Exploring Prehistoric Tuberculosis in Britain: A Combined Macroscopic and Biomolecular Approach

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Abstract

Tuberculosis (TB) is a bacterial, infectious disease, currently responsible for millions of deaths worldwide. Although the aetiology of the disease in its current form is well documented in the clinical literature, little is known of the form the disease took in earlier times, or the time at which it first entered Britain. This study aimed to test the hypotheses that TB was present in British prehistory, (as it was in Europe), prior to that previously identified in the Iron Age (Mays and Taylor, 2003) and that the infection was caused by both *M. tuberculosis* and *M. bovis*; the latter most commonly contracted from cattle. The objective of the project was to use ancient DNA (aDNA) from human skeletons to study the bacteria responsible for TB (*M. tuberculosis* complex) in order to then study the origin and evolution of the strains of the bacteria causing TB in prehistoric Britain. Thirteen individuals from Neolithic, Bronze and Iron Age sites in the south of England (comprising inhumations of reasonable preservation), were selected for inclusion in the project, based on non-specific evidence of infection, potentially representative of early tuberculous skeletal involvement. A biocultural approach was employed in order to better understand the environmental and social context from which the samples originated. The geographical area under study was limited to the south of Britain, (with the exception of Wetwang Slack in Yorkshire) because of the direct contact between Britain and the continent in this region. Biomolecular analysis did not produce positive results for TB, the reasons for which may include poor preservation of pathogen aDNA, and thus, no conclusive evidence was found of the presence of TB in prehistoric Britain prior to that already identified. Problems encountered during the project were highlighted in an effort to improve efficiency of future projects, with suggestions as to how this study may be extended in order to allow development of a much more comprehensive history of TB in Britain to be formed; its origins, spread and possible impact on ancient British populations.

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For My Parents

1 INTRODUCTION

Tuberculosis (TB) is a disease that has plagued both humans and animals alike, throughout documented history, and across the world. Evidence increasingly demonstrates that many suffered from the disease prior to records, during prehistory. This project is borne out of the need to delve further into the British past of this deadly disease, using the primary evidence available – the remains of the people who suffered. Traditional macroscopic identification methods, combined with biomolecular techniques, allow for comprehensive analyses of skeletal material. Fusing these with a biocultural approach throughout the course of this project, to understand both the environmental and social conditions to which people were subject, enabled greater appreciation of the potential for the presence and transmission of the TB pathogen in prehistoric Britain.

1.1 Aims:

The aim of this project was to test the hypothesis that TB existed in Britain prior to the Iron Age and that the infection was caused by both *M. tuberculosis* (the primary cause of TB in humans today) and *M. bovis* (the primary cause of TB in animals).

1.2 Objectives:

The objectives were to use pathogen ancient DNA (aDNA) from human skeletons to study the bacteria responsible for TB (*M. tuberculosis* complex) in order to then study the origin and evolution of the strains of the bacteria causing TB in prehistoric Britain.

1.3 Background:

TB is currently a major world health threat, particularly in populations where people's immune systems are weakened by other diseases such as HIV (human immunodeficiency virus) and AIDS (acquired immunodeficiency syndrome), and where healthcare is often

limited. As a reemerging disease responsible for millions of deaths every year, a great deal of modern clinical study surrounds the disease in its current form, but little is known of its origins and development throughout prehistory, particularly in Britain.

The earliest skeleton with TB recorded in Britain dates from the Iron Age (400–230 BC), at a site called Tarrant Hinton in Dorset (Mays and Taylor, 2003). The disease is visible in proceeding time periods with examples identified from the Roman period onwards (Stirland and Waldron, 1990, Anderson, 2001), with frequencies seeming to increase through time (Roberts and Buikstra, 2003: 132). Many earlier populations throughout the world also exhibit evidence of the disease. Globally, the earliest accepted evidence of TB originates in Italy during the Neolithic period (5800+-90BP) (Canci *et al*., 1996) and other early examples include human remains in Egypt (4500BC) (Morse, 1967). Given the presence of TB during domestication of plants and animals elsewhere in the world, including Europe, it is thought that TB is likely to have been present in Britain during the Neolithic and Bronze Ages. There are, however, a number of reasons why such evidence may be lacking, not least because there are relatively limited human remains available for study from such early periods. Other reasons may be that, with little immunity to the infection, people did not live long enough for evidence of the disease to manifest in the bones, or even that when many skeletons were originally excavated workers had had little or no training in identifying disease in skeletons, and therefore examples remain undocumented.

Until recently, many theorized that *M. bovis* predated *M. tuberculosis* in humans, the latter evolving from the former due to the domestication of animals. Evolutionary studies of TB have disproven this theory and the reverse shown to be true (Brosch *et al*., 2002). However, it is unknown which strains of the disease affected people throughout British prehistory and beyond. Only with the development of aDNA analysis has the opportunity to study the specific cause of TB in human skeletons become possible. The first confirmed cases of *M. bovis* causing TB in human remains date from the Iron Age in Siberia and were reported in 2007 (Taylor *et al*., 2007).

This study hopes to add to what is currently a sparsely documented history of TB in prehistoric Britain. Investigating whether any evidence of the disease can be found

predating the current earliest example, and identifying the specific infecting agent, may allow for development of a much more comprehensive history of the disease to be formed; its origins, spread and possible impact on ancient British populations.

The project forms part of a wider NERC funded project: *Biomolecular archaeology of ancient tuberculosis in Britain and Europe* (http://www.dur.ac.uk/archaeology/research/projects/?mode=project&id=353) joint between Durham University and Manchester University.

1.4 Methods

There are two main elements to the study; macroscopic analysis and pathogen aDNA analysis. Skeletons were selected for aDNA analysis based on the outcome of the macroscopic analysis, i.e. those exhibiting possible or probable evidence of the disease. TB can manifest itself on the human skeleton in many different ways, and thus there are many different skeletal indicators that must be identified before TB can be suggested as the cause. This may include both bone formation and/or destruction (Roberts and Buikstra, 2003: 88) and possible evidence of the disease includes both direct and indirect skeletal indicators. Direct evidence such as Potts disease (destruction and collapse of the vertebral bodies, leading to kyphosis) is perhaps the most recognisable and convincing of all skeletal indicators. TB affects the skeleton in 3-5% of people with the disease, and it is thought to affect the spine 25-50% of the time in those untreated (Resnick and Niwayama, 1995: 2462). Second to spinal involvement, weight bearing areas of the body, particularly the hip and knee, are also commonly affected, the hip accounting for 20 per cent of skeletal involvement (Aufderheide and Rodríguez-Martín, 1998: 138). Indirectly, lesions on the visceral surfaces of ribs, indicative of a respiratory infection, may also be suggestive of TB (Roberts, 1999).

A number of studies have provided evidence of skeletal TB based on macroscopic study alone and many provide convincing evidence of the disease (Anderson, 2001, Formicola *et al*., 1987, Pfeiffer, 1984), but differential diagnoses must always be considered, and there

are no known skeletal indicators differentiating infection caused by *M. bovis* or *M. tuberculosis,* (although, of the two, *M. bovis* is more likely to cause skeletal changes (Stead, 2000), making advances in aDNA analysis invaluable.

Ancient DNA analysis of organisms responsible for disease in skeletons from archaeological sites has been practised for over 18 years in the UK and elsewhere in the world, although the UK has been one of the leaders in the field (Brown, 2000, Brown and Brown, 1992, Hagelberg *et al*., 1989, Kaestle and Horsburgh, 2002, Stone, 2000, Herrmann and Hummel, 1994). With the development of modern genomic studies, it is also now possible to look at the strains of organisms causing disease, and thus consider how they have evolved and changed. The vast majority of aDNA work has involved TB, possibly because the pathogen is believed to survive much better than others over long periods of time (Zink *et al*., 2002, Donoghue *et al*., 2004), and much of this work has been done in the UK (Mays *et al*., 2001, Taylor *et al*., 1999, Taylor *et al*., 2005). This study is important for understanding TB's place in British prehistory and elsewhere in Europe, and is well placed, drawing from a body of experience and continually improving techniques.

1.5 Benefits:

Biomolecular archaeology can make great contributions to the study of past disease, not just in terms of disease frequency, but also the evolution of pathogenic organisms. In conjunction with the work of other groups who are attempting to progress the history of TB towards evolutionary issues, the project aims to establish biomolecular archaeology as an important means of studying the evolutionary biology of human disease. The biocultural approach taken here will also, it is hoped, encourage others to work with those whose expertise is outside of bioarchaeology, and who can be complementary to gaining a greater understanding of disease in the past.

The cross-disciplinary nature of the research means that the work will also be relevant to a variety of end-users/beneficiaries:

- **Archaeologists and historians** (information will supplement what little we know about the origin and evolution of TB in Britain)
- The data and new methods will provide source material for other **biomolecular archaeologists** wishing to conduct similar studies
- **Epidemiologists** and **clinical researchers** concerned with the recent resurgence of TB will benefit from the greater understanding of the recent evolution of the causative organisms, including the possible identification of key factors influencing the disease in the past that might be relevant today
- The **general public** will benefit from a project which, because of its underlying foundation in human history, and because of the increasing importance of TB today, it will be of interest to many sectors of the community, including those most vulnerable to TB such as the poor and those with HIV/AIDS.

1.6 Chapter Outlines:

Chapter one states the aims and objectives of the project, an introduction to the subject and the methods employed during the course of this project. The benefits of such work are presented, along with chapter outlines.

Chapter two presents a comprehensive and up to date commentary of TB, including its known history, global impact, pathogenesis, transmission and the methods used to identify and combat the disease today. The history of the disease at first takes a global view and tackles evidence of the disease both in the Old World and New Worlds. Focus is then placed on Britain and what is currently known about its long and uneasy relationship with TB from the Iron Age up to 21st century outbreaks in our inner city schools. The pathogen itself is also considered and, crucially, the effects of the disease on the body are summarised (and expanded upon in chapter four).

The history of the use of aDNA is also summarised here, and an explanation provided as to how previous research has led to this point in our understanding of the technique and its

wide range of possible applications. Other biomolecular methods are also touched upon, in order to place the advantages and disadvantages of aDNA techniques into a wider context.

Chapter three explores the prehistoric periods to which the study pertains, being the British Neolithic, Bronze Age and Iron Age, with a clear focus on the aspects most connected with disease susceptibility, transmission and maintenance of the pathogen. Areas discussed, include the role of climate, diet, population, migration and, crucially, the health impacts of the transition to agriculture. Modern studies are combined with archaeological evidence to explore the health impact of the many social, economic and environmental changes seen over the long time span of these periods and their relevance to TB.

It is hoped that this chapter will enable the reader to better appreciate the need for interdisciplinary studies of this kind, and the need to collate data from a wide range of sources in order to re-evaluate current levels of understanding of past human health, in this case, specifically the past human relationship with TB, based on the material available for analysis.

Chapter four presents the materials and methods utilised during the course of the study. This chapter is split into three main sections. Firstly, the sites from which the skeletal material originates are briefly described, with a focus on their geographical location and the details surrounding the recovery of the specific individuals chosen for analysis. The bone sample selected for aDNA analysis is then presented including a basic description, the reason why it was selected, and the method by which it was removed from the skeleton.

The second section presents the methods employed during the macroscopic element of the study. This includes basic osteological techniques for estimating biological sex, age at death and recognition of pathology. Many of these techniques have been modified since their original inception, the reasons for this, and their reliability are discussed.

Section three refers specifically to the aDNA analytical methods employed, with a detailed step by step account of the process, from preparation and extraction through to PCR

(polymerase chain reaction), gel electrophoresis, and direct sequencing. Steps taken to prevent contamination with modern DNA and alien PCR products are described.

Chapter five presents the results of both the macroscopic and aDNA methods. Details of each individual studied macroscopically, provide details as to sex, age at death, and overall pathology – the primary pathology, that is the pathological lesions which led to their being selected for inclusion in the study, is highlighted.

The aDNA results are presented in clear, tabulated form and include details of repeat analyses. Original data is referred to in the appendix.

Chapter six is the discussion. In this chapter, the information presented in the preceding four chapters, is synthesised, to enable a more coherent interpretation of the results. This begins with a more in depth look at the primary pathological lesions of all the individual skeletons included in the project, with some comments as to possible differential diagnoses. These results are then placed in a wider context by discussing them within their specific time period and, where possible, the specific site from which they were excavated.

Chapter seven (the conclusion), summarises the key findings of the project, followed by limitations faced within the study and in a wider context; specific details of many of the experiences in locating and accessing skeletal material are referred to. This section hopes to act as a cautionary tale to others but also highlights aspects which need to be addressed. Suggestions for further work follow, with many of the limitations presented previously, acting as a springboard for improved efficiency of future studies. Possible extensions to the study are discussed with explanations as to why particular suggestions are offered.

Finally, concluding remarks will present the original aims and objectives of the study, the results achieved and possible reasons for them, followed by a reiteration of why changes need to be made to improve future research and how this project, in particular, would benefit from further work.

2 TUBERCULOSIS

2.1 Background of the Disease

2.1.1 Tuberculosis Today

Tuberculosis (TB) has plagued humans and animals alike for thousands of years and is now considered an "emerging infectious disease" (EID) - defined as 'infections that have newly appeared in a population or have existed previously but are rapidly increasing in incidence or geographic range' (Morse, 1995:7) – or under this umbrella term, specifically, a "re-emerging/resurging" infectious disease; we are in effect 'in the midst of a war with the microbial world…' (Fauci, 2004: 1887). The acquired immune deficiency syndrome (AIDS) (a "newly emerging' disease") is now looking likely to exceed the death toll of the Black Death and the 1918 Influenza pandemic (Morens *et al*., 2004), and that the peak incidence rates of EIDs occurred in the 1980s, simultaneously with the HIV (Human Immunodeficiency Virus) pandemic, is no coincidence (Jones *et al*., 2008). In addition to biological factors, a number of socio-economic, environmental and ecological factors have been proven to be closely associated with the occurrence of EIDs (Jones *et al*., 2008) (including TB), which are summarised in Table 2-1.

Factor	Examples of specific factors
Ecological changes (including those due to economic development and land use)	**Agriculture**; dams, changes in water ecosystems; **deforestation/reforestation; flood/drought; famine; climate changes**
Human demographics, behaviour	Societal events: **Population growth and migration** (movement from rural areas to cities); **war or civil conflict**; urban decay; sexual behaviour; intravenous drug use; **use of high-density facilities**
International travel and commerce	Worldwide **movement of goods and people**; air travel
Technology and industry	Globalization of food supplies; changes in food processing and packaging; organ or tissue transplantation; drugs causing immunosuppression; widespread use of antibiotics
Microbial adaptation and change	**Microbial evolution**, response to selection in environment
Breakdown in public health measures	Curtailment or reduction in prevention programs; **inadequate sanitation** and vector control measures

Table 2-1 Factors Affecting the Emergence/Re-emergence of Infectious Disease. Data adapted from the Summary presented in Morse (1995: Table 2).

Examples highlighted are factors also likely to have impacted on the emergence of TB during prehistory discussed further in Chapter 2.

TB has been recognised as a serious global health problem for many years and it is clear that the re-emergence of the disease is a global emergency. Attention really refocused on the problem of TB, from a modern perspective, at the beginning of the 1990's when America suffered outbreaks of multidrug-resistant TB (MDR-TB) (Corbett and Raviglione, 2005: 3), and the World Health Organisation (WHO) declared a global health emergency (WHO, 2004). A number of studies were published, detailing incidence, mortality, prevalence, trends and reasons for the re-emergence of TB around the world (Raviglione *et al*., 1995, Dye *et al*., 1999, Bloom and Murray, 1992), although questions have been asked about the reliability, quality and possible biases behind the data (van der Werf and Borgdorff, 2007, Walker *et al*., 2007a). Suggestions as to how to control, or rather "combat" the disease were also offered (Rieder, 1998, Young and Robertson, 1998).

TB control programmes, from the 1980's onwards, such as DOTS (directly observed treatment short course) were employed to try and reverse the global rise of TB and, according to the most recent WHO report on *Global Tuberculosis Control,* DOTS has been responsible for up to 6 million lives being saved and 41 million patients being treated successfully between the years of 1995 and 2009 (2010a). In 2006, WHO launched a new initiative, the 'Stop TB Strategy' which aims to reduce the global burden of TB by 2015. Its objectives include: achieving worldwide access to 'high quality diagnosis and patient-centred treatment', reducing suffering and alleviating the socioeconomic burden associated with the disease, protecting poor and vulnerable populations against TB, and supporting the development of new tools and putting them to effective use (WHO, 2006: 6). Previously, in 2002, the *Global Fund for Aids, Tuberculosis and Malaria* was created to provide grants for programs to fight the disease (Fluck, 2004), and the European Commission also invested heavily in combating these three communicable diseases, having set aside around 130 million euros for research between 1999 and 2002 (European Commission, 2001). The target set by the Millennium Development Goals (MDGs) is that TB incidence should be falling by 2015 and The Stop TB Partnership's recent, additional targets, are to halve rates of prevalence and mortality by 2015 compared with 1990 levels (WHO, 2010a). It does now appear that after many years of increasing prevalence rates, progress is being made (Figure 2-1).

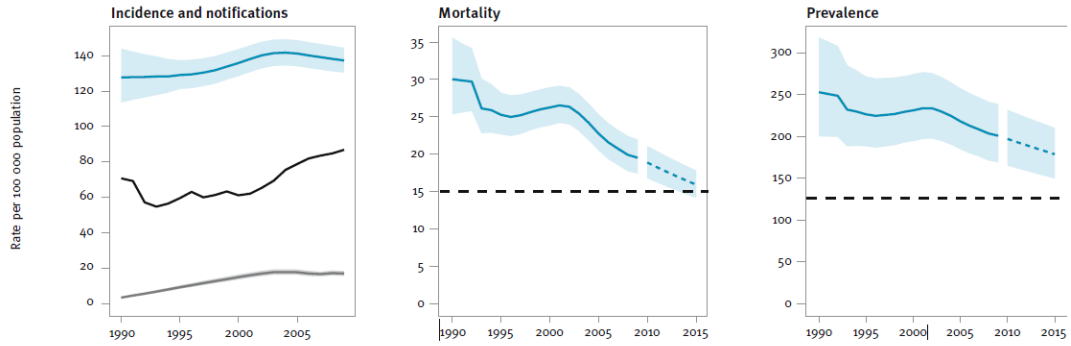

Figure 2-1 Global Trends in TB Notification Rates, Estimated Incidence, Mortality and Prevalence. (WHO, 2010: Figure 25).

(Left) The blue line represents estimated incidence rate including HIV-positive TB, the grey line, the estimated incident of HIV-positive TB and the black, all forms of TB including new and relapse cases. (Centre and right) Trends in global TB mortality and prevalence between 1990 and 2009, plus forecast figures between 2010 and 2015. Dashed lines represent the 2015 target levels. Mortality excludes deaths amongst HIV-positive people.

2.1.2 Current Global TB Trends

According to the WHO, TB was responsible for 1.6 million deaths in 2005 and, in 2008, a new person was infected every second with the disease (2008a). Incidence and death rates of TB in Africa and South East Asia far outstrip those seen elsewhere in the World, most notably when the rates are directly compared between Europe and The Americas (Table 1.2). With the exception of the South East Asia region, incidence rates are now declining globally (WHO, 2010a) (Figure 2-1) and, based on current data, it seems as though all WHO regions may achieve the 50% mortality and prevalence reduction target as set out by The Stop TB Partnership; Africa is the exception to this but a drop in mortality rates is still evident (WHO, 2010a). Despite this success, the disease remains a significant health crisis with one third of the world's population estimated to be infected with the disease. Not all of those carrying the infection, however, will develop active infection due to the methods by which the infection is triggered (See 2.1.6).

	Incidence[1]			Prevalence[2]		Mortality (excl. HIV)	
WHO region	No. in thousands	% of global total	Rate per 100 000 pop[3]	No. in thousands	Rate per 100 000 pop[3]	No. in thousands	Rate per 100 000 pop[3]
Africa	2 800	30%	340	3 900	450	430	50
The Americas	270	2.9%	29	350	37	20	2.1
Eastern Mediterranean	660	7.1%	110	1 000	180	99	18
Europe	420	4.5%	47	560	63	62	7
South-East Asia	3 300	35%	180	4 900	280	480	27
Western Pacific	1 900	21%	110	2 900	160	240	13
Global total	9 400	100%	140	14 000	164	1 300	19

[1] Incidence is the number of new cases arising during a defined period.
[2] Prevalence is the number of cases (new and previously occurring) that exists at a given point in time.
[3] Pop indicates population.

Table 2-2 Estimated Global, Incidence, Prevalence and Mortality Rates, 2009 (WHO, 2010).

Summarised Data Table from most recent WHO Report (2010) available, this version available at: **http://www.who.int/mediacentre/factsheets/fs104/en/**

2.1.3 TB in Britain Today

Britain is spared the high death toll seen in other regions of the world, but TB has not been eradicated in this country as once hoped. Occasional outbreaks have been noted, in recent years, in certain British schools, which may be linked to foreign travel, particularly to India (Hodgson, 2008, BBC News, 2001); comparison of TB data demonstrates the high burden of the disease in India in comparison to the UK (Table 1.3). The UK, and thus Britain, has a low burden of TB in a global context but there are problems in densely populated cities, and this is clearly demonstrated in London. London accounts for almost half of the cases of TB in Britain, and a 2007 study reported an increase in reported cases in the 15 years prior (Anderson *et al.*, 2007). Of note is the fact that the vast majority of patients with tuberculosis in London were born abroad, with India, again, being the most frequently stated country of birth. Earlier studies also noted this increased burden in urban areas and high rates of TB in those recently arriving from high prevalence areas of the world (Rose *et al.*, 2001, Crofts *et al.*, 2008). Others have found that socio-economic status has more bearing on TB rates than ethnic origin (Bhatti *et al.*, 1995) although these two factors are

interlinked and, as Grange *et al*, state in relation to the rise in TB seen in London and other European capitals, 'the emergence of the disease brings into sharp relief the interconnectedness of global concerns, including poverty, inequality and exclusion.'(2009).

Estimates of burden * 2009	Number (thousands)	Rate (per 100 000 pop)
Mortality (excluding HIV)	0.35 (0.28–0.47)	0.57 (0.46–0.77)
Prevalence (incl HIV)	9.1 (2.9–16)	15 (4.8–25)
Incidence (incl HIV)	7.4 (6.6–8.6)	12 (11–14)
Incidence (HIV-positive)	0.26 (0.12–0.47)	0.43 (0.19–0.76)
Case detection, all forms (%)	94 (81–110)	

Estimates of burden * 2009	Number (thousands)	Rate (per 100 000 pop)
Mortality (excluding HIV)	280 (160–430)	23 (14–36)
Prevalence (incl HIV)	3 000 (1 300–5 000)	249 (105–419)
Incidence (incl HIV)	2 000 (1 600–2 400)	168 (137–202)
Incidence (HIV-positive)	130 (54–240)	11 (4.5–20)
Case detection, all forms (%)	67 (56–83)	

Table 2-3 Comparison of TB Burden between the UK (above) and India (below) (Adapted from WHO, 2010).

2.1.4 Current TB Frequencies in Relation to HIV

One of the most dangerous developments intimately associated with the global rise in TB, has been the rise of HIV and AIDS (Havlir and Barnes, 1999); people who have HIV infection and carry the TB infection are 20 to 30 times more likely to develop active TB (Centres for Disease Control and Prevention, 2011). Since 1990 HIV has shown to be the single most important contributing factor to the rise in TB incidence in Africa (WHO, 2010a) and in 1999, and in Sub-Saharan Africa where the majority of HIV infected individuals live, two thirds were co-infected with both TB and HIV (Dye *et al*., 1999). This high burden of co-infection in Africa was still evident in 2009 (Figure 2-2). In 2000, it was estimated that, globally, 11.4 million people were infected with both HIV and *M. tuberculosis* (30% of the world's population), with the largest numbers of co-infected adults occurring in South Africa, (2 million), India, (1.7 million) and Nigeria, (0.9 million). In that year another half a million new cases (511,000) of active TB associated with HIV occurred (Corbett *et al*., 2003); by 2005, this figure had reached 526,000 (WHO; 2007).

Such high rates of co-infection stem from the synergistic nature of the interaction between HIV and TB, each increasing the pathogenicity of the other (de Jong *et al*., 2004). The two diseases have been called 'the cursed duet' (Sanduzzi *et al*., 2001: 294), as those with HIV can progress more quickly to active TB if infected with the disease, and those already hosting latent TB are more like to progress to active TB if infected with HIV (Parrish *et al*., 1998). Infection with HIV depresses a person's immune system, putting them at greater risk of infection with TB, and vice versa. To complicate matters further, not all drug regimes used to treat TB are recommended for those who are co-infected with both diseases (American Thoracic Society, 2003). However, in light of the global financial crisis, better integration of HIV and TB services has been suggested which, given the close association between the two diseases, may in fact strengthen the control of both TB and HIV infection (Maher, 2010).

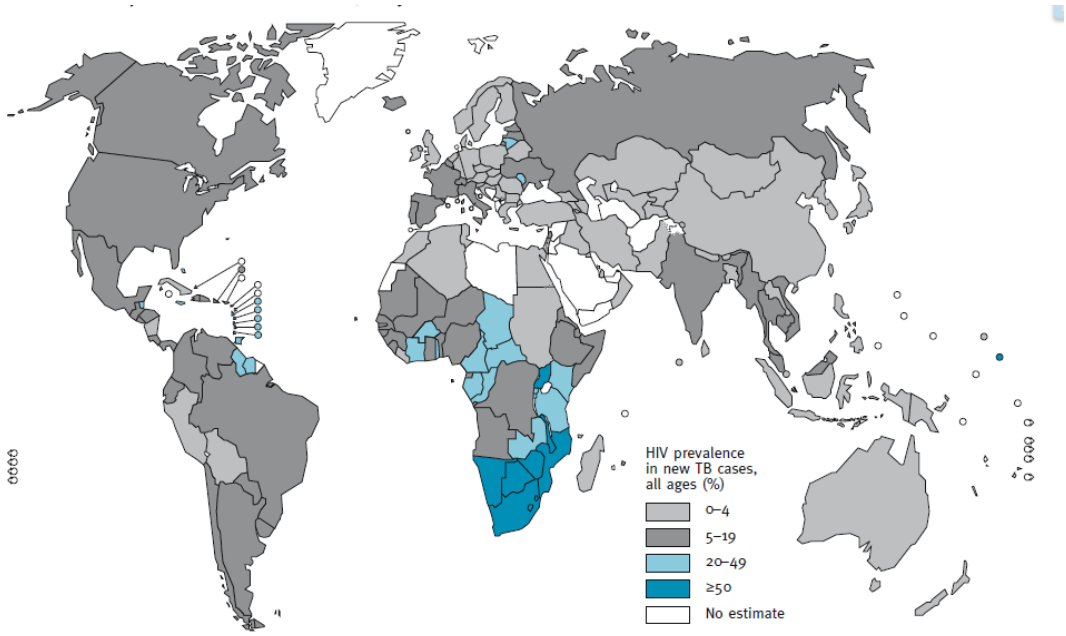

Figure 2-2 Estimated HIV Prevalence in New TB Cases, 2009 (WHO, 2010)

To illustrate the speed at which HIV has been, and continues to be, such a dangerous factor in increased susceptibility to TB, even outside of Africa and other high burden areas, a study of TB in Russia stated that 'Between 1987 and 1993, there have been 647 infected people [with HIV]…According to the Russian data, there have not been cases of TB among HIV positive people, and this is not a factor in the increase in tuberculosis in Russia, unlike in other parts of the World.' (Aksenova *et al*., 1995: 28). Just five years after this study was

published, over a thousand cases of TB in Russia were attributable to HIV (Corbett *et al*., 2003).

2.1.5 The development of drug resistant TB

There are many strains of TB that are resistant to drug therapy, and the two most dangerous types of drug resistance TB as outlined by WHO (2010a) are MDR-TB (multi drug resistant TB) and extensively drug-resistant (XDR). MDR-TB is resistant to the two most effective TB drugs: isoniazid (INH) and rifampizin (RIF) but XDR-TB is resistant to these two drugs in addition to others, with at least one being a second line defence drug. According to the same report, drug resistant strains have been documented in every country surveyed, and by 2010, 58 countries had reported at least one XDR-TB case. China, India, the Russian Federation and South Africa demonstrated the largest number of estimated cases if of MDR-TB in 2008.

Treatment failure is one of the key factors behind the evolution of antibiotic resistant strains of the disease. A person is especially susceptible to drug resistant strains if they have a history of previous treatment for TB, particularly if that treatment was inadequate or incomplete (Chakrabarti and Do Davies, 2007, Dias-Baptista *et al*., 2008, Ganguly and Katoch, 2000). Treatment that is not completed, or is interrupted, allows secondary drug resistance to occur; primary drug resistance appears when a person is initially infected with already resistant bacilli (Fluck, 2004: 17). In addition, the problem itself may be the drugs themselves being of poor quality, or being unavailable, termed “low bioavailability” (Fluck, 2004: 17). Placing patients on four-drug combination therapy reduces the chance of tubercle bacilli developing resistance, as they would have to develop resistance to the four separate drugs (Fluck, 2004: 20), although the rise of XDR-TB clearly demonstrates this to be an increasing possibility.

2.1.6 Pathogenesis of TB

TB is a bacterially induced infectious disease which when passed between humans (sometimes between humans and animals), is done so via inhaling droplets containing the

bacteria. Sneezing, coughing, talking, singing and spitting can all lead to the release of infectious particles into the air (Figure 2-3) (WHO, 2010b) which can remain there for hours; the infectious dose is highly efficient, being just one to ten bacilli (Russell *et al*., 2009). *M. tuberculosis* is the primary cause of TB in humans and *M. bovis* is the primary cause of TB in animals although, as a zoonotic disease, both humans and animals can be affected by either, most usually *M. bovis* being passed to humans from animals. There are numerous animals which can be affected by TB but cattle are considered to pose the greatest infection threat to humans (Grange, 1995). Although humans are more likely to acquire the disease caused by *M. bovis* by drinking infected milk or eating infected meat, it can also be acquired through droplet infection. Depending on the type of infecting bacillus and its pathway into the body, the bacilli will lodge themselves in the lungs or gastrointestinal tract and from there may spread around the body via the blood or lymphatic system.

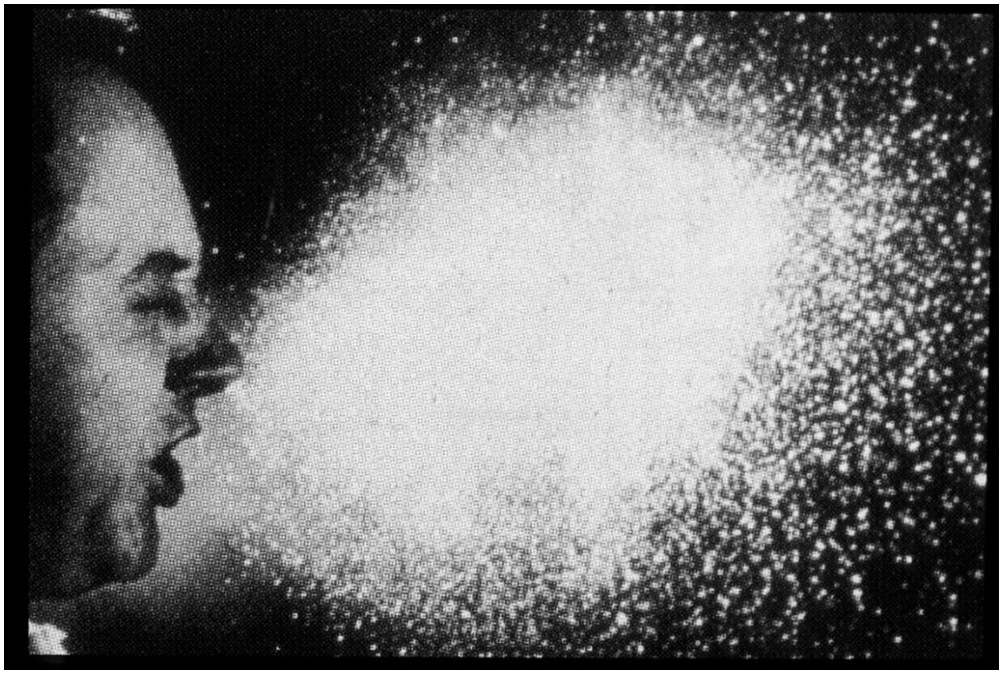

Figure 2-3 Release of sputum during sneezing; TB bacilli can also be released into the air via this method by a person suffering from active TB. (Source unknown, supplied by Charlotte Roberts).

Known as a biphasic disease, primary infection (where a patient has not been infected before) occurs usually in childhood and a secondary infection can follow (also referred to as post-primary infection) by a process of reactivation of dormant bacilli or re-infection (Chan

and Flynn, 2004, Chan and Flynn, 2000, Schinazi, 2003). *M. tuberculosis* has evolved in such a way that, if an individual is re-infected or the infection is reactivated, the body will potentially respond with an even more aggressive immune response (Aufderheide and Rodríguez-Martín, 1998: 120), and nearby organ tissues may be destroyed by this hyperimmunity (Ortner, 2003: 227). The infection can, however, lie dormant and never progress to "active" TB.

M. tuberculosis now infects one third of the global human population in its latent form (Ahmad, 2010). Parrish *et al*. (1998: 108) presents figures of a 30% infection rate of those exposed to the disease, then a 40% development to primary active TB, and a 60% latency, which can remain so or be reactivated with a two to 23% chance per lifetime. Those co-infected with HIV are at greater risk, as dormant bacilli can reactivate and cause active infection if a disruption to immune response occurs; the risk is then 5%-15% every year, similar to 50% over a lifetime (Ahmad, 2010). Bacilli that lie dormant in the body without causing active disease is known as latent TB (Parrish *et al*., 1998) although, according to Young and Robertson, 'The mode of microbial persistence between initial infection and subsequent reactivation of disease is unknown' (1998: 650). They suggest an alternative theory to tuberculosis bacilli lying dormant; that equilibrium might be established whereby bacteria are killed by the host's immune system at the same rate as it is able to replicate. More recently, the development of *M. tuberculosis* has been described by (Russell *et al*., 2009) and is summarised here: the inhaled bacilli are engulfed (phagocytosed) by white blood cells (macrophages) in the alveolar tissue of the lungs. The "closing in" of the bacilli causes an inflammatory response, causing the macrophages to invade local tissue, and attract neighbouring mononuclear cells which ultimately form the core, or cellular matrix, of the developing granuloma; the granuloma is considered the primary characteristic of the disease. The bacilli are then trapped in a capsule of differing cell types and, essentially, the infection is contained but, over time, it is suggested that increase in "foamy macrophages" (one of the type of cells forming the capsule) *within* the fibrous capsule, is responsible for the caseous debris in the centre of the granuloma. A progressive infection causes the caseous, necrotic centre of the granuloma to liquefy, and the thousands of bacilli (which replicated during initial infection) are released into the airway, causing the person to become infectious to others. The damage to the lungs triggers a cough, which helps release

the bacilli more effectively into the air in order that they may find a new host. This process is visually represented in Figure 2-4.

Miliary TB can also occur, as the result of bacilli being released into the bloodstream (haematogenous dissemination) and/or lymphatic system. Miliary TB is the effect of all forms of progressive haematogenous TB, whatever the ensuing pathology (Kim *et al*., 1990, Maartens *et al*., 1990, Baker and Glassroth, 1996) and develops in 1–2% of patients with tuberculosis (Kim *et al*., 2008). According to Sharma *et al*. (2005) the epidemiology of miliary tuberculosis has changed due to the presence of HIV/AIDS and the use of immunosuppressive drugs, with impaired cell-mediated immunity underlying the disease's development.

Once in the bloodstream, tubercle bacilli tend to locate in areas of red marrow in the bones which, according to Ortner (2003: 228), has a high circulatory and metabolic rate. Infants and children have a greater distribution of this type of marrow, but higher quantities are found in the vertebrae, ribs and sternum at all ages (Ortner, 2003: 229). This is demonstrated by the frequency of TB in the different bones of the body (see Section 4.3.1.6). If the bacilli enter through the intestinal tract, they may cause tubercular ulceration, although infection via this route is thought to be less effective, because the infecting bacilli are sensitive to gastric acid and therefore do not survive as well (Smith and Moss 1994: 48 cited in Roberts and Buikstra, 2003: 5).

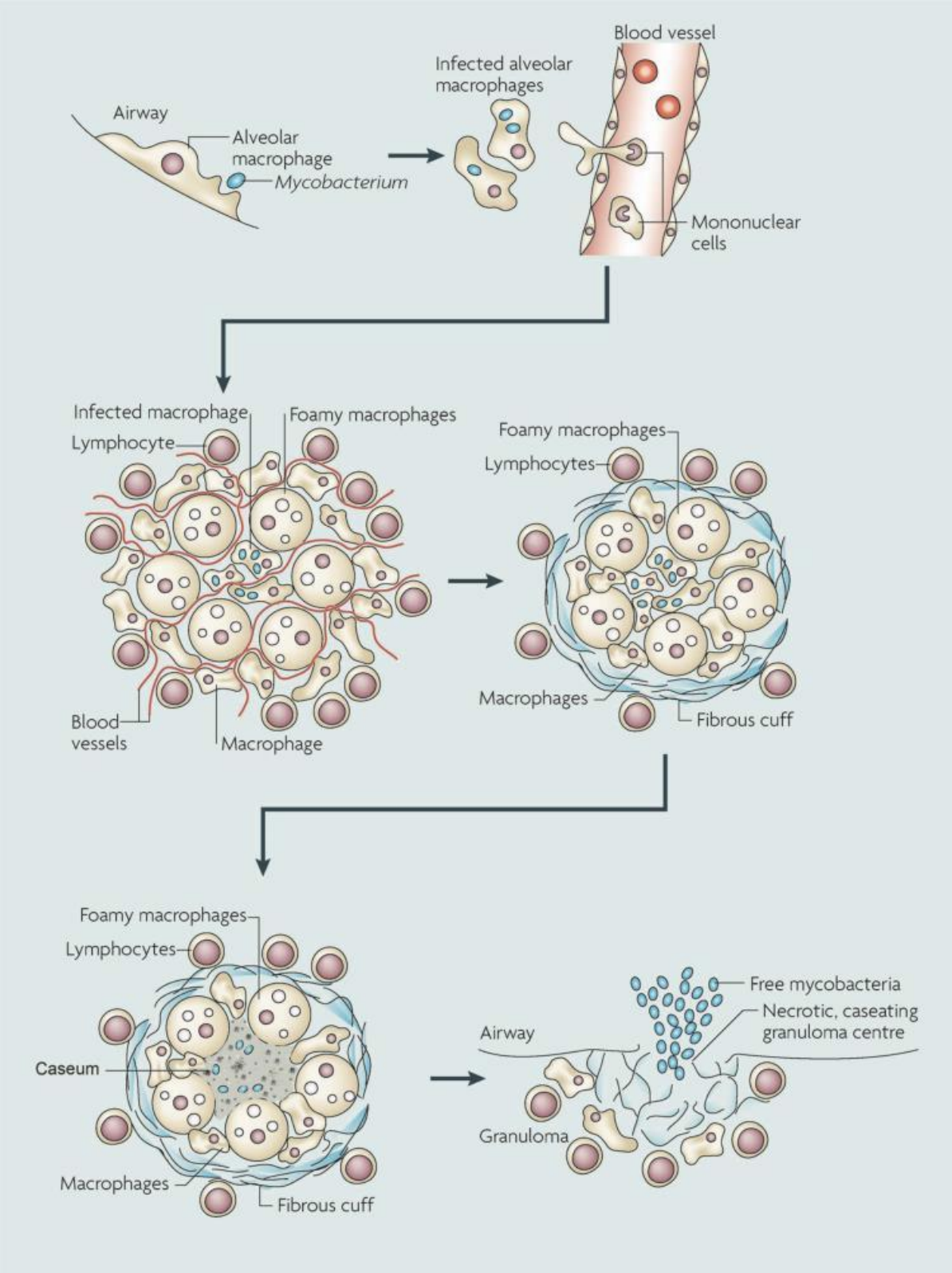

Figure 2-4 Histological Progression of the Human Tuberculosis Granuloma (Russell *et al*. 2009)

2.2 Factors Affecting TB Occurrence

There are a number of factors associated with a person's susceptibility to TB infection, and chances of progression to active infection. These are summarised in Figure 2-5 and discussed further in the following sections. It is important to appreciate the level to which these factors are associated.

