THE PRESENCE OF TUBERCULOSIS IN DANISH SKELETONS AD 800 - 1800
– from skeletal data to paleoepidemiological analysis

PhD thesis
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Photo on cover:  TB-related lesion on lateral body of ilium, skeleton G113, Ribe Grey friars.
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Preface

In 2012 a collaborative project between Sydvestjyske museer (Museum of Southwestern Jutland), CHART (Cultural Heritage and Archaeometric Research Team, University of Southern Denmark) and ADBOU (Unit of Anthropology, Department of Forensic Medicine, University of Southern Denmark) was awarded a grant from the Velux foundation. The project was aimed at exploring the population of the Danish town Ribe through 1,000 years by means of archaeological, archaeometric and anthropological studies. I was awarded a PhD scholarship proposing an anthropological project that would explore the impact and dynamics of chronic infectious diseases in the past.

The original project proposal was set out to investigate the associations of the three chronic infectious diseases tuberculosis (TB), leprosy and treponematosis. Including all three diseases in the study was found early on to be too ambitious as the osteological and epidemiological methods only were well-defined for leprosy (Boldsen, 2007). The focus instead was turned to the mycobacterial disease TB. The presence of the disease has had devastating social and economic consequences within the past three centuries worldwide. Though a decline in prevalence the past century it has re-emerged within the last three decades. The knowledge about its presence in the past is fragmentary due to lack of well-developed methods for detecting and estimating its presence in skeletal material. A Master thesis by Jørgensen (2010) was a preliminary attempt to define skeletal lesions ready to apply to population studies of TB. Such an approach was further explored in a Master thesis by Pedersen (2014) in skeletons from the Danish town of Odense. The extent of the Master theses was limited and further work was needed to get more detailed understanding of the skeletal expression of TB. Moreover methods still needed to be developed to enable paleoepidemiological analyses concerning TB.

With the present thesis existing osteological methods of detecting TB in skeletons are refined and methods that allow performing population-based studies of the disease in the past are developed. The methods are applied to data from Ribe to provide insights into the health and lives of people in Ribe, during the time period AD 800 - 1800. The great contribution of the present thesis is thus to bring the research of TB in the past from recordings of skeletal lesions to performing paleoepidemiological analysis. This will be of great value when exploring the presence and impact of TB in the past.
List of articles

This PhD thesis is based upon the work presented in the following three studies. The article manuscripts are available in chapter 9.

Study I:

Study II:
Pedersen DD, Kolmos HJ, Boldsen JL, 2016. Lesion patterns related to tuberculosis in identified and archaeological skeletal samples.

Study III:
Acknowledgements

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I wish to thank Dr. Ben Krause-Kyora and his students at the aDNA laboratory, CAU-Kiel for a very pleasant collaboration. Unfortunately the results of the microbiological analysis did not make it in time to be included in the thesis. I am however very excited about getting this work done and I look forward to collaborating with you on the many other projects we have talked about. I also need to thank archaeologists Morten Søvsø and Maria Knudsen from Sydvestjyske museer for your help with the archaeological contexts and Professor Dr. George R. Milner for proofreading the article manuscripts. Also thank you to Dr. David Hunt at Robert J. Terry Skeletal Collection, Smithsonian Institution in Washington D.C., Dr. Dawnie Steadman at Bass Donated Skeletal Collection, University of Tennessee, Knoxville and Dr. Niels Lynnerup at Unit of Forensic Anthropology, Department of Forensic Medicine, University of Copenhagen. They made skeletons in their collections available for data collection.

I appreciate the support from students and colleagues at ADBOU - Pernille Dahl Pedersen, Vicki Kristensen, Trine Bottos Olsen, Marianne Lauritzen, Dr. Svenja Weise, Peter Tarp and Bodil Theilade. I thank you for letting me share my excitement and worries about the work with you. The late Ulla Freund was responsible for my first practical experiences with bones at ADBOU in 2001. I had many discussions and talks with Ulla about how chronic diseases evolved and spread in the past. She was there for the first six months of the thesis work - I had hoped she would have seen it finished.

Finally I wish to thank my parents and parents in law – you were always ready to help. I am grateful for having four wonderful men in my life – Villiam, Kalle, Otto and Lars. Thank you to Lars for your support and for listening to all my thesis talk.

Dorthe Dangvard Pedersen, Odense 2016
List of abbreviations

aDNA  Ancient deoxyribonucleic acid
HLA  Human leukocyte antigen
HIV  Human immunodeficiency virus
M. bovis  Mycobacterium bovis
MDR-TB  Multidrug-resistant tuberculosis
MTBC  Mycobacterium tuberculosis complex
M. tuberculosis  Mycobacterium tuberculosis
TB  Tuberculosis
TDR-TB  Totally drug-resistant tuberculosis
WHO  World Health Organization
XDR-TB  Extensively drug-resistant tuberculosis
Summary

This thesis sets out to explore the presence of the chronic mycobacterial disease tuberculosis (TB) in skeletons from the Danish town Ribe in the 1,000 year period AD 800 - 1800. The work comprises a review text and three article manuscripts prepared for publication.

In recent centuries TB has caused the deaths of millions of people. From before the 18th century only few historical records are available and the impact of TB is not known. TB is potentially bone involving. The examination of skeletal remains can therefore provide information about the presence of TB in the past when no written records are available. The existing methods for detecting TB in skeletons and for data analyses are not applicable for population-based studies. We need to have methods refined and developed to be able to gain insights into the impact of TB in past populations.

Aims

• Refining the osteological methods for detecting TB in skeletons (study I). This is done in a case-control study that examines the association of 13 assumed TB-related bone lesions and TB diagnosis in skeletons with known TB status.

• Exploring lesion patterns related to pulmonary TB across time (study II). Lesion patterns in modern skeletons and skeletons from medieval and post-medieval periods in Ribe are compared. The aim is to get insights into possible changes in causative pathogens.

• Developing a method that enable paleoepidemiological studies of TB (study III). Lesion probability measures (sensitivities and specificities) and osteological lesion data are incorporated into a model for estimating frequency of TB at death in skeletal samples. The disease frequency at death is explored in Viking, medieval and post-medieval periods and among socially stratified samples in medieval period.

• Exploring male and female response to TB infection across time through studies of lesion patterns in modern skeletons and archaeological skeletons from Ribe.

• Getting insights into mortality patterns in relation to TB across time, in males and females and among socially stratified sub-samples in Ribe.
Skeletal material
A total of 1,637 skeletons were examined. Due to selection criteria concerning preservation and age not all skeletons were included in the different studies of the thesis.

From Ribe, 1,451 archaeological excavated skeletons were examined. The skeletons were excavated from three main sites – Grey friars, Black friars and Ribe Cathedral. Of these 160 complete adult skeletons were included in study II, 752 adult skeletons were included in study III and 738 adult skeletons were included in the additional analyses of mortality patterns.

From reference collections 186 identified skeletons were examined. From Robert J. Terry Skeletal Collection dated to first half of 20th century 124 skeletons were examined. Of these 62 were included as cases in study I and 95 were included in study II. From William M. Bass Donated Skeletal Collection 62 skeletons dated to 1988 - 2012 were included as controls in study I.

Methods
Data concerning sex, age and absence (0) or presence (1) of 13 TB-related lesions were recorded in the skeletons. Distribution of lesions in relation to TB diagnosis, time periods and males and females were tested by Pearson’s $\chi^2$ tests or if only few observations Fisher’s exact tests were used.

The paleoepidemiological tool developed in study III was based upon an approach developed for the study of leprosy by Boldsen (2001, 2005a, 2008, 2013). Statistics were modified for TB using lesion probability data derived from study I. Using the osteological lesion frequencies from Ribe the model estimated the probability of TB ($\tau$) and frequency of TB at death ($\rho$). Two models, one including and one not including rib lesions, were applied due to assumed difference in lesion patterns in the samples.

Mortality patterns in relation to TB were analyzed by Kaplan-Meier survival analysis and differences in survival were tested by log rank tests.

Results
In study I 6 of 13 lesions were found significantly more often among cases compared to controls - periosteal reaction and osteolytic lesions of visceral surface of ribs, ventral vertebral bodies, lateral iliac body, acetabulum, iliac auricular surface and ulnar olecranon process. Two types of lesions on ribs were found with different distributions. Soft nodules and vascular grooves were
found in 17.5% of both cases and controls. Periosteal changes and osteolytic lesions were found in 59.7% of TB cases and 3.2% of controls. Different patterns were also found for two types of vertebral lesions. Lesions on intervertebral surfaces of thoracic and lumbar vertebral bodies were found in 11.3% of cases and 4.8% of controls. Lesions on the ventral part of vertebral bodies were found in 58.1% and 3.2%, respectively.

In study II lesion patterns related to TB were found to change across time in medieval and post-medieval Ribe and in modern skeletons from Terry Collection. Significantly different rib lesion frequencies were found in the modern and archaeological samples and in the temporally different groups from Ribe. Rib lesions were identified in 13.9% of the skeletons from medieval Ribe, 26.9% from post-medieval Ribe, and 65.3% from the Terry Collection. Significant differences were also found for the number of lesions. Most skeletons with 1-2 lesions were found in the modern (75.9%) and post-medieval (84.6%) Ribe samples. Most skeletons with 3 and more lesions were seen in the Terry Collection (36.8%). The additional studies of male and female lesion patterns showed significantly different lesion frequencies on iliac auricular surface where females (57.6%) had more lesions than males (33.0%).

In study III the fit of the model including and not including rib lesions varied among the skeletal samples. Estimates when rib lesions are not included fitted medieval, post-medieval and high status skeletons. When rib lesions were included, the model fitted the Viking sample. Neither model fitted the low/common status sample. The frequency of TB at death remained unchanged from medieval (49%) to post-medieval period (51%). Differences were found in the frequency of disease among low/common (63%) and high status (19%) subsamples.

The additional analyses of mortality patterns showed that individuals that unlikely, probably and likely died with TB died at the same ages in Viking age and Post-medieval periods but that individuals that likely had TB died at higher ages in medieval period. Males with the three TB statuses died at the same ages. Females with likely TB survived longer than females with unlikely and probable TB. Skeletons with the three probabilities of TB in high social status graves died at the same ages. Low/common social skeletons with likely TB survived longer than those in the same social group that unlikely and probably had TB.

Discussion and conclusion
Six bone lesions are associated with TB diagnosis. Recording of these is recommended when performing paleoepidemiological studies of TB. Soft nodules on ribs can be dismissed as a TB
The presence of tuberculosis in Danish skeletons AD 800 - 1800

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indicator. Such are assumed to be cough related associated with chronic pulmonary conditions and long-term exposure to smoke or dust particles.

There is a change in the skeletal expression in post-medieval period where rib lesion involvement increases and individuals die with few lesions compared to medieval period. This is explained as a change from a great proportion of *M. bovis* infection in medieval period towards more pulmonary TB infection in post-medieval period. There seems to be a difference in male and female response to TB infection that may be connected to differential host susceptibility and immune reaction to disease.

The frequency of TB at death is high in medieval and post-medieval Ribe where 50% of people that died had TB at death. Great social differences in the presence of TB in medieval Ribe indicate that as assumed there is great social inequality in the population.

The osteological method presented enables systematic and uniform recordings of TB lesions in skeletons. The detailed criteria and descriptions for recording of lesions are easy to apply to data collection in skeletal samples. The τ-statistics developed estimates the probability of TB and from this the frequency of TB at death in samples. The contribution of the thesis enables population-based studies of TB in skeletons. This makes it possible to gain insight into the consequences and mechanisms of the presence of TB in the past.
Resumé

Indeværende afhandling undersøger forekomsten af den mykobakterielle kroniske infektionssygdom tuberkulose (TB) i skeletter fra den danske by Ribe dateret til 800 – 1800 e.kr. Afhandlingen består af en oversigtstekst samt tre artikelmanuskripter forberedt til publicering.

I de seneste århundreder har TB kostet millioner af menneskeliv. Fra før 1700-tallet kendes kun få historiske kilder om sygdommen og omfanget og konsekvenserne af dens udbredelse er derfor ikke kendt før denne tid. Da TB kan skabe forandringer i knogler kan undersøgelsen af skeletter bidrage med information om forekomsten af sygdommen i fortiden. De nuværende metoder til at spore TB i skeletter og til dataanalyse er ikke brugbare til analyser af sygdommens udbredelse på befolkningsniveau. Der er derfor et behov for at forbedre og udvikle metoder, der gør det muligt at undersøge de palæoepidemiologiske forhold for TB ved brug af skeletdata.

Formål

• Forbedre de osteologiske metoder til at spore TB i skeletter (undersøgelse I). Dette sker i et case-control studie, der undersøger sammenhængen mellem 13 formodede TB-relaterede knogleforandringer og TB-diagnose i skeletter med kendt TB status.
• Undersøge knogleforandringsmønstre relateret til lungetuberkulose over tid (undersøgelse II) med det formål at få indsigt i mulige ændringer i de sygdomsfremkaldende patogener.
• Udvikle en metode, der gør det muligt at udføre palæoepidemiologiske studier af TB (undersøgelse III). Sandsynlighedsmål for knogleforandringer (sensitivitet og specificitet) og data om knogleforandringsfrekvenser benyttes til at opstille en model til at estimere hyppigheden af TB ved døden i skeletsamlinger. Sygdomsfrekvensen ved døden undersøges i vikingetid, middelalder og efterreformationisk tid samt blandt socialt stratificerede grupper i middelalderen.
• Undersøge mulige forskelle i mænd og kvinders reaktion på TB infektion over tid. Dette gøres ved at undersøge knogleforandringsmønstre i moderne skeletter og de arkæologiske skeletter fra Ribe.
• Undersøge dødelighedsmønstre relateret til TB i Ribe over tid, hos mænd og kvinder samt blandt de socialt stratificerede grupper.
**Skeletter**

Skeletter fra i alt 1637 skeletter blev undersøgt i forbindelse med afhandlingen. På grund af krav til bevaring og alder indgår ikke alle skeletter i afhandlingens undersøgelser.


Fra referencesamlinger er undersøgt 186 moderne skeletter. Fra Robert J. Terry Skeletal Collection, der er dateret til første halvdel af 1900-tallet, er 124 skeletter undersøgt. Heraf indgår 62 skeletter af individer, der døde af TB, i undersøgelse I og 95 indgår i undersøgelse II.

Fra William M. Bass Donated Skeletal Collection er undersøgt 62 skeletter, der ikke var smittet med TB. Skeletterne, der er dateret til 1988-2012, indgår alle i undersøgelse I.

**Metoder**

Data vedrørende køn, alder og fravær(0) eller tilstedeværelse(1) af 13 TB-relaterede knogleforandringer blev registreret i skeletterne. Fordelingen af knogleforandringer i forhold til TB diagnose, tidsperioder samt mænd og kvinder blev testet ved brug af Pearson’s χ² test. Hvis kun få observationer var til stede blev Fisher’s exact test benyttet i stedet.


Dødelighedsmønstre i relation til TB blev undersøgt med Kaplan-Meier overlevelsesanalyse og forskellen i overlevelse blev testet med log rank.

**Resultater**

I Undersøgelse I blev 6 af 13 knogleforandringer fundet signifikant mere hos skeletter, der døde af TB I forhold til skeletter, der ikke var smittet med TB. Der er tale om forandringer af
periostal og osteolytisk karakter på den viscerale del af ribben, forandringer på den ventrale del af corpus vertebrae, laterale del af corpus ossis ilii, acetabulum, facies auricularis os ilium og olecranon. To typer knogleforandringer på ribben blev fundet med forskellige frekvensfordelinger. Afrundende noduli og vaskulære fordybninger blev fundet hos 17,5% af både cases og kontroller. Periostale reaktioner og osteolytiske forandringer på ribben blev fundet hos 59,7% af cases og 3,2% af kontroller. Forskelige forandringer var til stede for de to typer forandringer på ryghvirvler. Forandringer på facies intervertebralis blev fundet hos 11,3% af cases og 4,8% af kontroller. Forandringer på den ventrale del af corpus vertebrae blev fundet hos 58,1% af cases og 3,2% af kontroller.

I undersøgelse II blev fundet en ændring i knogleforandringsmønstret over tid i middelalder og efterreformatorisk tid i Ribe og i de moderne skeletter fra Terry samlingen. Signifikante forskelle blev fundet i frekvensen af ribbensforandringer hos moderne og arkæologiske skeletter og i de tidsmæssigt adskilte skeletter fra Ribe. Ribbensforandringer blev fundet hos 13,9% af skeletter fra middelalder, 26,9% fra efterreformatorisk tid og 65,3% fra Terry samlingen. Signifikante forskelle blev også fundet for antallet af forandringer i skeletter fra Ribe og fra Terry samlingen. Der blev fundet flest skeletter med 1 og 2 forandringer i middelalder (75,9%) og efterreformatorisk tid (84,6%) i Ribe. Der blev fundet flest skeletter med 3 eller flere forandringer i Terry skeletterne (36,8%). De supplerende studier af knogleforandringsmønstre hos mænd og kvinder viste signifikant forskellig frekvens for forandringer på facies auricularis os ilium, hvor kvinder (57,6%) havde flere forandringer end mænd (33,0%).

I undersøgelse III blev fundet en varierende ’goodness of fit’ for modellen med ribben og modellen uden ribben. Beregninger foretaget uden ribbensforandringer passer bedst til middelalder, efterreformatorisk tid og skeletter med høj social status. Beregninger foretaget med ribbensforandringer passer bedst til vikingetidsskeletter. Ingen af modellerne passer godt ved beregninger foretaget på lav/almindelige social status skeletter. Udbredelsen af TB ved døden var uforandret fra middelalder (49%) til efterreformatorisk tid (51%). Forskelle blev fundet i udbredelsen af TB hos skeletter med lav/almindelige (63%) og høj (19%) social status.

De supplerende analyser af dødelighedsmønstre viste at individer der usandsynligt, sandsynligt og meget sandsynligt havde TB, havde samme aldersfordeling ved døden i vikingetid og efterreformatorisk tid. Individer, der meget sandsynligt havde TB blev ældre i middelalderen. Mænd døde i samme alder med forskellig sandsynlighed for TB. Kvinder der meget sandsynligt havde TB blev ældre end kvinder med mere tvivlsom TB status. Høj-status
individer med forskellig sandsynlighed for TB døde i samme alder mens lav/almindelig status individer, der meget sandsynligt havde TB ved døden blev ældre end individer i samme sociale gruppe med tvivlsom TB status.

Diskussion og konklusion

Der sker en forandring i det skeletale udtryk for TB i efterreformatorisk tid hvor graden af ribbensinvolvering øges og individer dør med færre forandringer end i middelalderen. Dette forklares som en ændring fra en stor grad af infektion med M. bovis i middelalderen mod mere lungetuberkulose infektion i efterreformatorisk tid. Der synes at være en forskel i reaktionen på TB infektion hos mænd og kvinder. Dette kan være forbundet med forskelle hos de to køn i modtagelighed og immunreaktion over for sygdom.

Hyppigheden af TB ved døden er høj i middelalder og efterreformatorisk tid i Ribe, hvor 50% af de døde havde tegn på TB. De store sociale forskelle i hyppigheden af TB i middelalderens Ribe viser, at der som formodet er stor social ulighed i befolkningen.

1. Introduction

In recent centuries tuberculosis (TB) has caused the deaths of millions of people. The prevalence declined from c. 1850 and by the end of the 1950s the disease was almost eradicated in developed countries (Lillebaek et al., 2002). In the past 25 years TB has re-emerged. This is in developing countries caused by among others crowding, malnutrition and coinfection with human immunodeficiency virus (HIV) (Porter and McAdam, 1994). On global scale it is caused by migration from countries with high incidence rates to low-burden nations (Lillebaek, 2002; de Vries et al., 2014). Further on multidrug-resistant (MDR-TB) and totally drug-resistant (TDR-TB) strains make it difficult to treat the disease (Klopper et al., 2013). Surveillance and research into modern epidemiology and genetics of TB are used in the struggle to prevent and control the spread of TB in modern societies.

As TB potentially will affect bones the presence of the disease in the past can be studied in skeletons. In pre-antibiotic eras the directions of the spread TB and other infectious diseases are influenced by dynamics of host-pathogen interactions, socio-economy and environment without intervention from medical treatment. Getting insights into these interactions and understanding the mechanisms behind will contribute greatly to take measures to handle the TB crisis of the post-antibiotic era of the near future. We therefore need to have methods refined and developed to be able to gain insights into TB in the past.

The current paleoepidemiological TB research undertakes both individual and population-based analyzes, the main approach however still relies on counts of skeletal lesions without a quantitative appraisal of the degree to which various kinds of lesions can serve as TB indicators. There is no consensus on what skeletal lesions to record and what approaches to be used for data collection. The lack of uniform osteological methods for recording lesions makes it impossible to compare the results of different studies (Waldron, 1999). Current studies provide evidence of the presence of TB in the past in certain time periods and geographic locations. Insights into how prevalent the disease was and thus its impact in the past cannot be derived from the studies. We need to be able to deduce from skeletal data to infer about skeletal samples in the past. We need to relate the counts of lesions to probability measures of lesions and hereby take into account that lesions have varying ability of detecting TB. With such weighted probability of disease it is possible to get better estimates of both probability of TB diagnosis in individuals and further on bring the studies to population-based level.
1.1. Aims and objectives

This thesis has two main focus areas:

- The work of the thesis is focused on contributing to primary research into the presence of TB in the past. The thesis is set out to clear the road for performing population-based studies of TB in the past. The osteological methods for recording TB in skeletons are refined. Paleoepidemiological tools are developed to estimate the frequency of TB at death in skeletal samples.

- The methods are used to explore and gain insights into the impact, mechanisms and consequences behind the spread of TB in the Danish town Ribe throughout a 1,000 year period AD 800 – 1800. Studies of TB lesion patterns, the frequency of TB at death across time and among individuals with differing social background, and the mortality of TB will make it possible to examine the life and health in Ribe.

The two main focus areas are explored in three studies presented in article manuscripts prepared for publication. Additional studies of lesion patterns in males and females and mortality patterns in relations to TB are presented in the review text. A list of the three studies is found on page 3 and the full article manuscripts are available in chapter 9. The aims and objectives of the studies are described in the following sections.

1.1.1. Study I

The aim of this case-control study is to examine the association of assumed TB-related osteological lesion and TB diagnosis. The 124 skeletons in the study are from modern reference collections with known TB status at death. The Information about disease status is used for assessing the ability of the lesions to detect TB by calculating sensitivities and specificities. If the sensitivity of a lesion is greater than one minus specificity the probability of having a lesion is greater among those with disease than those without disease (Boldsen, 2001).

1.1.2. Study II

This study is aimed at examining the differences in lesion patterns across time. The differences in lesion patterns are studied in 95 modern skeletons of individuals that died of pulmonary TB in the first half of the 20th century and in 160 archaeological excavated Danish skeletons from Ribe dated to medieval and post-medieval periods. It is speculated whether lesion patterns can help determine route of transmission or causative TB pathogens.
1.1.3. Study III
This paper presents a paleoepidemiological tool that uses statistical modelling and osteological lesion data to estimate the individual probability of TB ($\tau$) and the frequency of TB at death at skeletal sample level. The tool is based upon the paleoepidemiological approach developed for the study of leprosy by Boldsen (2001, 2005a, 2005b, 2008 and 2013). Statistics are modified for TB data and estimations are made from two models (including and not including ribs) due to assumed different lesion patterns in the samples. The aim is to investigate the impact of disease across time and among socially stratified groups in Ribe.

1.1.4. Additional studies
In addition to the analyses presented in study I, II and III lesion patterns in males and females and mortality patterns in relation to probability of TB are studied. The aim is to get insights into host reactions to infection by studying possible differences in male and female response to TB infection and to investigate mortality of individuals with different TB status in the three time periods, among males and females and among social stratified sub-samples.

1.2. Limitations of the thesis
The thesis is restricted within a temporal and geographic frame. The skeletons available from the town of Ribe on the southwestern coast of Denmark are dated to AD 800 – 1800. More recent skeletons of modern reference collections are included to test associations of lesions and diagnosis, and to explore the change of lesion patterns across time. The methods presented are however applicable to skeletal samples of other time periods and geographic locations.

The approaches of the thesis are paleoepidemiological and not paleopathological. The thesis does not present in depth studies of specific cases of TB in individual skeletons. The overall picture of the presence of TB is the focus both in relation to general methodological questions and concerning more specifically the presence of TB in Ribe.

The work is aimed at refining the macroscopic methods for detecting TB in skeletons. Hence, the internal structure of bone in connection to lesions is not studied as part of the thesis. The archaeological bones that the methods presented are applied to will often due to influence of taphonomic processes have exposed internal structures. The study of internal and microscopic structures using methods such as x-ray, examination of thin-ground sections and CT-scanning
will be beneficial to understand how TB lesions are formed (Coqueugniot et al., 2015; Schultz and Schmidt-Schultz, 2015). This was however beyond the scope of the present thesis.

Samples were taken for microbiological analyses by Dr. Ben Krause-Kyora and his research team at the aDNA laboratory at Institute of clinical microbiology, Christian Albrechts University in Kiel. The analysis did unfortunately not yield results in time to be included in the work of the present thesis. The analyses planned combining paleoepidemiology and microbiological approaches are described more detailed in section 7.3.
2. Background

In the following sections an introduction is given to the topics that are discussed in the thesis.

2.1. Tuberculosis

2.1.1. Origin

Tuberculosis is a mycobacterial infectious disease that in humans primarily is caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). Humans can also contract TB from infection with *Mycobacterium bovis* (*M. bovis*). *M. tuberculosis* and *M. bovis* are part of the *Mycobacterium tuberculosis* complex (MTBC) that further includes the bacteria *M. africanum*, *M. canetti*, *M. caprae*, *M. microti* and *M. pinnipedii* that are pathogenic in a variety of animals (O’Reilly and Daborn, 1995).

![Out-of-Africa expansion of MTBC](image_url)

**Figure 1.** Out-of-Africa expansion of MTBC. The grey line represents the early progenitor *M. prototuberculosis* and the colored arrows represent the seven *M. tuberculosis* lineages. The numbers are years in thousands since divergence of lineages. (Comas et al., 2013).

The antiquity of *M. tuberculosis* and when it established itself in the human host are debated (Bos et al., 2014; Wirth et al., 2008; Comas et al., 2013). The different pathogens of MTBC most likely evolved in Africa from the early progenitor *M. prototuberculosis* (Gutierrez et al., 2005, Wirth et al., 2008). Genetic evidence of the variability and evolution of genetic markers
suggests its presence in East Africa 3 million years ago where it may have infected early hominids (Gutierrez et al., 2005). *M. prototuberculosis* probably co-migrated with modern humans out of Africa and spread from the African continent reaching Mesopotamia 64,000-46,000 years ago (Gutierrez et al., 2005). From here two major lineages emerged. One thought to be exclusively in humans spread with human migration to Asia, Europe and the African continent where it diverged into seven lineages as seen in figure 1 (Wirth, 2008).

*Mycobacterium bovis* is thought to be a late derived lineage (Brosch et al., 2002; Mostowy et al., 2005). It is thought to have evolved from a transition from human to animal host that very likely occurred in the early plant and animal domestication process in Mesopotamia c. 13,000 years ago (Wirth et al., 2008).

Studies of the mutation rate of *M. tuberculosis* suggest that the present diversity of the lineages in MTBC occurred between 250 and 1,000 years ago (Hirsh et al., 2004).

### 2.1.2. The white plague

The industrial development and rapid urbanization in Western Europe and North America from the 18th century caused a great increase in the prevalence of TB known as the white plague (Daniel, 2009). TB thrived in the poor and polluted environment of the overcrowded towns. At this time records of TB mortality start to be systematically provided from hospitals and national surveys (Davies et al., 1999; Bello et al, 1999; Ferlinz, 1999; Vuorinen, 1999). The TB mortality rates were similar in the countries and larger towns of Europe. London is an exception (Ferlinz, 1999). Here industrialization began already from the early 18th century and caused a very rapid growth in population. People lived under polluted, crowded and very poor conditions causing spread of TB. The disease peaked in London in the 18th century nearly 100 years earlier than in other European and Northern American cities. Here TB mortality rates peaked at 3-5 deaths per 1,000 around 1850. Fifty years later in 1900 the mortality was reduced to half (fig. 2) (Davies et al., 1999).

The decline of TB started before antibiotics became available for medical treatment in 1940s and before the BCG- vaccine was introduced in 1924 (Davies et al, 1999). Data from England and Wales show that in the time period 1853 to 1910 TB mortality rates declined more rapidly than improvements in social conditions (Davies et al., 1999). The decline is explained as a multifactorial process of environmental and social changes combined with natural selection of immunity towards TB infection (Davies et al, 1999; Grange et al., 2001). The process of natural
selection may have happened when the fertility of susceptible families was reduced and naturally resistant families produced children that survived. In this way acquired immunity could develop through successive generations of natural selection (Davies et al., 1999).

![Graph showing TB deaths and total deaths over time](image_url)

This figure illustrates data from Wales and England. The left-hand scale (●) shows death rates for TB per million per annum, while the right-hand scale (○) shows death rates for all causes per million per annum. (Davies et al., 1999).

### 2.1.3. Global health emergency

Tuberculosis has re-emerged within the last 25 years causing the WHO to declare the disease a global health emergency in 1993 (Grange and Zumla, 2002). The latest report from the World Health Organization (WHO) gives an estimate of 9.6 million new cases of TB in 2014 (fig. 3). In some African countries more than 5 cases per 1,000 population is reported (WHO, 2015).

In the worlds most impoverished countries co-infection with HIV is the greatest risk factor for developing active TB infection (Porter and McAdam, 1994). Of the new cases of TB in 2014 12% was found in individuals co-infected with HIV (WHO, 2015). The cumulative lifetime risk of reactivation of latent TB-infection is 30% for those co-infected compared to 5-10% in those not infected with HIV (Sharma et al., 2005). The development of active TB after infection very much depends on the composition and strength of the immune response of the infected. In HIV-infected persons the cells of the immune response is gradually suppressed and cannot resist TB infection (McMichael and Rowland-Jones, 2001).
The conflicts of war and poor standards of living make people of the poorest regions of the world migrate to developed countries. In this way TB is brought to low-burden TB countries by refugees and immigrants from high-burden countries (Lillebaek et al., 2002). In this way TB rates have increased in Europe within recent years (de Vries et al., 2014). London has in the past 20 years seen a doubling of rates. In Denmark several studies have shown that the rate of TB in immigrants is high compared to native Danes (Andersen et al., 2011; Lillebaek et al., 2002).

