	58	
(Roberts and Buikstra, 2003)	as above		100-300	200		1										Mature	50.0	F	2	58	
as above	Ancaster, England		100-300	200		1										Mature	50.0	M	2	2	
as above	as above		100-300	200															2	2	1 other
(Stirland and Waldron, 1990)	Ashton, England	ASH 237	100-300	200		1										20-30	25.0	F	2	297	
as above	as above	ASH 261	100-300	200		1										20-30	25.0	M	2	297	

(Roberts and Buikstra, 2003)	Cirencester, England		100-300	200		1	1							17-25	21.0	M	5	362	
as above	as above		100-300	200		1								old	60.0	F	5	362	
as above	as above		100-300	200		1								old	60.0	M	5	362	
as above	as above		100-300	200													5	362	2 other
(Roberts and Buikstra, 2003)	Queensford Mill, England		100-300	200		1										F	1	81	
as above	Tolpuddle Hall, England		100-300	200		1										M	1	48	
as above	Victoria Rd., England		100-300	200		1	1									F	1	201	
(Jankauskas, 1998)	Veršvai, Lithuania	4	200-300	250		1										M			
as above	as above	69	200-300	250						1		1				M			
(Wells, 1964)	West Hall, England		330-350	340							1	1		25	25.0	M	1	7	
(Jankauskas, 1998)	Plinkaigalis, Lithuania	177	400-500	450		1								25-30	27.5	F			
as above	Obeliai, Lithuania	16	400-500	450		1								45-50	47.5	M			
as above	Pagrybis, Lithuania	189	400-500	450	1									20-30	25.0	M			
(Roberts and Buikstra, 2003)	Vaud, Switzerland		400-600	500		1										F			
(Évinger et al., 2011)	Szekszárd-Tószegi dűlő, Hungary	1074	500-600	550				1						25-30	27.5	M	1	1384	
(Marcsik et al., 2006)	Kiskundorozsma-Daruhalom, Hungary	176	600-700	650		1										M		94	
(Marcsik et al., 1999)	Sükösd, Hungary	Sü-19	600-700	650		1		1						19-20	19.5	F		363	*
(Pálfi, 1991)	Bélmegyer-Csömöki, Hungary	Be-65	600-700	650		1								30-40	35.0	F	2	239	*
(Pálfi and Marcsik, 1999)	Bélmegyer, Hungary	Be-90	600-700	650		1		1								M		239	
(Molnár et al., 1998)	Pitvaros, Hungary	Pi-12	600-700	650		1		1								M		209	
as above	as above	Pi-110	600-700	650								1				M		209	
(Pálfi et al., 1999)	as above	Pi-215	600-700	650		1								50-60	55.0	M		209	*
(Pálfi and Marcsik, 1999)	Bélmegyer, Hungary	Be-215	600-700	650							1							239	
as above	Szeged-Makkoserdo, Hungary	209	600-700	650		1												152	
as above	as above	307	600-700	650		1												152	
(Marcsik and Pálfi, 1993)	as above	241	600-700	650					1									152	
(Pálfi and Marcsik, 1999)	Csólyospálos, Hungary	17	600-700	650		1												244	

as above	Székkutas, Hungary	343	600-700	650		1										518	
as above	as above	385	600-700	650		1										518	
as above	as above	531	600-700	650		1										518	
as above	Hetényegyháza, Hungary	156	600-700	650		1										263	
as above	Sükösd cemetery, Hungary	208	600-700	650		1										363	
as above	as above	218	600-700	650		1										363	
(Roberts and Buikstra, 2003)	Avar period, Hungary		600-700	650		1		2				20	20.0	F			
(Molnár et al., 1998)	Mélykút, Hungary	28	600-700	650				1							1	45	
(Ulrich-Bochsler et al., 1982)	Oberwil Kirche, Switzerland		600-800	700	1	1						18-23	20.5	F	1	28	
(Roberts and Buikstra, 2003)	Walkringen, Switzerland		600-900	700		1								M	1	20	
(Marcsik et al., 2006)	Felgyõ-Ürmös tanya, Hungary	205	700-800	750		1								F		64	
as above	Orosháza-Béke tsz, Hungary	63	700-800	750				1						M		98	
as above	Ároktõ-Csík gát, Hungary	80	700-800	750		1								M		228	
as above	as above	74	700-800	750		1								F		228	
(Jankauskas, 1998)	Šukioniai, Lithuania	1	700-800	750		1						20-30	25.0	M			
(Roberts and Buikstra, 2003)	Whithorn, Scotland		>600	800		1	1							M	3	59	
as above	as above		>600	800		1									3	59	
as above	as above		>600	800		1								M	3	59	
as above	Saltovo-Mayatsk culture, Russia		800-900	850				1				18-20	19.0	M	1	330	
(Évinger et al., 2011)	Zalavár-Városziget-Hadrianus templom, Hungary	135/01	800-900	850				1		1		15-17	16.0		3	381	*
as above	as above	50/01	800-900	850								22-24	23.0	M	3	381	*
as above	as above	39/02	800-900	850								30-40	35.0	F	3	381	*
(Acsádi et al., 1962)	Zalavár Castle, Hungary	92	800-1000	900		1						14-15	14.5		2	744	
(Byock et al., 2005)	Kirkjuhóll, Iceland		1000	1000	1							20-29	24.5	M	1	14	
(Gernaey et al., 2001)	Addingham, England	A134	1000	1000		1	1					25-30	27.5	M	1	30	*
(Marcsik et al., 2006)	Magyarhomoróg-Kónyadomb, Hungary	94	900-1100	1000		1								F		368	

(Roberts and Buikstra, 2003)	Sarkel-Belaya Vezha, Russia		900-1100	1000				1									
(Mays et al., 2001)	Wharram Percy, England	NA112	890-1160	1025				1				>50	60.0	M	9	687	*
as above	as above	NA046	890-1170	1030	1							>50	60.0	M	9	687	*
(Roberts and Buikstra, 2003)	Mlodzikowo, Poland		900-1200	1050				1								101	
(Marcsik et al., 2006)	Derekegyháza-Ibolyás domb, Hungary	6	1000-1200	1100	1									M		56	
(Pálfi and Marcsik, 1999)	Szegvár-Oromdülo, Hungary	275	1000-1200	1100	1											259	
as above	Püspökladány, Hungary	383	1000-1200	1100	1											371	
(Pálfi and Marcsik, 1999)	Bátmonostor, Hungary	9	1000-1200	1100	1											85	
as above	Csongrád-Felgyö, Hungary	1	1000-1200	1100				1				35-40	37.5	F		38	
as above	Hajdúdorog, Hungary	434	1000-1200	1100	1											612	
(Mark et al., 2010)	Csongrád-Ellés, Hungary	183	1000-1200	1100	1							40-45	42.5	M			
(Roberts and Buikstra, 2003)	Czersk, Poland		1000-1200	1100						1	1					620	
(Évinger et al., 2011)	Zalavár-Városziget-Kápolna, Hungary	17/03	1000-1200	1100	1							15-16	15.5		3	263	*
as above	as above	32/02	1000-1200	1100	1							35-40	37.5	M	3	263	*
as above	as above	74/03	1000-1200	1100	1							20-40	30.0	M	3	263	*
(Mays et al., 2001)	Wharram Percy, England	G438	1060-1170	1115	1	1	1			1		25-35	30.0	F	9	687	*
as above	as above	G482	1060-1170	1115	1							>50	60.0	M	9	687	*
(Roberts and Buikstra, 2003)	Alton, England		1000-1300	1150	1							24-27	25.5	M	1	41	
as above	Bedhampton, England		1000-1300	1150	1							17-25	21.0	M	2	87	
as above	as above		1000-1300	1150	1							17-25	21.0	F	2	87	
as above	Binchester, England		1000-1300	1150	1			1						M	1	54	
as above	Butler's field, England		1000-1300	1150	1							30-35	32.5	M	1	219	
as above	Cannington, England		1000-1300	1150	1									M	2	348	
as above	as above		1000-1300	1150											2	2	1 other
as above	Edix Hill, England		1000-1300	1150	1									M	1	148	
as above	Great Chesterford, England		1000-1300	1150	1									M	1	167	
as above	Nazeingbury, England		1000-1300	1150	1									F	2	153	

as above	as above		1000-1300	1150	1										M	2	153	
as above	Raunds, England	5218	1000-1300	1150	1	1						1		17-25	21.0	M	1	356	
as above	School St., England		1000-1300	1150	1									20	20.0	M	2	95	
as above	as above		1000-1300	1150	1									21-24	22.5	M	2	95	
as above	South Acre, England		1000-1300	1150	1											M	1	116	
(Mays et al., 2001)	Wharram Percy, England	EE056	900-1400	1150	1	1								>50	60.0		9	687	*
as above	as above	NA026	900-1400	1150	1	1				1				35-45	40.0	F	9	687	*
as above	as above	NA197	900-1400	1150				1						>50	60.0	M	9	687	*
(Marcsik et al., 2006)	Nagylak-Határsáv, Hungary	12	1100-1200	1150				1								F		243	
as above	as above	111	1100-1200	1150	1											F		243	
(Roberts and Buikstra, 2003)	Świeck, Poland		1100-1200	1150	1											M	1	506	
as above	Bedzin, Poland		1100-1200	1150	1											F			
(Weber et al., 2004)	Stuttgart, Germany	72	1100-1300	1200	1									>12	18.0			3200	spine only
(Marcsik et al., 2006)	Nyárlõrinc-Hangár út, Hungary	82	1100-1300	1200	1											F		483	
(Roberts and Buikstra, 2003)	Czarna Wielka, Poland		1100-1300	1200	1											F	4	250	
as above	as above		1100-1300	1200	1									25-35	30.0	F	4	250	
as above	as above		1100-1300	1200				2				1		18-20	19.0	M	4	250	
(Donoghue and Spigelman, 2008)	Saint Stephen's Basilica, Székesfehérvár, Hungary	VI.54	1000-1600	1250	1									20-22	21.0	F	3	935	
		VI.84	1000-1600	1250	1	1								27-36	31.5	F	3	935	
as above	as above		1000-1600	1250				1									3	935	
as above	as above																		
(Roberts and Buikstra, 2003)	Olbin, Poland		1200-1300	1250									1			M	1		
(Spigelman and Lemma, 1993)	England	sample 3	1000-1600	1300									1						*
(Mays et al., 2001)	Wharram Percy, England	WCO142	900-1700	1300	1		1	1		3			3			M	9	687	*

(Roberts and Buikstra, 2003)	Sypniewo, Poland		1200-1400	1300							1			F	1	160		
(Mays et al., 2001)	Wharram Percy, England	SA013	1279-1410	1344.5	1	1							35-45	40.0	F	9	687	*
(Roberts and Buikstra, 2003)	Oude en Nieuwe Gasthuis, Netherlands		1265-1433	1349	1										M	1	52	infirmary
as above	Waszawa, Poland		1200-1500	1350	1										M	1	93	
as above	Skrwilno, Poland		1200-1500	1350	1											1	310	
as above	Nänikon, Switzerland		1300-1400	1350	1								30-40	35.0	M			
as above	as above		1300-1400	1350	1													
as above	Battle of Wisby cemetery, Sweden		1361	1361	1											4	1185	
as above	Battle of Wisby cemetery, Sweden		1361	1361							1					4	1185	
as above	Whithorn, Scotland		1300-1450	1375	1		1								F	4	1553	
as above	as above		1300-1450	1375														3 other
(Jankauskas, 1998)	Diktarai, Lithuania	16	1300-1500	1400				1					50-55	52.5	M			
(Faerman et al., 1997)	Kražiai, Lithuania	K-16	1400-1500	1450	1			1		1	1		18-20	19.0	M			*
(Horáčková et al., 1999)	Kŕtiny, Czech Republic		1200-1700	1450	1											2	975	*
as above	as above	K4	1200-1700	1450					1				Adult	25.0		2	975	*
(Faerman et al., 1997)	Kraziai, Lithuania	K-16	1400-1500	1450	1			1		1	1		18-20	19.0	M		18	*
(Pálfi and Marcsik, 1999)	Gerla-Monostor, Hungary	32	1300-1600	1450	1												47	
(Roberts and Buikstra, 2003)	Kecskemét-Ferences, Hungary	125	1300-1600	1450				1									323	
as above	Blackfriars Friary, England		1300-1700	1500	1								21-23	22.0	F	2	681	
as above	Chelmsford Dominican Priory, England		1300-1700	1500	1								45-50	47.5	M	1	135	
as above	Chichester, England		1300-1700	1500	1	1									M	8	306	
as above	as above		1300-1700	1500	1								18-20	19.0	M	8	306	
as above	as above		1300-1700	1500	1								>30	35.0		8	306	
as above	as above		1300-1700	1500												8	306	5 other
as above	Hickleton, England		1300-1700	1500	1								25-30	27.5	M	1	68	
as above	Jewbury, England		1300-1700	1500	1			1							M	6	412	

as above	as above		1300-1700	1500	1					M	6	412	
as above	as above		1300-1700	1500	1					F	6	412	
as above	as above		1300-1700	1500	1					F	6	412	
as above	as above		1300-1700	1500	1						6	412	
as above	as above		1300-1700	1500	1						6	412	
as above	St. Andrew, England		1300-1700	1500	1	1	1	18-30	24.0	M	5	402	
as above	as above		1300-1700	1500	1	1		18-30	24.0	M	5	402	
as above	as above		1300-1700	1500	1	1		18-30	24.0	M	5	402	
as above	as above		1300-1700	1500	1	1		40-50	45.0	M	5	402	
as above	as above		1300-1700	1500							5	402	1 other
as above	St. Helen-on-the-Walls, England		1300-1700	1500	1	1					5	1042	
as above	as above		1300-1700	1500	1						5	1042	
as above	as above		1300-1700	1500	1						5	1042	
as above	as above		1300-1700	1500	1						5	1042	
as above	as above		1300-1700	1500							5	1042	1 other
as above	St. Oswald's Priory, England		1300-1700	1500	1					M	3	487	
as above	as above		1300-1700	1500	1					F	3	487	
as above	as above		1300-1700	1500							3	487	1 other
as above	Stratford Langthorne, England		1300-1700	1500	1			>35	40.0	M	1	28	
as above	Thetford, England		1300-1700	1500							2	99	2 other
(Jankauskas, 1998)	Alytus, Lithuania	222	1400-1600	1500	1			45-50	47.5	M		1345	*
as above	as above	228	1400-1600	1500	1			50-55	52.5	F		1345	*
as above	as above	257	1400-1600	1500	1			50-55	52.5	M		1345	*
as above	as above	61	1400-1600	1500	1			15-20	17.5			1345	
as above	as above	66	1400-1600	1500			1	20-25	22.5	M		1345	
as above	as above	315	1400-1600	1500	1			25-30	27.5	F		1345	
as above	as above	285	1400-1600	1500	1			40-45	42.5	F		1345	

as above	as above	54	1400-1600	1500		1									50-55	52.5	F		1345	
as above	as above	5	1400-1600	1500		1											M		1345	
(Jankauskas, 1998)	Arglaičiai, Lithuania	12	1400-1600	1500			1	1							45-50	47.5	F			
(Roberts and Buikstra, 2003)	Farringdon Street, England		1300-1700	1500		1											F	4	533	
as above	as above		1300-1700	1500		1											F	4	533	
as above	as above		1300-1700	1500		1						1					M	4	533	
as above	as above		1300-1700	1500														4	533	1 other
as above	Royal Mint, England		1300-1700	1500		1												5	940	
as above	as above		1300-1700	1500		1												5	940	
as above	as above		1300-1700	1500														5	940	3 other
as above	St. Saviour, England		1300-1700	1500		1											M	1	193	
as above	Newcastle infirmary, England		1300-1700	1500		1	1											2	210	
as above	as above		1300-1700	1500		1												2	210	
as above	Ensay, Scotland		1300-1700	1500		1									18-20	19.0	F	5	416	
as above	as above		1300-1700	1500		1									40	40.0	F	5	416	
as above	as above		1300-1700	1500		1									45	45.0	F	5	416	
as above	as above		1300-1700	1500		1											F	5	416	
as above	as above		1300-1700	1500														5	416	1 other
(Ôz et al., 2009)	Szeged, Hungary	483	1350-1713	1531		1									30-40	35.0	F	4	641	
as above	as above	561	1350-1713	1531		1									50-60	55.0		4	641	
as above	as above	487	1350-1713	1531	1			1			1			1	40-60	50.0	M	4	641	
as above	as above	152	1350-1713	1531	1	1					2		1					4	641	
(Roberts and Buikstra, 2003)	Oude en Nieuwe Gasthuis, Netherlands		1433-1652	1542		1											M	1	49	infirmary
as above	Gdańsk, Poland		1500-1600	1550		1											F	1	44	
(Merczi, 2001)	Visegrád-Diós, Hungary	85	1500-1600	1550		1									56-60	58.0	F	1	228	
(Roberts and Buikstra, 2003)	St. Agnes monastery, Netherlands		1573-1574	1573		1												1	13	
(Zink et al., 2005)	Southern Germany	R 535	1400-1800	1600							1						M		2547	*, ossuary

as above	as above	R 136	1400-1800	1600			1					M		2547	*, ossuary
as above	as above	R 393	1400-1800	1600			1							2547	*, ossuary
as above	as above	R 166	1400-1800	1600			1					M		2547	*, ossuary
as above	as above	R 93	1400-1800	1600			1					F		2547	*, ossuary
as above	as above	R 340	1400-1800	1600			1							2547	*, ossuary
as above	as above	R 285	1400-1800	1600			1							2547	*, ossuary
as above	as above	R 302	1400-1800	1600			1							2547	*, ossuary
as above	as above	R 343	1400-1800	1600			1							2547	*, ossuary
as above	as above	R 87	1400-1800	1600			1					M		2547	*, ossuary
(Jankauskas, 1998)	Drǫseikiai, Lithuania	20	1600	1600				1	>55	60.0	F				
(Molnár et al., 2005)	Bácsalmás cemetery, Hungary	32	1600-1700	1650	1				25-30	27.5	M	14	173	*
as above	as above	35	1600-1700	1650	1				18-20	19.0		14	173	*
as above	as above	83	1600-1700	1650	1				50-55	52.5	M	14	173	*
as above	as above	106	1600-1700	1650	1				40-45	42.5	M	14	173	*
as above	as above	128	1600-1700	1650	1				18-23	20.5	F	14	173	*
(Pálfi and Marcsik, 1999)	Bácsalmás cemetery, Hungary	Ba-53	1600-1700	1650	1				18-20	19.0	M	14	173	*
(Molnár and Pálfi, 1994)	Bácsalmás-Homokbánya cemetery, Hungary	Ba-39	1600-1700	1650	1						M	14	173	*
(Haas et al., 2000)	Bácsalmás cemetery, Hungary	Ba-48	1600-1700	1650	1				16-18	17.0	M	14	173	*
(Molnár and Pálfi, 1994)	Bácsalmás-Homokbánya cemetery, Hungary	Ba-61	1600-1700	1650		9			20-25	22.5	M	14	173	
as above	as above	Ba-85	1600-1700	1650		3			50-60	55.0	M	14	173	*
(Haas et al., 2000)	Bácsalmás cemetery, Hungary	Ba-115	1600-1700	1650	1						M	14	173	
as above	as above	Ba-118	1600-1700	1650	1				20-21	20.5	M	14	173	*
as above	as above	Ba-160	1600-1700	1650		1					F	14	173	
(Pálfi and Marcsik, 1999)	Bácsalmás cemetery, Hungary	Ba-142	1600-1700	1650	1							14	173	