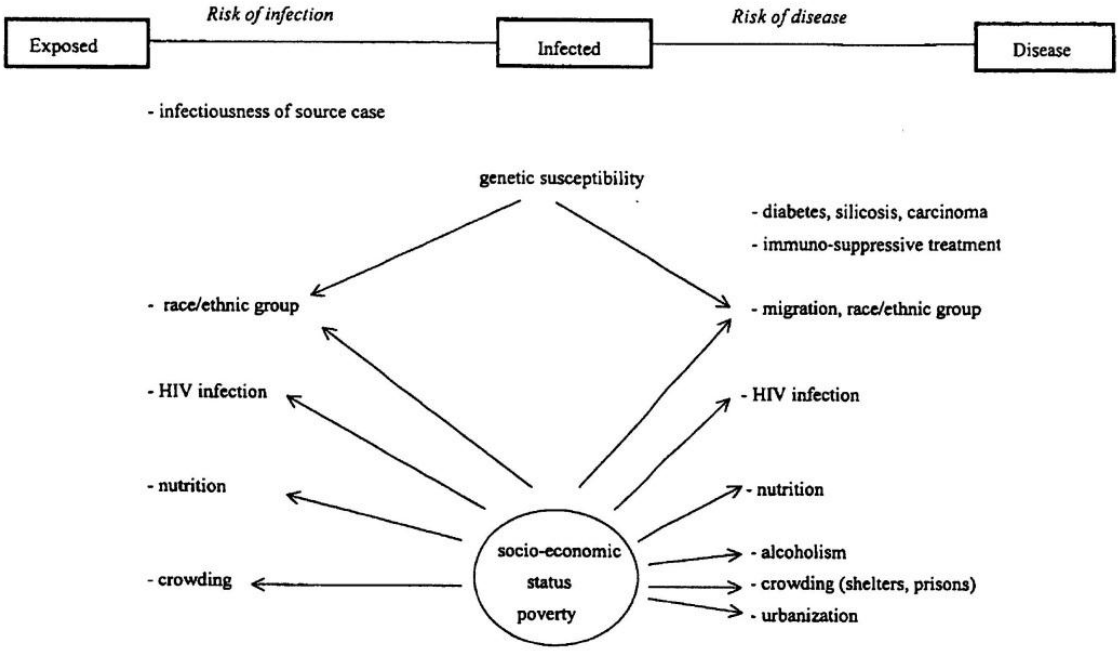

Figure 2-5 Impact of environmental factors on the risk of infection and development of disease after infection and their link with host-related factors. (Lienhardt, 2001: Fig. 3)

2.3 Intrinsic

2.3.1 Age

Age can play a major role in the contraction of TB because today, advancing age is associated with a decline in immune system function (Kumar and Burns, 2008), making an individual more susceptible to TB. Increased life expectancy also makes an individual more

likely to experience additional health problems which, in turn, can lead to an increased susceptibility to TB; this is reflected clinically where elderly patients with TB often exhibit atypical radiographic appearances present with non-specific complaints (Chan *et al*., 1995). Older people also have more adverse drug reactions, and greater TB-related mortality than younger people (Salvadó *et al*., 2010). It has been thought that children do not contribute significantly to the TB burden, but this has been shown to be an inaccurate assumption, especially in endemic areas (Marais *et al*., 2006). The risk of disease after primary infection is greatest in children less than four years of age, declining slowly between the ages of five and ten, a rapid increase in risk between 15 and 19 years and, finally, a second peak associated with the ages of 20 to 30 years (Marais *et al*., 2004). Overall, there is a difference in both response to infection and clinical features of the disease between different age groups (Donald *et al*., 2010). One of the reasons is that tubercle bacilli tend to locate in areas of high red marrow content in the bones and Infants and children have a greater amounts (Ortner, 2003: 229)

Age can also impact on the way in which people live. It could be argued that young and middle aged adults, as a rule, travel more extensively, perhaps for business. This will expose them to a greater diversity of people than in their home communities, varying prevalence rates of TB in different countries and therefore, increased transmission risk. Age may also dictate the occupation of a person, and as a result, there may be differing levels of occupationally related health risks, in addition to different standards of living.

2.3.2 Biological Sex

Biological sex also appears to affect susceptibility to TB as do many diseases. More male TB cases are diagnosed and reported to WHO each year than female (Diwan and Thorson, 1999) (Figure 2-6) for which there may be many reasons, biological or more socially constructed. Infection rates are similar, however, between males and females until adolescence, after which males demonstrate a distinct increase (Holmes *et al*., 1998).

Some studies clearly demonstrate this sex bias in relation to TB susceptibility (Salim *et al*., 2004) but it has been shown that the higher figure of reported cases for men, is not

generally as a result of differential access to health care (Borgdorff *et al*., 2000). Because only five to ten percent of those infected with M. *tuberculosis* will develop active TB infection, Neyrollles *et al* have suggested that, as a whole, the human population is 'remarkably resistant' to the bacillus, but that because up to 70 per cent of the people do develop it are men, this appears to suggest that women are more resistant (2009: 4).

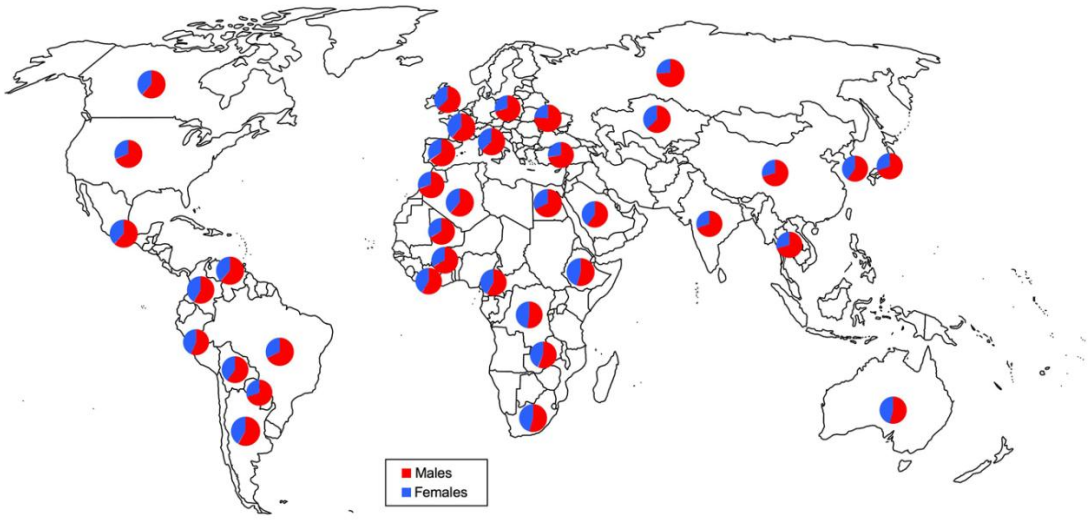

Figure 2-6 Sex distribution for new smear-positive TB case notification in 2007 in various countries (WHO, 2009)

it is possible that an X chromosome susceptibility gene may contribute to the excess of males with TB (Bellamy *et al*., 1999) but it is also possible that one of the key reasons for different male/female prevalence rates, is due to differential immune reactivity between men and women; women's immune systems are generally stronger and more effective (Ahmed *et al*., 1985, Talal *et al*., 1982). For this reason, as Ortner suggests, in the absence of other factors, women may be expected to survive chronic stages of the disease more often than men (1998: 82). In archaeological populations, however, the evidence is complicated as it is not possible to 'control' for these variables, particularly sex related differences in exposure (Ortner, 1998). These variables, even in living populations, pose a difficult interpretive task.

Despite the fact that differences in male and female rates of TB infection are, to some degree, a reflection of biologically determined factors, they can also be as a result of more socially constructed conditions. Hudelson highlights that social and economic roles and

activities, influenced by gender, could lead to different levels of exposure to the disease (1996). Although the terms biological sex and gender are often used interchangeably, their definitions are distinct, even if in practice their application to past and present can be ambiguous. Gender is socially constructed and is distinct from sex, whereas sexually dimorphic features are biologically determined (Uplekar *et al*., 2001). Failure to make this distinction has previously been reported as a growing, and worrying, trend in bioarchaeology, where such distinction is perhaps more important than in other fields, as it is (often) possible to determine the sex of an individual based on skeletal morphology and extract information on gender roles through analyses of artefacts (Walker and Cook, 1998).

> *…[gender] creates different risks and protections for physical illnesses, produces different behaviour when ill, elicits different responses in health care personnel, affects the social worth of patients, and influences priorities of treatment, research and financing.*
>
> (Lorber and Moore, 2002: 3).

Many of the variables referred to above, are culturally related, as in many countries women are not on equal social footing with men, and thus their health needs are often not prioritised. A number of studies have focussed attention on sex and gender issues in TB control (Thorson and Diwan, 2001, Holmes *et al*., 1998, Hudelson, 1996, Uplekar *et al*., 2001, Borgdorff *et al*., 2000) and highlighted the need for control programmes to take into account such issues.

Studies where there was not found to be a substantial difference between male and female ratios, in terms of prevalence and notified cases (thus suggesting epidemiological differences rather than unequal access to health care), do admit that access to diagnosis does not necessarily mean access to effective treatment (Borgdorff *et al*., 2000). In addition, HIV which is a significant burden in many countries, may well be having an impact on female rates of TB as there is often a higher prevalence of HIV in young women than in men (Diwan and Thorson, 1999). The relationship between these two diseases is, as previously discussed, synergistic, potentially putting many HIV positive women between two and hundred times higher risk of contracting TB (Nunn *et al*., 1994). Because the general health

of an individual influences their chances of progression to disease, areas where female health is generally poor, particularly in relation to malnutrition and HIV, may influence an increased risk of developing the disease (Hudelson, 1996). The relationship between sex, gender and TB development is clearly complicated in modern societies, and there is no reason to think it was any, or much, less so, in earlier populations. A genetic predisposition to TB linked with sex will be considered further below.

2.3.3 Genetic predisposition

> *Mycobacterial infections in man will prove to be as much dependant on the genetic make up of the host, as on the virulence of the bacteria.*
>
> (Newport and Levin, 1999: 120)

Research has demonstrated that genetic factors may play a part in the susceptibility of humans to infection, progression to, and outcome of, disease (Casanova and Abel, 2007, Hill, 2006, Puffer, 1944). An understanding of paediatric TB has seen recent progress in terms of evidence for genetic susceptibility (Malik and Godfrey-Faussett, 2005, Alcaïs *et al*., 2005, Casanova and Abel, 2005) but, on the whole, 'The molecular basis of genetic predisposition to pulmonary tuberculosis in adults remains largely elusive' (Baghdadi *et al*., 2006).

Many polymorphisms in various candidate genes have been purported as being linked to pulmonary TB, but only a few have been replicated independently (Casanova and Abel, 2002). Recently, a genome-wide linkage study looking at a total of 96 Moroccan families with proven pulmonary TB demonstrated that a single region of chromosome 8q12-q13 was significantly linked to TB, indicating the presence of a major tuberculosis susceptibility gene. This link was stronger in families where one parent was also affected by TB, and much lower in families without affected parents, which indicates that 'the genetic component involves at least one major locus with a dominant susceptibility allele' (Baghdadi *et al*., 2006: 1679).

There are other genetic factors such as the effect of sex specific steroid hormones which may influence the immune system and its subsequent responses to disease (Bouwman and Brown, 2005, Grossman, 1985, Whitacre, 2001, Marriott and Huet-Hudson, 2006). It has been demonstrated that women are more at risk of active disease progression, following infection, during reproductive years, and men at older ages (Olakowski, Grzybowski and Allen, 1964, Comstock *et al*., 1974, Groth-Petersen *et al*., 1959). In one study, females had a 130 per cent higher risk between 10 and 44, with men having up to a two and a half times greater risk than women of the same age (Olakowski, 1973). Differences to account for these results are many and varied (Holmes *et al*., 1998) but they may reflect the differing levels of sex hormones associated with age because differences in male/female levels of TB only really become apparent after sexual maturity (Neyrolles and Quintana-Murci, 2009).

2.4 Extrinsic

2.4.1 Poverty and Housing

> *Tuberculosis is a social disease, and presents problems that transcend the conventional medical approach…Its understanding demands that the impact of social and economic factors on the individual be considered as much as the mechanism by which tubercle bacilli cause damage to the human body.*
>
> (Dubos and Dubos, 1952)

Diseases such as AIDS, malaria and TB trap families and households in a vicious cycle of both poverty and disease (European Commission; 2001). TB has long been known as a disease of poverty and remains so, which has been established in a number of studies (Spence *et al*., 1993, Bhatti *et al*., 1995). The exact nature of how poverty causes TB is not entirely clear, although links with poor nutrition and overcrowding are considered factors (Spence *et al*., 1993). The effects poverty related overcrowding and sub-standard living conditions have on TB are seen around the world (Antunes and Waldman, 2001, Coker *et al*., 2006, Padmavathy *et al*., 2008).

The link with TB and poverty is also well illustrated as a result of research into the resurgence of TB in New York City, reaching its height between 1984 to 1992; the incidence of TB was intimately associated with the level of poverty in any given area (Figure 2-7) (Barr *et al*., 2001).

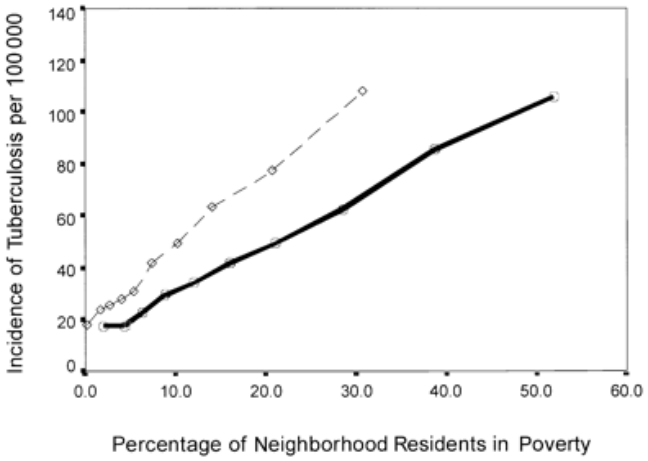

Figure 2-7 Tuberculosis incidence in New York City in 1992 plotted by decile of neighbourhood rates of poverty and abject poverty (Barr *et al*, 2001).

Bhatti *et al*. demonstrated a national rise in TB in England and Wales between 1980-92 that only affected the poorest areas, with an increase of 35% in the very poorest 10th of the population, 13% in the following two poorest 10^{ths,} and for the remaining 70% of the population no increase at all (1995). It should be noted that this study found socioeconomic factors to play a major role in the increase of TB but much less so for migrants from endemic areas. A study into the relationship between the decline of TB and improving social conditions during the Victorian period in England and Wales demonstrated that, in addition to improving living conditions, other factors must have played a part, of which natural selection was suggested as probably the most significant (Davies *et al*., 1999). Natural selection in this case, was based on the assumption that TB primarily affected young adults, those susceptible to TB and of child bearing age, and thus the morbidity and mortality rates of the disease would limit the number of children born to those susceptible to TB, or would deprive young children of susceptible parents so that child mortality rates would increase. Ultimately this would result in fewer children born of people susceptible to TB, and would leave others naturally resistant to the disease to reproduce (Davies *et al*., 1999: 91).

Because of the way in which TB is transmitted between humans, overcrowding and poor living conditions result in a greater opportunity for the bacilli to be passed between an individual with active TB and another; being in contact with many people in confined spaces, as demonstrated in longhouse dwellings in Sarawak (Chen, 1988), can have a significant impact on the spread of the disease. A limited or lack of space, ventilation and sunlight can encourage emergence and development of pathogenic organisms, as can poor sanitation (Roberts and Buikstra, 2003: 59).

Substandard living conditions, as a result of poverty, can also create unhygienic environments, making people more susceptible to illness, with those of lower socioeconomic status consistently demonstrating poorer health (Marmot, 1999, Smith, 1999), and this may result in greater susceptibility to TB. Low socio-economic status can also limit access to health care, compounding an already significant problem, particularly if treatment regimens are started but not completed (see 2.1.5). Poverty can also result in poor nutritional status and malnutrition through limited food access which could lead to increased susceptibility to TB.

2.4.2 Diet

Poor diet and nutrition have been shown to induce immunodeficiency and compromised antimicrobial resistance. The results of one study, conducted on mice, concluded that protein calorie malnutrition, 'selectively compromises several components of the cellular immune response that are important for containing and restricting tuberculosis infection …' (Chan *et al*., 1996: 14857). In addition, the response of the mice, suffering from malnutrition, to the infection, was reversed when the diet was restored to a full protein diet (Chan *et al*., 1996: 14857).

It is generally accepted that malnutrition is a risk factor for TB development because of lowered cell-mediated immunity (Myrvik, 1994, Chandra, 1991, Gershwin *et al*., 1987); as Cegielski and McMurray state: 'It makes biological sense.' (2004: 286). Extensive clinical evidence to support this notion, however, is lacking and more detail is required in order to fully understand how TB and malnutrition are linked; if the link is definitively confirmed,

malnutrition 'may account for a greater population attributable risk of TB than HIV infection, and certainly a much more correctable one.' (Cegielski and McMurray, 2004: 286).

Vitamin D deficiency has been linked with TB rates in studies which focus on foreign-born people living in Britain (Ustianowski *et al*., 2005). It was shown that vegetarian diets may contribute to deficiencies in Vitamin D and thus a create a greater susceptibility to TB, as demonstrated by a study of the comparison between Hindus who are mainly vegetarian, and Muslims, although dietary practices were not specifically recorded (Ustianowski *et al*., 2005). Other research has recorded a statistically higher TB incidence between Hindus and Muslims considered to be as a consequence of vegetarianism (Strachan *et al*., 1995).

2.4.3 Climate

Current climate change is a major cause for concern regarding global health (Costello *et al*., 2009, Myers and Bernstein, 2011, Woodward *et al*., 2011) with the general acceptance that it is has been caused, or at least encouraged by, the effect of human industrialisation. Around 80 per cent of Co_2 in the atmosphere is currently caused by industrialisation and the rest by land use such as deforestation but, since the rise industrial human activity, approximately 900 billion tons of CO2 has been released into the atmosphere around which 450 billion tonnes remains there (Costello *et al*., 2009: 1698). It is also important to note that those who have contributed least to these emissions are the people who will most feel the effects of its negative impact (Costello *et al*., 2009).

The extreme nature of weather changes predicted with current climate change, poses numerous 'acute' and 'chronic' health risks, the speed of which may limit the effectiveness of our ability to adapt (Davis and Topping Jr, 2008: 39).

> *Some of the health consequences of climate change are straightforward: warmer temperatures, changes in the hydrologic cycle, increased ground-level ozone, and enhanced pollen production will increase exposure to heat stress, alter patterns of infectious disease, and compromise air quality. These [are] direct impacts of climate change…we argue that it is the indirect impacts of climate change – large scale*

> *alterations to Earth's natural systems – that pose the greatest risks to human health. The changes are curtailing access to water and food and are undermining the very concept of stable homes.*
>
> (Myers and Bernstein, 2011)

2.4.4 Effect of Temperature and Seasonality on Disease

Whatever the cause of climate change, the effects of it, such as rising temperatures, can have disastrous health impacts on societies, as are being witnessed in the world today (Costello *et al*., 2009); temperatures tend to demonstrate a u-shaped temperature-mortality relationship, with mortality increasing in colder and hotter conditions (McMichael *et al*., 2008). A recent example of this were the effects of heatwaves generated across Europe in 2003, which caused in excess of 70,000 deaths (Robine *et al*., 2003). In Germany, mortality rates were increased particularly from respiratory causes (Hoffmann *et al*., 2008). It is not known to what extent climate change will affect those with pre-existing respiratory disease, but it is thought that the frequency of TB will be particularly affected, which may be partly caused by overcrowding through migration (Ayres *et al*., 2009). 'Extreme temperature events (both hot and cold), changes in air pollution, flooding, damp housing, thunderstorms, changes in allergen disposition and consequent allergies, forest fires and dust storms' are identified as key factors attributable to climate change that could influence respiratory disease (Ayres *et al*., 2009: 296).

In 1991 it was suggested that the effects of climate change on infectious disease would be unknown until greater detail was known about the temperature and humidity changes that would be experienced (Shope, 1991). It was suggested, however, that diseases most likely to increase in their distribution and severity were those which demonstrated 'three-factor' (agent, vector and human being) and 'four-factor' (plus vertebrate reservoir host) ecologies (Shope, 1991: 171), with an emphasis placed on diseases such as yellow fever, dengue and cholera. Perhaps once the entirety of the MTB complex is considered, this may also be considered a 'three-factor' ecological disease, although transmission of human strains of the diseases need not depend on an additional animal vector. Twenty years later, we are

however, in a better position to suggest the effects of climate change on health, of which infectious disease will play a key role; (see: Costello *et al*., 2009).

Seasonal effects can also be seen in the transmission of some diseases such as influenza (Lipsitch and Viboud, 2009) and, of course, TB (Naranbat *et al*., 2009, Chan, 2000, Douglas *et al*., 1996, Nagayama and Ohmori, 2006); climate may have played a role in the origin and development of *M. tuberculosis* itself, although more work needs to be done in this area (Nerlich and Lösch, 2009). Two of the explanations offered by Naranbat *et al.* (2009), to explain seasonal fluctuation findings of TB rates in Mongolia (Figure 2-8) may well be applicable to prehistoric populations – that temperature and precipitations dictate how much time is spent indoors and that seasonally-dependent nutrition and metabolism could influence the response to infection. Time spent indoors would also have exposed individuals and families to increased risk of communicable diseases by prolonging the contact period between individuals, or even increased smoke inhalation of biomass fuels potentially, although not yet demonstrated entirely (see 3.2.1), compounding the problem of person to person transmission through increased susceptibility of the lungs to respiratory infection.

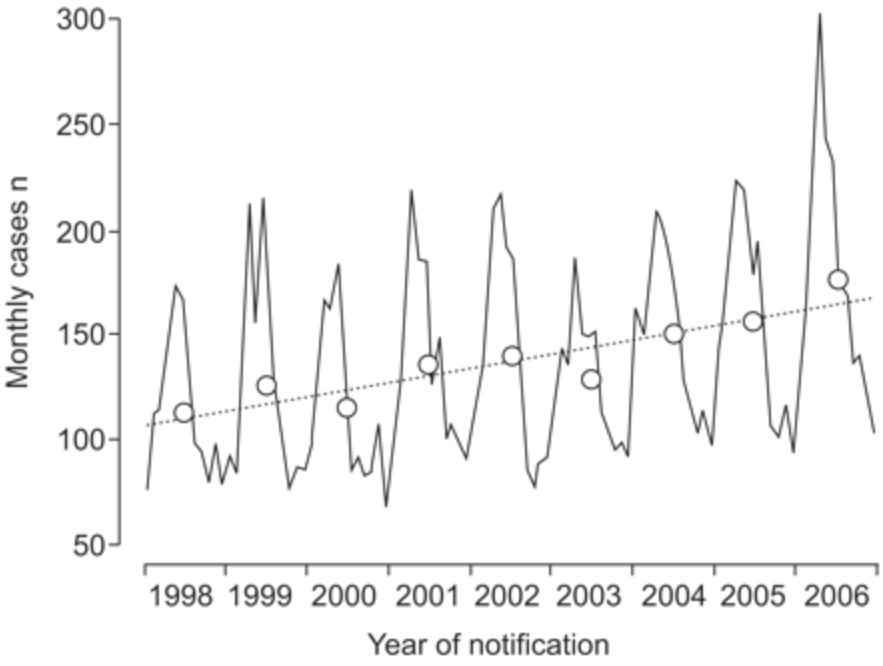

Figure 2-8 Seasonal variation in notification of sputum smear-positive tuberculosis in Mongolia 1998-2006 (Naranbat *et al.* (2009).

Seasonality and sunlight exposure are invariably linked and these factors are also associated with vitamin D production which has been shown to have an impact on susceptibility to TB (Ustianowski *et al*., 2005). Vitamin D stimulates anti-mycobacterial activity and thus a deficiency in the vitamin is associated with a greater susceptibility to TB. Vitamin D deficiency can even cause growth retardation in the skeleton and encourage skeletal deformities leading to an increased risk of fractures (Holick, 2007). One such study, which demonstrates the link between sunlight exposure and TB susceptibility was between 1983-1992, where there were peaks of TB notifications during the summer months and troughs during winter (Douglas *et al*., 1996). Winter months result in people being outside less frequently, for shorter periods, and with heavily clothed skin (Chan, 2000), so the level of solar ultraviolet radiation that reaches the skin during these months is reduced, and thus the synthesis of Vitamin D from the skin is reduced. A number of studies have indicated that the prevalence of many diseases are associated with areas of higher northern or lower southern latitudes, which has led to the suggestion that vitamin D may play a part in their pathogenesis or may even be influential in their prognosis (Grant, 2003, Holick, 2003). Recent research however, using ecologic meta-regression analysis, suggests that levels of vitamin D globally, are dependant on age, gender and skin colour with no overall influence of latitude (Hagenau *et al*., 2009) which is in contrast to previous cross-sectional research findings (Lips, 2001); the influence of latitude, and thus relationship to TB susceptibility via vitamin D levels, is clearly not straight forward.

2.4.5 Travel / Migration

The speed and scale of travel in 2011 is exceptional; a person can travel from the UK to Australia in less than 24 hours and many flights across Europe are cheap, encouraging regular travel. This poses a significant threat regarding the transmission of infectious disease from country to country (and subsequently within a given country). In 2010, 101.5 million passengers arrived in the UK (Home Office, 2011a) and in 2011 (ending March), just under half a million visas were granted for immigrants entering the UK (Home Office, 2011a), added to just over 22,000 applications made for asylum (Home Office, 2011b). The scale of those also living illegally in the UK can only be estimated, but in 2005 a Home Office study offered a figure of between 310,000 and 570,000 (BBC News, 2005), which is likely to be

even higher in 2011. These figures reflect large numbers of people entering the UK, not only for short term visits but in order to become resident. Important to note is that, in 2006, of those requesting and being granted British Citizenship, one of the largest groups was from India (Mensah, 2007) which, in 2009, had the highest incidence rate of TB in the world at 1.6-2.4 million (WHO, 2010a).

The dangers of global travel to disease transmission have also been highlighted in recent years by outbreaks of TB in UK schools which may be linked to foreign travel, and in particular to India (Hodgson, 2008, BBC News, 2001). Recently, Andrew Lansley, the Government Health Secretary, directly blamed Immigration on the soaring rates of TB seen in inner city Birmingham (Dayani, 2010). TB is not the only disease to highlight the dangers of global travel, with other examples including the international spread of SARS (severe acute respiratory syndrome), to which the WHO issued a number of official Global Alerts and Responses (2003), and avian influenza (H5N1) (2011), which was first isolated in a human patient in 1997 and, by September 2004, was suspected as being transmitted between humans (Nature, 2011).

2.4.6 Occupation

Bowden and McDiarmid identify three types of occupation associated with contracting TB: unskilled labouring, those that increase susceptibility to the disease and those that expose workers to TB (1994). The first category may include migrant farm workers or food handlers, the second category includes those that are at risk from inhaling particles and dust produced by their occupation such as woodworkers, sandblasters, potters and miners, the latter often working in unventilated and cramped conditions which may increase their chance of developing pulmonary infections due to irritation and inflammation in the lungs (Roberts and Buikstra, 2003: 69). The third category includes those working with animals, particularly cattle, who in the past as well as lower paid workers now, have always had a greater propensity for contracting bovine TB via the respiratory tract. For example, tanners, veterinary surgeons and farmers can be susceptible. It was also common in the past for people to live in close contact with their animals, often in the same house; such conditions would have made contracting the disease even easier via the respiratory tract. Occupations

such as veterinary surgeons fall into the third category along with those who, for example, work in hospitals; even in the 1940's TB was so common amongst healthcare workers in America that medical schools would admit six extra pupils every year as they expected to lose that many to TB (Rosenthal, 1992).

Healthcare workers in particular have received attention due to their obviously increased exposure to TB infection. Some studies have suggested that, as a rule, their rates of infection are similar to the general population, with the exception of a few specialist areas such as inhalation therapists (those who work with people experiencing cardiology or pulmonary dysfunctions) and lower paid workers (McKenna *et al*., 1996); others demonstrate a "significantly" high risk of TB infection for female nurses and clinical lab technicians (Usui *et al*., 2000). That lower paid workers are at greater risk of infection illustrates that socio-economic factors also have a part to play.

Infection need not always be passed from infected, *living* patients. There have been reports of pathologists contracting the disease through autopsy of infected individuals (Ussery *et al*., 1995), and of funeral directors during the process of embalming (Lauzardo *et al*., 2001, Sterling *et al*., 2000). In one case, suggestions as to the process by which the transmission occurred, included an expulsion of infected air from the chest cavity during handling (although this was considered unlikely due to the limited number of droplet nuclei that would be expelled), or bacilli present in bodily fluids extracted, aerosolising once being mixed with water and splashed in the sink; the latter decided as being the more likely process of transmission from those with disseminated disease. This author would question the assumption regarding unlikeliness of the former pathway (that not enough droplet nuclei would be expelled to cause infection), because the number required is only, in fact, between one and ten (WHO, 2010a).

It is also interesting to note that, despite the inversely proportional nature of TB to socio-economic status (McKenna *et al*., 1996), funeral directors enjoy a high socio-economic status (Lauzardo *et al*., 2001) and thus it is a combination of factors, associated with occupation, that will impact on a person's exposure and susceptibility to disease in any given occupation.

2.4.7 TB from Animal Hosts

Bovine TB can be contracted by humans via the gastrointestinal tract but also via contact with infected urine and dung, and this is why those who are in close contact with, or even live with their animals, are at a greater risk of contracting the disease. In addition, in some parts of the world, these animal waste products are used in washing, building, and as fuel or fertilizer, thus making the potential exposure to *M. bovis* higher (Roberts and Buikstra, 2003: 77). *M. bovis* can be contracted through drinking infected milk and eating infected meat, but there are strict regulations on TB testing and control in cattle today, although this has not always the case (see 2.6).

Tuberculosis (particularly bovine) can affect a wide range of species and, whilst bovine TB (bTB) is most commonly associated with cattle which are highly susceptible to *M. bovis* (Menzies and Neill, 2000), it can also be passed to human hosts (Evans *et al*., 2007). Bovine TB also affects many animal species found in Britain today (Morris *et al*., 1994), including the badger, antelope, fox and hare and domestic animals such as horses, dogs cats and sheep (Moda *et al*., 1996, O'Reilly and Daborn, 1995). Despite this range of animal hosts, it is cattle which are considered to pose the greatest infection threat to humans (Grange, 1995). Despite elimination of TB from most cattle herds in Britain (with the exception of certain areas of south west England) by around 1960, due to a herd testing and slaughter policy (Proud, 2006), there has been a gradual rise in the number of cases seen across the country for the last three decades or so (Krebs, 1997); this is a problem that is also affecting other areas of Europe (Humblet *et al*., 2010) and areas of America (Knust, 2008).

There are factors which influence the chance of infection amongst cattle; for example, dairy herds are more at risk than those raised for beef due to their longer lifespan because they will have a longer exposure to bovine TB (Olea-Popelka *et al*., 2004, Ramírez-Villaescusa *et al*., 2009, Humblet *et al*., 2009), because they are brought together twice a day for milking allows respiratory spread of the disease (Menzies and Neill, 2000). The badger, however, officially known as *Meles meles*, is the most important of the wildlife species to be affected as they have been identified as the primary wildlife reservoir species by which cattle are infected with the disease (Cheeseman *et al*., 1989), supported by similarities in the *M. bovis*

strain types isolated from cattle and associated badgers (Woodroffe *et al*., 2009); this is proving to be the main sticking point in eradicating the disease (Vial and Donnelly, 2011), and the "Randomised Badger Culling Trial" was launched in 1998 in response to this (Bourne *et al*., 1998). Ultimately, research tends to suggest that, unless the culling were implemented over wide areas and consistently, overall benefits are unlikely (Donnelly *et al*., 2007), as any benefits are outweighed by the massive financial costs to the UK (Jenkins *et al*., 2010) – culls in small, localised areas, infact add to the problem of MTB cattle transmission from badger populations (Donnelly *et al*., 2003, LeFevre *et al*., 2005).

2.1 Recognition of the Disease in Skeletal Human Remains

A discussion of the skeletal changes expected as the result of TB infection, and recognised in archaeological skeletons through macroscopic investigation, can be found in the methods section (4.3.1.6) as an accompanying explanation of the pathology used to identify possible or probable TB in this study. It would be beneficial here however, to briefly summarise some of the primary skeletal manifestations expected and offer a brief discussion of the difficulties applicable to diagnosis of the disease in archaeological skeletal remains.

TB can affect almost any part of the human body, the exceptions being teeth and hair (Fluck, 2004: 4). Both *mycobacterium tuberculosis* and *mycobacteriu*m *bovis* can cause skeletal changes in the skeleton but it is *M. bovis* which is the more likely of the two, to do this (Stead, 2000). Essentially, the pattern of TB is influenced by its specific portal of entry; the TB bacillus will become established in the lungs if inhaled, or in the gastrointestinal tract if ingested. Infection in the gastrointestinal tract can be due to either ingestion of bacilli from swallowed human sputum, from infected milk, haematogenous spread or by local extension, although this form of TB is now generally uncommon in the indigenous populations of developed countries (Gordon and Mwandumba, 2008: 169). Changes associated with TB infection can be specific, in that they are as a direct cause of the invading pathogen, or non-specific, which means they can occur as a secondary process in response to the pathogen, and according to Resnick and Nimayawa, (1995: 2462), it is generally accepted that the haematogenous route, is that by which most skeletal involvement occurs.

The most reliable specific lesion of TB in skeletal remains is usually Potts Disease, which is an angular kyphosis of the spine due to the demineralisation of a vertebra, and subsequent collapse (Figure 2-9); it is evidence of this pathology, which is most usually relied upon in archaeological human remains, to provide a firm diagnosis (Roberts and Buikstra, 2008: 5). The spine is the most common site of skeletal involvement, and, in living populations, the most serious (Rezai *et al*., 1995, Boachie-Adjei and Squillante, 1996); the spine accounts for approximately half of all bone involvement (Hodgson and Ormerod, 1990, Davies *et al*., 1984). Archaeologically, the earliest case of spinal involvement in a confirmed case of TB (by aDNA analysis) in British skeletal remains dates to the Iron Age period in Britain, from the site of Tarrant Hinton in Dorset (Mays and Taylor, 2003) (Figure 2-10).

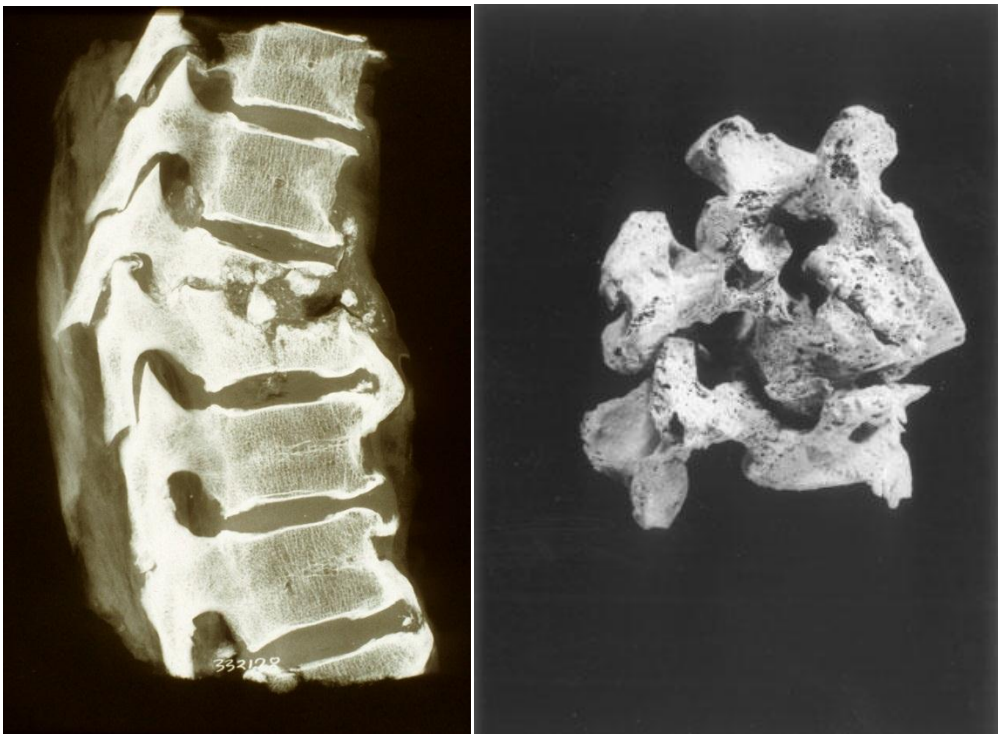

Figure 2-9 (Left) Clinical X-Ray of vertebral destruction caused by TB. Photo by Bruce Ragsdale.

Figure 2-10 (Right) Re-articulation of the first three lumbar vertebrae showing angular kyphosis – Tarrant Hinton Dorset (Mays and Taylor, 2003: Figure 4).

Overall, it is thought that skeletal involvement occurs in three to five per cent of untreated people (Resnick and Niwayama, 1995: 2462) and that bone and joint TB, as a result of respiratory TB, generally occurs three to five years after the initial infection (Wallgren, 1948) through haematogenous spread from the initial site of infection. Evidence of TB on an archaeological skeleton therefore suggests a chronic condition and one which indicates long

term survival alongside the pathogen. It is this situation which gives rise to the paradoxical situation whereby skeletons who exhibit pathology consistent with chronic disease may reflect a healthier individual than one who did not live long enough to exhibit such changes (Wood *et al*., 1992). This creates a serious problem for bioarchaeologists attempting to reconstruct the health of past populations, compounded by the fact that those skeletons excavated, generally represent only a tiny fraction of any given population (Figure 7-1).

Nevertheless, bioarchaeologists must work with the visible evidence, unless of course, biomolecular techniques can be employed, which may be able to detect the presence of pathogens where no pathology exists (see 2.2). Recognition of TB in archaeological skeletal remains also requires the ability to distinguish true pathology from normal variations and post-mortem changes (Roberts and Buikstra, 2008: 4). Funerary rituals and taphonomic changes may also impact on both the level of survival and structural integrity of bones, and evidence of disease can be destroyed through the process of cremation (Roberts and Buikstra, 2008: 5). This is of particular relevance for this study, as the number of human remains able to be considered for inclusion in the project was limited, in part by the changing funeral practises over the Neolithic, Bronze and Iron Ages of Britain, during which, cremation came in and out of fashion at various times. Certain pathology, such as the "plaque" type of new bone formation on the visceral surfaces of ribs, often associated with TB (see below) and described by Pfeiffer (1991), can often be delicate and easily detached from the surface of bone; damage, often inflicted by poor handling and storage of bones (Caffell *et al*., 2001) can also therefore impact on the ability to recognise disease in archaeological human bone.