The global TB prevalence can potentially rise further in the near future due to development of drug-resistant TB strains. Spontaneous mutations in the genome of *M. tuberculosis* result in evolvement of multidrug-resistant (MDR-TB) and even totally drug-resistant (TDR-TB) strains (Gandhi et al., 2010). Many different antibiotics are used for treatment to prevent development of resistance but when the program of treatment is not followed correctly some bacteria will survive and potentially mutate. In 2008 440,000 cases of MDR-TB (3.6% of total TB cases) occurred worldwide (Gandhi et al., 2010). In 2013 approximately 7% of the global TB infections were MDR-TB (Fiske and Haas, 2013). The world is presently at the brink of a post-antibiotic era were the spread of TDR-TB bacilli may have devastating consequences.
2.1.4. Transmission and developing active disease

*Mycobacterium tuberculosis* is spread through infected airborne droplets expelled from the lungs by coughing, sneezing or talk (Fiske and Haas, 2013). *M. bovis* is transmitted to humans either through consumption of unpasteurized milk from infected cattle or by inhalation of airborne droplets (Francis, 1950).

Not all those infected will develop active disease. The risk of developing active disease is linked to host-pathogen interactions that can be influenced by route of transmission, pathogen virulence, the ability of the immune response to suppress the progression of disease (Bellamy et al. 1998; Bloom and Small, 1998; Valway et al. 1998; Maliarik and Iannuzzi, 2003; Malik and Godfrey-Faussett, 2005). Genetic predisposition towards TB infection has been studied in twin-pairs (Comstock, 1978). In 202 twin-pairs the prevalence of TB in monozygotic twins was twice the prevalence found in dizygotic pairs. Genetic predisposition is also assumed likely in Inuit populations in Greenland (Ladefoged, 2011).

![Pathogenic life cycle of *M. tuberculosis*]( Cambier et al., 2014)

Figure 4. Pathogenic life cycle of *M. tuberculosis* (Cambier et al., 2014).

When TB is contracted the bacteria undergo various cellular processes that are illustrated in the pathogenic life cycle of *M. tuberculosis* in figure 4. Bacteria enter the host via small aerosol droplets with a few bacteria in each. The aerosols settle in the alveolar spaces of the lower or middle regions of the lungs with most airflow (Fiske and Haas, 2013). The bacteria are ingested by macrophages forming a ghon-complex. Here the disease remains latent when bacteria multiplication is suppressed and encapsulated by phagosomes (Fiske and Haas, 2013). *M. tuberculosis* has a mycolic acid membrane that protects it from the toxic substances that
cells of the immune response attempt to kill it with (Cambier et al, 2014). The bacteria therefore can survive within the macrophages and may multiply in the lung alveoli developing active TB (Fiske and Haas, 2013). When active disease has developed the host acts with inflammatory response to the multiplied bacteria within the alveoli. This results in the formation of open pulmonary cavities with large numbers of bacteria (Fiske and Haas, 2013). Coughing and sneezing now spread bacteria from lesions to air. Primary TB infection develops when bacteria multiplication happens in direct continuation of bacterial transmission. Secondary TB develops when the bacteria are endogenous reactivated within macrophages or when exogenous reinfection cause bacteria to multiply in lung alveoli (Fiske and Haas, 2013).

When untreated and unhealed the bacteria will disseminate by hematogenous or lymphohematogenous route from the pulmonary region and develop extra-pulmonary infection. In extra-pulmonary TB most organs and tissues of the body can get affected. The most common sites are lymph nodes, abdominal cavity, meninges, urinary tract, gastrointestinal tract and the skeleton (Fiske and Haas, 2013). Infection with \textit{M. bovis} through the gastrointestinal tract can cause direct extra-pulmonary infection. Here primary infection form in the intestinal wall and mesenteric lymph nodes (Ortner, 2003: 227).

2.2. \textbf{Detecting tuberculosis in past populations}

In time periods before the 19\textsuperscript{th} century historical sources about TB are few. Insights into the presence of TB are therefore mainly available through studies of either mummified or skeletal remains.

The earliest known cases of TB-related osteological lesions date to the neolithic period (Roberts, 2015). Skeletons from an adult female and infant with skeletal lesions were found in the Neolithic site of Atlit-Yam in the Eastern Mediterranean dated to BC 7,250 – 6,160 (Hershkovitz et al., 2008). The osteological lesions are not characteristic TB-related lesions but \textit{M. tuberculosis} DNA was found in both the infant and the female skeleton (Wilbur et al., 2009). More convincing TB-related lesions are present in skeletons from Central Germany dated to 5,400-4,800 (Nicklisch et al., 2012) and the site of Alsónyeék-Bátaszék in the western part of Hungary dated to BC 5,000 – 4,000 (Köhler et al., 2012). The literature presenting skeletal TB findings are very extensive and it is not possible to provide a full review in the present thesis. Such reviews are available in Holloway et al. (2011) and Roberts and Buikstra (2003).
The skeletal expression of TB is studied by different research approaches. Lesions are presented in clinical studies of radiological images and autopsies (e.g. Sorrel and Sorrel-Dejerine, 1932; Davidson and Horowitz, 1970; Tuli, 1975; Thijn and Steensma, 1990), in studies of skeletons in modern reference samples (e.g. Kelley and El-Najjar, 1980; Kelley and Miccozzi, 1984; Roberts et al., 1994; Santos, 2000; Ortner, 2003; Santos and Roberts, 2006; Steyn et al., 2013) in archaeological skeletal material (e.g. Buikstra, 1976; Murray et al., 1990; Baker, 1999; Roberts and Buikstra, 2003; Holloway et al., 2011; Hajdu et al., 2012; Nicklisch et al., 2012), using microbiological methods of detecting TB bacteria DNA in skeletal material (e.g. Salo et al., 1994; Mays et al., 2001; Brosch et al., 2002; Zink et al., 2005; Hershkovitz et al., 2008; Baker et al., 2015; Pálfi et al., 2015) and X-ray and CT scanning are used for microscopic analysis of internal bone and soft tissue alterations (Nicklisch et al., 2012; Coqueugniot et al., 2015; Schultz and Schmidt-Schultz, 2015). Profound descriptions of most possible TB skeletal lesion sites are provided by Aufderheide and Rodriquez-Martin (1998), Ortner (2003) and Roberts and Buikstra (2003).

2.2.1. Skeletal lesions

Tuberculosis may affect all parts of the skeleton. The bacteria are disseminated to bones either through hematogenous route from infected organs and lymph nodes or by direct contact dissemination from infected soft tissues (Ortner, 2003). The bacteria primarily settle in highly vascularized areas. This means that areas of the skeleton with trabecular bone structure as e.g. metaphysis and epiphysis of long bones, vertebrae and ribs potentially will see high involvement rates (Ortner, 2003: 228). The shoulder region, head and neck of ribs and bones of hands and feet are reported as low frequency lesions (Aufderheide and Rodriquez-Martin, 1998; Ortner, 2003). So are lesions reported more frequently in infants and sub-adults than adults (Thijn and Steensma, 1990; Santos and Roberts, 2001; Ortner, 2003: 247; Roberts and Buikstra, 2003:101; Dawson and Brown, 2012; Pálfi et al., 2012). Child-specific lesions are among others found on the endocranial surface of the flat bones of the cranial vault and on long tubular bones where an expanded shell of periosteal reactive bone can be formed (Hershkovitz et al., 2002; Ortner, 2003: 245).

Pathognomonic lesions in the sense of lesions that are only present in individuals infected with TB are not described in the literature. Pott’s kyphosis however is with all probability TB diagnostic. Kyphosis of the spine is not a lesion type but a consequence of advanced progress of
disease when erosive lesions in vertebrae cause the vertebrae and consequently the spine to collapse (Kelley and El-Najjar, 1980). If recording TB with Pott’s kyphosis as the primary diagnostic feature as done by Morse (1967) very low frequencies of TB of the spine will be obtained (Kelley and El-Najjar, 1980).

In the following sections the common sites of TB lesions that are considered for data collection in this thesis are introduced.

2.2.1.1. Ribs
The lungs are the primary infection site of pulmonary TB and in a chronic stage of disease lesions can be formed on the ribs. The association of rib lesions and TB diagnosis have been demonstrated in skeletons of individuals known to have died from or been infected by TB in identified skeletal collections (Kelley and Micozzi, 1984; Robert et al., 1994; Santos and Roberts, 2001; Matos and Santos, 2006; Santos and Roberts, 2006) and in skeletons from archaeological collections (Lambert, 2002; Mays et al., 2002; Nicklisch et al., 2012). In the archaeological cases the presence of *Mycobacterium tuberculosis* in bone samples supported the osteological diagnosis.

The visceral surface of the body is the most common site of lesions on ribs. Four different types of lesions are observed on ribs. Soft nodules are found along the costal groove on the visceral surface of ribs. Internal intercostal muscles attach in the costal grooves. These muscles cause downward depression of ribs doing forced expiration (Köft-Maier, 2000: 71). Cough provokes severe forced expiration and accompanying stress on costal grooves from muscle contraction. Vascular grooves indicate an infectious or inflammatory process in the pulmonary region. These are formed when excess bone cells are produced in the inner osteogenic layer of the periosteum leaving depressions from veins, arteries and nerves bound in the fibrous layer of the periosteum (Marieb et al. 2008: 130). Periosteal changes with additional bone formation are either formed due to contact dissemination from pulmonary infection of the thoracic wall or by hematogenous dissemination from intercostal lymph nodes to vertebral end of ribs (Harisinghani et al. 2000). The final bone lesions are osteolytic erosions that also most likely are formed due to contact or hematogenous dissemination (Kelley and El-Najjar, 1980).

2.2.1.2. Spine
The spine is a common site of skeletal changes in TB (Kelley and El-Najjar, 1980; Resnick and Nawayama, 1995; Hajdu et al., 2012). The lower thoracic and upper lumbar regions are most
frequently involved. In most cases at least two adjacent vertebrae are affected (Kastert and Uehlerger 1964:486). Two different sites in the spine can be affected – the cranial and caudal surfaces of vertebral bodies and the ventral part of vertebral bodies.

On the intervertebral surfaces erosive pitting and cavitation have been associated with TB infection (Kelley and El-Najjar, 1980; Aufderheide and Rodriguez-Martin, 1998; Ortner, 2003). The cavities can in severe cases result in a collapse of the vertebral body and the formation of an angular kyphosis of the spine. Such cases are very rarely seen (Morse, 1967). The lesions on the intervertebral surfaces are most likely formed from either hematogenous dissemination or by direct extension from lesions in the lungs or urinary system. The extension of TB infection between vertebrae occurs through perforation of the pulposus of intervertebral discs (Ortner, 2003: 231).

Baker (1999) suggested that deep cavities with irregular or oval shape and sharp edges on ventral part of vertebral bodies are linked to hypervascularity formed in early-stage TB (Baker, 1999; Spekker et al., 2012). The lesions presented as such are however most likely related to the hypervascularity found in not yet fully developed bones of children and young adults (Roberts and Buikstra, 2003: 127). Lesions on the ventral part of vertebral bodies can however be related to TB (Kelley and El-Najjar, 1980; Ortner, 2003). In such cases the lesions are seen as deep pits with rounded edges accompanied by pitting and periosteal changes in adjacent bone tissues (Kelley and El-Najjar, 1980; Haas et al., 2000; Hajdu et al., 2012). The lesions are explained as either drainage channels for pus produced in tubeculi in soft tissues of the central part of vertebral bodies or as caused by psoas abscesses (Ortner, 1999). Psoas abscesses are formed in the psoas muscle from hematogenous dissemination from infected organs in its vicinity. The abscesses are extended through the ligament of attachment to lower thoracic and lumbar vertebrae that can be secondary affected. Such destructions with abscess formation on the ventral part of vertebral bodies can lead to collapse and formation of an angular kyphosis (Ortner, 2003: 234-235).

2.2.1.3. Joints

Lesions in and around the hip joint are presented as indications of TB infection (Kelley and El-Najjar, 1980; Ortner, 2003). The sites involved are the lateral body of ilium adjacent to the acetabulum, the acetabulum itself and the proximal end of femur. On the lateral body of ilium changes are seen as pits with rounded edges accompanied by pitting and periosteal changes.
Such most likely correspond to the changes found on the ventral part of the ventral vertebral bodies and represent drainage channels from pus and secondary periosteal changes formed as direct extension by contact with psoas abscesses from soft tissues (Ortner, 2003: 236). In severe degree an abscess can be present in the area (Roberts and Buikstra 2003:93; Zhang et al., 2015). In the acetabulum the articular surface and the cartilage free center of the acetabular fossa can be affected (Ortner, 2003). Spread of infection will usually happen through a hematogenous route but can also occur from psoas abscesses in the soft tissues. The changes to the acetabular fossa are distinguished from what Rissech et al. (2006) present as variable 7 of age-related changes to the acetabulum. TB-related changes are seen as deep cavities (fig. 12.B) or a woven structure consisting of dense trabeculae (fig. 12.C). Changes on proximal femur are most often found on either caput of femur or on greater trochanter (Ortner, 2003: 239). The lesions are characterized as areas of pitting and erosive cavities (Kelley and El-Najjar, 1980). In progressive stages a dislocation of the neck of the femur can arise due to the weakening of the neck due to destructions of bone tissues. The femur is affected due to extension of primary synovial TB that reaches the bone along the synovial attachments on the neck of femur. In severe cases of dislocation the lesions in and around the hip joint can be mistaken for congenital dislocation. The erosion of the head and neck will in TB-related lesions show more extensive erosions. Further on the infection will cause pitting and erosive lesions in the new formed acetabulum (Kelley and El-Najjar, 1980).

The sacroiliac joint is a common site of skeletal involvement in relation to TB (Ortner, 2003: 237). The lesions are found on the iliac and sacral auricular surfaces. Skeletal changes in the sacroiliac joint are believed to develop through direct extension from spinal involvement (Ortner, 2003: 239). The lesions are unlike most other lesion sites commonly bilateral (Aufderheide and Rodriguez-Martin, 1998: 139). Changes are seen as pitting and erosive cavities (Kelley and El-Najjar, 1980).

TB of the knee joint is believed to be initiated as synovial infection that is extended along the capsular insertions of femur and tibia (Ortner, 2003: 240). The lesions probably develop due to extensive erosion of the articular cartilage resulting in pitting and erosion of the bone of the joint (Kelley and El-Najjar, 1980). Lesions are seen in both distal end of femur and proximal end of tibia.

The elbow joint is described as the most involved site of TB in the upper extremities (Ortner, 2003: 243). Both distal end of humerus, proximal end of radius and proximal end of ulna can be
affected. The lesions are found as pitting and slight erosion as in the hip and knee joint (Kelley and El-Najjar, 1980). In progressive stage of disease ankyloses can occur. In such cases it is difficult to differentiate the lesions from sequelae in erosive arthropathy or septic arthritis (Ortner, 2003:243).

2.2.2. Differential diagnosis
Differentiation of TB lesions from other bone involving conditions or pseudopathology is one of the greatest challenges in the study of the osteological expression of TB (Buikstra, 1976). In archaeological excavated skeletons distinguishing TB lesions can be particular challenging (Waldron, 1987). Taphonomic processes due to biotic or abiotic factors will cause structural changes to human remains (White and Hannus, 1983; Nawrocki, 1995). The eroded bone surfaces and joint ends from such processes can be mistaken for the often erosive, porous and pitted appearance of TB related lesions.

Many bone involving conditions caused by pathological processes can leave lesions similar to TB-related lesions in the skeleton (Buikstra, 1976; Kelley and El-Najjar, 1980; Aufderheide and Rodriguez-Martin, 1998). In the spine compression fractures both traumatic induced or caused by osteoporosis can cause collapse of vertebral bodies (Ortner, 2003: 411). Such will often unlike TB involve a single vertebral body. Brucellosis and echinococcosis can also produce lesions in the vertebral bodies. Such will unlike TB involve vertebrae in several restricted areas of the spine (Ortner and Putschar, 1985). Lesions on caudal and cranial surfaces of vertebral bodies can resemble Schmorl’s nodes but are contrary such not localized or found centrally on the intervertebral surfaces (Sonne-Holm et al., 2013). The lesions have also contrary Schmorl’s nodes no sclerotic boundaries and the pitting and erosions are accompanied by exposure of the trabecular bone tissue (Sajula et al. 1986). TB arthritis of the joints can be difficult to distinguish from other arthritic conditions such as rheumatoid and pyogenic arthritis (Resnick and Niwayama 1995: 2484; Tseng et al., 2014). The disease process in TB arthritis is contrary to pyogenic arthritis slowly progressing (Resnick and Niwayama, 1995: 2484). Problems of differential diagnosis of lesions can be avoided when recording lesions in the entire skeleton. TB often leaves skeletal involvement in multiple sites and the patterns of skeletal expression are unique (Kelley and El-Najjar, 1980; Kelley and Micozzi, 1984). Using precise and standardized criteria for recording lesions further eliminate the risk of differential diagnosis.
2.2.3. Paleoepidemiology

When data about TB lesions has been recorded from skeletons the challenge is to study the epidemiological properties of the disease with skeletal data as only source of information. In contrast to epidemiological studies many unknown parameters are present in the skeletal samples that affect the possibilities interpreting the paleoepidemiological findings. Besides the introduced problems of detecting the lesions related to TB some considerations are needed when using skeletal data for analysis.

The data available are from samples of dead. The samples are the accumulated dead throughout a long period of time and are highly selected parts of the once living population. Those that died were the frailest in a population at a given age. When it comes to the study of bone involving diseases the absence of lesions are not necessarily a sign of absence of disease. Many die of disease before skeletal development. Very different rates of bone involvement are reported and this number is assumed to vary greatly between individuals due to the complexity of host-pathogen interactions (Cheyne, 1911; Davidson and Horowitz, 1970; Kelley and El-Najjar, 1980; Matos and Santos, 2006). Sub-adults, older individuals and otherwise immune depressed individuals will have a high risk of dying of TB before skeletal changes develop (Wood et al., 1992; Jaspan et al., 2005). Such will be underrepresented in the skeletal record (Wood et al, 1992; Roberts and Buikstra, 2003: 129). Results of studies of the presence of TB derived from analysis of skeletal data cannot be directly transferred to conclusions about the presence of TB in the once living population.

Another topic to consider when performing paleoepidemiological studies of TB is preservation of the skeletons (Waldron, 1999; Dutour, 2008). The archaeological excavated skeletons are the main source of skeletal material for most studies. In such samples skeletons may be partly preserved due to reburial on cemeteries within existing graves. Bones may also be altered by taphonomic processes. This leaves incomplete skeletons and partly preserved bones that make it impossible to look at individual patterning of lesions. Some areas of the skeleton are more vulnerable to postmortem erosion than others. Small bones of the hand and feet and ribs will easily be destroyed by taphonomic processes (Bello, 2000 as presented by Dutour, 2008). This mean that in relation to TB the possibilities of studying rib lesions are skewed compared to other sites in the skeleton. To minimize the issues of preservation it is an advantage to study larger skeletal samples.
The lack of agreement about what bone lesions to record and what criteria to use for the recording is a great concern when it comes to detection of TB in skeletons. Several have demanded standardization methods for data collection in order to have uniform methods that enable comparability of studies between skeletal samples (Waldron, 1999, Zuckerman et al., 2015). Zuckerman et al. (2015) emphasize the importance of having evaluation of TB lesions integrated into widely used diagnostic criteria. Recently Mariotti et al. (2015) presented a study of skeletal evidence of TB in the identified skeletal collection of Certosa Cemetery in Bologna, Italy. They address the importance of defining a set of TB-specific lesions. The current state of the research makes it difficult to compare the results of different studies and ultimately restricts the possibilities of assessing the spread of TB in the past.

In recent years the studies have taken on more paleoepidemiological approaches trying to examine the presence of TB in a population based setting (Maczel, 2004; Holloway, 2013; Mariotti et al., 2015; Sparacello et al, 2016). The picture of the presence of TB in the past provided by such is however very fragmentary. The studies are a reflection of either the intensity of studies by certain researchers in specific geographic regions or simply the lack of skeletal material in other regions (Holloway et al, 2011). Population-based approaches to the study of TB in the past have been performed by e.g. Buikstra (1999), Jankauskas (1999), Pálfi and Marcside (1999), Maczel (2004) and Sparacello et al. (2016). Though studies are brought from individual-based to population-based analysis the main approach is still the count of osteological lesions without considering the ability of the different lesions to detect TB.

2.3. Ribe
The present thesis is based upon data collected from skeletons of individuals buried in the Danish town Ribe. The available skeletons and the archaeological and historical sources about life in the town bring about a unique possibility to study the presence of TB in connection to the socioeconomic aspects of life from Viking age to early modern times.
2.3.1. Viking period (AD 700 – 1050)

Ribe is one of the earliest urban settlements in Scandinavia. It was established as a seasonal trading place that became a permanent settlement in AD 705-710 (Hybel and Poulsen, 2007: 229). The town had a favorable geographic location connected to the northwestern European trading routes via the North Sea and connected to continental Europe to the south by the arterial road Oksevejen (oxen road) across land (Roesdahl, 2001: 87) (fig. 5).

Early Ribe consisted of narrow separated plots from where craftsmen worked and lived. The plots were situated north of the small river (Ribe Å) close to the riverside. The town was extended south of the river in the 9th century. Here a church was erected by the missionary Ansgar in c. AD 860 (Møller and Nyborg, 1979). The marketplace on the northern bank of Ribe Å was in use into the 10th century at this time a rampart and wide moat were built to the north to protect the town (fig. 6).

The main activities in Ribe in the Viking period were related to trade. Products from both the European continent and local products manufactured in Ribe were traded. Local products consisted of e.g. amber and glass beads, ceramics and combs. Coins (sceattes) were presumably minted in Ribe and used for trade with Frisian and French merchants (Kieffer-Olsen, 2008). The coin minting and archaeological findings of trade goods from as far away as the Mediterranean
witness long distance trade that must have been organized and controlled by a central authority. This authority was probably a chief controlling a larger area of Jutland or an early king of the Danish territory (Kieffer-Olsen, 2008).

The Viking period population in Ribe consisted of those involved in trade - foreign and local merchants travelling in and out of town - and craftsmen engaged in manufacturing products for trade. Besides these, a group of local inhabitants lived in and on the outskirts of town. This group made a living from farming, fishing and very likely doing construction work of roads and trenches related to the market area (Kieffer-Olsen, 2008).

2.3.2. Medieval period (AD 1050 – 1536)

Danish Viking kings ruled the Danelagen in England in the 11th century. The death of the last Danish ruler of England Hardeknud in 1042 marked the end of the Viking age (Roesdahl, 2004: 12). The importance of trade endured in Ribe into the medieval period and the contact to Western Europe is reflected in the clerical presence in the town (Nielsen, 1985). Further, the architectonic constructions and details of adornment in Ribe Cathedral dated to c. 1150 resemble the architecture in the tuff built churches in the Rhine region (Feveile et al., 2010: 57).

The town expanded during the medieval period as the part of town on the south bank of the river was extended. In AD 1300 the medieval town on the southern bank was fortified and a defense wall, moats and city gates were built. The expansion of the medieval town most likely meant that the population grew as it did elsewhere in Denmark in 12th and 13th centuries (Hybel and Poulsen, 2007: 113). By the 14th century societal changes happened throughout the
Northern and Western European regions. The plague reached Denmark and Ribe around AD 1350 (Enemark, 1999). The size of the population was reduced dramatically.

Fluctuating temperatures and wet weather through several years put stress on agricultural production (Hybel and Poulsen, 2007: 74). As a consequence farming was reorganized in many parts of Europe and agricultural production was changed from grain to meat. As the importance of meat production and cattle trade, including export to other parts of Europe, grew, cattle markets were established in Ribe during the 15th century to facilitate the new lucrative opportunities (Enemark, 1999). The cattle trade peaked during the first part of the 16th century (Willerslev, 1952).

The social differences between the people living in Ribe are thought to have been great (Feveile et al., 2010: 51). During the 14th century, national legislation was changed and people were more clearly divided than before into social classes, each with clear legal positions (Jakobsen and Madsen, 2001: 139). The late medieval population most likely comprised the overall social structure as present in Ribe during the post-medieval period (Degn, 1981: 201). Here an upper class consisted of the Bishop, nobility and wealthy merchants, a middle class of craftsmen, traders and clergy and a lower class consisted of day laborers, unskilled laborers and servants (Degn, 1981).

2.3.3. Post-medieval period (AD 1536 – 1800)

Following the Protestant Reformation in 1536, the clerical institutions all over Denmark were reorganized, churches were taken out of use and demolished and parishes were consolidated. The ten churches and four monasteries present in medieval Ribe were reduced to two parish churches in post-medieval period - the Cathedral and Sct. Catharinae (Black friars) (fig. 7).

Cattle trade and economic growth persisted in Ribe in the wake of the Reformation but from late 16th century the town was struck by external factors that changed the life in town (Bitsch Christensen, 2010). In 1580 a great fire destroyed 213 houses in Ribe. Furthermore, flooding and sand drifting changed the access to trade routes by sea via the river. In early 17th century larger ships were built that were able to sail North of Jutland to reach Copenhagen and the Baltic Sea. The trade routes across sea were consequently changed, and by 1660 the cattle export in Ribe was redirected to Copenhagen and Aalborg in Northern Jutland (Bitsch Christensen, 2010). Further plague epidemics and wars during the 17th century put life in town
under pressure. This influenced the social structure in the town and social unrest arose as the social inequalities grew (Degn, 1981)

There was a slight increase in the population of Ribe in the second half of the 16\textsuperscript{th} century as the population peaked at c. 5,000 inhabitants. From 1610 a decline was seen, evident in among others the number of taxpayers and baptized babies in town (Degn, 1981). The population decline continued until the late 18\textsuperscript{th} century where the population comprised 2,000 inhabitants (Bitsch Christensen, 2010).

Figure 7. Plan of Ribe in the year AD 1651. Two churches remain after the demolition of churches after the Protestant Reformation in 1536 - the Cathedral in the center of town and Sct. Catharinae to the east. (Mejer, 1652)
3. Skeletal material

Data was collected from a total of 1,637 skeletons – 1,451 archaeological excavated skeletons from Ribe, 124 modern skeletons from Robert J. Terry Anatomical Skeletal Collection and 62 modern skeletons from William M. Bass Donated Skeletal Collection.

3.1. Archaeological skeletal samples

Since the 1950s large parts of the Viking age and medieval town has been archaeological excavated in Ribe (Bencard, 1981). The history of Ribe and the socioeconomic conditions in the town are well-known. Excavation activities of demolished churches, monasteries and their associated cemeteries have yielded skeletons dated to a 1,000 year period AD 800 – 1800.

Skeletal remains were examined from 1,451 individuals dated to the time period approx. AD 800 – 1800 for the present thesis. The skeletons are from three main sites - Grey friars, Black friars and Ribe Cathedral (Our lady). The sites are marked on the map in figure 8.

Figure 8. The churches in medieval Ribe. Skeletons used in this thesis were excavated from Grey friars (marked with red circle), Black friars (marked with green circle) and Ribe Cathedral (marked with blue circle) (Nielsen, 1985:46).
3.1.1. Grey friar’s monastery

It was established in 1232 as the first Franciscan monastery in Denmark. The church St. Laurentii was consecrated in 1280 (Møller et al., 1984b). Parts of the medieval parish cemetery outside the monastery complex and parts of the monastery complex itself were excavated in 1993. The archaeological excavation (ASR 1015) revealed that burial activities began from approx. AD 1250 (Jantzen et al., 1995; Andersen, 2003). From historical sources it is known that the monastery was in use until the Protestant reformation in 1536. The demolition of the church began the year after (Møller et al., 1984b). All 584 skeletons from Grey friars are dated to the medieval period. The skeletons are stored at ADBOU, the skeletal collection, Institute of Forensic Medicine, University of Southern Denmark in Odense, Denmark.

3.1.2. Black friar’s monastery

This Dominican monastery was founded in AD 1228. The earliest burials are from approx. AD 1250. From the late 15th century the monastery was run as a home for the elderly. In 1543 the buildings of the complex was turned into a hospital and the church and cemetery were kept as a parish cemetery and church (Møller et al., 1984a). The skeletons included in this study were unearthed in two separate excavations. In 1983 parts of the monastery courtyard was excavated (ASR 327). The skeletons found are dated to the first half of the 16th century (Madsen et al., 1984). In 1988 an area southeast of the monastery church was excavated (ASR 808). Written sources of the burial practices in this area of the cemetery revealed that the burials likely are from the period AD 1754-1807 (Frandsen, 1988). The 98 skeletons available from Black friars are stored at the Anthropological Collection, Unit of Forensic Anthropology, Department of Forensic Medicine, University of Copenhagen, Denmark.

3.1.3. Ribe Cathedral (Our Lady)

The present cathedral in Ribe was built in the first half of 12th century but it had several predecessors. The earliest church at the site was erected by the missionary Ansgar in AD 860 (Møller and Nyborg, 1979; Søvsø, 2009). Two main excavations have been carried out in the vicinity of the Cathedral – south of the cathedral in 2008/2009 (ASR 13) and 2011/2012 (ASR 13II) and small excavation fields around the cathedral in 2012 (ASR 2391) (Søvsø, 2009; Søvsø, 2010; Madsen and Søvsø, 2010). The so far oldest known Christian Viking Age burials in Scandinavia are among the excavated graves and are dated to the 9th and 10th centuries. The area south of the cathedral was without burials in the period AD 1050-1225, whereas the area
around the cathedral was used as cemetery in continuity from Viking Age until 1800. The 745 skeletons available from the area near the cathedral are stored at ADBOU, Unit of Anthropology, Department of Forensic Medicine, University of Southern Denmark.