(Jankauskas, 1998)	Didieji Likiškiai, Lithuania	12	1600-1700	1650		1			2				25-30	27.5	M			
(Roberts and Buikstra, 2003)	Zienki, Poland		1600-1700	1650			1									1	163	
(Spigelman and Lemma, 1993)	Scotland	sample 10	1600-1700	1650		1												*
(Horáčková et al., 1999)	Jilhava, Czech Republic	111	1720	1720			1						30-40	35.0	F	1	5	*
(Évinger et al., 2011)	Zsámbék – Premontrei templom, Hungary	161	1700-1800	1750		1							50-60	55.0	F	1	394	*
(Bachmann et al., 2008)	Kaiserebersdorf Castle, Austria	KE20	1700-1800	1750		1	1						Mature	50.0	F			
as above	as above	KE23	1700-1800	1750						1			Mature	50.0	F			*
(Jankauskas, 1998)	Buivydai, Lithuania	4	1700-1800	1750			1						>55	60.0	M			
(Roberts and Buikstra, 2003)	Bern-Bärengraben, Switzerland		1700-1800	1750		1												
(Bachmann et al., 2008)	Weisbach collection	WB 565	1800-1900	1850	1								22	22.0	M			*
(Bachmann et al., 2008)	Weisbach collection	WB 354	1800-1900	1850	1								24	24.0	M			*
as above	as above	WB 594	1800-1900	1850	1								22	22.0	M			*
New World																		
(Sáez, 2008)	Puqeldon 1, Los Lagos		417-297 BCE	-60		1										3	22	
as above	as above		417-297 BCE	-60						1						3	22	
as above	as above		417-297 BCE	-60		1										3	22	
(Ortner, 2003)	Illinois	NMNH 381853	400	400		1							22	22.0	M			
(Arriaza et al., 1995)	Borders of Atacama Desert, Chile	AZ71 T194	500	500		1							25+	27.0	M	4	483	
as above	as above	AZ71	500	500		1						1	40	40.0	F	4	483	
(Friedrich et al., 2009)	Laguna de los Cóndores, Peru	47206	500-1000	750		1							20-40	30.0	M	2	219	

as above	as above	47199	500-1000	750	1			1				1	20-40	30.0	F	2	219	
(Ritchie, 1952)	Durkee farm, New York	AP 526	500-1300	900	1								26-30	28.0	F			
as above	Peter Rapp farm, New York	AP 529	500-1300	900	1								40	40.0	M			
as above	Kipp Island, New York	AP 530	500-1300	900	1													
(Lombardi and Caceres, 2000)	Nasca, Peru	67466	900	900	1								50	50.0	M			*
(Williams and Snortland-Coles, 1986)	Jamestown Mound, Illinois	12	860-1000	930			1						35-45	40.0	F	1	75	
(Morse, 1967)	Nashville, Tennessee	17223	prehistoric	1000	1	1												
(El-Najjar, 1979)	Chavez Pass, Arizona	78/445N/339E	900-1100	1000	1	3							20-25	22.5	M	4		
as above	as above	78/82N/172E	900-1100	1000	1								25-30	27.5	M	4		
as above	as above	77/80B/61	900-1100	1000	1								25-35	30.0	F	4		
as above	as above	78/82N/174/6E	900-1100	1000	1								40-50	45.0	M	4		
(Arriaza et al., 1995)	Borders of Atacama Desert, Chile	AZ140 T113	1000	1000	1								18-20	19.0	M	4	483	
(Klaus et al., 2010)	Illimo, Peru	ILL-22	900-1100	1000	1								20-35	27.5	M		52	
(Buikstra, 1977)	Schild Cemetery, Illinois	SB-201	820-1310	1065	1	13	1			10	1	4	21-22	21.5	F	14	422	*
as above	as above	SA-4	820-1310	1065						4			22-24	23.0	M	14	422	
as above	as above	SA-96a	820-1310	1065	1	1	1	1		4	1	2	25-30	27.5	F	14	422	
as above	as above	SA-100a	820-1310	1065	1						1		20-25	22.5	F	14	422	
as above	as above	SB-165	820-1310	1065	1							1	35-40	37.5	M	14	422	
as above	as above	SB-194	820-1310	1065	1								25-26	25.5	M	14	422	
as above	as above	SB-195	820-1310	1065	1		1						20-22	21.0	F	14	422	
as above	as above	SB-233	820-1310	1065	1					1			50+	55.0	F	14	422	
as above	as above	SB-256	820-1310	1065	1					3			50+	55.0	M	14	422	
as above	as above	SB-262a	820-1310	1065					2	1			Teens	18.0		14	422	
as above	as above	SB-269	820-1310	1065	1	8				1			50+	55.0	F	14	422	*, ribs
(Raff et al., 2006)	Schild Cemetery, Illinois	SA41	1000-1200	1100		5									M	14	422	*, ribs
as above	as above	SB297	1000-1200	1100		2							27-30	28.5	M	14	422	*, ribs

as above	as above	SB250	1000-1200	1100			1					42-47	44.5	M	14	422	*, ribs
(Etxeberria et al., 2000)	Soacha, Colombia		1100-1300	1200		1											
as above	as above		1100-1300	1200		1											
as above	as above		1100-1300	1200		1											
as above	as above		1100-1300	1200		1											
as above	as above		1100-1300	1200		1											
as above	as above		1100-1300	1200		1											
(Buikstra, 1977)	Fulton County, Illinois		1200-1300	1250		1						36	36.0	M			
as above	as above		1200-1300	1250		1						25	25.0	M			
(Etxeberria et al., 2000)	Valle del Cauca, Colombia	T1	1000-1500	1250	1	1	1	1				35-40	37.5	M			
(Martinez et al., 2010)	Sátivanorte, Colombia	SO10-IX	1200-1300	1250		1						25-30	27.5	M			
(Pfeiffer, 1984)	Ontario		1275	1275		1											
(Buikstra, 1977)	Turpin Site, Ohio		1125-1425	1275		1						20-25	22.5	F			
as above	as above		1125-1425	1275		1						40+	50.0	M			
as above	as above		1125-1425	1275		1						19-20	19.5	F			
(Lichtor and Lichtor, 1957)	Clarksville, Tennessee		1200-1400	1300		1											
(Powell, 1987)	Irene Mound, Georgia		1150-1450	1300		1								F	10	176	
as above	as above		1150-1450	1300		1								F	10	176	
as above	as above		1150-1450	1300		1								M	10	176	
as above	as above		1150-1450	1300					1					F	10	176	

as above	as above		1150-1450	1300					1						10	176
as above	as above		1150-1450	1300					1						10	176
as above	as above		1150-1450	1300					1						10	176
as above	as above		1150-1450	1300	1	1							F	10	176
(Morse, 1967)	Memphis, Tennessee		1027-1617	1322	1						30	30.0	M		
(Buikstra, 1977)	Fulton County, Illinois		1130-1530	1330	1										
as above	as above		1150-1550	1350	1										
(Perzigian and Widmer, 1979)	Ohio		950-1750	1350	1						20-25	22.5	F		
as above	as above		950-1750	1350	1						>35	40.0	M		
as above	as above		950-1750	1350	1	1					>35	40.0	M		
as above	as above		950-1750	1350	1	1					16-18	17.0	F		
as above	as above		950-1750	1350	1						16-18	17.0	F		
as above	as above		950-1750	1350			1						F		
(Buikstra, 1988)	Estuquina, Peru	M6-336a	1350	1350	1						39+	40.0	M	13	414
as above	as above	M6-1002	1350	1350	1						17-19	18.0	M	13	414
as above	as above	M6-1021	1350	1350	1				1		45+	50.0	F	13	414
as above	as above	M6-1183a	1350	1350	1						35-39	37.0	M	13	414
as above	as above	M6-2256a	1350	1350	1						30-40	35.0	M	13	414
as above	as above	M6-2297	1350	1350	1						37-30	28.5	M	13	414
as above	as above	M6-3198a	1350	1350	1						20-21	20.5	M	13	414
as above	as above	M6-3215	1350	1350	1	1		1		1	25-30	27.5	M	13	414

as above	as above	M6-3644a	1350	1350	1							25-26	25.5	F	13	414	
as above	as above	M6-4165	1350	1350	1							27-30	28.5	M	13	414	
as above	as above	M6-4176	1350	1350			1	1				22-24	23.0	M	13	414	
as above	as above	M6-4256	1350	1350	1	7						45+	50.0	M	13	414	
as above	as above	M6-5390	1350	1350	1	17		1			1	50+	55.0	F	13	414	
(Buikstra, 1977)	Bennet Site, Canada		1300-1400	1350	1							adult	20.0	M			
as above	Fairty Ossuary, Canada		1400	1400	1												
(Etxeberria et al., 2000)	Marin, Boyacá, Colombia		<1500	1400	1									F			
as above	Incan site, Peru		<1500	1400	1												
as above	Guane, Colombia	Mom-0003	<1500	1400	1			1						M			
(Klaus et al., 2010)	La Caleta de San José, Peru	CSJ-21	1375-1450	1412	1							>45	50.0	M		26	
as above	as above	CSJ-F1	1375-1450	1412	1											26	
(Buikstra, 1977)	Fulton County, Illinois		1220-1620	1420	1												
(Braun et al., 1998)	Uxbridge Ossuary, Ontario	P48	1410-1483	1447	1												*
(Lahr and Bowman, 1992)	Kechipawan, New Mexico		1300-1600	1450					1						3	54	
(Hogue, 2007)	Lyon's Bluff	II/68b	1280-1690	1485	1										2	74	
as above	as above	1/01	1280-1690	1485	1										2	74	
(Pfeiffer, 1984)	Uxbridge Ossuary, Ontario		1490	1490			1					25-30	27.5	F	26	457	ossuary
as above	as above		1490	1490	1		1					35-40	37.5	F	26	457	ossuary
as above	as above		1490	1490			1					35-44	39.5	M	26	457	ossuary
as above	as above		1490	1490	1							17-25	21.0		26	457	ossuary
as above	as above		1490	1490	1							17-25	21.0		26	457	ossuary
as above	as above		1490	1490	1							17-25	21.0		26	457	ossuary
as above	as above		1490	1490	1							17-25	21.0		26	457	ossuary
as above	as above		1490	1490	1							17-25	21.0		26	457	ossuary
(Gerszten et al., 2001)	South America		1500	1500	1							32	32.0	F			
(Kim, 2011)	Anse Sainte-Marguerite, Guadeloupe	S207	Colonial	1500	1							Adult			15	148	*

(Dutour et al., 2001)	as above	S10	Colonial	1500		1	1		>25	30.0	M	15	148
as above	as above	S103	Colonial	1500		1			>25	30.0	M	15	148
as above	as above	s107	Colonial	1500		1			>25	30.0	M	15	148
as above	as above	S115	Colonial	1500		1			>25	30.0	M	15	148
as above	as above	S116	Colonial	1500				1	>25	30.0	M	15	148
as above	as above	S131	Colonial	1500		1			<25	20.0	M	15	148
as above	as above	S152	Colonial	1500				1	>25	30.0	M	15	148
as above	as above	S157	Colonial	1500		1	1		>25	30.0	M	15	148
as above	as above	S19	Colonial	1500	1	1			15-20	17.5	F	15	148
(Dutour et al., 2001)	as above	S4	Colonial	1500		1			>25	30.0	M	15	148
as above	as above	S79	Colonial	1500		1			>25	30.0	F	15	148
as above	as above	S86	Colonial	1500		1			<25	20.0	F	15	148
as above	as above	S90	Colonial	1500		1			<25	20.0	F	15	148
(Aceves-Avila et al., 1998)	Hospital Real de San Jose de los Naturales, Mexico		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443
as above	as above		1500-1600	1550		1					M	19	443

as above	as above		1500-1600	1550	1									M	19	443	
as above	as above		1500-1600	1550	1									F	19	443	
as above	as above		1500-1600	1550	1									F	19	443	
as above	as above		1500-1600	1550	1									F	19	443	
as above	as above		1500-1600	1550	1									F	19	443	
(Ortner, 2003)	Pecos Pueblo, New Mexico	59811	1300-1838	1569				1				40	40.0	F			
as above	Yukon River, Alaska	NMNH 345394	<1630	1600			1					20	20.0	F	1		
(Buikstra, 1976)	Hudson Bay, Canada	XIV-C-313	<1700	1600	1							20-21	20.5	F	3	28	both
as above	as above	XIV-C-316	<1700	1600	1							17-18	17.5	F	3	28	both
as above	as above	XIV-C-323	<1700	1600	1							18-19	18.5	M	3	28	both
(Klaus et al., 2010)	Chapel of San Pedro de Mórrope, Peru	U12 05-15	1539-1750	1644	1							15-20	17.5	F		870	
(Hogue, 2007)	Protohistoric sites near Mississippi	22OK904	1400-1900	1650	1										1	85	
(Lambert, 2006)	Eaton's Estate, North Carolina	Burial 14	1830-1850	1840		1							20.0	M	3	17	
as above	as above	Burial 9	1830-1850	1840	1								35.0	M	3	17	
as above	as above	Burial 8	1830-1850	1840	1								44.0	F	3	17	
(Wescott et al., 2010)	Lexington, Missouri	Lot C-41, plot 2	1854	1854		1						20-30	25.0	F			*
(Kelley and El-Najjar, 1980)	Hamann-Todd collection	114	1853-1938	1896						1			77.0	M		3100	TB record
as above	as above	448	1853-1938	1896		1							31.0	M		3100	TB record
as above	as above	470	1853-1938	1896		1							35.0	M		3100	TB record
as above	as above	708	1853-1938	1896	1		1			1			32.0	M		3100	TB record
as above	as above	813	1853-1938	1896						1			22.0	M		3100	TB record
as above	as above	1014	1853-1938	1896	1	1							22.0	M		3100	TB record
as above	as above	1062	1853-1938	1896					1				35.0	M		3100	TB record
as above	as above	1069	1853-1938	1896				1					49.0	M		3100	TB record

as above	as above	1070	1853-1938	1896						1						42.0	F		3100	TB record
as above	as above	1084	1853-1938	1896	1	1										24.0	M		3100	TB record
as above	as above	1091	1853-1938	1896		1										25.0	M		3100	TB record
as above	as above	1116	1853-1938	1896	1	1										31.0	M		3100	TB record
as above	as above	1178	1853-1938	1896	1											73.0	M		3100	TB record
as above	as above	1184	1853-1938	1896	1			1								37.0	M		3100	TB record
as above	as above	1204	1853-1938	1896	1			1								22.0	M		3100	TB record
as above	as above	1271	1853-1938	1896	1	1										39.0	M		3100	TB record
as above	as above	1480	1853-1938	1896	1	1										50.0	M		3100	TB record
as above	as above	1556	1853-1938	1896					1							38.0	M		3100	TB record
as above	as above	1799	1853-1938	1896	1											22.0	M		3100	TB record
as above	as above	1888	1853-1938	1896	1											21.0	M		3100	TB record
as above	as above	2044	1853-1938	1896	1	1		1			1	1				25.0	M		3100	TB record
as above	as above	2562	1853-1938	1896	1											33.0	M		3100	TB record
as above	as above	3030	1853-1938	1896	1											45.0	M		3100	TB record
as above	as above	3540	1853-1938	1896	1											22.0	M		3100	TB record
as above	as above	E14	1853-1938	1896								1				15.0	F		3100	TB record
as above	as above	E410	1853-1938	1896								1				20.0	M		3100	TB record