As previously stated, TB can, and does affect many other areas of the skeleton; the variety of skeletal sites which can demonstrate TB involvement is well illustrated by South Asian patients in England (Figure 2-11) (Hodgson and Ormerod, 1990). There are clinical examples of both single (Parkinson *et al*., 1990) and multiple joint involvement (Valdazo *et al*., 1990) and occasionally, there are so many sites of involvement that the disease manifestations mimic metastatic bone disease (Ormerod *et al*., 1989).

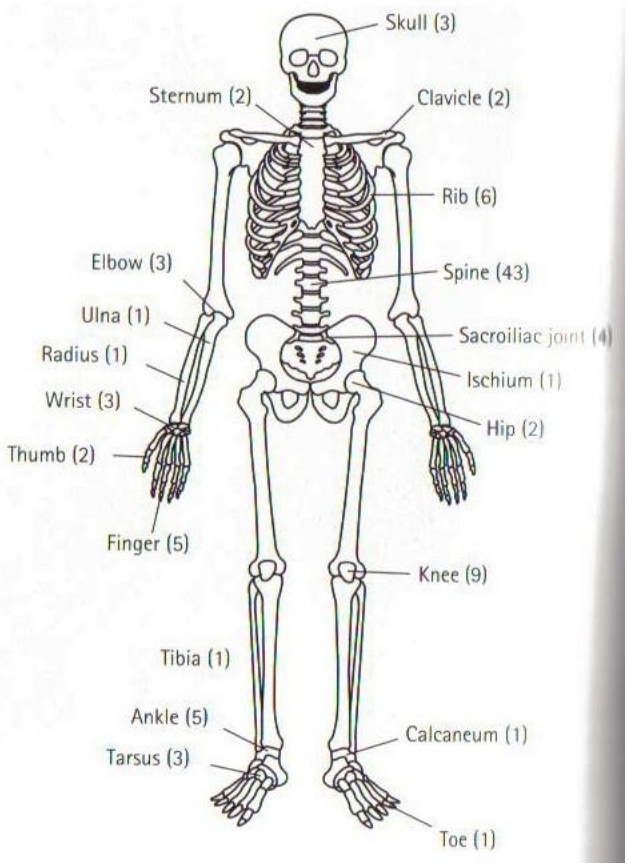

Figure 2-11 Sites of bony tuberculosis in Asian patients. Number of patients for each site in parentheses (Hodgson and Ormerod, 1990)

Tuberculosis of the hip constitutes around 15% of all cases of osteoarticular tuberculosis (Babhulkar and Pande, 2002) and is usually very destructive with foci in the femoral head and neck and possibly the acetabulum. Teklali *et al.* (2003) reviewed 106 cases of paediatric peripheral osteoarticular TB, excluding vertebral TB, and demonstrated that 63% of osteoarticular foci was the result of hip and knee TB combined. TB infection in the ribs however, is one of the most discussed sites of involvement in the bioarchaeological literature, as studies have demonstrated strong correlations between rib pathology and deaths known to be caused by TB (Roberts *et al.*, 1994, Santos, 2000, Kelley and Micozzi, 1984), yet pathological changes on the ribs cannot be considered pathognomonic for TB. This is because any chronic respiratory infection could stimulate similar skeletal changes in the ribs, therefore even rib lesions found in conjunction with pathology considered diagnostic for TB, such as Potts disease, or identified in skeletons for whom aDNA analysis

has confirmed the presence of *mycobacterium tuberculosis* or *M. bovis*, still cannot be definitively attributed to TB; a person may suffer from more than one disease at any one time. The presence of rib lesions therefore are a nonspecific skeletal indicator, but may well be considered suggestive of TB infection (Roberts, 1999). Based on destructive lesions in the ribs (which occur less frequently than new bone formation in archaeological skeletons) the clinical literature reports TB in the ribs as being rare (Johnson and Rothstein, 1952, Rechtman, 1929, Leader, 1950, Sinoff and Segal, 1975), but it is possible that rib involvement may actually be as high as nine percent in patients with TB (Kelley and Micozzi, 1984, Roberts *et al*., 1994).

Other possible sites of skeletal manifestation of TB infection include the endocaranium, most commonly affecting the cranial vault (Ortner, 2003: 247), and although cranial involvement is generally rare, there are numerous examples and studies to be found in the clinical literature (Le Roux *et al.*, 1990; Gupta *et al.*, 1989; Kang *et al.*, 2008; Shameem *et al.*, 2009; Barton, 1961; Mohanty *et al.*, 1981; Patankar *et al.*, 2000; Ramdurg *et al.*, 2010; Diyora *et al.*, 2009). Facial bones, the shoulder joint, sternum and elbow joint are also affected; the latter occurring most commonly in those aged 20-30 (Ganguli, 1963). Joints in the wrist can be affected, as can bones in the hands and feet, where a bony shell is formed around the affected bone and which is referred to as *spina ventosa* (Ortner, 2003: 242). The Sacroiliac Joint is affected in up to 2% of cases of skeletal tuberculosis, (Aufderheide and Rodríguez-Martín, 1998: 139), and the ilium, ischium and pubic symphysis can all be involved, the former usually through extension from a psoas abscess and, the latter two, which are both rarely affected (Ortner and Putschar, 1981: 49-50), usually suffer destructive lesions (Ortner, 2003: 239). The shafts of long bones are not usually involved but where they are, the tibia is most commonly involved and the fibula the least (Konschegg 1934: 422 cited in Ortner, 2003: 245). TB can manifest in the ankle joint and bones of the feet, usually by expansion from an adjacent tubercular soft tissue focus or via haematogenous dissemination; if the origin of infection is the tibia, destruction and subsequent healing ankylosis of the tibiotalar joint can occur (Ortner, 2003: 241).

In general, the invading pathogen tends to settle in areas high in red marrow content and gradually destroy the bony tissue (Roberts and Buikstra, 2008: 5) but both bone destruction

and formation can occur as part of the TB infection, sometimes both together. Roberts and Buikstra (2008: 5) add emphasis to a point that has been repeatedly expressed by others (Wood *et al*., 1992, Roberts and Manchester, 2005, Ortner, 2003, Buikstra and Ubelaker, 1994) – that pathological lesions need to be accurately recorded, including their presence and distribution, along with possible differential diagnoses. This action allows verification or re-evaluation of the remains in the future (Roberts and Buikstra, 2008: 5).

> *The whole problem of diagnosis lies in the fact that, as in syphilis, the bone may 'react' to the bacillus in a very characteristic way, as shown by a typical Pott's disease of the spine, but in other parts of the skeleton the bony changes may be far from distinctive.*
>
> (Brothwell, 1961: 325)

For the reasons discussed above, TB as recognised in the archaeological record is likely to have been significantly under reported but possible early examples from Britain do exist. Nonspecific skeletal changes constituting possible TB are recorded from the Neolithic (e.g. McKinley, 1996) to the late medieval period onwards (e.g. Roberts, 1999) but no definite evidence has been presented for any period earlier than the Iron Age, other than that previously described from Tarrant Hinton in Dorset (Mays and Taylor, 2003). Unless clear diagnostic pathology is identified, such as Pott's disease, in Neolithic / Bronze Age skeletons not yet excavated, or in skeletons previously excavated but where pathology has been overlooked, it appears that biomolecular techniques of pathogen identification are likely to be the crucial step forward needed to shed light on the early history of TB in Britain.

2.2 Alternative Techniques of Identification of TB in Human Remains:

2.2.1 A Short History on the Use of aDNA

It was the 1980's that brought about the realisation that DNA in ancient remains could be extracted and studied (O'Rourke *et al*., 2000); the first work was on a museum specimen of a quagga, an extinct member of the horse family (Higuchi *et al*., 1984). A year later, Pääbo

(1985, 1986) extracted DNA sequence data from a 2400 year old Egyptian mummy. In 1989 the development of PCR being used to amplify ancient DNA (aDNA) occurred (Hagelberg *et al*., 1989), but it was not until the early 1990's that the process of extracting DNA for diagnosis of disease in human remains was employed, with the identification of *M. tuberculosis* (Spigelman and Lemma, 1993, Salo *et al*., 1994) and *M. leprae* (Rafi *et al*., 1994). Pathogen DNA remains in the body after death (Mulligan, 2006) and can therefore be extracted as human DNA can, dependant of course on its survival. Since these initial studies, there has been a large increase in the number of published pathogen aDNA studies, summarised most recently by Tsangaras and Greenwood (in press) (Figure 2-12).

	1993	1995	2000	2005	2011 [A]
Ancient Pathogen Publication Timeline	1	2	17	23	16

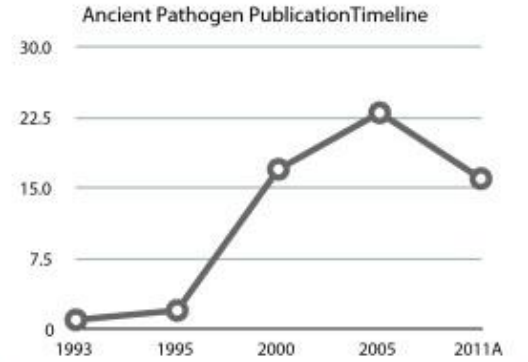

Figure 2-12 Graph depicting the increase in ancient pathogen studies in the last 17 years (Tsangaras and Greenwood, in press: Fig. 1)

The potential of pathogen aDNA studies was recognised early in the history of aDNA studies, not just because of its ability to identify the presence of a particular pathogen, but also because of its ability to address wider research questions:

> *DNA analysis of museum or archaeological specimens can provide a more exact temporal and spatial perspective on the initiation and spread of disease.*
>
> (Wayne *et al*., 1999)

The majority of pathogen aDNA analysis has to date focused on TB (Salo *et al*., 1994, Gernaey *et al*., 2001, Mays and Taylor, 2003, Arriaza *et al*., 1995, Donoghue *et al*., 1998, Fletcher *et al*., 2003b, Haas *et al*., 2000, Zink *et al*., 2001, Zink *et al*., 2003b, Donoghue, 2011, Taylor *et al*., 2007, Spigelman *et al*., 2005); this may be due to the increased likelihood of survival of the pathogen because of a resistant mycolic acid component of the cell wall (Donoghue *et al*., 2004, Zink *et al*., 2002, Mark *et al*., 2010). Other diseases which can result in distinct skeletal morphology, have also received attention, with recent aDNA work concerning leprosy, having helped to shed light on the pattern of the disease's transmission in Europe from analysis of medieval skeletons (Watson and Lockwood, 2009) and another identifying the disease in archaeological remains from Japan (Suzuki *et al*., 2010). Syphilis is a disease which was reportedly identified in an early paper (Kolman, 1999), but has since, not able to have been isolated (von Hunnius *et al*., 2007, Barnes and Thomas, 2006, Bouwman and Brown, 2005). TB remains the most studied disease in the pathogen aDNA literature.

A study in 1996 (Baron *et al*.) showed that M. *tuberculosis* complex DNA could be detected in both macroscopically affected bone and unaffected bone, leading to the conclusion that any infectious agent should be identifiable if it is carried through the bloodstream or lymphatic system to the bone. Use of aDNA to identify specific pathogens has therefore been employed to identify diseases that do not manifest in the skeleton such as malaria (Taylor *et al*., 1997) and plague (Drancourt *et al*., 1998, Raoult *et al*., 2000, Wiechmann and Grupe, 2005). In addition, aDNA analysis has been used to identify the causative bacteria of non-specific pathological lesions (Haas *et al*., 2000), to confirm a diagnosis suggested via other diagnostic techniques (Taylor *et al*., 2000, Mays and Taylor, 2002, Baxarias *et al*., 1998), and to identify the specific organism causing a disease (Zink and Nerlich, 2004).

Before analysis of aDNA, it had only been possible to identify TB through macroscopic analysis, where skeletal lesions existed, but neither *M. tuberculosis* nor *M. bovis* leave any species specific skeletal indicators, although it has been suggested that *M. bovis* may affect the skeleton more frequently (Stead, 2000). It is therefore not possible, using macroscopic methods, to confidently differentiate the two and identify the specific organism causing infection. Although it faces many difficulties (see 2.2.2), the use of PCR (polymerase chain

reaction) to amplify aDNA from samples of bone and teeth has been shown to be a useful tool for diagnosis of disease when used in conjunction with macroscopic analysis, depending of course upon whether pathogen aDNA survives in human remains excavated and is not contaminated with other DNA. The development of the PCR process (Mullis and Faloona, 1987) and then applied to aDNA studies has been referred to as the "second wave" of progress in this field (Millar *et al*., 2008).

The majority of TB aDNA studies use PCR primers that target an MTBC specific, repetitive locus: IS*6110* (Eisenach *et al*., 1990). The sequence length of IS*6110* is relatively short, (123bp) meaning that the effect of DNA fragmentation is limited and because IS*6110* is present in multiple copies, the chance of detection is increased (Pääbo *et al*., 2004), even more so when a nested PCR, targeting a sequence length of an even smaller 92bp, is employed (Donoghue, 2009). IS*1081*, another repetitive insertion element (Collins and Stephens, 1991) is also present in most MTBC specific sequences and is often utilised, usually in conjunction with IS*6110*, in MTB aDNA analyses (Taylor *et al*., 2007), as it has been in this study (for further discussion of these loci, see 2.2.4).

The last few years have seen the development of new techniques which are likely to impact dramatically on aDNA studies in general and to which Millar *et al* (2008) refer to as the "third wave" of progress. "Next-generation" sequencing techniques (massively parallel DNA sequencing) are opening opportunities in other fields of research such as biomedicine (Ansorge, 2009) but are well suited to aDNA work as they involve sequencing fragments up to approximately 250bp in length, which is comparable to that found in the majority of ancient genomes (Millar *et al*., 2008); work on the Neanderthal genome has already begun (Green *et al*., 2006). The new sequencers are able to detect sequences as they are generated, by SBS (sequencing by synthesis) (Millar *et al*, 2008); the process is quick and provides high throughput sequencing. In addition, because of the high capacity of the new sequencers, whilst contaminating DNA will also be sequenced, any target aDNA, even if present in small amounts, will still be sequenced in significant quantities (Ansorge, 2009) although there are of course limitations, such as the degradation aDNA is often subject to (Millar, *et al*, 2008). Summaries of the commercial platforms available for massively parallel DNA sequencing can be found in Mardis (2008) and papers referred to above (Ansorage,

2009; Millar, 2008). The potential of these "next-generation" techniques for medical research is clear, in regards to exploring the causes of disease, drug development and in diagnostics (Ansorage, 2009) and it is therefore a promising move towards the potential for greater understanding of ancient pathogens, including tuberculosis.

2.2.2 Limitations and Potential Solutions

There are numerous reviews available concerning the problems and progress of aDNA research (Mulligan, 2006), some being TB specific (Donoghue *et al*., 2009), all of which highlight the problems and the potential of the technique in light of previous research. Problems include possible poor survival of the ancient DNA although some studies suggest that despite the difficulty of estimating aDNA survival by macroscopic inspection, bones that are hard, heavy and compact, are more likely to yield aDNA such as the diaphyses of long bones rather than more porous bones such as the vertebrae (Bollongino *et al*., 2008); although the study does not specifically refer to pathogen aDNA, it seems likely that the principle would apply to all aDNA. This creates an additional problem for aDNA analysis of TB, as it is in these "spongy bones" such as the vertebrae that the infection most usually affects, and samples are therefore usually taken from the infected bone or as close to it as possible in order to maximise aDNA retrieval as pathogen aDNA is unlikely to be found in all cells in the body:

> *The diagenetic processes that make ancient DNA research generally difficult are also impediments to ancient pathogen research and perhaps more so given that their DNA may represent an even rarer proportion of the remaining nucleic acids in a fossil sample than host DNA.*
>
> (Tsangaras and Greenwood, in press).

In addition, studies suggest that the "thermal history" of fossil bones plays a key role in biomolecule survival (Bollongino *et al*., 2008, Smith *et al*., 2003) and some processes to which bones were subject, prior to deposition, may also impact on aDNA survival, with

cremated remains unlikely to yield any aDNA (Ottoni *et al*., 2009). With regards to pathogen aDNA specifically, there are however, numerous factors which will impact on the ability to retrieve sequence data in addition to degraded aDNA, including the amount of pathogen actually present in the person at time of death (Tsanagara and Greenwood, in press). Evidence also suggests that freshly excavated bones are favoured for aDNA retrieval as in one study, during 57 years of storage 'at least as much amplifiable DNA was lost as during the previous 3,200 years of burial' (Pruvost *et al*., 2007: 739). The study focussed on auroch bones but it would again, seem likely that these results would be applicable to all excavated skeletal remains and it would therefore be prudent to take note of the study's suggestion for revision of the post-excavation treatment of excavated remains.

Because of the problems concerning pathogen aDNA survival, PCR is favoured above hybridisation analysis, because of its sensitivity (Brown, 2000), and its ability to amplify even very small surviving pieces of aDNA. However, because of this, contamination is a major problem; this includes the environment from which the human remains are excavated (Gilbert *et al*., 2004, Gilbert *et al*., 2005b), from humans who have been in contact with the skeletal material, and from old PCR products. As Bouwman *et al.* suggest (2006), the assumption should be that the sample will be contaminated and an attempt made to recognise and distinguish this contaminating DNA. This is in contrast to earlier researchers who assumed that contamination with other (modern) human DNA would not affect the results of studies on pathogenic DNA (Bouwman and Brown, 2005) although some maintain that contamination with modern human DNA is more of a problem for human aDNA studies than for pathogen aDNA studies (Donoghue and Spigelman, 2006, Taylor *et al*., 2010). A study by Bouwman *et al*. (2006) has shown that ancient and contaminating DNA can be distinguished by rigorous techniques of analysis.

A number of suggestions have also been made for best practice during excavation such as the wearing of sterile gloves (Brown, 2000) and some argue that sterile excavation conditions are necessary to limit contamination post excavation (Roberts and Ingham, 2008), although others remain unconvinced of the necessity of this system for pathogen aDNA studies and question the feasibility of applying such methods on the small chance any given excavated remains may be subject to aDNA research (Taylor *et al*., 2010). In addition,

a potential problem associated with the retrieval and identification of TB specific aDNA is that soil may contain bacterial sequences which are very similar to pathogenic DNA, particularly those of TB (Pääbo *et al*., 2004, Wayne *et al*., 1999, Tsangaras and Greenwood).

Some papers have been critical of the process of DNA extraction and the subsequent analytical process (Richards *et al*., 1995, Cooper and Poinar, 2000, Yang *et al*., 2003) with some questioning the necessity of such invasive techniques (Stoneking, 1995), even suggesting that aDNA work should not continue at all as the results could never be substantiated (Cooper, 1994). The possibility of bacterial pathogen DNA preservation and the analytical methods used were strongly called into question in a study by Barnes and Thomas (2006). According to the study, ancient pathogen DNA analyses had, (amongst other things), 'rejected technical standards employed in other ancient DNA work', and not reported the replication of results in independent laboratories or differences that are found between the replications (2006: 645). Fifty nine individuals from the eighteenth and twentieth centuries, known to have suffered from TB or treponemal disease were analysed in order to test the survival of bacterial pathogen DNA; no reproducible evidence was obtained. The study suggests a number of reasons for these results such as poor DNA preservation and inappropriate or poor extraction techniques being used. They warn that previous studies should be considered and interpreted with caution (Barnes and Thomas, 2006). The study placed a greater emphasis on the need for understanding how pathogen DNA survives and how it is analysed.

In order to validate results of aDNA studies, some have advocated tests of authenticity (eg Pääbo *et al*., 2004); a number of criteria have been outlined in order to authenticate data and have been available for aDNA studies for over a decade, the most referred to being perhaps that of Cooper and Poinar (2000). The guidelines were proposed for all aDNA work, not specifically targeted at pathogen DNA work and this has since resulted in modified guidelines being produced (Donoghue, 2008) by those who do not consider all of the criteria to be as relevant for MTBC aDNA work, such as test for the persistence of human aDNA and preservation assessment. Others too have questioned the validity of reliance on a checklist system and suggest a more "cognitive" approach to data analysis, approaching results critically to gauge their reliability (Gilbert *et al*., 2005a).

Over the years however, a number of standardisation processes have been repeatedly emphasised as being important for aDNA work, some of which follow. It is advised that work should take place in specialised aDNA laboratories (Bouwman and Brown, 2005, Bramanti *et al*., 2003) without analytical instruments ever being exchanged between laboratories (Herrmann and Hummel, 1994, Yang *et al*., 2003). Protective clothing should also be worn whenever entering the aDNA work area (Bramanti *et al*., 2003, Yang *et al*., 2003). As many negative controls and samples should also be analysed (Stoneking, 1995, Yang *et al*., 2003), and independent replication of data in a different laboratory should be attained, extraction, amplification and sequencing should all be completed in this second laboratory (Cooper and Poinar; 2000, Yang; 2003).

Unfortunately, it does not appear that the precautionary steps outlined above are, as yet, being extensively used and this has been highlighted in a recent study by Roberts and Ingham, (2008). Using 15 criteria, a total of 65 papers were reviewed, all published between 1993 and 2006. Ninety per cent of these papers failed to recognise the use of basic contamination control and 85% did not validate results independently; 34% did not mention contamination removal although Roberts and Ingham comment that it is unknown as to whether this process did occur and was not discussed, or did not take place at all; Stoneking (1995) advocates at least the removal of the outer surface layer of bone or teeth in order to reduce the possibility of contamination. Such a lack of standardisation between research in this area opens up the technique to serious criticism which it can ill afford but as mentioned previously, not all believe that such rigorous adherence to aDNA guidelines such as those published by Cooper and Poinar is necessary for pathogen DNA studies, or an enitrely useful guage of the validity of results (Gilbert *et al*, 2005; Donoghue, 2008; Taylor *et al*, 2010; Tsangaras and Greenwood, in press) although all agree that there is great merit and necessity in many of the criteria. For this reason, the most recent review of published papers concerning pathogen aDNA studies considered seven out of the nine criteria proposed by Cooper and Poinar (Tsanagars and Greenwood, in press) but still found that the majority of studies failed to adhere to these guidelines.

The field of aDNA research holds huge potential but in order 'to move forward, population studies and global patterning of disease are recommended as a key focus with specific questions to ask and hypotheses to test' (Roberts and Ingham, 2008); population studies however, though commendable, pose greater challenges (Taylor *et al*, 2010). Whilst a lot of work has focussed on individual case studies, there are a number which have, or are, addressing questions pertaining to particular populations, and specifically TB (Bachmann *et al*., 2008, Murphy *et al*., 2009) with a number of papers concerning Hungarian populations (Fletcher *et al*., 2003a, Fletcher *et al*., 2003b) and the current work taking place across Europe as part of the *Biomolecular archaeology of ancient tuberculosis in Britain and Europe* project, (http://www.dur.ac.uk/archaeology/research/projects/?mode=project&id=353) joint between Durham University and Manchester University.

Debate continues as to best practice between researchers from multiple disciplines (given the range of subjects to which aDNA analyses can contribute, see Cipollaro *et al*, 2005, it is not surprising), all with a vested interest in the development and continual improvement of this valuable technique (Donoghue *et al*., 2009, Gilbert *et al*., 2005a, Wilbur *et al*., 2009, Taylor *et al*., 2010, Roberts and Ingham, 2008). It is hoped that whilst some consensus can be reached regarding the validation of results in order to better standardise practice and encourage better reporting of findings, debate and discussion will continue to play in a key role in pushing the boundaries of pathogen aDNA analyses, in order to shed more light on past human health.

2.2.3 Ethical Implications

As aDNA can theoretically be recovered from any organic material such as bone, teeth and soft tissue (O'Rourke *et al*., 2000), human remains in curatorial care are obviously a vital resource for aDNA studies of past disease. There are, however, ethical implications with the process which must be addressed by both curator and researcher. As a destructive and irreversible process utilising finite resources, bound by human courtesy, respect and future scientific discovery to be preserved and cared for, it evokes strong reaction from numerous interested parties including specific cultural or religious groups (Ubelaker and Grant, 1989, Jones *et al*., 1999, Webb, 1987, Balter, 2000).

> *…researchers have an obligation to use scientific principles to protect sample materials that are in the public trust, and which are of sufficient antiquity to not be reliably associated with modern populations.*
>
> (O'Rourke *et al*., 2000: 223)

Certainly, curators must limit such research to those studies which will best contribute to our knowledge of past human societies and in this case, the evolution of disease which may have a significant impact on current disease prevention and control research. The researcher must also take a responsible and minimalist approach to their work regarding sampling, taking no more than is needed and to address only well formed research questions, the results of which should be made available to all. This has been highlighted in recent years, specifically in reference to aDNA analyses concerning TB, (Wilbur *et al*., 2009):

> *…in general, the justification for destructive analysis depends on the linkage between two factors: the stage to which the research area has progressed and the nature of the question being asked…the destructive nature of biomolecular studies of ancient TB places a responsibility on researchers to ensure that all components of their projects – palaeopathological and biomolecular – are carried out to high scientific standards.*
>
> (Wilbur *et al*, 2009: 1991-1995)

The study was critical of research they did not consider was justifiable (Hershkovitz *et al*., 2008) given that previous research had already demonstrated the antiquity of the disease (Brosch *et al*., 2002, Gutierrez *et al*., 2005, Pfister *et al*., 2008). This view was contested by the original authors who viewed the research question posed, not as "a hunt for the world's oldest case of TB", and rather an investigation into the health of an early coastal community in the Levant (Donoghue *et al*., 2009). The exchange of views between these papers further highlights the discrepancies within the pathogen aDNA community as to best practice, but what is quite clear is that Wilbur *et al*.'s assertion that destructive sampling must be

undertaken 'only if the research questions justify it' (2009: 1991) is something all researchers must critically consider before the start of any project.

2.2.4 The Clinical Literature

> *The rapid and specific diagnosis of tuberculosis is one of the most pressing needs in efforts to eradicate the disease.*
>
> (Eisenach *et al*., 1988: 2240)

Due to the current global problem of TB, the extensive clinical literature produced on *Mycobacterium tuberculosis* complex and its specific genetic identities has been invaluable to the progression of aDNA techniques. This work, of course, has the distinct advantage of data from living patients and modern DNA of structural integrity. Biological research on modern TB samples supports efforts to compare different strains, and their geographical movement, which in turn may allow identification of strains with specific properties such as high infectivity, virulence and even multi-drug resistance (Van Embden *et al*., 1993), all of which may be key to understanding how to potentially eradicate or at least control the disease now and in the future.

The techniques discussed in the clinical literature on TB, as in the aDNA literature, have inevitably evolved over two decades and, whilst the applications and/or purpose may differ between modern and ancient research, the data provided by current clinical literature is of significant advantage to the bioarchaeologist. The determination of DNA sequences for the IS element, IS*6110* (along with other, virtually identical IS elements such as IS986), from different *M. tuberculosis* strains, has enabled extensive study of the MTB complex to occur, employing techniques such as RFLP (restriction fragment length polymorphism). Alland *et al.* (2003) have also studied the evolution and genetics of IS*6110* using SNP (single nucleotide polymorphism) markers and its CGM-based (comparative-genome markers) tree, with SNPs able to act as 'evolutionary clocks'. Filliol *et al.* (2006) also utilised SNPs in order to shed light on the evolution of the disease. Out of hundreds of MTB complex (*M. tb, M. bovis, M. africanum, M. microti,* M. canettii) isolates studied, a few have been found devoid of the IS*6110* element (Hermans *et al*., 1990, Van Soolingen *et al*., 1993, van Soolingen *et al*., 1991,

McAdam *et al*., 1990, Yuen *et al*., 1993). This has raised questions as to the usefulness of IS*6110* in population genetics and phylogenetic analyses (Fang *et al*., 1999).

Repetitive elements and insertion sequences are commonly used as target sequences in order to identify differences between mycobacterial strains; in the case of tuberculosis, this is the irregular insertion of IS*6110* into the genome (Cave *et al*., 1991, Alland *et al*., 1994, Eisenach *et al*., 1988, Van Embden *et al*., 1993). IS*6110* is certainly the most extensively used element for epidemiological studies (Kanduma *et al*., 2003). As many as 25 copies of IS*6110*, which is related to members of the enterobacterial IS3 family (McAdam, *et al.* 1990), are present in the genomes of clinical isolates of MTB and the presence of this element has been closely linked to patients with clinically diagnosed TB (Rollo and Marota, 1999), making it an excellent focus for aDNA studies where a more definite diagnosis of TB than macroscopic analysis can provide is being sought. The number of copies of IS*6110* is also species and strain dependant; *M. africanum* and MTB tend to contain more copies than *M. bovis* (Kanduma *et al*., 2003).

Another IS element, IS*1081* (1324bp) is significantly different to IS*6110* and the IS3 family (Collins and Stephens, 1991). Work in this area has shown that differentiation between MTB BCG and MTB can be achieved in modern clinical samples using IS*1081*. This work has also shown that of 99 strains of MTB complex studied, all contained five or six IS*1081* copies. This is of great use for aDNA work as this provides an additional insertion sequence with which to attempt to confirm the pathogen's presence or absence, particularly as strains lacking this element are not believed to exist (Van Soolingen *et al*., 1997), although there are many closely related elements that are found in environmental bacteria (Picardeau *et al*., 1996), Wilbur *et al* (2009) therefore advise that PCR products be cloned and sequenced to ensure a positive result is truly IS*1081* and not a closely related sequence. Park (2000) suggest that this IS sequence may also be useful for epidemiological studies due to the conserved copy number and low discriminatory power it demonstrates, although because the copy number is lower than IS*6110* others suggest its use in epidemiological studies is limited. However, it remains another useful genetic marker to identify presence or absence of TB in bioarchaeological studies.

By far, one of the most useful techniques to emerge from the literature and which has enabled aDNA analysis to progress, extending the time frame for which aDNA is viable, has been the development of PCR, as discussed earlier. PCR, which allows for specific amplification of discrete fragments (Eisenach *et al*., 1988) and from only a few target molecules (Mullis and Faloona, 1987), has enabled bioarchaeologists to extract aDNA of limited integrity and target pathogen specific insertion sequences, as in the case of tuberculosis, but this technique is certainly not limited to TB. PCR, however, has many limitations common to both modern and ancient research, the largest of which, as previously described, is that of foreign DNA contamination.

2.2.5 Alternative Biomolecular Techniques

Although aDNA is the most utilised approach in the study of ancient pathogens (Drancourt and Raoult, 2008), analysis of nonnucleotidic biomolecules from ancient microbes also has the ability to complement such work and is being increasingly utilised (Thi-Nguyen-Ny *et al*., 2011) (Figure 2-13). Evidence suggests that proteins may demonstrate a higher resistance to taphonomic decay than aDNA (Heaton *et al*., 2009, Nielsen-Marsh *et al*., 2005, Haensch *et al*., 2010) making them very useful in ancient pathogen research. The techniques applied to nonnucleotidic biomolecules include immunochromatographic detection, enzyme-linked immunosorbent assay (ELISA) and immunohistochemical analysis (the applications of which are summarised in Thi-Nguyen-Ny *et al* (2011) (Figure 2-14). The techniques which have been most employed in *mycobacterium tuberculosis* research however, are mass spectrometry, and lipid analysis. Matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI-TOF MS) has been applied to Hungarian skeletal samples (Boros-Major *et al*., 2010, Mark *et al*., 2010), the former study analysing microbial proteins and the latter, microbial lipids. The results of the latter study, which used this method to identify mycolic acids in bones samples from five different periods (600AD – 1600AD) (Mark *et al*, 2010), have however been called into question after reinterpretation (Minnikin *et al*., 2010); the original authors have since stated '…the clinical protocols and standards cannot directly be used for the biomolecular palaeopathological investigations…' (Mark *et al*, 2011).

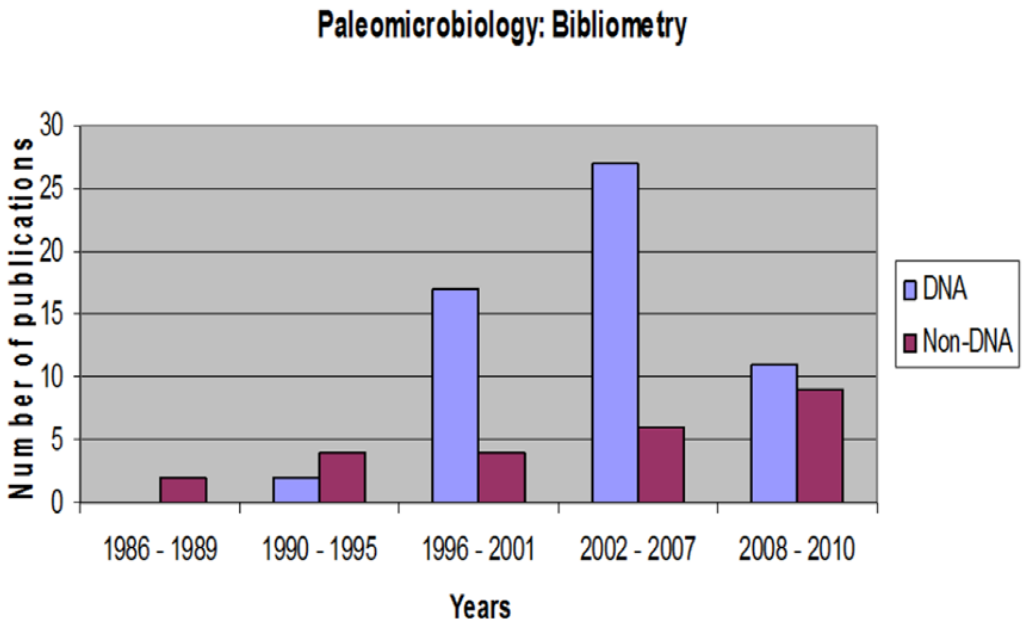

Figure 2-13 Paleomicrobiology: bibliometry. There is a growing number of reports based on the use of nonnucleotidic biomolecules in ancient specimens (Thi-Nguyen-Ny *et al*, 2011: Fig 1).

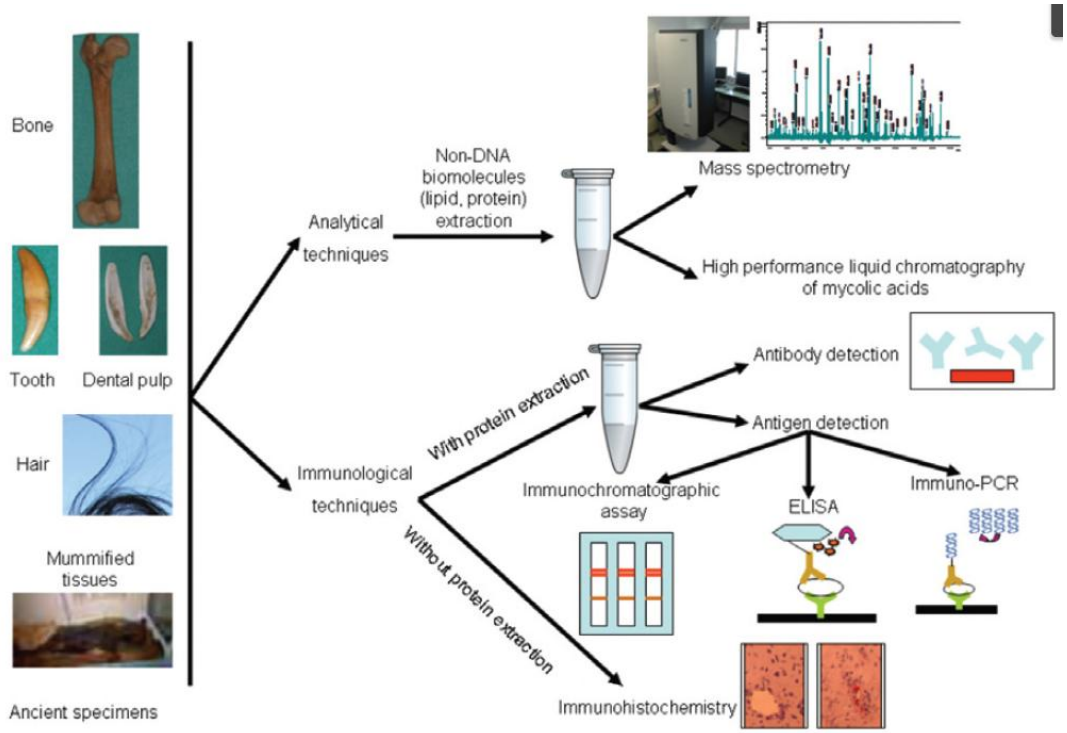

Figure 2-14 Suitable source materials and methods used for nonnucleotidic detection in paleomicrobiology (Thi-Nguyen-Ny *et al*, 2011: Fig 2).

The second form of analysis to receive significant attention for *mycobacterium tuberculosis* studies is the detection of microbial lipids (Donoghue, 2008, Donoghue *et al*., 2010, Donoghue *et al*., 1998, Gernaey *et al*., 2001). Mycolic acids which are contained within the waxy coat of mycobacteria such as TB have the potential for long term survival (Gernaey *et al*., 1999) (Figure 2-15 and Figure 2-16) and are detectable in minute amounts (Donoghue *et al*, 2010). High-performance liquid chromatography (HPLC) (Thi-Nguyen-Ny *et al*., 2011) has been applied to a fragment of 1400 year old lung tissue (calcified pleura) from the Negev desert at Karkur, Israel (600AD) (Donoghue, 1998), alongside aDNA extraction, and both successfully identified *M. tuberculosis* (Donoghue *et al*., 1998, Spigelman and Donoghue, 1999). However, as mentioned, calcified pleura itself cannot be definitively attributed to TB. The technique employed (HPLC) was also applied to an 11th century rib from the site of Addingham in England which demonstrated new bone formation with the skeleton also demonstrated Pott's disease (Gernaey *et al*, 2001); the presence of TB was confirmed both by aDNA analysis and the mycolic acid analyses. Ribs and long bones from the site of Atlit-Yam, Israel (9250-8160 years BP) were also subject to HPLC, and 19th/20th century ribs from Coimbra, Portugal (Redman *et al*., 2009). Research on the Coimbra material showed that out of eight patients, four had died of TB. TB specific mycolic acids were identified in all, but aDNA in only two; however, both DNA and mycolic acids were each identified in an individual who did not die of TB (in Roberts and Buikstra, 2003: 176). Finally, a study was carried out of a skeletal population from Newcastle Infirmary burial ground (1753-1845AD), where 27% of the people buried there were known to have TB at death, as seen in historical data (Gernaey *et al.*, 1999). The results showed identification of mycolic acids, specific to *M. tuberculosis* in 24% of the individuals, much higher than the 0.95% (2/210) of individuals with identified morphological changes consistent with Potts disease via osteological examination (Boulter *et al*., 1998, Gernaey *et al*., 1999).

Analyses of nonnucleotidic biomolecules, present many opportunities for the study of ancient pathogens and can complement aDNA work as demonstrated above. Thi-Nguyen-Ny *et al* (2011) also refer to a technique developed for the diagnosis of modern infectious diseases (Lepidi *et al*., 2006) called autoimmunohistochemistry which, because antibodies can survive for centuries, they suggest may be applicable to ancient samples. It is clear that:

…with the foreseeable developments of technology…the analysis of nonnucleotidic biomolecules is becoming a standard approach to complement aDNA analysis in the field of paleomicrobiology.