3.2. Identified skeletal collections

3.2.1. Robert J. Terry Skeletal Collection
The Robert J. Terry Skeletal Collection housed at the Department of Anthropology, National Museum of Natural History, Smithsonian Institution in Washington D.C. curates 1,728 skeletons (Hunt and Albanese, 2005). The individuals died between 1920 and 1967 and were unclaimed by relatives. For most individuals data about sex, ethnicity, year of birth, year of death, and cause of death are available. The Terry collection was chosen to provide cases for the present study because many individuals - more than 15% - had TB as the death diagnosis from the pre-antibiotics period. This made it possible to select cases according to age restriction. The temporal setting of the skeletons of the collection assumingly made the lesion patterns related to TB more comparable to the patterns found in the archaeological samples. Data was recorded from 124 skeletons from Terry.

3.2.2. William Bass Donated Skeletal Collection
The William M. Bass Donated Skeletal Collection is housed at University of Tennessee in Knoxville. It curates more than 1,000 individuals that died in the time period 1981 to the present (Jantz and Jantz, 2008). The collection is continuously growing when people donate their bodies to the body donation program. The bodies are left for naturally decomposition of soft tissues in the Body farm. Due to the risk of spread of infections individuals dying of infectious diseases are not considered for whole body donations. The possibility of individuals in the collection being infected with TB is therefore very low. Data was collected from 62 skeletons of individuals that died between 1988 and 2012.

3.3. Selection of skeletons
Data from all examined skeletons were not included in the studies of the thesis. Both sub-adult and adult skeletons were examined in the samples from Ribe. Children will due to their immature immune response to a larger degree than adults die with TB before osteological
lesions develop (Wood et al., 1992; Jaspan et al., 2006). This is difficult to take into account if adult and sub-adult frequencies of TB are compared. Sub-adults at ages below 16 years at death were therefore not included in any of the analyses of the thesis.

The skeletons were besides age restrictions subjected to different selection criteria in each of the studies of the thesis. Table 1 gives an overview of the skeletons examined for the thesis and the skeletons included in the different studies. The criteria are described in the following sections.

<table>
<thead>
<tr>
<th>Table 1. Overview of skeletal material recorded for the present thesis and the number of skeletons included in the three studies.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Collection</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Ribe</td>
</tr>
<tr>
<td>Terry Collection</td>
</tr>
<tr>
<td>Bass Collection</td>
</tr>
<tr>
<td><strong>Time period</strong></td>
</tr>
<tr>
<td>AD 800 – 1050</td>
</tr>
<tr>
<td>AD 1050-1536</td>
</tr>
<tr>
<td>AD 1536 – 1800</td>
</tr>
<tr>
<td>AD 1920-1967</td>
</tr>
<tr>
<td>AD 1988-2012</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Child</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
</tbody>
</table>

**3.3.1. Study I**

The study included 62 cases from Terry Collection with TB as listed cause of death. Individuals younger than 25 and older than 55 years at death were not included in the study in order to minimize the effect of age on the immune response and, hence, its influence on the development of active disease (Giefing-Kröll et al., 2015). From Bass Collection 62 age and sex matched controls were included that unlikely had TB. The Bass controls were of the same sex as the Terry cases, and their ages were within 2.5 years of their respective Terry skeletons.

**3.3.2. Study II**

Study II included 95 skeletons from Terry Collection with all six sites preserved of which at least one had a positive score. From Ribe 160 complete adult skeletons were included. Skeletons
with known dating and with all six sites preserved of which at least one had a positive score were included.

3.3.3. Study III

In study III data from 752 adult skeletons was included. Skeletons with at least one of the sites for TB indicator recording preserved were included. Furthermore only skeletons that could be dated to either Viking, medieval or post-medieval period were included.

From Grey friars 351 adults were included of which 252 were buried on parish cemetery and are regarded as low/common social status grave. The remaining 99 were buried inside the church and monastery complex and are regarded as high status graves. The skeletons are all dated to the medieval period. Skeletons of 73 adults from Black friars were included all of which are dated to the post-medieval period. The social affiliation of the individuals is not known. From Ribe cathedral 328 adult skeletons were included. Of these 155 are dated to the medieval period and 105 are regarded as low/common individuals based on their modest burial types and location in the parish cemetery and 42 individuals are regarded as high status individuals because they were either buried in brick coffins in the parish cemetery or within the cathedral building complex.

3.3.4. Additional studies

Male and female differences in lesion patterns were analyzed in addition to the analyses of lesion patterns in study II. Of the 255 skeletons in the study 145 were males and 110 were females. From Ribe there were 94 males and 66 females. From Terry Collection 51 were males and 44 were females.

Mortality patterns in the time periods, in males and females and in the socially stratified sub-samples in relation to probability of TB were analyzed in addition to the analyses of study III. Of the 752 skeletons in study III 738 had known sex and were included in the study of mortality patterns.
4. Methods

4.1. Osteology

4.1.1. Sex assessment

Sex was assessed in the skeletons to examine paleodemographical aspects in relations to the presence of TB.

The sexual characteristics of males and females do not develop before adulthood. The sex of sub-adults was therefore not assessed. When ilium, ischium and the pubic bones have fused in acetabulum of the pelvis at an age of 15-16 years the sex characteristics have developed and sex was assessed. If pelvic bones were not preserved the degree of epiphyseal fusion of the long bones was assessed. Here complete fusion of the bones in the elbow joint and partly fusion of distal femur and both distal and proximal end of tibia correspond to an age at death of approx. 16 years (Scheuer and Black, 2000).

The sex of adult individuals was estimated from the morphology of the cranium and pelvic bones as described by Buikstra and Ubelaker (1994). In addition the size and robustness of the post-cranial skeleton were considered.

Sex was recorded on a seven point scale as described in table 2 (Boldsen et al., 2010).

Table 2. Sex assessment scores.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distinct male morphology</td>
</tr>
<tr>
<td>2</td>
<td>Predominantly male morphology</td>
</tr>
<tr>
<td>3</td>
<td>Slight male morphology</td>
</tr>
<tr>
<td>4</td>
<td>Indeterminable/children</td>
</tr>
<tr>
<td>5</td>
<td>Slight female morphology</td>
</tr>
<tr>
<td>6</td>
<td>Predominantly female morphology</td>
</tr>
<tr>
<td>7</td>
<td>Distinct female morphology</td>
</tr>
</tbody>
</table>

4.1.2. Age estimation

The most likely age at death was estimated to examine age distribution and mortality in relations to the presence of TB.

Age in sub-adults was estimated according to dental development, epiphysis fusion and the length of long bones (Ubelaker, 1989; Scheuer and Black, 2000).

Age in adults was estimated according to Experience-based Age Assessment as discussed by Milner and Boldsen (2012) and by Milner et al. (2016). Here round edges of limbus acetabulum,
round edges of intercondylar areas of proximal tibia, rounded linea aspera, and smooth areas on fossa trochanteria and caput fovea of proximal femur were considered young traits. Contrary sharp and pointed areas of these elements were considered old traits. Each skeleton was given an age range of minimum and maximum age at death.

4.1.3. Osteological lesions related to tuberculosis

The absence and presence of 13 osteological lesions were recorded in the archaeological skeletons from Ribe and identified skeletons from Terry and Bass Collection.

It is important to consider what to record, what not to record and how to record it. The lesions were selected based upon an evaluation of the published literature presenting skeletal lesions as described in section 2.2. To ensure reproducibility on both intra- and interobserver level and to avoid risks of differential diagnosis the criteria for scoring lesions were written up in a registration manual. The cutoff point for the criteria of positive lesion scores in the present study were set at a mild stage of disease progression to include early signs of disease and thus detect as many of the affected as possible (Murray et al., 1990; Baker, 1999; Roberts and Buikstra, 2003: 127). The descriptions of lesions and illustrations of such are included in the article manuscript of study I (section 9.1.). The lesions except those on ribs and vertebrae were recorded in both left and right side of the skeleton. The scoring criteria for the 13 lesions are described in short below.

**Soft nodules and vascular grooves on visceral surface of ribs (RIB1).** Lesions were recorded on visceral surface of the body of ribs. If fragmented only pieces measuring more than 5 cm were considered. The lesions were given one of the following scores: /) No information. Less than three pieces of ribs larger than 5 cm were preserved. 0) No bone changes related to TB. 1) Two or more pieces of ribs had either soft nodules covering more than 5 cm or two or more vascular grooves.

**Periosteal reaction and osteolytic lesions on visceral surface of ribs (RIB2).** Lesions were recorded on visceral surface of the body of ribs. If fragmented only pieces measuring more than 5 cm were considered. The lesions were given one of the following scores: /) No information. Less than three pieces of ribs larger than 5 cm were preserved. 0) No bone changes related to
TB. 1) Two or more pieces of ribs had either periosteal reactions with new bone formation or one or more shallow or deep osteolytic lesions measuring more than 5 mm in diameter.

Cranial and caudal surfaces of thoracic and lumbar vertebral bodies (VER). Lesions were recorded on cranial and caudal surfaces of thoracic and lumbar vertebral bodies. The lesions were given one of the following scores: 1) No information. Less than two vertebral bodies were preserved or more than 50% of the bone surface of those preserved was damaged by post mortem changes. 0) No bone changes related to TB. 1) Two or more thoracic or lumbar ventral surfaces had Clustered pitting or erosive cavities in two or more areas each measuring at least 3 mm or in one area measuring more than 10 mm.

Ventral part of thoracic and lumbar vertebral body (VEN). Lesions were recorded on ventral surface of thoracic and lumbar vertebral bodies. The lesions were given one of the following scores: 1) No information. Less than two vertebral bodies were preserved or more than 50% of the bone surface of those preserved was damaged by post mortem changes. 0) No bone changes related to TB. 1) Two or more thoracic or lumbar ventral surfaces had periosteal changes with woven or lamellar structure on at least 50% of the bone surface or three or more large pits measuring at least 3 mm in diameter were present. In severe cases an abscess could be present.

Lateral body of ilium (BOD). Lesions were recorded on lateral body of ilium between lower gluteal line and the upper acetabular margin. The lesions were given one of the following scores: 1) No information. Less than 50% of body of ilium was preserved. 0) No bone changes related to TB. 1) At least 50% of the bone surface had periosteal changes or three or more large pits measuring more than 3 mm were present. In severe cases an abscess could be present.

Acetabulum (ACE). Lesions were recorded on acetabular fossa and articular surface of acetabulum. The lesions were given one of the following scores: 1) No information. Less than 50% of fossa and/or lunate surface were preserved. 0) No bone changes related to TB. 1) Pitting or erosive cavities were present on articular surface in two or more areas each measuring more than 3 mm or in one area measuring more than 10 mm. In severe cases an abscess could be
present. In acetabular fossa two or more deep cavities were present each measuring more than 3 mm or more than 50 % of the area had a woven structure with dense trabeculae.

**Proximal femur (PF).** Lesions were recorded on caput of femur and greater trochanter of proximal femur. The lesions were given one of the following scores: /) No information. Less than 50% of caput of femur and/or greater trochanter are preserved. 0) No bone changes related to TB. 1) One of the following lesions is present Clustered pitting or erosive cavities on caput of femur in two or more areas each measuring more than 3 mm or in one area measuring more than 10 mm. Clustered pitting or erosive cavities on greater trochanter in two or more areas each measuring more than 3 mm or in one area measuring more than 10 mm.

**Iliac auricular surface (ILI).** Lesions were recorded on iliac auricular surfaces. The lesions were given one of the following scores: /) No information. Less than 50% of the iliac auricular surfaces were preserved. 0) No bone changes related to TB. 1) Clustered pitting or erosive cavities were present in two or more areas each measuring more than 3 mm or in one area measuring more than 10 mm.

**Distal femur (DH).** Lesions were recorded on articular surface of distal femur. The lesions were given one of the following scores: /) No information. Less than 50% of articular surface were preserved. 0) No bone changes related to TB. 1) Clustered pitting or erosive cavities were present in two or more areas each measuring more than 3 mm or in one area measuring more than 10 mm.

**Proximal tibia (PT).** Lesions were recorded articular surface and intercondylar areas of proximal tibia. The lesions were given one of the following scores: /) No information. Less than 50% of articular surface and/or intercondylar areas were preserved. 0) No bone changes related to TB. 1) One of the following lesions was present: Clustered pitting or erosive cavities on the articular surface in two or more areas each measuring more than 3 mm or one area measuring more than 10 mm. Three or more large pits on intercondylar areas each measuring more than 3 mm and/or periosteal changes with woven or lamellar structure on at least 50 % of intercondylar areas.
Distal humerus (DH). Lesions were recorded on condylar areas and articular surface of distal humerus. The lesions were given one of the following scores: /) No information. Less than 50% of condylar area or articular surface is preserved. 0) No bone changes related to TB. 1) One of the following lesions was present: Clustered pitting on lateral and/or medial posterior condylar areas covering more than 1 cm²; clustered pitting or erosive cavities in two or more areas on articular surface measuring more than 3 mm or in one area measuring more than 10 mm.

Radial tuberosity of proximal radius (PR). Lesions were recorded on radial tuberosity. The lesions were given one of the following scores: /) No information. Less than 50% of radial tuberosity is preserved. 0) No bone changes related to TB. 1) Clustered pitting or erosive cavities were present in two or more areas measuring more than 3 mm or in one area measuring more than 10 mm.

Olecranon process of ulna (PU). Lesions were recorded on olecranon process of proximal ulna. The lesions were given one of the following scores: /) No information. Less than 50% of proximal ulna was preserved. 0) No bone changes related to TB. 1) Clustered pitting or erosive cavities on olecranon process were present in two or more areas measuring more than 3 mm or in one area measuring more than 10 mm.

4.2. Statistics

The data management and analysis were carried out using STATA 13 for windows and IBM SPSS Statistics version 22. In study I and II STATA 13 was used. In study III and for Kaplan Meier survival analysis IBM SPSS was used.

Data was computed and recoded into new variables to prepare data for statistical analysis. Sex assessment scores were merged into a sex variable with three categories. The scores 1 and 2 were merged into male, scores 3, 4 and 5 were merged into unknown sex and scores 6 and 7 were merged into female. An age midpoint variable was further calculated from the minimum and maximum age range. The 13 lesions except those on the ribs and vertebrae were recorded in both left and right side of the skeleton. For data analysis one variable for each lesion was computed from scores on the left side. When the left side was missing the score on the right side was used instead.
4.2.1. Associations

Associations were tested by crosstabs and applying Pearson’s \( \chi^2 \) test statistics. Fisher’s exact test statistics were applied when analyses included only few observations. In all analyses the significance was set at the 0.05 level.

Differences in frequencies of lesions assumed related to TB in Terry and Bass Collections in study I were tested by Fisher’s exact test. Fisher’s exact test was further used for analysis of the interrelationship of the 13 lesions in all skeletons and in the six TB-detectors grouped in cases and controls. In all analyses a 0.05 significance level was applied.

The lesion patterns were in study II examined by testing the differences in lesion frequency in relation to Terry Collection and medieval and post-medieval periods in Ribe and in relation to males and females. The distributions were tested by Pearson’s \( \chi^2 \) test.

Differences in frequency of number of lesions in Terry Collection and Medieval and Post-medieval periods and in males and females were also tested by Pearson’s \( \chi^2 \).

In the additional studies, differences in frequencies of skeletons, that unlikely, probably and very likely were infected with TB, in the three time periods and among males and females, were tested by Pearson’s \( \chi^2 \) tests.

4.2.2. Epidemiological diagnostic measures

Diagnostics of disease are based upon probabilities. The epidemiology of disease is characterized by parameters that describe such probabilities. The characteristics of a diagnostic test are expressed through the parameters sensitivity and specificity or by negative or positive predictive values. Predictive values are used in clinical studies to estimate the probability that a patient is truly not sick or sick if the diagnostic test is either negative or positive. Predictive values depend on population prevalence of disease (Kirkwood and Sterne, 2003). Sensitivity and specificity do not depend on the population prevalence of disease and are related to the biology of the disease. The concern in the thesis is the principle association of bone lesions and disease diagnosis. Measures of sensitivity and specificity are therefore used to assess the lesions ability to detect disease. Sensitivity describes the probability of having a given lesion given the individual had the disease - the ability of the presence of a given lesion to detect the true positive. Specificity describes the probability of not having a given lesion given the individual did not have the disease - the ability of the absence of a given lesion to detect the true negative. If disease status is known, as it was in study I, the sensitivity and specificity can
be calculated for lesions from the absence or presence of lesions in those with and those without TB (table 3). Lesions are related to TB if the sensitivity of a lesion is greater than one minus specificity (Boldsen, 2001).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th># sick</th>
<th># not sick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>True positive (TP)</td>
<td>False positive (FP)</td>
</tr>
<tr>
<td>Absent</td>
<td>False negative (FN)</td>
<td>True negative (TN)</td>
</tr>
</tbody>
</table>

Table 3. Diagnostic criteria and definitions of sensitivity and specificity.

Sensitivity = \( \frac{TP}{sick} \)

Specificity = \( \frac{TN}{not \, sick} \)

4.2.3. Probability of tuberculosis at death - \( \tau \)-statistics

For the paleoepidemiological study of leprosy Boldsen (2001, 2005a, 2013) has developed a purely statistical approach to estimating the epidemiological characteristics of leprosy at death from skeletal lesion frequencies. The method was applied to the study of TB in Ribe across time and between social stratified sub-samples as presented in study III. The individual probability of TB (\( \tau_k \)) was estimated given either positive or negative scores of six osteological lesions presented as TB indicators in study I. The epidemiological measures were calculated from lesion scores found in identified skeletons from Terry Collection and modern skeletons from Bass Collection.

The probability of having TB (\( \tau_k \)) was calculated as a weighted sum of lesion scores (\( \Delta_{jk} \)) as expressed in equation (1). Here the \( \tau \)-statistics for individual number ‘K’ was estimated from the combined log-likelihood estimate assuming the \( k \)th skeleton was affected by TB (enumerator) and assuming the \( k \)th skeleton was not affected by TB (denominator). Sensitivity and specificity of lesions were derived from measurements presented in study I (table 5 in section 5.1.). The lesion scores (\( \Delta_{jk} \)) are derived from the data recorded in the skeletons from Ribe that are found in table 3 for the time periods and in table 7 for the social stratified sub-samples in the manuscript of study III.

\[
\tau_k = \sum_{j=1}^{6} \ln \left( \frac{sens_j^{\Delta_{jk}} (1 - sens_j)^{1 - \Delta_{jk}}}{spe_j^{1 - \Delta_{jk}} (1 - spe_j)^{\Delta_{jk}}} \right)
\]
4.2.4. Frequency of tuberculosis at death

From the individual probability of TB at death ($\tau_k$) the frequency of TB at death on sample level can be estimated. This is done by the function $f(p)$ in equation (2). This combines the information about the frequency of TB at death ($p$) contained in mean and variance for the $\tau$-values. Observed mean ($\bar{\tau}_0$) is expressed as the weighted mean of simulated $\tau$-values in a sample where no one ($\bar{\tau}_+$) and a sample where every one ($\bar{\tau}_-$) suffered from TB. This comprises the numerator of the first part of equation (2). Likewise observed variance ($s_0^2$) is expressed as the weighted variance of simulated $\tau$-values in a sample where no one ($s_+^2$) and a sample where every one ($s_-^2$) suffered from TB. Here an additional part expresses the difference between the means of the two sets of simulated $\tau$-values. This comprises the numerator of the second part of equation (2). In this the simulated negative values and simulated positive values are used to estimate the maximum likelihood of the frequency of TB ($\hat{p}$). The value of $p$ that minimizes $f(p)$ is termed $\hat{p}$. This is a $\chi^2$ distribution with one degree of freedom that evaluates the hypothesis that the mean and the variance for $\tau$-values indicate the same $\hat{p}$. The hypothesis must be rejected when $f(\hat{p}) > 3.84$ ($p < 0.05$). This means that $\hat{p}$ is not a valid estimator of the TB frequency. The interval of $p$-values that fulfills the inequality $f(p) - f(\hat{p}) < 3.84$ belongs to the 95% confidence interval for $\tau$. From this the goodness of fit of the models can be tested.

$$f(p) = \left(\frac{\tau_0 - (p \cdot \bar{\tau}_+ + (1-p) \cdot \bar{\tau}_-)}{se(\bar{\tau}_0)}\right)^2 + \left(\frac{s_0^2 - (p \cdot s_+^2 + (1-p) \cdot s_-^2 + p \cdot (1-p) \cdot (\bar{\tau}_+ - \bar{\tau}_-)^2)}{se(s_0^2)}\right)^2$$ (2)

In study II it was shown that the lesion pattern in Terry Collection is different from the pattern seen in skeletons from medieval and post-medieval Ribe. The differences are most obvious concerning rib lesions. It was therefore assumed that the epidemiological measures based upon the identified skeletons were not applicable when studying the archaeological skeletons from Ribe. Therefore $\tau$-statistics were estimated from two models – one including the sensitivity and specificity measures for rib lesions and one not including such measures for rib lesions. Estimates were done for Viking age, medieval and post-medieval periods in Ribe and for social stratified sub-samples in medieval period.

4.2.5. Mortality patterns related to probability of tuberculosis

To be able to study the probability of TB at death as a categorical variable in connection to survival analysis it was necessary to compute the estimated probability measures of TB ($\tau$) into
a TB status variable. Before this was done the $\tau$-values were however transformed from individual to population measure by adding the log of the odds of TB in the population to the raw data (equation (3)). With this the sample frequency of TB is taken into account. The individual disease status is assessed based upon knowledge about the sample from which the skeleton came (Boldsen, 2005a). The values of $\tau_k(pop)$ are categorized within the TB status variable. TB status is grouped into TB-: TB unlikely ($\tau(pop) < -1$); TB?: TB possible ($-1 \leq \tau(pop) < 1$); and TB+: TB likely ($\tau(pop) \geq 1$).

$$\tau_k(pop) = \tau_k + \ln\left(\frac{p}{1-p}\right)$$ (3)

Mortality patterns in relations to TB status were studied in skeletons in the three time periods, in males and females and in the social stratified sub-samples in Ribe. Mortality patterns were analyzed by Kaplan-Meier survival analysis using $\tau_k(pop)$ estimated from the model including ribs lesions and the model without rib lesions. Kaplan-Meier survival analysis gives an exact description of the survival sequence in the sample as survival rates for each individual is estimated (Kirkwood and Stern, 2003: 276). The survival rates are visualized in Kaplan-Meier curves. The differences in survival are tested by log-rank tests.
5. Results

5.1. Skeletal lesions related to tuberculosis

Thirteen lesions assumed to be TB-related were found with different frequencies in identified skeletons from Terry Collection that died of TB and Bass Collection that were not infected with TB (table 4 and figure 9A and 9B). All lesions recorded were found in individuals that died of TB but with varying frequency. A great difference was found in the distribution of the two types of rib lesions. Soft nodules and vascular grooves (RIB1) were found equally frequent in the cases (17.7%) and controls (17.7%). Periosteal changes and erosive osteolytic lesions (RIB2) were found significantly more in cases (59.7%) compared to controls (3.2%). The two lesion types on vertebrae were also found with different frequencies. Lesions on cranial and caudal surfaces of vertebral bodies (VER) were found in 11.3% of cases and in 4.8% of controls. Lesions on ventral part of vertebral bodies (VEN) were contrary found in 58.1% of cases and in 3.2% of controls.

Six of the lesions were found significantly more in cases compared to controls.

| Table 4. The frequency distribution of the lesion in cases from Terry Collection and controls from Bass Collection. |
|---|---|---|---|
| Lesion | Cases (N=62) | Controls (N=62) | Fisher’s exact |
| RIB11 | Unaffected | 51 (82.3) | 51 (82.3) | 1.000 |
| | Affected | 11 (17.7) | 11 (17.7) | 0.000 |
| RIB22 | Unaffected | 25 (40.3) | 60 (96.8) | 0.323 |
| | Affected | 37 (59.7) | 2 (3.2) | 0.001 |
| VER3 | Unaffected | 55 (88.7) | 59 (95.2) | 0.001 |
| | Affected | 7 (11.3) | 3 (4.8) | 0.001 |
| VEN4 | Unaffected | 26 (41.9) | 60 (96.8) | 0.001 |
| | Affected | 36 (58.1) | 2 (3.2) | 0.001 |
| BOD5 | Unaffected | 44 (71.0) | 61 (100.0) | 0.001 |
| | Affected | 18 (29.0) | 0 (0.0) | 0.001 |
| ACE6 | Unaffected | 45 (72.6) | 58 (95.1) | 0.114 |
| | Affected | 17 (27.4) | 3 (4.9) | 0.114 |
| PF7 | Unaffected | 56 (90.3) | 60 (98.4) | 0.001 |
| | Affected | 6 (9.7) | 1 (1.6) | 0.001 |
| ILI8 | Unaffected | 39 (62.9) | 58 (95.1) | 0.001 |
| | Affected | 23 (37.1) | 3 (4.9) | 0.001 |
| DF9 | Unaffected | 59 (95.2) | 61 (100.0) | 0.244 |
| | Affected | 3 (4.8) | 0 (0.0) | 0.244 |
| PT10 | Unaffected | 60 (96.8) | 61 (100.0) | 0.496 |
| | Affected | 2 (3.2) | 0 (0.0) | 0.496 |
| DH11 | Unaffected | 58 (93.5) | 62 (100.0) | 0.119 |
| | Affected | 4 (6.5) | 0 (0.0) | 0.119 |
| PR12 | Unaffected | 59 (95.2) | 62 (100.0) | 0.244 |
| | Affected | 3 (4.8) | 0 (0.0) | 0.244 |
| PU13 | Unaffected | 56 (90.3) | 62 (100.0) | 0.028 |
| | Affected | 6 (9.7) | 0 (0.0) | 0.028 |

1RIB1: soft nodules and vascular grooves on visceral surface of ribs; 2RIB2: Periosteal reaction and osteolytic lesions on visceral surface of ribs; 3VER: caudal and cranial surface of thoracic and lumbar vertebral bodies; 4VEN: Ventral part of thoracic and lumbar vertebral bodies; 5BOD: Lateral body of ilium; 6ACE: Acetabulum; 7PF: Proximal femur; 8ILI: Iliac auricular surface; 9DF: Distal femur; 10PT: Proximal tibia; 11DH: Distal humerus; 12PR: Radial tuberosity of proximal radius; 13PU: Olecranon process of proximal ulna.
Sensitivity and specificity measures were calculated for each of the 13 lesions (table 5). Periosteal reaction and osteolytic lesions on visceral surface of ribs (RIB2) and lesions on ventral part of vertebral bodies (VEN) were found to be good detectors of TB. The presence of RIB2 lesion provided a 59.7% probability of a valid TB-diagnosis. The absence of a lesion here provided a 3.2% probability of TB. For VEN the presence provided a 58.1% probability of a valid diagnosis and the probability of TB when absence of lesion was 4.8%. The presence of lesions on proximal tibia (PT) gave little evidence of TB. Here the presence of lesion provided a just 3.2% probability of TB-diagnosis. The absence of lesion provided a 0% probability of TB-diagnosis. For all lesions the criteria of being related to TB when sensitivity is greater than one...
minus specificity were met. When specificity is 1 none of skeletons with negative TB status had the lesions. In lesions that are found significant more in cases than controls the specificity of 1 means that these lesions are only found in TB cases and that their presence on their own gives a likely TB detection. For the other significant lesions they are present also in controls. This means that they are found more in TB but they can be caused by other conditions.

<table>
<thead>
<tr>
<th>Table 5. Sensitivity and specificity of the TB-related lesions.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>RIB1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>RIB2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>VER</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>VEN</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>BOD</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>ACE</td>
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<tr>
<td></td>
</tr>
<tr>
<td>PF</td>
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<tr>
<td></td>
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<tr>
<td>ILI</td>
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<tr>
<td></td>
</tr>
<tr>
<td>DF</td>
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<td>PT</td>
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<td></td>
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<tr>
<td>DH</td>
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<td></td>
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<tr>
<td>PR</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PU</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

1RIB1: soft nodules and vascular grooves on visceral surface of ribs; 2RIB2: Periosteal reaction and osteolytic lesions on visceral surface of ribs; 3VER: caudal and cranial surface of thoracic and lumbar vertebral bodies; 4VEN: Ventral part of thoracic and lumbar vertebral bodies; 5BOD: Lateral body of ilium; 6ACE: Acetabulum; 7PF: Proximal femur; 8ILI: Iliac auricular surface; 9DF: Distal femur; 10PT: Proximal tibia; 11DH: Distal humerus; 12PR: Radial tuberosity of proximal radius; 13PU: Olecranon process of proximal ulna.

5.2. Tuberculosis-related lesion patterns

The results of the analysis of lesion patterns in Terry Collection and in Ribe in study II are given in figure 10. Significant differences were present for the frequency distributions of rib lesions in Terry Collection, Medieval and Post-medieval Ribe (p = <0.001). The frequency of rib lesions
was low in medieval Ribe (13.9%) compared to post-medieval Ribe (26.9%) and Terry Collection (65.3%). The differences were significant between both Terry and medieval and post-medieval Ribe but also among the two time periods in Ribe (table 6).

Table 6. Associations of frequency of lesions in the three time periods. Values in bold are significant at the 0.05 level.

<table>
<thead>
<tr>
<th></th>
<th>Medieval (N=108)</th>
<th>Post-medieval (N=52)</th>
<th>1920-1967 (N=95)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>RIB (^1)</td>
<td>0.045</td>
<td>&lt;0.001</td>
<td>0.740</td>
</tr>
<tr>
<td>VEN (^2)</td>
<td>0.267</td>
<td>0.740</td>
<td>0.962</td>
</tr>
<tr>
<td>BOD (^3)</td>
<td>0.056</td>
<td>0.962</td>
<td>0.915</td>
</tr>
<tr>
<td>ACE (^4)</td>
<td>0.144</td>
<td>0.915</td>
<td>0.906</td>
</tr>
<tr>
<td>ILI (^5)</td>
<td>0.845</td>
<td>0.106</td>
<td>0.992</td>
</tr>
<tr>
<td>PU (^6)</td>
<td>0.074</td>
<td>0.992</td>
<td>0.076</td>
</tr>
</tbody>
</table>

\(^1\)RIB: Periosteal reaction and osteolytic lesions on visceral surface of ribs; \(^2\)VEN: Ventral part of thoracic and lumbar vertebral bodies; \(^3\)BOD: Lateral body of ilium; \(^4\)ACE: Acetabulum; \(^5\)ILI: Iliac auricular surface; \(^6\)PU: Olecranon process of proximal ulna.