Asia

(Douglas, 1996)	Non Nok Tha, Thailand	1-2	2500-2000 BCE	-2250	1									40-45	42.5	F	5	180	
as above	as above	1-12	2000-1000 BCE	-1500			1							35-40	37.5	M	5	180	
as above	as above	2-32	2000-1000 BCE	-1500	1									25-30	27.5	M	5	180	

as above	as above	1-40	1000 BCE	-1000		1					55-60	57.5	F	5	180	
as above	as above	1-47	1000 BCE	-1000		1							F	5	180	
(Suzuki and Inoue, 2007)	Aoyakamijichi, Japan	31711	454 BCE-124	-289		1									109	
as above	as above	30616	454 BCE-124	-289		1									109	
(Roberts and Buikstra, 2003)	Issyk-Kul Lake, former Soviet Union		1000 BCE to 700 CE	-150		1							M			
(Suzuki et al., 2008)	Nukdo site, Korea	117	100 BCE-0	-50		1	3				15-20	17.5	F		200	
(Roberts and Buikstra, 2003)	Biisk, Siberia		100 BCE to 0	-50		1		2	1							
(Murphy et al., 2009)	Aymyrlyg, Tyva, Siberia	XXXI. 34	300 BCE-300	0		1					25-35	30.0	F	9	202	*
as above	as above	XXXI. 77	300 BCE-300	0		1			1		25-35	30.0	M	9	202	*
as above	as above	XXXI.85 . Sk. 1	300 BCE-300	0		1					25-35	30.0	F	9	202	*
as above	as above	XXXI. 163	300 BCE-300	0		1					35-45	40.0	F	9	202	*
as above	as above	XXXI. 63	300 BCE-300	0		1	1				15-17	16.0	F	9	202	
(Tayles and Buckley, 2004)	Noen U-Loke site, Thailand	B36	0-200	100	1	1							F	1	89	
(Roberts and Buikstra, 2003)	Kobyakovo, Russia		200-300	250				1								
as above	Jyoyama, Japan		300-700	500		1		1					M			
as above	Unoki, Japan		300-700	500		1							M			
as above	Asahi-Dai, Japan		300-700	500		1							F			
(Ortner, 2003)	Unoki, Japan	3	300-700	500		1							F			
(Spigelman and Lemma, 1993)	Borneo	sample 5	<600	500					1							*
(Roberts and Buikstra, 2003)	Saragash, Siberia		1000-1100	1050		1										
as above	Ainu, Japan		1600-1900	1750		1							F			sacrum
as above	Kakako		1853-1854	1853		1					25-30	27.5	F			
Mediterranean																
(Al-Sarie et al., 1996)	'Ain Ghazal, Jordan		7250 BCE	-7250		1					30	30.0	M	3	75	
as above	as above		7250 BCE	-7250		1					20-25	22.5		3	75	

as above	as above		7250 BCE	-7250	1			>40	50.0	F	3	75	
(Hershkovitz et al., 2008)	Atlit-Yam site, Mediterranean		7250-6160 BCE	-6705		1		25	25.0	F			*
(Canci et al., 1996)	Arma dell'Aquila cave, Italy		5800 BCE	-5800	1			30	30.0	F			
(Roberts and Buikstra, 2003)	Neolithic cave, Spain		5000-4000 BCE	-4500	1					M			
(Formicola et al., 1987)	Arene Candide cave, Liguria, Italy	AC 5	4000-3500 BCE	-3750	1			15	15.0	M	1	13	
(Morse, 1967)	Sakkara, Egypt		3300 BCE	-3300	1			Old	50.0	F			
(Zink et al., 2003)	Abydos, Egypt	U-623	3500-2650 BCE	-3075	1							250	
as above	as above	Q-W5	3500-2650	-3075	1							250	*
as above	as above	U 559 Abydos	3500-2650	-3075	1			20-40	30.0	F		250	*
(Morse, 1967)	Nubia, Egypt		3000 BCE	-3000	1					M			
as above	as above		3000 BCE	-3000	1					F			
as above	as above		3000 BCE	-3000	1					M			
as above	as above		3000 BCE	-3000	1					M			
as above	as above		3000 BCE	-3000	1					M			
as above	Nagada, Egypt	B107	4500-1069 BCE	-2785	1								
as above	as above	T52	4500-1069 BCE	-2785	1								
as above	as above	T7	4500-1069 BCE	-2785	1								
as above	as above	586	4500-1069 BCE	-2785	1								
as above	as above	753	4500-1069 BCE	-2785	1								
as above	as above	853	4500-1069 BCE	-2785	1								
as above	as above	60	4500-1069 BCE	-2785	1								
(Ortner, 1979)	Bab edh-Dhra, Jordan		3150-2200 BCE	-2675	1			18	18.0	M	2	92	
(Morse, 1967)	Nubia, Egypt		2000 BCE	-2000	1			21	21.0	F			
(Malnasi, 2005)	Dayr Al-Barshā, Egypt	Individua l 26	2050–1750 BCE	-1900	1			20-24	22.0	M	1	57	

(Morse, 1967)	Nubia, Egypt	182C	2050-1700 BCE	-1875	1						
as above	as above	182B	2050-1700 BCE	-1875	1						
as above	as above	20A	2050-1700 BCE	-1875	1						
as above	as above	182E:a	2050-1700 BCE	-1875	1						
as above	as above	182E:b	2050-1700 BCE	-1875	1						
(Zink et al., 2001)	Thebes-West, Egypt	TT196-M163	2050-1650 BCE	-1850	1				M		*
as above	as above	DAN 46 K95.1	2050-1650 BCE	-1850	1						
(Zink et al., 2003)	Thebes-West, Egypt	TT196-2-25	2100-1550 BCE	-1825	1					183	
as above	as above		2100-1550 BCE	-1825	1					183	
as above	as above		2100-1550 BCE	-1825	1					183	
(Roberts and Buikstra, 2003)	Madonna di Loreto, Italy		1700-1500 BCE	-1600	1				M		
as above	Olmo do Nogara, Italy		1700-1500 BCE	-1600	1				M		
as above	Nubia, Egypt		1500 BCE	-1500	1	Old	50.0	F			
(Zink et al., 2001)	Thebes-West, Egypt	TT84-70	1450-1250 BCE	-1350	1	20-30	25.0	M	620		*
as above	as above	TT-85-2-51	1450-1250 BCE	-1350	1				620		*
as above	as above	DAN 33 K93.11	1450-1250 BCE	-1350	1			M	620		
(Zink et al., 2005)	as above	Tomb TT95	1450-1250 BCE	-1350	1				620		*
as above	as above	Tomb TT95	1450-1250 BCE	-1350	1			M	620		
(Zink et al., 2001)	as above	TT453-15	1450-1250 BCE	-1350	1				620		*
(Zink et al., 2005)	as above	Tomb TT95	1450-1250 BCE	-1350	1			M			
as above	as above	TT183	1450-1250 BCE	-1350	1						*
(Zink et al., 2001)	as above	TT183-29	1550-1080 BCE	-1315	1						*
as above	as above	TT84-4	1550-1080 BCE	-1315	1						

as above	as above	DAN-M7-K93.11	1550-1080 BCE	-1315		1							30-50	40	F			
as above	as above	TT95-35	1550-1080 BCE	-1315			1								M			
as above	as above	DAN 3 K93.11	1550-1080 BCE	-1315	1								20-30	25	M			*
as above	as above	DAN 74 K93.11	1550-1080 BCE	-1315	1										M			*
as above	as above	DAN-106-K94.1	1550-1080 BCE	-1315		1												
(Zink et al., 2003)	as above	DAN 93.11-33	1550-1080 BCE	-1315		1												
(Nerlich et al., 1997)	as above		1550-1080 BCE	-1315	1	1							<35	30	M			*
(Strouhal, 1995)	Serra 400, Nubia, Egypt	15/6	1550-1070 BCE	-1310		1							18	18	F			
(Brier, 2004)	Egypt	Nespereh an	1085-945 BCE	-1015		1												
(Ortner, 2003)	as above		1000 BCE	-1000		1												
(Roberts and Buikstra, 2003)	Oliena-Nuoro, Sardinia		1000-300 BCE	-650		1												
(Littleton, 2003)	Bahrain, Denmark	964.A	300 BCE-250 CE	-25			1								M	2	17	
as above	as above	963.M	300 BCE-250 CE	-25	1								15-25	20	F	2	17	
(Matheson et al., 2009)	Tomb of the Shroud, Jerusalem	SC1	0-100	50							1				M	3	11	*
Henneberg and Henneberg	Pompeii, Italy		79 CE	79		1										2	500	
as above	as above		79 CE	79		1										2	500	
(Roberts and Buikstra, 2003)	Saraçhane, Istanbul		300-600	450		1										3	250	
as above	as above		300-600	450		1										3	250	
(Spigelman and Lemma, 1993)	Turkey	sample 1	300-600	450		1												*
(Molnár et al., 1998)	St.-Martin-de-Cadillan, France	8	400-600	500		1									M			
as above	Porte d'Orée, France	2	600-700	650		1			1							1	8	
(Strouhal, 1995)	Nubia, Egypt	10/15	350-1500	925		1							50-60	55	F			
(Roberts and Buikstra, 2003)	Santa Cristòfol de la Castanya, Spain		1100-1200	1150						2			14-16	15				*

(Molnár et al., 1998)	La Roquebrussanne, France	1	1100-1200	1150	1		1	1			51-57	54	M			*
(Roberts and Buikstra, 2003)	Abbaye de la Celle, France		1100-1200	1150										25	80	7 both
as above	Santa Maria del Hito, Spain		1300-1500	1400	1								M			
as above	Santa Maria Ripoll, Spain		1300-1400	1400	1								M			
(Giuffra et al., 2009)	Florence, Italy	Cardinal Carlo de' Medici	1595-1666	1630.5	1						71	71	M			
(Roberts and Buikstra, 2003)	Santa Eulalia de Riuprimer, Spain		1600-1800	1700	1								F			
(Campbell and Ackermann, 2010)	Makgope, South Africa	UP 49	1682-1745	1713		2		2			>45	50	M	5	947	
(Roberts and Buikstra, 2003)	L'Observance Series, France		1722	1722	1									1	216	
(Campbell and Ackermann, 2010)	Henkries, South Africa	SAM-AP 1271	1652-1911	1781	1						>35	40	F	5	947	
as above	Cape Town, South Africa	UCT 552	1755-1827	1791	1	1		1			30-35	32.5	M	5	947	
as above	as above	SAM-AP 3738	1755-1827	1791	1	2					35-49	42	M	5	947	
as above	Gladstone cemetery, South Africa	GLD N8.3	1897-1900	1899	1			1		1	35-45	40	M	5	947	

Appendix 2: For manuscript 1: "Evolution of human tuberculosis: A systematic review and meta-analysis of paleopathological evidence."

Details of sampling for cases confirmed using ancient DNA.

Ref.	Geographic Location	ID/grave No.	Date	Pathology	Area sampled
Northern and Central Europe					
(Mays and Taylor, 2003)	Tarrant Hinton, England	7	400-230 BCE	L1, L2, L3	T12, L1, L2, rib
(Jankauskas, 1998)	Marvelė, Lithuania	917	100-200	Middle/lower T vertebrae, metatarsals, hip	?
(Haas et al., 2000)	Sükösd, Hungary	Sü-19	600-700	T3-T6	?
(Haas et al., 2000)	Bélmegyer, Hungary	Be-65	600-700	T and L vertebrae	?
(Haas et al., 2000)	Pitvaros, Hungary	Pi-215	600-700	L and S vertebrae	?
(Évinger et al., 2011)	Zalavár-Városziget-Hadrianus templom, Hungary	135/01	800-900	L2, L3, iliac	?
(Évinger et al., 2011)	Zalavár-Városziget-Hadrianus templom, Hungary	50/01	800-900	L3/L4	?
(Évinger et al., 2011)	Zalavár-Városziget-Hadrianus templom, Hungary	39/02	800-900	T1-3, T7-8, L1-3	?
(Mays et al., 2001)	Wharram Percy, England	NA112	890-1160	Hip	Pathological bone
(Mays et al., 2001)	Wharram Percy, England	NA046	890-1170	L vertebrae	Pathological bone
(Gernaey et al., 2001)	Addingham, England	A134	1000	Spine, ribs	Ribs
(Évinger et al., 2011)	Zalavár-Városziget-Kápolna, Hungary	17/03	1000-1200	T8-L2	?
(Évinger et al., 2011)	Zalavár-Városziget-Kápolna, Hungary	32/02	1000-1200	T1-L2	?
(Évinger et al., 2011)	Zalavár-Városziget-Kápolna, Hungary	74/03	1000-1200	T6-T8	?
(Mays et al., 2001)	Wharram Percy, England	G438	1060-1170	T/L vertebrae, ulna, ribs, ilium	Pathological bone
(Mays et al., 2001)	Wharram Percy, England	G482	1060-1170	L vertebrae	Pathological bone

(Mays et al., 2001)	Wharram Percy, England	EE056	900-1400	T/L vertebrae, ribs	Pathological bone
(Mays et al., 2001)	Wharram Percy, England	NA026	900-1400	T vertebrae, ribs, scapula	Pathological bone
(Mays et al., 2001)	Wharram Percy, England	NA197	900-1400	Hip	Pathological bone
(Spigelman and Lemma, 1993)	England	sample 3	1000-1600	Talus	Talus
(Mays et al., 2001)	Wharram Percy, England	WCO142	900-1700	L/S vertebrae, hip, ilium, femora, tibiae, fibula, metatarsal, calcanei, foot phalanx	Pathological bone
(Mays et al., 2001)	Wharram Percy, England	SA013	1279-1410	T vertebrae, ribs	Pathological bone
(Horáčková et al., 1999)	Kŕtiny, Czech Republic		1200-1700	Spine	Spine
(Horáčková et al., 1999)	Kŕtiny, Czech Republic	K4	1200-1700	Tibia	Tibia
(Jankauskas, 1998)	Alytus, Lithuania	222	1400-1600	T11, T12	?
(Jankauskas, 1998)	Alytus, Lithuania	228	1400-1600	T12, L1	?
(Jankauskas, 1998)	Alytus, Lithuania	257	1400-1600	L3, L4	?
(Zink et al., 2005)	Southern Germany	R 535	1400-1800	Femur	Femur
(Zink et al., 2005)	Southern Germany	R 136	1400-1800	Femur	Femur
(Zink et al., 2005)	Southern Germany	R 393	1400-1800	Humerus	Humerus
(Zink et al., 2005)	Southern Germany	R 166	1400-1800	Tibia	Tibia
(Zink et al., 2005)	Southern Germany	R 93	1400-1800	Fibula	Fibula
(Zink et al., 2005)	Southern Germany	R 340	1400-1800	Femur	Femur
(Zink et al., 2005)	Southern Germany	R 285	1400-1800	Tibia	Tibia
(Zink et al., 2005)	Southern Germany	R 302	1400-1800	Tibia	Tibia
(Zink et al., 2005)	Southern Germany	R 343	1400-1800	Tibia	Tibia
(Zink et al., 2005)	Southern Germany	R 87	1400-1800	Tibia	Tibia
(Faerman et al., 1997)	Kražiai, Lithuania	16	1500-1700	T11, T12, L1, L2, shoulder, elbow, hip	T12, femur
(Haas et al., 2000)	Bácsalmás cemetery, Hungary	Ba-39	1600-1700	C/T/L vertebrae	?
(Haas et al., 2000)	Bácsalmás cemetery, Hungary	Ba-48	1600-1700	T/L vertebrae	?
(Haas et al., 2000)	Bácsalmás cemetery, Hungary	Ba-85	1600-1700	Ribs	?
(Haas et al., 2000)	Bácsalmás cemetery, Hungary	Ba-118	1600-1700	L vertebrae	?
(Pálfi and Marcsik, 1999)	Bácsalmás cemetery, Hungary	Ba-53	1600-1700	Vertebrae	?
(Molnár et al., 2005)	Bácsalmás cemetery, Hungary	32	1600-1700	Vertebrae	?

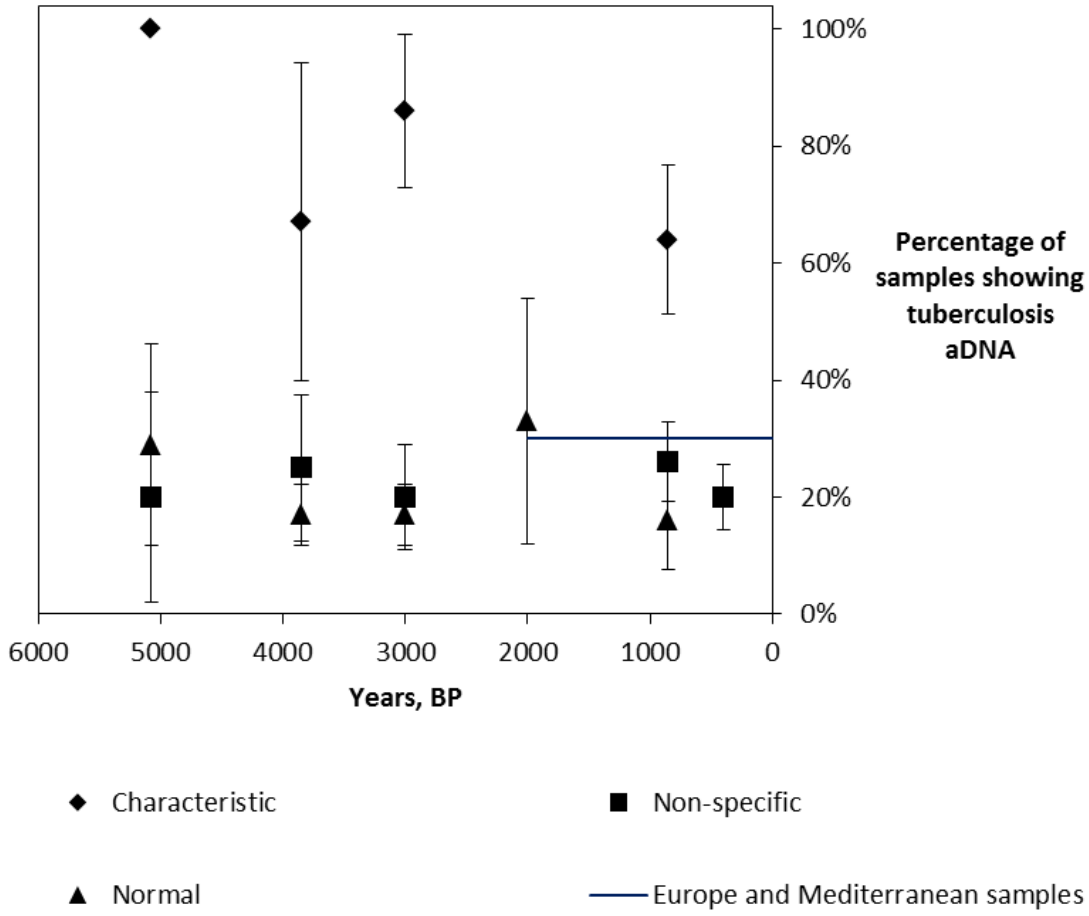
(Molnár et al., 2005)	Bácsalmás cemetery, Hungary	35	1600-1700	Vertebrae	?
(Molnár et al., 2005)	Bácsalmás cemetery, Hungary	83	1600-1700	Vertebrae	?
(Molnár et al., 2005)	Bácsalmás cemetery, Hungary	106	1600-1700	Vertebrae	?
(Molnár et al., 2005)	Bácsalmás cemetery, Hungary	128	1600-1700	Vertebrae	?
(Spigelman and Lemma, 1993)	Scotland	sample 10	1600-1700	L/S vertebrae	L/S vertebrae
(Horáčková et al., 1999)	Jihlava, Czech Republic	111	1720	Hip	Hip
(Évinger et al., 2011)	Zsámbék – Premontrei templom, Hungary	161	1700-1800	L3-L5, sacrum	?
(Bachmann et al., 2008)	Kaiserebersdorf Castle, Austria	KE23	1700-1800	Elbow	Mid-shaft of femur
(Bachmann et al., 2008)	Weisbach collection	WB 565	1800-1900	Skull	Non-pathological region of skull
(Bachmann et al., 2008)	Weisbach collection	WB 354	1800-1900	Skull	Non-pathological region of skull
(Bachmann et al., 2008)	Weisbach collection	WB 594	1800-1900	Skull	Non-pathological region of skull
New World					
(Lombardi and Caceres, 2000)	Nasca, Peru	67466	900	T10, T11, T12	Soft tissue
(Braun et al., 1998)	Schild Cemetery, Illinois	SB-201	820-1310	T11	T11
(Raff et al., 2006)	Schild Cemetery, Illinois	SA41	1000-1200	Ribs	Ribs
(Raff et al., 2006)	Schild Cemetery, Illinois	SB297	1000-1200	Ribs	Ribs
(Raff et al., 2006)	Schild Cemetery, Illinois	SB269	820-1310	Ribs	Ribs
(Raff et al., 2006)	Schild Cemetery, Illinois	SB250	1000-1200	Ribs	Ribs
(Braun et al., 1998)	Uxbridge Ossuary, Ontario	P48	1410-1483	L3, L4	L3, L4
(Kim, 2011)	Anse Sainte-Marguerite, Guadeloupe	S207	Colonial	Spine	Left ulna
(Wescott et al., 2010)	Lexington, Missouri	Lot C-41, plot 2	1854	Left 6th rib	Manubrium, sternum, left 1st/2nd ribs
Asia					