(Thi-Nguyen-Ny *et al*, 2011: 378)

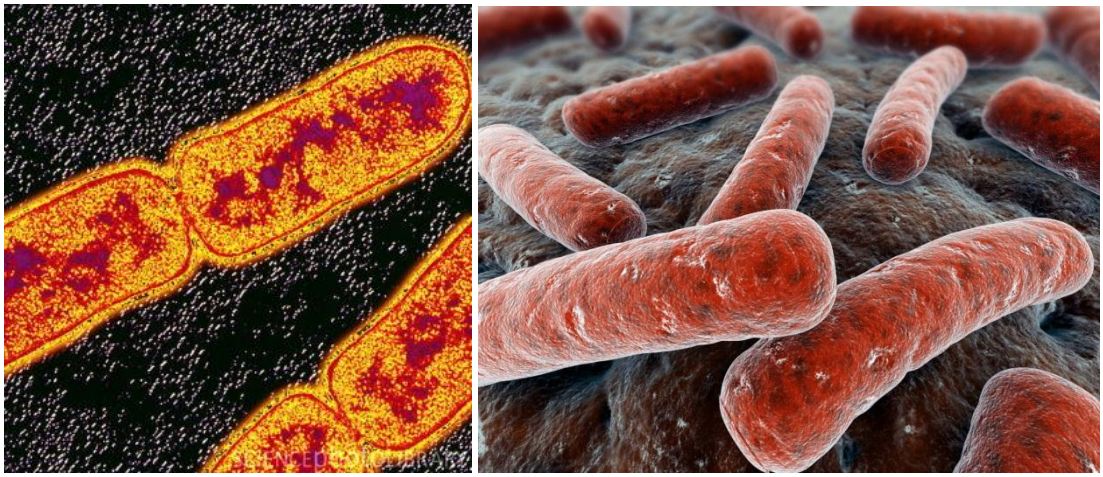

Figure 2-15 (Left) Transmission electron micrograph (TEM) of *Mycobacterium tuberculosis* bacteria. Magnification: x50,000 at 6x6cm size. (www.Sciencephoto.com)

Figure 2-16 (Right) Magnified image of *Mycobacterium tuberculosis* bacteria showing their rod shape and waxy outer coat (http://www.bioquellus.com/technology/microbiology/mycobacterium-tuberculosis/)

2.3 Tuberculosis in the Old World

The earliest skeletal case of TB from the Old World may date from 7250B.C. in Jordan (El-Najjar *et al*., 1996) but there could be numerous differential diagnoses for the lesions seen in these skeletal remains (Roberts and Buikstra, 2003: 176). A much more recent case has made the news claiming to date from 500,000 years ago (Kappelman *et al*., 2008) but this diagnosis has since been challenged based on the limited evidence of documented diagnosed cases associated with the type of cranial lesions described, and the possibility of alternative diagnoses; the challenging authors suggest ectopic arachnoid granulations (AG) may be a more fitting diagnosis (Roberts *et al*., 2009). All that was recovered were skull fragments; on the endocranial surface of part of the frontal bone, pathological lesions which were 'numerous small, roundish and ellipsoidal, flat granular impressions with a relatively smooth surface' were identified (Kappelman *et al*., 2008: 112). These lesions are thought to

be consistent with a form of TB called *Leptomeningitis tuberculosa*, which attacks the meninges of the brain. Although theoretically possible that the lesions were caused by tuberculous infection, without additional accompanying skeletal evidence there is certainly no definite evidence that TB caused the lesions, and TB should only be considered as one possible cause. In addition, the remains are thought to be from a person aged somewhere between 15 and 40; the majority of these cases related to TB occur in children below the age of 10 and those lesions are usually second to, or are coexistent with, other tuberculous skeletal foci (Sorrel *et al*., 1932: 79-80). Again, this cannot be seen here. Hershkovitz *et al*, (2008) also report evidence of *Mycobacterium tuberculosis* from the site of Atlit-Yam in the Eastern Mediterranean, dating from 9250-8160 years ago. The results of the study demonstrate identification of the disease based on both morphological (macroscopic) and biomolecular methods but these too have been questioned, based on unconvincing palaeopathological evidence, with the lesions described as being 'indistinguishable from other infectious diseases'; amongst other criticisms, insufficient procedures to prevent contamination are also reported (Stone *et al*., 2009: 80).

The earliest accepted evidence of TB currently originates in Italy from the Neolithic period, 5800+_90 BP from Liguria (Canci *et al*., 1996). Here a female skeleton within a cist grave had lumbar vertebrae affected; the area is very close to that of Arene Candide where TB was reported for the first time in Italy by Formicola *et al.* (1987). As Roberts and Buikstra comment (2003: 174), the proximity of these sites may indicate a commonality of culture leading to the presence of the infection. Evidence has also been reported from Egypt, possibly from as early as 4500BC (Derry, 1938, Morse *et al*., 1964), Neolithic Poland (Gladykowska-Rzeczycka, 1999) and Neolithic Spain (Sanoja; 1975 in Roberts and Buikstra, 2003). Although not as early as elsewhere in the world, a recent discovery of TB in the Aneolithic Yayoi period (ca. 300BC to AD300) in Japan is now the earliest to be found there, the remains dating to between 454BC and AD124 (Suzuki and Inoue, 2007). Human remains with evidence of TB in Egypt include the "The Priests and Priestesses of Amun", dating from the Twenty First Dynasty (c. 1069 – 945BC), discovered by Grebart in 1891. One of the 44 mummies showed unmistakable evidence of TB, that of Nesperehan, with angular kyphosis of the lower thoracic and upper lumbar vertebrae (Morse; 1967: 263). Furthermore, tubercle bacilli have been isolated in bone from a mummy of a child from the site of Dra

Abu el Naga (1314-1085BC) (Zimmerman, 1979), and aDNA of TB has been identified and sequenced from an infected lung of a mummy (Zink *et al*., 2000) dated to between 1550 and 1080BC. As Lynnerup states:

> *mummified remains may sometimes in themselves be indicative of good molecular preservation, and furthermore, allow for direct comparison with soft tissue pathological changes.*
>
> (2007: 175).

More studies by Zink *et al.* using biomolecular techniques (Zink *et al*., 2001, Zink *et al*., 2004, Zink *et al*., 2003a, Zink *et al*., 2003b) have identified Egyptian human remains as having been infected with the disease. Even more recently, (Zink *et al*., 2005) identified numerous individuals with typical tuberculous morphology, non-specific alterations, and no macroscopic morphology as being infected with TB from the period 1450-500BC and, in 2007, an additional set of Egyptian samples from the period 3500-5000BC showed 23% positive for MTB aDNA.

In Britain, the earliest reported TB comes from an individual buried at Tarrant Hinton in Dorset (Mays and Taylor, 2003). The study utilised both osteological examination and aDNA analysis, the latter of which supported the original diagnosis of TB. It was unfortunate in this study that confirmation of the causative pathogen having been *M. tuberculosis* or *M. bovis* could not be obtained. Further aDNA analyses were applied to the sample however, and the causative strain was found to be *M. tuberculosis* (Taylor *et al*.2005). Of Iron Age date (400 – 230BC) this find gives proof of the infection being present prior to the Roman invasion. Although relatively few cases of bone TB have been recorded from the Roman period (43-410AD), it is present (Farwell and Molleson, 1993, Stirland and Waldron, 1990, Cox, 1989, Anderson, 2001, Wells, 1982). The first confirmed evidence of human TB caused by *M. bovis* was located in four out of five individuals studied from the Iron Age cemetery of Aymyrlyg, South Siberia (Taylor *et al*., 2007), Not only did the genotyping performed on the material establish that *M. bovis* was the cause, but it also provided yet more support for the phylogenetic model of the *M. tuberculosis* complex being due to specific deletions and polymorphisms and *M. bovis* being a later development than *M. tuberculosis* (Brosch *et al*.,

2002). In addition, *M. africanum*, another member of the *M. tuberculosis* complex, has been found in very early Egyptian material which may suggest that it too had a role in the evolutionary process of TB, perhaps even parallel to *M. tuberculosis* (Zink *et al*., 2007).

Evidence from Britain during the early medieval period (c.410-c.1050AD) is also apparent and throughout the country; two sites from Hampshire, at Alton (Powers and Brothwell, 1988) and Bedhampton (Manchester and Roberts, 1986, Shennan, 1978) revealed one male, and one male and one female skeleton, respectively, to have evidence of TB in the spine (Figure 2-17). Other sites include Addingham in Yorkshire (Boylston and Roberts, 1996), and Raunds in Northamptonshire (Powell, 1996), the affected individual from the latter site also having evidence of knee involvement, and both sites showing evidence of rib lesions. Additionally, from the site of Addingham in Yorkshire, confirmation of TB via aDNA and mycolic acid analyses was recorded for a male with Potts disease and rib lesions (Gernaey *et al*., 2001). A number of other sites also present individuals with evidence of TB, mostly single individuals (Duhig *et al*., 1998, Brothwell, 2000, Boyle *et al*., 1998, Norton and Boylston, 1997, Waldron, 1988, McKinley, 1996), with the exceptions of Nazeingbury in Essex where two individuals were affected (Putnam, 1978), as at School Street, Ipswich in Suffolk (Mays, 1989). In the Late Medieval period (c.1050-c.1550AD), an increasing number of sites have revealed individuals with the disease. In total, 18 sites showed evidence of affected individuals and in higher frequencies than in the early medieval period (Roberts and Buikstra, 2003). For example, the site of St Helen-on-the-Walls in York included four people with evidence of TB in the spine and one with evidence elsewhere on the skeleton (Dawes and Magilton, 1980), and the well known site of Wharram Percy in Yorkshire revealed seven individuals with spinal TB, two affected elsewhere in the body, and some people also showed evidence of rib lesions (Mays, 2007). Other sites from throughout the country also show affected individuals in numbers ranging from one to dozens (Ortner and Bush, 1993, Molleson *et al*., 1993, Stroud and Kemp, 1993, Manchester and Roberts, 1986, Gernaey *et al*., 1999, Miles, 1989, Boulter *et al*., 1998, Heighway, 1980, Rogers, 1999, Mays, 1991, Stuart-Macadam, 1986, Magilton and Boylston, 2008, Waldron, 1993, Brothwell and Browne, 1994). However, a number of individuals from a total of twelve sites (some of which are included above), had individuals with non-specific changes such as new bone

formation on the ribs, or calcified pleura, which may or may not have been caused by TB (Chundun and Roberts, 1995, Roberts, 1999, Henderson, 1990, Chundun, 1992).

Figure 2-17 TB in a spine from the early medieval site of Bedhampton, Hampshire. (Photo by and courtesy of Charlotte Roberts).

2.4 Tuberculosis in the New World

TB studies in the New World have helped to create a more comprehensive understanding of the global presence of TB in the past, and offered proof that the disease was present in the Americas before Columbus (Salo *et al,* (1994). Studies have also employed biomolecular analyses in the search for TB and as such have contributed to the continual progression of the discipline. A general overview of research so far performed on New World material, is therefore essential in order to better appreciate the global scale of ancient TB, the methods employed in its detection to recognise the influence of such studies on current research.

Ales Hrdlička, a 20th century physical anthropologist and medical doctor stated 'as yet no bones of undoubtedly pre-Columbian origin have been found that show tuberculous lesions…' (Hrdlička, 1909: 1). In 1886, however, three individuals had been identified with possible TB (Whitney, 1886) but, because of doubt surrounding the diagnosis of these examples (Morse, 1961), Hrdlička's word on the matter stood firm. By the middle of the 20th century there had been numerous reports of skeletal TB, particularly Potts disease, originating from North America (Lichtor and Lichtor, 1957, Crane and Griffin, 1959, Ritchie, 1952, Judd and Allen, 1954); South America was also represented (García-Frías, 1940, Requeña, 1945). Not all examples could be dated to the pre-Columbian period with absolute certainty (Hooton *et al*., 1930, García-Frías, 1940) and, coupled with what was considered the "extreme" nature of the examples, Morse remained sceptical on the subject of pre-Columbian TB, also questioning the archaeological provenance of the remains and suggesting that the examples seen were too few (Morse, 1961, Morse, 1969, Morse *et al*., 1964, Morse, 1967). Morse's diagnostic criteria, which originated from clinical data, may not have been an accurate model for identifying pathological changes from earlier skeletal examples (Roberts and Buikstra, 2003), and many have noted the restrictive nature of this clinical standard (Buikstra, 1976, Buikstra, 1981, Buikstra, 1977, Ortner and Putschar, 1985). In the same era Cockburn (1963) also discounted TB in this period due to insufficiently large, settled population figures existing but, as Roberts and Buikstra (2003: 189) comment, major population centres did exist in late prehistoric North America, and evidence of TB is found in these areas; communities of thousands may not even have been needed in order to maintain the disease (Black, 1975, Daniel *et al*., 1981). Others disagreed with Morse's viewpoint entirely (Ackerknecht, 1955) and Wells' opinion on the matter was that Morse 'rejects it for the New World whilst accepting it for the Old.' (1964: 100). Later examples, however, "gradually eroded that scepticism" (Aufderheide and Rodríguez-Martín, 1998) and demonstrated convincing evidence (Buikstra and Cook, 1981, Allison and Pezzia, 1973, Hartney, 1981).

A total of 44 sites in North America have been reported to have evidence of affected individuals, some of which report numerous examples such as those by Powell (1988); two juveniles had affected ribs, one male and two females had vertebral involvement, and four males and four females of young-middle age also had affected ribs. A great many of the

sites are located in the east but this may be a reflection of the greater number of those working in bioarchaeology in the region (Roberts and Buikstra, 2003: 190). South America hosts 15 sites with evidence of TB, in Peru (Buikstra and Williams, 1991, García-Frías, 1940, Allison *et al*., 1981, Allison *et al*., 1973, Burgess, 1992, Owen, 1993, Lombardi and Guido, 1992), Venezuela (Requeña, 1945), Chile (Allison et. al.; 1981) and Columbia (Arregoces, 1989, Arateco, 1998, Boada, 1988, Correal and Flórez, 1992, Martinez de Arateco Hoyo, 1999, Rodriguez, 1988). It should also be noted that in South America there is an imbalance of sex ratios, unlike in North America. Males appear to have had a higher risk of dying with skeletal TB than females and this is particularly evident at the site of Estuquina (Buikstra and Williams, 1991). It has been suggested that stresses due to warfare may account for this (Arriaza *et al*., 1995), although occupational factors may be more likely (Roberts and Buikstra, 2003: 207).

The use of aDNA analysis in more recent years seems to have finally given a definitive answer to the question as to whether or not TB existed in the Americas, pre-Columbus. The work of Salo *et. al.* (1994) involved naturally mummified remains of a female from Peru who demonstrated pathology suggestive of primary pulmonary TB in the soft tissues (no evidence could be found skeletally). Again, using biomolecular techniques, evidence of TB has also been found in North American Pre-Columbian remains by Braun *et. al.;* (1998). There is however, according to Braun *et. al.*, no evidence for an association between the Peruvian tubercular aDNA recovered and that from the North American example; thus, it is possible that more than one New World strain was in existence. In addition, Arriaza *et. al.* (1995) amplified bacterial DNA from a young girl who dates to around the same time as that reported by Salo *et. al.* (1994). Stead *et. al.* (2000) emphasised that the techniques used by Salo *et. al.* could not differentiate between M. *tuberculosis* and M. *bovis*. It has been argued convincingly (Daniel, 2000) that the earliest TB in America was caused by M. *tuberculosis* and that it originated from its earliest immigrants, or those from Asia, between 10 or 20 (possibly even earlier) thousand years ago. Before this, during the 1990's, a number of papers (Bates and Stead, 1993, Haas and Haas, 1996) suggested that M. *bovis* was the cause of the disease, although opinion may have changed in light of Brosch *et al*.'s (2002) study already discussed. Mackowiak *et. al.* (2005) also supports the view that it was more likely that TB was transported to the New World via infected humans than animals. Although

theories abound there are no conclusive answers to the questions posed as yet: which strain of TB was responsible for the pre-Columbian cases, and when did it arrive or how did it arrive – via nomadic peoples crossing the Bering Land Bridge or across the Ocean (Mackowiak *et al*., 2005). As Mackowiak *et. al.* suitably state 'Thus, after >1 century of debate, PCR methods have finally firmly established the presence-but not yet the complete identity –of mycobacterial infection prevalent among pre-Columbian Americans.' (2005: 517) How TB came into existence in the New World has clearly been a source of debate, although it is of course now evident that it did not arrive with Christopher Columbus.

Pre-Columbian evidence, however, still lacks in Mesoamerica despite the numerous examples from both North and South America (Roberts and Buikstra, 2003: 193). Sea travel has been suggested as one explanation for the "passing over" of Mesoamerica in transmission of the disease between the north and south (Roberts and Buikstra, 2003: 193-194). There are many sites from which large groups of human remains have been discovered, for example the site of Teotihuacan, once home to thousands of inhabitants, has produced hundreds of skeletons and yet no pathology indicative of TB has been recorded (Sempowski *et al*., 1994, Storey, 1992). Buikstra does draw attention to the argument that people in Mesoamerica may not have been living long enough for the disease to manifest in the skeleton (Roberts and Buikstra, 2003) and refers to various stressors which have been identified (Martin, 1994, Whittington, 1989, Wright, 1994) that may have impacted on peoples immune systems. However, conversely, there were higher frequencies of skeletal TB in groups from North America who also suffered from such stressors (Eisenberg, 1986). Other reasons may account for the lack of skeletal evidence in the area, for example poor preservation of remains (Mackowiak *et al*., 2005), although it is likely that Potts disease would still be evident in this scenario (Roberts and Buikstra, 2003), or that those with such skeletal deformities may have been buried elsewhere (Mackowiak *et al*., 2005). The latter may stem from particular death rituals afforded to those with special physical conditions which may have been destructive (Roberts and Buikstra, 2003, Buikstra and Charles, 1999). The presence of TB in Mesoamerica may be implied through a figurine, one of seven found in a decayed Mayan town called Dzibilchaltun. This particular figurine may show evidence of Potts disease but, as Mackowiak *et. al.* (2005) warns, this and other artistic representations possibly depictive of acute angular kyphosis found in indigenous

American iconography is, at best, circumstantial. Maya vases commonly depict figures with a rounded curve in the upper spine and this may well be an artistic convention (Roberts and Buikstra, 2003, Buikstra, 1999) as suggested with some artistic representation in Egypt, or represent another spinal condition.

2.5 Historical, Literary and Artistic Evidence for TB:

TB has acquired a multitude of names throughout its long history and has no doubt been confused with other illnesses or missed altogether along the way (Dormandy, 1999: 1). In addition to "Phthisis", "King's Evil" (of Medieval origin), "Scrofula", and "Consumption" have all been used to describe what we know to be TB (Roberts and Buikstra, 2003: 7). This variety of names is important to note when attempting to identify the disease in historical writings. Known also as the "White Plague", TB was associated with 'childhood, innocence and even holiness' (Dormandy, 1999: xiv)

Literary sources should be approached with utmost caution and with:

> *'the full knowledge that ancient views of the medical cosmos provided no stimulus to separate out various diseases producing similar symptoms or to recognise the common cause of a condition…'*
>
> (Aufderheide and Rodríguez-Martín, 1998: 125)

TB has a long written history, the earliest examples including a medical text in China (2700BC) describing TB of the lymph glands and associated symptoms and treatment (Keers, 1981), the Egyptian medical papyrus of Ebers (1500BC) (Evans, 1998: 6) and the religious Sanskrit hymns from India (1500BC) which describe phthisis (Evans, 1998). Cave also comments that the medical papyri quite possibly hold reference to TB in 'still obscure passages…' (1939: 144). Evans also identifies numerous Greek authors who appear to be describing conditions resembling TB, including Hippocrates (c. 450BC). It was from

Hippocratic times that TB was known as “Phthisis” (Daniel *et al*.); it was described as a shrinking of the body involving ulcers in the lungs along with a fever and cough (Meachen, 1936, Clarke, 1962). Hippocratic authors also described Potts deformity of the spine with surprising specificity (Aufderheide and Rodríguez-Martín, 1998: 127). Homer, living around 800BC, may also have commented upon the disease but these writings are, to say the least, ambiguous (Homer, The Odyssey: 11.100-101 and 5.396-399). Aristotle (384-322BC), however, recognised the contagious nature of the disease, as did Galen (AD131-200) (Evans, 1998, Chalke, 1959). Plato (430-347BC) also commented on the disease, recommending no treatment as it was of no benefit to the patient or state (Meachen, 1978 cited in Evans, 1998). Other Greek and Roman writers include Herodotus (484-425BC), Pliny (AD 23/24-79), Seneca, Ovid and Celsus. Columella (AD50) and Vegetius (AD420-?) also noted that consumption affected animals (Meinecke, 1927). It is odd that TB is not mentioned in earlier writings such

as in the Beers medical papyrus or the Semitic Code of Laws of Hammurabi of Babylon as many examples of the disease have been found in skeletal material from these regions (Mercer, 1964: 245). Morse, however, discusses documents dated to 675BC from Mesopotamia which may describe TB (Morse, 1967).

There are numerous possible artistic representations of TB in Egyptian art such as that of a serving maid appearing to show involvement of the mid-thoracic spine (Morse; 1967: 263), a servant boy from an Old Kingdom tomb scene appearing to suffer from TB of the cervico-thoracic region (Morse; 1967: 263), and the figure of an emaciated human found crouching in a clay vessel which also shows angular kyphosis of the thoracic spine (Morse, 1967: 261). It has been suggested that the latter was an artist’s representation of a style of burial rather than a comment on any particular disease; it must also be taken into account the possibility of stylistic conventions (Evans, 1998). This illustrates the difficulty in interpreting art as sources of disease in the past.

In addition to Egyptian examples, TB and its effects have also been captured in art such as the paintings and etchings by the famous artist Edvard Munch, the subject of which was inspired by his sister Sophie who died from the disease aged just sixteen (Dormandy, 1999).

In addition, The Stagecoach by Hogarth depicts a person with kyphosis of the spine which may have been due to TB, and one of the most famous paintings in the world, Botticelli's The Birth of Venus (Figure 2-18), where the subject is shown with long fair hair, whitish-pink skin, small shoulders, a narrow thorax and low placed, close breasts, are all signs of phthisis as represented in the Renaissance; the model for this picture, Simonetta Catanea, died of TB aged 23 (Chevrolet, 1924 in Boetsch, 1999).

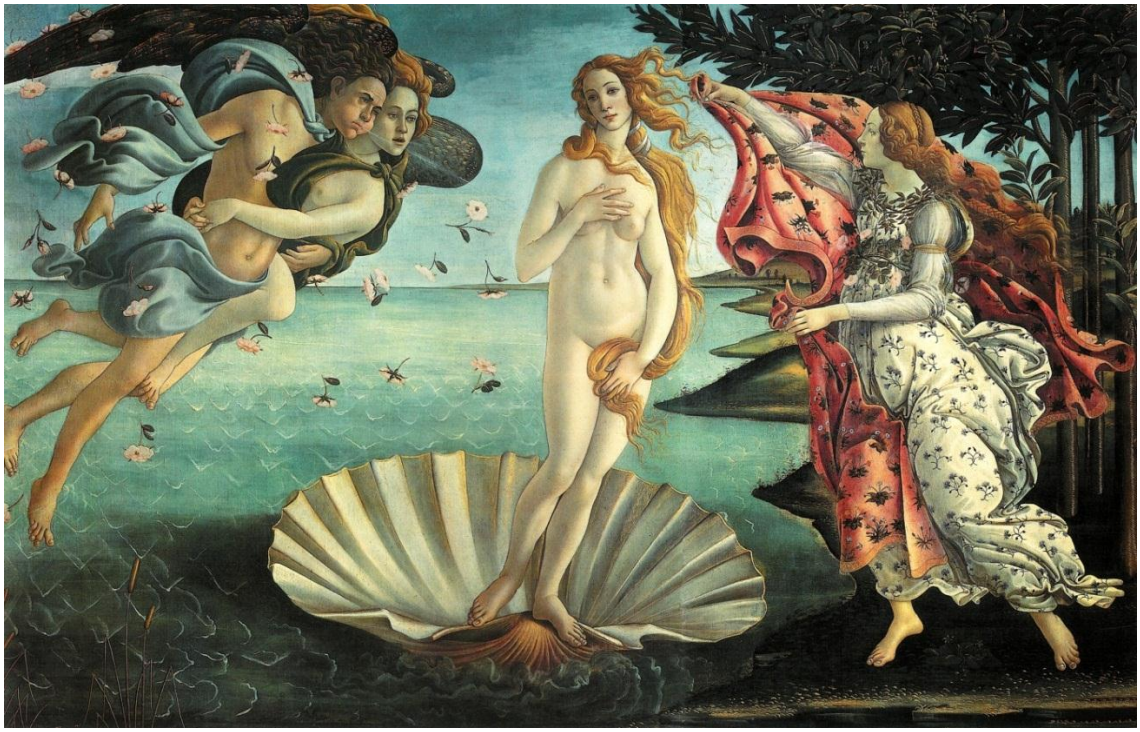

Figure 2-18 Botticelli's, The Birth of Venus
(http://upload.wikimedia.org/wikipedia/commons/4/47/La_nascita_di_Venere_%28Botticelli%29.jpg)

Later written evidence also includes further Chinese medical texts from the Sui (AD 581-618) and Tang (AD 618-907) Dynasties which describe pulmonary symptoms which may be TB (Morse, 1967). In England, in the Anglo Saxon period, Bede may have been describing TB with his words: the pestilential state of the air which destroyed thousands of men and cattle (Chalke, 1959: 84). Later in the Medieval period, Paracelsus (1493-1541) described phthisis (Rosen, 1943), Fracastorius (1483-1553) suggested this may have been due to invisible 'germs', whilst Spigelius (1578-1625) said that consumption in women may be brought about by wearing tight dresses, constricting the chest. During the Middle Ages, it was common for those suffering from TB to be touched by the ruling monarch, Edward the Confessor being the first to perform such a duty; Charles II is thought to have touched 4000

people (on average) per year (Mercer, 1964: 248). The London Bills of Mortality, recorded causes of death and the figures suggest that TB was becoming common by the early 17th Century (Roberts and Buikstra, 2003: 216), and "Consumption", which implies pulmonary TB, was apparently responsible for twenty per cent of all deaths (in London) in non plague years during the 17th century (Clarkson, 1975). It is unlikely that all those recorded as having the disease actually suffered (Roberts and Manchester, 2005), and it is likely that many had other illnesses whose manifestations resembled that of TB, such as coughing up blood, which may have been due to bronchitis and other pulmonary illnesses. However, the argument has also been put forward that there may be an underestimation of deaths from historical data due to the stigma attached to the infection at the time (Evans, 1998). There is, however, no reason to consider historical evidence of this sort to be 'widely inaccurate' (Roberts and Cox, 2003: 338).

Rates of TB began to decline in the early 20th century but, throughout the 19th century, pulmonary TB was probably the biggest cause of death (Roberts and Cox, 2003: 338) and this was no doubt exacerbated by poor standards of living. The annual mortality rate in Britain peaked at around 400-500 / 100,000 population, the rates being higher in urban areas. Much of the later 'romanticised' literature (Boetsch, 1999) was written during these centuries of dominance by TB infection, and give detailed accounts of the disease, its symptoms and effect on the body. British history also has its own long list of literary greats who contracted and eventually succumbed to the disease, such as Keats, D H Lawrence, George Orwell, and at least four Bronte family members, to name but a few (Daniel *et al*., 1994: 17). As a trained doctor, Keats himself was aware of his condition after coughing up blood; he apparently stated 'This is arterial blood: I cannot be deceived by its colour. It is my death warrant.' (Cook, unknown date, cited in Dormandy, 1999: 13).

2.6 Developments in Prevention and Cure

Sir Percival Pott, after whom "Pott's Disease" is named, is considered to be the first to describe the spinal changes associated with TB, and is better known than Dalechamps who had previously discussed displacement of the vertebrae (Mercer, 1964: 246). It was,

however, Koch's (Figure 2-19) discovery of the infecting bacillus in 1882 which has influenced the study of TB beyond that of any previous discovery or description. Koch defied previous thoughts on the disease, such as those held by Virchow who continued to believe TB was bought on by, and developed as a result of, other diseases (Mercer, 1964: 249). Interesting to note however, is that rates of TB had begun to subside even before Koch identified its cause (Figure 2-21). In 1840, George Boddington had argued that patients suffering from TB should be exposed to airy environments, and amongst other things, an atmosphere free of damp. The first sanatorium was opened in 1859 in Germany and, according to Evans (1998: 10), the sanatorium era lasted almost 100 years; Koch's discovery giving "fresh impetus" to the movement. Admission to a sanatorium was usually voluntary and there were no obvious effects on the rate of decline of TB by their introduction (Aufderheide and Rodriguez-Martin, 1998: 130). In 1898 the "National Association for the Prevention of consumption and other forms of Tuberculosis" (NAPT) was formed and a vast cohort of literature was distributed that attempted to educate individuals to be personally responsible for not contracting the disease, a movement that did not prove to be particularly successful (Evans, 1998: 10). France and Germany had already established anti-tuberculosis associations before the NAPT, as had America and Belgium; other countries such as Canada, Denmark and Australia were not far behind (Bryder, 1988: 18). The NAPT's propaganda was often based on "scare tactics" which may have been somewhat counter-productive; an exhibition held in 1907 was described as a 'cabinet of horrors' (NAPT Council Minutes, 1939 in Bryder, 1988: 20).

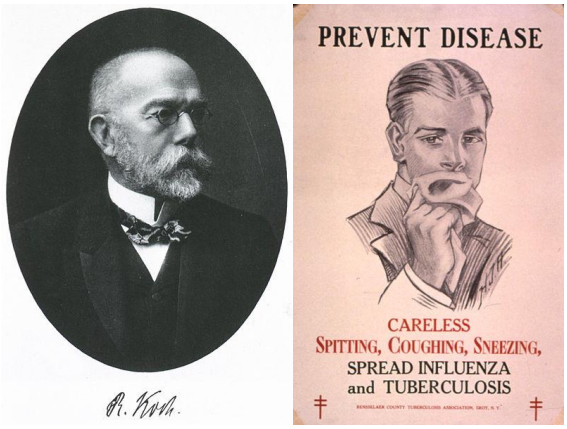

Figure 2-19 (Left) Dr. Robert Koch discovered the TB bacillus in 1882 (http://ihm.nlm.nih.gov/luna/servlet/view/search?q=B016691)

Figure 2-20 (Right) Poster produced as part of a public health campaign to stop the spread of TB (http://profiles.nlm.nih.gov/ps/retrieve/ResourceMetadata/VCBBBH)

The vaccine, BCG (Bacille Calmette et Guerin), developed in 1924 (Evans, 1998: 16), had been shown to protect up to 80 per cent of the population at risk but was not administered in Britain until the late 1950's (Bryder, 1988: 194). By this time, after the introduction of antituberculous drugs such as streptomycin, there had been a sharp decline in the incidence of active TB (Bryder, 1988: 194).

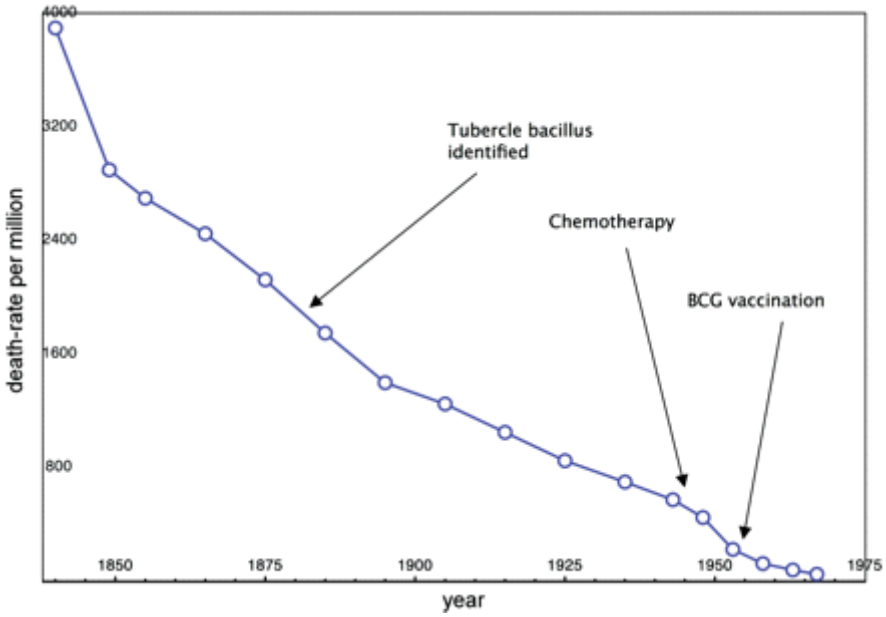

Figure 2-21 Respiratory tuberculosis death-rates in England and Wales (based upon McKeown, 1979, sourced from Selgelid, 2008: Fig 1).

When milk was introduced into schools in 1937, rates of TB had increased (Roberts and Buikstra, 2003: 83) through the contraction of *M. bovis*. This was despite various attempts to reduce TB amongst cattle, such as the Tuberculosis Order in 1913 which meant affected and unaffected cattle were kept separately, and affected cows were slaughtered, after the passing of the Milk Act in 1925 (Dormandy, 1999). Cattle tested after the 1935 introduction of Tuberculous Attested Herds Scheme were done voluntarily, but by 1950 it had become compulsory (Hardie and Watson, 1992). Atkins discusses the incredibly slow implementation of TB control through this nationwide slaughter policy, given that it was known and widely accepted that 40% of the milking herd was infected with the disease in the 1920s and 1930's – it is referred to as a "slow and torturous progress of policy" (Atkins, 2009). After 1960 in Britain, all milk produced was tested or pasteurised and, since the 1990s, any untreated milk

must display a health warning and herds must be tested if milk is sold to the public (Roberts and Buikstra, 2003: 83-3).

The advances seen in TB control early in the 20th century, led to a decline in TB rates and, in a 1964 paper, it was stated: 'But at long last the disease is being wiped out…to see the enemy in retreat is one of the rare and most exhilarating experiences reserved for our generation.' (Mercer, 1964: 253); a poem dedicated to the "Last Dying Speech and Confession of BACILLUS TUBERCULOSIS, Esq." was even found amongst the papers of a Professor Sir Robert Johnstone after his death (assumed to be the writer) (Appendix 1). This judgement was unfortunately premature for many of the reasons described in 2.1.4 and 2.1.5, which include the unforeseen development and rise of HIV and of MDR-TB.

In order to tackle the current global problem of disease, focus rests firstly on prevention, with the BCG vaccine still one of the most widely employed of all current vaccines (more than 80% of neonates and infants receive the vaccine in countries that have a national vaccination scheme) (WHO, 2004). Secondly, extensive screening for the disease amongst high risk individuals in high TB burden areas is employed via the application of the Mantoux test (also known as the Tuberculin Sensitivity Test) which is a skin test to identify latent TB; those infected will display a hypersensitivity response to a TB extract (Kumar *et al*., 2007) but this can only be used with caution on those previously immunized as similar responses can occur (Rothel and Andersen, 2005). There are also more recently developed blood tests employed, called interferon-γ release assays which measure a person's immune reactivity to the TB bacterium and which are not affected by immunization.

Diagnostic procedures for individuals suspected of having TB include a number of tests such as physical examination, consideration of medical history and x-rays, but the only way to definitively diagnose the disease is through a positive TB culture result which is where a clinical sample is extracted from a patient, such as pus or sputum, and any tuberculosis bacteria is grown in laboratory conditions. The primary disadvantage with this method however, is the length of time it takes for the cultures to grow as the bacterium is slow-growing. Because of this, recent development of new tests include PCR for the detection of the pathogen DNA (Reddy *et al*., 2002), and the MTD, Gen-Probe test (amplified *mycobacterium tuberculosis* direct test), which is used to test smears (from sputum) for the

presence of TB bacilli (Guerra *et al*., 2007). These new tests are both fast and sensitive and should positively impact further, on the current world decline in TB (See Figure 2-1). According to WHO, DST (drug susceptibility testing) is also used to identify drug resistant strains of the disease but a “lack of diagnostic capacity“ in laboratories has resulted in only seven per cent of the estimated global burden of MDR-TB and even less XDR-TB cases being detected (2011); this is a major challenge given the rise in these strains of the disease (see 2.1.5). In countries where TB is not common, the disease can go undetected as it is rarely the first consideration, something which has been highlighted in a recent case in in Britain (Paduano, 2011).

Finally, treatment is primarily through the use of antibiotics, or, less frequently, surgical intervention. Treatment with antibiotics is a long process, typically around 6 to 24 months to completely remove mycobacteria from the body (Centres for Disease Control and Prevention, 2003), and multiple combinations of drugs are employed (with the exception of latent TB where single drugs may be used) in order to reduce the possibility of resistance to the antibiotics developing (O'Brien, 1994). In regards to the treatment of TB in bones, joints and the spine specifically, Tuli (2007) identifies three phases of development: the pre-antitubercular era, the post-antituburcular era where all patients were treated with both surgery and antitubercular drugs, and the post-antitubercular era when patients were treated with anti-tubercular drugs and surgery only if necessary; those treated with surgery in the pre-antitubercular era experienced serious complications and a mortality of nearly 50%. Recently, surgery has been employed (alongside drug therapy), in order to combat MDR-TB. Removal of infected nodules in cases of pulmonary TB is thought to reduce the number of bacilli in the lungs whilst better exposing those left to drugs within the person’s bloodstream, aimed at fighting the infection (Lalloo *et al*., 2006).

3 Prehistoric Britain

3.1 The Neolithic, Bronze and Iron Ages in Britain

3.1.1 Introduction

Any study which aims to explore the possible presence of infectious disease in past populations and its subsequent impact on these populations, must explore the various aspects of the period and populations in question, pertinent to the spread of infectious disease. Placing the biological evidence for disease within its cultural context is known as a biocultural approach and is advocated by Roberts (2000a). Without this context, it is difficult to draw rational conclusions, because data derived from a particular period or population cannot be interpreted without at least some understanding of the environment and conditions which led to its occurrence. Equally, the balance between reasonable interpretation and speculation must be maintained; "…interpretation and speculation are fine so long as their bounds are confined by the limits of the observations upon which they are based…" (Waldron, 1994: 102).

It is also not acceptable to directly compare modern data with archaeological, nor advisable to apply assumptions derived from modern data onto past populations, without adequate consideration of the potential differences between the periods from which the data sets originate. For the study of disease this is particularly relevant as, apart from the changing environmental and social conditions over time, the changing nature of the pathogen and it's potentially differential effects on individuals or groups of people must also be taken into consideration.

The following chapter aims to introduce the reader to some key aspects of the Neolithic, Bronze and Iron Ages and the people who inhabited Britain at these times, with some suggestions as to how the changing environmental and social conditions, could have played a part in the history of TB in Britain. This chapter is not, and need not be, a compendium of

all changes which occurred between the early Neolithic and late Iron Ages in Britain. It is written with the range of extrinsic and intrinsic factors that make a person potentially more vulnerable to TB as the key focus. It is arranged chronologically *within* defined topic areas and draws on the many fields of study which have led to our understanding of prehistoric Britain and its people thus far, and of the specific nature of the TB pathogen.

3.1.2 Overview of the Periods:

As Britain "emerged" from the Mesolithic, significant changes as to the way people lived and organised their lives marked what has been termed, the 'Neolithic Revolution' (Cole, 1965), a period dating from c.4000 to c.2500/2200BC (Haselgrove, 1999: 58). Farming and monument building are two of the primary features commonly accepted as distinguishing this period from the Mesolithic. However, debate continues as to the speed and nature of this change; was it a rapid transition as once thought (Childe, 1936) or a slower and less defined shift (Dennell, 1983) that gave rise to one of Britain's most significant and influential periods. Analysis of diet is playing a key part in this debate (Richards, 2003, Richards and Hedges, 1999) as has a consideration of what has been termed the "Neolithic Package" (Thomas, 2003, Schulting, 2000), and even analysis of sea travel conditions combined with radiocarbon dates (Garrow and Sturt, 2011). With regard to the former, however, (Thomas, 2007: 423) warns:

> *…as soon as we define the Neolithic in terms of a particular mode of subsistence, the question as to whether the economy is in all cases the base onto which other elements are built is placed beyond the scope of investigation.*

The Neolithic is a certainly a period which presents both continuities and contrasts to the preceding Mesolithic period and to the periods which followed (Haselgrove, 1999: 59).