Figure 10. Frequency (%) of lesions in skeletons from Ribe dated to medieval and post-medieval periods and from Terry Collection dated to 1920-1967.
The presence of tuberculosis in Danish skeletons AD 800 - 1800

The distribution of skeletons with 1-2 lesions and 3 or more lesions in skeletons from Ribe dated to medieval and post-medieval periods and in Terry Collection is given in figure 11. Significant different distribution of the number of lesions was found between skeletons from Terry Collection and medieval Ribe ($p = 0.048$) and between Terry Collection and Post-medieval Ribe ($p = 0.006$). Many of the skeletons from Terry Collection had 3 or more lesions (36.8%) compared to Medieval (24.1%) and Post-medieval Ribe (15.4%). The difference between the two time periods in Ribe was not statistically significant ($p = 0.208$).

![Figure 11. Frequency (%) of skeletons with 1-2 positive lesions and 3 or more positive lesions in skeletons from Ribe dated to medieval and post-medieval periods and in Terry Collection.](image)

The distribution of lesions in 145 males and 110 females in Ribe and in Terry Collection is given in figure 13A and 13B. The lesion frequency of males and females on iliac auricular surface (ILI) was significantly different in Ribe ($p = 0.002$). Females (57.6%) had more lesions than males (33.0%). There was no difference in lesion frequencies between males and females in Terry Collection. There seems to be greater variability in lesion frequencies in males and females in Ribe compared to males and females in Terry Collection (figure 12A and 12B).

Figure 13 gives the distribution of males and females with 1-2 lesions and 3 or more lesions in medieval and post-medieval Ribe and Terry Collection. None of the distributions were significant. It is however seen that in both time periods in Ribe more females (28.5 and 25.0% respectively) compared to males (21.2 and 7.1% respectively) have 3 or more lesions. In Terry Collection more males (41.2%) compared to females (31.8%) have 3 or more lesions.
Figure 12. Lesion frequency distribution in males and females in skeletons from Ribe (A) and Terry Collection (B).

RIB: Periosteal reaction and osteolytic lesions on visceral surface of ribs; VEN: Ventral part of thoracic and lumbar vertebral bodies; BOD: Lateral body of ilium; ACE: Acetabulum; ILI: Iliac auricular surface; PU: Olecranon process of proximal ulna.

Figure 13. Distribution of males and females with 1-2 lesions and 3 or more lesions in Ribe and Terry Collection.
5.3. Tuberculosis in Ribe AD 800 – 1800

Individual probability of TB (τ) and frequency of TB at death in the three time periods from Ribe were estimated from a model including rib lesions and one not including rib lesions. The analysis included 752 adult skeletons and the estimates are given in table 7 and 8 and illustrated in figure 14A and 14B. The frequency of TB at death (p) ranged between 0.26 (26%) in medieval period and 0.53 (53%) in Viking age when including ribs. Only 29 skeletons were available from Viking Age and the confidence interval had a great span between 31% and 83%. The maximum likelihood estimate of the frequency of TB (p) was very similar for Medieval (26%) and Post-medieval periods (30%). Excluding rib lesions gave rise to higher frequency of TB at death (p) in all time periods (table 7 and figure 15B). In Viking Age the maximum likelihood estimate of p was larger than 0.99 (99%), in medieval period the prevalence was 0.49 (49%) and in post-medieval period it was 0.51 (51%).

<table>
<thead>
<tr>
<th>Time period</th>
<th>N</th>
<th>τ</th>
<th>TB prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>Viking Age</td>
<td>29</td>
<td>Mean</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>4.85</td>
</tr>
<tr>
<td>Medieval</td>
<td>507</td>
<td>Mean</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>7.84</td>
</tr>
<tr>
<td>Post-medieval</td>
<td>216</td>
<td>Mean</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>8.57</td>
</tr>
</tbody>
</table>

Table 7. τ-distribution properties and estimated TB frequencies for the three time periods modeled including rib lesions.

<table>
<thead>
<tr>
<th>Time period</th>
<th>N</th>
<th>τ</th>
<th>TB prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>Viking Age</td>
<td>26</td>
<td>Mean</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>4.11</td>
</tr>
<tr>
<td>Medieval</td>
<td>479</td>
<td>Mean</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>7.58</td>
</tr>
<tr>
<td>Post-medieval</td>
<td>201</td>
<td>Mean</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>6.75</td>
</tr>
</tbody>
</table>
The presence of tuberculosis in Danish skeletons AD 800 - 1800

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Figure 14. Sample frequencies of TB in the three time periods. A: Including rib lesions. B: Not including rib lesions. Grey - Viking age, dark grey - medieval period and black - post-medieval period.

The results of the goodness of fit of the estimated model to the $\chi^2$ distributions of the $\tau$-values for the three time periods including and excluding rib lesions are given in table 9. In Viking Age mean and variance indicate equal distribution of population prevalence ($\rho$) when ribs are included. Here the $p$-value for the $\chi^2$ distribution is statistical insignificant. In the model not including rib lesions the mean and variance indicate equal distribution of population prevalence ($\rho$) for both medieval and post-medieval periods. The model including ribs fit the Viking Age data whereas the model not including ribs fit medieval and post-medieval data. There seems to be a difference in the expression of TB in the three time periods as the weighted contributions of lesions influence the estimates in Viking Age differently from medieval and post-medieval periods. Few Viking age skeletons are available which gives very wide confidence intervals for TB prevalence. Hence, the estimates contribute with limited information about the presence of TB at death in Viking age. All that can be concluded is that some individuals had TB and some did not have TB.

Table 9. Goodness of fit test of the estimation model to the distribution of $\tau$-values for the three time periods.

<table>
<thead>
<tr>
<th>Time period</th>
<th>Including ribs</th>
<th>Not including ribs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$f(\rho)$</td>
<td>df</td>
</tr>
<tr>
<td>Viking Age</td>
<td>0.19</td>
<td>1</td>
</tr>
<tr>
<td>Medieval</td>
<td>8.54</td>
<td>1</td>
</tr>
<tr>
<td>Post-medieval</td>
<td>8.32</td>
<td>1</td>
</tr>
</tbody>
</table>
Individual probability of TB ($\tau$) and frequency of TB at death were estimated from the model including rib lesions and the model not including rib lesions in the two social stratified sub-samples in medieval Ribe. The analysis included 499 adult skeletons and the estimates are given in tables 10 and 11 and are illustrated in figure 15A and 15B. In the model including rib lesions the frequency of TB at death ranged between 0.11 (11%) for the high status sub-sample and 0.33 (33%) for the low/common status sub-sample. The confidence intervals for the frequency distributions were narrow for both groups. In the model not including rib lesions higher frequencies of TB at death ($p$) were obtained in both low/common and high status graves. The lower social status group had a maximum likelihood estimate of 0.63 (63%) and the high status group had an estimate of 0.19 (19%).

Table 10. $\tau$-distribution properties and estimated TB frequencies for the skeletons with common social status and with high social status from medieval period including rib lesions.

<table>
<thead>
<tr>
<th>Social status</th>
<th>N</th>
<th>$\tau$</th>
<th>TB prevalence</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Simulated</td>
<td>Simulated</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>negative</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>358</td>
<td>Mean</td>
<td>0.35</td>
<td>-1.58</td>
<td>3.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>8.90</td>
<td>1.64</td>
<td>10.20</td>
</tr>
<tr>
<td>High</td>
<td>141</td>
<td>Mean</td>
<td>-0.89</td>
<td>-1.83</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>4.51</td>
<td>1.23</td>
<td>12.66</td>
</tr>
</tbody>
</table>

Table 11. $\tau$-distribution properties and estimated TB frequencies for the skeletons with common social status and with high social status from medieval period not including rib lesions.

<table>
<thead>
<tr>
<th>Social status</th>
<th>N</th>
<th>$\tau$</th>
<th>TB prevalence</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Simulated</td>
<td>Simulated</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>negative</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>Common</td>
<td>341</td>
<td>Mean</td>
<td>0.90</td>
<td>-1.07</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>8.66</td>
<td>1.14</td>
<td>6.52</td>
</tr>
<tr>
<td>High</td>
<td>131</td>
<td>Mean</td>
<td>-0.35</td>
<td>-1.23</td>
<td>2.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>4.08</td>
<td>0.74</td>
<td>8.47</td>
</tr>
</tbody>
</table>
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The results of the goodness of fit test of the model for estimation of population prevalence in the different social status burials are given in table 12. When including rib lesions the mean and variance indicate significant different distributions of $f(\hat{p})$ for both social stratified sub-samples. When not including rib lesions mean and variance indicate different values of $p$ for low/common social status sample. For the high social status sample the mean and variance indicate the same estimate of $p$. None of the models fit the sample of low/common social status very well. The model not including ribs fit the sample of high social status. There seems to be a difference in the expression of TB in the two sub-samples as the weighted contributions of lesions influence the estimates differently in the two samples.

### Table 12. Goodness of fit test of the estimation model to the distribution of $\tau$-values for the social status groups.

<table>
<thead>
<tr>
<th>Social status</th>
<th>Including ribs</th>
<th>Excluding ribs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$f(\hat{p})$</td>
<td>df</td>
</tr>
<tr>
<td>Common</td>
<td>7.33</td>
<td>1</td>
</tr>
<tr>
<td>High</td>
<td>6.02</td>
<td>1</td>
</tr>
</tbody>
</table>

#### 5.4. Tuberculosis and mortality

Mortality patterns in skeletons with different probability measures of TB ($\tau$) were explored in the three time periods, in males and females and in the social stratified sub-samples in Ribe. The analyses were performed using $\tau$-estimated from the model including ribs lesions and the model without rib lesions. No differences were found between the results of the two models.
and the model not including rib lesions is therefore presented as this model showed the best fit for the estimates of the frequency of TB at death in study III.

Table 13. Distribution of time periods, sex and social status in skeletons that unlikely had TB (TB-: $\tau$(pop) < -1), probably had TB (TB?: -1 ≤ $\tau$(pop) < 1) and likely had TB (TB+: $\tau$(pop) ≥ 1). Model not including rib lesions.

<table>
<thead>
<tr>
<th></th>
<th>TB- (N=234)</th>
<th>TB? (N=233)</th>
<th>TB+ (N=228)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time period</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viking Age</td>
<td>1 (4.2)</td>
<td>14 (58.3)</td>
<td>9 (37.5)</td>
<td>0.024</td>
</tr>
<tr>
<td>Medieval</td>
<td>164 (34.7)</td>
<td>153 (32.4)</td>
<td>155 (32.8)</td>
<td></td>
</tr>
<tr>
<td>Post-medieval</td>
<td>69 (34.7)</td>
<td>66 (33.2)</td>
<td>64 (32.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>145 (37.2)</td>
<td>123 (31.5)</td>
<td>122 (31.3)</td>
<td>0.085</td>
</tr>
<tr>
<td>Female</td>
<td>89 (29.2)</td>
<td>110 (36.1)</td>
<td>106 (34.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Social status</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low/common</td>
<td>101 (30.1)</td>
<td>106 (31.5)</td>
<td>129 (38.4)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>62 (48.1)</td>
<td>43 (33.3)</td>
<td>24 (18.6)</td>
<td></td>
</tr>
</tbody>
</table>

Table 13 gives the overall distribution of the three time periods, sex and social status in skeletons that unlikely (TB-), probably (TB?) and very likely (TB+) had TB for estimates derived from the model without including rib lesions. Significant differences are seen in the probability of TB in the three time periods. There is a difference between the distributions in Viking skeletons compared to the two other periods. Few Vikings age skeletons fall in the TB unlikely group (4.2%) compared to medieval (34.7%) and post-medieval (34.7%) periods. The distributions of TB probability in medieval and post-medieval periods are almost identical. The frequency distribution of the probability of TB in males and females is the same. There is a significant difference in the probability of TB in the social status sub-samples where many in the low/common sub-sample (38.4%) likely have TB compared to high status individuals (18.6%). The distribution of males and females in the categories of TB probability within Viking, medieval and post-medieval periods were analyzed and no differences were found.
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Figure 16 gives the Kaplan-Meier survival plots for the probability of TB in the three time periods using estimates from the model not including rib lesions. A difference in survival is present in medieval period where those that very likely had TB die at higher ages than in the two other TB probability categories. The differences in medieval period are statistically significant as seen from the results of the log rank test given in table 14.

Table 14. Log rank test of equality of survival distributions for the different levels of TB in the three time periods. Model not including rib lesions.

<table>
<thead>
<tr>
<th>Time period</th>
<th>Log Rank $\chi^2$</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viking Age</td>
<td>0.213</td>
<td>2</td>
<td>0.899</td>
</tr>
<tr>
<td>Medieval</td>
<td>25.705</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post-medieval</td>
<td>0.226</td>
<td>2</td>
<td>0.893</td>
</tr>
</tbody>
</table>

Figure 16 gives the Kaplan-Meier survival plots for the probability of TB in the three time periods using estimates from the model not including rib lesions. A difference in survival is present in medieval period where those that very likely had TB die at higher ages than in the two other TB probability categories. The differences in medieval period are statistically significant as seen from the results of the log rank test given in table 14.
Figure 17 gives the Kaplan-Meier survival plots for the probability of TB in males and females in the model not including rib lesions. A statistical difference in survival of females are found (table 15), where those that likely had TB die at higher ages than females that unlikely and probably had TB.

![Image](image_url)


<table>
<thead>
<tr>
<th>Sex</th>
<th>Log Rank Chi-Square</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2.094</td>
<td>2</td>
<td>0.351</td>
</tr>
<tr>
<td>Female</td>
<td>11.278</td>
<td>2</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 15. Log rank test of equality of survival distributions for the different levels of TB in males and females. Model not including rib lesions.

Figure 18 gives the Kaplan-Meier survival plots for the probability of TB in the social stratified sub-samples in the model not including rib lesions. A statistical difference was found in survival of low/common status individuals with different probability of TB (table 16). Low/common status individuals that very likely had TB die at higher ages than individuals that unlikely and probably had TB.
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Figure 18. Kaplan Meier survival plots of probable TB status of low/common and high social status. Blue: TB-: TB unlikely (τ(pop) < -1). Green: TB?: TB possible (-1 ≤ τ(pop) < 1). Sand: TB+: TB likely (τ(pop) ≥ 1). Model not including rib lesions.

Table 16. Log rank test of equality of survival distributions for the different levels of TB in low/common and high social status sub-samples. Model not including rib lesions.

<table>
<thead>
<tr>
<th>Social status</th>
<th>Log Rank Chi-Square</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low/common</td>
<td>31.306</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High</td>
<td>3.023</td>
<td>2</td>
<td>0.221</td>
</tr>
</tbody>
</table>
6. Discussion

6.1. Skeletal lesions related to tuberculosis

All 13 osteological lesion types, recorded in skeletons from Terry Collection that died of TB and the uninfected controls from Bass Collection in study I, were present in the cases that died of TB. Six of the lesions were significantly more frequent in cases than controls.

Lesions on ribs were recorded as two types of lesions in study I. The two types were found with very different distributions in cases and controls. Soft nodules and vascular grooves (RIB1) were found equally frequent in cases and controls and are not well-related to TB-diagnosis. In contrast, periosteal reaction and osteolytic lesions on ribs (RIB2) were significantly more frequent in Terry Collection and is considered a good TB indicator. Ten of eleven skeletons with positive scores of RIB1 in Bass Collection had soft nodules. These are very likely signs of non-specific changes related to chronic pulmonary conditions as suggested by Roberts et al. (1994), Eyler et al. (1996) and Matos and Santos (2006). The formation of nodules is linked to stress on ribs from muscle activity during forced expiration and are thus thought to be cough-related (Köft-Maier, 2000: 71). Aside from chronic pulmonary conditions such as bronchitis, long term exposure to airborne dust or smoke particles will cause chronic cough. In Bass Collection some of the skeletons are listed as smokers in the medical records. This could explain the presence of nodules on ribs in the control group. Unfortunately, information about smoking status is not available in connection to all individuals examined in Bass Collection. The association of smoking and assumed cough-related nodules cannot be studied using the data recorded for the study.

The two types of lesions in the spine are likewise found with very different distributions in cases and controls. Lesions on caudal and cranial surface of thoracic and lumbar vertebral bodies (VER) are found with non-significant different frequencies in the two groups of skeletons. The lesions on the surface of vertebral bodies do not detect TB very well. These are in other studies presented as ‘classic’ signs of skeletal TB (Kelley and El-Najjar, 1980; Aufderheide and Rodriquez-Martin, 1998; Ortner, 2003). This assertion is often put forward in severe cases of skeletal TB as by e.g. Haas et al. (2000) and Hajdu et al. (2012). In such examples the erosive lesions are related to the destruction of the trabecular tissue within vertebrae that may cause the vertebral body to collapse and an angular kyphosis to form (Ortner, 2003). The lack of association of this lesion type and TB diagnosis in the present study may have a
connection to the cutoff point for the criteria of scoring a positive lesion (table 5 and fig. 5 of study I). Better results of associations are likely obtained if the scoring criteria for intervertebral surface of vertebral body (VER) are raised to a more severe expression of a disease process. In such the erosive lesions are with great certainty related to the destruction of the trabecular tissue within vertebrae that happen in disease progression stages of Pott’s disease before formation of kyphosis (Ortner, 2003). Lesions on ventral part of vertebral bodies (VEN) are in contrast to lesions on the surfaces of vertebral bodies found significantly more in cases compared to controls and are considered good TB indicators. The greater degree of involvement of ventral parts compared to intervertebral surfaces on vertebral bodies can, besides the mentioned definition of cutoff points for recording, be related to how TB is disseminated in the spine as explained in section 2.2.1. of this thesis. The ventral part of vertebral bodies (VEN) is closely connected to soft tissues of the lower back and abdomen. This leaves an accessible route of dissemination of bacteria via the nutritional supply of the spine and soft tissue lesions in lungs and psoas muscle in contrast to dissemination via the pulposus of intervertebral discs that cause changes to intervertebral surfaces.

Tuberculosis of joints was recorded in hip, sacroiliac, knee and elbow joints. Lesions in hip and sacroiliac joint were as expected from other studies found with high frequencies (Aufderheide and Rodriguez-Martin, 1998; Ortner, 2003). All lesions recorded on the pelvis - lateral body of ilium, acetabulum and iliac auricular surface - were found significantly more in the Terry Collection cases than in the controls. These are considered to be good TB indicators. Knee joint lesions are elsewhere reported as frequently involved in relation to TB (Aufderheide and Rodriguez-Martin, 1998: 138). The low frequency of knee joint lesions found in this thesis may be explained as variability of the skeletal expression of TB. Skeletal involvement varies across time and between populations depending on e.g. host resistance, virulence of pathogen, route of transmission and age of the infected (Buikstra, 1976; Aufderheide and Rodriguez-Martin, 1998: 133; Maliarik and Iannuzzi, 2003). The identified skeletons from Terry Collection died of pulmonary TB. The ratio of pulmonary TB versus extra-pulmonary TB shifts towards more extra-pulmonary involvement in populations with high rates of infection with M. bovis through the gastrointestinal tract (Cormigan and Flynn, 1992). In such populations greater degree of involvement of joints may be found. The results of the present study show that the epidemiological value of lesions in knee and elbow in relation to pulmonary TB is limited.
Lesions, that are TB-related, are found more often in individuals affected by TB than in individuals not affected by TB. The sensitivity and specificity measures of study I evaluate such in relations to the 13 lesions recorded. Lesions with low sensitivities provide limited epidemiological information about the probability of having TB. Some lesions have high sensitivities, but none are 100% diagnostic of TB on their own. Lesions on the lateral body of ilium and the olecranon process of the ulna are found only in people who died from TB. Based on data from this study, these lesions can be characterized as pathognomonic lesions with specificities of 1 and sensitivities above 0.

Recording of the presence of one of the six good TB indicators on its own does not prove the presence of TB. Diagnosing disease is a statistical process based upon probabilities. Hence, the epidemiological parameters derived in study I can be used to estimate the probability of diagnosis.

6.2. Tuberculosis-related lesion patterns

The lesion patterns of the six good TB indicators from study I were examined in modern skeletons infected with pulmonary TB and in archaeological skeletons with unknown infections. Differences were found between the two groups of skeletons, as well as between the medieval and post-medieval samples.

The greatest differences were found for rib lesions. These were present with significant different distributions between the modern and archaeological skeletons and between the two time periods in Ribe. The frequency increased across time. In Ribe greater degree of rib involvement is evident from the post-medieval period where significant more skeletons have rib lesions compared to the previous medieval period. The increased rib involvement across time very likely reflects changes towards more pulmonary involvement related to a change in the causative pathogen. Pulmonary TB is to a great extent caused by *M. tuberculosis* infection (Domingo et al. 2014). *M. bovis* is transmitted from cattle to humans both via lungs and the gastrointestinal tract. If transmitted via airborne particles inhaled in lungs *M. bovis* will cause the same disease process in its host as *M. tuberculosis* (Francis, 1950). If transmitted via the gastrointestinal tract *M. bovis* will if causing active disease to a larger degree than *M. tuberculosis* result in extra-pulmonary involvement (Cormican and Flynn, 1992).
The ratio of *M. tuberculosis* and *M. bovis* infection in humans and thus also assumingly the ratio of pulmonary versus extra-pulmonary TB vary between populations and across time (Cormigan and Flynn, 1992; Cosivi et al. 1998). National surveys of TB in Denmark from 1932 and onwards show that there was a distinct difference in the presence of bovine infection in humans living in rural and urban areas (Madsen et al., 1942). During 1931-1935, 4.4% of the 15-30 year olds residing in Jutland’s towns with positive TB in sputum tested positive for bovine TB. During the same period, the corresponding figure for that age group in rural Jutland was 17% (Madsen et al., 1942: 59). Ribe is situated on the southwestern coast of Jutland. In 1935 this region had the largest recorded occurrence of TB in cattle in Denmark and also the largest recorded frequency of *M. bovis* infection in humans. Cattle have played an important role in economy and food production in and around Ribe since the medieval period (Enemark, 2003). The areas around Ribe were ideal for cattle grazing. Markets for cattle trade were as mentioned previously in this thesis established in Ribe from the 15th century (Willerslev, 1952; Enemark, 1999). The risk of contracting *M. bovis* from close contact between cattle and the population was thus inevitable in late medieval and early post-medieval Ribe. The low degree of rib involvement in skeletons from medieval Ribe and to some extent post-medieval Ribe is assumed to reflect a great proportion of extra-pulmonary TB.

From post-medieval period a change in the skeletal expression of TB seem to happen. There is as mentioned an increase of rib lesions in post-medieval period compared to the previous medieval period, which indicates more pulmonary TB infection. At the same time, more of post-medieval skeletons have 1-2 lesions and less have 3 or more lesions compared to both medieval period and modern skeletons. This indicates that individuals in this time period to a larger degree die of TB before lesions develop (Wood et al., 1992). With declining importance of cattle trade in Ribe the risk of contracting bovine TB decreased. With this, the risk of contracting the more virulent pulmonary transmitted *M. tuberculosis* increased (Francis, 1950). The population in post-medieval Ribe probably had little resistance towards this bacterial exposure. Many individuals therefore died of infection before chronic stage of disease. In contrast, the greater proportion of skeletons in Terry Collection with 3 or more lesions indicates chronicity of disease as individuals survive a long time with active disease allowing skeletal lesions to develop (Wood et al., 1992). Individuals in Terry Collection were from a poor social background compared to what was found across much of the United States in the first half of the 20th century. Compared
to conditions in Ribe, however, conditions were likely sufficient for people to have stronger immune responses, helping them survive longer with a TB infection.

When comparing the lesion patterns of males and females in Ribe and in Terry Collection there seems to be a greater variability in the lesion patterns of males and females in Ribe compared to Terry Collection. In Ribe males have a tendency for more rib and ventral parts of vertebral body involvement compared to females that have more involvement in hip and sacroiliac joint compared to males. Male and female lesion frequencies are very similar in Terry Collection. The lesion patterns found in Ribe may be related to the presence of cattle. Girls, more than boys, were in contact with potentially infected milk through their daily chores of milking cows and preparing food. Girls therefore to a larger degree than boys got naturally vaccinated against the more virulent pulmonary infecting \( M. \text{tuberculosis} \) (Francis, 1950). Non-vaccinated adult females to a larger degree than males handled infected milk through food production. Females therefore more likely contracted TB through the gastrointestinal tract and had greatest risk of developing the less virulent extra-pulmonary infection. Non-vaccinated boys and males did more than females contract pulmonary TB either through \( M. \text{bovis} \) infection from airborne particle due to chores of handling the cattle in stables or \( M. \text{tuberculosis} \) due to lack of protection from natural vaccination. This explains the apparent greater involvement of the ribs and spine in males. This also explains the tendency of differences in the number of lesions in males and females in Ribe. Here, males die with few lesions and females seem to survive for more lesions to develop. Natural vaccination and infected with a less virulent pathogen have a positive effect on female risk of dying with TB.

Changes in the ratio of \( M. \text{tuberculosis} \) and \( M. \text{bovis} \) infection, in host-pathogen interactions, and in living conditions over time probably resulted in the differences in lesion patterns identified between Ribe and Terry Collection skeletons. It is possible to gain important insights into the impact of TB in the past through studies of the presence of skeletal lesions.

6.3. Tuberculosis in Ribe AD 800-1800

The estimated frequency of TB at death in medieval and post-medieval Ribe is very similar. For both time periods the frequency of TB at death is c. 50\% based upon the model not including ribs. This does however not mean that half of the living population in Ribe had active bone involving TB. The epidemiological properties of TB-related lesions give an estimate of the frequency of TB among the individuals of a sample of dead. Half of those that died in Ribe had
skeletal TB at death. The individuals that died were a strongly selected group of the frailest individuals in the population at a given age. These were not in terms of health equivalent to the living in the same age groups (Wood et al. 1992). The high frequency of TB found may be due to the route of transmission of TB. The study of lesion patterns indicates a great prevalence of extra-pulmonary TB contrary pulmonary TB in Ribe. Extra-pulmonary infection is not as virulent as infection in the lungs (Francis, 1950). With extra-pulmonary infection it is possible to survive longer and skeletal lesions have time to develop. This is also confirmed by the poor fit of the model including ribs in the present study.

The influence of social factors on the risk of TB and risk of dying of TB has been stressed by several scholars in studies from different geographic regions and time periods (Schoeman et al., 1991; Ferlinz, 1999; Souza et al., 2000; Grange et al., 2001; Gupta et al., 2003; Ladefoged et al., 2011 and Olson et al., 2012). In a study of mortality rates in different districts of Paris in the time period 1865 – 1934 rates were found to be particularly high for the poor and very poor districts throughout the period studied (Bello et al., 1999). Such social differences in the frequency of TB at death are also found in Ribe in the medieval period. Here the low/common status individuals have three times greater frequency of TB at death compared to high status individuals. The standards of living were presumably very different in the social classes represented in the population of Ribe (Jakobsen and Madsen, 2001: 139). The divergent TB frequencies in the two social stratified sub-samples in Ribe are very likely a result of such social differences and consequent differences in living conditions. The difference in the fit of the models either including or not including rib lesions indicates that not only the frequency of disease but also the expression of disease is different in the two groups. The majority of people in town belonged to the low/common sub-sample which spanned great diversity in standards of living. This would cause variability of skeletal expression due to divergent immune reactions and risks of contracting TB in this social group. In contrast, a minor part of the people in town belonged to the upper class. The people of the high status sub-sample, being a minority, likely had more uniform standards of living and therefore also assumingly had the same risk of TB exposure.

The sensitivity and specificity measures used in the τ-statistics are based upon the frequency of lesions in identified skeletons from Terry and Bass Collections as analyzed in study I. These modern individuals died of pulmonary TB and show great degree of rib involvement compared to the archaeological excavated skeletons from Ribe as demonstrated in study II. A change in
skeletal expression and possibly the causative pathogen is assumed from medieval to modern period. Viking age estimates of frequency of TB at death fit the model including ribs, in contrast to the fit of models in data from medieval and post-medieval periods. Even though there are few Viking age skeletons in the study and the estimated frequency of TB at death have wide confidence intervals, the differences indicate a change towards less rib involvement from Viking age to medieval period (from AD 1050). Likely causes are complex processes of evolution of both pathogen genetic makeup and host immunogenetics (Bloom and Small, 1998; Malik and Geodfrey-Faussett, 2005; Weiss and McMichael, 2004; Narasimhan et al., 2014). In Viking age and the early part of medieval period there is no cattle trade in Ribe and the town is less densely populated than later parts of the medieval period (Kieffer-Olsen, 2008; Feveile et al., 2010). Such societal changes may explain the possible changes in the frequency of TB at death. The small sub-sample of Viking Age skeletons included makes it difficult to explore the differences further.

6.4. Tuberculosis and mortality

The medieval period was the only time period where significant different mortality patterns were present in individuals with different TB status. Individuals in medieval Ribe with likely TB died at higher ages than those with unlikely and probable TB. This shows what is explained in the osteological paradox that lesions will occur when individuals survive for lesions to develop (Wood et al., 1992). Whether those that died without lesions died of TB before lesions developed or they died of some other cause is difficult to deduce from the skeletal data. The survival with lesions in medieval period can be linked to the presence of bovine TB as discussed in the section about lesion patterns. Infection with M. bovis seems to be particular frequent in medieval period. It causes a greater proportion of extra-pulmonary TB, that is less virulent than pulmonary TB. Therefore individuals in medieval period, to a larger degree than in the previous Viking period and later post-medieval period, survive for lesions to develop which results in the difference in survival among individuals with different probability of TB.