(Murphy et al., 2009)	Aymyrlyg, Tyva, Siberia	XXXI. 34	300 BCE-300	Spine	L3, L4
(Murphy et al., 2009)	Aymyrlyg, Tyva, Siberia	XXXI. 77	300 BCE-300	Spine, knee	L5, L6
(Murphy et al., 2009)	Aymyrlyg, Tyva, Siberia	XXXI.85. Sk. 1	300 BCE-300	L5	L4, L5
(Murphy et al., 2009)	Aymyrlyg, Tyva, Siberia	XXXI. 163	300 BCE-300	L vertebrae	L4, L5
(Spigelman and Lemma, 1993)	Borneo	sample 5	<600	Ulna	Ulna
Mediterranean					
(Hershkovitz et al., 2008)	Atlit-Yam site, Mediterranean		7250-6160 BCE	Tibia	Ribs, tibia
(Zink et al., 2001)	Abydos, Egypt	Q-W5	3500-2650	T6	?
(Zink et al., 2001)	Abydos, Egypt	U 559 Abydos	3500-2650	L2	?
(Zink et al., 2001)	Thebes-West, Egypt	TT196-M163	2050-1650 BCE	L3	?
(Zink et al., 2001)	Thebes-West, Egypt	TT84-70	1450-1250 BCE	L4, L5	?
(Zink et al., 2001)	Thebes-West, Egypt	TT-85-2-51	1450-1250 BCE	L4, L5, S1	?
(Zink et al., 2005)	Thebes-West, Egypt	Tomb TT95	1450-1250 BCE	L1, L2, L3, L4	?
(Zink et al., 2001)	Thebes-West, Egypt	TT453-15	1450-1250 BCE	T8, T9, T10, T11, T12, L1, L2	?
(Zink et al., 2005)	Thebes-West, Egypt	TT183	1450-1250 BCE	L3	?
(Zink et al., 2001)	Thebes-West, Egypt	TT183-29	1550-1080 BCE	L2	?
(Zink et al., 2001)	Thebes-West, Egypt	DAN 3 K93.11	1550-1080 BCE	Temporal bone	?
(Zink et al., 2001)	Thebes-West, Egypt	DAN 74 K93.11	1550-1080 BCE	Skull	?
(Nerlich et al., 1997)	Thebes-West, Egypt		1550-1080 BCE	L vertebrae	Soft tissue
(Matheson et al., 2009)	Tomb of the Shroud, Jerusalem	SC1	0-100	Phalange	Phalange
(Spigelman and Lemma, 1993)	Turkey	sample 1	300-600	L/S vertebrae	L/S vertebrae

(Roberts and Buikstra, 2003)	Santa Cristòfol de la Castanya, Spain		1100-1200	Both knees	?
(Molnár et al., 1998)	La Roquebrussanne, France	1	1100-1200	L1, L2, L3, ilium, femur	?

Appendix 3: For manuscript 1: "Evolution of human tuberculosis: A systematic review and meta-analysis of paleopathological evidence."

Percentage of bone samples with lesions characteristic of tuberculosis, non-specific or none showing positive results for *Mycobacterial* ancient DNA. Generated from data presented in (Bouwman et al., 2011; Fusegawa et al., 2003; Zink et al., 2005; Zink et al., 2007).



Appendix 4: For manuscript 3: "Skeletal Lesions in Human Tuberculosis may sometimes heal: An aid to palaeopathological diagnoses."

Additional cases of tuberculosis diagnosed in Galler Collection patients where skeletal lesions have developed. The cases are organised into first (before 1946), second (1946-1950) and third (after 1950) time periods.

Second time period (1946-1950)

Autopsy Number: 739

Autopsy Year: 1948

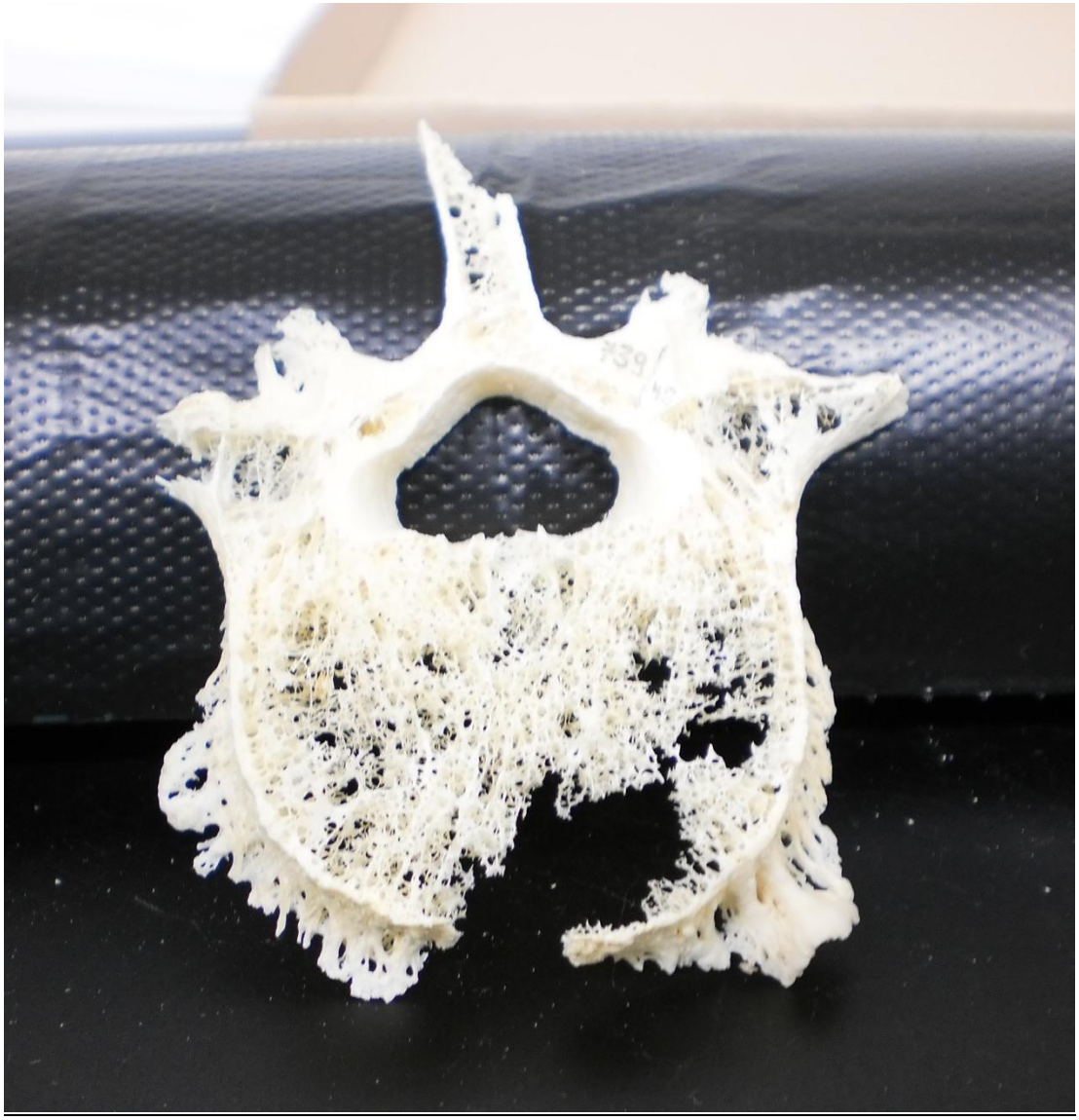
Age: 76

Sex: Female

Description: A single lumbar vertebra of a76 year old female was available for study and shows a lytic lesion in the anterior region of the vertebral body. The size of this lesion is approximately 7 mm x 9 mm. No healing has occurred in this case.

Since this individual only had one vertebra available for study, this complicates any potential differential diagnosis. The medical records include information regarding the presence of TB spondylitis as well as an aortic aneurysm. There is a description of vertebral erosion due to the aortic aneurism rather than through TB. However, the spondylitis of TB may have been a confounding factor in the destruction of this vertebra. The bone destruction is on the anterior region of the vertebra, as would be

expected in TB. We could not provide a clear diagnosis for this case, but it is possible TB was at least partially responsible for the lesion observed.



Third time period (after 1950)

Autopsy Number: 411

Autopsy Year: 1955

Age: 34

Sex: Male

Description: The image shows the section extending from cervical vertebra six to thoracic vertebra seven of a 34 year old male. Spondylitis of thoracic vertebrae one to three has resulted in extensive fusion along the surface of the vertebral bodies. Intervertebral discs spaces are no longer present. This individual had pulmonary TB in the right lung as the primary focus and the lymph node in the neck was the secondary focus (starting in 1922, 33 years before death). The lymph nodes were later removed surgically. The individual also had meningeal TB (of the inner ear). The meningeal and spinal TB began around 1 year of age. At age 3, this individual was hospitalized and placed in a plaster corset. At the age of 19, the urogenital system had been affected by TB. As a result of this, the right kidney was removed. During the next 20 years, the individual had several operations to help with the problems caused by urogenital TB. Healing in this case has occurred through fusion of the affected vertebrae.

The medical records for this patient do not describe compression fractures, Paget's disease, osteomyelitis or neoplasms. Osteoporosis is mentioned, but this would not have impacted the development of the bone lesions because it is very unlikely this individual had osteoporosis at the age of 1 year, when the spinal TB begun. The anterior regions of the bodies of three vertebrae were destroyed, leading to collapse of the spine. This is consistent with TB and we considered this as a very likely case.

SEE FIGURE 1B IN MANUSCRIPT 3 FOR IMAGE

Autopsy Number: 60

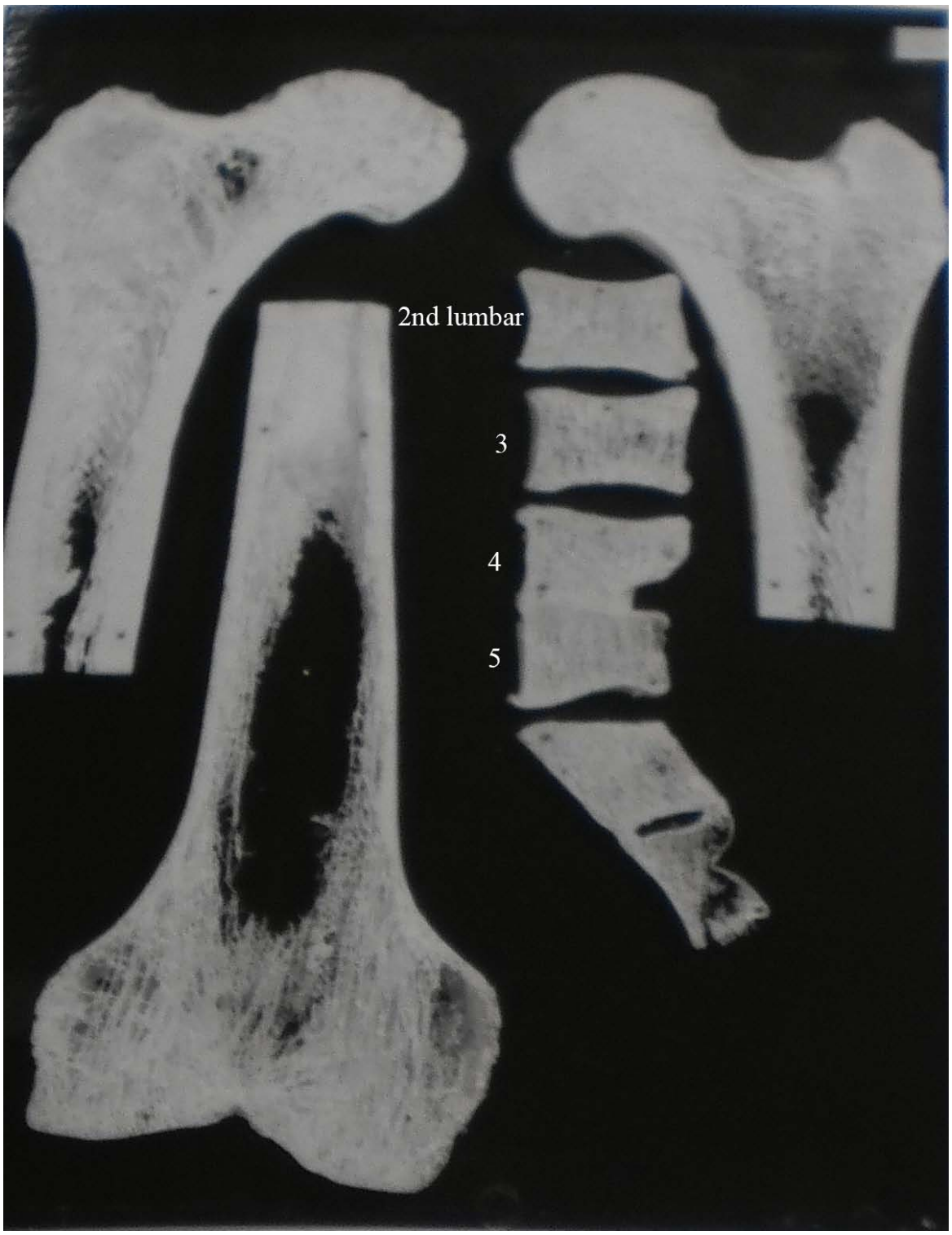
Autopsy Year: 1956

Age: 69

Sex: Male

Description: The image shows the proximal third of both femora, the distal third of the left femur and the second to fifth lumbar vertebrae as well as two sacral vertebrae of a 69 year old male. The fourth and fifth lumbar vertebrae have fused together through their vertebral bodies. There is no intervertebral disc between these vertebrae. The sacral vertebrae were also involved in the disease process. This individual had general subacute miliary TB, as well as meningitis of tuberculous origin. The left femur was also involved as well as the wing of the ilium. Both were atrophied. The left hip was diagnosed with TB in 1954 (2 years before death) when the individual complained of pain in the hip joint and lower extremities. The femoral head had necrotic areas that extended down into the medial part of the compact bone. The joint cartilage had been completely eroded away. Healing occurred through fusion of the affected vertebrae in this case.

The medical record of this individual does mention the presence of Paget's disease of the femur and third lumbar vertebra. However, pathological changes are noted on lumbar vertebrae four and five, rather than lumbar three. Additionally, the medical records report the presence of tubercles in tissues surrounding lumbar vertebra four, extending further towards the sacrum. There is also complete destruction of the vertebral disc between lumbar vertebrae four and five. These characteristics indicate that TB was likely responsible for the pathology around lumbar vertebrae four and five.