Towards the end of the Neolithic, further economic and social changes led to archaeologists identifying what is commonly known as the Bronze Age. The period can be split into three broad time periods; Early Bronze Age, 2000-1500, Middle Bronze Age, 1500-1100 and Late Bronze Age, c.1100-800 (Parker Pearson, 1999: 77). There is, however, a distinct overlap

between the Late Neolithic and Early Bronze Age periods; metalwork found in the Late Neolithic demonstrates that clear distinctions are impossible.

With the arrival of the Iron Age (800-43BC) came further changes, as could be expected from a growing population and with continued advancing technological and social achievements. There is, unsurprisingly, no clear end to the Bronze Age and the start of the Iron Age for the same reasons as stated for the Neolithic / Bronze Age transition. This is mainly because many aspects of the two periods overlapped, and attributes once considered of purely Iron Age origin can now be traced back to the Late Bronze Age, such as the construction of hillforts (Haselgrove, 1999: 113).

What we recognise as the Iron Age came to an end with the Roman invasion in AD43. It was originally thought that the Roman period was the first in Britain to demonstrate clear, unequivocal evidence of TB (Stirland and Waldron, 1990) but it has since been proven that the disease was here prior to the Roman period (Mays and Taylor, 2003). The question as to how much earlier it is possible for the disease to have been in Britain is the key focus of this study.

3.2 Population, Settlement Patterns and Housing

3.2.1 Introduction

Archaeological evidence of settlement patterns, occupation sites and housing can aid in the task of building a better picture of the living environments experienced by prehistoric peoples. The result of this is that the possibility, method and mode of disease transmission between communities can be hypothesised. Discussion regarding intra-community, and intra-familial disease presence and transmission, can also be considered when consulting the evidence for the structures of houses and their spatial relationship to each other. Before any of these factors can be discussed, however, it is useful to consider population estimates for the periods in question as TB is well known to be a density dependant disease.

3.2.2 Neolithic

Population figures can only ever be rough estimates; it is not possible even in 2011 to account for every individual in the world although the U.S. Census Bureau has, through analyses based on assumed trends in fertility, mortality, and migration, attempted to estimate the current world population – it is not intended to be, nor can it be entirely, accurate, but was created to illustrate the speed at which the population is growing. To demonstrate, the estimated total population of the World on 26/07/11 at 17:04 UTC: 6,951,472,951 (http://www.census.gov/population/popclockworld.html). Global population figures were not thought to have exceeded 15 million before the adoption of agriculture (Tellier, 2009: 26).

> *If a generalization may be risked about the population growth in the agricultural period, it is that it was much faster than the rate that preceded it and much slower than the one that followed.*
>
> (McKeown, 1988: 57)

Cohen has suggested, that the population growth witnessed with the adoption of agriculture was not just a result of technological change but, rather was a cause (1977). In essence, it was population pressure which led to the adoption and acceptance of agriculture but, in return, agriculture was the mechanism which enabled much larger numbers of people to be fed (McKeown, 1988: 44). These views run parallel to, and are highly reflective of, the two schools of archaeological theory regarding cause and effect of population change, summarised by Chamberlain (2006: 4), the first view being that asserted by Childe (1936) whereby under normal circumstances, a population's capacity is dictated by its resources, and it is sociocultural innovations that enable periodic growth in population size and density - the reverse view being that cultural change is mainly a consequence of population growth, rather than a trigger for, first proposed by Boserup (1965) and Dumond (1965).

Recent research using radiocarbon dating has identified a "dramatic increase" in population density between 6100 cal BP and 5400 cal BP (4150BC-3450BC) (Figure 3-1) which coincides with the appearance of cultigens around 6000 cal BP (4050BC); before this, all regions of Britain were sparsely populated (Collard *et al.*, 2010). This dramatic increase in population ties in well with the results of a recent study identifying Britain's first "Building Boom"

(Bayliss *et al*., 2011, Ghosh, 2011). The study created and utilised a new method of dating, combining radio-carbon dating with other dating sources and applying statistical analyses (Bayliss *et al*., 2011). The primary focus was on causewayed enclosures and demonstrates that they spread rapidly after their introduction, within 10-75 years (95% probability) (Figure 3-1 and (Bayliss *et al*., 2011: 690). This is right in the middle of the dates for the population increase identified above (Collard *et al*., 2010). No doubt the population estimates for the period offered below, will be re-considered in the next few years, in light of this new information – Collard *et al.* demonstrate that the average population increase was 0.37 per cent annually (2010).

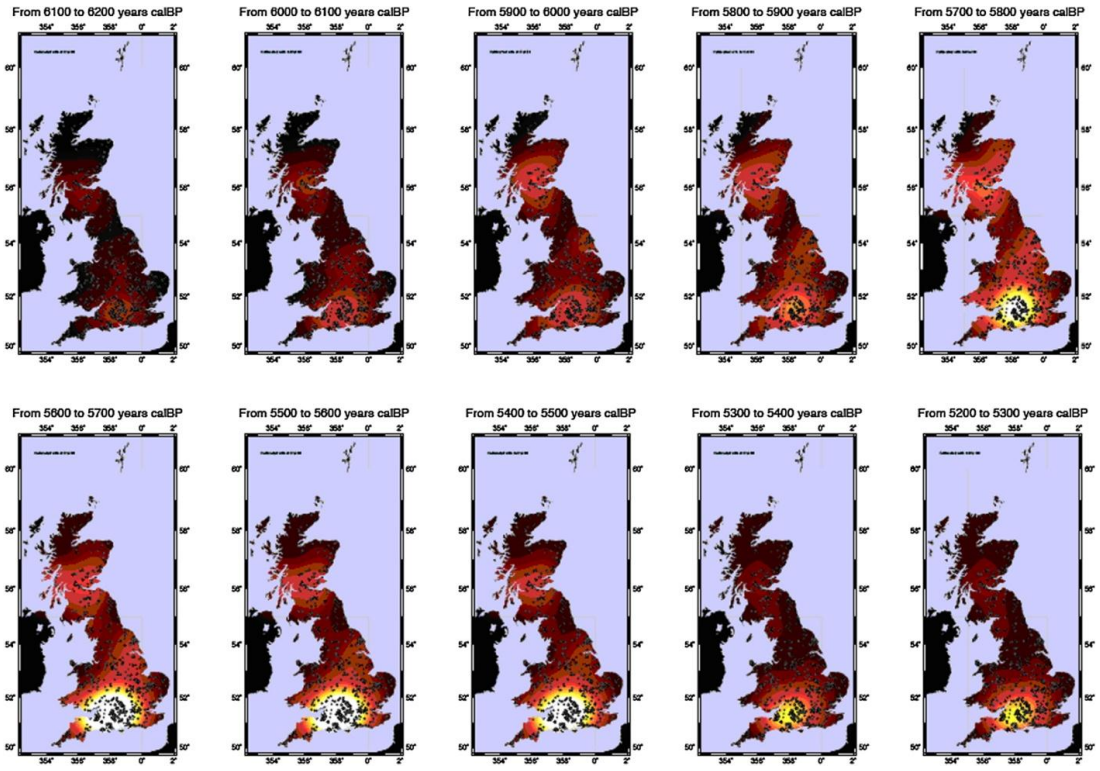

Figure 3-1 Spatial density of full set of dates between 6100 and 5300 cal BP in 100-year time slices (Collard *et al*, 2010: Fig 2)

Estimates of population figures in Britain during 2010 stood at 60,462,575 (Office for National Statistics, 2010). Population estimates for the Neolithic period in Britain vary significantly, with Brothwell (1972) suggesting, perhaps conservatively, somewhere in the region of 10,000 to 40,000 and McEvedy and Jones (1978) up to 50,000 in England and Wales. Darvill (1987) goes so far as to suggest a population of around 200,000 for the

period, possibly consisting of small scale groups, and possibly extended family units spread throughout the countryside.

Such diverse population estimates for the period create great difficultly when considering the effect population density may have had on the transmission of TB and other communicable diseases, if it is assumed they were present. There have been attempts to estimate the required population sizes for the maintenance of human infections such as measles (Fenner, 1971, Cockburn, 1977, Black, 1966), which clearly demonstrate that most human infections would not have been able to establish themselves until many thousands of people were in regular contact (McKeown, 1988: 50). This may suggest that the people of the early Neolithic, who were perhaps not yet leading entirely sedentary lifestyles and still resembled their earlier hunter-gatherer ancestors, and who lived lives that minimised their exposure to infection (McKeown, 1988: 20), were also safe from the threat of infectious disease. This may to some degree be true but, unlike diseases such as measles, smallpox, and whooping cough, which could not have been maintained under such minimal population figures, TB (amongst but a few others) is characterised by chronicity and recurrent excretion and infection (McKeown, 1988: 38). This means that, once present, it would have the ability to stay dormant in an individual and thus have the potential to recur in an individual, causing active infection or reinfection in others.

Understanding the population densities required for certain diseases however, is still difficult to translate into studies of prehistoric populations as calculating population densities is highly problematic. Chamberlain (2006) presents the methods by which population figures can be estimated using house sizes and floor areas such a 1962 study (Naroll, 1962) which calculated an approximate average ratio of 10m2 of roofed space per person, working from data attained through the study of a number of sedentary societies from across the world. Chamberlain warns however, that in applying such formulae to archaeological evidence, it is necessary to be able to distinguish between habitation space and storage and livestock space, in addition to considering the proportion of rooms or building that would be occupied at any one time (2006: 126); with prehistoric archaeological sites, this is often a complex task.

By 3000bc farming settlements were extensive, but over the course of the Neolithic period there were numerous developments in terms of settlement pattern and design such as in the Orkney Islands where isolated farmsteads were replaced by 'small village-type settlements' (Reid, 1993: 34). Of those found in southern England, most appear to be similar in size to those found in Orkney. The earliest houses seem to be part of small, isolated farmsteads, but more substantial settlements in the form of causewayed enclosures are also in evidence, suggesting a move towards larger settlements and 'communal centres' (Reid, 1993: 35). Early farming communities, however, were unlikely to be isolated geographically, with rivers and walkways being used to provide communication (Darvill, 1987: 71).

The possible number of people living in one structure becomes a useful context in which to consider TB transmission in particular (in its various forms), given the links with overcrowding and living conditions seen even in the modern era around the world (Coker *et al*., 2006, Antunes and Waldman, 2001, Padmavathy *et al*., 2008). Of the Neolithic houses that existed, Darvill suggests that up to 12 people may have slept and worked in one house, which may have consisted of one or two rooms (Darvill, 1987: 57). This would have created a suitable environment for any airborne infection present to spread between hosts. Research has shown that there is a significant relationship between TB and overcrowding (Elender *et al*., 1998), and there is certainly a strong foundation for TB being regarded as a disease of poverty, to which overcrowding is commonly accepted as being a resulting factor. Chen (1988) studied longhouse dwellings in Sarawak and concluded that those living in clustered individual dwellings were less likely to contract TB, compared to those who lived in the larger long houses, due to increased and prolonged contact with other individuals. Evidence also suggests that humans often shared their accommodation with animals and that poor sanitation, lack of space, sunlight and ventilation was highly likely; such conditions could have led to the 'appearance and maintenance of pathogenic organisms' (Roberts and Buikstra, 2003: 59). Cohen (1989) also discusses the accumulation of refuse and poor ventilation as being two of the negative effects seen in health with the evolution of permanent housing which is, in turn, an effect of the adoption of agriculture.

It is also suggested, however, that not all communities of the Neolithic lived an entirely sedentary lifestyle and in fact remained relatively mobile (Darvill and Thomas, 1996). If this

was the case then, theoretically, such people may not have been exposed to the same level of poor conditions (such as constant poor ventilation and overcrowding) as those leading a more sedentary lifestyle. Thus, the latter were not exposed to the same levels of threat regarding TB transmission, as mentioned previously (McKeown, 1988: 20). More nomadic, hunter-gatherer lifestyles, however, may have brought their own risks in terms of regular opportunities for zoonotic transmission of the disease from wild animals, and the potential for being brought into contact with a greater range of people and places, thus increasing the possibility of contracting a new infection and consequently becoming carriers of said infection.

There are other aspects of living in more permanent structures which may make a person more likely to contract diseases, particularly those that affect the respiratory system. In the past, people would have used biomass for fuel, being defined as '…any material derived from plants or animals which is deliberately burnt by humans.' (Bruce *et al*., 2000). Biomass fuels would have been readily available in the prehistoric periods and are still the major primary fuel source, particularly in rural households in developing countries (World Resource Institute, 1998). This has enabled a number of studies to be conducted into the possible links between respiratory diseases and solid fuel (biomass) use and of particular importance is the relationship between this type of fuel use and the risk of TB (Gupta *et al*., 1997, Mishra *et al*., 1999, Perez-Padilla *et al*., 2001, Hazra *et al*., 2007, Crampin *et al*., 2004, Shetty *et al*., 2006). However, each of these studies was systematically reviewed (Slama *et al*., 2010) and only three (Perez-Padilla *et al*., 2001, Crampin *et al*., 2004, Shetty *et al*., 2006) were considered to demonstrate a significant effect of exposure to solid fuel smoke and TB. The authors did not consider this review to convincingly demonstrate a clear link between the two at this time, which contradicts Lin *et al.*'s (2007) earlier review of the literature and subsequent conclusion. Slama *et al.* do acknowledge, however, that, while as yet, there are not enough studies to support a link between the two, their conclusions are 'highly modifiable' if more high quality studies involving case-control or cohort studies are published (2010: 10).

Assuming that a link between the burning of biomass fuels and TB is perhaps not unlikely to be demonstrated in the future, it would seem prudent to at least consider the evidence for

indoor air pollution in prehistory, to some degree. As Bruce *et al.* suggest (2000: 1084), given the immunosuppressive effect seen in the lungs by the inhalation of smoke (Houtmeyers *et al*., 1999), an increase in the risk of developing TB is 'quite conceivable', despite the current lack of evidence from biomass smoke. In addition, if this link were confirmed, the exposure to biomass smoke could be 'an additional factor in the relationship between poverty and tuberculosis, hitherto explained by malnutrition, overcrowding and inadequate access to healthcare.' (Bruce *et al*., 2000: 1084).

Bernofsky summarises the factors that can affect indoor air pollution: …house type, plan and structure, building materials, amount of dust and animal dander, type of fuels, how the fuels are used… (2010: 50). Archaeological evidence suggests that the majority of houses in the Neolithic would have been timber-framed, rectangular or sub-rectangular and either single units or larger houses being divided into a number of rooms – roofs were likely to be thatched, although turf, shingles or animal hides could have been used (Reid, 1993: 17) (Figure 3-2 and Figure 3-3). Presumably thatching, which would allow smoke to pass through it (Bernofsky, 2010: 56) would offer relatively good ventilation. Examples of stone built houses are evident in Orkney and these demonstrate a 'central square shaped stone hearth' (Darvill, 1987: 57) which likely resemble many of the hearths still seen in developing countries today (Smith, 1987). These houses also demonstrate partitions, which Darvill has tentatively suggested to be separate cooking, sleeping and working areas (Darvill, 1987: 57). If this is the case, then at least in the larger houses of the period, again, perhaps smoke inhalation was limited by separation. There is evidence of periostitis on ribs from individuals from the Neolithic sites of both Hazelton North and Hambeldon Hill. Rib periostitis is linked with chronic pulmonary infections (Guttentag and Salwen, 1999) so these examples are likely evidence of infection in the lungs, but the specific cause has not and cannot be identified (Roberts and Cox, 2003: 60), and it is certainly not possible, on the basis of rib lesions alone, to diagnose TB, as mentioned previously.

It is likely that all peoples, those now living in more settled communities and those continuing to travel, at least periodically, were likely to find themselves in a situation where, if present, TB could certainly have had the opportunity, if not the virulence at this time, to cause infection. Evidence for poor air quality in the Neolithic is difficult to confirm and it

seems likely that it did not play a major part in the transmission of infectious disease, given the very limited evidence for the period (Roberts and Cox, 2003).

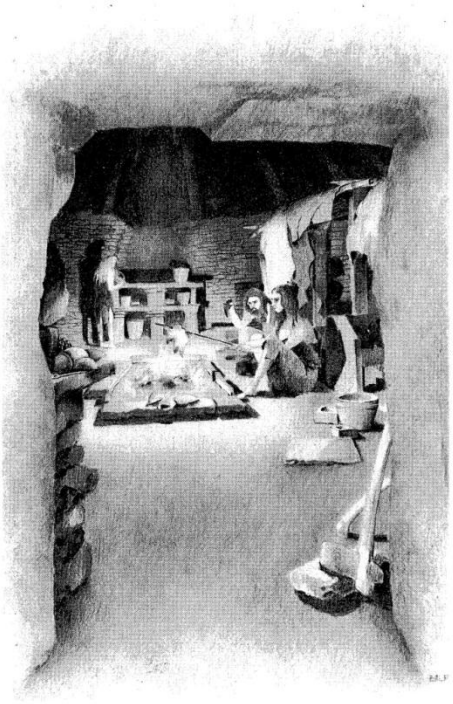

Figure 3-2 Inside a Neolithic house at Skara Brae. (Parker Pearson, 1993: Fig44)

Figure 3-3 Reconstruction of a Neolithic house at Horton, near Windsor (http://www.wessexarch.co.uk/blogs/news/2008/06/30/stone-age-house-found)

3.2.3 Bronze Age

A distinct change in settlements and houses can be seen within the Bronze Age period. Whilst Early Bronze Age houses are difficult to identify as they left little trace, by this period,

many houses were built of stone rather than timber and have thus left a more permanent record of their presence (Parker Pearson, 1999: 84). There is also an increase in the frequency of settlement sites which is probably as a result of an increased population; figures for the Bronze Age are obviously higher, ranging between 20,000 to 100,000 for England and Wales (Brothwell, 1972, McEvedy and Jones, 1978). Settlement sites are more widely distributed and are more substantial than their Neolithic predecessors. Most houses were circular (Reid, 1993: 35) and formed small groups rather than large settlements; hearths continued to be placed within the structure (Parker Pearson, 1999: 85). Experimental archaeology has demonstrated that smoke holes in circular houses tend to cause a fire hazard as the drafts created can send sparks into the thatching, and thus it is likely that these houses did not have smoke holes (Parker Pearson, 1999: 103). This may seem to suggest that poor air quality would have been a problem but, as discussed for the Neolithic, thatched roofs probably allowed for at least reasonable ventilation. There is also evidence that a build up of soot on the inside of thatched roofs can reduce the possibility of fire (Letts, 1999 in Bernofsky, 2011: 55). Perhaps smoke holes may have featured in these houses, providing additional ventilation, if the assumption for their not being present is based solely on experimental findings. There is, again, very limited evidence of problems associated with high levels of air pollutants with only one case of rib periostitis having so far been identified from the period (Roberts and Cox, 2003: 86). That these round houses were also grouped or clustered, perhaps demonstrates a change in settlement pattern that would have favoured communicable disease transmission, although it is the number of individuals in one house which is perhaps of greater importance as demonstrated by Chen (1988). These settlements were also becoming more diverse even including other structures such as granaries which perhaps reflects these increasing clusters of communities.

Whilst the nature of settlements began to change from more isolated groups to more community based, meaning a greater pool of potential hosts for communicable diseases, there was another highly significant change seen during the Bronze Age. With emphasis taken away from monumental architecture, the focus was now on fortifying settlements (Darvill, 1987: 137). This began during the middle Bronze Age with ditches, and by the end of the Bronze Age had become extensive, with undefended hill top enclosures being established. These developments suggest that there should have been density dependent

diseases, particularly given the increased population, increased settlements and a 'closing in' of those settlements with fortifying architecture (such as being surrounded by a ditch). It is assumed that infections could have been a real problem, especially given the likely unsanitary conditions such settlements would encourage (Roberts and Cox, 2003: 86) but, as yet, there is still no supporting evidence, which may suggest that the population level was not yet high enough for such a disease to pose a problem (Roberts and Cox, 2003: 87) or that conditions were not favourable to the spread of TB; morbidity for some diseases, particularly infectious ones (such as TB), are very much dependant upon population size and density (Chamberlain, 2006). There are, of course, many reasons why evidence of specific infections has not been identified yet and not necessarily because they didn't exist; absence of evidence is not evidence of absence (Wood *et al*., 1992), as discussed in the previous chapter.

3.2.4 Iron Age

Population figures for the Iron Age are unsurprisingly higher again, ranging from a very conservative 50,000 (Brothwell, 1972) to a somewhat more substantial 1 million (Darvill, 1987); a mid way estimate lies between 200,000 and 600,000 (McEvedy and Jones, 1978). The 3000 plus hill forts recorded for the Iron Age period clearly dominate the pattern of settlement for this period. It is suggested that this 'hill-fort based society', which was established around 650 BC, was a response to earlier tensions and in some areas this appears to be true (Darvill, 1987: 133), but Darvill also questions whether or not this development was one of demonstrable power and prestige rather than purely defence.

The Iron Age, after c.600 BC, witnessed a breakdown of the previous 1st millennium Bronze Age regional groups, evident, amongst other things, by increasingly regionalised artefact styles and settlement types. As with other developments, there is a difference between different areas of England; the settlement pattern of the south and east shows a hierarchical pattern with small farmsteads evident as well as large focal sites (Millett, 1990: 5). Despite the numerous hill-forts evident by c.300BC, they remain rare in the east and it is these smaller villages, hamlets and farmsteads that dominated, many of which were

connected with trackways, and are thought to have been able to support 35-85 people (Darvill, 1987: 47). These connections are clear evidence of the interaction between communities, meaning diseases such as TB, already identified for the period, would have had the opportunity to spread from settlement to settlement. The houses themselves do not appear to change much from the Late Bronze Age, apart from being larger (Figure 3-4) and, given the limited evidence of respiratory diseases so far seen, it is suggested that internal living conditions may not have been such a health risk as once assumed (Roberts and Cox, 2003: 87). Bernofsky (2010) conducted a study into rates of chronic maxillary sinusitis and rib periostitis (both linked with poor air quality) from the Iron Age to the Post Medieval period in southern England and recorded only one rib lesion from the Iron Age. It was suggested that Iron Age houses offered relatively good ventilation which would account for such low levels of respiratory disease. However, the same populations demonstrated very high levels of chronic maxillary sinusitis – meaning that a factor other than air quality must account for the difference.

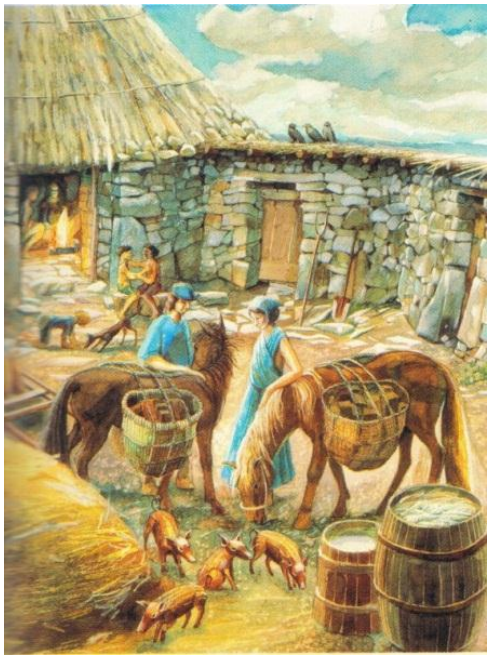

Figure 3-4 Reconstruction of a later Iron Age stone-built house at Carn Euny, Cornwal (Cunliffe, 2004: Fig 33)

3.2.5 Conclusion

Overall, there was a clear increase in population figures from one period to the next with settlements and housing evolving over time. Original settlement patterns were very much focused on smaller, more mobile groups but, with an increasing population, life became

more sedentary and community focused. It is clear however that contact was a common theme between communities across all periods, allowing for the transmission of communicable disease and aided by multiple occupants in one house. Population increases are often associated with the occurrence of density dependent diseases (Roberts and Cox, 2003: 58) but again, there is no specific evidence of this from either the Neolithic or the Bronze Age even by the later period (Roberts and Cox, 2003: 58); this changed during the Iron Age with the appearance of TB but this still remains a single incident out a period which extended many hundreds of years.

3.3 Social Hierarchy

3.3.1 Introduction

TB is considered a "disease of poverty" in relation to the poor social and environmental conditions which enable the disease to flourish. Despite this, it is also a disease that crosses social boundaries, affecting rich and poor, but it is the continually deprived conditions to which the "poor" are subject that lead them to be the most regular recipients of the disease. This is reflected in historical accounts where different levels of TB were seen across the social strata in London in 1842 (Dormandy, 1999: 77) and is still the case today where the highest incidence rates are seen in the highly populated and developing regions such as Africa and South-East Asia (WHO, 2010b).

Social hierarchies often come with a distinction in living or social conditions between the different tiers of 'importance'. This is often seen reflected in the health of a population because those with greater power often have access to greater quantity and quality of food, with greater biological stresses placed on the poor (Roberts and Cox, 2003: 58). These factors have a major impact on the susceptibility of an individual to TB and, thus, status in prehistoric populations must be considered. This is a phenomenon not confined to the past but found throughout human history and even, disappointingly, in the present – those of lower socioeconomic status, consistently demonstrate poorer health (Marmot, 1999, Smith, 1999), also reflected by TB incidence, as mentioned above.

> *Hierarchical position may have a direct effect on health as well as indirect effects through SES-related [Socioeconomic status] differences in the physical and social environment, health behaviours, or personality. In other words, one's relative position in the SES hierarchy, apart from the material implications of one's position, may affect risk of disease.* (Adler *et al*., 1994: 20)

The presence of social hierarchies may be inferred from archaeological societies, and consequently, health inferences may be made if the skeletal evidence is in support. In prehistory, social hierarchies may be attested to through social activities such as evidence of warfare, construction of fortified settlements, occupation, trade and exchange or monumental building, all of which require some level of organisation and, thus, social structure. While these elements may suggest that a hierarchical structure was in existence, it is often difficult to identify the specific nature of the hierarchy and impossible to identify an individual's particular place within it, without speaking in the broadest of terms. As such, making suggestions as to potential health inequalities due to hierarchical status can only be based on skeletal evidence, combined with archaeological contextual data, and this must be done within the context of the particular population under study. For example, contrasting evidence of what may be considered 'high status' food has been found between populations in Anglo Saxon England (Privat *et al*., 2002) and Viking Sweden (Linderholm *et al*., 2008), discussed in more detail below.

Burial evidence for status must also be approached with caution – 'high status' burials are but an interpretation of the material culture associated with skeletal remains or of the manner of burial. The interpretation of grave goods (Härke, 2000) and the relationship between grave goods, rank, power and status are extremely complex (Parker Pearson, 1999). Differential, stress-induced pathologies within a population may be evidence of differential placement within a SES hierarchy but the many other factors which can affect health must also be considered, whether direct or indirect, such as those discussed in sections 2.2 to 2.4.7. One study noted that some pathologies associated with biological stress such as enamel hypoplasia and cribra orbitalia were found not to be related to status in one population (as would be expected) (Robb *et al*., 2001).

Furthermore, position within an archaeological social hierarchy need not always be thought of in a vertical or linear fashion; different groups within a hierarchy may group together, not necessarily reflecting high or low rank but based perhaps on sex, gender, age or abilities. Of course, sometimes being a member of one of these groups may automatically place a person at the top or bottom of the hierarchy; discrimination against many females in India "…starts right from her birth and continues to her last breath.' (Bhasin *et al*., 1994: 76). Gender is a key concern when considering possible division of labour and possible health effects in the past. Gender as opposed to biological sex is used here because, as Roberts *et al.* note, 'It cannot be assumed that males and females in the past acted within gender roles similar of those today.' (1998: 93). It has been found that SES exposures during childhood and adolescence can subsequently impact on adult health outcomes (Cohen *et al*., 2010).

Malnutrition is a key concern where access to food is limited, be this by SES status, hierarchical position, or other factors (see 2.2). Malnutrition has been linked to increased susceptibility to TB (2.4.2), but there are of course instances where access to more plentiful and richer foods can have a detrimental effect on the state of health, causing higher rates of obesity in higher SES individuals, as demonstrated in modern day Latin America (Rosero-Bixby and Dow, 2009). This serves to demonstrate the complex nature of diet / health relationships.

The development of stable isotopes to identify diets, is also contributing to the study of status. For example, analyses of individuals buried at Poundbury Camp, Dorset in Dorchester revealed different dietary intakes between high status burials (in lead coffins), and those buried in wooden coffins; the former regularly consumed marine foods (Richards *et al*., 1998). Privat *et al.* (2002) identified differences in diet between different age groups of males, between wealthy and poor burials, and between burials containing weapons and those which did not, in an Anglo Saxon population from Wally Corner, Berinsfield, Oxfordshire; male burials with and without weapons showed little difference between nitrogen values - a contrary pattern to that found by Linderholm *et al.*, (2008) looking at Swedish Viking graves whose results demonstrated statistically significant differences in diet between individuals buried with weapons as opposed to those buried without. This same

study demonstrated that female burials with artefacts associated with trade, shared dietary values demonstrating some of the lowest levels in sulphur, which contrasted with female burials without the trade associated artefacts; this may imply a common area of origin or habitat (between those women buried with artefacts associated with trade) in the 10-15 years prior to death (Linderholm *et al*., 2008). Differential dietary patterns between various groups in many past populations serves to highlight the complexity of social hierarchy. The dietary intake of these groups may reflect preferential or disadvantaged access to food which, in turn, could lead to health inequalities with the ultimate consequence being that of greater susceptibility of infectious disease due to reduced immune system strength.

3.3.2 Neolithic

Many of the social and economic developments seen in the Neolithic would have required a change in organisational structure, indicating the rise of a social hierarchy. As LaFreniere and Charlseworth (1983) discuss, hierarchical social structures are in evidence in almost all human social groups (a consequence of which is reduced intra-group aggression), but the Neolithic period would perhaps have required a more defined hierarchy than that which had developed before because the Neolithic demonstrates greater contact and cooperation with different communities, outside of an extended family contingent. In fact, it has been argued that status differentiation and agriculture occur at virtually the same time (at least in the Near East) (Price and Bar-Yosef, 2010). Monument building and the development of structures such as chambered tombs all indicate the need for a social structure whereby the division of labour, storage and distribution of supplies and a central point of control, would be required. Archaeological evidence of 'ranking' in early farming communities is, however, limited with regard to burials. There are very few individuals of any given community who were formally interred, but this practice does imply some form of preferential criteria (Darvill, 1987: 71). Roberts and Cox warn, however, that it is impossible to suggest whether any differences in health were the result of different social status because social status is not reflected in the burial record, unless it is assumed that those recovered from long barrows and chambered tombs were of higher status (Roberts and Cox, 2003: 58). If this is

assumed, they pose the interesting question of where the poor were buried (Roberts and Cox, 2003: 58).

New research may also add weight to the theory of hierarchical society development within the British Neolithic. Evidence of violence, both inter-personal and collective, has been identified at a number of causewayed enclosure sites in the South of Britain (Figure 3-5). The site at Crickley Hill in Gloucestershire demonstrated great numbers of arrowheads found in addition to evidence of the settlement being attacked and burnt (Darvill, 1987: 76). Hambledon Hill in Dors*et al*so provides convincing evidence of an attack, as do the sites of Hembury in Devon and Carn Brea in Cornwall (Pollard, 1997: 15). At least one contemporary burial has been found with an arrowhead imbedded in the spine at Fengate, Peterborough (Pollard, 1997: 15). It may be prudent at this point to compare this pattern with that of the extensive population expansion identified in this time period. It was once said that if a population increases faster than its means of subsistence, it will be subject to the checks of war, famine and disease (Malthus, 1798). Given the concurrence of dates between these two Neolithic features, the similarity in the maps may be a manifestation of at least the first element of this theory. This is of course speculation. There is only very limited evidence of malnutrition, or at least periods of biological stress for the Neolithic in the form of Harris Lines and enamel hypoplasia (Roberts and Cox, 2003: 67).

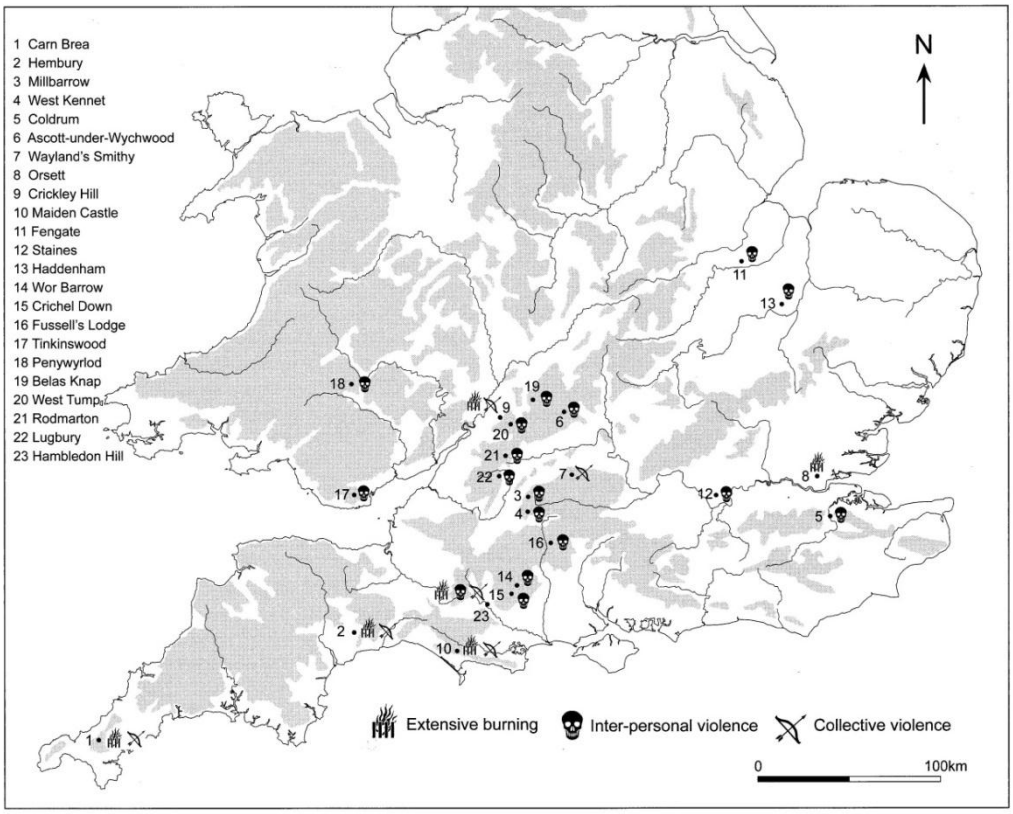

Figure 3-5 Map of violent episode in southern Britain in the fourth millennium cal BC. Interpersonal violence includes both cranial trauma and arrowhead wounds (Bayliss *et al*, 2011: Fig. 14.37).

3.3.3 Bronze Age

There were significant changes during the course of the Bronze Age to suggest a change and development in social hierarchies. It is during the Early Bronze Age that archaeologists have postulated the rise of an elite who controlled 'chiefdoms' (Darvill, 1987: 75). One of the primary pieces of evidence in the debate is the rich grave goods associated with specific burials in Wessex known as the 'Wessex Culture' (Parker Pearson, 1999: 94). For example, close to Stonehenge in Wiltshire is the Normanton Down group in which the Bush Barrow is considered one of the most impressive from the Wessex Culture (Parker Pearson, 1999: 89). The Bush Barrow contained a gold lozenge, a stone mace, three large bronze daggers and a baton which were all associated with the burial of a single elderly man who, by inference, has been assumed to be one of the ruling elite (Parker Pearson, 1999: 89). It has also been suggested that grave goods during this period can suggest hierarchical differences in the division of men and women, with male graves containing grave goods such as arrowheads and daggers, and female graves containing shale and jet beads, amongst others such as picks and hoes (Parker Pearson, 1999: 89) but, as previously stated, the interpretation of

grave goods is highly complex (Parker Pearson, 2003: Chapter 4). This is no better demonstrated than in the case of a recently excavated male skeleton found in a position usually seen with female skeletons. The individual had no gender specific grave goods, leading the researchers to suggest the individual was that of a 'third gender', viewed as male, female or both but which the media promptly referred to as the first 'gay caveman' (Daily Mail, 2011).

What also developed during the Bronze Age was increased evidence for warfare with the presence of weapons not for purely ceremonial purpose, protective armour (Champion, 1999: 109), and importantly the development of defensive structures, not seen in the Neolithic. Developments such as these, particularly evidence for fortifications and warfare, demonstrate changing needs and concerns over the course of the Bronze Age. As mentioned previously, preparation for, defence against, and general participation in, warfare requires central organisation. The possibility of a social collapse in this period has been considered but Bradley (2001: 222) sees no need to subscribe to this view:

> *'It is true that there were certain tensions towards the middle of the second millennium BC – fluctuations in the supply of metals, changes of burial customs, the settlement of increasingly marginal land- but it is better the think in terms of transformation rather than catastrophe'.*

As the Bronze Age progressed there was, a change in monument building and burial rites and much of this discontinuity is more evident in the South of Britain than the North (Darvill, 1987: 75). There is a distinct lapse in emphasis on mortuary monuments and formal burials than previously, which may demonstrate changes in hierarchical structure. However, as Bradley explains:

> *'There is nothing to indicate that social distinctions had disappeared; rather they were expressed in different ways…The disappearance of mortuary monuments suggests a less stable society, perhaps one that placed less weight on genealogy.'*
>
> (2001: 223)

3.3.4 Iron Age

For the Iron Age, whilst settlements may reflect specific types of social and economic organisation, there is still relatively little that can be said regarding the possible differences in health between people of different social classes. This is mainly because human remains for the period are limited, as previously discussed, but also the many ways in which they were buried or disposed of still creates little in the way of obvious distinctions. There is evidence of some high status burials such as at Wetwang Slack in Yorkshire, where a few graves were accompanied by two wheeled carts (Dent, 1985). Isotopic analyses were applied to 62 individuals from Wetwang Slack from across the status spectrum and different burial contexts, but no dietary difference were identified which the authors attribute to a population with a homogenous diet and limited mobility (Jay and Richards, 2006).

It is clear, however, from developments in production, trade and exchange that social hierarchies played a key role in the development and organisation of this period. The complexity of Iron Age society must not be underestimated as it is this period that witnesses the emergence of specific tribal zones. Central southern Britain is 'where the evidence suggests the development of a complex social system based on territorial units dominated by developed hillforts' (Cunliffe, 2004: 53). It was probably this period, more than any of the previous, which gave rise to greater inequalities, partly because of a greatly increased population density and because of the now highly sophisticated and extensive tribal system.

3.4 Diet and Economy

3.4.1 General Health Impact of Agriculture

The adoption of agriculture has had a profound effect on human societies. In Britain, agriculture was first witnessed in the Neolithic and, as a species, we are still largely dependent on domesticated products, especially plants (Larsen, 2011). Research shows that there is no 'universal biological response to agriculture' (Larsen, 2011), but past populations

around the world have generally shown a repeated trend of declining health associated with the adoption of agriculture, although there are often complex patterns (Cohen and Armelagos, 1984, Lukacs, 2007, Starling and Stock, 2007, Pechenkina *et al*., 2007, Roberts and Cox, 2007, Smith and Horwitz, 2007, Larsen, 1982, Larsen, 1984). A specific focus has also centred on dental pathology where women's oral health appears to decline more than that of men (Hillson, 2001, Lukacs, 2008, Lukacs and Largaespada, 2006, Steckel *et al*., 2002, Watson *et al*., 2010, Fields *et al*., 2009). It is believed by many that the disadvantages of agriculture outweigh the benefits and even go so far as to suggest that the development of agriculture was '...a backwards tumble in as far as human health was concerned' (Kiple, 2000: 1705).