Females with likely TB survive longer than females without. The difference in the risk of dying with and without lesions for females can, as for medieval period be explained in the light of the great proportion of bovine infection in females, as discussed in relations to lesion patterns. Males to a larger degree compared to females die with few lesions which explain the lack of excess survival for males with lesions. There is a difference in exposure to bovine TB and
thus in this way females are more than males natural vaccinated and contract the less virulent bovine infection through gastrointestinal tract. Males are in general more susceptible than females to infectious diseases, also including TB, and have greater risk of dying of the infections when experiencing the same exposure (Owens, 2002; Rettew et al., 2010: 125). Physiological factors are believed to be important causes of the sex differences (Nhamoyebonde and Leslie, 2015). Animal models have shown how castration of guinea pigs and mice enhanced TB survival and reduced severity of disease (Yamamoto et al., 1991). Human parallels have been observed in a study of patients in an institution for the mentally ill (Hamilton and Mestler, 1969). Of the castrated males 8.1% died from TB, compared to 20.6% of intact males. It is thus believed that sex hormones influence the immune response to TB bacilli and physiological differences in males and females influence their different susceptibility to infections (Nhamoyebonde and Leslie, 2015).

In the socially stratified sub-samples only the low/common group shows significant differences in mortality in individuals with different TB probability. Individuals with lesions die at higher ages than individuals without lesions. This indicates as for the medieval period and females that those that had lesions survived longer than those without because those without lesions died before lesions developed. The low/common status group has a different mortality profile than the high status group in relation to the probability of TB. So some differences are present between the socioeconomic sub-samples as also demonstrated in the estimated frequencies of TB. Lesion patterns were not studied in the social stratified sub-samples because the skeletons needed to be complete, which left few skeletons with information about social status for analysis. In contrast to the study of male and female differences, there are no additional studies of lesion patterns available, that can be used to explain the mortality patterns found in the socially stratified groups.
7. Conclusion

7.1. Main findings

7.1.1. Paleoepidemiological properties of tuberculosis

- Six lesions were identified as good TB indicators; periosteal reaction and osteolytic lesions on ribs, ventral part of vertebral bodies, lateral body of ilium, acetabulum, iliac auricular surface and ulnar olecranon process.
- Soft nodules on visceral surface of ribs are not related to TB and are assumed cough related. The presence of such are besides chronic pulmonary conditions likely related to long-term exposure to smoke or dust particles.
- The epidemiological value of lesions of the knee joint and mild stage of disease progression on intervertebral plate of vertebral bodies is limited in relation to pulmonary TB.
- Lesion patterns can be used to identify causative pathogens and explain changes in the expression of TB across time.
- Social conditions are confirmed as likely risk factors for TB already in medieval period.
- The difference in male and female response to TB infection is confirmed.

7.1.2. Tuberculosis in Ribe

- There may have been a change in skeletal expression from Viking age to medieval period. Viking age skeletons seem to have more rib lesions and greater frequency of TB at death than the later time periods.
- Approx. 50% of people that died in medieval and post-medieval Ribe had TB at death.
- Great social differences in the presence of TB in medieval Ribe confirm that there as assumed was great social inequality in the population.
- A great proportion of TB in medieval Ribe is assumed to have been caused by \textit{M. bovis}.
- There is a change in the skeletal expression in post-medieval period where rib lesion involvement increases and individuals die with few lesions compared to medieval period. This is explained as a change towards more pulmonary TB infection.
7.2. Overall contribution

With the results presented in this thesis it is possible to perform population-based studies of TB in the past.

The osteological method enables systematic and uniform recordings of TB lesions in skeletons. The detailed criteria and descriptions for recording of lesions are easy to apply to data collection in skeletal samples. The $\tau$-statistics developed estimates the probability of TB and from this the frequency of TB at death in samples. With such paleoepidemiological approaches it is possible to explore and gain insights into the impact, mechanisms and consequences behind the spread of TB in different temporal and geographical settings.

The potential of the methods presented are exemplified through the studies of TB in the skeletons from Ribe. Here interesting insights have been gained both into some fundamental conditions concerning TB in the past and specifically concerning the life and health in Ribe.

The work of the thesis introduces new fields of research that potentially will change our understanding of the presence of TB in past populations. Further the research may help clarify concepts of host-pathogen interactions and susceptibility to disease in different demographic and socioeconomic sub-samples that may contribute to direct treatment and societal efforts to control and prevent the disease in modern societies.

7.3. Perspectives

Uniform osteological methods as presented are applicable to skeletal samples and with such it will be possible to compare studies of different skeletal samples performed by the same and different observers. Intra- and inter observer studies will be beneficial to test the possibilities of reproducing data collected according to the registration manual. Descriptions of scoring criteria needs to be detailed enough for reproducibility of data and intra- and inter observer studies will contribute to refining the scoring criteria even more.

Lesions on caudal and cranial surfaces of vertebral bodies were surprisingly not associated with TB diagnosis. The recording of these lesions with different cutoff points in relation to disease progression and to analyze the association of such to TB diagnosis should be considered. The lesions are characteristic and frequently occurring and including these in the osteological method with more severe cutoff points than done in this thesis will be reasonable.

Pars petrosal bone and teeth have been sampled from skeletons from Ribe to extract mycobacterial DNA. The idea is to study the presence of $M.\ tuberculosi$s and $M.\ bovis$ in the
archaeological samples. This will be done to test the hypothesis that there is a shift from *M. bovis* to *M. tuberculosis* across time and to relate the pathogens to certain lesion patterns. Further microbiological analyses of the bone and teeth samples from Ribe will provide insights into the presence of human leukocyte antigens (HLA). This will make it possible to relate the profile of parts of the immunogenetic composition of individuals with and without TB to differences in sex, age and socioeconomic affiliation across time.

Data about the presence of leprosy-related osteological lesions as described by Boldsen and Freund (2006) were recorded in the Ribe skeletons but were not included in the present thesis. These data will be used in a study that investigates the degree of co-infection of TB and leprosy in Ribe. It will also be interesting to study the lesion patterns in relation to leprosy as done for TB. There might be differences in how the skeleton is affected across time and between different demographic groups that can contribute to the interpretation of the presence of leprosy before and during the great medieval epidemics.

Lesions, that as mentioned in section 2.2.1, are considered to be child-specific – endocranial lesions and lesions on neural arches, cervical vertebrae and diaphysis of long bones were recorded in the skeletons from Ribe as part of the thesis. The data was not used and sub-adults were excluded in all three studies. Studies of the lesion frequencies and lesion patterns in children are however needed to incorporate in the data collection and paleoepidemiological analysis of TB in the past. An investigation of TB-related lesions in sub-adults compared to adults is planned. Here, the presence of child-specific lesions and the six general TB-related lesions will be compared in adults and sub-adults to find out if children, when they have skeletal TB, have child-specific or the lesions found in adults.

The τ-statistics opens up for studies into the impact of TB on population level which enables a large scale picture to be drawn of the impact of TB. Hence, it will be very interesting to apply τ-statistics to the prehistoric skeletal record. Data from the Viking age skeletons apparently showed differing frequency of TB at death and divergent lesion patterns compared to medieval and post-medieval periods. Through studies of TB in prehistoric and early urbanized communities it will be possible to explore the role of infectious diseases in the urbanization processes.

Several of the studies mentioned are planned to be incorporated into the research project ‘TheCityDwellers’ that will explore the life in and around the Danish town Viborg in medieval period. In this project, financed by the Danish Research Council, TB-related lesions will be
recorded in more than 1,000 skeletons from both rural and urban sub-samples and comparative studies of lesion patterns and the frequency of TB at death in Viborg and Ribe will be performed. This will make it possible to look further into the host-pathogen interactions in socially stratified groups.

The results of the studies presented confirm the complexity of studying TB in the past using skeletal remains (Waldron, 1999; Dutour, 2008). The multifactorial causation of the disease and the changes in host-pathogen interactions cause great differences in the expression of disease both between and within populations. In further studies of the paleoepidemiological properties of TB it will be necessary to take into account the differences in probability measures of lesions between populations and across time.
8. References

8.1. Unpublished work


8.2. Published work


The presence of tuberculosis in Danish skeletons AD 800 - 1800

Dorthe Dangvard Pedersen


The presence of tuberculosis in Danish skeletons AD 800 - 1800

Dorthe Dangvard Pedersen


Maczel, 2004. *On the traces of tuberculosis. Diagnostic criteria of tuberculous affection of the human skeleton and their application in Hungarian and French anthropological series.* PhD thesis, University of La Méditerranée – Aix-Marseille II, Faculty of Medicine, Marseille, France and University of Szeged, Faculty of Sciences, Szeged, Hungary.


The presence of tuberculosis in Danish skeletons AD 800 - 1800

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8.3. Weblinks

9. Article manuscripts
9.1. Study I

Skeletal lesions and tuberculosis – a case-control study

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ABBREVIATED TITLE

TB-related skeletal lesions

KEY WORDS

bone lesions; skeletal diagnosis; identified skeletons; paleoepidemiology

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ABSTRACT

**Objectives**: Skeletal lesions in 13 parts of the skeleton are analyzed to establish their association with a diagnosis of tuberculosis. Lesion sensitivity and specificity are estimated to quantify their relation to this particular disease.

**Materials and Methods**: A case-control study was performed with 124 skeletons from documented skeletal collections: 62 skeletons from individuals that died from tuberculosis, and 62 age and sex-matched skeletons that most likely did not have the disease, which were included as controls. The association of 13 lesions distributed throughout the skeleton and tuberculosis was tested by comparing lesion distributions in the people who suffered from tuberculosis and controls.

**Results**: Six of 13 lesions were found significantly more often among cases compared to controls - periosteal reaction and osteolytic lesions of visceral surface of ribs (RIB2), ventral vertebral bodies (VEN), lateral iliac body (BOD), acetabulum (ACE), iliac auricular surface (ILI) and ulnar olecranon process (PU). Two types of lesions on ribs were found with different distributions. RIB1 (soft nodules and vascular grooves) was found in 17.5 % of both cases and controls. RIB2 (periosteal changes and osteolytic lesions) was found in 59.7 % of tuberculosis cases and 3.2 % of controls. Different patterns were also found for two types of vertebral lesions. Lesions on intervertebral surfaces of thoracic and lumbar vertebral bodies were found in 11.3 % of cases and 4.8 % of controls. Lesions on the ventral part of vertebral bodies were found in 58.1 % and 3.2 %, respectively.

**Discussion**: Recording of the six bone lesions that were associated with diagnosed tuberculosis is recommended when performing paleoepidemiological studies of tuberculosis. Seven bone lesions were not found to have a significant relation to a diagnosis of tuberculosis. They provide limited information about the epidemiology of tuberculosis in skeletal samples, and therefore should not be considered tuberculosis markers.
The mycobacterial disease tuberculosis (TB) is a chronic infectious disease known to have had devastating consequences on humans in the past. (Davies et al., 1999; Grange et al., 2001; Grange and Zumla, 2002; Daniel, 2006). The disease can affect bone, so it is possible to detect TB in archaeological skeletons. The detection of TB in skeletons enables paleopidemiological studies to be carried out, thereby providing insights into the impact of TB in the distant past where historical sources are absent, scarce, or otherwise uninformative.

Paleomicrobiological analyses have established an association between certain skeletal lesions and the bacteria responsible for TB in archaeological bone or tooth samples (Mays et al., 2001; Hershkovitz et al., 2008; Nicklisch et al., 2012). Thus, lesions on vertebral bodies and the visceral surface of ribs have been related to infection with *Mycobacterium tuberculosis* (Arriaza et al., 1995; Taylor et al., 2007; Pósa et al., 2012). Despite the impressive strides forward in ancient DNA (aDNA) research, the results only provide evidence of TB infection, not whether a person was affected by active disease. To understand what the absence or presence of specific bone lesions mean in terms of TB, it is necessary to study the patterning of those lesions in individuals with and without the disease.

The association of lesions with specific diseases can be tested by recording lesions in skeletons from individuals known to have experienced those diseases. Fortunately, such information is available in identified skeletons from several modern reference samples where disease status is either listed as the cause of death or mentioned in extensive medical history records. Previous studies of TB in reference collections have been conducted using data from the Hamann-Todd Skeletal Collection, Cleveland Museum of Natural History, USA (Kelley and Micozzi, 1984); Robert J. Terry Skeletal Collection (Roberts et al., 1994); Coimbra Identified Skeletal Collection, Portugal (Santos and Roberts, 2001; Santos and Roberts, 2006); Human Identified Skeletal Collection, Museum Bocage, Lisbon, Portugal (Matos and Santos, 2006); and Italian Identified Collection from Certosa Cemetery, Bologna, Italy (Mariotti et al., 2015). In these studies the presence of lesions was compared in skeletons of individuals that died of TB and those listed as having experienced other causes of death. Much of the population of Europe and North America was infected by TB during the 18th to 20th centuries (Daniel, 2009). Those not listed with TB as the cause of death in reference collections very likely were exposed to TB at some point in their lives. Some of the infected had active disease, although they died of some other cause. This is reflected in the high frequencies of rib lesions found in groups of individuals that were not reported as dying from TB in these studies (Mariotti et al., 2015). Here the information about cause of death cannot be used to differentiate between individuals with different TB disease status.
Another strategy is needed to relate bone lesions to TB-diagnoses, and that is the aim of this paper. This case-control study examines the association of bone lesions plausibly related to TB and documented TB status. A total of 124 skeletons are examined with different outcomes: 62 individuals are said to have had TB and 62 skeletons, included as controls, are from people who were unlikely to have had the disease. We test whether 13 osteological lesions are found with significantly different frequencies in the two groups. Those with the disease came from the Robert J. Terry Skeletal Collection, and individuals in the William M. Bass Donated Skeletal Collection were assumed to not have had tuberculosis. Information on disease status is used to assess the ability of the lesions to detect TB by calculating each lesions sensitivity and specificity.

With these epidemiological probability measures, it is possible to test whether the lesions are, in fact, related to TB. Sensitivity describes the probability of having a given lesion given that the individual had the disease. Specificity describes the probability of not having the lesion given that the individual did not have the disease. If the sensitivity of a lesion is greater than one minus specificity, the probability of having a lesion is greater among those with disease than those without it (Boldsen, 2001).

MATERIAL AND METHODS

Skeletons

The cases with TB were selected from the Robert J. Terry Skeletal Collection at the Department of Anthropology, National Museum of Natural History, Smithsonian Institution in Washington, D.C. The skeletal remains of 1728 individuals who died between 1920 and 1967 are included in the collection (Hunt and Albanese, 2005). The bodies of these individuals were unclaimed by relatives. For most individuals, data on sex, ethnicity, year of birth, year of death, and cause of death are available. The Terry collection was chosen to provide cases for the present study because many individuals - more than 15 % - had TB as the death diagnosis. This made it possible to select cases according to their age. The temporal setting of the skeletons in the pre-antibiotic era presumably means the tuberculous lesion patterns are roughly comparable to what might be found in archaeological samples. Skeletons from Terry collection were not suited to provide data for the group of controls because the absence of TB as a cause of death was not evidence of an absence of TB in these individuals. That is because TB was rampant in the United States at that time, especially in the disadvantaged socioeconomic groups that gave rise to this skeletal collection. Data were collected from 62 skeletons based upon availability and a random procedure outlined in table 1.
Controls, also 62 skeletons, were selected from the William M. Bass Donated Skeletal Collection at the Forensic Anthropology Center, University of Tennessee, Knoxville. More than 1,000 individuals who died from 1981 to the present are included in that well-documented collection (Jantz and Jantz, 2008). The United States is considered a TB low-burden country, and that has been true of the last half of the 20th century (Centers for Disease Control and Prevention, 2008). Therefore it is highly unlikely that individuals in Bass collection had developed TB, although the oldest ones might have been exposed to the pathogen when young. Data were collected from 62 skeletons of individuals that died between 1988 and 2012 and were not diagnosed with TB. The Bass controls were the same sex as the Terry cases, and their ages were within 2.5 years of their respective Terry skeletons.

Individuals younger than 25 and older than 55 years at death were not included in the study in order to minimize the effect of age on the immune response and, hence, its influence on the development of active disease (Giefing-Kröll et al., 2015). An overview of the distribution of sex, age group and status of TB infection in the 124 skeletons in the study is found in table 2.

<table>
<thead>
<tr>
<th>Selection criteria</th>
<th># available</th>
<th># excluded</th>
<th>% excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cause of death TB</td>
<td>240</td>
<td>1488</td>
<td>86.1</td>
</tr>
<tr>
<td>Age 25 - 55</td>
<td>164</td>
<td>76</td>
<td>31.7</td>
</tr>
<tr>
<td>Random selection</td>
<td>62</td>
<td>102</td>
<td>62.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Assumed TB status</th>
<th>Terry collection (N=62)</th>
<th>Bass collection (N=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>62 (50.0)</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>62 (50.0)</td>
<td>62</td>
</tr>
</tbody>
</table>

**Table 1. Selection of Terry collection skeletons.**

**Table 2. Distribution of sex, age group and TB status in 62 cases from the Terry collection and 62 controls from the Bass collection.**

**Osteological methods**

The skeletal expression of TB is studied by different research approaches. Lesions are presented in clinical studies of radiological images and autopsies by Sorrel and Sorrel-Dejerine (1932), Davidson and Horowitz (1970), Tuli (1975) and Thijn and Steensma (1990); in studies of
modern skeletal reference samples by Kelley and El-Najjar (1980), Roberts et al. (1994), Santos (2000), Ortner (2003) and Steyn et al. (2013); in archaeological skeletons by Roberts and Buikstra (2003), Holloway et al. (2011), Hajdu et al. (2012), and Nicklisch et al. (2012); and in analyses featuring microbiological methods of detecting TB bacterial DNA in bones by Salo et al. (1994), Mays et al. (2001), Brosch et al. (2002), Zink et al. (2005), Hershkovitz et al. (2008), Baker et al. (2015), and Pálfi et al. (2015). The selection of lesions to be included was based upon these studies of TB-related bone lesions. Besides the research referred to above, descriptions by Buikstra (1976), Steinbock (1976), Zimmerman and Kelley (1982), Kelley and Micozzi (1984), Murray et al. (1990), Aufderheide and Rodriguez-Martin (1998), Baker (1999), Ortner (2003), and Santos and Roberts (2006) were also reviewed.

Differentiating lesions attributable to TB from other pathological conditions is one of the greatest challenges facing osteologists when studying TB in skeletal remains (Buikstra, 1976). Such problems are particularly difficult when trying to identify early signs of disease, although that is critical to studies of their impact on past communities (Murray et al., 1990; Baker, 1999; Roberts and Buikstra, 2003: 127). The cutoff points for positive lesion scores in this study were intentionally set at a low level; that is, a mild stage of the disease’s progression. The patterns of skeletal expression related to TB are often distinctive, minimizing difficulties in differential diagnosis (Kelley and El-Najjar, 1980; Kelley and Micozzi, 1984). That allowed us to include lesions that represented most of the skeleton, not just focus on classic examples of TB-related bony involvement, notably collapsed vertebral columns.

The resulting list of potentially TB-related skeletal lesions included 13 different sites in the skeleton (table 17). Lesions on cervical vertebrae, neural arches, and long bone diaphyses are reported as infrequent and child-specific, so they were not included (Thijn and Stennisma, 1990; Santos and Roberts, 2001; Ortner, 2003: 247; Roberts and Buikstra, 2003:101; Dawson and Brown, 2012; Pálfi et al., 2012). Lesions on the skull, in the shoulder region, head and neck of ribs, and bones of the hands and feet are also considered to occur infrequently, so they were not included (Aufderheide and Rodriguez-Martin, 1998; Ortner, 2003; Roberts and Buikstra, 2003).

A recording manual describing the criteria for location, degree of preservation, extent of lesions, and types of bony reactions was prepared for use in the case-control study. The lesions were recorded in the left and right sides of the skeleton according to a dichotomous recording system - 0 (absent) and 1 (present). This ensured an operational tool that was manageable when applied to large skeletal samples. Recording criteria are described in tables 3-15, and pictures of lesions can be found in figures 1-20. The illustrations of bones are part of an ongoing project that seeks to identify TB in archaeological skeletons from Denmark.
**Soft nodules and vascular grooves on visceral surface of ribs (RIB1).** Baker (1999) suggests that soft nodules and vascular grooves on visceral surface of ribs are probable signs of early stage TB. Criteria for recording the lesions are found in table 3 and illustrated in figures 1 and 2.

| Table 3: Recording criteria for soft nodules and vascular grooves on visceral surface of ribs. |
| Location: | Potential TB lesions are recorded on visceral surface of rib bodies. |
| Scores: | 1: No information. Less than three pieces of ribs larger than 5 cm are preserved. |
| | 0: No bone changes related to TB are present (figures 1.A and 2.A). |
| | 1: Two or more pieces of ribs larger than 5 cm have either: |
| | • Soft nodules covering more than 5 cm (figure 1.B and C). |
| | • Two or more vascular grooves (figure 2.B and C). |

**Figure 1.A-C: TB-related bone lesions on ribs. A. Without bone changes related to TB. B. Nodules. C. Extensive area with nodules. Photos by DD Pedersen.**

**Periosteal reaction and osteolytic lesions on visceral rib surface (RIB2).** These bone changes are presented as related to TB by Kelley and Micozzi (1984), Robert et al. (1994), Santos and Roberts (2001), Lambert (2002), Mays et al. (2002), Matos and Santos (2006), Santos and Roberts (2006), and Nicklisch et al. (2012). Criteria for recording the lesions are found in table 4 and illustrated in figures 3 and 4.

| Table 4: Recording criteria for periosteal reaction and osteolytic lesions on visceral surface of ribs. |
| Location: | Potential TB lesions are recorded on visceral surface of the rib bodies. |
| Scores: | 1: No information. Less than three pieces of ribs larger than 5 cm are preserved. |
| | 0: No bone changes related to TB are present (figure 3.A and 4.A). |
| | 1: Two or more pieces of ribs larger than 5 cm have either: |
| | • Periosteal reactions with new bone formation (figure 3.B and C). |
| | • One or more shallow or deep osteolytic lesion measuring more than 5 mm in diameter (figure 4.B and C). |
Cranial and caudal surfaces of thoracic and lumbar vertebral bodies (VER). On the intervertebral surfaces erosive pitting and cavitation have been associated with TB infection (Kelley and El-Najjar, 1980; Aufderheide and Rodriquez-Martin, 1998; Ortner, 2003). These lesions are unlike Schmorl’s nodes because they are not localized and found centrally on the intervertebral surfaces (Sonne-Holm et al., 2013). Unlike Schmorl’s nodes, the possible TB-related lesions lack sclerotic borders and the pitting and erosion are accompanied by exposure of the trabecular bone tissue (Sajula et al. 1986). Recording criteria are given in table 5 and illustrated in figure 5.

Table 5: Recording criteria for cranial and caudal surfaces of thoracic and lumbar vertebral bodies.

<table>
<thead>
<tr>
<th>Location</th>
<th>Potential TB lesions are recorded on cranial and caudal surfaces of thoracic and lumbar vertebral bodies. Thoracic and lumbar vertebrae are recorded separately.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scores:</td>
<td>/: No information. Less than two thoracic and lumbar vertebral bodies are preserved or more than 50% of the bone surface of those preserved is damaged by postmortem changes.</td>
</tr>
<tr>
<td>0:</td>
<td>No bone changes related to TB are present (figure 5.A).</td>
</tr>
<tr>
<td>1:</td>
<td>Two or more vertebral bodies have either:</td>
</tr>
<tr>
<td></td>
<td>• Clustered pitting or erosive cavities in two or more areas each measuring at least 3 mm (figure 5.B).</td>
</tr>
<tr>
<td></td>
<td>• Clustered pitting or erosive cavities in one area measuring more than 10 mm (figure 5.C).</td>
</tr>
</tbody>
</table>

Figure 3.A-C: TB-related bone lesions on ribs. A. Without bone changes related to TB. B. Slight periosteal reaction with new bone formation. C. Severe periosteal reaction with new bone formation. Photos by DD Pedersen.

Figure 4.A-C: TB-related bone lesions on ribs. A. Without bone changes related to TB. B. Deep osteolytic lesions exposing the trabecular tissue. C. Shallow osteolytic lesions in the compact tissue. Photos by DD Pedersen.
Ventral part of thoracic and lumbar vertebral bodies (VEN). Lesions on ventral part of the vertebral bodies have been suggested to be related to TB (Kelley and El-Najjar, 1980; Haas et al., 2000; Ortner, 2003; Hajdu et al., 2012). In fact, these lesions may be signs of early stage TB (Baker, 1999; Spekker et al., 2012). Criteria for recording the lesions are found in table 6 and illustrated in figures 6 and 7.

Table 6: Recording criteria for ventral vertebral body.

| Location: Potential TB lesions are recorded on ventral surface of thoracic and lumbar vertebral bodies. Thoracic and lumbar vertebrae are recorded separately. |
| Scores: |
| /: No information. Less than two thoracic and lumbar vertebral bodies are preserved or more than 50 % of the bone surface of those preserved is damaged by postmortem changes. |
| 0: No bone changes related to TB are present (figure 6.A and 7.A). |
| 1: Two or more thoracic or lumbar ventral surfaces have either: |
| • Periosteal changes with woven or lamellar structure on at least 50 % of the bone surface (figure 6.B and C). |
| • Three or more large pits with circular shape and rounded edges measuring at least 3 mm in diameter (figure 7.B). In severe cases an abscess/cloaca is formed (figure 7.C). |
Lateral body of ilium (BOD). Lesions on the lateral body of ilium adjacent to the acetabulum are suggested to be related to TB (Ortner, 2003). The changes likely represent drainage channels from pus and secondary periosteal changes formed as direct extension by contact with psoas abscesses from soft tissues (Ortner, 2003: 236). The recording criteria for lateral body of ilium are given in table 7 and illustrated in figures 8 and 9.

Table 7: Recording criteria for lateral body of ilium.

| Location: Potential TB lesions are recorded on lateral body of ilium between lower gluteal line and the upper acetabular margin. |
| Scores: |
| /: No information. Less than 50% of body of ilium is preserved. |
| 1: One of the following lesions is present: |
| • Periosteal changes with woven or lamellar structure on at least 50% of the bone surface (figure 8.B). In severe cases an abscess can be present (figure 8.C). |
| • Three or more large pits with circular shape and rounded edges measuring more than 3 mm (figure 9.B and C). |

Figure 7.A-C: TB-related bone lesions on the ventral part of the vertebral body. A. Without bone changes related to TB. B. Large pit with circular shape and rounded edges. C. Large pits and abscess/cloaca. Photos by DD Pedersen.

Figure 8.A-C: TB-related lesions on lateral body of ilium. A. Without bone changes related to TB. B. Periosteal changes with woven structure. C. Periosteal changes with lamellar structure and abscess. Photos by DD Pedersen.
Acetabulum (ACE). In the acetabulum, the articular surface and the cartilage-free center of the acetabular fossa can be affected (Ortner, 2003). The changes on acetabular fossa are distinguished from what Rissech et al. (2006) present as variable 7 of age-related changes to the acetabulum. Recording criteria for the acetabulum are found in table 8 and illustrated in figures 10 and 11.

Table 8: Recording criteria for acetabulum.

<table>
<thead>
<tr>
<th>Location</th>
<th>Scores</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential TB lesions are recorded on acetabular fossa and articular surface of acetabulum.</td>
<td>/</td>
<td>No information. Less than 50% of fossa and/or lunate surface are preserved.</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>No bone changes related to TB are present (figure 10.A and 11.A).</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>One of the following lesions is present:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Clustered pitting or erosive cavities on articular surface in two or more areas, each measuring more than 3 mm or in one area measuring more than 10 mm (figure 10.B and C). In severe cases an abscess can be present (figure 10.C).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Two or more deep cavities in the acetabular fossa, each measuring more than 3 mm or more than 50% of the area, with borders consisting of a woven structure with dense trabeculae (figure 11.B and C).</td>
</tr>
</tbody>
</table>

Figure 9.A-C: TB-related bone lesions on lateral body of ilium. A. Without bone changes related to TB. B. Large pits. C. Large pits and periosteal changes. Photos by DD Pedersen.

Figure 10.A-C: TB-related bone lesions on articular surface of acetabulum. A. Without bone changes related to TB. B. Clustered pitting and shallow erosive lesions. C. Deep erosive cavities and abscess. Photos by DD Pedersen.
Proximal femur (PF). Changes on the caput of the femur and the greater trochanter are suggested to be related to TB (Kelley and El-Najjar, 1980; Ortner, 2003: 239). Criteria for recording the lesions are found in table 9 and illustrated in figures 12 and 13.

Table 9: Recording criteria for proximal femur.

| Location: Potential TB lesions are recorded on the caput and greater trochanter of the femur. |
|---|---|
| Scores: /
| No information. Less than 50% of the caput of the femur and/or the greater trochanter are preserved. |
| 0: No bone changes related to TB are present (figure 12.A and 13.A). |
| 1: One of the following lesions is present: |
| • Clustered pitting or erosive cavities on the caput of the femur in two or more areas, each measuring more than 3 mm or in one area measuring more than 10 mm (figure 12.B and C). |
| • Clustered pitting or erosive cavities on the greater trochanter in two or more areas, each measuring more than 3 mm or in one area measuring more than 10 mm (figure 13.B and C). |
The sacroiliac joint is a common site of skeletal involvement in relation to TB (Ortner, 2003: 237; Aufderheide and Rodriguez-Martin, 1998: 139). The recording criteria for iliac auricular surface are found in table 10 and illustrated in figure 14.

Table 10: Recording criteria for iliac auricular surfaces.

| Location: | Potential TB lesions are recorded on iliac auricular surfaces. |
| Scores:   | /: No information. Less than 50% of the iliac auricular surfaces are preserved. |
|           | 0: No bone changes related to TB are present (figure 14.A). |
|           | 1: Clustered pitting (figure 14.B) or erosive cavities (figure 14.C) in two or more areas, each measuring more than 3 mm or in one area measuring more than 10 mm. |

Distal femur (DH). The distal part of femur is involved in TB that affects the knee joint (Kelley and El-Najjar, 1980; Ortner, 2003: 240). Recording criteria for the distal femur are given in table 11 and illustrated in figure 15.
Table 11: Recording criteria for distal end of femur.