2nd lumbar
3
4
5

Autopsy Number: 487

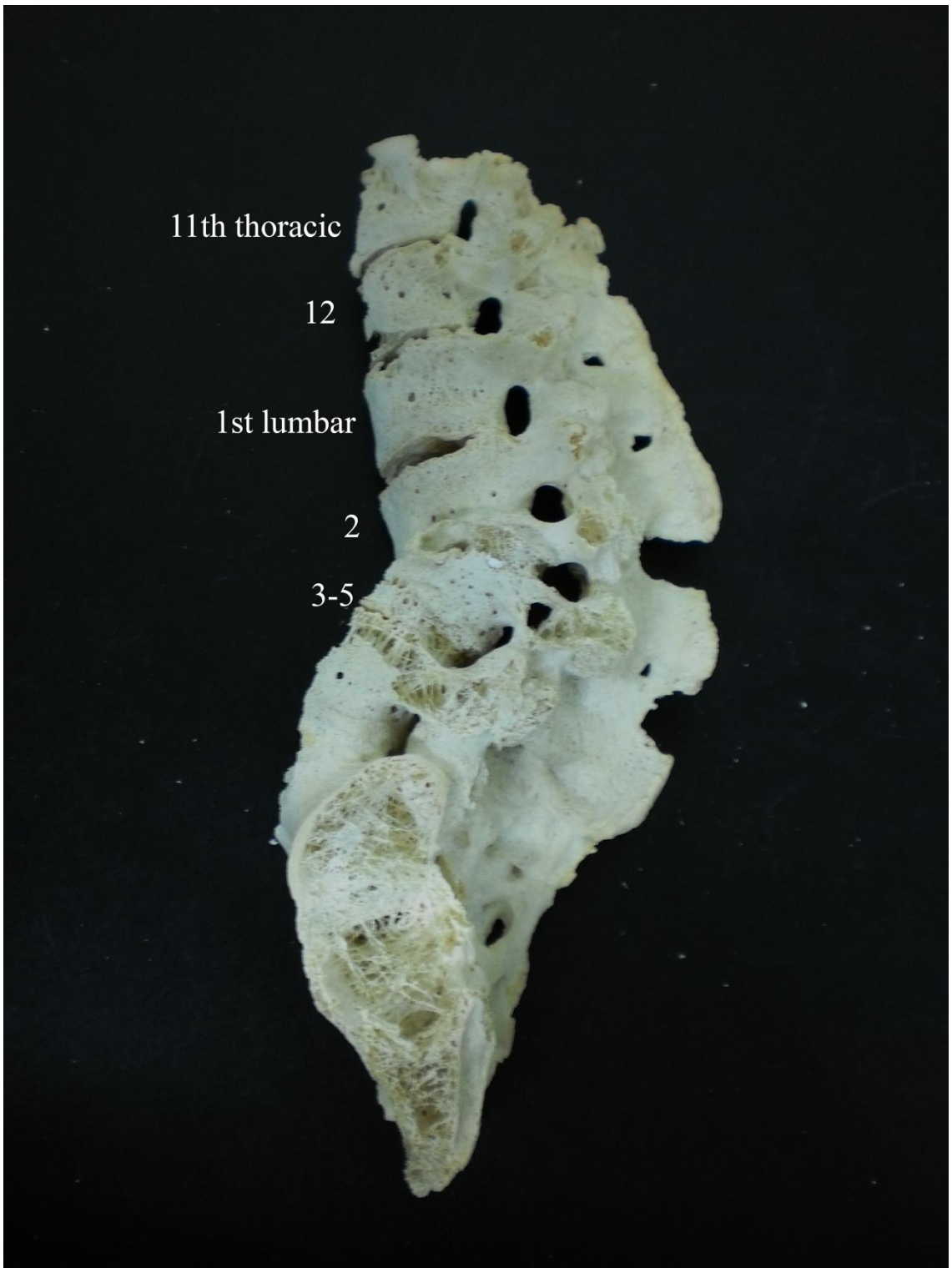
Autopsy Year: 1958

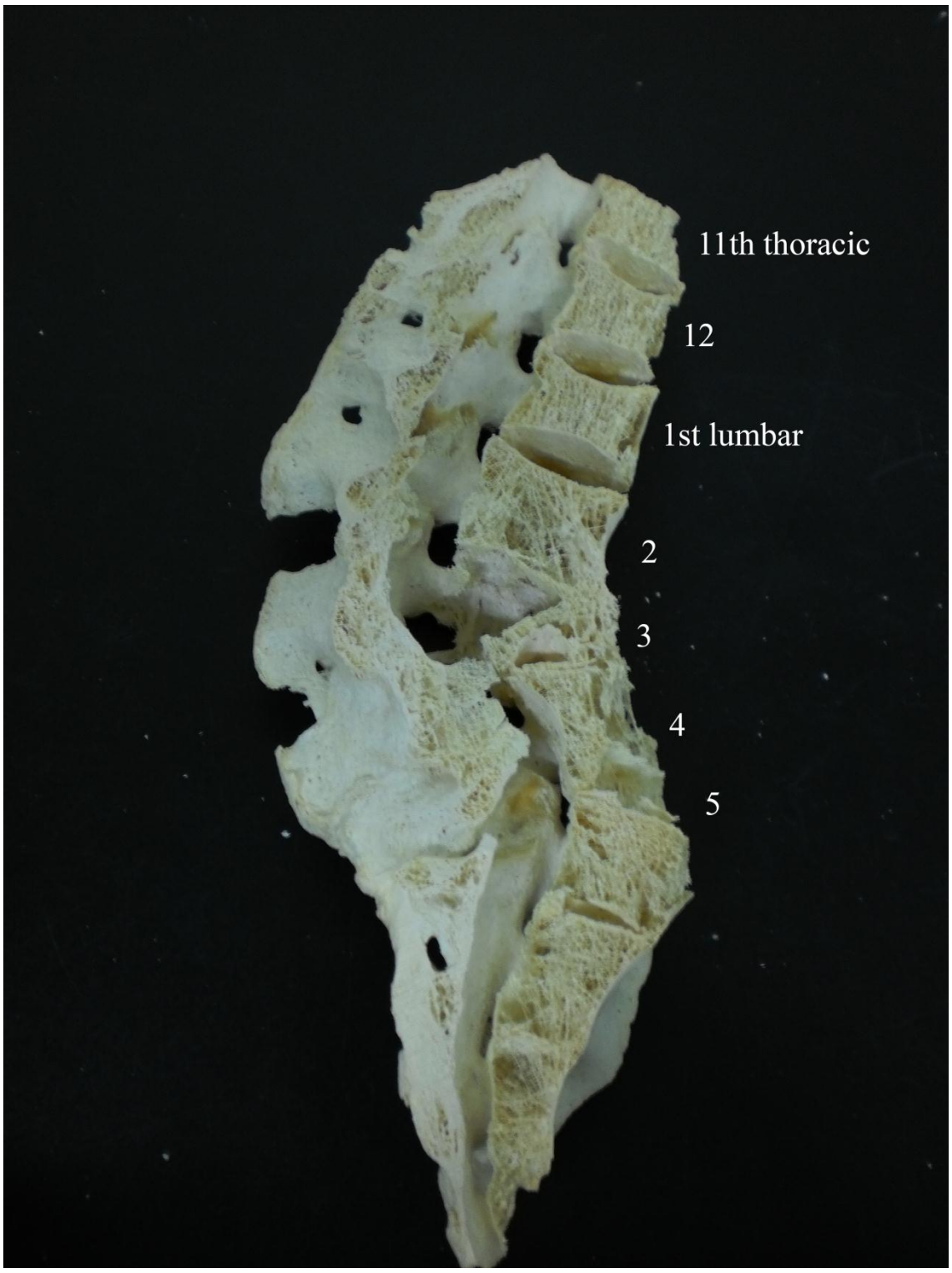
Age: 80

Sex: Male

Description: The image shows the section including thoracic vertebrae eleven to lumbar vertebra five (and the sacrum) of an 80 year old male. The anterior regions of lumbar vertebrae three to five have been almost completely destroyed. Lumbar vertebra two has also been involved and is fused to lumbar vertebra 3 on the anterior edge of the vertebral body. Vertebral bodies are no longer distinct from one another. This individual had pulmonary TB, resulting in spinal lesions. As well as extensive vertebral fusion, healing has occurred by fusion of spinous processes.

The medical records of this individual do not mention compression fractures, Paget's disease, osteomyelitis or neoplasms but does include hip luxation. However, the lesions we were interested in were on the spine, not the hip. The records reported TB as well as osteoporosis. There is destruction of the anterior part of the vertebral body of lumbar vertebrae three to five with consequent collapse of the spine. Although posterior elements have been involved, there are no indications of other diseases that would have caused this type of lesion.


11th thoracic
12
1st lumbar
2
3-5


11th thoracic
12
1st lumbar
2
3
4
5

Autopsy Number: 1441

Autopsy Year: 1954

Age: 80

Sex: Male

Description: The image shows several vertebrae of an 80 year old male, but we were unable to determine specifically which ones these were. Some of the vertebrae have been compressed. Healing has occurred between two vertebrae via fusion of their vertebral bodies. There is also a minor amount of lipping on the vertebra anterior to these two fused vertebrae. The bony deposit protrudes approximately 6 mm from the vertebral body. The fusion is complete leaving no space between vertebrae.

The medical records for this individual state that he had TB spondylitis but not any of the other diseases we considered in our differential diagnoses (compression fractures, Paget's disease, osteomyelitis or neoplasms). We had a limited number of bones available for examination, however we do notice the involvement of only two vertebrae. Fusion has occurred between the vertebral bodies of these. Based on the vertebrae, there would have been a mild degree of kyphosis. Based on medical records and these limited observations, we can suggest a possible cause of the vertebral fusion as TB, but we cannot be certain.

Autopsy Number: 2420

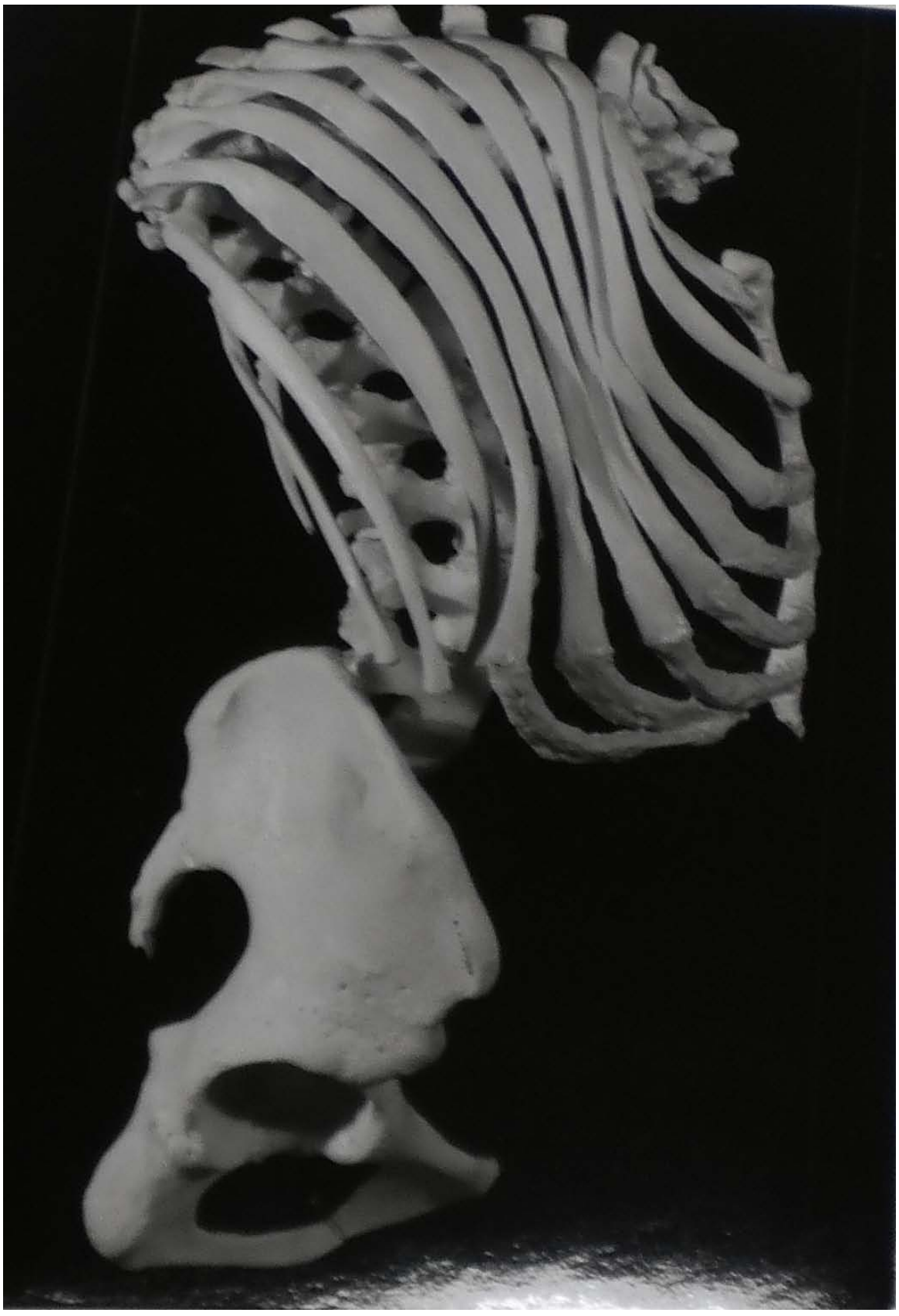
Autopsy Year: 1965

Age: 37

Sex: Male

Description: The image shows a view of the right side of the thorax and pelvis of a 37 year old male. The individual had TB spondylitis from age 7, resulting in kyphosis and fusion of thoracic vertebrae nine and ten. The angle of collapse is approximately 90 degrees. Despite the spinal collapse, there is minimal scoliosis. However, the ribs have deformed in order to compensate for the change in the vertebral column. There is also compensation in the lumbar vertebrae. This individual developed measles at the age of 7, followed by pneumonia in both lungs, ultimately resulting in the development of TB and spondylitis. He spent a great amount of time in sanatoria as a child and had to wear a steel corset to keep his chest straight. Healing in this case has occurred by stabilisation of the spine through fusion of thoracic vertebrae.

Medical records for this individual describe severe TB spondylitis, with resulting kyphosis of the spine at an angle of almost 90 degrees, typical of TB. The individual was treated for TB throughout his life, so we were certain that the spinal lesions in this case were the result of TB and not another cause (such as compression fractures, Paget's disease, osteomyelitis or neoplasms).



Autopsy Number: 1219

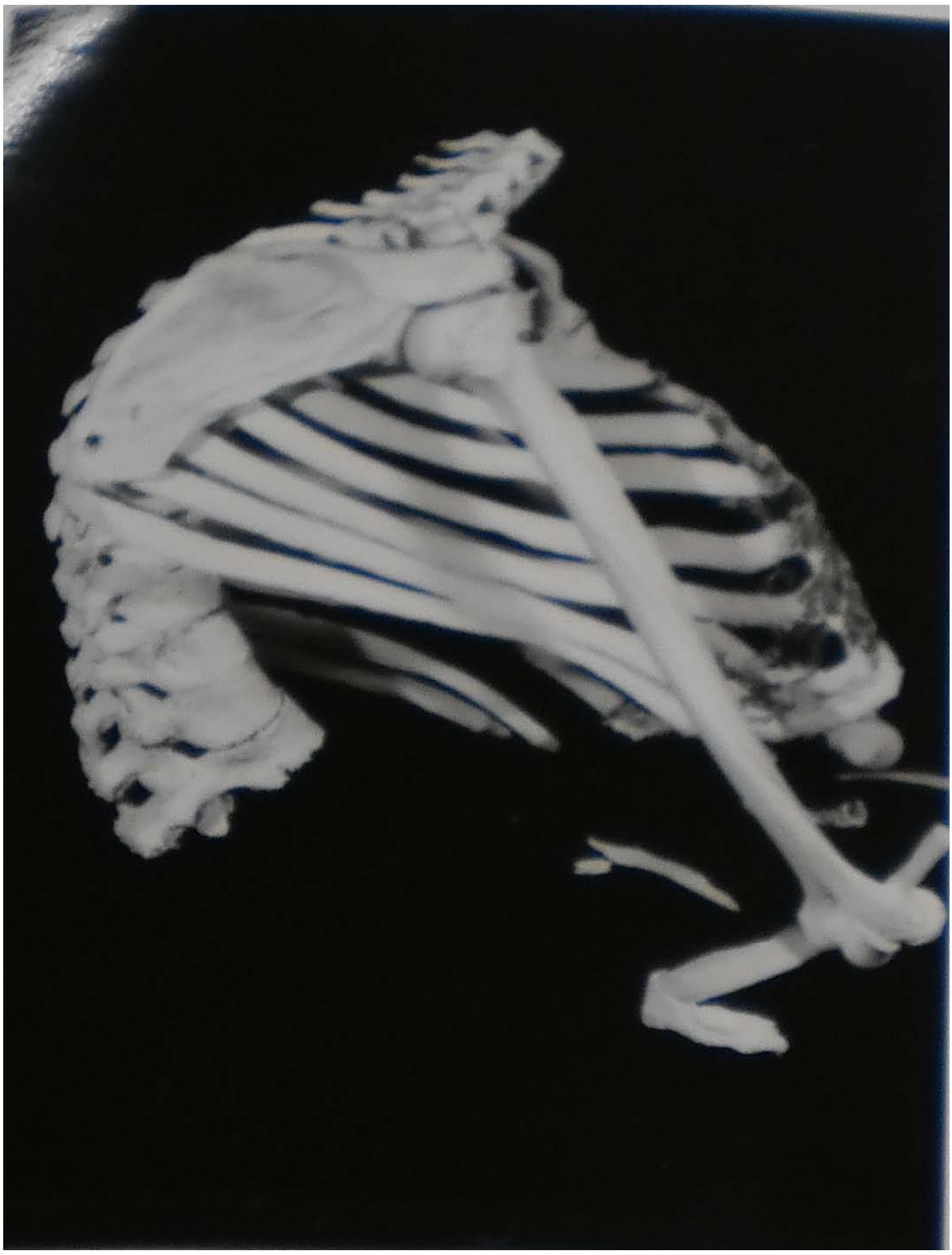
Autopsy Year: 1969

Age: 69

Sex: Female

Description: The image shows the right side of the thorax and right humerus of a 69 year old female. In 1918 (51 years before death), this individual had an inflammation of the vertebrae (TB spondylitis), resulting in collapse and fusion of the fifth and sixth thoracic vertebrae. This consequently caused severe kyphoscoliosis in the thoracic and upper lumbar regions of the spine and compensation in the cervical and remaining lumbar regions. The kyphosis/scoliosis involved seven of the thoracic and three of the lumbar vertebrae. The ribs have been deformed as a result of the spinal collapse. This individual had extensive pulmonary TB. Healing of the spinal lesions in this case has occurred through fusion of the affected vertebrae.

Medical records do not mention any of the other diseases we considered to be the most likely cause of spinal lesions in this sample (compression fractures, Paget's disease, osteomyelitis or neoplasms). The lesions we observed are consistent with TB being the cause. There was anterior destruction of vertebral bodies, leading to a collapse of the spine in the thoracic region. Only two vertebrae were affected by the disease process. Posterior regions of the vertebrae were unaffected. In this case, the lesions of the spine would be caused by TB spondylitis.



Autopsy Number: 1645

Autopsy Year: 1957

Age: 43

Sex: Female

Description: The image shows a single lumbar vertebra of a 43 year old female. We were unable to determine specifically which vertebra. A circular lytic lesion is present on the right side of the vertebral body measuring approximately 12 mm x 14 mm. This individual had pulmonary TB of the left lung. In this case, there is no evidence of healing.

The medical records for this individual do not mention any of the other diseases we considered (compression fractures, Paget's disease, osteomyelitis or neoplasms). There is mention of TB spondylitis, however. In this case, we only had a single lumbar vertebra available for examination. Destruction of the central part of the vertebral body is not uncommon in TB and the destruction affected only a single lumbar vertebra. We could not tell anything about potential kyphosis for this case, however, with the bone destruction observed, it would be unlikely that the spine would have collapsed. This individual died at an earlier than average age of 43 years (average was 62±2 years) of a haemorrhage in the brain. It is possible that this individual died before bone lesions resulting from TB could develop further.