Logically it might seem that improved health would be the result of the more 'stable' nature of agriculture (compared to the reliance on wild foods through hunting and gathering). The reduction in health, however, is considered to be an effect of eating more carbohydrate-rich foods and living more sedentary lifestyles (Larsen, 2011) and, whilst crop cultivation enables growing and storing produce in quantities sufficient to feed an increasing population (seen over all chronological periods), a less varied diet, along with the need to tend to crops all year round, are two other major disadvantages of an agricultural system (Cohen, 1989). The more sedentary nature of agricultural societies has significant, associated, negative health impacts. McKeown identifies four aspects of both the agricultural and industrial periods that led to the eventual predominance of infectious disease: (a) large populations (large enough to allow 'some human infections to become established and others to be amplified)', (b) defective hygiene and crowding, (c) insufficient food and (d) close contact with domesticated and other animals (1988: 48), all of which are intimately associated with the adoption of this more sedentary lifestyle. Effects (a), (c) and (d) are discussed in sections (2.4.1, 2.4.2 and 2.4.7) and (b), below.

Agriculture brings with it a vulnerability to human societies. Reliance on a system means that, if the system fails, entire social networks feel the consequences, often with major impacts on health, although the impact could rest more heavily on particular groups in a hierarchical society. Crop failures, and insufficient storage of foods, can lead to episodes of famine, placing great biological stress on individuals. Malnutrition can occur as a result of

reduced food availability and, as a direct consequence of famine. It is generally accepted that malnutrition is a risk factor for TB development because of lowered cell-mediated immunity (Chandra, 1991, Chandra and Newberne, 1977, Myrvik, 1994, Gershwin *et al*., 1987); as Cegielski and McMurray state, 'It makes biological sense.' (2004: 286). Extensive, clinical evidence to support this notion, however, is lacking and more detail is required in order to fully understand how TB and malnutrition are linked; if the link is definitively confirmed, malnutrition 'may account for a greater population attributable risk of TB than HIV infection, and certainly a much more correctable one.'(Cegielski and McMurray, 2004: 286).

The young are particularly vulnerable to long term health effects of famine. It has been shown that malnutrition suffered whilst in utero, lowers kidney functioning (Brenner and Chertow, 1994) and cardiac health (Hoet and Hanson, 1999) and those suffering malnutrition during the first two years of life demonstrate health effects such as increased blood pressure, an increased chance of mental illness and reduced adult height (Walker *et al*., 2007b, Victora *et al*., 2008); the 1959-1961 famine in China resulted in individuals of the 1959 birth cohort growing to a height 3.03cm less than normal (Chen and Zhou, 2007). A study of people who experienced the Dutch Potato Famine (1846-47) showed than men exposed to severe famine for at least 4 months in utero and directly after birth, had a 'significant lower residual life expectancy at age 50 than others' (Van Den Berg *et al*., 2009).

3.4.2 Skeletal Evidence Reflecting Biological Stress

> *The morphology and composition of the human skeleton reflect the direct interaction of humans with either environment and therefore provide an empirical basis for evaluating the biological costs and benefits of subsistence regimens.*
>
> (Lambert, 2009: 603)

The associated risk of malnutrition and even famine, as a result of reliance on an agricultural system has been discussed above, but it is necessary to understand the skeletal manifestations that allow bioarchaeologists to infer these potential periods of biological

stress in an individual's life. These biological stress indicators seen in skeletal remains, if interpreted with caution, can allow consideration of associated negative health impacts the adoption of agriculture may have had on an individual. In particular, the ability to recognise biological stress in an individual, may suggest at least a period of increased likelihood of susceptibility to disease, particularly infectious disease such as TB, as biological stress is likely to have resulted in weakened immune strength; it is weakened immune strength that is presumed to be the primary influence to susceptibility to infectious disease (Cohen and Williamson, 1991).

Agriculture is considered to have had an effect on bone metabolism as its adoption in the Neolithic is thought to have reduced animal protein consumption and created a diet reliant on foods lacking in nutritional variety (Larsen, 2003). Most of the skeletal manifestations of biological stress fall into the category of metabolic diseases, which reflect 'abnormalities of deficiency or excess… of dietary constituents… [and of] …hormones…' below (Roberts and Manchester, 2005: 221). The enamel defect, enamel hypoplasia, is of great use regarding the inference of biological stress, which could be an indication of malnutrition, but this, along with other accepted skeletal markers of biological stress, are subject to problems which have been previously highlighted (Lewis and Roberts, 1997). Nevertheless, they remain useful indicators if interpreted with caution, and continue to be utilised in studies pertaining to agricultural transitions (Temple, 2010, Starling and Stock, 2007). The following are skeletal markers of "stress" that have been identified and used as indicators of reduced health with the adoption of agriculture:

- Harris lines can be seen most commonly on the tibia, fibula and radius in individuals who were subject to periods of biological stress causing growth arrest during childhood (Roberts and Manchester, 2005: 24). In order to occur, however, an individual must experience this stress and subsequently recover, so the lines will not be visible on an individual subject to continual stress, for example by persistent malnourishment as a result of poor diet (Roberts and Manchester, 2005: 24). Additional problems include the continual remodelling bone is subject to, and that the formation of Harris Lines has been recorded when no disease episode is documented and vice versa (Gindhart, 1969). Studies also suggest that the formation

of Harris Lines can be a result of very minor, or very major stresses such as malnutrition (Dreizen *et al*., 1964).

- Enamel hypoplasia is a dental enamel defect, is non-specific in aetiology, and also considered an indicator of stress. An enamel thickness deficiency causes a disruption of the usually smooth contour of the crown surface (Hillson, 2002: 165) and it forms when growth is disrupted by a systemic disturbance, often dietary related (Goodman *et al*., 1980, Goodman, 1991). The defects, usually lines, pits or grooves on the surface of the tooth can only be seen if people are subject to periods of biological stress during the time when the teeth were developing – unlike Harris Lines, these defects are permanent (Roberts and Manchester, 2005: 75) as teeth are not subject to the continual remodelling, as seen in bone (Parfitt, 2009).

- Osteoporosis is one of the metabolic bone diseases often considered when studying diet in past agricultural populations as it may offer insights into the composition of diet. However, data must be interpreted with caution due to the wide range of risk factors associated with its development (Agarwal, 2008).

- Vitamin C and D deficiencies can also be expressed in the skeleton; it is possible that the transition to agriculture reduced the amount of Vitamin C available in the diet, in part due to the processes of cooking and storage (Roberts and Manchester, 2005: 234). Vitamin C deficiency may manifest as scurvy and Vitamin D deficiency, as rickets. Vitamin D deficiency has already been demonstrated as a potentially influencing factor of increased susceptibility to TB (Ustianowski *et al*., 2005).

- Reduced stature is one of the effects that may be seen in the skeleton archaeologically as there is a strong correlation between this and an individual's nutritional status (Roberts and Manchester, 2005: 39). Differences in stature often accurately reflect differential health status within a population (Steckel, 1995: 1910). Decrease in stature during agricultural transitions has been repeatedly

demonstrated, with global studies since 1984 recently reviewed, showing that the trend holds true for the majority (Mummert et al., 2011).

Until recently, it was commonly accepted within the bioarchaeological community that porotic hyperostosis and cribra orbitalia (areas of pitting and porosity on the external surface of the skull and the roof of the orbital sockets, respectively) were an effect of iron deficiency anaemia, often accepted as suggesting poor sanitation, dietary deficiencies and infectious disease (Hengen, 1971, Walker, 1986, Blom *et al*., 2005). Walker *et al.* (2009) argue that iron deficiency cannot be the cause of these lesions, as haematological research demonstrates that iron deficiency anaemia partly results from reduced red blood cell production (Brugnara, 2003, Arndt *et al*., 2005, Thomas and Thomas, 2005, Kempe *et al*., 2006). This makes it an impossible cause of haemapoietic marrow expansion, recognised in these pathological lesions (Walker *et al.*, 2009); previous studies should now be interpreted with caution.

3.4.3 Stable Isotopes and Diet

Diet plays a key role in the consideration of human health (and thus human health in the past) and the relationship between the two has been discussed previously (2.4.2). The development of techniques such as isotopic analysis is allowing new insights into questions of cultural and economic shifts, and a clear distinction between Mesolithic and Neolithic diet is apparent. Some of the earliest studies using isotopic analyses were done in North America in order to gain a better understanding of the change in subsistence that occurred with the introduction of maize agriculture (Vogel and Van Der Merwe, 1977, Van der Merwe and Vogel, 1978, Bender *et al*., 1981). More recently, European studies have used stable isotope analysis not only to investigate diet (Richards *et al*., 2001) but also to explore possible links between nutrition and disease (Papathanasiou, 2003), combining macroscopic techniques for diet such as dental evidence with stable isotope analysis (Lubell *et al*., 1994).

Isotopic analysis enables the study of palaeodiet because individuals are, in effect, what they eat, and the technique is being increasingly employed in archaeology (Chisholm *et al*.,

1983). A uniform diet eaten over a period of time results in the chemical components of human tissue maintaining a stable isotopic value (Schwarcz and Schoeninger, 1991). Collagen is the primary protein and key component of bone; it contains carbon and, as it is thought that the isotopic composition of an individual's diet controls the δ^{13} C-value of collagen (Lovell *et al*., 1986), changes to diet can thus be identified and characterised. The nitrogen isotope ^{15}N can also be found in collagen and its values can reflect different trophic levels of diet and distinguish between marine and terrestrial protein consumption (Papathanasiou, 2003). Collagen survives quite well, due to its relative insolubility, so residues can often be found in even degraded bone (Schwarcz and Schoeninger, 1991), making it a retrievable and exploitable tool for palaeodietary studies. However, results gained using carbon isotope ratios have been questioned because of the possible effects of physiological variables such as age or sex on values, but experiments demonstrate a very small impact, thus supporting results as reflecting genuine dietary differences (Lovell *et al*., 1986). Stable carbon and nitrogen isotope analyses can be used to measure the consumption of C4 plants such as maize (Buikstra and Milner, 1991, Katzenberg *et al*., 1995, Larsen *et al*., 1992) and to explore marine versus terrestrial diets (Lubell *et al*., 1994, Richards *et al*., 2003, Lidén *et al*., 2004, Milner *et al*., 2004). Katzenberg warns, however, that palaeodietary studies in particular must be aware of their own limitations as the consumption of a number of different foods, in various combinations and with various emphasis, could result in similar stable isotope ratios being acquired; Katzenberg therefore advocates the use of multiple elements and familiarity with other sources of dietary information in order to "unravel" the problem (2008: 432)

3.4.4 Neolithic

Study of prehistoric diet is a key factor behind the changing attitude that there was a quick transition between the British Mesolithic and Neolithic. Richard and Hedges (1999: 891) summarise the changes in subsistence practices seen during the Neolithic as being:

…a change from exclusive use of wild foods in the Mesolithic to the increased use of domesticated plants and animals in the Neolithic, introduced to the diets either gradually or rapidly.

The Neolithic certainly demonstrates cultivation of cereals and wild food plants, although the level to which diet was comprised of these wild foods is debated (Robinson, 2000: 89). Fairbairn believes that, based on the minimal evidence available, cereals appeared in, and their use subsequently spread across Britain, in less than 400 years (2000: 108) but that the range of cultivated species was limited in comparison to sites in continental Europe (2000: 109). Figure 3-6 shows the 'founder crops', or crops that did not exist in northern Europe before humans, and highlight those that have been identified in Britain (Fairbairn, 2000: 107).

Archaeological evidence for agriculture can demonstrate what species of plant were being cultivated via seed and grain impressions on pottery, plus carbonised seeds and other plant remains from archaeological sites (Darvill, 1987: 52). Crops grown in Britain during the Neolithic period include emmer, einkorn, bread and spelt wheat, six-row barley, and flax (Helbaek, 1952, Smith, 1981: 186-9) (Figure 3-6). However, they are found in different quantities at a number of sites, which demonstrates different soil and other environmental factors in favour of specific crops (Dennell, 1976 cited in Darvill, 1987: 52). For example, in Southern England, on the chalklands, a greater quantity of emmer wheat has been found compared to einkorn wheat (Darvill, 1987: 52). In the future, it is hoped that differing d15N values in archaeobotanical remains (primarily in cereal cultivation, less so in pulses), identified as being due to manuring, will shed more light on evidence of crop growing conditions and crop husbandry practices, to form a key element of palaeodietary studies (Fraser *et al*., 2011).

Cereals		Pulses	
English name	***Latin Name***	***English name***	***Latin Name***
Einkorn wheat*	*Triticum monococcum*	Pea	*Pisum sativum*
Emmer wheat*	*Triticum dicoccum*	Lentil	*Lens culinaris*
Macaroni wheat	*Triticum durum*	Bean	*Vicia faba*
Bread Wheat *	*Triticum aestivum*	Bitter vetch	*Vicia ervila*
2–row hulled barley	*Hordeum vulgare*	Chickpea	*Cicer arietinum*
6–row hulled barley*	*Hordeum hexastichum*		
Naked barley*	*Hordeum hexastichum* var *nudum*	**Other**	
Rye	*Secale cereale*	Flax/linseed*	*Linum usitatissimum*

Figure 3-6 Principal crops of the Old World Neolithic. (Fairbairn, 2000:Table 11.1).

These crops did not exist in northern Europe before introduction by humans (*denotes unambiguous finds in British Neolithic contexts – nomenclature follows van Zeist 1984).

Richards undertook an isotope analysis of individuals at four Neolithic sites in the south of Britain: Hambledon Hill, Dorset, and three of the Cotswold Severn tombs: Parc le Breos Cwm, South Wales, Hazleton, Gloucestershire, and West Kennet, Wiltshire. The results demonstrated very different patterns of diet, with some sites showing that the vast amount of protein in the diet was derived from animal sources, and the reverse at others (2000). The results contradict notions of a typical Neolithic subsistence economy '…different subsistence regimes were followed in different areas by different communities and at a regional and national scale the picture is more of a 'mosaic' of adaptations. (2000: 132). Another study, also employed stable isotope analysis to characterise the diets of individuals at Hazleton North in Gloucestershire where it was found that they shared a very similar pattern of isotopic composition, from which it can inferred they shared a similar diet; all diets demonstrated very high meat or animal product intake (Hedges *et al*., 2008).

Evidence of previously unknown foods in Britain such as the grape (*Vitis vinifera L*.) found in Neolithic Dorset (Jones and Legge, 1987: 454) may also suggest that the people of the Neolithic were not limited to the natural flora of Britain. Although evidence is limited to this site, it is not necessarily proof of its absence elsewhere during the period. Due to a lack of extensive sampling at British Neolithic sites and the reduced preservation potential, it is also possible that the grape was more regularly cultivated during the period; its presence in a causewayed enclosure', however, may imply something about the status of the plant (Jones and Legge, 1987: 454) and, as such, it may not have been available to those considered lower in any social hierarchy.

In addition to cereals and pulses, many of the animals domesticated during the Neolithic period are also known not to be native to Britain and thus must have been transported from mainland Europe (Barker, 1985: 203). The most common and well known examples include the sheep and goat, meaning that these animals were again imported from the mainland via the sea as there was of course no land bridge between the continent and Britain by the Neolithic period. Other animals found in Britain at this time include the pig, its native wild ancestor being the wild boar, and cattle, preceded by the auroch (who was to die out during the course of the Bronze Age). Despite both of these animals having native ancestors, modern genetic studies indicate that pigs of Near Eastern ancestry were introduced to

Europe during the Neolithic, although were rapidly replaced by European pigs after their domestication (Larson et al, 2007). Likewise, genetic evidence now offers significant support for a Near Eastern decent of modern European cattle based on distributions of clustered haplotypes (Troy et al., 2001).

Not only does this evidence demonstrate complex interactions with other populations, it also shows that infectious diseases had an opportunity to diffuse through the British population. This is highly significant with regard to TB. As discussed, there is evidence of very early TB from Europe (Canci *et al*., 1996), and thus it was perfectly possible for the disease to have reached Britain during this period if it is assumed that it wasn't already present. However, it has yet to be demonstrated, and proof of contact is certainly not enough to guarantee its transmission; there are many other factors still to think about when considering the potential success of any infectious agent.

Cattle, sheep, pigs and goats were all integral to early Neolithic farming (Copley *et al*., 2003). Archaeological evidence shows a predominance of cattle in settlements in southern England, with fewer numbers of sheep and pig, sheep perhaps being less represented (Darvill, 1987: 51). The predominance of cattle is significant to note as cattle are believed to be the most infectious to humans when animals are the reservoir for human TB (Grange, 1995).

In Britain, the use of stable isotopes, in addition to archaeological evidence, has been invaluable as a method of identifying a significant change in diet at the beginning of the Neolithic. It is, of course, not possible for isotopic analysis to distinguish between wild and domesticated resources (Thomas, 2003), and thus the exact nature of this dietary, and possibly cultural and economic, change is hard to detail. A shift to a primarily terrestrial as opposed to a marine diet has, however, been convincingly demonstrated (Richards and Hedges, 1999) even to the point of marine foods being 'comprehensively abandoned' (Richards *et al*., 2003: 366). This in itself is an important development and is emphasised by the fact that it is not purely recognised in inland populations but also seen in those of the coastal. Interestingly, similar findings have been demonstrated in Denmark (Tauber, 1981, Richards *et al*., 2003) and Portugal (Lubell *et al*., 1994). As to the question of whether this

dietary shift was gradual or rapid, results certainly suggest the latter as all 78 radiocarbon dated individuals included in the study demonstrated this shift to a terrestrial diet, regardless of the site from which they were excavated (Richards and Hedges, 1999, Richards *et al*., 2003).

> *It may be possible that this [a move away from marine resources] is due to a rapid change in diet away from wild foods to one in which the new Neolithic domesticated plants and animals were predominant.*
>
> (Richards and Hedges, 1999: 896)

Reasons as to why there was such a 'rejection' of marine resources are discussed by Thomas who suggests that it may have been a change in the relationship between humans and the sea, or even between people and people, i.e., there was a cultural shift which rendered marine foods obsolete (2003). If it is assumed that the results reflect the dietary shift of indigenous British peoples then such a dramatic change in protein intake could have had a significant impact on health. As mentioned previously, poor diet, particularly one low in protein, can have an impact on the susceptibility of an individual to contracting diseases such as TB due to immunodeficiency, and compromised antimicrobial resistance (Chan *et al*., 1996). However, "the question of what is an acceptable daily protein intake is complicated by age, health, sex and other aspects of the diet." (Leach *et al*., 2006). A previously hunter-gather lifestyle suddenly replaced by an agricultural one alters the diet considerably, and the transition to agriculture is often associated with a decrease in health of a population (Cohen and Armelagos, 1984). A shift away from marine resources does not necessarily indicate a move away from wild foods to be replaced predominantly by domesticated plants and animals, although it is possible (Richards and Hedges, 1999).

'But an important by-product of this early stockbreeding must eventually have been dairy products which certainly introduced a new dimension into the range of foods.' (Brothwell, 1971: 82). There is evidence for the exploitation of secondary animal products, including milk (Copley *et al*., 2003, Copley *et al*., 2005b). Analysis of fat residues on pots dating to the Mesolithic and the Neolithic also suggests that dairying was in fact an important element of early Neolithic 'farming' from the mid-fifth millennium BC onwards (Copley *et al*., 2005b,

Copley *et al*., 2003). The data collated from the 2003 study demonstrated 'direct evidence for the exploitation of domesticated ruminant animals for dairy products at all Neolithic, Bronze Age and Iron Age settlements in Britain' (Copley *et al*., 2003). As another example of secondary product exploitation, sheep, which were probably the earliest herd animal domesticated from the wild (Gade, 2000), were likely used for wool, milk and meat (See 2.4.6 for a discussion on occupation related disease risks).

3.4.5 Bronze Age

The Bronze Age witnessed an intensification of agriculture and, along with population growth, became even more vulnerable to the health implications of such a system. According to Champion, the agricultural economy in the later Bronze Age was mixed, 'exploiting crops and animals in more complex and more intensive ways than before' (1999: 103). There is also clear evidence of continued dairying (Copley *et al*., 2003, Copley *et al*., 2005b) and further exploitation of secondary products of animals, all of which brought their own threats to health (See 2.4.7).

This period shows a decrease in wild animals associated with settlements and deposits with a predominance of domesticates. However, all the wild species seen in the Neolithic are accounted for in the Bronze Age, although the auroch is known to have survived only until the mid Bronze Age (Darvill, 1987:133). At this time there is also evidence of a previously unknown species – the squirrel (Darvill, 1987: 75).

Whilst the crops cultivated in the Bronze Age remained similar, with both wheat and barley being grown (barley being the main crop), there was the addition of oats (Roberts and Cox, 2003: 77). This can be viewed as an advantageous development given that they are a good source of energy, providing high quality protein, minerals, essential fatty acids and high concentration of soluble fibre (Roberts and Cox, 2003: 77), all of which would have contributed to a strong immune system, assuming of course that the crop was successful. From a faunal perspective, there is an increase of bird remains found archaeologically, but still a lack of fish (Jay, 2008: 201) and, through other deposits, it is clear that domesticated

animals seemed to take precedence over wild (Maltby, 2001: 20). This could have had both positive and negative effects on health, limiting contact with wild animals but putting a greater reliance on the domesticates.

3.4.6 Iron Age

The Iron Age demonstrates a mixed and varied economy and a general geographic expansion of agriculture (Huntley and Birks, 1983). Whilst there is an increase in the amount of data available from faunal studies for this period, there are still areas from which there is little evidence, due to either a lack of preservation or a lack of attention paid (Maltby, 2001: 17).Central and southern England have produced much of the larger collections of animal bones for the period, and assemblages are dominated by the primary domesticated species including cattle, sheep, pig, horse and dog, although there is evidence for much intra-site variability (Maltby, 2001: 20). Whilst goats are not represented in any great number, they do also show a presence in Iron Age faunal remains. Bradley (1978: 183-5) also suggest an increase in the importance of sheep during the period, and evidence does suggest they dominate faunal assemblages for the Iron Age (King, 1991: 16), particularly in chalk and limestone Areas. Cattle, however, continued to play a major role in Iron Age agricultural practises as 'Cattle can be valued for their meat, skins, manure, dairy commodities and transport and draught qualities.' (Maltby, 2001: 21).

Interestingly, a site dating to the Iron Age (Skeleton Green) demonstrates a higher quantity of domestic fowl in its faunal assemblage than other British Iron Age sites (Ashdown and Evans, 1981). This is more in keeping with finds in Europe for the period and thus is taken to demonstrate continental influences; the same is also true of the prevalence of pig bones (Grant, 1984: 112-3). The site also shows a deviation from the general Iron Age trend regarding butchery of cattle limb bones (Maltby, 1985). The pattern identified (Ashdown and Evans, 1981: 214-5) actually shows closer affinities with urban Romano-British deposits identified which may, again, support a continental influence being particularly strong at this site (Maltby, 2001: 22).

Significant variations in mortality profiles from different sites serve to highlight the different uses of cattle during this period (Maltby, 2001: 21). Suggestions have arisen regarding differences in mortality profiles being the product of cattle management systems (Grant, 1984: 107-10). Much the same suggestion has arisen with regard to crop cultivation based on the plant evidence; management of arable production, processing and distribution between hill forts and arable and pastoral farmsteads, although this is questioned elsewhere (Maltby, 2001: 21).

A wide range of crops are evident for the Iron Age and it has even been suggested that Iron Age cereal cultivation involved 'a more diverse range of species and land races than any other cereal regime before or since.' (Jones, 2001: 31). Domesticated plant species for the Iron Age are known to include spelt wheat, which is only very rarely seen previously, emmer wheat, known to have been a major crop of the Neolithic and Bronze Age but less prevalent for this period, bread wheat which was becoming the dominant species of wheat at a small number of sites in the Iron Age (Jones, 1988), and six-row barley, not new in the Iron Age but an important and highly adaptable crop (Jones, 2001). Oats, which made their first appearance in the Late Bronze Age, were continuing to be cultivated although wild forms may also have been eaten, and rye, shown to be grown from the Bronze Age onwards was still present (Jones, 2001). Legumes and brassicas were also being cultivated at this time (Jones, 2001: 31-33), all of which serve to demonstrate the truly diverse crop range witnessed by the Iron Age, but with clear roots from the Bronze Age.

> *The Iron Age economy was in its entirety rooted in plant management in some form, be it directly for human food, or indirectly for animal food of fuel…The enclosed landscape that was thus expanding during the iron age involved fixing the boundaries of managed vegetation…It is hard to find a period of time, either earlier or later in which so much of the human plant diet is contained within the annual cycle of seed bearing herbs, predominantly grasses (including cereals) and legumes.*
>
> (Jones, 1988: 29)

The consumption of fish, which forms a large part of any discussion of diet for the prehistoric periods of Britain, is once again called into question at Glastonbury Lake Village in Somerset. Measuring carbon and nitrogen isotopes, little evidence has been found from the Iron Age to indicate utilisation of aquatic resources; this is true even of sites whose geographical location would lend weight to such assumption; (Jay and Richards, 2007, Jay, 2005). However, this particular site, which has been extensively studied due to its excellent preservation, has demonstrated the presence of fish bones along with other aquatic birds and mammals (Coles and Minnitt, 1995: 194-5), and this suggests a more varied diet for people at this site than at others. The isotopic data do not complement this assumption (Jay, 2005: 213) but rather supports the consumption of a largely terrestrial diet based on domesticated plants and animals, consistent with other contemporary examples. The data may also indicate that, in comparison to other sites, the occupants of Glastonbury Lake Village were consuming slightly less animal protein, although this is somewhat ambiguous (Jay, 2005: 208).

3.4.7 Conclusion

Roberts and Cox (2007) provided a general overview of health in Britain from the Neolithic period to the mid-nineteenth century, collating previously reported data. Overall, what they found was broadly consistent with the idea of a general trend of declining health with intensification of agriculture and increasing social complexity; the prehistoric periods specifically demonstrated minimal evidence of disease, possibly due to their relatively small populations (Roberts and Cox, 2007). However, the first evidence of non-specific infection is found in the Neolithic, as well as increasing dental disease, trauma, metabolic and joint diseases; metabolic disease also increases in the Bronze Age, and the Iron Age witnesses a number of new diseases (Roberts and Cox, 2003). Stature generally increased through time for males, which is interpreted as potentially representing adaptation of the population to changing circumstances, and to dietary and disease stress (Roberts and Cox, 2007). Again, it must be emphasised that exploring health in past populations may be hampered by the

effect of the osteological paradox (Wood *et al*., 1992), and that it is therefore likely that many diseases are underestimated in the bioarchaeological literature. Wood *et al.* (1992) argued that the identification of populations with limited or no skeletal pathologies does not necessarily reflect a healthy population – the reverse could be true. Those that developed disease but lived long enough to develop skeletal changes may be representative of better immunity, whereas a lack of pathology could mean that individuals died before skeletal changes could occur, and thus cannot be considered as healthy. It is simply not possible to assess what is not skeletally visible.

Finally, despite the general decline in health with agriculture entails, there is apparently a more positive effect which may be a biological driving force behind its universal adoption. Lambert (2009) argues that the question of reproductive fitness has been missing from past measurements as to the 'cost-benefit equation' of the transition to agriculture, and that a Darwinian model might be better suited to explaining the pace and scale of its uptake, the motivation being 'a positive feedback relationship between cultural behaviour and reproductive fitness' (2009: 607). This reproductive fitness has however come at a price.

> *Health decline could have been a costly by-product of an economic system that enhanced fertility and led to population growth…people would likely be unaware of the association between health decline and agriculture, at least in terms of problems such as bacterial infection and its link to dental caries and other infectious diseases.*
>
> (2009: 607)

Richards (2002) too recognises this trade off seen between declining health and extensive population expansion humans experienced as a result of the Neolithic revolution. This paradoxical relationship has caused Lambert to question the 'measures bioarchaeologists use to assess the biological costs and benefits of agriculture' (Lambert, 2009: 603).

3.5 Climatic Conditions and Landscape

3.5.1 Introduction

Climate is a natural phenomenon but can also be influenced by the actions of humans and the way they interact with their environment. From a biological perspective, a stable climate to which a population is adjusted, is integral to maintaining a balance of health and changes to this climate, require adaption in order to sustain health. Sometimes climate can be directly associated with disease. For example, it may dictate where in the world certain vector dependent diseases can flourish such as malaria (Hay *et al*., 2002), which is spread to humans via mosquitoes and, for this reason, is commonly found in warm climates which favour the breeding cycles and survival of mosquitoes. Climatic changes, and the human response to them, can also have indirect effects on health. For example, wetter conditions may lead to waterlogging of soils, leading to a decrease in crop yield (Thomasson, 1982) and to an economy dependant on this resource, possibly famine, leading to malnutrition and subsequent health problems. The relationship between climate change and human health is highly complex, and no doubt, always has been. Research into the deterioration of climate in the past has explored the impact on human societies, including disruptions and changes to social, and economic conditions specifically in Britain (Amesbury *et al*., 2008, Tipping *et al*., 2008) and further afield (Finsinger and Tinner, 2006, Turney *et al*., 2006, Berglund, 2003, Edwards *et al*., 2007).

3.5.2 Food Access

It is necessary to consider what effect climate may have on human access to food, how it may dictate what foods can to be produced and subsequent nutrition availability (See 2.4.2 for a full discussion of nutrition and related health effects). Climate change can threaten human health through the direct impact it has on food availability which may translate into under nutrition and food insecurity (Brown and Funk, 2008). Malnutrition, which can occur as a result of poor nutrition from reduced food ability is often cited as a factor in TB development, due to lowered cell-mediated immunity (Chandra, 1991, Myrvik, 1994,

Gershwin *et al*., 1987), although clinical evidence of this, particularly in humans, is sparse and more detail is required in order to fully understand how TB and malnutrition are linked (Cegielski and McMurray, 2004). Nevertheless, negative health effects of known famines are well documented, specifically in relation to the young (Brenner and Chertow, 1994, Walker *et al*., 2007b, Chen and Zhou, 2007, Hoet and Hanson, 1999, van den Berg *et al*., 2007, Victora *et al*., 2008).

3.5.3 Neolithic

It has even been suggested that it was climate change that triggered agricultural expansion into the north-west fringe of Europe, including Britain, around 4000 years BC (Bonsall *et al*., 2002). Whatever the driving force behind the adoption of agriculture in Britain, extensive manipulation of the landscape was a key factor in its application, and human 'land-use legacy' during these prehistoric times may well be an important contributor to the variation in biodiversity seen in modern times, based on research in Estonia (Partel *et al*., 2007: 574). Bogs appeared due to the extensive clearing of forests and the grazing of animals; removing plants and allowing grazing animals to keep grasses short leads to a reduction in transpiration of water and subsequent water-logging of the ground. Waterlogging can have a detrimental effect on crop yields as it will inhibit germination and retard growth in cereals (Thomasson, 1982). Whilst the development of technology enabled greater and more efficient land clearance (as seen in pollen diagrams - Darvill, 1987: 75), with this came the problems of wind erosion of soils and, whilst the fauna seemed mainly unchanged, aurochs became extinct during the 2nd millennium BC (Darvill, 1987: 75).

That clearing and deforestation witnessed in the Neolithic was pro-active, has however been questioned, given that society was highly dependent on these resources (Jones, 1988, Moffett *et al*., 1989). Brown (1997) offers an alternative model to a purely purposive one previously assumed, and asserts that previous studies have failed to appreciate the role of ecological processes and change. Here it's argued that the inhabitants of Mesolithic and Neolithic Britain may have employed a more opportunistic model of clearance use (Figure 3-7).

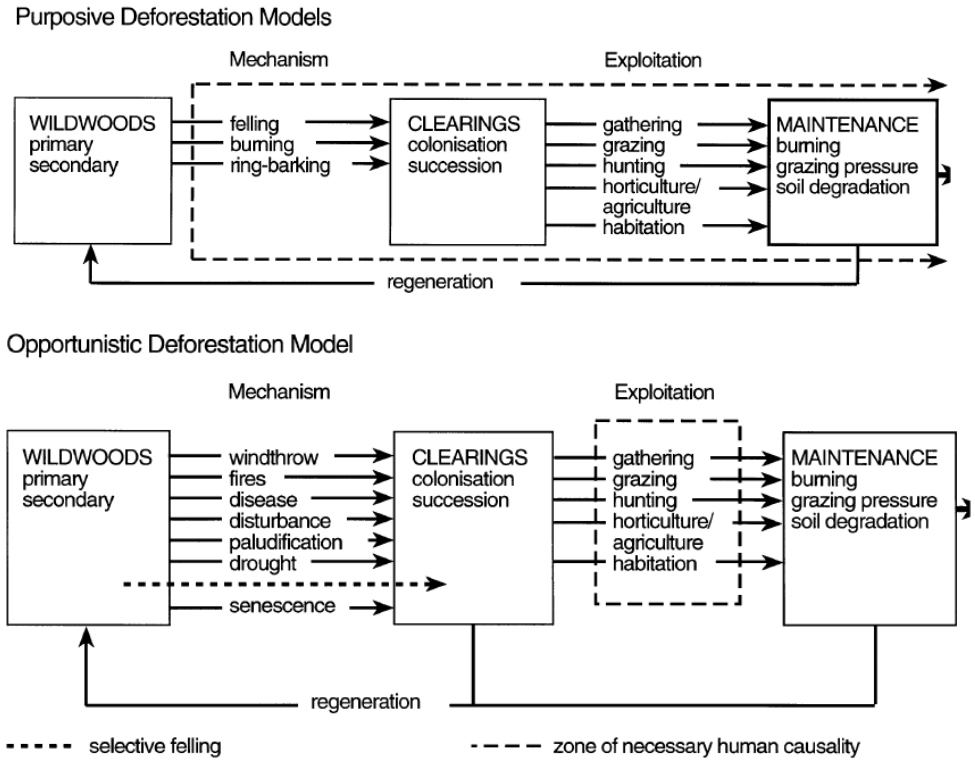

Figure 3-7 'A diagrammatic conceptualisation of the purposive and opportunistic models of deforestation.' (Brown, 1997: Figure 2).

These changes to the landscape, whether they were instigated by humans or just exploited, could have affected, either indirectly or directly, exposure to or transmission of TB. The depleted soils (due to clearance and grazing) may well have led to manuring as a means of replenishing nutrients into the soil. The importance of soil has been emphasised by Minami who goes so far as to state that soil collapse can lead to 'the collapse of human culture, civilization, livelihood and health.' (2009: 603).

Enhancing the quality of the soil, using animal dung as fertiliser or manure may have presented a health threat in terms of zoonoses (Roberts and Buikstra, 2003: 77; Roberts and Cox, 2003: 57). Evidence shows that the TB bacterium can survive for up to five months in cow dung during the winter period (O'Reilly and Daborn, 1995), for 4 months in autumn and two months in summer (Williams and Hoy, 1930), for up to seven months in manure, in a variety of environmental conditions (Duffield and Young, 1985), and six months in slurry (Hahesy *et al*., 1992). Storing manure and manure spreading all year round are two risk factors identified for TB transmission amongst cattle herds today (Ramírez-Villaescusa *et al*.,

2009), and manure also poses a risk of infectious disease for human workers (Arinaminpathy and McLean, 2009).

3.5.4 Bronze Age

Although the start of the Bronze Age showed little environmental change, this altered significantly as time progressed. The start of the Bronze Age has been suggested as being the beginning of a separation between people from nature (Evans, 1999: 65) but, towards the end of the period, nature certainly asserted its influence on the Bronze Age way of life. The climate began to deteriorate, becoming colder and wetter and, perhaps in conjunction with intensified land clearance, there was also an increase in peat bogs and standing water in highland areas (Roberts, 1998: 168). Changes such as these impacted on what crops could grow (Cunliffe, 1995: 16); a temperature reduction of two degrees in upland areas would have resulting in a reduction of five weeks of the growing season (Burgess, 1985), and the wetter conditions would have had a detrimental effect on soil and subsequent crop yield (Balaam *et al*., 1982).

One of the effects of current climate change is likely to be the displacement of people from inhospitable areas, the effect of which is likely to be reduced health through the stresses such migration would entail (Costello *et al*., 2009). During this period sophisticated division of the land is seen, and one such example is that of the moor-wide boundaries, known as reaves on Dartmoor. Deteriorating climate has been argued as being an influencing factor in their abandonment (Fleming, 1988, Parker-Pearson, 1993). This hypothesis was recently tested and the results do demonstrate that the shift to a cooler and wetter climate around ca. 1395 to 1155 BC (demonstrated by palaeoclimatic reconstruction using testate amoebae and peat humification analyses) that coincides with the apparent abandonment of the reaves (Amesbury *et al*., 2008). It is emphasised, however, that the abandonment of the reaves may not be as a direct result of this climate change, although the results demonstrate 'compelling support' that it played a part, and that other factors must also be considered (Amesbury *et al.*, 2008: 96).

Similar questions have been asked of north-east Scotland farming communities, where it was proposed that in the face of the climate deterioration at the end of the Bronze Age, agricultural activities were relocated (Tipping *et al*., 2008). As with Dartmoor, the results demonstrated socio-economic changes around the time of climatic deterioration. Changes were seen in terms of land use, but they may be interpreted as being adaptive responses to the climate and do not indicate settlement abandonment in the face of climatic stress (Tipping *et al.*, 2008).

3.5.5 Iron Age

The beginning of the Iron Age was subject to the same climatic deterioration as that witnessed at the end of the British Iron Age, and it was not until c.400BC that things started to improve (Darvill, 1987: 133). It can perhaps be inferred from this that the possible health impacts seen in the Bronze Age, thus continued into the Iron Age. An ever growing population, however, is likely to have increasing pressure on food availability and space which, in turn, may have led to greater biological stresses and thus weakened immune systems during this period. To combat the colder conditions, populations may turn to animal products such as dung to help with insulation and for burning as fuel (Roberts and Buikstra, 2003: 63) (Figure 3-8), and this is likely to have been the case throughout the later Bronze Age and early Iron Age. Dung, as an animal by-product would be readily available in an agricultural community but the risk of contamination from infected material must again be emphasised (Arinaminpathy and McLean, 2009).

Crop failure and consequently under-nutrition no doubt continued to pose a risk to health. Again, this was probably seen across both periods during the course of this colder, wetter climate. More time would also have been spent indoors and thus increased contact time between people and exposure to higher levels of air pollution, both of which may have influenced the possibility of TB transmission, as previously demonstrated. It is of course in this period, that we find the first confirmed individual with human TB (Mays and Taylor, 2003) and thus the discussion is no longer hypothetical.

Figure 3-8 A woman forms patties of cow dung to be dried and used for fuel in New Delhi, India (http://edition.cnn.com/2008/WORLD/asiapcf/01/07/eco.about.manure/index.html)

The later Iron Age however, shows evidence for a warmer and drier climate which then transformed into a climate not unlike today, and this enabled people to expand into previously uncultivated areas of land (Darvill, 1987: 133); temperatures are shown to have risen by around two degrees (Dark and Dark, 1997). Cultivation of the land gathered speed and agriculture intensified. Forest and woodland clearance was extensive, although less so in northern regions and in Scotland. The result, certainly for the more southern areas of Britain, was a very open landscape with field systems and boundaries (Turner, 1981: 267).

3.5.6 Conclusion

It has been said that Britain has no climate given that climate is described as the average state of the weather (Stamp and Beaver, 1963: 71), but what these prehistoric periods demonstrate is a palaeoclimate which was subject to relatively frequent change to which the inhabitants of Britain had to adapt in order to survive. Increased population expansion would seem to suggest that, on the whole, they were successful. Woodward *et al.* (2011) recognise the need to adapt to climate change in order to sustain health, and adaptation is the key concern of current health initiatives in the face of rapid 21st century climate change (Costello *et al*., 2009).