<table>
<thead>
<tr>
<th>Location:</th>
<th>Potential TB lesions are recorded on the articular surface of distal femur.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scores:</td>
<td>/: No information. Less than 50% of articular surface is preserved.</td>
</tr>
<tr>
<td></td>
<td>0: No bone changes related to TB are present (figure 15.A).</td>
</tr>
<tr>
<td></td>
<td>1: Clustered pitting or erosive cavities on two or more areas each measuring more than 3 mm (figure 15.B) or in one area measuring more than 10 mm (figure 15.C).</td>
</tr>
</tbody>
</table>

![Figure 15.A-C: TB-related bone lesions of distal femur. A. Without bone changes related to TB. B. Clustered pitting and erosive cavities. C. Extensive pitting and small erosive cavities. Photos by DD Pedersen.](image)

Proximal tibia (PT). Tuberculosis of the knee joint can also affect the proximal tibia (Kelley and El-Najjar, 1980; Ortner, 2003: 240). The recording criteria for the proximal tibia are found in table 12 and illustrated in figure 16.

Table 12: Recording criteria for proximal end of tibia.

<table>
<thead>
<tr>
<th>Location:</th>
<th>Potential TB lesions are recorded on the articular surface and intercondylar areas of the proximal tibia.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scores:</td>
<td>/: No information. Less than 50% of articular surface and/or intercondylar areas are preserved.</td>
</tr>
<tr>
<td></td>
<td>0: No bone changes related to TB are present (figure 16.A).</td>
</tr>
<tr>
<td></td>
<td>1: One of the following lesions is present:</td>
</tr>
<tr>
<td></td>
<td>• Clustered pitting or erosive cavities on the articular surface in two or more areas, each measuring more than 3 mm (figure 16.B) or one area measuring more than 10 mm (figure 16.C).</td>
</tr>
<tr>
<td></td>
<td>• Three or more large pits on intercondylar areas, each measuring more than 3 mm (figure 16.B and C) and/or periosteal changes with woven (figure 16.B) or lamellar structure (figure 16.C) on at least 50 % of intercondylar areas.</td>
</tr>
</tbody>
</table>
Distal humerus (DH). The elbow joint is described as the most common site of tubercular lesions in the bones of the upper extremity (Ortner; 2003: 243). The humerus may be affected at the elbow joint (Kelley and El-Najjar, 1980). Recording criteria for the distal humerus are given in table 13 and illustrated in figures 17 and 18.

Table 13: Recording criteria for the distal end of the humerus.

| Location: Potential TB lesions are recorded on the condylar areas and articular surface of the distal humerus. |
| Scores: | 1/: No information. Less than 50% of condylar area or articular surface is preserved. |
| 0: No bone changes related to TB are present (figure 17.A and 18.A). |
| 1: One of the following lesions is present: |
| - Clustered pitting on lateral and/or medial posterior condylar areas covering more than 1 cm² (figure 17.B and C). |
| - Clustered pitting or erosive cavities in two or more areas on the articular surface measuring more than 3 mm or in one area measuring more than 10 mm (figure 18.B and C). |

Figure 16.A-C: TB-related bone lesions on proximal tibia. A. Without bone changes related to TB. B. Clustered pitting on articular surface and periosteal changes in intercondylar areas. C. Erosive cavities on articular surface and intercondylar area. Photos by DD Pedersen.

Figure 17.A-B: TB-related bone lesions on posterior condylar areas of distal humerus. A. Without bone changes related to TB. B. Pitting on lateral condylar area. C. Extensive pitting on lateral area and small erosive cavity on articular surface. Photos by DD Pedersen.
Radial tuberosity of the proximal radius (PR). Tuberculosis of the elbow joint can also affect the proximal radius (Kelley and El-Najjar, 1980; Ortner, 2003: 243). Based upon observations made in the ongoing project on archaeological Danish skeletons, the radial tuberosity of the proximal radius is scored. The recording criteria for radial tuberosity are found in table 14 and illustrated in figure 19.

**Table 14: Recording criteria for the radial tuberosity.**

<table>
<thead>
<tr>
<th>Location</th>
<th>Potential TB lesions are recorded on the radial tuberosity.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scores:</td>
<td>/: No information. Less than 50% of radial tuberosity is preserved.</td>
</tr>
<tr>
<td></td>
<td>0: No bone changes related to TB are present (figure 19.A).</td>
</tr>
<tr>
<td></td>
<td>1: Clustered pitting (figure 19.B) or erosive cavities (figure 19.C) in two or more areas measuring more than 3 mm or in one area measuring more than 10 mm.</td>
</tr>
</tbody>
</table>

Olecranon process of the proximal ulna (PU). Proximal end of the ulna is involved in TB of the elbow joint (Kelley and El-Najjar, 1980; Ortner, 2003: 243). Based upon observations of archaeological Danish skeletons, the olecranon process on proximal ulna is scored. Recording criteria for the proximal end of the ulna are given in table 15 and illustrated in figure 20.
Table 15: Recording criteria for the proximal end of the ulna.

<table>
<thead>
<tr>
<th>Location:</th>
<th>Potential TB lesions are recorded on the olecranon process of proximal ulna.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scores:</td>
<td>/: No information. Less than 50% of proximal ulna is preserved.</td>
</tr>
<tr>
<td></td>
<td>0: No bone changes related to TB are present (figure 20.A).</td>
</tr>
<tr>
<td></td>
<td>1: One of the following lesions is present:</td>
</tr>
<tr>
<td></td>
<td>• Clustered pitting (figure 20.B) or erosive cavities (figure 20.C) on the olecranon process in two or more areas measuring more than 3 mm or in one area measuring more than 10 mm.</td>
</tr>
</tbody>
</table>

Figure 20.A-C: TB-related bone lesions on olecranon process. A. Without bone changes related to TB. B. Erosive cavities. C. Clustered pitting and erosive cavities. Photos by DD Pedersen.

**Statistical methods**

The data management and analyses were carried out using STATA 13 for windows. All lesion sites, except those on ribs and vertebrae, were recorded for both the left and right sides. In the data analysis, one variable for each lesion was computed from scores of the left side. When the left side was missing, the score for the right side was used. Differences in frequencies and associations of the 13 lesions in skeletons from Terry collection and Bass collection were tested by Fisher’s exact tests.

The characteristics of a diagnostic test are expressed through sensitivity and specificity, or by negative or positive predictive values. Predictive values are used in clinical studies to estimate the probability that a patient is truly negative or positive if the test is either negative or positive. Predictive values depend on the population prevalence of disease (Kirkwood and Sterne, 2003). Sensitivity and specificity do not depend on the population prevalence of disease, and are related to the biology of the disease. In the present study the concern was the principle association of bone lesions and disease diagnosis, so sensitivity and specificity are estimated to assess the lesions ability to detect the disease.
Disease diagnosis is an estimate based upon probabilities and defined by the sensitivities and specificities of specific symptoms of disease. The sensitivity and specificity for each lesion were calculated according to the definitions listed in table 16.

Table 16: Diagnostic criteria and definitions of sensitivity and specificity.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th># sick</th>
<th># not sick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>True positive (TP)</td>
<td>False positive (FP)</td>
</tr>
<tr>
<td>Absent</td>
<td>False negative (FN)</td>
<td>True negative (TN)</td>
</tr>
</tbody>
</table>

\[
\text{Sensitivity} = \frac{TP}{\text{sick}}
\]

\[
\text{Specificity} = \frac{TN}{\text{not sick}}
\]

RESULTS

Lesion site frequencies are shown in table 17. In the Terry collection, most lesions were found as periosteal reactions and osteolytic lesions on the visceral surfaces of ribs (RIB2) (59.7 %) and on the ventral part of vertebral bodies (VEN) (58.1 %). The least affected site in the Terry skeletons – the individuals who were identified as suffering from TB – was the proximal tibia (PT) (3.2 %). In the Bass collection, 11 skeletons had soft nodules and vascular grooves on the visceral surface of ribs (RIB1) (17.7 %). The exact same frequency rate for this lesion type was found in Terry skeletons. For all remaining lesions, the Bass control group had less involvement compared to the Terry case group. No of Bass collection skeleton had a lesion on the lateral body of the ilium (BOD), or a lesion in the knee (DF and PT) or elbow (DH, PR, PU) joints. Figures 21 and 22 illustrate the frequencies of lesions in the cases and controls. In 6 of 13 lesion sites, the difference between the frequency of lesions in the case and control groups was statistically significant (table 17). For these lesions, it was assumed there was an association between the bony involvement and a positive disease diagnosis. For the remaining non-significant lesion distributions, except for soft nodules and vascular grooves on ribs (RIB1), there were few affected in both the case and the control group. Little, therefore, could be done with the results.
Table 17: The frequency distribution of the 13 lesions in skeletons from the Terry and Bass Collections.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Terry Collection (N=62)</th>
<th>Bass Collection (N=62)</th>
<th>Fisher’s exact</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIB1</td>
<td>Unaffected: 51 (82.3)</td>
<td>51 (82.3)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Affected: 11 (17.7)</td>
<td>11 (17.7)</td>
<td></td>
</tr>
<tr>
<td>RIB2</td>
<td>Unaffected: 25 (40.3)</td>
<td>60 (96.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Affected: 37 (59.7)</td>
<td>2 (3.2)</td>
<td></td>
</tr>
<tr>
<td>VER1</td>
<td>Unaffected: 55 (88.7)</td>
<td>59 (95.2)</td>
<td>0.323</td>
</tr>
<tr>
<td></td>
<td>Affected: 7 (11.3)</td>
<td>3 (4.8)</td>
<td></td>
</tr>
<tr>
<td>VEN4</td>
<td>Unaffected: 26 (41.9)</td>
<td>60 (96.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Affected: 36 (58.1)</td>
<td>2 (3.2)</td>
<td></td>
</tr>
<tr>
<td>BOD5</td>
<td>Unaffected: 44 (71.0)</td>
<td>61 (100.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Affected: 18 (29.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>ACE6</td>
<td>Unaffected: 45 (72.6)</td>
<td>58 (95.1)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Affected: 17 (27.4)</td>
<td>3 (4.9)</td>
<td></td>
</tr>
<tr>
<td>PF7</td>
<td>Unaffected: 56 (90.3)</td>
<td>60 (98.4)</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>Affected: 6 (9.7)</td>
<td>1 (1.6)</td>
<td></td>
</tr>
<tr>
<td>ILI8</td>
<td>Unaffected: 39 (62.9)</td>
<td>58 (95.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Affected: 23 (37.1)</td>
<td>3 (4.9)</td>
<td></td>
</tr>
<tr>
<td>DF9</td>
<td>Unaffected: 59 (95.2)</td>
<td>61 (100.0)</td>
<td>0.244</td>
</tr>
<tr>
<td></td>
<td>Affected: 3 (4.8)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>PT10</td>
<td>Unaffected: 60 (96.8)</td>
<td>61 (100.0)</td>
<td>0.496</td>
</tr>
<tr>
<td></td>
<td>Affected: 2 (3.2)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>DH11</td>
<td>Unaffected: 58 (93.5)</td>
<td>62 (100.0)</td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td>Affected: 4 (6.5)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>PR12</td>
<td>Unaffected: 59 (95.2)</td>
<td>62 (100.0)</td>
<td>0.244</td>
</tr>
<tr>
<td></td>
<td>Affected: 3 (4.8)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>PU13</td>
<td>Unaffected: 56 (90.3)</td>
<td>62 (100.0)</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>Affected: 6 (9.7)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

1RIB1: soft nodules and vascular grooves on the visceral surface of ribs; 2RIB2: Periosteal reaction and osteolytic lesions on the visceral surface of ribs; 3VER: caudal and cranial surfaces of thoracic and lumbar vertebral bodies; 4VEN: Ventral part of thoracic and lumbar vertebral bodies; 5BOD: Lateral body of ilium; 6ACE: Acetabulum; 7PF: Proximal femur; 8ILI: Iliac auricular surface; 9DF: Distal femur; 10PT: Proximal tibia; 11DH: Distal humerus; 12PR: Radial tuberosity; 13PU: Olecranon process of the ulna.
Figure 21: Distribution of lesion frequencies in cases from Terry collection.

Figure 22: Distribution of lesion frequencies in controls from Bass collection.
Table 18: Fisher’s exact test of the interrelationship of the 13 lesions. Values in bold are significant at the 0.05 level.

<table>
<thead>
<tr>
<th></th>
<th>RIB1</th>
<th>VER</th>
<th>VEN</th>
<th>BOD</th>
<th>ACE</th>
<th>PF</th>
<th>ILI</th>
<th>DF</th>
<th>PT</th>
<th>DH</th>
<th>PR</th>
<th>PU</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIB1</td>
<td>0.802</td>
<td>1.00</td>
<td>1.00</td>
<td>0.839</td>
<td>0.756</td>
<td>0.350</td>
<td>0.564</td>
<td>1.00</td>
<td>1.00</td>
<td>0.547</td>
<td>1.00</td>
<td>0.288</td>
</tr>
<tr>
<td>RIB2</td>
<td></td>
<td>1.00</td>
<td></td>
<td>&lt;0.001</td>
<td>0.006</td>
<td>0.019</td>
<td>0.391</td>
<td>&lt;0.001</td>
<td>0.236</td>
<td>0.099</td>
<td>0.009</td>
<td>0.233</td>
</tr>
<tr>
<td>VER1</td>
<td></td>
<td></td>
<td>0.009</td>
<td>0.039</td>
<td>0.010</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.017</td>
<td>0.074</td>
</tr>
<tr>
<td>VEN4</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>0.029</td>
<td>0.008</td>
<td>0.028</td>
<td>0.094</td>
<td>0.085</td>
<td>0.027</td>
<td>0.010</td>
</tr>
<tr>
<td>BOD3</td>
<td></td>
<td></td>
<td></td>
<td>0.065</td>
<td></td>
<td>&lt;0.001</td>
<td>0.383</td>
<td>0.274</td>
<td>0.010</td>
<td>0.381</td>
<td>0.212</td>
<td></td>
</tr>
<tr>
<td>ACE6</td>
<td></td>
<td></td>
<td>0.322</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>0.418</td>
<td>0.302</td>
<td>0.013</td>
<td>0.068</td>
<td>0.252</td>
<td></td>
</tr>
<tr>
<td>PF7</td>
<td></td>
<td></td>
<td>0.037</td>
<td>0.162</td>
<td>1.00</td>
<td></td>
<td>0.211</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILI8</td>
<td></td>
<td></td>
<td></td>
<td>0.114</td>
<td>0.382</td>
<td></td>
<td>0.002</td>
<td>0.009</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.064</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.064</td>
<td>1.00</td>
<td>0.182</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DH11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1RIB1: soft nodules and vascular grooves on visceral surface of ribs; 2RIB2: Periosteal reactions and osteolytic lesions on visceral surface of ribs; 3VER: caudal and cranial surfaces of thoracic and lumbar vertebral bodies; 4VEN: Ventral part of thoracic and lumbar vertebral bodies; 5BOD: Lateral body of ilium; 6ACE: Acetabulum; 7PF: Proximal femur; 8ILI: Iliac auricular surface; 9DF: Distal femur; 10PT: Proximal tibia; 11DH: Distal humerus; 12PR: Radial tuberosity; 13PU: Olecranon process of the ulna.

Table 18 shows the test results of Fisher’s exact test of interrelationship of lesions. Lesions found to be non-significant in table 17 are related to only a few, or none, of the other lesions. That was not true of lesions that had significantly different frequencies in the Terry and Bass skeletons. Soft nodules and vascular grooves on visceral surface of ribs (RIB1) and proximal tibia (PT) were not found to be significantly related to any other lesion.

Table 19 shows the estimated sensitivities and specificities of the lesions. For all lesions, the criteria of being related to TB when sensitivity is greater than one minus specificity was met. The presence of periosteal reactions and osteolytic lesions on the visceral surfaces of ribs (RIB2) provided a 59.7% probability of a valid TB-diagnosis. An absence of those lesions provided a 3.2% probability of TB. For the ventral part of vertebral bodies (VEN) the probability of a valid diagnosis was 58.1% and the probability of TB when the lesion was absence was 4.8%. In contrast, the presence of proximal tibia (PT) lesions provided only a 3.2% probability of TB-diagnosis. Their absence provided a 0% probability of TB-diagnosis.

Table 20 gives the results of the Fisher’s exact test of independence of the six lesions that are significantly more common in cases than controls. Lesions in the pelvic area – the lateral body of ilium (BOD), acetabulum (ACE) and iliac auricular surface (ILI) – were significantly related both in cases and controls. The ventral part of the vertebral body and acetabulum were significantly related in controls. The iliac auricular surface and olecranon process were significantly related in cases.
Table 20: Fisher’s exact test of independence of the six lesions that are found significantly more in cases compared to control. Values in bold are significant at the 0.05 level.

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Group</th>
<th>VEN</th>
<th>BOD</th>
<th>ACE</th>
<th>ILI</th>
<th>PU</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIB21</td>
<td>Cases</td>
<td>0.203</td>
<td>1.000</td>
<td>0.777</td>
<td>0.110</td>
<td>0.387</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>0.200</td>
<td>1.000</td>
<td>0.591</td>
<td>0.105</td>
<td>0.500</td>
</tr>
<tr>
<td>VEN2</td>
<td>Cases</td>
<td>0.053</td>
<td>0.381</td>
<td>0.794</td>
<td>0.387</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>0.230</td>
<td>0.013</td>
<td>0.055</td>
<td>0.191</td>
<td></td>
</tr>
<tr>
<td>BOD3</td>
<td>Cases</td>
<td>&lt;0.001</td>
<td>0.020</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>&lt;0.001</td>
<td>0.027</td>
<td>0.434</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE4</td>
<td>Cases</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.648</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.648</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILI5</td>
<td>Cases</td>
<td>0.023</td>
<td>0.023</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>0.115</td>
<td>0.115</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1RIB2: Periosteal reaction and osteolytic lesions on visceral surface of ribs; 2VEN: Ventral part of thoracic and lumbar vertebral bodies; 3BOD: Lateral body of ilium; 4ACE: Acetabulum; 5ILI: Iliac auricular surface; 6PU: Olecranon process of the ulna.
DISCUSSION

In this study, 6 of 13 possibly TB-related lesions are indeed associated with a diagnosis of TB when the individuals were alive. These lesions, therefore, are considered to be TB indicators. The remaining seven lesions were non-significantly distributed between cases and controls, did not show great relation with other lesions, and had low sensitivity estimates. These lesions provide limited epidemiological information about TB, and are not considered to be TB indicators.

The two types of rib lesions had very different distributional patterns. Soft nodules and vascular grooves (RIB1) were found equally frequently in cases and controls, hence are not an indicator of TB. Ten of eleven skeletons with positive scores of RIB1 had soft nodules. These are very likely signs of non-specific changes related to chronic pulmonary conditions, as suggested by Roberts et al. (1994), Eyler et al. (1996) and Matos and Santos (2006). Nodules are found along the costal groove on the visceral surface of ribs. Internal intercostal muscles attach in the costal grooves. These muscles cause downward depression of ribs doing forced expiration (Köft-Maier, 2000: 71). Cough provokes severe forced expiration and accompanying stress on costal grooves from muscle contraction. Chronic coughing, therefore, is a likely cause of the formation of rib nodules. Besides chronic pulmonary conditions such as bronchitis, long-term exposure to airborne dust or smoke particles can cause chronic coughing. In the Bass collection, some skeletons are documented smokers. That could explain the presence of rib nodules in the control group. Unfortunately information about smoking is not available for all individuals examined in Bass collection. That means the association of smoking and cough-related nodules cannot be studied using the available data.

![Figure 23: Periosteal changes on rib of individual UT17-91D from Bass collection. Photo by DD Pedersen.](image-url)

In contrast, periosteal reaction and osteolytic lesions on ribs (RIB2) differently distributed in the two skeletal groups. Therefore, they are considered TB indicators. Two skeletons in the control group have periosteal reaction on ribs. One skeleton (UT17-91D) has periosteal changes
on the visceral surface of the sternal ends and midshafts of ribs (Fig. 23). This individual was diagnosed with non-specified cancer and also has periosteal changes on scapulae, shaft of femur and proximal humerus. It is assumed that the lesions found on the ribs are related to a metastatic cancer (Tatelman and Drouillard, 1953).

The two types of lesions in the spine are likewise found with very different distributions in cases and controls. Significant frequency distributions are found for lesions on ventral parts of vertebral bodies (VEN). Lesions on the caudal and cranial surfaces of thoracic and lumbar vertebral bodies (VER) are found with non-significantly different frequencies in the two groups of skeletons. The lesions on surface of vertebral bodies (VER) cannot be considered related to TB-diagnosis. In other studies, however, these lesions are presented as a ‘classic’ sign of skeletal TB (Kelley and El-Najjar, 1980; Aufderheide and Rodriguez-Martin, 1998; Ortner, 2003). This assertion is often put forward in severe cases of skeletal TB (e.g., Haas et al., 2000; Hajdu et al., 2012). In such examples the erosive lesions are related to the destruction of the trabecular tissue within vertebrae that may cause the vertebral body to collapse and an angular kyphosis to form (Ortner, 2003). The lack of association of this lesion type and TB diagnosis in the present study may have a connection to the cutoff point for the criteria of scoring a positive lesion. The lesion illustrated in figure 5.B may have other causes besides TB, whereas the lesion in figure 5.C. is similar to the ‘classic’ sign of TB. Better results of associations are likely obtained if the scoring criteria for intervertebral surface of vertebral body (VER) are raised to a more severe expression of a disease process. Lesions on ventral part of vertebral body (VEN) are considered early signs of TB infection (Baker, 1999; Spekker et al., 2012). Dissemination of TB in the spine will occur through three routes: between vertebrae through perforation of the pulposus of intervertebral discs (Ortner, 2003: 231); direct extension from soft tissue lesions, notably from the lungs and psoas muscle (Aufderheide and Rodriguez-Martin, 1998: 122); and hematogenous dissemination via the nutritional supply of the spine (Ortner, 2003). The ventral part of vertebral bodies (VEN) is closely connected to soft tissues. Here there is an accessible route of dissemination of bacteria compared to crossing the intervertebral discs. This also explains the greater degree of involvement of ventral parts compared to intervertebral surfaces on vertebral bodies found in the present study.

Besides the mentioned lesions on ribs and on vertebral bodies, five lesions are found with non-significant distributions in cases and controls. All are found in low frequencies in both the Terry Collection and Bass Collections, although for all lesions the case group is more heavily affected. Including more skeletons in the study may result in significant distributions of some of these lesions. We find low frequency rates for lesions such as the knee joint that elsewhere are
reported to be frequently involved in relation to TB (Aufderheide and Rodriguez-Martin, 1998: 138). This may be due to the variability of the skeletal expression of TB. Skeletal involvement varies across time and between populations depending on host resistance, virulence of pathogen, route of transmission, and age of the infected individual (Buikstra, 1976; Aufderheide and Rodriguez-Martin, 1998: 133; Maliarik and Iannuzzi, 2003). The identified skeletons from Terry Collection died of pulmonary TB. This explains the high involvement rates of ribs found in the present study, as also found in other studies using skeletons from contemporary reference samples (Kelley and Micozzi, 1984; Roberts et al., 1994; Santos and Roberts, 2001; Matos and Santos, 2006; Santos and Roberts, 2006; Mariotti et al., 2015). The ratio of pulmonary TB versus extra-pulmonary TB shifts towards more extra-pulmonary involvement in populations with high rates of infection with M. bovis through the gastrointestinal tract (Cormigan and Flynn, 1992). In such populations a greater degree of involvement of joints may be found. The results of the present study show that the epidemiological value of lesions in the knee and elbow in relation to pulmonary TB is limited.

The design and choice of skeletons in the present study makes it possible to assess the ability of the lesions to detect TB. The calculated sensitivities and specificities vary. Lesions with low sensitivities provide limited epidemiological information about the probability of having TB. Some lesions have high sensitivities, but none are 100 % diagnostic of TB on their own. Lesions on the lateral body of ilium and the olecranon process of the ulna are found only in people who died from TB. Based on data from this study, these lesions can be characterized as pathognomonic lesions with specificities of 1 and sensitivities above 0.

Diagnosing disease is a statistical process based upon probabilities. Hence, the epidemiological parameters derived in the present study can be used to estimate the probability of diagnosis. Including the estimated parameters of all lesions in one model will enable the estimation of the frequency of TB at death for the skeletal sample as a whole, as has been done for leprosy by Boldsen (2001, 2005, 2008, 2013). The prevalence of TB is influenced by demographic, socioeconomic and immunogenetic aspects of the particular population analyzed (Davies et al. 1999, Daniel, 2009). Therefore varying lesion patterns and the ability of lesions to detect TB must be considered when modeling the frequency of TB at death in different skeletal samples.

This study shows that 6 of 13 lesions initially assumed to be related to TB are, in fact, useful indicators of TB in skeletal samples. Periosteal reaction and osteolytic lesions on ribs (RIB2) and lesions on the ventral part of thoracic and lumbar vertebral bodies (VEN), lateral body of ilium (BOD), acetabulum (ACE), iliac auricular surface (ILI), and the olecranon process of the ulna
(PU) should be recorded when seeking to identify TB in skeletal samples. Soft nodules and vascular grooves on ribs (RIB1), lesions on the caudal and cranial surfaces of vertebral bodies (VER), lesions in knee joint (DF and PT), and lesions on the humerus (DH) and radius (PR) at the elbow are not consistent with a diagnosis of TB. These lesions apparently can be caused by other pathological conditions. The descriptions of the six good TB indicators serve as a model for uniform and effective data collection in large skeletal samples. This is the first step towards performing paleoepidemiological studies of TB in archaeological skeletons to gain insights into the impact of this disease in the past.

ACKNOWLEDGEMENTS
We wish to thank Dr. Dawnie Wolfe Steadman from William M. Bass Donated Skeletal Collection and Dr. David Hunt from Robert J. Terry Skeletal Collection for providing access to skeletons. Thank you to Dr. George R. Milner (Department of Anthropology, Pennsylvania State University) for proofreading the final version of the manuscript.

LITERATURE CITED


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9.2. Study II

Lesion patterns related to tuberculosis in identified and archaeological skeletons

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ABBREVIATED TITLE
Lesion patterns related to TB

KEY WORDS
Lesion patterns; pulmonary TB; Danish skeletons, identified skeletons

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Velux foundation as part of the Ophelia project and the Faculty of Health Sciences, University of Southern Denmark.
ABSTRACT

Objectives: This paper focuses on lesion patterns in 95 modern skeletons assumed to be infected with pulmonary tuberculosis and in 160 archaeological Danish skeletons of unknown tuberculosis status.

Materials and methods: Skeletons from the Robert J. Terry Skeletal Collection, which dates to the early to mid-20th century, and medieval to post-medieval Ribe were examined. Data on tuberculosis-related lesions in six locations of the skeleton were recorded. Skeletal lesion frequencies were compared between samples and across time.

Results: Significantly different rib lesion frequencies were found in the modern and archaeological samples and, in the latter, temporally different groups of skeletons. Rib lesions were identified in 13.9% of the skeletons from medieval Ribe, 26.9% from post-medieval Ribe, and 65.3% from the Terry Collection. Significant differences were also found for the number of lesions in the Ribe and Terry Collection skeletons. Most of the skeletons with 1-2 lesions were found in the medieval (75.9%) and post-medieval (84.6%) Ribe samples, and most skeletons with 3 and more lesions were seen in the Terry Collection (36.8%).

Discussion: Rib lesions in post-medieval Ribe resemble the pattern in the 20th century Terry Collection. Post-medieval skeletons, however, have the least involvement of all other lesion sites. The results indicate differences in the expression of tuberculosis. That was presumably caused by changes in pathogens, host immunogenetics, and living conditions, all of which would have varied through time.
Tuberculosis (TB) is a chronic infectious disease that in humans is primarily caused by *Mycobacterium tuberculosis*. The bacteria are spread through infected airborne droplets expelled from the lungs by coughing. Humans can also contract TB from infection by *M. bovis* transmitted to humans through unpasteurized milk from infected cattle or by inhalation of airborne droplets (Francis, 1950). *M. tuberculosis* and *M. bovis* are part of the *M. tuberculosis* complex that also includes the bacteria *M. africanum, M. canetti, M. caprae, M. microti* and *M. pinnipedii* that cause disease in a variety of animals (O’Reilly and Daborn, 1995; Grange, 2009).

When exposed to TB bacteria, a person may either resist infection or be infected (Maliarik and Iannuzzi, 2003). Socioeconomic factors, as well as the host and pathogen genetic makeup, influence the likelihood of TB transmission (Bellamy et al. 1998; Valway et al. 1998; Maliarik and Iannuzzi, 2003). Approximately 25% of those exposed will be infected. The impact of infection on the host depends on the route of transmission, pathogen virulence, and the ability of the immune response to suppress the disease’s progression (Bloom and Small, 1998; Malik and Godfrey-Faussett, 2005). The disease may stay latent when bacteria multiplication is suppressed and is encapsulated by the immune response; alternatively, the bacteria will multiply and an active form of the disease will develop (Fiske and Haas, 2013). The most common route of transmission is the pulmonary tract where in the active stage of the disease caseous necroses are formed in the lung apices (Fiske and Haas, 2013). When untreated and unhealed, the bacteria will disseminate from the pulmonary region through the bloodstream, and extra-pulmonary infection will develop. Infection with *M. bovis* through the gastrointestinal tract can also develop extra-pulmonary infection. In extra-pulmonary TB, most organs and tissues of the body can be affected, notably the lymph nodes, abdominal cavity, meninges, urinary tract, gastrointestinal tract, and the skeleton (Fiske and Haas, 2013).

Great variability is seen in the parts of the skeleton affected by skeletal TB. The bacteria settle in highly vascularized areas; that is, places rich in trabecular bone such as the metaphyses and epiphyses of long bones, vertebrae, and ribs (Ortner, 2003: 228). Depending on the route of transmission and host-pathogen interactions, however, TB-victims will be affected in different ways and degrees. Documenting TB-related lesion patterns in skeletons across time and in populations from different socioeconomic settings makes it possible to gain insights into what influences how the skeleton is affected by TB (Steyn et al., 2013).

With the overall aim of studying the skeletal expression of TB across time, this paper examines six kinds of TB-related lesions in modern skeletons from the Robert J. Terry Collection and in historic period skeletons from Danish archaeological sites. Skeletal lesion patterns in 95 people who were infected by pulmonary TB and died in the early to mid-20th
century are compared with those seen in 160 medieval and post-medieval adults of unknown TB status.