SEE FIGURE 1A IN MANUSCRIPT 3 FOR IMAGE

Autopsy Number: 785

Autopsy Year: 1963

Age: 64

Sex: Male

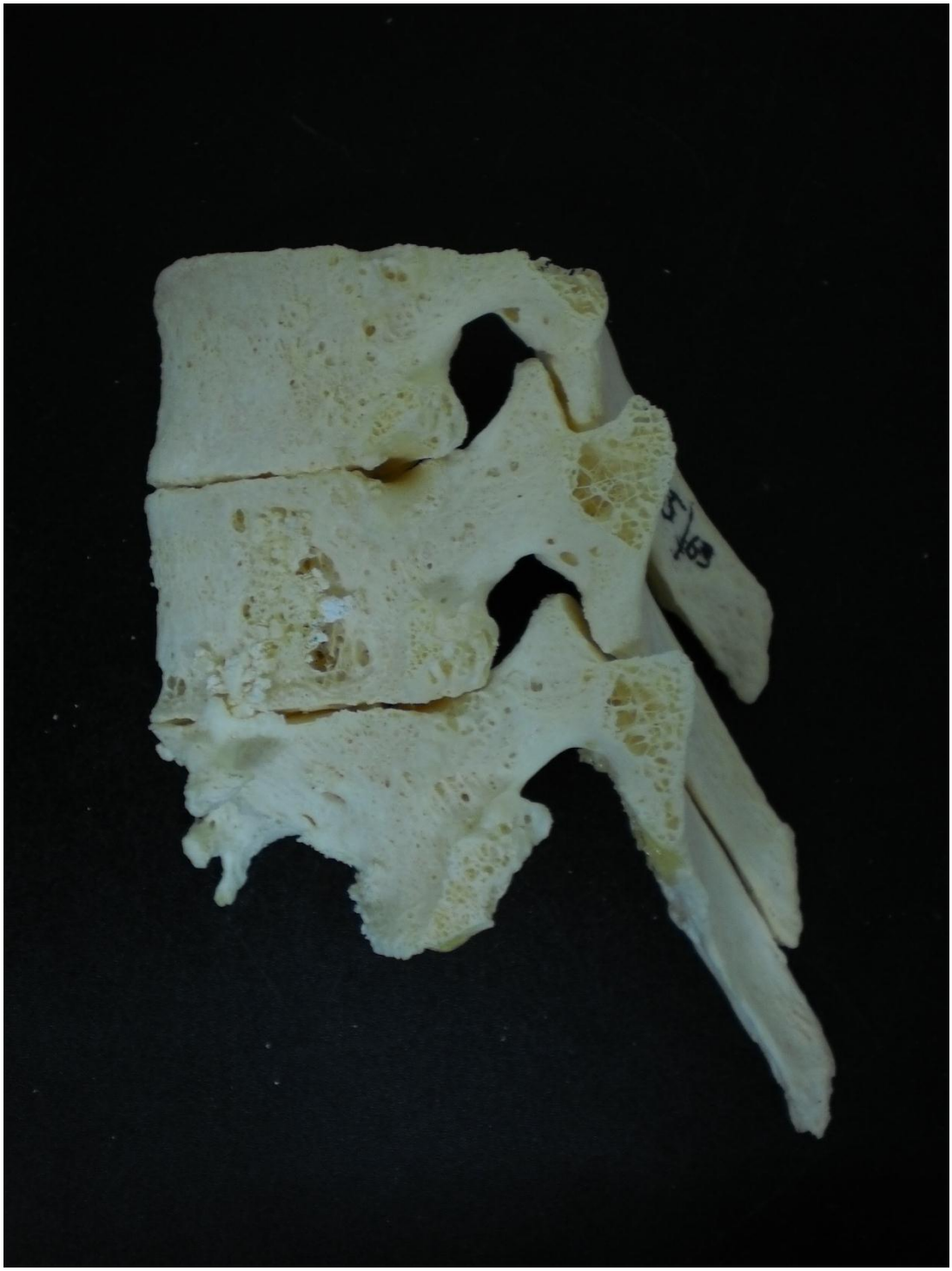
Description: The first image shows the seventh to tenth thoracic vertebrae and the second shows three higher thoracic vertebrae of a 64 year old male. The individual had pulmonary TB as well as TB spondylitis of the eighth thoracic vertebra, which also later affected the ninth and tenth thoracic vertebrae. The anterior region of thoracic vertebra nine has been almost completely destroyed. The eighth and tenth thoracic vertebrae have fused to the ninth through the surface of their vertebral bodies. Thoracic vertebra seven appears to have two lytic lesions on the anterior region of the vertebral body. The first (positioned superiorly) measures approximately 1.5 mm x 2.5 mm. The second measures 3.5 mm x 2.5 mm.

There is also bone deposition and lipping on the other lumbar vertebrae (though these are not fused together). The vertebra in the centre of the second image has a small, circular lytic lesion on the left side of the vertebral body. It measures approximately 2.2 mm x 1.8 mm. Thoracic vertebrae eight to ten have healed through fusion of the vertebrae as well as posterior elements.

The medical records for this individual describe TB spondylitis of multiple vertebrae in detail as well as the presence of osteoporosis. There were two separate foci for skeletal lesions; the first of four thoracic vertebrae and the second involved two lumbar vertebrae. Although a total of six vertebrae were affected, atypical for TB, these vertebrae were not adjacent to one another, making TB still a potential cause of the lesions. Additionally, for the thoracic vertebrae, there was extensive destruction of the

anterior region of the vertebral bodies. One vertebra has been almost completely destroyed. This has led to collapse of the spine and fusion of the affected vertebrae. Although posterior elements were involved, the most probable cause of these lesions is likely to be TB.

SEE FIGURE 1D IN MANUSCRIPT 3 FOR FIRST HALF OF THIS IMAGE



Autopsy Number: 1167

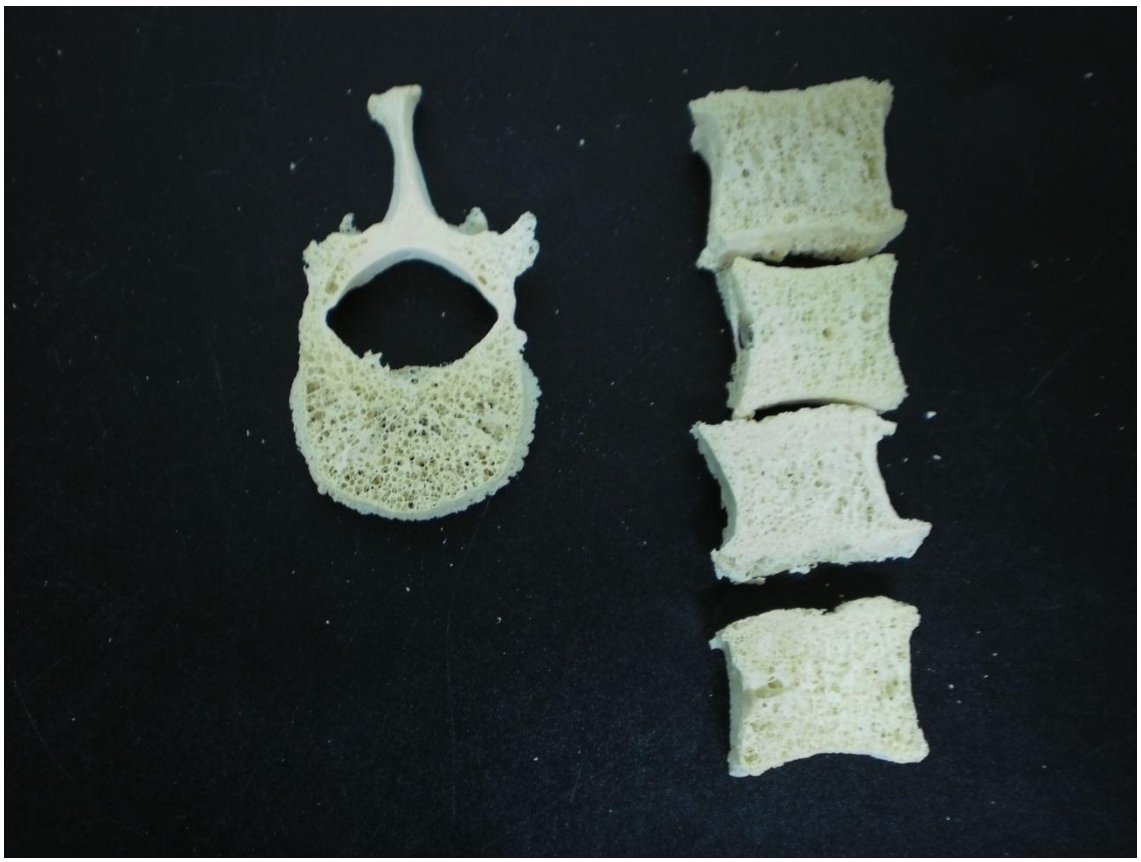
Autopsy Year: 1960

Age: 94

Sex: Female

Description: The image shows a lumbar vertebra as well as four other vertebrae (possibly thoracic) of a 94 year old female. The individual had bone TB causing some damage to lower thoracic and upper lumbar vertebrae. The medical records also describe kyphoscoliosis of the second to fourth lumbar vertebrae. In this case, healing has occurred via bone remodelling and deposition.

Medical records for this individual report bone TB, osteoporosis and kyphoscoliosis of lumbar vertebrae two to four. There is no mention of compression fractures, Paget's disease, osteomyelitis or neoplasms. It may be that the visible vertebral malformations are a result of aging. We had limited skeletal material available for examination in this case, which excluded lumbar vertebrae two to four. Based on the material we did have available, we observed some damage to the vertebrae and no evidence of other diseases. In this case, we had to base most of the diagnosis from the medical records, but considered this individual to have TB as described in the reports.



Autopsy Number: 1227

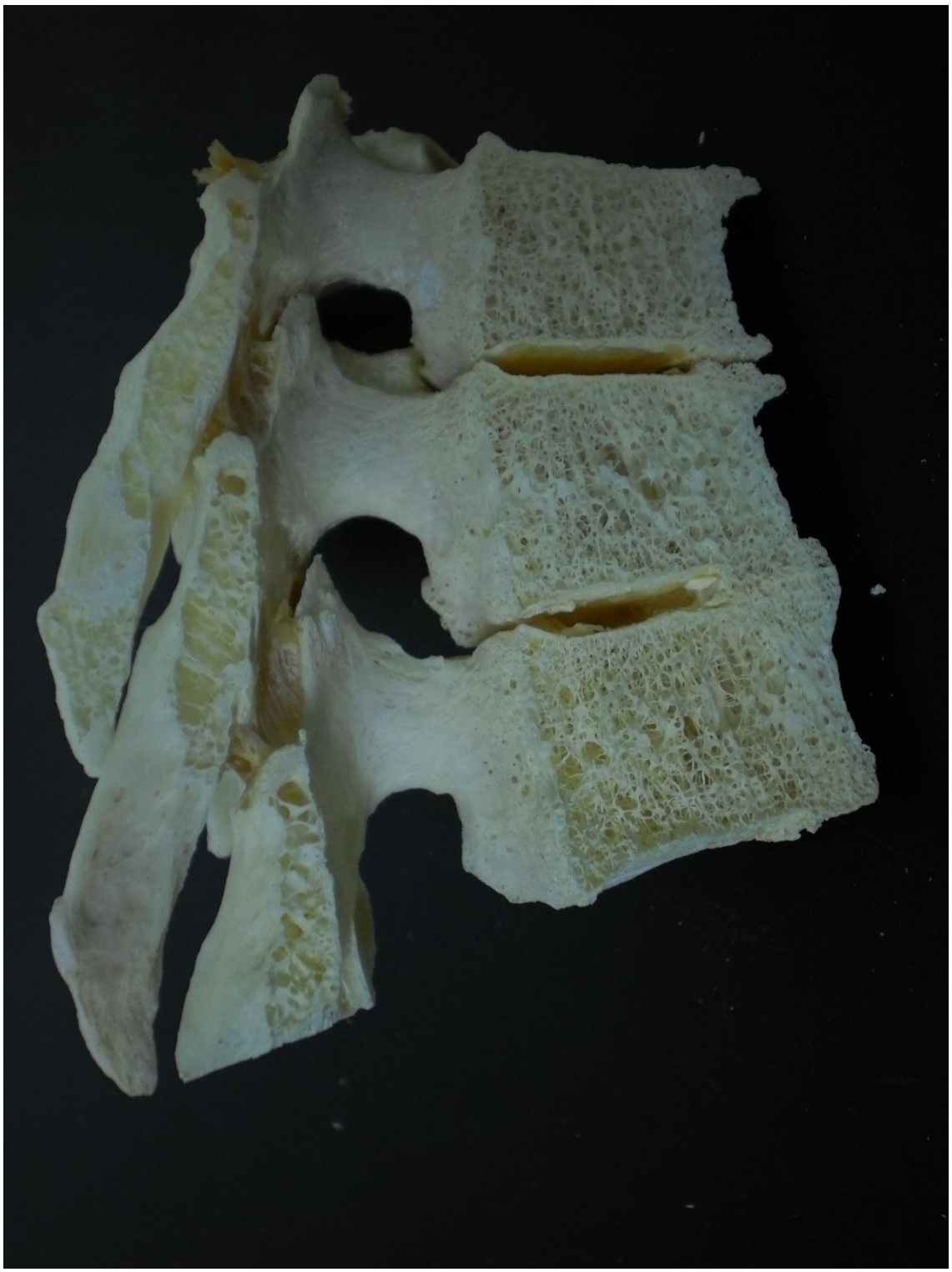
Autopsy Year: 1969

Age: 89

Sex: Female

Description: The image shows three thoracic vertebrae of an 89 year old female. We were unable to determine which vertebrae these were specifically, however, none of them were the fifth thoracic vertebra. There was fusion and bone deposition around several costovertebral joints as well as small amounts of bone deposition between vertebrae. The anterior region of two thoracic vertebrae (lower two shown in the image) have fused together, however, this fusion is not complete between vertebral bodies. The medical records also describe TB spondylitis of the fifth thoracic vertebra, with small abscesses and kyphosis. The vertebra is almost completely destroyed. Healing in this case is a result of bone deposition.

Medical records for this individual describe TB spondylitis of the fifth thoracic vertebra as well as osteoporosis, but not compression fractures, Paget's disease, osteomyelitis or neoplasms. The fifth thoracic vertebra was not available for examination; the medical reports indicated that it had been almost completely destroyed by the disease process. Kyphosis resulted from this event. Observations from the vertebrae available revealed evidence of vertebral fusion. Due to the limited number of vertebrae involved, reports of vertebral destruction and spinal collapse, this can be considered a case of TB.



Autopsy Number: 1466

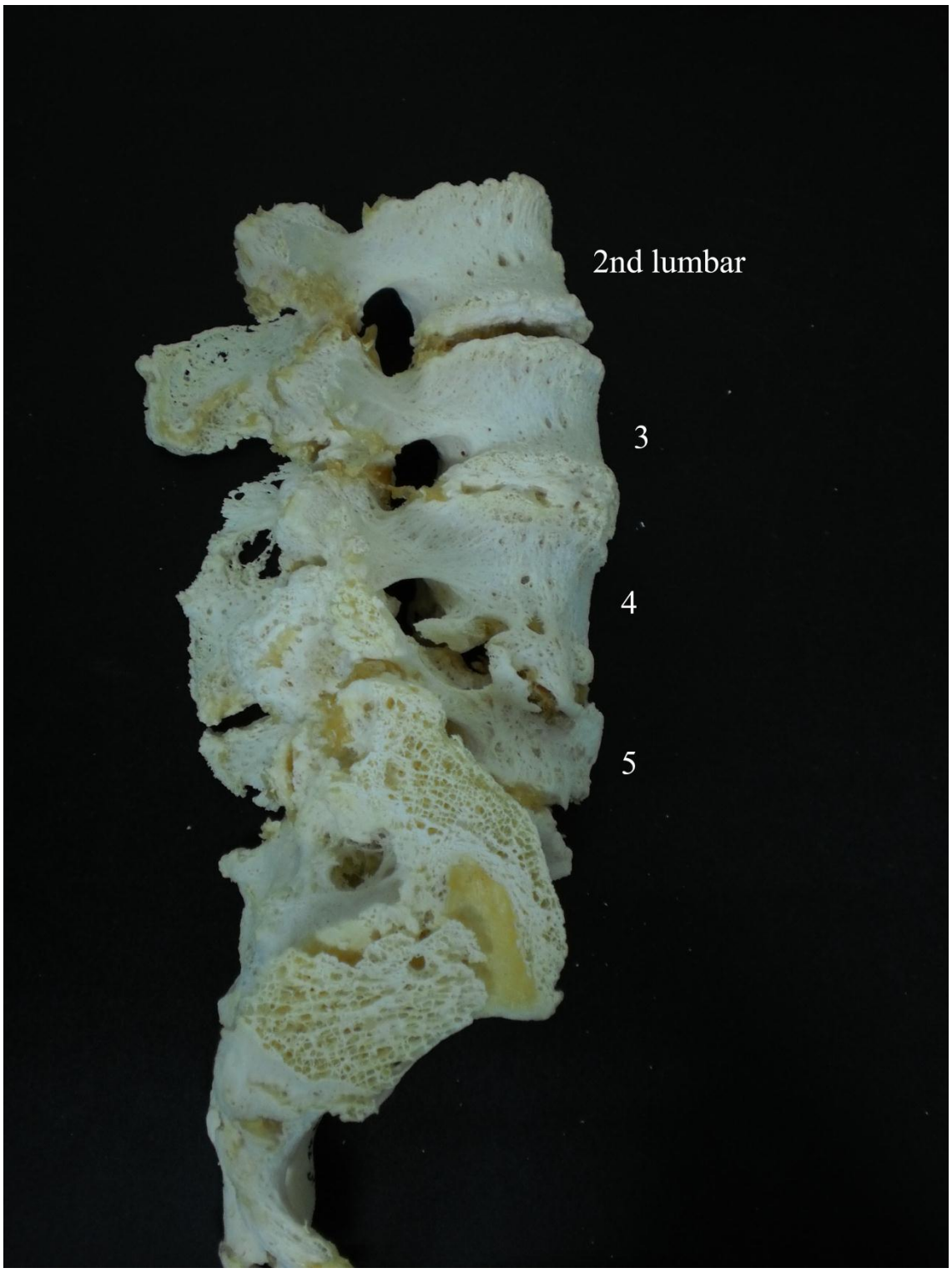
Autopsy Year: 1966

Age: 85

Sex: Female

Description: The image shows the second to fifth lumbar vertebrae and the sacrum of an 85 year old female. The individual had TB spondylitis resulting in destruction of the anterior region of the fifth lumbar vertebra. The vertebral body is almost completely destroyed. Lipping and bone deposition has occurred on several vertebrae. This included the inferior edge of the vertebral body of the third and fourth lumbar vertebrae. Some fusion of posterior elements of lumbar vertebrae three to five have occurred.

This individual was reported to have spondylitis TB, osteoporosis, kyphosis and gonarthrosis. The lower vertebrae were available for examination and anterior destruction of a single vertebral body was observed. Since this was the fifth lumbar vertebra, collapse of the spine was very minimal. Lytic lesions are present on the fourth lumbar vertebra. There was a large amount of bone deposition in this case, however, the individual was 85 years at the time of death. Thus these lesions could be the result of many years of healing after the disease was arrested.