Along with these changes seen in the Neolithic, Bronze and Iron Ages, would have come effects that are intimately associated with human health and that either directly or indirectly affected a person's susceptibility to infectious disease transmission. An example of this is temperature increase or decrease, causing shifts in lifestyle patterns (such as more time spent indoors or increased use of dung for fuel or insulation) which may reflected in the seasonal patterns of some diseases today (Lipsitch and Viboud, 2009, Naranbat *et al*., 2009, Douglas *et al*., 1996, Nagayama and Ohmori, 2006, Chan, 1999), or it may have been poor crop yields due to increased precipitation and poor soils. Whether the TB seen in the Iron Age is a reflection of climatic conditions and the human response to these, or other socio-economic, or cultural factors explored in this chapter, is unknown but it is clear that climate change can play its part in poor health and increased risk of respiratory infection and it will continue to do so.

3.6 Funerary Context and Health

3.6.1 Introduction

What is so difficult about studying the health effects of such environmental and social, changes is the lack of skeletal evidence available for the Neolithic period. Whilst there is certainly more material to draw upon than from the previous Mesolithic period (Roberts and Cox, 2003: 56), there is distinctively less than from later periods, particularly from the Roman period onwards. Importantly, many of the remains that have been found from the period originate from chambered tombs or ditches or pits of monuments, and they are often disarticulated (Darvill, 1987: 68), making study of discrete individual skeletons a difficult if not impossible process. Individual formal burials for the period are a rare occurrence (Whittle, 1999: 66) but an increase is seen from the Middle Neolithic onwards (Whittle, 1999: 66). Human remains for the period are also often in a very fragmentary state due to taphonomic changes, again, making comprehensive study challenging.

3.6.2 Neolithic

The difficulties of working with skeletal remains from the Neolithic period, due to funerary context is discussed in section 3497.2, with particular reference to the Neolithic skeletal remains included in this study, but it should be noted here that the majority of Neolithic remains are excavated from long barrows or frequently associated with causewayed enclosures. Evidence of infectious disease is so far limited to only a few examples and in many cases can only be considered non-specific in aetiology such as two individuals (one from Hazleton North, Gloucestershire and one from Hambledon Hill, Dorset) displaying periostitis on the ribs, possibly indicative of some form of respiratory infection which could be TB (Roberts and Cox, 2003: 60-1). Evidence of specific infection is very limited and the diagnoses cautious; an individual with possible meningitis from Hambledon Hill (Roberts and Cox, 2003: 64) and another with suggested poliomyelitis from Cissbury County (Rolleston, 1879 cited in Roberts and Cox, 2003: 65) are indicated. In the case of the latter, Roberts and Cox (2003: 65) are careful to point out that many conditions could cause the pathological lesions seen on this individual skeleton. Whilst it may appear that TB as an infectious disease could have potentially established itself, if not perhaps thrived, there is as yet no evidence for the disease.

3.6.3 Bronze Age

This is also true of the Bronze Age, despite a number of factors potentially exposing communities to a greater risk of contracting infectious disease than had been seen before. Infections are only seen in eight people from the Bronze Age, all of which are non-specific in aetiology, including two people with maxilliary sinusitis and one with rib inflammation (Roberts and Cox, 2003: 86). There is also some evidence of mastoiditis (Brothwell, 1961, McKenzie and Brothwell, 1967) which is rarely reported in the past but not perhaps due to a lack of occurrence (Roberts and Cox, 2003: 87).

Whilst the burial of individual bodies did traverse the Neolithic / Early Bronze Age transition with individuals often being interred under round mounds or pits, cremations became the predominant burial rite by the middle Bronze Age (Parker-Pearson, 1993: 50). During the middle Bronze Age there was an overlap of burial rites so much so that both inhumations and cremations can be found within the same funerary context (Bradley, 2007: 162).

However, it was the Middle Bronze Age that saw the development of major cemeteries that can start to be linked with specific settlements such as that found at Itford Hill in Sussex containing a group of cremations associated with a round barrow (Bradley, 2007: 197). Cremated bone can and should be analysed but is a challenge, and any metrical or pathological evidence is compromised because of fragmentation (McKinley, 2004). Adding to this difficulty is the fact that, during the Later Bronze Age, there was a move away from organised cemeteries towards a variety of alternative methods. Examples of this include individuals being 'dumped' in rivers (Parker Pearson, 1999: 87), and in the East Anglian Fens bodies have been found placed in bogs and pools with associated artefacts (Healy and Housley, 1992); some have even been found in caves in the North of England (Barnatt and Edmonds, 2002).

3.6.4 Iron Age

There are numerous changes in burial customs seen throughout the Iron Age. In the early Iron Age, there are limited remains evident which may, in part, be due to the continuation of alternative methods of disposal such as in rivers seen in the Later Bronze Age (Darvill, 1987: 158). Types of burial varied between regions with cist graves appearing in the southwest from around the 5th century BC, and formal cemeteries in about the 1st century BC (Hill, 1995). Shrines and sanctuaries become visible in the south of Britain during the later Bronze Age, during which time excarnation and the scattering of remains was also common (Haselgrove, 1999: 123). Remains from the Iron Age are generally limited to a handful of formal cemeteries such as Wetwang Slack (Dent, 1984) so there is a limited number of remains for study which, in many ways, makes study of this period as challenging as its predecessors already considered.

The Iron Age, whilst demonstrating an increase in population actually shows a decrease in the frequency of certain diseases. Although remains for the period are also quite limited, and direct comparisons in health data between this period and others may be to some extent ambiguous, the data must be discussed in the context of evidence available. Since Roberts and Cox (2003) reported on the state of health in Britain, from prehistory to the present, there has been one major development of specific importance for this study. TB

has been convincingly demonstrated from a mid Iron Age site (400 – 230BC) at Tarrant Hinton, Dorset (Mays and Taylor, 2003). The diagnosis was confirmed by ancient DNA analysis, although the specific causative agent (*M. tuberculosis* or *M. bovis)* could not be identified. Despite this, the occurrence of TB in Britain at this time has now demonstrated that the disease was not brought to Britain with the Romans, at least not with the Roman invasion and occupation of AD43.

Despite this evidence, this skeleton remains the earliest and only confirmed case of TB in Britain at this time which is still perhaps surprising given the assumption that the risk of density dependent diseases was likely to be continually increasing due to an ever increasing population and change in settlement patterns as discussed earlier. Prior to this evidence, there were only nine individuals demonstrating non-specific infection out of the 591 individuals considered by Roberts and Cox (2003: 92-3). Only one of these had inflammatory lesions on the ribs, four showed periostitis on a long bone, two demonstrated infection on the endocranial surface of the skull and two showed osteitis - there was at this point, not a single occurrence of specific infection (Roberts and Cox, 2003: 93).

3.7 Occupation

3.7.1 Introduction

> *Probably no environment impinging on the health of man has so much effect and is so malleable as the environment in which he works.*
>
> (Enterline, 1964: 973)

Occupationally related infections are those contracted during “employment” (Lim, 2009: 1) but, when looking into the distant past, the definition of “employment” must be more loosely interpreted to include general subsistence practices such as agriculture.

Links between specific occupations and increased risk of TB have been clearly demonstrated (McKenna *et al*., 1996) (see 2.4.6), and generally diseases influenced through occupation are

because of an individual being directly placed in a situation where they are more likely to be exposed to disease, or through associated living conditions (Lim, 2009).

3.7.2 Neolithic

The adoption of agriculture in Britain marked the transition to the Neolithic, and both crop cultivation and animal husbandry has been shown to have played a significant part of life over the course of the period. Agriculture cannot fail to play a prominent role in any discussion pertaining to occupationally related health; respiratory diseases associated with agriculture were some of the first to be recognised as occupational hazards (Schenker *et al*., 1998). Farmers are at risk of lung irritation due to very fine particles that result in the air from working with crops, and exposure can result in inflammation of the respiratory system (Schenker *et al*., 1998). As a result, increased susceptibility to pulmonary infection, including TB, often results.

Farmers are also at direct risk of infection from the land they work, including organic (naturally occurring) dust containing microorganisms which can lead to a number of respiratory disorders; 'Infectious bioaerosols have long been the bane of human existence' (Schenker *et al*., 1998: 57). Exposure to these threats can occur when dealing with animals, harvesting, processing or storing grains, amongst others.

Farmers need not just be subject to increased susceptibility to TB infection through other pulmonary problems; farmers are amongst the most at risk occupation groups for direct M. *bovis* exposure (O'Reilly and Daborn, 1995), as are zookeepers (Dalovisio *et al*., 1992). The range of wild animals affected has been outlined in section 2.4.7.

> *Humans must have very soon become aware of the dangers linked to contacts with animals…the harvesting of crops and the exploitation of animals for food were also hazardous and those involved in these activities would surely have individually or collectively experienced the risks connected with obtaining such food. It can be hypothesised that any form of human occupational relationship with animals has been associated with specific diseases.*
>
> (Battelli, 2008: 602).

Of course, by its very nature as a zoonotic disease, TB can also be passed from humans to animals which, in the long term, can bring negative health impacts to humans again. Infected domesticated animals can, amongst others, lead to animal production being reduced in yield and quality (Schenker *et al*., 1998), which can create difficult conditions for those dependant on this resource for food and/or income; resulting stress could also increase disease susceptibility.

Of particular relevance to Neolithic societies, where this may have been equally if not even more pronounced than today, unlike like many occupational settings, farms are often the focus of both work and residence; family members may be incidentally exposed to similar (although reduced) risks as those working the land which 'increases the risk to the population by several-fold' (Schenker *et al*., 1998: 52).

The exploitation of secondary animal products, such as milk obtained through dairying, offers increased threat of exposure to TB by the close contact required to manage cattle for this purpose, in addition to the actual extraction and consumption of milk. The processing of other secondary products may also have placed individuals at risk from TB infection; leather tanners were known to be at an increased risk of TB in the working classes of Buenos Aires during the late 19th and early 20th centuries (Reber, 1999), and the use of manuring and the associated health threats have previously been explored (See 3.5.3).

3.7.3 Bronze Age

'The textile industry is one of the occupation[s] which affects health of workers, especially the spinning and weaving section' (Babel and Bala, 2008: 98). Evidence of hides and textiles are amongst the exchange items found in the Bronze Age, and in 2008 textile production was the second largest industry after agriculture, in India (Babel and Bala, 2008: 98). There is explicit indication of textiles being manufactured as a regular feature of Bronze Age life with loom weights, spindle whorls and combs all having been discovered at settlements (Parker-Pearson, 1993: 104). Whilst it is known that such equipment was in use from c.2500BC, it is only from the middle Bronze Age that the material evidence really survives.

Textile production is one of the occupations associated with increased risk of TB infection due to the production of particulate pollution (Bowden and McDiarmid, 1994). As with some farming activities, particles can enter the lungs and cause irritation, making a person more susceptible to respiratory infection such as TB and, if already suffering from TB, can also increase their level of contagion – coughing encourages the release of bacilli into the air. For example, Babel and Bala's study of 160 workers in a textile mill in Rajasthan, India reported air 'thick with cotton dust' and TB, amongst numerous other ailments, was directly attributable to these poor conditions (2008: 98-101). Bronze Age people would not have been subject to such intensive industry, but the presence of particles as a result of textile production would likely have occurred.

Mining would have increased during the Bronze Age in order to acquire the raw materials required for metal manufacture. Miners seeking metal ores would have been at risk from particulates in the air but also to toxic metals in the atmosphere, soil and water, again weakening their immune systems and making them vulnerable to communicable diseases. The link between TB and mining is documented in living populations, particularly in areas of Africa (Stuckler *et al*., 2011, Hayumbu *et al*., 2008); miners in southern Africa experience TB infection rates up to ten times that of the general population (Basu *et al*., 2009). Silica dust exposure is a product of mining, and this increases the risk of pulmonary TB, but living and working conditions also impact on the high rates of TB seen (Basu *et al*, 2009).

3.7.4 Iron Age

Mining and agriculture of course remained significant occupations throughout the Iron Age, and any sort of intensive or exhausting work can work to lower resistance to TB (Johnston, 1993: 1061). There is also greater evidence for warfare in the Iron Age, but also for a diversification and development of occupations and crafts such as bronze and iron smithing, woodworking, pottery and stonework, and even limited evidence of glasswork; salt was also mined (Morris, 2007). It is, however, unlikely that many craft workers were full time but

rather mixed their crafts with standard agricultural duties during quieter periods (Haselgrove, 1999: 125).

Artefacts would have been traded and exchanged as in previous periods, and probably on an even greater scale, but along with many of these developments came increased or at least different risks to health. The most obvious of these and which may be evident in the archaeological record is injuries. Out of the 591 individuals reviewed by Roberts and Cox (2003), a total of 28 people showed fractures, an increase from the Bronze Age. As with the Bronze Age, however, the smithing, woodworking, mining and textile industries would have exposed workers to an increased risk of respiratory infection, risks they could ill afford in light of the evidence for TB identified for the period – if this was not limited to the site from which it was identified. There is at least evidence that many of these crafts were performed away from the main settlement sites which may have proved useful in reducing the risks to both the workers and lay people in the settlements.

What this period shows more evidence of, than either the Neolithic or Bronze Ages, is warfare. Defended hilltop enclosures dominate the period, and evidence of skeletal stress associated with violent conflict has been suggested as the cause of skeletal changes observed in prehistoric cemetery populations (Larsen, 1997, Klaus and Tam, 2009). However, recent research has focussed on populations who lived in known conflict zones to see if this link could be substantiated (Jennings, 2010). Comparison of four medieval cemetery population along the Anglo-Scottish border (described as conflict-zone) with four contemporary “unstressed” populations did not find a difference in overall mortality or morbidity. The only differences were found in non-adults (higher prevalence rates of cribra orbitalia, periosteal bone lesions, and vitamin C deficiencies) and higher rates of enamel hypoplasia in young adults of the “stressed” population were identified. That no overall difference of morbidity and mortality was identified, may suggest that identifying areas and periods of conflict in archaeological populations where documentary or other specifically archaeological evidence is not available, may be extremely difficult.

3.8 Trade and Contact

3.8.1 Introduction

> *It is a common misapprehension that because Britain is an island it was cut off from the continent, its communities developing in grand isolation, only occasionally being jolted from their comfortable complacency by incoming bands of warriors prepared to brave the dangers of the Channel and the North Sea. Throughout prehistory, the reality was, in all probability, very different.* (Cunliffe, 2004: 5).

Many aspects of Neolithic, Bronze and Iron Age life point towards interaction between, and movement of people, both within Britain itself and further afield and this certainly developed over time. Archaeological evidence for mobility can be found through the distribution of artefacts, manipulation of the landscape, occurrence of specific foods and even direct evidence of movement of people themselves; interactions can also be inferred based on similarities between monument styles and building techniques. The level of mobility seen in British prehistory may not rival that of the modern era (see 2.4.5 for a more in depth discussion), for obvious reasons, but it was, in fact, substantial and would have offered its own dangers. The interaction of communities with others could have presented a real threat and contact with the continent could have exposed British inhabitants to disease from elsewhere.

3.8.2 Neolithic

Debate continues as to whether the Mesolithic inhabitants of Britain had contact with their Irish or Continental neighbours with some suggesting this to be highly unlikely (Sheridan, 2007a, Tresset, 2003) and others suggesting that the coastal communities of this period would have been highly capable of such travel (Thomas, 2007, Whittle, 2003, Tolan-Smith, 2009). There is little doubt, however, that the Neolithic inhabitants of Britain made use of

the sea as a means by which to travel, although Garrow and Sturt draw attention to the fact that the North Sea and English Channel maritime routes have not been considered in as much detail as those of the Normandy/Brittany to south-west England, Wales, Northern Ireland and western Scotland routes (2011). Certainly, around 6000BC, the western seaways are thought to have represented 'a clear and open maritime route…that had been open and in existence for 5000 years. By contrast, the North Sea was still undergoing considerable change…' (Garrow and Sturt, 2011: 63).

Evidence suggests that small boats were able to make journeys across open water during this period (Farr, 2006, Robb and Farr, 2005) and there is specific evidence that such journeys did take place across the channel once the evidence for artefacts is examined (Peacock *et al*., 2010). It is not thought that such journeys would be easy (Peacock *et al*., 2010), but use of the tidal flows would have been employed (McGrail, 2001) and navigators would have used environmental clues from the sky and inherited knowledge and experience (McGrail, 1993).

The evidence for Neolithic sea-faring is inevitably tied in with the Mesolithic/Neolithic transition. Early discussions of the Neolithic often debated the possibility of large scale immigration from Europe or indigenous adoption (Cole, 1965, Fox, 1932, Case, 1969), the former of which would clearly have required significant ability to travel. Although theories of large scale immigration fell out of fashion for a period, the debate continues into the 21st century (Sheridan, 2000, Sheridan, 2003, Sheridan, 2004, Thomas, 2003, Thomas, 2007, Cooney, 2007, Whittle, 2007, Callaghan and Scarre, 2009). The changes demonstrated in the Neolithic period started to be considered as representing a 'Neolithic Package', that is to say an 'all or nothing' development with little overlap between the Mesolithic and Neolithic, thus in many ways, supporting to the view of a possibly rapid introduction from Europe (Richards and Hedges, 1999, Schulting, 2000). This all or nothing approach was elsewhere questioned (Thomas, 2003) although the appearance of a sudden 'cultural repertoire' was recognized. Emphasis here is placed on the increased range of contact, movement and the formation of new social relationships rather than full scale migration or invasion, a theory which allows for a greater degree of indigenous 'inventiveness' as not all aspects of the 'repertoire' have continental parallels (Thomas, 2003).

Pailler and Sheridan, (2009: 32 cited in Garrow and Sturt, 2011) suggest that early colonisation (between 4300-4000BC) occurred from Brittany, around the western seaways, to south-west England, Wales and Western Scotland. Garrow and Sturt argue that the evidence actually suggests '…not full-blown population movement from the Continent, but rather general patterns of connectivity within the fifth millennium – involving small scale, relatively routine movement in both directions…' (2011: 68). The relatively late evidence of the 'Neolithic' arriving on the Islands around Britain within the western seaways (3750-3300BC, but 4000-3800BC for Britain) may suggest that this was attributable to the more local connections between the Islands and Britain, rather than an arrival directly from the continent (Garrow and Sturt, 2011). It may also suggest that any colonisation from the continent was more likely to have been via the English Channel, the North Sea, or possibly that the evidence lends itself to the argument for indigenous adoption (Garrow and Sturt, 2011).

Figure 3-9 Map showing dates estimates for the start of Neolithic activity area by area across Britain and Ireland, at 95% probability (68% probability in brackets).

'Dragons lurk over areas not modelled in this study'. (Bayliss *et al*, 2011: Fig. 14.177).

Recent developments touched upon earlier in the chapter had added significant weight to the theory of migrant farmers. A massive surge in population apparent around the start of the Neolithic lends itself to this theory especially once the dietary (Richards *et al*., 2003) and material culture evidence (Sheridan, 2003, Sheridan, 2004, Sheridan, 2000, Sheridan, 2007b) are considered (Collard *et al*., 2010). This research also suggests that farming was introduced independently to England and Scotland, suggested by Sheridan (2007b, 2003, 2004, 2000) but also indicates that the migrations to south-west England took place before that of Scotland, based on demographic patterns (Collard *et al*., 2010). The recent work by Bayliss *et al.* (2011) using Bayesian statistical modelling of radiocarbon dates could be seen

to support these findings based on dates alone. An important point to note, however, is that although the former study identifies the south-west of England as the starting point of the population increase, the latter identifies the south-east as the earliest point of Neolithic activity, not moving to the south-west area until the later 39th or earlier 38th century cal BC, roughly the same time as seen in Southern Scotland (Figure 3-9) (Bayliss *et al*., 2011: 836).

The Neolithic period, whether considered a revolution or not, certainly brought about a number of changes, many of which could have had a significant impact on human health. Immigration and invasion models from continental Europe to Britain present an opportunity for communicable diseases such as TB to arrive in Britain, if it was not already present, and to consequent spread between and within communities. Even if large scale immigration is discounted, which in light of recent work seems unlikely (Collard *et al*., 2010, Bayliss *et al*., 2011), it is clear that there was a greater degree of contact between communities and populations throughout the Neolithic which only continued to increase with time. One of the more recent bioarchaeological developments, which demonstrates this clearly in the Neolithic, is the use of isotopes in order to also track the movement of people during their lifetime. Strontium isotopes vary amongst different types of rock and enter the body via diet, then being deposited in the skeleton, both in the tooth enamel and the bones (Price *et al*., 2004). Whilst the bones changes continually throughout a person's life, the tooth enamel which forms during early childhood does not, and thus differences in isotopic readings between an individual's tooth enamel and their bones, can indicate a change in residence (Price *et al*., 2004). An example of this can be found in a study which took strontium and lead isotope readings from four associated burials dating from the late fourth millennium BC (early Neolithic), that of an adult female and three juveniles (Montgomery *et al*., 2000). The burials were excavated in 1997, from Monkton-up-Wimbourne in Dorset and arranged in a shared burial pit. The results indicate that over the adult's lifetime she moved at least 80km between early childhood and death. The juvenile data also indicated that they had moved a significant distance during their shorter lives, but it is unclear as to whether these four individuals travelled together.

The evidence presented above clearly points to wide ranging contact, both within Britain and between Britain and the continent, and from possibly as far back as the Mesolithic

period. Although this contact extended far beyond the area selected for study here – the south of Britain - reaching as far as Scotland, its proximity to the continent and the geography of its coast lends itself to contact within both the western seaways and the English Channel.

The close juxtaposition of Northern Gaul and Southern Britain has ensured that the two lands have remained in contact, albeit sporadically, throughout much of the prehistoric period

(Cunliffe, 1984:3)

Evidence for trade and exchange also come from the study of artefacts and food. Evidence of previously unknown foods in Britain such as the grape (*Vitis vinifera L*.) found in Neolithic Dorset (Jones and Legge, 1987: 454) may imply networks of communication and trade with other areas of Europe; it is possible that such an unusual and rare item to be found in Britain was imported (Jones and Legge, 1987: 454). Darvill (1987: 71) divides artefacts into two groups, everyday and prestige items, the most common example belonging to the former group, flint. Flint, as a raw material was carried from its origins in the chalklands of southern and eastern England to the west and north (Darvill, 1987: 71). Known for its axe production sites, there are also numerous examples of axes being distributed over significant distances in Britain. This was by either direct acquisition, direct and indirect exchange, and possibly even trade (Whittle, 1999: 65), an example of which includes Cornish axes being located in Essex (Darvill, 1987: 71). Interestingly, jadeite axes, made in the foothills of the Alps have been found distributed through Britain from the south coast right up to areas in Northern Scotland, a distance of over 1200km away (Petrequin *et al*., 2002 cited in Bradley, 2007), further demonstrating contact with contemporary societies in Europe. Darvill (1987: 72-3) offers the suggestion that certain objects were considered of special significance because of limited access to flint mines, which supports the notion of a gift exchange system being implemented (Clark, 1966) as a form of trade whereby groups in effect become obligated to one another and those objects considered rare would be most sought after (Darvill, 1987: 73). This also implies contact outside of the family of a community group, and extra-community contact in addition to, or rather than, intra-group contact.

Monument building was a key feature of the Neolithic and there are great similarities between monuments of the period within Britain in terms of type, size and associated artefacts. For example, Bradley and Holgate (1984) discuss the similarity between double-ditched henges found in the north east of England and those found in the upper Thames Valley.

> *If communities were seeking to emulate each other achievements, that process was geographically extensive. It seems to have extended from Orkney to southern Britain and drew in people on either side of the Irish Sea.*
>
> (Bradley, 2007: 133)

There is little doubt that the monumental building which played such an important part in the lives of the Neolithic people can also shed light on the movement and potential contact between communities. It served to bring people together and there is evidence to suggest that monuments acted as meeting places, again perhaps for neighbouring communities. However, evidence of increased communication between communities is not isolated to large monumental buildings but also on a smaller, settlement level. For example, a change in settlement pattern such as that demonstrated at Skara Brae in the Orkney Islands suggests that "…these changes clearly point to the development of an hierarchical society, with a strong sense of group identity, but with a wide network of external contacts." (Reid, 1993: 34)

3.8.3 Bronze Age

The discovery of a Bronze Age boat in 1992, known as the Dover boat, also highlighted this close geographical position of what is England's busiest port to the shortest sea crossing to the continent (Clark, 2009: 4). "Transmanche" is a term often used to describe the area of contact between south-eastern England, northern France and the Low Countries, all of which did not have the raw materials needed for the manufacture of bronze and thus would

have required all the materials to be imported, presumably on boats, through river systems and across the sea (Clark, 2009: 6).

Needham (2009) draws attention to the potential of these many rivers that 'tap deeply into the landmass', which may indicate that, by extension, the British population was exposed to more than simply the inhabitants of France and the Low Countries, but was, in fact, connected through complex networks to the much wider continent. These rivers extend from the CSNS (The Channel/southern Northern Sea) Mariority (Figure 3-10) which Needham describes as a "geographic system" rather than a "region" or "area". He defines this as being a '…definable zone of privileged or relatively high-flux interaction used for the execution of certain specialist maritime exchanges' (2009:18). What is important to note is that he also draws attention to the fact that these exchanges need not be limited to objects, but can include people (2009: 18). Again this has special significance regarding the potential for infectious disease. Travelling as a marriage partner, for example, suggests mobility with the aim of perhaps settling permanently and integrating with a distant community; Cunliffe suggest it to be highly likely that small-scale movements took place for the purpose of intermarriages (2009: 84).

> *'…as the urban revolution gathered speed, mating patterns changed, and there were perhaps greater movements of people over shorter periods of time and larger groups socially interacting'.*
>
> (Brothwell, 1969: 86)

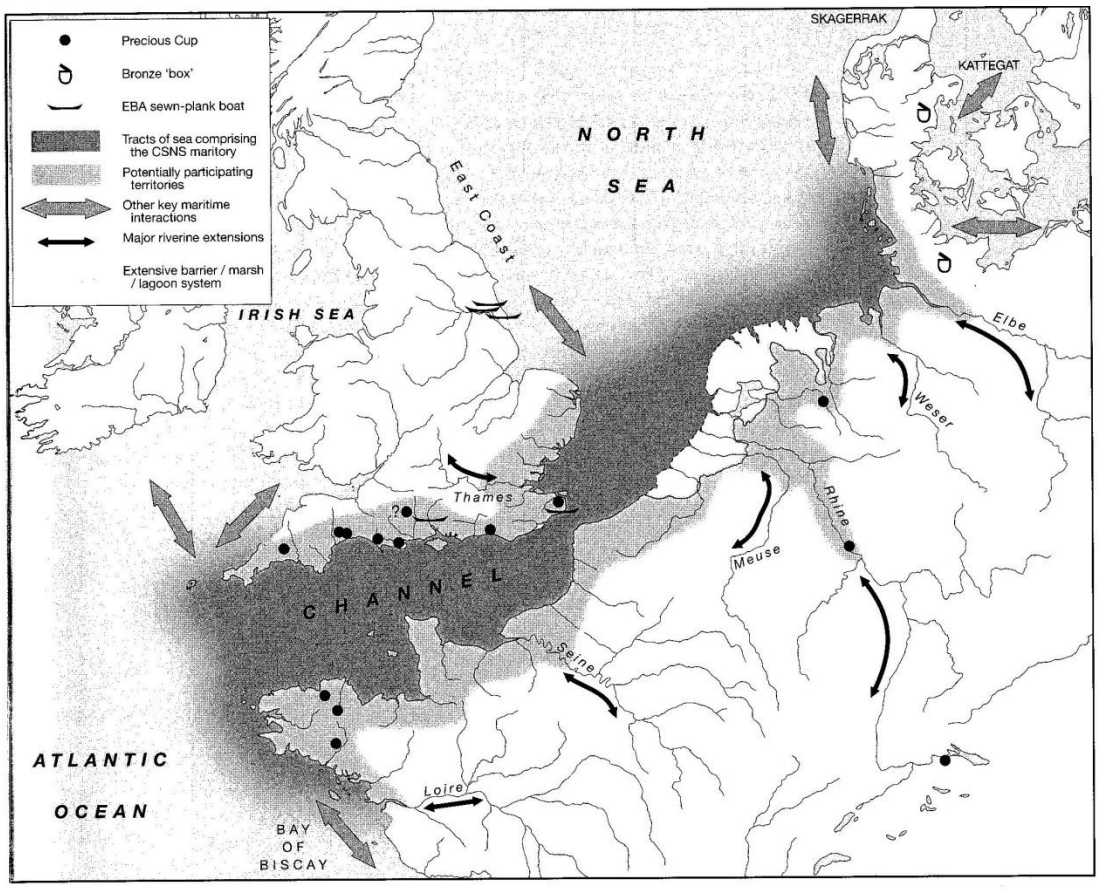

Figure 3-10 The EBA Channel / Southern North Sea mariority including the larger inflowing rivers (Needham, 2009: Figure 2.5)

Rather than possibly fleeting meetings of traders, this would allow carriers of a disease to introduce it to the family with whom they live and, subsequently, the community in which they settle. The transmission of disease, particularly TB, which McKeown describes as being characterised by chronicity and recurrent excretion and infection (1988: 38), need not be such a rapid process as seen by some diseases today, but could have essentially entered Britain 'under the radar', to have been reactivated by some stimulus later in life.

It would be impossible to discuss mobility in the Bronze Age without reference to what have commonly been referred to as 'The Beaker People' or 'The Beaker Folk'. A new style of pottery known as Beaker ware became evident during the Bronze Age and was in use between 2700 – 1700BC (Parker-Pearson, 1993: 85). Perhaps not unsurprisingly, as with the 'arrival' of agriculture, there were similar theories surrounding 'invasion' from foreign peoples with regard to 'Beaker Folk' who supposedly brought new ideas with them and who

were identifiable by their burial practises and the intricately decorated 'beakers' which with which they were associated (Crawford, 1912). It was, for a long time, thought that the Beaker folk were a tribe who had 'swept across Europe from the East', also bringing with them knowledge of metal work (Parker Pearson, 1993: 85). They were also considered identifiable by different cranial morphology (Crawford, 1912: 188) and, although this is a theory that has been discounted by many (Darvill, 1987: 89), it is quite possible that this theory does indeed support the argument for an influx of distinctive people (Brothwell, 2000: 3). Studies of British crania, using a multivariate analysis of measurements, demonstrate that the original differences noted between Neolithic and Bronze Age skulls are accurate (Brothwell and Krzanowski, 1974, Brodie, 1994) (Figure 3-11). Whilst some argue that the comparative material is too far removed in time (most Neolithic crania are derived from early or middle Neolithic contexts) and thus offer the suggestion that natural morphological changes had time to occur (Burgess and Shennan, 1976), the few later Neolithic crania used in Brodie's study followed the same morphological pattern as the earlier Neolithic crania – they were morphologically different to the Bronze Age (Brodie, 1994). Mays suggests that it could be argued that there were other factors at work; non-genetic factors such as climate or diet could be considered but he also points out that none of the changes seen in the crania are consistent with what is known about the Neolithic/Bronze Age transition and, at this stage, the arrival of migrants does seem to be the most logical explanation (2000: 282-3). Some argue that beaker pottery in Britain can be accounted for by a mixture of migration, trade and emulation by local communities (Parker Pearson, 1993: 85).

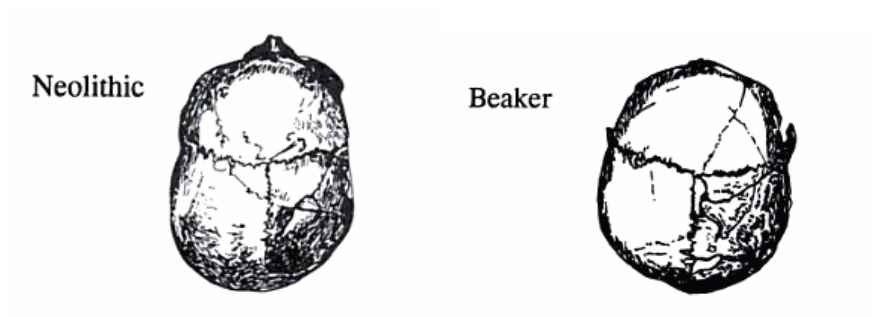

Figure 3-11 Superior view of typical long-headed Neolithic and round-headed Beaker skulls from Britain (adapted from Harrison, 1980).

There is a great deal of evidence to suggest that the Beaker People or 'Bell Beaker' people were highly mobile throughout the continent. Using strontium isotopes, one study demonstrated that, of 69 individuals analysed in southern Bavaria, 17.5-25% changed residence during their lifetime; the methods used are also likely to have underestimated this movement (Grupe *et al*., 1997). An earlier study on individuals from the Bavarian area also demonstrated that three out of eight individuals spent their childhood in a different place to their eventual burial (Price *et al*., 1994), and a wider study which analysed skeletons from southern Germany, Austria, the Czech Republic and Hungary revealed that, out of 81 Bell Beaker individuals, 51 had moved since their childhood (Price *et al*., 2004). A long distance journey directly related to southern Britain can be inferred from the Bronze Age remains of one of Britain's best known archaeological individuals – the Amesbury Archer (2470-2280 cal BC). The Amesbury Archer and his companion (a younger man thought to be related due to them sharing an unusual, genetic non-metric trait of the foot bones) were found buried close to Stonehenge, with multiple grave goods in the style of the Bell Beaker tradition common across much of Europe at this time (Fitzpatrick, 2009: 177) Oxygen isotope analysis of the archer's teeth suggested that he lived much of his childhood in central Europe or Scandinavia, refined to the Alpine region – although his companion could well have been raised in southern England (Fitzpatrick, 2009: 177). As Fitzpatrick warns, it may be mistaken to think that the Archer made the journey alone or that these two burials represent his entire familial group (Fitzpatrick, 2009: 177). It would also be a mistake to assume that the archer, and perhaps his family, were the only ones to make such a journey, and this is clear evidence of travel from an area far inland on the continent to Britain. This is of particular significance when the proximity to Italy, of the area from which the archer originated, is considered. Italy provides clear evidence of early TB, with the disease having been identified from the Neolithic period in the north-western part of the country (Formicola *et al*., 1987, Canci *et al*., 1996).

Not far from the burial site of the Amesbury Archer also lies the burial site of the well known "Boscombe Bowmen" at Boscombe Down, Wiltshire. Strontium and oxygen isotope analyses applied to seven individuals from a single grave (three adults, one sub-adult, two juveniles and one infant) show an important migratory pattern (Evans *et al*., 2006). All three adults originated from an area different to where they were buried, and all three moved to

another area during their adolescence and, at some point later in their lives; all three came to Wiltshire but each at different times. What is so important about this pattern is that it is:

> *the first case of a systematic, reproducible migration pathway from several individuals…which may indicate an established route and pattern of residence, with men spending their childhood near their birth place until they were about 6 years old, and then moving – for some social or age- or work-related reason – to another area, before finally travelling to Wiltshire in later life.*
>
> *(Evans et al., 2006: 316)*

If this suggestion is an accurate reflection of migratory patterns, the implication is that there may have been a regular influx of migrants to Britain, who travelled great distances before arriving in the south of Britain, all with the potential to bring infectious disease with them from the continent. Contrary to the results of the adults from the burial group, the two juveniles of the group shared the same isotopic signatures and were thus raised in the same environment, but it was not the same as that of the adults.

There is also considerable evidence for long distance movement of artefacts and metals for the Bronze Age and this is primarily due to the metals which give the period its name. At this time, there was an "explosion" in the quantity and quality of metallurgy, primarily involving copper and tin to make bronze (Harding, 2001: 309). The copper is known to have been exploited in the Alpine, Carpathian, Balkan and Irish regions, in addition to smaller deposits (Harding, 2001: 310), and thus both the raw materials and artefacts were traded to meet demand over large distances. Whether or not "Beaker" ware found during this period represents people invading, or mass migrations, it is clearly evidence of extensive networks of communication and, importantly, the phenomenon of beaker ware that is something that links many places across Europe; Bell beakers have been found as far east as Hungary and as far west as Portugal (Salanova, 2000). In addition, great numbers of items associated with beakers in graves are known to have "travelled" large distances themselves, such as amber beads originating from the Baltic (Parker Pearson, 1989: 85). In a similar vein, the Wessex culture, described above, has led some archaeologists to create cultural links with places

and people as far away as Greece and the Mycenaeans and, at one point, it was believed that the Mycenaeans even had a hand in the building of Stonehenge (Parker Pearson, 1989: 94-5). However, as Parker Pearson explains:

> *…we must not forget that people forged contacts over considerable distances. Europe was criss-crossed by long distance trade routes. Objects could travel a long way along these routes without there being direct connections between the ends of the routes…Europe was a complex web of networks and not a continent of isolated communities.*
>
> (1989: 95)

From a Bronze Age perspective, whilst contact with continental Europe has already been demonstrated via artefacts, monuments to have their place in this aspect of prehistoric development. Stonehenge, at the time of its transformation from a timber to a stone structure, coincides with Bell Beakers appearing in graves and the appearance of the first metalwork in southern Britain (Bradley, 2007: 142). Differences between stone monuments such as Stonehenge and other timber monuments are evident not just in their building material but also in their associated artefacts; whilst timber circles often contained animal bones and portable artefacts, Stonehenge had very few objects but did have associated human remains (Bradley, 2007: 141).

> *The growth of later Neolithic monuments was sometimes associated with the promotion of long distance relationships. Now that process extended beyond Britain and Ireland, and both of these Islands were drawn into an international network.*
>
> (Bradley, 2007: 142)

One project is currently at the forefront of this research, investigating the immigration versus indigenous Beaker question in Britain. *The Beaker isotope project: mobility, migration and diet in the British Early Bronze Age* (http://www.sheffield.ac.uk/archaeology/research/beaker-isotope/index.html*)* is an AHRC

(Arts and Humanities Research Council) funded, collaborative study between staff at The University of Sheffield (Andrew Chamberlain and Mike Parker Pearson) and the Max Planck Institute for Evolutionary Anthropology in Germany (Mike Richards) (with Patrick Mahoney – Sheffield, and Mandy Jay – Durham/Leipzig). In light of pilot studies demonstrating a high level of mobility amongst certain individuals associated with the use of Beakers, the project is fusing macroscopic methods of skeletal analysis with isotopic analysis to shed light on diet, mobility and health.

3.8.4 Iron Age

Links with Italy are seen in the Late Iron Age, when north Italian wine-drinking equipment was amongst many items of exchange entering Britain (Cunliffe, 2005, Cunliffe and De Jersey, 1997), although that is not to say that travellers from Italy were accompanying them. The Early Iron Age, however, shows clear evidence of Britain is continued contact with the continent, mostly based on archaeological evidence of objects being widely distributed across Britain, Ireland and the continent, such as specific sword types (Cunliffe, 2004: 447-452). Interestingly, by the Middle Iron Age, (500-150BC), Cunliffe points to a reduction in the number of recognisable items of elite exchange in Britain which could be considered evidence of reduced trading across the sea, supported by the development of "a distinct rationality among the communities of southern Britain" (2009: 85). Pottery styles found in Kent, however, bear an extremely close resemblance to that of the adjacent continent, demonstrating that certainly some degree of contact was maintained during this period, particularly in the channel area (Cunliffe, 2009: 85). The development of coins in the Late Iron Age also highlights continued contact, with a clear point of entry being that of the Thames estuary (Cunliffe, 2009: 87).