MATERIAL AND METHODS

Skeletal material

The study included 255 skeletons. Of that total, 95 were known age and sex individuals from the Robert J. Terry Skeletal Collection housed at the Department of Anthropology, National Museum of Natural History, Smithsonian Institution in Washington, D.C. The remainders were 160 archaeological skeletons from the Danish town of Ribe in Jutland.

Due to their immature immune systems, children have a higher risk of dying of TB before skeletal changes develop than adults (Jaspan et al., 2005). Hence, subadult skeletons do not show skeletal signs of TB to the extent of adults. Individuals that died at ages below 16 years with unfused pelvic bones in acetabula or unfused elbow joint epiphyses were therefore excluded from this study.

To be included, adult skeletons needed to have the six sites of the skeleton preserved where TB-related lesions were scored (Pedersen et al., 2016). They also had to have a positive score (1) in at least one of the six locations. Selection of skeletal material was based on the availability of skeletons, as listed in table 1.

Identified skeletal collection. The 1728 individuals in the Robert J. Terry Skeletal Collection died in St. Louis, Missouri, during the 1920 to 1967 period, and their bodies were unclaimed (Hunt and Albanese, 2005). In this study, 124 complete skeletons were examined. They were selected as a random stratified sample consisting of 62 people reported on death certificates as dying from TB, and 62 where some other cause of death was recorded. Of the 124 skeletons, 95 had at least one positive TB-related lesion, so they were included in the study. Fifty-two died of pulmonary TB. The remaining 43 died of a non-tuberculous cause. These individuals, however, were quite likely infected with TB as the disease was very prevalent when the individuals were alive, especially among low socioeconomic groups.

Archaeological excavated skeletal samples. The Danish town Ribe is located on the south-western coast of Denmark. Founded around AD 710, it was one of the earliest urban settlements in Scandinavia (Feveile, 2006). In the early medieval period, many churches were erected and cemeteries were established within the town. Some of those cemeteries have recently been excavated, yielding 1,451 skeletons dating to AD 800 – 1800. Of 1,098 adult skeletons examined
from Ribe, 160 individuals were complete and had at least one TB-related lesion. These skeletons are dated to AD 1050-1800, and can be assigned to the medieval (AD 1050-1536) and post-medieval periods (AD 1536-1800). The skeletons came from three main locations – Grey friars, Black friars and Our Lady (Ribe Cathedral). Those from Grey friars and Ribe Cathedral are housed at the Unit of Anthropology (ADBOU), Department of Forensic Medicine, University of Southern Denmark. Skeletons from Black friars are housed at the Anthropological Collection, Unit of Forensic Anthropology, Department of Forensic Medicine, University of Copenhagen.

Table 1. Selection criteria and availability of the skeletal material from the Terry Collection and from Ribe.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Selection criteria</th>
<th># available</th>
<th># excluded</th>
<th>% excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terry Collection</td>
<td>Age of death &gt;16</td>
<td>124</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Known time period of death</td>
<td>124</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>All six sites preserved</td>
<td>124</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>One or more positive lesions</td>
<td>95</td>
<td>29</td>
<td>23.4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>95</td>
<td>29</td>
<td>23.4</td>
</tr>
<tr>
<td>Ribe</td>
<td>Age of death &gt;16</td>
<td>1,451</td>
<td>353</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>Known time period of death</td>
<td>1,098</td>
<td>258</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td>All six lesion sites preserved</td>
<td>840</td>
<td>474</td>
<td>56.4</td>
</tr>
<tr>
<td></td>
<td>One or more positive lesions</td>
<td>366</td>
<td>206</td>
<td>56.3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>160</td>
<td>1,291</td>
<td>89.0</td>
</tr>
</tbody>
</table>

Osteological methods

Data concerning age, sex and TB-related lesions were recorded for each skeleton. Sex was estimated from the morphology of the cranium and pelvic bones, as described by Buikstra and Ubelaker (1994). The size and robustness of the postcranial skeleton were also considered. Age was estimated according to experience-based age assessment, as discussed by Milner and Boldsen (2012) and by Milner et al. (2016).

Six osteological lesions related to TB were recorded in the skeletons. The lesions are described in detail and illustrated in Pedersen et al. (2016). The recording criteria are described below.

An overview of the skeletons is provided in table 2.

Visceral surfaces of ribs (RIB). Lesions were recorded on the visceral surface of rib bodies. If fragmented, only pieces measuring more than 5 cm were considered. The lesions were given one of the following scores: /) No information. Less than three pieces of ribs larger than 5 cm were preserved. 0) No bone changes related to TB. 1) Two or more pieces of ribs had either periosteal
reactions with new bone formation or one or more shallow or deep osteolytic lesions measuring more than 5 mm in diameter.

**Ventral part of thoracic and lumbar vertebral bodies (VEN).** Lesions were recorded on the ventral surface of thoracic and lumbar vertebral bodies. Lesions were given one of the following scores: /) No information. Less than two vertebral bodies were preserved or more than 50% of the bone surface of those preserved was damaged by postmortem changes. 0) No bone changes related to TB. 1) Two or more thoracic or lumbar ventral surfaces had periosteal changes with woven or lamellar structure on at least 50% of the bone surface or three or more large pits measuring at least 3 mm in diameter were present. In severe cases an abscess could be present.

**Lateral body of the ilium (BOD).** Lesions were recorded on the lateral body of the ilium between the lower gluteal line and the upper acetabular margin. Lesions were given one of the following scores: /) No information. Less than 50% of body of ilium was preserved. 0) No bone changes related to TB. 1) At least 50% of the bone surface had periosteal changes, or three or more large pits measuring more than 3 mm were present. In severe cases an abscess could be present.

**Acetabulum (ACE).** Lesions were recorded on the acetabular fossa and articular surface of the acetabulum. These lesions were given one of the following scores: /) No information. Less than 50% of fossa and/or lunate surface were preserved. 0) No bone changes related to TB. 1) Pitting or erosive cavities were present on the articular surface in two or more areas, each measuring more than 3 mm or in one area measuring more than 10 mm. In severe cases an abscess could be present. In the acetabular fossa, two or more deep cavities were present, each measuring more than 3 mm or more than 50% of the area had a woven structure with dense trabeculae.

**Iliac auricular surface (ILI).** Lesions were recorded on iliac auricular surfaces. Lesions were given one of the following scores: /) No information. Less than 50% of the iliac auricular surfaces were preserved. 0) No bone changes related to TB. 1) Clustered pitting or erosive cavities were present in two or more areas, each measuring more than 3 mm or in one area measuring more than 10 mm.

**Olecranon process of the ulna (PU).** Lesions were recorded on the olecranon process of the ulna. Lesions were given one of the following scores: /) No information. Less than 50% of proximal ulna was preserved. 0) No bone changes related to TB. 1) Clustered pitting or erosive
cavities on the olecranon process were present in two or more areas measuring more than 3 mm or in one area measuring more than 10 mm.

| Table 2. Distribution of sex, age and the frequency of TB-related bone lesions in Terry collection and Ribe skeletons. |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|----------------------------------|
| N (%)  | Terry Collection (N=95) | Ribe (N=160) | P-value  |
| Sex    |                               |              |         |
| Female | 110 (43.1)                   | 66 (41.3)    | 0.403   |
| Male   | 145 (56.9)                   | 94 (58.7)    |         |
| Age group (years) |                               |              |         |
| 16-30  | 55 (21.6)                    | 35 (21.9)    | 0.537   |
| 30-50  | 151 (59.2)                   | 91 (56.9)    |         |
| 50-70  | 47 (18.4)                    | 32 (20.0)    |         |
| 70-90  | 2 (0.8)                      | 2 (1.3)      |         |
| Time period |                               |              |         |
| Medieval | 108 (42.4)                 | 108 (67.5)   |         |
| Post-Medieval | 52 (20.4)          | 52 (32.5)    |         |
| AD 1920-1967 | 95 (37.2)              | 0 (0.0)      |         |
| Lesion  |                               |              |         |
| RIB¹   | Unaffected 164 (64.3)        | 131 (81.9)   | <0.001  |
|        | Affected    91 (35.7)        | 29 (19.1)    |         |
| VEN²   | Unaffected 156 (61.2)        | 101 (63.1)   | 0.407   |
|        | Affected    99 (38.8)        | 59 (36.9)    |         |
| BOD³   | Unaffected 171 (67.1)        | 110 (68.7)   | 0.456   |
|        | Affected    84 (32.9)        | 50 (31.3)    |         |
| ACE⁴   | Unaffected 158 (62.0)        | 101 (66.1)   | 0.619   |
|        | Affected    97 (38.0)        | 59 (36.9)    |         |
| ILI⁵   | Unaffected 156 (61.2)        | 91 (56.9)    | 0.067   |
|        | Affected    99 (38.8)        | 69 (43.1)    |         |
| PU⁶    | Unaffected 230 (86.3)        | 140 (87.5)   | 0.460   |
|        | Affected    35 (13.7)        | 20 (12.5)    |         |

¹RIB: Periosteal reaction and osteolytic lesions on the visceral surface of ribs; ²VEN: Ventral part of the thoracic and lumbar vertebral bodies; ³BOD: Lateral body of ilium; ⁴ACE: Acetabulum; ⁵ILI: Iliac auricular surface; ⁶PU: Olecranon process of the proximal ulna.

**Statistical methods**

Data management and analysis were carried out using STATA 13 for windows.

All lesions, except those on the ribs and vertebrae, were recorded for the left and right sides. For the purposes of data analysis, scores were merged into one for each site. The left side score was used when present; when absent, the right side score was used.
Lesion frequency distributions and associations of lesions were tested by Pearson’s $\chi^2$-tests. Analyses were performed between the modern and archaeological skeletons, and through time for the medieval, post-medieval, and AD 1920-1967 periods.

**RESULTS**

In the identified skeletons from the Terry collection, ribs were the most affected (65.3%) followed by vertebrae (42.1%) (table 2). In the Ribe skeletons, the iliac auricular surface was most affected (43.1%) followed by vertebrae and acetabula where 36.9% of the skeletons were affected (table 2). The difference in frequencies in the two samples was significant for rib lesions. More than three times as many skeletons from Terry Collection had rib lesions than skeletons from Ribe. For all lesions, the highest frequencies were found in the Terry Collection, except for those of the iliac auricular surface.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Score</th>
<th>Ribe Medieval (N=108)</th>
<th>Ribe Post-Medieval (N=52)</th>
<th>Terry Collection (N=95)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIB$^1$</td>
<td>Unaffected</td>
<td>93 (86.4)</td>
<td>38 (73.1)</td>
<td>33 (34.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>15 (13.9)</td>
<td>14 (26.9)</td>
<td>62 (65.3)</td>
<td></td>
</tr>
<tr>
<td>VEN$^2$</td>
<td>Unaffected</td>
<td>65 (60.2)</td>
<td>36 (69.2)</td>
<td>55 (57.9)</td>
<td>0.388</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>43 (39.8)</td>
<td>16 (30.8)</td>
<td>40 (42.1)</td>
<td></td>
</tr>
<tr>
<td>BOD$^3$</td>
<td>Unaffected</td>
<td>69 (63.9)</td>
<td>41 (78.8)</td>
<td>61 (64.2)</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>39 (36.1)</td>
<td>11 (21.2)</td>
<td>34 (35.8)</td>
<td></td>
</tr>
<tr>
<td>ACE$^4$</td>
<td>Unaffected</td>
<td>64 (59.3)</td>
<td>37 (71.2)</td>
<td>57 (60.0)</td>
<td>0.308</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>44 (40.7)</td>
<td>15 (28.8)</td>
<td>38 (40.0)</td>
<td></td>
</tr>
<tr>
<td>ILI$^5$</td>
<td>Unaffected</td>
<td>62 (57.4)</td>
<td>29 (55.8)</td>
<td>65 (68.4)</td>
<td>0.184</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>46 (42.6)</td>
<td>23 (44.2)</td>
<td>30 (31.6)</td>
<td></td>
</tr>
<tr>
<td>PU$^6$</td>
<td>Unaffected</td>
<td>91 (84.3)</td>
<td>49 (94.2)</td>
<td>80 (84.2)</td>
<td>0.174</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>17 (15.7)</td>
<td>3 (5.8)</td>
<td>15 (15.8)</td>
<td></td>
</tr>
</tbody>
</table>

$^1$RIB: Periosteal reaction and osteolytic lesions on visceral surface of ribs; $^2$VEN: Ventral part of thoracic and lumbar vertebral bodies; $^3$BOD: Lateral body of ilium; $^4$ACE: Acetabulum; $^5$ILI: Iliac auricular surface; $^6$PU: Olecranon process of the proximal ulna.

Table 3 shows the frequencies of lesions in skeletons from the Terry Collection and the archaeological samples from Ribe. Frequency distributions are shown in figure 1. Ribs showed statistical significant differences in lesion frequencies in the three time periods. The frequency of rib lesions was low in medieval Ribe (13.9%) compared to post-medieval Ribe (26.9%) and the Terry collection (65.3%). For all remaining lesions, except for the iliac auricular surface, the frequencies in the medieval Ribe and Terry samples were almost the same, and greater than the frequencies in post-medieval skeletons (figure 1). There are significantly different distributions
for rib lesions between skeletons from Ribe dating to the medieval and post-medieval periods, between medieval Ribe and the Terry Collection, and between post-medieval Ribe and the Terry Collection (table 4).

![Graph showing frequency (%) of TB-related lesions in Ribe and Terry Collection](image)

**Figure 1**: Frequency (%) of lesions in skeletons from Ribe dated to the medieval and post-medieval periods and from the Terry Collection dated to 1920-1967.

**Table 4**: Associations of frequency of lesions in the three time periods. 
*Values in bold are significant at the 0.05 level.*

<table>
<thead>
<tr>
<th></th>
<th>Medieval (N=108)</th>
<th>Post-Medieval (N=52)</th>
<th>1920-1967 (N=95)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>RIB</td>
<td>0.045</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>VEN</td>
<td>0.267</td>
<td>0.740</td>
<td></td>
</tr>
<tr>
<td>BOD</td>
<td>0.056</td>
<td>0.962</td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>0.144</td>
<td>0.915</td>
<td></td>
</tr>
<tr>
<td>ILI</td>
<td>0.845</td>
<td>0.106</td>
<td></td>
</tr>
<tr>
<td>PU</td>
<td>0.074</td>
<td>0.992</td>
<td></td>
</tr>
<tr>
<td>RIB</td>
<td>-</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>VEN</td>
<td>-</td>
<td>0.176</td>
<td></td>
</tr>
<tr>
<td>BOD</td>
<td>-</td>
<td>0.066</td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>-</td>
<td>0.178</td>
<td></td>
</tr>
<tr>
<td>ILI</td>
<td>-</td>
<td>0.127</td>
<td></td>
</tr>
<tr>
<td>PU</td>
<td>-</td>
<td>0.076</td>
<td></td>
</tr>
</tbody>
</table>

1RIB: Periosteal reaction and osteolytic lesions on visceral surface of ribs; 2VEN: Ventral part of thoracic and lumbar vertebral bodies; 3BOD: Lateral body of ilium; 4ACE: Acetabulum; 5ILI: Iliac auricular surface; 6PU: Olecranon process of the proximal ulna.
Table 5 and figure 2 give the frequency of skeletons with 1 and 2 positive lesions, and 3 or more positive lesions. The distributions of skeletons with few and with multiple lesions were significantly different in the three time periods. More skeletons with 3 or more lesions were found in the Terry Collection (36.8%) than in the medieval (24.1%) and post-medieval Ribe (15.4%) samples. As seen in table 6, the differences were significant for skeletons from both medieval and post-medieval Ribe when compared to those in the Terry Collection. The difference in frequency between the two time periods in Ribe was not significant.

Table 5. Frequency distribution of skeletons with 1 and 2 positive lesions and 3 or more positive lesions in the three time periods.

<table>
<thead>
<tr>
<th></th>
<th>Ribe Medieval (N=108)</th>
<th>Ribe Post-Medieval (N=52)</th>
<th>1920-1967 (N=95)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 2 lesions</td>
<td>82 (75.9)</td>
<td>44 (84.6)</td>
<td>60 (63.2)</td>
<td>0.013</td>
</tr>
<tr>
<td>3+ lesions</td>
<td>26 (24.1)</td>
<td>8 (15.4)</td>
<td>35 (36.8)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: Frequency (%) of skeletons with 1-2 positive lesions and 3 or more positive lesions in skeletons from the medieval and post-medieval Ribe and Terry Collection samples.

Table 6: Associations of frequency of number of skeletons with 1-2 positive lesions and 3 or more positive lesions in the three time periods.

<table>
<thead>
<tr>
<th></th>
<th>P-value (Medieval)</th>
<th>P-value (Post-Medieval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medieval (N=108)</td>
<td>0.208</td>
<td>0.048</td>
</tr>
<tr>
<td>Post-Medieval (N=52)</td>
<td>-</td>
<td>0.006</td>
</tr>
</tbody>
</table>
DISCUSSION

Skeletal lesion patterns related to TB were examined in modern skeletons infected with pulmonary TB and in archaeological skeletons with unknown infections. Differences were found between those two groups of skeletons, as well as between the medieval and post-medieval samples.

Rib lesions show the greatest differences; the modern and archaeological sample frequencies were significantly different, as were the medieval and post-medieval sample frequencies. Lesion frequency increased markedly through time. The Terry Collection skeletons were certainly infected by pulmonary TB. The increased rib involvement across time very likely reflects changes towards a greater pulmonary involvement. In Ribe, more rib involvement is evident during the post-medieval period when significantly more skeletons have rib lesions than those from the medieval period.

The change towards pulmonary involvement may have been caused by a change in the pathogen. Pulmonary TB is to a great extent caused by *M. tuberculosis* infection (Domingo et al. 2014). *M. bovis* is transmitted from cattle to humans both via lungs and the gastrointestinal tract. If transmitted via airborne particles inhaled in lungs, *M. bovis* can elicit the same disease process as *M. tuberculosis* (Francis, 1950). The gastrointestinal tract, however, is the most common route of *M. bovis* transmission. *M. bovis* from infected milk will result in extra-pulmonary TB to a larger extent than *M. tuberculosis* (Cormican and Flynn, 1992). A study from England and Wales focusing on 1901-1932 showed that 20% of cases of bone and joint TB were caused by *M. bovis* (Griffith, 1937).

The ratio of *M. tuberculosis* and *M. bovis* infection in humans, thus quite likely the ratio of pulmonary versus extra-pulmonary TB, varies among populations and over time (Cormigan and Flynn, 1992; Cosivi et al. 1998). For example, national surveys for TB were carried out in Denmark by the Tuberculosis Department of the State Serum Institute beginning in 1932 (Madsen et al., 1942). These surveys showed that there was a distinct difference in the presence of bovine infection in humans living in rural and urban areas. During 1931-1935, 4.4% of the 15-30 year olds residing in Jutland’s towns with positive TB in sputum tested positive for bovine TB. During the same period, the corresponding figure for that age group in rural Jutland was 17% (Madsen et al., 1942: 59). Ribe is situated on the southwestern coast of Jutland. In 1935, this region had the largest recorded occurrence of TB in cattle in Denmark and also the largest recorded frequency of *M. bovis* infection in humans. Cattle have played an important role in Ribe’s economy and food production since the medieval times (Enemark, 2003). The Ribe area was ideal for cattle grazing.
Farming was reorganized in many parts of Europe in the wake of the plague epidemics that reached Denmark around AD 1350 (Enemark, 1999). The size of the population was reduced dramatically, and fluctuating temperatures and wet weather put stress on agricultural production (Hybel and Poulsen, 2007: 74). As a consequence, agricultural production changed from grain to meat. As the importance of the cattle trade, including export to other parts of Europe, grew, cattle markets were established in Ribe during the 15th century to facilitate the new lucrative opportunities (Enemark, 1999). The cattle trade peaked during the first part of the 16th century (Willerslev, 1952). The risk of contracting *M. bovis* from close contact between cattle and people was thus inevitable in late medieval Ribe. The comparatively low rib involvement in skeletons from medieval Ribe, and to some extent the post-medieval population, might be a reflection of a greater proportion of extra-pulmonary TB.

The high frequency of lesions on the iliac auricular surface (ILI) in the archaeological skeletons may also be evidence of the presence of extra-pulmonary TB. The sacroiliac joint is closely linked to the intestinal walls and urinary system, which are some of frequently affected sites in extra-pulmonary TB infection.

The frequencies of the remaining four lesions – ventral vertebrae bodies (VEN), lateral body of ilium (BOD), acetabulum (ACE) and olecranon process of ulna (PU) – are strikingly similar in medieval Ribe and in the Terry Collection. In contrast, post-medieval skeletons have a rather low involvement of the four lesion types. This result, combined with the large proportion of skeletons with few lesions, indicate changes in TB expression in the post-medieval period. There is as mentioned an increase of rib lesions which indicate less bovine and correspondingly more pulmonary TB infection in post-medieval period. At the same time there is a general low involvement rate. This suggests that individuals at that time to a larger degree died of TB before lesions developed (Wood et al., 1992). This may be connected to an increase of pulmonary TB infection. The influence of TB on its host is a result of differences in pathogen virulence and the immunogenetic composition of infected individuals (Valway, 1998; Maliarik and Iannuzzi, 2003). With the eventual decline in the importance of the cattle trade in Ribe, the risk of contracting bovine TB decreased while that of the more virulent pulmonary form from *M. tuberculosis* increased (Francis, 1950). The population in post-medieval Ribe probably had little resistance towards this bacterial exposure. Many individuals therefore died of infection before TB developed into a chronic disease.

Unlike skeletons from medieval and post-medieval Ribe, many of those in the Terry Collection have 3 or more lesions. That indicates chronicity of the disease as individuals survived a long time with active infections, allowing recognizable skeletal lesions to develop.
Individuals in the Terry Collection were from a poor social background compared to what was found across much of the United States in the first half of the 20th century. Compared to conditions in Ribe, however, conditions were likely sufficient for people to have stronger immune responses, helping them survive longer with a TB infection.

Changes in the ratio of *M. tuberculosis* and *M. bovis* infection, in host-pathogen interactions, and in living conditions over time probably resulted in the differences in lesion patterns identified in the present study. The results show that important insights into the impact of TB in the past can be explored through studies of skeletal lesions. The possibility that there was a shift in disease patterns in Ribe from *M. bovis* towards *M. tuberculosis* could be examined by studies of mycobacterial DNA and lipid biomarkers. That could be the scope of future studies on the skeletal material examined here.

**ACKNOWLEDGEMENTS**

We wish to thank archaeologists Morten Søvsø and Maria Knudsen from Sydvestjyske museer in Ribe for assisting with the archaeological information concerning the skeletons from Ribe. We further wish to thank Professor Niels Lynnerup from the Anthropological Collection, Unit of Forensic Anthropology, Department of Forensic Medicine, University of Copenhagen for providing access to the skeletons from Black friars and Dr. David Hunt from the Robert J. Terry Skeletal Collection, Smithsonian Institution, Washington D.C. for providing access to the skeletons in the collection. We appreciate the help from Dr. George R. Milner (Department of Anthropology, Pennsylvania State University) who proofread the final version of the manuscript.

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9.3. Study III

Tuberculosis in the Danish town of Ribe, AD 800-1800 – a paleoepidemiological approach

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ABBREVIATED TITLE
Tuberculosis in Danish skeleton AD 800 - 1800

KEYWORDS

tuberculosis; osteological lesions; Danish skeletons; probability measures

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ABSTRACT

Objectives: In recent centuries, millions of people have died from tuberculosis. Before the 18\textsuperscript{th} century when few historical records are available, however, the impact of tuberculosis on European communities is largely unknown. Here statistical modelling and osteological lesion data are combined to estimate the frequency of tuberculosis in skeletons. The aim is to investigate the impact of disease across time and among socially stratified groups.

Material and methods: The paleoepidemiological approach adopted here was originally developed for studying leprosy. Statistics are modified for data derived from studies of modern skeletons of people who died of tuberculosis. The method is applied to 752 adult skeletons from the Danish town of Ribe, dated to AD 800-1800. Data are estimated from two models, one including ribs and the other where they are omitted, to accommodate different lesion patterns in the samples attributable to the bovine and pulmonary forms of tuberculosis.

Results: The fit of the models varies among the skeletal samples. Estimates when rib lesions are not included fit medieval, post-medieval and high status skeletons. When rib lesions are included, the model fits the Viking sample. Neither model fit the low/common status sample. The frequency of tuberculosis at death remained unchanged from the medieval (49\%) to post-medieval period (51\%). Differences are found in the frequency of disease among low/common (63\%) and high status (19\%) subsamples.

Discussion: This is the first attempt to apply a strictly paleoepidemiological approach to tuberculosis in archaeological skeletons. In historic period Ribe, frequency rates of tuberculosis at death were high in the medieval and post-medieval periods, and they were not uniform among people of different social status. The differences in the fit of the two models applied suggest that great variability in the skeletal expression of tuberculosis was present throughout the 1,000 year period in Ribe.
Tuberculosis (TB) caused the death of millions of people during the last three centuries (Daniel, 2006). Because historical sources are available for this time period, the prevalence and mortality rates of TB are well-known. The spread and impact of this disease in the more distant past, however, are not clear. TB is a chronic infectious disease that can affect the skeleton. Therefore, studies of mummified and skeletal remains provide information about this disease when few, if any, historical records are available. Besides identifying the presence of TB in the distant past, skeletal studies can provide insights into the progression of the disease in the pre-antibiotic era, the disease’s geographical, social, and temporal distribution, and its effect on communities. The risk factors that contribute to TB exposure, infection, and development are complex. Besides host and pathogen characteristics, risk factors are related to environmental and social settings, such as crowding and ventilation in houses, occupational load, and malnutrition (Narasimhan et al., 2014).

Archaeological skeletons with lesions related to TB date from Neolithic to modern times (Hershkovitz et al., 2008; Pósa et al., 2015; Sparacella, 2016). Microbiological analyses are often used to confirm a relation between lesions and the presence of Mycobacterium tuberculosis (Salo et al., 1994; Pálfi et al., 1999; Brosch et al., 2002; Roberts and Buikstra, 2003; Zink et al., 2005; Hershkovitz et al., 2008; Holloway et al., 2011). The picture of TB in the past provided by such evidence, however, is quite fragmentary. Uneven spatial and geographical coverages reflect the intensity of studies in specific geographic regions and the lack of skeletal material in other places (Holloway et al, 2011). Population-based approaches to the study of TB have been undertaken by Buikstra (1999), Jankauskas (1999), Pálfi and Marcšik (1999), Maczel (2004), and Sparacello et al. (2016). Though both individual and population-based analyses are undertaken, the main approach still relies on counts of skeletal lesions without a quantitative appraisal of the degree to which various kinds of lesions can serve as TB indicators.

What is needed in the study of TB in the past is a paleoepidemiological approach, as has been developed for leprosy (Boldsen, 2001, 2005a, 2008 and 2013). The idea behind this approach is that disease diagnoses are based upon lesion probability measures; that is, lesions related to a specific disease contribute unequally to the detection of that disease. Hence, the probabilities of a positive diagnosis depend on what lesions are present in the skeleton. Boldsen (2005a) has developed a method that incorporates the diagnostic probability measures sensitivity and specificity in analyses of archaeological skeletons. These measures, and the frequencies of lesions in the skeletons of interest, are then used to estimate how widespread leprosy was in the past through the disease’s frequency in skeletal samples. Like leprosy, TB is a chronic infectious disease that can affect the skeleton. Six bone lesions useful for quantitative analyses have been
associated with TB (Pedersen et al., 2016a). These lesions have been shown to have higher frequencies in skeletons of individuals who suffered from TB than they were in skeletons of people who were not infected by TB. Because the TB status of the individuals in this case-control study was known, lesion sensitivity and specificity could be estimated. These TB-related lesions and the diagnostic probability measures can now be applied to the study of TB in skeletal samples, much like what has already been done for leprosy.

This study presents a model for estimating the paleoepidemiological properties of TB, based on what has already been done for leprosy (Boldsen, 2001, 2005a, 2013). We model the estimation of individual probability of TB, termed \( \tau \) (tau). From \( \tau \) the frequency of TB at death at population level is estimated. The model is applied to 752 skeletons dated to AD 800 – 1800 from the Danish town of Ribe. We estimate the frequency of TB at death during the Viking, medieval, and post-medieval periods to identify change over time in TB in an urban setting. Furthermore, TB frequencies in socially stratified groups in medieval Ribe are compared to identify the role of the social background as a risk factor affecting mortality.

**MATERIAL AND METHODS**

**Skeletal material**

Data were recorded in skeletons from Ribe on the southwestern coast of Denmark. The town, dating back to AD 710, was one of the earliest urban settlements in Scandinavia (Feveile, 2006; Hybel and Poulsen, 2007: 229). The town grew from a craft and trade center situated along trading routes between Scandinavia to the north and the rest of Europe to the south (Kristensen and Poulsen, 2016: 40). One of the first churches in Scandinavia was erected in Ribe by the missionary Ansgar in AD 860 (Møller and Nyborg, 1979).

Ribe’s history and its role in the economy of its time are well-known. Since the 1950s, archaeologists have excavated large parts of the Viking and medieval town (Bencard, 1981). These excavations have focused on demolished churches and monasteries, along with their associated cemeteries. These burial grounds have yielded approximately 1,500 skeletons dating to a 1,000 year period from AD 800 – 1800. Data concerning TB were recorded in skeletons from three cemeteries: Grey friars, Black friars, and Ribe Cathedral (Our lady).

Grey friars’ (Franciscan) monastery was established in 1232, and continued to be used until the Protestant reformation in 1536 (Møller et al., 1984b). Parts of the medieval parish cemetery outside the monastery complex along with some of the monastery and church were excavated in 1993 (Jantzen et al., 1995; Andersen, 2003). All 351 adult skeletons included from Grey friars are dated to the medieval period. Of these individuals, 252 were buried in the parish cemetery.
and are regarded as people of low/common social status. The remaining 99 skeletons came from within the church and monastery complex, and they are regarded as people of high social status. The skeletons are stored at Unit of Anthropology (ADBOU), Department of Forensic Medicine, University of Southern Denmark in Odense, Denmark.