2nd lumbar
3
4
5

Autopsy Number: 1485

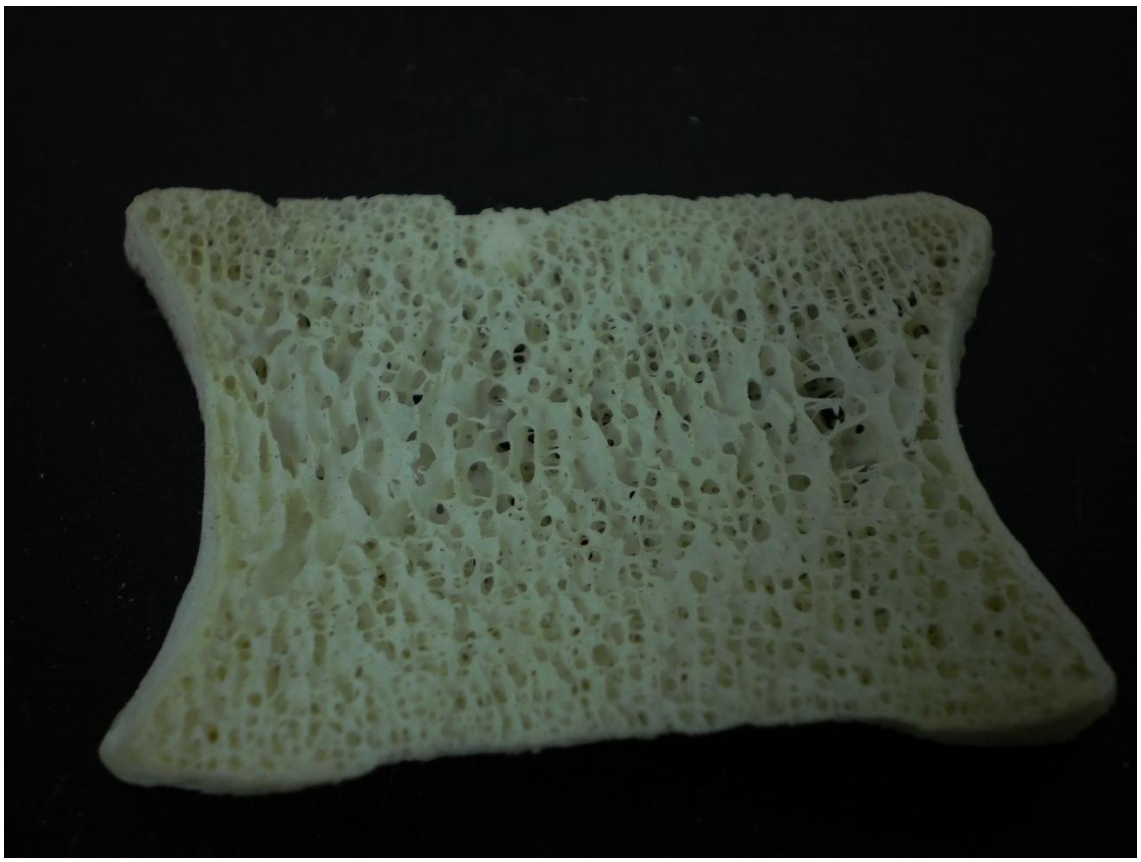
Autopsy Year: 1960

Age: 31

Sex: Female

Description: The image shows a single vertebra of a 31 year old female, though we were unable to determine the specific vertebra. It is slightly compressed but this may not be related to a tuberculosis disease process. This patient had chronic TB that also showed evidence of involvement of the psoas muscle, which had healed by bone deposition on the femur.

Medical records for this individual describe chronic TB as well as several cardiac conditions. This individual may not have developed spinal TB as she died at 31 years, much earlier than the average for the sample (62±2 years). In this case, we considered the individual to have TB of the hip joint and psoas muscle, but not of the spine.



Autopsy Number: 1959

Autopsy Year: 1966

Age: 76

Sex: Female

Description: The image shows twelve vertebrae (probably all thoracic) of a 76 year old female. This individual had pulmonary TB for many years. Five of the middle thoracic vertebrae have been destroyed in the anterior region of the vertebral body. This had led to collapse of the spine at a 90 degree angle. These five thoracic vertebrae have fused together into a solid mass, making it impossible to distinguish single vertebrae. Posterior elements near to the site of collapse have fused together, potentially as a means of mechanical stabilisation for the spine.

Medical records for this individual give details of both breast and lung cancers, which were surgically removed. “Old” pulmonary TB as well as a description of the spinal lesions observed were also given. Although it is possible that the metastases were responsible for the lesions observed, this is unlikely. There is specific destruction of the anterior regions of the vertebral bodies, with marked kyphosis (angle approximately 90 degrees) and fusion of the affected vertebrae. Five vertebrae were affected, however, this individual had TB for many years and bone destruction is extensive. Posterior elements are involved, however this would have occurred some time after the disease was arrested. Differential diagnosis in this case indicates the lesions were very likely to be a result of TB.

Autopsy Number: 2289

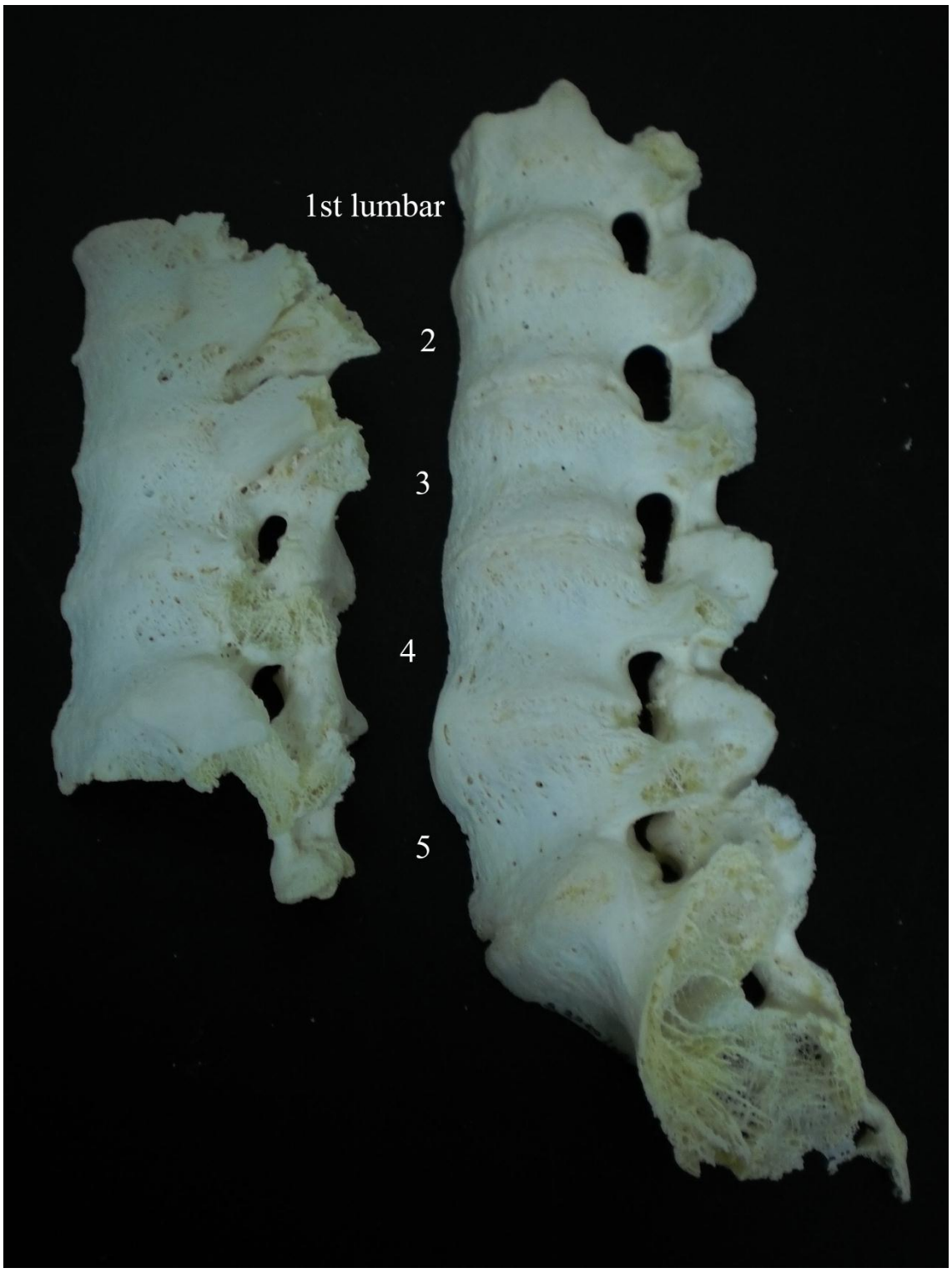
Autopsy Year: 1968

Age: 60

Sex: Male

Description: The image shows four thoracic vertebrae (left) and lumbar vertebrae one to five plus the upper part of the sacrum (right) of a 60 year old male. This is the only individual with the cause of death recorded as tuberculosis. He had pulmonary TB in the right lung and received a lobectomy of the upper lobe 3 years before death. There are lytic lesions on vertebral bodies. The individual also had ankylosing spondylitis which would have strengthened the spine after bone destruction from a tuberculosis related process.

This individual suffered from a compression fracture of thoracic vertebra twelve, although it could have also been a result of a metastatic growth. Ankylosing spondylitis as well as pulmonary TB, the latter of which, was the cause of death. In this case, it was difficult to determine the cause of any observed skeletal lesions because the ankylosing spondylitis covered any lytic lesions that may have been present.


1st lumbar
2
3
4
5

Autopsy Number: 2461

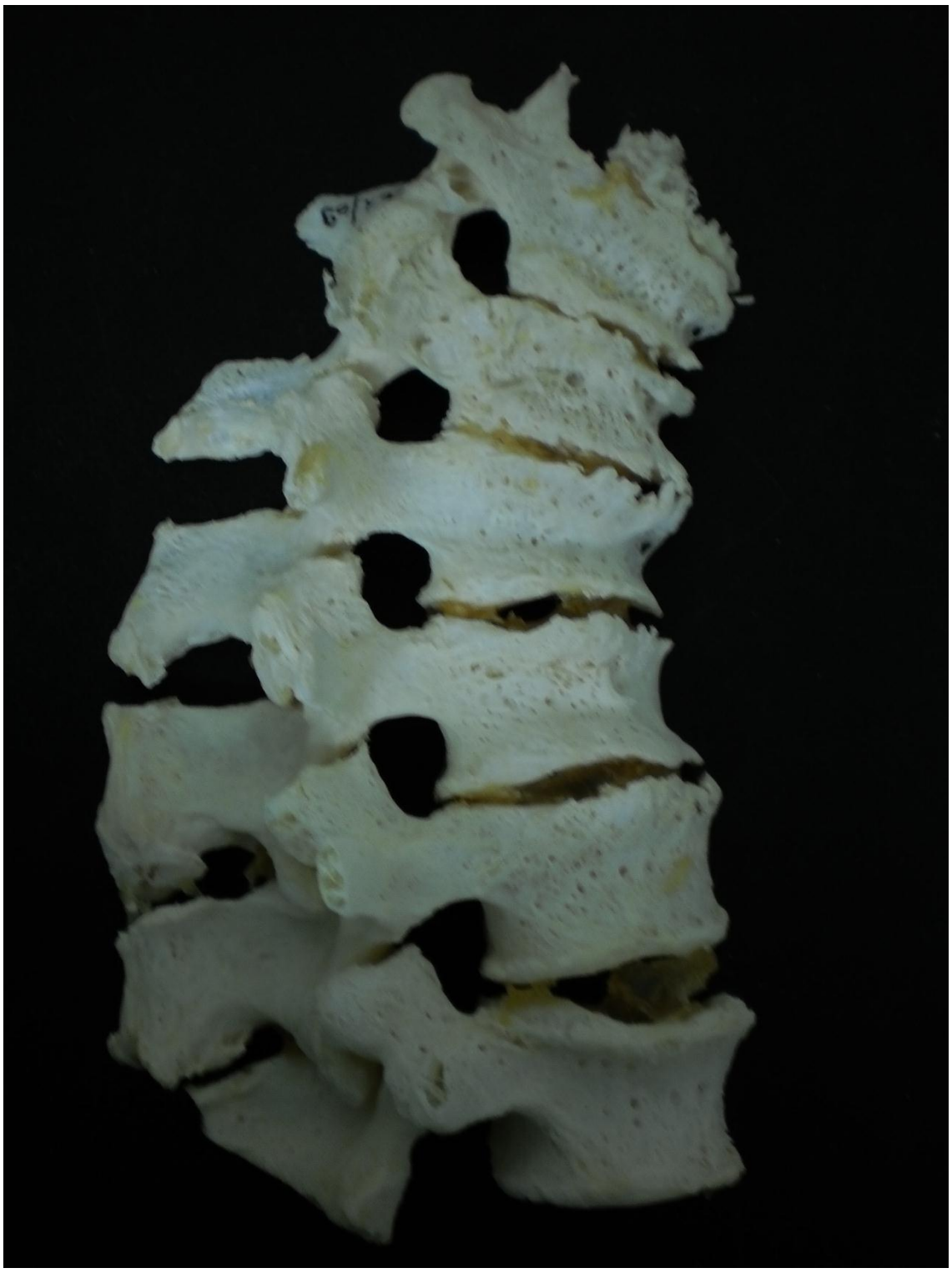
Autopsy Year: 1969

Age: 69

Sex: Female

Description: The images show six vertebrae (left) and five vertebrae (right), most likely thoracic and lumbar, of a 69 year old female. The individual had chronic TB and spondylitis. The cause of death was pneumonia and may have been the result of complications arising from TB. Lipping and bone deposition has occurred on several vertebrae. Between two vertebrae (right) enough bone has been deposited to fuse the two together at the anterior edge of the vertebral body. There is also bone deposition on several ribs. One costovertebral joint is fused.

Medical records for this individual reported osteoporosis, kyphosis, spondylosis and chronic TB, but not compression fractures, Paget's disease, osteomyelitis or neoplasms. Observations from the vertebrae show damage to vertebral bodies as well as bone deposition. There is a mild kyphosis (as observed in the left image). In the absence of any other evidence, it was difficult to diagnose this case. The most likely cause of these lesions is a combination of TB and osteoporosis.



SEE FIGURE 1C IN MANUSCRIPT 3 FOR SECOND PART OF THIS IMAGE

Literature for Appendices 1, 2 and 3

Aceves-Avila, F.J., Báez-Molgado, S., Medina, F., Fraga, A., 1998. Paleopathology in osseous remains from the 16th century. A survey of rheumatic diseases. J. Rheumatol. 25, 776-782.

Acsádi, G., Harsányi, L., Nemeskéri, J., 1962. The population of Zalavár in the Middle Ages. Acta Archaeologica (Hungary) 14, 113-141.

Al-Sarie, I., Al-Shiyab, A., El-Najjar, M., 1996. Cases of tuberculosis at 'Ain Ghazal, Jordan. Paléorient 22, 123-128.

Anderson, T., 2001. A case of skeletal tuberculosis from Roman Towcester. Int. J. Osteoarchaeol. 6, 444-446.

Arriaza, B.T., Salo, W., Aufderheide, A.C., Holcomb, T.A., 1995. Pre-columbian tuberculosis in Northern Chile: Molecular and skeletal evidence. Am. J. Phys. Anthropol. 98, 37-45.

Bachmann, L., Däubl, B., Lindqvist, C., Kruckenhauser, L., Teschler-Nicola, M., Haring, E., 2008. PCR diagnostics of Mycobacterium tuberculosis in historic human long bone remains from 18th century burials in Kaiserebersdorf, Austria. BMC Research Notes 1(83), doi:10.1186/1756-0500-1-83.

Bouwman, A.S., Müller, R., Roberts, C., Brown, T., 2011. An ancient DNA study of tuberculosis in Europe. American Association of Physical Anthropologists. Minneapolis, Minnesota.

Braun, M., Cook, D.C., Pfeiffer, S., 1998. DNA from Mycobacterium tuberculosis Complex identified in North American, Pre-columbian human skeletal remains. J. Archaeol. Sci. 25, 271-277.

Brier, B., 2004. Infectious diseases in Egypt. Infect. Dis. Clin. North Am. 18, 17-27.

Buikstra, J., 1977. Differential diagnosis: An epidemiological model. Yrbk Phys. Anthropol. [1976], 316-328.

Buikstra, J., 1988. Tuberculosis in the Americas: Current perspectives. In: Ortner, D.J., Aufderheide, A.C., (Eds.), Human Paleopathology: Current Syntheses and Future Options. Smithsonian Institution, Zagreb, Yugoslavia

Buikstra, J.E., 1976. The Caribou Eskimo: general and specific disease. Am. J. Phys. Anthropol. 45, 351-367.

Byock, J., Walker, P., Erlandson, J., Holck, P., Zori, D., Gudmundsson, M., Tveskov, M., 2005. A Viking-age valley in Iceland: The Mosfell Archaeological Project. Medieval Archaeology 49, 195-218.

Campbell, T.J., Ackermann, R.R., 2010. Evaluating the emergence of tuberculosis in South Africa.

Canci, A., Minozzi, S., Tarli, S.M.B., 1996. New evidence of tuberculous spondylitis from Neolithic Liguria (Italy). Int. J. Osteoarchaeol. 5, 497-501.

Donoghue, H., Spigelman, M., 2008. Examination of Mycobacterium leprae and Mycobacterium tuberculosis DNA in samples I/11, VI/24 and VI/27 of the Székesfehérvár material. In: Éry K, (Ed.), A Székesfehérvári Királyi Bazilika embertani leletei [Human skeletal remains from the Saint Stephen's Basilica, Székesfehérvár]. Ecclesia Beatae Mariae Virginis Albaeregalis I. Balassi Kiadó, Budapest, [in Hungarian.], pp. 171–174.

Douglas, M.T., 1996. Paleopathology in Human Skeletal Remains from the Pre-Metal, Bronze and Iron Ages, Northeastern Thailand. University of Hawaii, Hilo.

Dutour, O., Romon, T., Ardagna, Y., Tatilon, C., Courtaud, P., 2001. Paléoépidémiologie de la tuberculose en Guadeloupe: le cimetière d'esclaves de l'Anse Sainte-Marguerite. L'Homme et ses Images, GALF, 361-368.

El-Najjar, M.Y., 1979. Human treponematosis and tuberculosis: Evidence from the New World. Am. J. Phys. Anthropol. 51, 599-618.

Etxeberria, F., Romero, W.M., Herrasti, L., 2000. Angular kyphosis of the spine: Mal de Pott identification in a mummy prehispanic Guane Colombia. Chungara (Arica) 32, 41-48.

Évinger, S., Bernert, Z., Fóthi, E., Wolff, K., Kővári, I., Marcsik, A., Donoghue, H.D., O'Grady, J., Kiss, K.K., Hajdu, T., 2011. New skeletal tuberculosis cases in past populations from Western Hungary (Transdanubia). HOMO 62, 165-183.

Faerman, M., Jankauskas, R., Gorski, A., Bercovier, H., Greenblatt, C., 1997. Prevalence of human tuberculosis in Medieval population of Lithuania based on ancient DNA analysis. Ancient Biomolecules 1, 205-214.

Formicola, V., Milanesi, Q., Scarsini, C., 1987. Evidence of spinal tuberculosis at the beginning of the fourth millennium BC from Arene Candide cave (Liguria, Italy). Am. J. Phys. Anthropol. 72, 1-6.

Friedrich, K.M., Nemec, S., Czerny, C., Fischer, H., Plischke, S., Gahleitner, A., Viola, T.B., Imhof, H., Seidler, H., Guillen, S., 2009. The story of 12 Chachapoyan mummies through multidetector computed tomography. Eur. J. Radiol. 6, 6.

Fusegawa, H., Wang, B.H., Sakurai, K., Nagasawa, K., Okauchi, M., Nagakura, K., 2003. Outbreak of tuberculosis in a 2000-year-old Chinese population. Kansenshogaku Zasshi 77, 146-149.

Gernaey, A.M., Minnikin, D.E., Copley, M.S., Dixon, R.A., Middleton, J.C., Roberts, C.A., 2001. Mycolic acids and ancient DNA confirm an osteological diagnosis of tuberculosis. Tuberculosis 81, 259-265.

Gerszten, P.C., Gerszten, E., Allison, M.J., 2001. Diseases of the spine in South American mummies. Neurosurgery 48, 208-213.

Giuffra, V., Vitiello, A., Giusiani, S., Fornaciari, A., Caramella, D., Villari, N., Fornaciari, G., 2009. Rheumatoid arthritis, Klippel-Feil syndrome and Pott's disease in Cardinal Carlo de' Medici (1595-1666). Clin. Exp. Rheumatol. 27, 594-602.

Haas, C.J., Zink, A., Molnár, E., Szeimies, U., Reischl, U., Marcsik, A., Ardagna, Y., Dutour, O., Pálfi, G., Nerlich, A.G., 2000. Molecular evidence for different stages of tuberculosis in ancient bone samples from Hungary. Am. J. Phys. Anthropol. 113, 293-304.

Hershkovitz, I., Donoghue, H.D., Minnikin, D.E., Besra, G.S., Lee, O.Y.C., Gernaey, A.M., Galili, E., Eshed, V., Greenblatt, C.L., Lemma, E., Bar-Gal, G.K., Spigelman, M., 2008. Detection and molecular characterization of 9000-year-old Mycobacterium tuberculosis from a neolithic settlement in the Eastern mediterranean. PLoS ONE 3(10), e3426, doi: 10.1371/journal.pone.0003426

Hogue, S.H., 2007. Mississippian and Protohistoric/Early Contact diet and health: Biological and cultural continuity and change in Oktibbeha County, Mississippi. Southeastern Archaeology 26, 246-268.

Horáčková, L., Vargová, L., Horváth, R., Bartoš, M., 1999. Morphological, roentgenological and molecular analyses in bone specimens attributed to tuberculosis, Moravia (Czech Republic). In: Pálfi, G. (Ed.), Tuberculosis: Past

and Present. Golden Book Publishers and Tuberculosis Foundation, Budapest/Szeged, pp. 413-417.

Jankauskas, R., 1998. History of human tuberculosis in Lithuania: Possibilities and limitations of paleoosteological evidences. Bull et Mem de la Societe d' Anthropologie de Paris 10, 357-374.

Kelley, M.A., El-Najjar, M.Y., 1980. Natural variation and differential diagnosis of skeletal changes in tuberculosis. Am. J. Phys. Anthropol. 52, 153-167.

Kim, M-R., 2011. A Multi-technical approach of ancient DNA and results of tuberculosis of bone samples from slave burials on Guadeloupe (18th/19th century). Paleopathology Association. Minneapolis, Minnesota.

Klaus, H.D., Wilbur, A.K., Temple, D.H., Buikstra, J.E., Stone, A.C., Fernandez, M., Wester, C., Tam, M.E., 2010. Tuberculosis on the north coast of Peru: skeletal and molecular paleopathology of late pre-Hispanic and postcontact mycobacterial disease. J. Archaeol. Sci. 37, 2587-2597.

Lahr, M.M., Bowman, J.E., 1992. Paleopathology of the Kechipawan site: Health and disease in a south-western Pueblo. J. Archaeol. Sci. 19, 639-654.

Lambert, P.M., 2006. Infectious disease among enslaved African Americans at Eaton's Estate, Warren County, North Carolina, ca. 1830-1850. Mem. Inst. Oswaldo Cruz 10, 107-117.

Lichtor J, and Lichtor A. 1957. Paleopathological evidence suggesting pre-Columbian tuberculosis of the spine. J. Bone Joint Sur. Am. 39 A, 1398-1399.

Littleton, J., 2003. Unequal in life? Human remains from the Danish excavations of Tylos tombs. Arabian Archaelogy and Epigraphy 14, 164-193.

Lombardi, G.P., Caceres, U.G., 2000. Multisystemic tuberculosis in a pre-Columbian Peruvian mummy: four diagnostic levels and a paleoepidemiological hypothesis. Chungara (Arica) 32, 55-60.

Malnasi, C., 2005. Paleopathology in Ancient Egypt: Evidence from the sites of Dayr Al-Barsha and Sheikh Said. University of Central Florida, Orlando, Florida. PhD thesis?

Marcsik, A., Molnár, E., Szathmary, L., 2006. The antiquity of tuberculosis in Hungary: the skeletal evidence. Mem. Inst. Oswaldo Cruz 101, Suppl. 2, 67-71.

Marcsik, A., Pálfi, G., 1993. Data for the epidemiology of skeletal tuberculosis in ancient populations. Czlowiek w Czasie i Przestrzeni, Gdańsk, pp. 354-358.

Marcsik, A., Szentgyörgyi, R., Gyetvai, A., Finnegan, M., Pálfi, G., 1999. Probable Pott's paraplegia from the 7th-8th century A.D. In: Pálfi, G., Dutour, O., Deák, J., Hutás, I. (Eds.), Tuberculosis: Past and Present. Golden Book Publishers and Tuberculosis Foundation, Budapest/Szeged, pp. 333-336.

Mark, L., Patonai, Z., Vaczy, A., Lorand, T., Marcsik, A., 2010. High-throughput mass spectrometric analysis of 1400-year-old mycolic acids as biomarkers for ancient tuberculosis infection. J. Archaeol. Sci. 37, 302-305.

Martinez, A.F., Meléndez, B.F., Manrique, F.G., 2010. Bio-anthropology and paleopathology of the SO10-IX Muisca mummy from Sátivanorte, Boyacá, Colombia. Colombia Medica 41, 112-120.

Matheson, C.D., Vernon, K.K., Lahti, A., Fratpietro, R., Spigelman, M., Gibson, S., Greenblatt, C.L., Donoghue, H.D., 2009. Molecular exploration of the first-century Tomb of the Shroud in Akeldama, Jerusalem. PLoS ONE 4(12), e8319, doi: 10.1371/journal.pone.0008319.

Mays, S., Taylor, G.M., 2003. A first prehistoric case of tuberculosis from Britain. Int. J. Osteoarchaeol. 13, 189-196.

Mays, S., Taylor, G.M., Legge, A.J., Young, D.B., Turner-Walker, G., 2001. Paleopathological and biomolecular study of tuberculosis in a medieval skeletal collection from England. Am. J. Phys. Anthropol. 114, 298-311.

Merczi, M., 2001. Examination of pathological alterations in the Late Roman Age cemetery of Visegrád-Diós. [In Hungarian.]. A Wosinsky Mór Múzeum Évkönyve 23, 25-38.

Molnár, E., Maczel, M., Marcsik, A., Pálfi, G., Nerlich, A., Zink, A., 2005. A Csont-ízületi Tuberkulózis Molekuláris Biológiai Vizsgálata Egy Középkori Temető Embertani Anyagában. Folia Anthropologica 3, 41-51.

Molnár, E., Marcsik, A., Dutour, O., Bérato, J., Pálfi, G., 1998. Skeletal tuberculosis in Hungarian and French Medieval anthropological material. In: Guerci, A. (Ed.), La Cura Delle Malattie: Itinerari Storici; Treating Illnesses: Historical Routes. Erga Edizioni, Genova, pp. 87-99.

Molnár, E., Pálfi, G., 1994. Probable cases of skeletal infections in the 17th century anthropological series from Bácsalmás (Hungary). Acta Biologica Szegediensis 40, 117-132.

Morse, D., 1967. Tuberculosis. In: Brothwell, D., Sandison, A.T. (Eds.), Diseases in Antiquity. Charles C. Thomas, Illinois, pp. 249-271.

Murphy, E.M., Chistov, Y.K., Hopkins, R., Rutland, P., Taylor, G.M., 2009. Tuberculosis among Iron Age individuals from Tyva, South Siberia: palaeopathological and biomolecular findings. J. Archaeol. Sci. 36, 2029-2038.

Nerlich, A.G., Haas, C.J., Zink, A., Szeimies, U., Hagedorn, H.G., 1997. Molecular evidence for tuberculosis in an ancient Egyptian mummy. Lancet 350(9088), 1404.

Ortner, D.J., 1979. Disease and mortality in the Early Bronze Age people of Bab edh-Dhra, Jordan. Am. J. Phys. Anthropol. 51, 589-597.

Ortner, D.J., 2003. Identification of Pathological Conditions in Human Skeletal Remains. Academic Press, Elsevier, San Diego.

Ôz, B., Hajnal, K., Marcsik, A., Fogas, O., Horváth, F., Zádori, P., Kelemen, K., Vandulek, C., Schultz, M., Márk, L., Molnár, E., Pálfi, G., 2009. Preliminary report on the paleopathological research of the skeletal material from the Szeged medieval castle excavation. Acta Biologica Szegediensis 53, 125-138.

Pálfi, G., 1991. The osteo-archaeological evidence of vertebral tuberculosis in the 8th century. Acta Biologica Szegediensis 37, 101-105.

Pálfi, G., Ardagna, Y., Molnár, E., Dutour, O., Panuel, M., Haas, C.J., Zink, A., Nerlich, A., 1999. Coexistence of tuberculosis and ankylosing spondylitis in a 7-8th century specimen evidenced by molecular biology. In: Pálfi, G., Dutour, O., Deák, J., Hutás, I. (Eds.), Tuberculosis: Past and Present. Golden Book Publishers and Tuberculosis Foundation, Budapest/Szeged, pp. 403-409.

Pálfi, G., Marcsik, A., 1999. Paleoepidemiological data of tuberculosis in Hungary. In: Pálfi, G., Dutour, O., Deák, J., Hutás, I., (Eds.), Tuberculosis: Past and Present. Golden Book Publishers and Tuberculosis Foundation, Budapest/Szeged, pp. 533-539.

Perzigian, A.J., Widmer, L., 1979. Evidence for tuberculosis in a prehistoric population. J. A. M. A. 241, 2643-2646.

Pfeiffer, S., 1984. Paleopathology in an Iroquoian ossuary, with special reference to tuberculosis. Am. J. Phys. Anthropol. 65, 181-189.

Powell ML. 1987. On the Eve of the Conquest: Life and death at Irene Mound, Georgia. In: Thomas DH, editor. The archaeology of mission Santa Catalina de Guale. New York: American Museum of Natural History. pp 27-35..

Raff, J., Cook, D.C., Kaestle, F., 2006. Tuberculosis in the new world: A study of ribs from the Schild Mississippian population, West-Central Illinois. Mem. Inst. Oswaldo Cruz 101, Suppl. 2, 25-27.

Ritchie, W.A., 1952. Paleopathological evidence suggesting pre-columbian tuberculosis in New York State. Am. J. Phys. Anthropol. 10, 305-318.

Roberts, C.A., Buikstra, J., 2003. The Bioarchaeology of Tuberculosis: A Global View on a Reemerging Disease. University Press of Florida, Florida.

Sáez, A., 2008. Impacto Del Contacto Hispano-Indigena En La Salud De La Poblacion De Chiloe. Un Caso De Tuberculosis En El Cementerio Puqueldon 1. Magallania 36, 167-174.

Spigelman, M., Lemma, E., 1993. The use of the polymerase chain reaction (PCR) to detect Mycobacterium tuberculosis in ancient skeletons. Int. J. Osteoarchaeol. 2, 137-143.

Steinbock, R.T., 1976. Paleopathological Diagnosis and Interpretation: Bone Diseases in Ancient Human Populations. Charles C Thomas, Springfield, Illinois.

Stirland, A., Waldron, T., 1990. The earliest cases of tuberculosis in Britain. J. Archaeol. Sci. 17, 221-230.

Strouhal, E., 1995. Survey on the anthropological collection of the Finnish Nubia Expedition. Hippokrates (Helsinki) 12, 9-27.

Suzuki, T., Fujita, H., Jong, G.C., 2008. Brief communication: New evidence of tuberculosis from prehistoric Korea - Population movement and early evidence of tuberculosis in far East Asia. Am. J. Phys. Anthropol. 136, 357-360.

Suzuki, T., Inoue, T., 2007. Earliest evidence of spinal tuberculosis from the Aneolithic Yayoi period in Japan. Int. J. Osteoarchaeol. 4, 392-402.

Tayles, N., Buckley, H.R., 2004. Leprosy and tuberculosis in Iron Age Southeast Asia? Am. J. Phys. Anthropol. 125, 239-256.

Ulrich-Bochsler, S., Schäublin, E., Zeltner, T.B., Glowatzki, G., 1982. Invalidisierende Wirbelsäulenverkrümmung an einem Skelettfund aus dem Frühmittelalter (7./8. bis Anfang 9. Jh.): Ein Fall einer wahrscheinlichen Spondylitis tuberculosa. Scweiz med. Wchr. 112, 1318-1323.

Weber, J., Czarnetzki, A., Pusch, C.M., 2004. Paleopathological examination of medieval spines with exceptional thoracic kyphosis most likely secondary to spinal tuberculosis. Historical vignette. J. Neurosurg. Spine 1, 238-242.

Wells, C., 1964. An early case of birth injury. Multiple abnormalities in a Romano-British skeleton. Developmental Medicine and Child Neurology 89, 397-402.

Wescott, D., Brinsko, K., Faerman, M., Golda, S., Nichols, J., Spigelman, M., Stewart, B., Streeter, M., Tykot, R., Zamstein, L., 2010. A Fisk patent metallic burial case from Western Missouri: an interdisciplinary and comprehensive effort to reconstruct the history of an early settler of Lexington, Missouri. Archaeol. Anthropol. Sci. 2, 283-305.

Williams, J.A., Snortland-Coles, S., 1986. Pre-contact tuberculosis in a Plains Woodland mortuary. Plains Anthropologist 114, 249-252.

Zink, A., Haas, C.J., Reischl, U., Szeimies, U., Nerlich, A.G., 2001. Molecular analysis of skeletal tuberculosis in an ancient Egyptian population. J. Med. Microbiol. 50, 355-366.

Zink, A.R., Grabner, W., Nerlich, A.G., 2005. Molecular identification of human tuberculosis in recent and historic bone tissue samples: The role of molecular techniques for the study of historic tuberculosis. Am. J. Phys. Anthropol. 126, 32-47.

Zink, A.R., Grabner, W., Reischl, U., Wolf, H., Nerlich, A.G., 2003. Molecular study on human tuberculosis in three geographically distinct and time delineated populations from ancient Egypt. Epidemiol. Infect. 130, 239-249.

Zink, A.R., Molnár, E., Motamedi, N., Pálfi, G., Marcsik, A., Nerlich, A.G., 2007. Molecular history of tuberculosis from ancient mummies and skeletons. Int. J. Osteoarchaeol. 17, 380-391.

References Cited in Additional Thesis Text

Antunes JLF, and Waldman EA. 1999. Tuberculosis in the twentieth century: time-series mortality in São Paulo, Brazil, 1900-97. Cad Saúde Pública, Rio de Janeiro 15(3):463-476.

Condran GA, and Cheney RA. 1982. Mortality Trends in Philadelphia: Age- and Cause-Specific Death Rates 1870-1930. Demography 19(1):97-123.

Doege TC. 1965. Tuberculosis Mortality in the United States, 1900 to 1960. Journal of the American Medical Association 192(12):1045-1048.

Dormandy T. 1999. The White Death: A History of Tuberculosis. London: The Hambledon Press.

Fairchild AL, and Oppenheimer GM. 1998. Public health nihilism vs pragmatism: history, politics, and the control of tuberculosis. Am J Public Health 88(7):1105-1117.

Herzog H. 1998. History of tuberculosis. Respiration 65(1):5-15.

Johnston W. 1995. The Modern Epidemic: A History of Tuberculosis in Japan. Cambridge (Massachusetts): Harvard University Press.

Lewis M, Taylor R, and Powles J. 1998. The Australian mortality decline: all-cause mortality 1788-1990. Australian and New Zealand journal of public health 22(1):27-36.

Nathanson CA. 2007. Disease Prevention As Social Change: The State, Society, and Public Health in the United States, France, Great Britain, and Canada. New York: Russell Sage Foundation.

Omran AR. 1983. The Epidemiologic Transition Theory. A Preliminary Update. Journal of Tropical Pediatrics 29(6):305-316.

Ortner DJ. 2003. Identification of pathological conditions in human skeletal remains. USA: Elsevier.

Pálfi G, Dutour O, Deák J, and Hutás I, editors. 1999. Tuberculosis: Past and Present. Budapest/Szeged: Golden Book Publishers and Tuberculosis Foundation.

Preston SH, and Walle Evd. 1978. French Mortality in the Nineteenth Century. Population studies 32(2):275-297.

Puranen B. 1999. Tuberculosis and the Decline of Mortality in Sweden. In: Edited by:, Schofield R, Reher D, and Bideau A, editors. The Decline of Mortality in Europe. New York: Oxford University Press. p 97-117.

Ramos J. 1976. The history of tuberculosis in Mexico. Bulletin of the International Union against Tuberculosis 51(1):29-33.

Roberts C, Betsinger TK, Steckel RH, Larsen CS, Walker PL, Blondiaux J, Grupe G, Jankauskas R, Maat G, McGlynn G et al. . 2009. Understanding the Impact of Infectious Disease on European Populations: Contributions from the Global History of Health Project. American Association of Physical Anthropology Symposium 2009. Chicago, USA: American Journal of Physical Anthropology Supplement. p 222-223.

Roberts CA, and Buikstra J. 2003. The Bioarchaeology of Tuberculosis: A Global View on a Reemerging Disease. Florida: University Press of Florida.

Smith FB. 1988. The Retreat of Tuberculosis 1850-1950. New York: Croom Helm Ltd.

Steinberg J. 1996. Why Switzerland? Cambridge, UK: Cambridge University Press.

Steinbock RT. 1976. Paleopathological diagnosis and interpretation: Bone diseases in ancient human populations. Springfield, Illinois, USA: Charles C Thomas.

Waddington K. 2004. To Stamp Out "So Terrible a Malady": Bovine Tuberculosis and Tuberculin Testing in Britain, 1890-1939. Medical History 48(1):29-48.

Wolleswinkel-van den Bosch JH, Looman CW, Van Poppel FW, and Mackenbach JP. 1997. Cause-specific mortality trends in The Netherlands, 1875-1992: a formal analysis of the epidemiologic transition. Int J Epidemiol 26(4):772-781.

World Health Organization. 2012. World Health Organization Tuberculosis burden estimates.