Black friars’ (Dominican) monastery was founded in 1228 (Møller et al., 1984a). In 1543 the buildings were turned into a hospital, and the church and cemetery were kept as a parish cemetery and church (Møller et al., 1984a). In 1983, part of the monastery courtyard was excavated (Madsen et al., 1984). The skeletons that were found date to the first half of the 16th century. In 1988, the area southeast of the monastery church was excavated. Written sources about burial practices indicate that these burials likely date to the AD 1754-1807 period (Frandsen, 1988). All 73 adults included in the present study, therefore, belong to a post-medieval sample. The skeletons are stored at the Anthropological Collection, Unit of Forensic Anthropology, Department of Forensic Medicine, University of Copenhagen, Denmark.

The present cathedral in Ribe was built in the first half of 12th century, but it had several predecessors. The earliest church was erected by the missionary Ansgar during the Viking age (Møller and Nyborg, 1979; Søvsø, 2009). Two main excavations have been carried out in the vicinity of the Cathedral – south of the cathedral in 2008-2009 and 2011-2012, and additional pits around the cathedral in 2012 (Søvsø, 2009; Søvsø, 2010; Madsen and Søvsø, 2010). Christian Viking age burials are among those that have been excavated. In the AD 1050-1225 period, the area south of the cathedral was not used for burials. Aside from this, the cemetery around Ribe Cathedral was used for burials from Viking times until 1800. Of 328 adult skeletons in the present study, 155 date to the medieval period. Of these burials, 105 are regarded as low/common individuals based on their modest burial types and location in the parish cemetery. Skeletons of 42 individuals were either buried in brick coffins in the parish cemetery or within the cathedral building complex. They are considered people of high social status. The skeletons are stored at Unit of Anthropology (ADBOU), Department of Forensic Medicine, University of Southern Denmark in Odense, Denmark.

Of 1,427 skeletons examined from Ribe, 752 adult skeletons are included in the present study. Subadults that died at ages younger than 16 years are not included. Skeletons without information about dating are also not included. Figure 1 gives an overview of the selection of skeletons from the available skeletons from Ribe.
Osteological methods

Six skeletal lesions identified as TB indicators by Pedersen et al. (2016a) were recorded in the following locations: 1) Visceral surface of the ribs, 2) Ventral part of thoracic and lumbar vertebral bodies, 3) Lateral body of the ilium, 4) Acetabulum, 5) Iliac auricular surface, 6) Olecranon process of the ulna. The lesions are described in detail and illustrated in appendix 1.

Besides TB-related lesions, data concerning sex and age were recorded. Sex was estimated from the morphology of the cranium and pelvic bones, as well as the size and robustness of the postcranial skeleton (Buikstra and Ubelaker, 1994). Age in adults was estimated according to experience-based age assessment as discussed by Milner and Boldsen (2012) and by Milner et al. (2016). The ages of subadults were estimated through dental development and epiphysis fusion to long bones (Ubelaker, 1989; Scheuer and Black, 2000). Fusion of the ilium, ischium and pubis in the acetabulum distinguished adults from subadults who were less than 16 years old. If the pelvic bones were not preserved, the complete epiphyseal fusion of bones in the elbow joint or the beginning of fusion in other long bones were used to separate subadults from adults. An overview of the distribution of sex, age at death, sites and social status of skeletons from the Viking, medieval, and post-medieval periods is provided in table 1.
Diagnoses of TB are based upon an assessment of the probability of the disease occurring with particular skeletal features, as estimated from lesion sensitivity and specificity. Sensitivity describes the probability of having a particular lesion given that the individual actually had the disease. Specificity describes the probability of not having the lesion given that the individual did not have the disease. These diagnostic probability measures and the frequency counts of TB lesions can be used for developing $\tau$-statistics, as $\lambda$ was for leprosy by Boldsen (2001, 2005a). The individual probability of TB can then be estimated given either positive or negative scores of osteological lesions.

$$
\tau_k = \sum_{j=1}^{6} \ln \left( \frac{sens_j^{\Delta_{jk}} \cdot (1 - sens_j)^{(1-\Delta_{jk})}}{spe_j^{\Delta_{jk}} \cdot (1 - spe_j)^{\Delta_{jk}}} \right)
$$

(1)

The probability of having TB ($\tau_k$) can be calculated as a weighted sum of lesion scores ($\Delta_{jk}$), as expressed in equation (1). Here the $\tau$-statistic for individual number ‘$k$’ is estimated from the combined log-likelihood estimate, assuming the $k^{th}$ skeleton was affected by TB (numerator) and
assuming the $k^{th}$ skeleton was not affected by TB (denominator). Sensitivity and specificity estimates for the six lesions, which are from Pedersen et al. (2016a), are found in table 2. The lesion scores ($\Delta_{jk}$) are derived from data recorded in the skeletons from Ribe, where table 3 summarizes the three time periods, and table 7 the two socially stratified groups, also from medieval Ribe.

The epidemiological measures were calculated from lesion scores derived from skeletons in a documented sample, the Terry Collection that dates to the first half of the 20th century, and modern skeletons from the Bass Collection (Pedersen et al., 2016a). In a paper by Pedersen et al. (2016b), it was shown that the lesion pattern in the Terry collection differs from what is seen in skeletons from medieval and post-medieval Ribe. The differences are most obvious concerning rib lesions. This lesion type is much more prevalent in Terry than in the archaeological samples. This suggests a change in host-pathogen interaction over time. The epidemiological measures based upon the identified skeletons may not be applicable to the archaeological skeletons. Therefore the estimates of $\tau$ were done both with and without sensitivity and specificity measures of rib lesions. As it was for leprosy (Boldsen, 2005b), independence was assumed among TB lesion scores when conditioned on disease status.

The frequency of TB at death ($p$), a measure of population prevalence, can be estimated from the probability of disease ($\tau_k$). The mean and variance of the $\tau$ distribution contains information about the frequency of TB at death ($p$). Observed mean ($\bar{\tau}_0$) is expressed as the weighted mean of simulated $\tau$-values in a sample where nobody ($\bar{\tau}_+$) and a sample where everybody ($\bar{\tau}_-$)
suffered from TB (equation (2)). Likewise observed variance \((s_0^2)\) is expressed as the weighted variance of simulated \(\tau\)-values in a sample where nobody \((s_+^2)\) and a sample where everybody \((s_-^2)\) suffered from TB (equation (3)). Here an additional part of the equation expresses the difference between the means of the two sets of simulated \(\tau\)-values. Equation 2 and 3 each provide an estimate of \(p\) by rearrangement.

\[
\bar{\tau}_0 = p \cdot \bar{\tau}_+ + (1 - p) \cdot \bar{\tau}_-
\]

(2)

\[
s_0^2 = p \cdot s_+^2 + (1 - p) \cdot s_-^2 + p \cdot (1 - p) \cdot (\bar{\tau}_+ - \bar{\tau}_-)^2
\]

(3)

The function in equation (4) estimates \(f(p)\) that combines information about the frequency of TB at death \((p)\) that is contained in the mean and variance for the \(\tau\)-values. Simulated negative values and simulated positive values are used to estimate the maximum likelihood of the frequency of TB \((p)\). The value of \(p\) that minimizes \(f(p)\) is termed \(\hat{p}\). This is a \(\chi^2\) distribution with one degree of freedom that evaluates the hypothesis that the mean and variance for \(\tau\)-values indicate the same \(\hat{p}\). The hypothesis must be rejected when \(f(\hat{p}) > 3.84\) \((p < 0.05)\). This means that \(\hat{p}\) is not a valid estimator of the TB frequency. The interval of \(p\)-values that fulfills the inequality \(f(p) - f(\hat{p}) < 3.84\) belongs to the 95% confidence interval for \(\tau\).

\[
f(p) = \left(\frac{\bar{\tau}_0 - (p \cdot \bar{\tau}_+ + (1 - p) \cdot \bar{\tau}_-)}{se(\bar{\tau}_0)}\right)^2 + \left(\frac{s_0^2 - (p \cdot s_+^2 + (1 - p) \cdot s_-^2 + p \cdot (1 - p) \cdot (\bar{\tau}_+ - \bar{\tau}_-)^2)}{se(s_0^2)}\right)^2
\]

(4)

The probability of TB \((\tau)\) and frequency of TB at death \((p)\) are estimated for three time periods (Viking, medieval, and post-medieval) and for two medieval social groups. For all five subsamples the estimates are done based upon a model that includes rib lesions and another one that omits the rib lesions.
RESULTS

Table 3 shows the lesion scores for the three time periods. Estimates generated from the model including rib lesions are given in table 4, also for the three time periods. The observed τ-values fall within the range of simulated negative and simulated positive values of τ. The frequency of TB at death (p) ranges from 0.26 (26%) in the medieval period to 0.53 (53%) in the Viking age. Only 29 skeletons were available from Viking age contexts. Hence, the confidence interval spans 31% and 83%. The maximum likelihood estimate of the frequency of TB (p) is similar for the medieval (26%) and post-medieval (30%) samples. The observed and simulated τ-values and frequency of TB for each of the three time periods estimated without the rib lesions are given in table 5. The observed τ-values fall within the range of simulated negative and simulated positive values of τ. Excluding the ribs gives rise to higher frequencies of TB at death (p) in all time periods. In the Viking age sample the maximum likelihood estimate of p is larger than 0.99 (99%); for the medieval and post-medieval samples the estimates are 0.49 (49%) and 0.51 (51%), respectively. The graphs in figure 2 illustrate the distribution of the sample frequency in the three time periods when all lesions are included (fig. 2.A) and when rib lesions are omitted (fig. 2.B).
The results of the goodness of fit of the estimated model to the $\chi^2$ distributions of the $\tau$-values for the three time periods including and excluding rib lesions are provided in Table 6. In the

### Table 4. $\tau$-distribution properties and estimated TB frequency at death for the three time periods modeled with ribs.

<table>
<thead>
<tr>
<th>Time period</th>
<th>N</th>
<th>T</th>
<th>TB frequency</th>
<th>p</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Simulated negative</td>
<td>Simulated positive</td>
<td></td>
</tr>
<tr>
<td>Viking Age</td>
<td>29</td>
<td>Mean</td>
<td>0.50</td>
<td>-0.89</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>4.85</td>
<td>0.83</td>
<td>6.31</td>
</tr>
<tr>
<td>Medieval</td>
<td>507</td>
<td>Mean</td>
<td>0.00</td>
<td>-1.53</td>
<td>3.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>7.84</td>
<td>1.62</td>
<td>11.05</td>
</tr>
<tr>
<td>Post-medieval</td>
<td>216</td>
<td>Mean</td>
<td>0.22</td>
<td>-1.78</td>
<td>3.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>8.57</td>
<td>1.39</td>
<td>10.09</td>
</tr>
</tbody>
</table>

### Table 5. $\tau$-distribution properties and estimated TB frequency at death for the three time periods modeled without ribs.

<table>
<thead>
<tr>
<th>Time period</th>
<th>N</th>
<th>$\tau$</th>
<th>TB frequency</th>
<th>p</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Simulated negative</td>
<td>Simulated positive</td>
<td></td>
</tr>
<tr>
<td>Viking Age</td>
<td>26</td>
<td>Mean</td>
<td>0.90</td>
<td>-0.51</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>4.11</td>
<td>0.30</td>
<td>6.45</td>
</tr>
<tr>
<td>Medieval</td>
<td>479</td>
<td>Mean</td>
<td>0.55</td>
<td>-1.04</td>
<td>2.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>7.58</td>
<td>1.32</td>
<td>6.88</td>
</tr>
<tr>
<td>Post-medieval</td>
<td>201</td>
<td>Mean</td>
<td>0.54</td>
<td>-1.09</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variance</td>
<td>6.75</td>
<td>1.26</td>
<td>6.92</td>
</tr>
</tbody>
</table>

![Figure 2](image.png)

**Figure 2.** Sample frequencies of TB in the three time periods. A: Including rib lesions. B: Not including rib lesions. Grey - Viking, dark grey - medieval, and black - post-medieval.

The results of the goodness of fit of the estimated model to the $\chi^2$ distributions of the $\tau$-values for the three time periods including and excluding rib lesions are provided in Table 6. In the
Viking age sample, the mean and variance indicate an equal distribution of population prevalence ($p$) when ribs are included. That is not true for the model that omits ribs where the $p$-value for the $\chi^2$ distribution is statistical significant. The estimated models of $f(\hat{p})$ for the medieval and post-medieval samples provide significant $p$-values in the model that includes rib lesions. The mean and variance indicate different distributions of TB ($p$) in these two time periods. In the model without rib lesions the mean and variance indicate equal distributions of population prevalence ($p$) for both time periods. The model including ribs fits the Viking age data, whereas the model omitting ribs fits the medieval and post-medieval data. There seems to be a difference in the expression of TB in the three time periods as the weighted contributions of lesions influence the estimates in Viking age differently from the medieval and post-medieval periods.

<table>
<thead>
<tr>
<th>Time period</th>
<th>Including ribs</th>
<th>Excluding ribs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$f(\hat{p})$</td>
<td>$df$</td>
</tr>
<tr>
<td>Viking Age</td>
<td>0.19</td>
<td>1</td>
</tr>
<tr>
<td>Medieval</td>
<td>8.54</td>
<td>1</td>
</tr>
<tr>
<td>Post-medieval</td>
<td>8.32</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 6. Goodness of fit test of the estimation model to the distribution of $\tau$-values for the three time periods.

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Score</th>
<th>Social status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low/common (N=358)</td>
</tr>
<tr>
<td>RIB$^1$</td>
<td>positive</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>253</td>
</tr>
<tr>
<td>VEN$^2$</td>
<td>positive</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>188</td>
</tr>
<tr>
<td>BOD$^3$</td>
<td>positive</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>161</td>
</tr>
<tr>
<td>ACE$^4$</td>
<td>positive</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>203</td>
</tr>
<tr>
<td>ILI$^5$</td>
<td>positive</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>159</td>
</tr>
<tr>
<td>PU$^6$</td>
<td>positive</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>252</td>
</tr>
</tbody>
</table>

1RIB: Periosteal reactions and osteolytic lesions on visceral surface of ribs; 2VEN: Ventral part of thoracic and lumbar vertebral bodies; 3BOD: Lateral body of ilium; 4ACE: Acetabulum; 5ILI: Iliac auricular surface; 6PU: Olecranon process of the proximal ulna.
The raw data for lesion scores in the two socially stratified groups of medieval Ribe are given in table 7. The estimated paleoepidemiological properties of TB-related lesions in the two subsamples that include rib lesions are given in table 8. In both samples the observed \( \tau \)-values fall within the range of the simulated negative and positive values of \( \tau \). The frequency of TB at death ranges between 0.11 (11\%) for the high-status sample and 0.33 (33\%) for the low/common status sample. Confidence intervals for the frequency distributions are narrow for both groups. The estimated paleoepidemiological properties of TB in the two socially stratified subsamples when rib lesions are not included are given in table 9. In both samples the observed \( \tau \)-values fall within the range of the simulated negative and positive values of \( \tau \). Excluding the ribs results in a higher frequency of TB at death (\( p \)) in both low/common and high status graves. The lower social status group has a maximum likelihood estimate of 0.63 (63\%), and the high status group is 0.19 (19\%). The graphs in figure 3 show the sample frequency distributions for the social status groups when all lesions are included (fig. 3.A) and when rib lesions are omitted (fig. 3.B).

<table>
<thead>
<tr>
<th>Social status</th>
<th>N</th>
<th>( \tau )</th>
<th>TB frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Simulated negative</td>
</tr>
<tr>
<td>Low/common</td>
<td>358</td>
<td>0.35</td>
<td>-1.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.90</td>
<td>1.64</td>
</tr>
<tr>
<td>High</td>
<td>141</td>
<td>-0.89</td>
<td>-1.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.51</td>
<td>1.23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Social status</th>
<th>N</th>
<th>( \tau )</th>
<th>TB frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Simulated negative</td>
</tr>
<tr>
<td>Low/common</td>
<td>341</td>
<td>0.90</td>
<td>-1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.66</td>
<td>1.14</td>
</tr>
<tr>
<td>High</td>
<td>131</td>
<td>-0.35</td>
<td>-1.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.08</td>
<td>0.74</td>
</tr>
</tbody>
</table>
The results of the goodness of fit test of the model for estimating population prevalence in different social groups are given in table 10. When including rib lesions the mean and variance indicate significantly different distributions of $f(\hat{p})$ for both social samples. When not including rib lesions, the mean and variance indicate different values of $p$ for low/common social status sample. For the high-social status sample the mean and variance indicate the same estimate of $p$. No model fits the sample of low/common social status very well. The model not including ribs fits the sample of high social status. There seems to be a difference in the expression of TB in the two subsamples as the weighted contributions of lesions influence the estimates differently in the two samples.

**Table 10. Goodness of fit test of the estimation model to the distribution of $\tau$-values for the social status groups.**

<table>
<thead>
<tr>
<th>Social status</th>
<th>Including ribs</th>
<th></th>
<th>Excluding ribs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$f(\hat{p})$</td>
<td>df</td>
<td>p-value</td>
<td>$f(\hat{p})$</td>
</tr>
<tr>
<td>Low/common</td>
<td>7.33</td>
<td>1</td>
<td>&lt;0.01</td>
<td>11.75</td>
</tr>
<tr>
<td>High</td>
<td>6.02</td>
<td>1</td>
<td>0.01</td>
<td>1.37</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This paper presents the first attempt to provide insights into the prevalence of TB in archaeological samples using a strictly paleoepidemiological approach and two models (including ribs and not including ribs). The estimated frequency of TB at death in Viking age Ribe is 53% based upon the model including ribs. The confidence interval is wide because of the
small number of skeletons in the study. Hence, the estimate contributes only limited information about TB during the Viking age. All that can be concluded is that some individuals had TB, and others did not.

The frequency of TB at death in the medieval and post-medieval periods is very similar. For both time periods the frequency of TB at death is ca. 50% based upon the model without rib lesions. That does not mean that one-half of the living population in Ribe had active TB bone involvement. The epidemiological properties of TB-related lesions give an estimate of the frequency of TB among individuals in a sample of the dead. Half of those who died in Ribe had skeletal TB at death. They must have been a strongly selected group of the frailest individuals in the population at a given age. On average, these people were not as healthy as the living in the same age groups (Wood et al. 1992). The high frequency of TB found may be due to the route of transmission of TB. Extra-pulmonary TB is assumed to be prevalent in Ribe in the time periods studied. This assumption is based upon the lesion patterns found in Ribe (Pedersen et al., 2016b). This is also confirmed by the poor fit of the model including ribs in the present study. Cattle breeding and trade in and around Ribe during AD 1400-1600 is believed to have contributed to a high risk of gastrointestinal contraction of bacteria (Pedersen et al., 2016b). Extra-pulmonary infection is not as virulent as infection in the lungs (Francis, 1950). With extra-pulmonary infection it is possible to survive longer and skeletal lesions have time to develop.

There are no studies of archaeological skeletons available that present valid estimates of the frequency of TB at death in past populations. Reviews of reported cases are available (Roberts and Buikstra, 2003; Holloway et al., 2011). No studies of contemporary archaeological samples, however, are available that use comparative methods. The upper range of dating of skeletons from Ribe falls in the late 18th and early 19th centuries. From this time period historical records of TB mortality start to be systematically provided from hospitals and national surveys (Davies et al., 1999; Bello et al., 1999; Ferlinz, 1999; Vuorinen, 1999). Records are available from many European countries including Denmark (Lillebaek et al., 2002). The mortality rates are similar in the European countries. TB mortality peaks at 3-5 deaths per 1,000 around the year 1850. Fifty years later in 1900 the mortality is reduced to half. The decline of TB happens before antibiotics became available for medical treatment in the 1940s, and before the BCG vaccine was introduced in 1924 (Davies et al., 1999). The decline is explained as a multifactorial process of environmental and social changes combined with natural selection of immunity towards TB infection (Davies et al., 1999; Grange et al., 2001). In the present study, the skeletons from Ribe are dated broadly to the Viking through post-medieval periods. It would be of great interest to
apply the approach introduced here to skeletons dating to the 18th century because it would then be possible to compare estimates derived from skeletal data with those from historical records.

The influence of social factors on the risk of TB and risk of dying of TB has been stressed by many scholars (Schoeman et al., 1991; Ferlinz, 1999; Souza et al., 2000; Grange et al., 2001; Gupta et al., 2003; Ladefoged et al., 2011 and Olson et al., 2012). In a study of mortality rates in different districts of Paris during 1865 – 1934, the impact of social factors on TB are evident (Bello et al., 1999). The time span is divided into eight shorter time periods. In each of them the poor and very poor districts had the highest mortality rates. Such social differences in the frequency of TB at death are also found in Ribe in the medieval period. The estimates of the socially stratified groups in Ribe are very different. Low/common status individuals have a three times greater frequency of TB at death when compared to high-status individuals. During the 14th century, national legislation was changed and people were more clearly divided than before into social classes, each with clear legal positions (Jakobsen and Madsen, 2001: 139). The economic wherewithal of the classes was very different, and so were presumably also living conditions. It is therefore believed that the social inequality in Ribe during the AD 1560 – 1660 period reported by Degn (1981: 201) was largely unchanged from the medieval period. The different TB frequencies in the two social status samples in Ribe very likely resulted from variation in living conditions. The goodness of fit test of the two models applied (including ribs and not including them) show different results for the two groups. Neither model fit the low/common social status subsample very well. The model without ribs best fits the high-status group. This indicates that not only the frequency of disease but also its expression is different in the two groups. The majority of people in town belonged to the low/common sub-sample which spanned great diversity in standards of living. This would cause variability of skeletal expression due to divergent immune reactions and risks of contracting TB in this social group. In contrast a minor part of the people in town belonged to the high status sub-sample. The people within this group likely had uniform standards of living and therefore also presumably had the same risk of TB exposure.

The sensitivity and specificity measures used in the τ-statistics are based upon the frequency of lesions in well-documented skeletons from the Terry and Bass collections (Pedersen et al., 2016a). These individuals died of pulmonary TB, and had considerable rib involvement. A study by Pedersen et al. (2016b) demonstrated that differences in lesion frequencies between skeletons from the Terry collection and archaeological skeletons from Ribe were mainly due to rib involvement. The discrepancy of estimated frequency of TB at death when including and not including ribs is consistent with this interpretation. Only for the Viking age sample does the
mean and variance indicate the same frequency of TB at death when estimated with the model including ribs. The skeletal expression of TB in the medieval and post-medieval periods differs from modern skeletons from Terry collection. A change, however, may also have occurred from the Viking to medieval periods (after AD 1050) as indicated by the goodness of fit tests. The most reliable results for the Viking age are derived from the model including ribs, so the pattern of skeletal involvement resembles the modern sample more than it does those of the two other historical periods. Likely causes are complex processes of evolution of both the pathogen’s genetic makeup and host immunogenetics, along with changes in socioeconomic factors and living conditions (Bloom and Small, 1998; Malik and Geodfrey-Faussett, 2005; McMichael, 2004; Narasimhan et al., 2014). The small sample of Viking age skeletons makes it difficult to explore the differences further in this study. More skeletons dating to before AD 1050 need to be examined to confirm or reject changes in skeletal expression across time.

In summary, we find that the skeletal expression of TB may have changed from the Viking to medieval periods. Furthermore, the frequency rates of TB at death are great in medieval and post-medieval times. Finally there are great differences in the frequency of TB at death in socially stratified samples dating to the medieval period; that is, social status at that time seems to be an important risk factor for TB. The results confirm the complexity of studying TB in the past using skeletal remains (Waldron, 1999; Dutour, 2008). The multifactorial causation behind the disease and the evolution of host-pathogen interactions cause great differences in the expression of disease both between and within populations. The patterns of skeletal expression in the modern samples used for modelling $\tau$-statistics are not applicable to the archaeological populations in Ribe. In further studies of the paleopidemiological properties of TB it will be necessary to take into account the differences in probability measures of lesions between populations and across time.

ACKNOWLEDGEMENT

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APPENDIX 1

A recording manual for osteological lesions related to tuberculosis

Six osteological lesions are recorded in skeletons and provide data for the paleoepidemiological approach to the study of TB presented in this paper. The criteria for scoring the lesions as presented in this manual are reproduced from descriptions in Pedersen et al. (2016a). The lesions can be present in the bones with different degree of severity. For ensuring simplicity in the paleoepidemiological modeling of TB the lesions are scored dichotomous as absent (0) or present (1). None of the lesions are diagnostic of TB on their own. Each lesion contributes to the probability of TB and the more lesions present the more likely a diagnosis.

Visceral surface of ribs

Location: Potential TB lesions are recorded on visceral surface of the body of ribs.

Scores:

/: No information. Less than three pieces of ribs larger than 5 cm are preserved.

0: No bone changes related to TB are present (figure 4.A and 5.A).

1: Two or more pieces of ribs larger than 5 cm have either:

- Periosteal reactions with new bone formation (figure 4.B and C).
- One or more shallow or deep osteolytic lesion measuring more than 5 mm in diameter (figure 5.B and C).

Figure 4.A-C: Periosteal reactions on visceral surface of ribs. A. Without periosteal reaction (Δj1 = 0). B. Slight periosteal reaction with new bone formation (Δj1 =1). C. Severe periosteal reaction with new bone formation (Δj1 =1). Photos by DD Pedersen.

Figure 5.A-C: Osteolytic lesions on visceral surface of ribs. A. Without osteolytic lesions (Δj1 = 0). B. Deep osteolytic lesions (Δj1 =1). C. Shallow osteolytic lesions (Δj1 =1). Photos by DD Pedersen.
Ventral part of thoracic and lumbar vertebral body

Location: Potential TB lesions are recorded on ventral surface of thoracic and lumbar vertebral bodies. Thoracic and lumbar vertebrae are recorded separately.

Scores:
/l: No information. Less than two thoracic and lumbar vertebral bodies are preserved or more than 50% of the bone surface of those preserved is damaged by post mortem changes.
0: No bone changes related to TB is present (figure 6.A and 7.A).
1: Two or more thoracic or lumbar ventral surfaces have either:
- Periosteal changes with woven or lamellar structure on at least 50% of the bone surface (figure 6.B and C).
- Three or more large pits with circular shape and rounded edges measuring at least 3 mm in diameter (figure 7.B). In severe cases an abscess/cloaca is formed (figure 7.C).

![Figure 6.A-C: Periosteal changes on ventral part of vertebral body. A. Without periosteal changes ($\Delta_{ij} = 0$). B. Slight periosteal changes ($\Delta_{ij} = 1$). C. Severe periosteal changes($\Delta_{ij} = 1$). Photos by DD Pedersen.](image)

![Figure 7.A-C: Large pits on ventral part of vertebral body. A. Without pits ($\Delta_{ij} = 0$). B. Large pit with circular shape and rounded edges($\Delta_{ij} = 1$). C. Large pits and abscess/cloaca($\Delta_{ij} = 1$). Photos by DD Pedersen.](image)

Lateral body of ilium

Location: Potential TB lesions are recorded on lateral body of ilium between lower gluteal line and the upper acetabular margin.

Scores:
/l: No information. Less than 50% of body of ilium is preserved.

1: One of the following lesions is present:

- Periosteal changes with woven or lamellar structure on at least 50% of the bone surface (figure 8.B). In severe cases an abscess can be present (figure 8.C).
- Three or more large pits with circular shape and rounded edges measuring more than 3 mm (figure 9.B and C).

![Figure 8.A-C: Periosteal changes on lateral body of ilium. A. Without periosteal changes (Δj3 = 0). B. Periosteal changes with woven structure (Δj3 = 1). C. Periosteal changes with lamellar structure and abscess (Δj3 = 1). Photos by DD Pedersen.](image)

![Figure 9.A-C: Large pits on lateral body of ilium. A. Without large pits (Δj3 = 0). B. Large pits (Δj3 = 1). C. Large pits and periosteal changes (Δj3 = 1). Photos by DD Pedersen.](image)

**Acetabulum**

*Location:* Potential TB lesions are recorded on acetabular fossa and articular surface of acetabulum.

*Scores:*

1: No information. Less than 50% of fossa and/or lunate surface are preserved.

0: No bone changes related to TB is present (figure 10.A and 11.A).

1: One of the following lesions is present:

- Clustered pitting or erosive cavities on articular surface in two or more areas each measuring more than 3 mm or in one area measuring more than 10 mm (figure 10.B and C). In severe cases an abscess can be present (figure 10.C).
- Two or more deep cavities in acetabular fossa each measuring more than 3 mm or more than 50% of the area has a woven structure with dense trabeculae (figure 11. B and C).

![Figure 10.A-C: Clustered pitting and erosive cavities on articular surface of acetabulum. A. Without erosive cavities (Δj4= 0). B. Clustered pitting and shallow erosive cavities (Δj4= 1). C. Deep erosive cavities and abscess(Δj4= 1). Photos by DD Pedersen.](image1)

![Figure 11.A-C: Cavities and woven structure in acetabular fossa. A. Without cavities or woven structure (Δj4= 0). B. Deep cavity(Δj4 = 1). C. Woven structure(Δj4 = 1). Photos by DD Pedersen.](image2)

**Iliac auricular surface**

*Location:* Potential TB lesions are recorded on iliac auricular surfaces.

*Scores:*

1: No information. Less than 50% of the iliac auricular surfaces are preserved.

0: No bone changes related to TB is present (figure 12.A).

1: Clustered pitting (figure 12.B) or erosive cavities (figure 12.C) in two or more areas each measuring more than 3 mm or in one area measuring more than 10 mm.
Location: Potential TB lesions are recorded on olecranon process of proximal ulna.

Scores:

/: No information. Less than 50% of proximal ulna is preserved.

0: No bone changes related to TB is present (figure 13.A).

1: Clustered pitting (figure 13.B) or erosive cavities (figure 13.C) on olecranon process in two or more areas measuring more than 3 mm or in one area measuring more than 10 mm.

Figure 12.A-C: Clustered pitting and erosive cavities on iliac auricular surfaces. A. Without clustered pitting or erosive cavities ($\Delta_{j5} = 0$). B. Clustered pitting ($\Delta_{j5} = 1$). C. Erosive cavities($\Delta_{j5} = 1$). Photos by DD Pedersen.

Olecranon process of proximal ulna

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