As with the Beaker People of the Bronze Age, the Iron Age also has its own mystery culture, the Arras culture. Evidence is found only in a small area of Eastern Yorkshire (Dent, 1982), outside the primary focus area of this study but, in fact occurring at the site of Wetwang Slack from which a few samples were analysed. Burials from this culture are usually crouched inhumations, placed beneath square barrows and dating to between the 4th and

1st century (Mays, 2000: 283). There is evidence to suggest that the Arras culture represent a tribe from Northern France (Stead and Society, 1979), although some argue for an indigenous development (Higham, 1987). As with the "Beaker People", the osteological remains certainly seem to lend themselves to the theory of migrants as the crania appear to be distinct from contemporary, non Arras individuals (Dawes and Magilton, 1980, Leese, 1991), although Mays warns that there are relatively few Iron Age skeletal samples with which to compare the Arras crania and, therefore, caution should be taken in the interpretation of the results at this stage (2000: 284).

Isotopic evidence too, can play its part in understanding Iron Age mobility. There is isotopic evidence exploring diet from the Iron Age site of Galstonbury Lake Village which may demonstrate two 'incomers' to the site, due to both their differing nitrogen values and also their burial within a mound, rather than in close proximity to them, as with other individuals studied (Jay, 2008: 213).

Manufacture and exchange in the Iron Age became more complex (Morris *et al*., 1994) and Haselgrove (1999: 125) identifies three levels of craft activity: output that meets community or household requirements, specialised items for wider distribution, and luxury goods. The items produced and exchanged include, but are not limited to, pottery, stonework, woodwork and salt (Morris, 2007). Technological advances also made glasswork possible. However, the distribution and exchange of these goods is hard to identify:

> *What the archaeological record has shown is that there was a variety of different levels of production, or degrees of complexity in organisation, and that no single type of distribution can possibly describe the networks of exchange which took place during the Iron Age*
>
> (Morris, 2007: 57).

Nevertheless, it is true to say that, as trade and contact developed, so too did the risk associated with infectious disease but it is not until the Iron Age that we finally see archaeological evidence of Tuberculosis (Mays and Taylor, 2003), that may well be directly related to the extensive trade and contact witnessed in this period.

3.9 Summary

This chapter has attempted to bring together some of the key aspects of prehistoric life in Britain, with particular focus on those which could, either directly, or indirectly, have impacted on the health of prehistoric populations and individuals; the health status of a population is closely associated with its living conditions (Cohen and Armelagos, 1984; Steckel and Rose, 2002). Particular emphasis has been placed on those aspects of daily life which may have (for example), placed people at increased risk of transmission from infectious disease, such as increasing contact with other communities and individuals within Britain, and from across the waters of both the western seaways and the Channel. The possible negative impact of natural environmental conditions such as seasonality can have on human health, in relation to infectious disease, has also been considered, but particular attention has been paid to the agricultural transition in Britain, and the domino effect of resulting health impacts on the potential for TB to have established itself on this island. Modern study and knowledge has been drawn upon, in order to better understand the resulting skeletal manifestations viewed archaeologically. The following chapter will now discuss the methods employed in this particular study, in its attempt to identify TB from the early inhabitants of Britain, and to understand the pathogens' evolutionary place in the history of TB. Through subsequent chapters, it is necessary to bear in mind the complex interactions of lifestyles, environment, health and economics, as presented in this chapter and the resulting difficulties of interpretation, particularly in prehistoric populations.

4 MATERIALS AND METHODS

This section will introduce the geographical scope of the skeletal samples included in the project (
Figure 4-2) and then identify the individuals selected for inclusion in the project. Basic site background and skeletal information is presented wherever possible. The basic reasons for inclusion of these individuals in the project is presented, but further descriptions pertaining to their primary pathology, is presented in Chapter 5, with further discussion in Chapter 6. A description and photograph of the specific sample removed for biomolecular analysis from each individual is included here (but photographs do not necessarily show the primary pathology of interest, as not all samples were taken directly from the lesion - all photographs of specific pathology are presented in Chapter 5), which is of relevance for the records of curating institutions and for any subsequent discussion of aDNA limitations. A full list of all the sites included in the project, along with the museum or institution at which they are curated, and references for the relevant sources of information regarding skeletal remains, can be found in Appendix 2.

4.1 Identification of Skeletal Material

4.1.1 Skeletal Criteria

The criteria skeletons needed to meet (as far as was possible) in order to best identify and assist museums to locate human remains of potential use consisted of the following:

- **Originating from sites in the south of England.** This is the geographical area decided for inclusion in the project (Figure 4-1), the reason for which is discussed in more detail below.

- **Individual skeletons, or those that could be identified as such, rather than mixed remains (meaning mixed skeletal elements originating from two or more different individuals and which could not positively be attributed to one specific individual).**

Using individual skeletons would allow a full analysis of all bones present for that skeleton, which may indicate particular patterns of pathology linked with possible TB identification. Inclusion of mixed remains was considered of limited value as mixed skeletal elements could not be seen in the context of the individual, including their age and sex, both of which can have a bearing on susceptibility to infectious disease, as discussed previously.

- **Of prehistoric date (Neolithic, Bronze Age, Iron Age).** With the earliest evidence of TB originating from the Iron Age, it was considered important to extend the chronological time period under investigation, going as far back as the Neolithic. Skeletal remains available for study originating from earlier than this are few, due to deposition methods, and there are continental parallels for TB from the Neolithic, making this period a clear candidate for exploring the possibility of early cases of TB in Britain.

- **Of reasonable preservation both in quantity and quality (although this was in most cases based on individual judgment).** The more skeletal elements available for study relating to a single individual, the more comprehensive the skeletal analysis could be, potentially highlighting pathology indicative of infectious disease. It was important that, wherever possible, the 'quality' of the bones was such that accurate identification of pathology (if present) could occur. Often, when a bone is heavily degraded, it is harder to distinguish areas of subtle pathology, such as rib lesions, from the appearance of damage or natural erosion. It was also considered important that the bone was of sufficient quality, so that the extraction of aDNA was given the best possible chance of success, given the age of the bones.

- **Inhumations as opposed to cremations.** This criterion was primarily set in order to increase the chance of success for aDNA extraction as it is known that DNA denatures at high temperatures, such as those required for the cremation of bone (Ottoni *et al*., 2009). It was also taken in to consideration that cremated bone can be exceptionally difficult to extract information from, such as age, sex and pathology

and is very much dependent upon the level of fragmentation and skeletal recovery (McKinley, 2000).

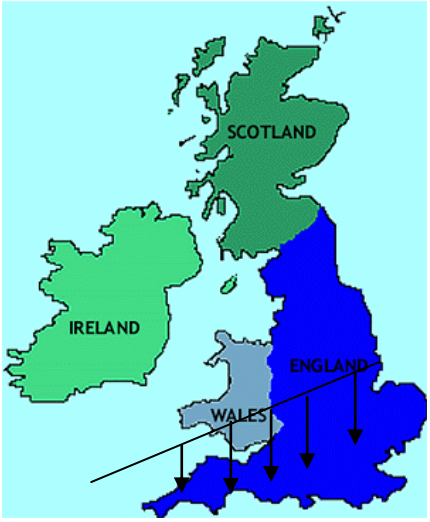

Figure 4-1 Map to show the southern areas from which human remains were sought (Map adapted from http://www.antor.org/great-britain.html).

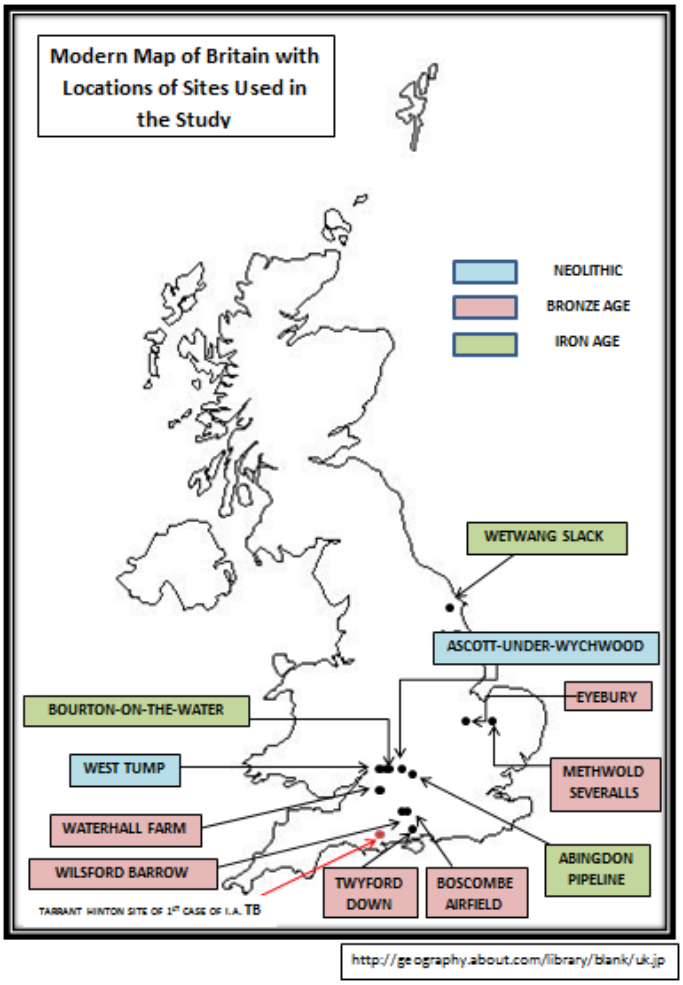

Figure 4-2 Map showing the geographical location of sites included in the project.

4.1.2 Locating Collections

To some extent the natural geography of the country dictated what was, for the purpose of the project, considered 'south'; running from the most northerly tip of the Norfolk region, across to the most southern portion of the England Wales border (Figure 4-1). Primarily this was influenced by research into trade and exchange routes and close contact between southern Britain and the continent during prehistory (Peacock *et al*., 2010, Needham, 2009, Cunliffe, 2009, Cunliffe, 1984). This contact, particularly with the south of Britain, could have resulted in a significant portal for tuberculosis to enter Britain, if not already present, as there is clear evidence for the disease on the continent from the Neolithic onwards (Canci *et al*., 1996, Gladykowska-Rzeczycka, 1999, Sager *et al*., 1972). It is also interesting to note that examples of the disease in Britain from the Roman period are all focused in the south and east (see Roberts and Buikstra, 2003: Table 3.8) which will be discussed in more detail later. It was also necessary to limit the quantity of material to be examined due to time restraints and, based on the research above, southern England was the most logical focal choice.

It is known, however, that the prehistoric periods do not offer a great deal of skeletal material for study. One of the reasons for this is the type of disposal of the dead seen from the Neolithic onwards. The Neolithic itself (where it must be remembered that the population was perhaps only as many as 200,000 - Darvill, 1987) demonstrates a relatively high proportion of visible graves due to the use of recognisable funerary monuments, such as the Ascott-Under-Wychwood long barrow (Benson and Whittle, 2007) included in this study. Remains from this period are however, from chambered tombs or ditches or pits of monuments and are often disarticulated (Darvill, 1987: 68), which limits the quantity of useful material although an increase in individual burials is seen from the Middle Neolithic (Whittle, 1999: 67). The Bronze Age saw the introduction of cremation burials (although individual inhumation was still practised), meaning that fewer skeletons are of use for this study due to the complex nature of disease recognition in cremated remains and the resulting denaturation of useful material for aDNA analysis. Bronze Age burial practises show an overlap of inhumation and cremation, the latter becoming the predominant burial rite by the Middle Bronze Age (Parker Pearson, 1993: 50). This period also sees more unusual forms of deposition, including individuals being 'dumped' in rivers (Parker Pearson,

1999: 87), placed in bogs and pools with associated artefacts (Healy and Housley, 1992) or placed in caves (Barnatt and Edmonds, 2002). These more unusual practices undoubtedly mean that there are a great number of individuals who are currently unaccounted for. Cremation practises continued through to the Iron Age but were later replaced by inhumations once more, and the use of formal cemeteries begins to occur, although cremation was to become a popular burial rite once again, by the time of the Roman invasion. It is clear that during the Iron Age period, there was a great range of burial rites being practised but, surprisingly, there are still limited numbers of burials available for study (Roberts and Cox, 2003: 90). Generally, due to the various methods of disposal seen over these periods and the vast period of time since deposition, skeletal material is often poorly preserved, a problem which can also be compounded by poor storage methods, post excavation.

Through previous contacts and extensive searching for museums/other curating institutions holding human remains from these periods, it was possible to locate material of potential use for the project. Using elements of the WGHR *Scoping Survey of Historic Human Remains In English Museums* (2003) it was possible to identify museums which held human remains; although this was a useful starting point, it was acknowledged that this may now have some inaccuracies due to the year of publication. Contact was also made with other projects and people concerning skeletal collections for the prehistoric period in Britain such as the Beaker Project (http://www.shef.ac.uk/archaeology/research/beaker-isotope) in order to better locate collections of use.

Museums selected for contact would in the first instance be sent an email with details of the project and criteria for selection of samples; this was followed up by telephone if no response was received. To those museums who responded positively to the request, a subsequent email was sent including a copy of the research outline which included a brief background to the history of tuberculosis in Britain, rationale for the project, the sampling procedure and bibliography. Details of the project's Principal Investigator and funding body were also clearly stated, as this study was funded by a studentship tied to the main project (http://www.dur.ac.uk/archaeology/research/projects/?mode=project&id=353).

4.1.3 University Regulations

Prior to contact with museums, a full risk assessment was provided for the Durham University's Archaeology Department and the project and sampling methods were both approved by the Departmental Ethics Committee.

4.1.4 Contact with Museums / Institutions

When requested, a letter of reference was provided by the Principal Investigator of the larger research project, Professor Charlotte Roberts. Upon arrival at a museum or institution the point of contact was met and the purpose and methods of the study were further discussed if requested.

When databases of human remains held by the museum or institution were provided by email or by post, these were used to create a shortlist of human remains which fit the original criteria. Where information or notes regarding pathological lesions displayed on an individual skeleton were included, this allowed for a greater detection of potential skeletons for the project. Although these notes did not specifically identify TB there were certain indications of infectious disease possibly attributable to TB such as, for example, periosteal new bone formation or destructive lesions on vertebral bodies, as discussed previously. Where information on disease was not available, skeletons identified were based purely on the original criteria which allowed skeletons to be prioritised according to potential in preparation for the research visits, especially when time was limited.

Where suitable collections were identified, visits, in some cases exploratory visits, were arranged and applicable forms were completed when requested by individual museums. Exploratory trips generally consisted of one to two day visits, usually only when databases of collections could not be provided via email.

4.2 Sites Included in the Project

4.2.1 Abingdon Pipeline, Oxford

4.2.1.1 Site Information and Skeletal Recovery:

Name: Abingdon Pipeline
Code: APO 03
Period (From which Sk. Originates): Iron Age

During archaeological evaluation work, carried out by Cotswold Archaeology in 2003 along the route of the Pipeline, an inhumation was identified and excavated (Cotswold Archaeology, 2003: 18). The site itself yielded four burials in total, one adult inhumation (Group 30), one neonate (Group 44) and two cremation burials (Groups 23 and 21). Whilst it has been suggested that the neonate bones date from the Roman period and the cremations, one from the Bronze Age and one the Late Iron Age / Early Roman, the adult inhumation discussed here can be dated to the Iron Age (Figure 4-3).

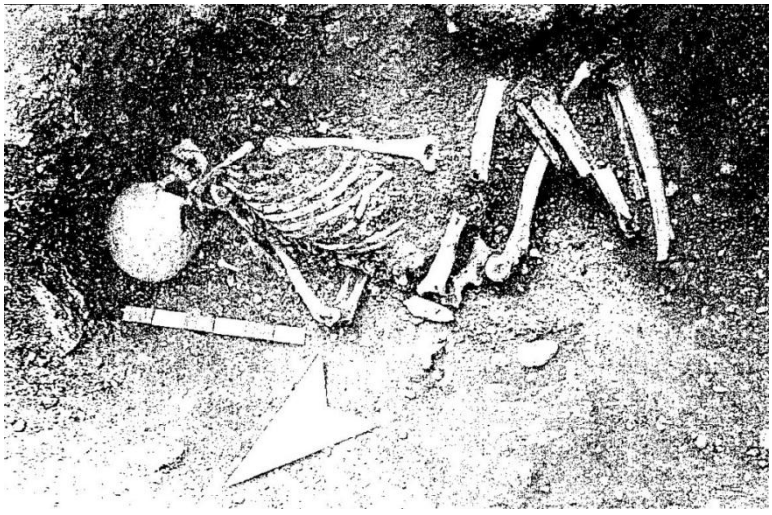

Figure 4-3 Photograph of inhumation burial 4197 from group 30 (Cotswold Archaeology, with permission).

The site revealed evidence of use throughout numerous periods; the earliest artefacts included Mesolithic blades and Neolithic arrowheads. Further finds demonstrated use up to the Roman period with pottery, a stone drain, double ditched enclosure and the bones of a neonate, as mentioned above (Cotswold Archaeology, 2003: 20). In total, 11 middle Iron Age

settlements were identified in the archaeological evaluation area, many of which were still in use through the Romano-British Period (Cotswold Archaeology, 2003: 12). Abingdon and its locale are host to a number of Iron Age farming settlements, particularly to the South West in the Vale of the White Horse (Hearne, 2001), indicating significant activity for this period.

The Pit (Group 30) in which the adult inhumation was located was truncated and a dark sandy silt covered the remains (Figure 4-4). Found in a tightly flexed position and almost complete, it is suggested that the skeleton was covered quickly. Worked flint, hazelnut shells and pottery found in Pit 31 directly beneath the inhumation has hinted at the possibility of being considered 'a type of ritual deposit' associated with the burial (Cotswold Archaeology, 2003: 18).

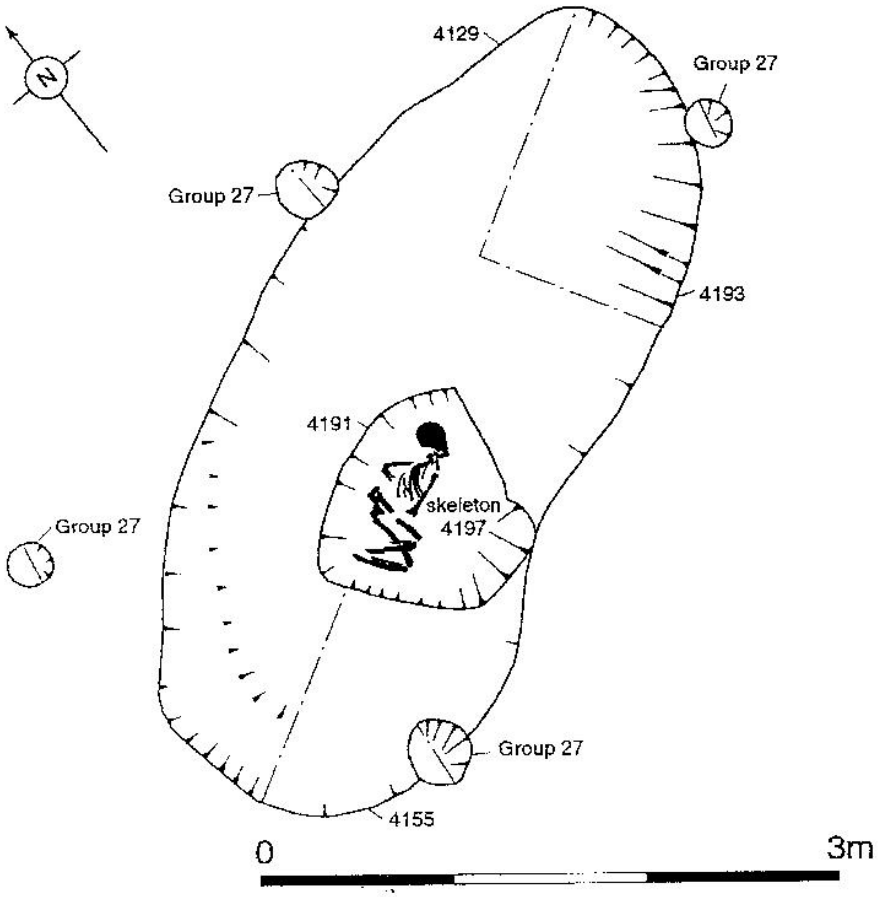

Figure 4-4 Plan of inhumation burial 4197 from group 30, (Cotswold Archaeology, with permission).

4.2.2 Ascott-under-Wychwood Long Barrow, Oxford

4.2.2.1 Site Information and Skeletal Recovery:

Name:	Ascott-Under Wychwood
Code:	N/A
Period (From which Sk. Originates):	Neolithic

This Neolithic Long Barrow lies to the North West of Oxford in the Oxfordshire Cotswolds (Figure 4-5) and is one of numerous prehistoric sites of interest in the region. Excavation of the site began in 1965 under the direction of Don Benson as a result of plans to straighten part of a local road (Benson and Roe, 2007: 1), which in fact never took place, rendering the barrow and immediate area still available for limited study (Benson and Roe, 2007: 21). The long barrow shows evidence of Mesolithic occupation due to the presence of Mesolithic worked flint (McFadyen *et al*., 2007: 25) but more than one interpretation regarding the sequential development of the site has been offered in light of recent, additional radiocarbon dating evidence (Bayliss *et al*., 2007b). The preferred model of the authors, demonstrates a gap between the early occupation (including timber structures and a midden) 'long enough to allow a turfline to form' and the subsequent initiation of the cists and deposition of human remains starting in 3755-3690 cal. BC (Bayliss *et al*., 2007a: 29).

The cists and human remains were located and excavated in 1968, and upon initial excavation there appearing to be six or seven undisturbed burial groups, "the potential importance…and the opportunity for significant advance in knowledge and understanding of Neolithic burial practice" being "readily apparent" (Benson and Roe, 2007: 17) although their interpretation was contested after an early publication (Chesterman, 1977, Benson and Clegg, 1978). There were in fact four stone cists and six deposits of human remains which were located either within or between these stone cists (Whittle *et al*., 2007b: 137, Chesterman, 1977: 22) (Fig.3.5). Initial reports suggested a total MNI of 49 (Chesterman, 1977: 24) but more recent analysis puts this figure at a more conservative MNI of 21 (Galer, 2007: 189).

The 1965-1969 excavations produced a great deal of data and there have been significant post-excavation analyses performed (Benson and Roe, 2007: 21), and certainly with regard to the human remains more recent re-examination has been done (Galer, 2007: 189), enabling an extremely thorough, over-all publication to be produced (Benson and Whittle, 2007).

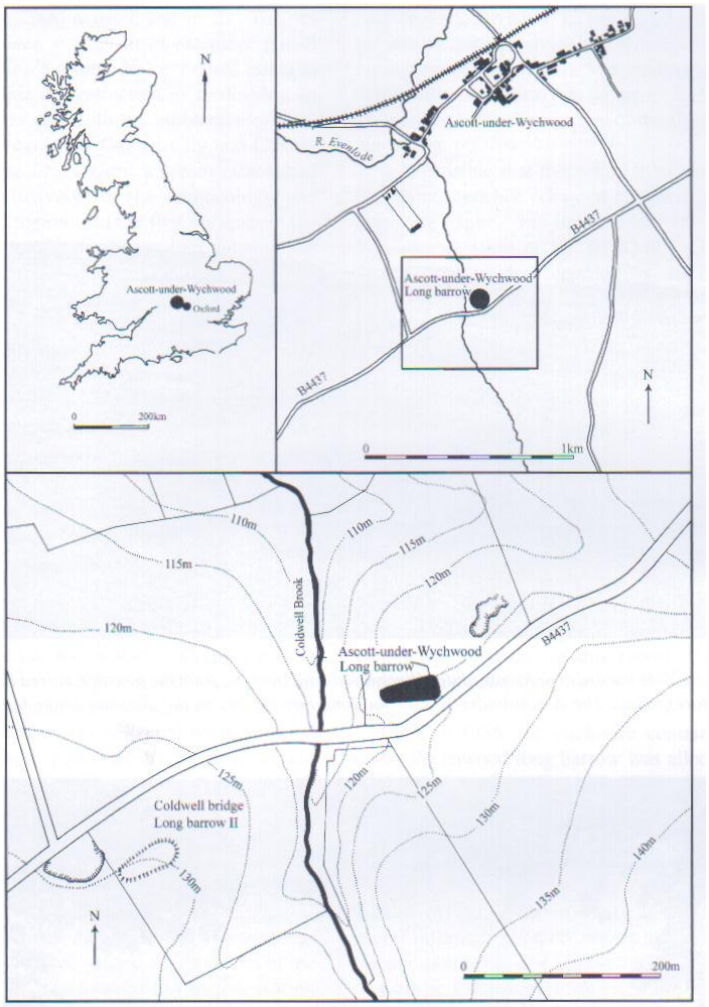

Figure 4-5 Map showing the location of the Ascott-under-Wychwood long barrow, locally and nationally (Benson and Roe, 2007) Fig.1.2).

Individual A3, the remains of who were selected for study here, was located in Deposit A in the southern inner cist (Figure 4-6), and also within this cist were individuals A1 (juvenile), A2 (adult) and a considerable number of skeletal elements which could not be assigned to

any particular individual (Galer, 2007). The pathology noted during this study is the same as that which was highlighted in the most recent publication regarding the human bone from Ascott-under-Wychwood (Galer, 2007: 196).

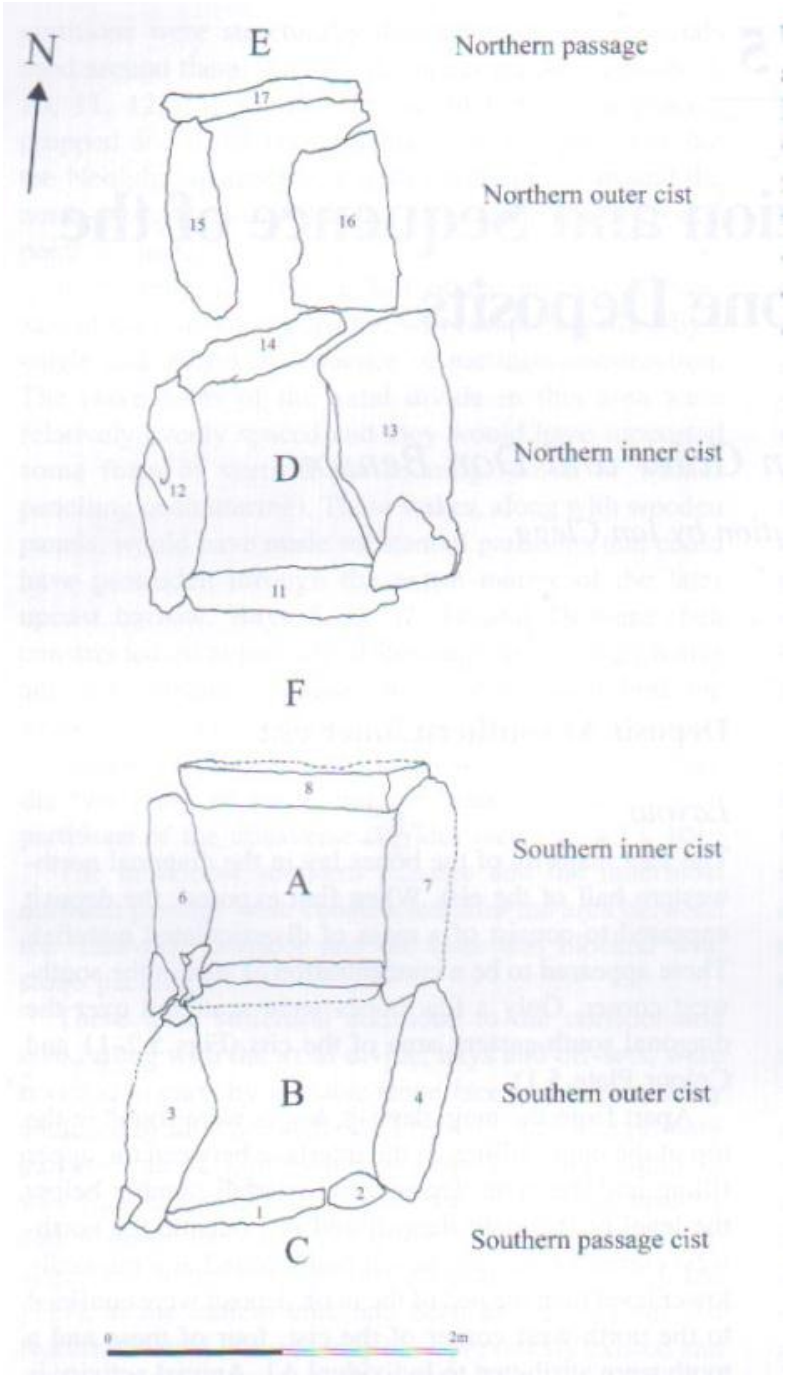

Figure 4-6 Plan of the cists including in deposit A in which the human remains of individual A3 were located (Whittle *et al.*, 2007b): 138, Fig 5.1)

4.2.3 Boscombe Airfield Fire Hydrant, Wiltshire

4.2.3.1 Site Information and Skeletal Recovery:

Name: Boscombe Airfield Fire Hydrant
Code: 62260
Period (from which Sk. originates): Early Bronze Age (cal. 1747 BC – 1607 BC)

Boscombe Airfield and the area of Boscombe Down are 'situated within a rich archaeological landscape' and lie to the east of Stonehenge and Avebury World Heritage Site (Wessex Archaeology, 2005). A number of important prehistoric finds have been identified in the area in recent years, including a collection of inhumations dating from the Early Bronze Age which have come to be known as the 'Boscombe Bowmen' (WA, 2005b cited in WA, 2005a), in addition to the famous 'Amesbury Archer'. The picture that has emerged though extensive excavation of the area (mainly due to modern development) has been one of a significant mortuary landscape with over 36 inhumations and cremations dating from the prehistoric periods being recorded (Wessex Archaeology, 2005a). More than 60 entries have been recorded in the Wilshire Sites and Monuments Record originating just from the airfield (Manning *et al*., 2010).

This particular burial found in 2006, during a watching brief for a new fire hydrant water main on the airfield has been considered a significant discovery because of both its local position in relation to other known features and its potential association with other burials in the nearby area (Manning *et al*., 2010). The burial was located in a shallow, oval shaped grave in the southern section of the water main and was significantly disturbed, with only approximately 45% of the skeleton able to be recovered from four contexts (mainly from in situ positions, some from spoil) (Manning *et al*., 2010).

Radiocarbon dating places the burial towards the end of the Early Bronze Age which suggests that this may be one of a significant group of post-Beaker burials and cremations to be located in the Boscombe Down area (Manning *et al*., 2010).

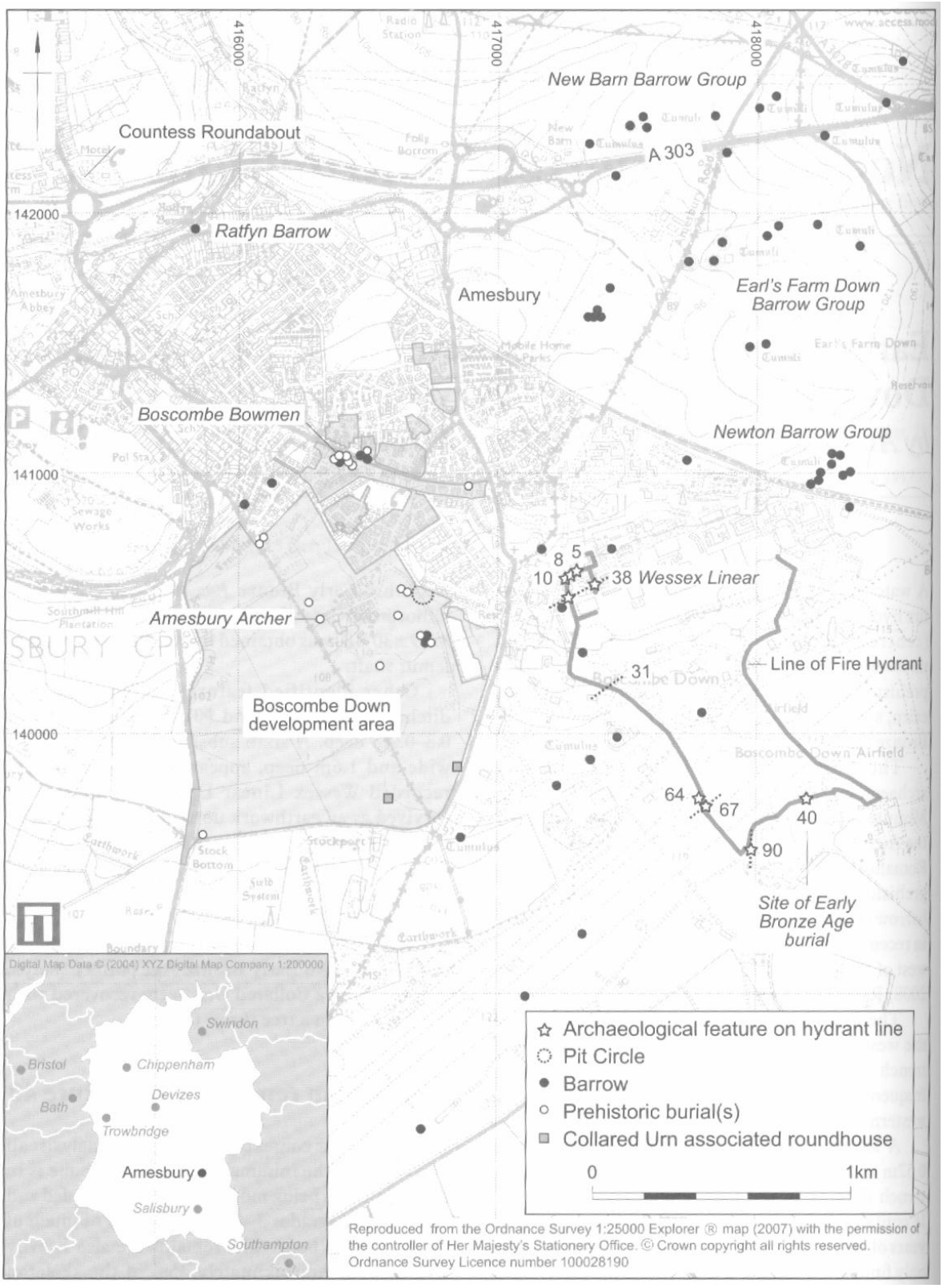

Figure 4-7 Map showing area of Boscombe Down Fire Hydrant (Barclay *et al*, 2010)

4.2.4 Bourton-on-the-Water Primary School, Gloucestershire

This skeleton forms part of the group of skeletons selected for sampling by a member of the research team for the wider NERC funded project, incorporating European skeletons across all time periods. For this reason, there is not the same level of detail available for the individual skeleton regarding the pathology noted which led to the decision to take an aDNA sample, other than that noted in a separate report which will be discussed further in the results section (Roberts, 2000b). Pictures taken by the researcher however, are included for clarity.

4.2.4.1 Site Information and Skeletal Recovery:

Name:	Bourton-on-the-Water Primary School
Code:	BPS00
Period (from which Sk. originates):	Middle Iron Age

Excavations at Bourton-on-the-Water Primary School have taken place since 1995, some by Cotswold Archaeology (CA; formerly Cotswold Archaeology Trust), and the others by Gloucestershire County Council Archaeological Service (GCCAS) in advance of successive building developments at the school (Piper and Catchpole, 1996, Wills, 2001, Wills, 2002, Wills, 2003, Wills, 2004) (Figure 4-8). The Primary School site lies next to The Cotswold School, where a number of excavations have also taken place by both CA and GCCAS, again in advance of building developments. Locally, the schools are close to two significant archaeological sites, Salmonsbury Camp and Bourton Bridge. Excavations of the former site demonstrate multiple periods of activity from the Neolithic to Saxon periods (Marshall, 1995, Marshall, 1996, Dunning, 1976). Excavations of the latter, at Bourton Bridge have primarily focussed on the Fosse Way where a possible Roman posting house forming part of a roadside settlement was discovered (O'Neil, 1968).

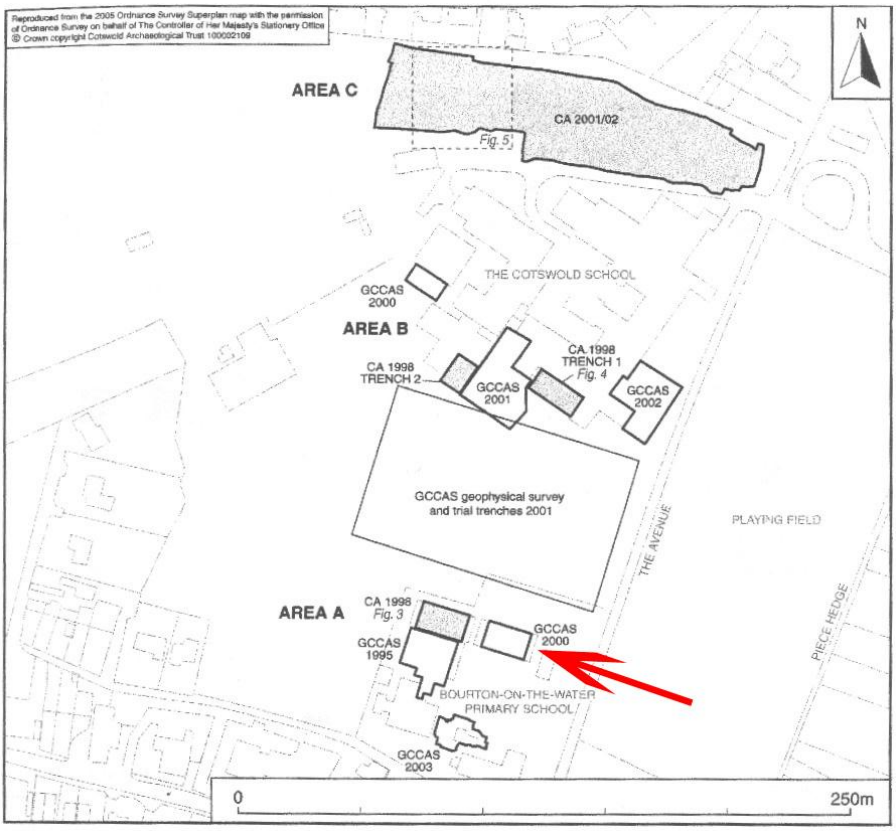

Figure 4-8 Plan to show the location of both CA and GCCAS excavation trenches at Bourton-on-the-Water Primary School and The Cotswold School (Lang, 2008: Fig 2)

The 1995 excavation by GCCAS identified evidence of early Iron Age occupation at the site, demonstrating several periods of re-building and nine pits dated specifically to the early Iron Age (Piper and Catchpole, 1996: 24-25). Excavations in 1998 and 2001/2 by CA incorporated three distinct areas; A, B and C (Wills and Rawes, 1999, Barber and Leah, 1998) of which A is the most significant for this study as it is located specifically at the site of the Primary School, as opposed to The Cotswold School just to the North. The 1998 CA excavations were flanked by the earlier GCCAS 1995 excavation (Piper and Catchpole, 1996) and later excavations in 2000 to the east (Wills, 2001) and 2003 to the south (Wills, 2004). The skeleton in question was located during the 2000 GCCAS excavation.

The 2000 GCCAS excavation consisted of a watching brief and excavation in advance of new classrooms and a toilet block being built (Wills, 2001). Most features located during the course of the excavation dated to the middle Iron Age, although the Middle Bronze Age was

signified by a at least one pothole and sherds of Neolithic pottery were also evident (Wills, 2001).

The skeleton in question was located in a pit with pottery placing the date as Middle Iron Age (Wills, 2001) (Figure 4-9). Original excavation records demonstrate that the skeleton was in a tightly flexed position, on their right side in the west side of Pit 76. The surrounding deposit was primarily composed of “silty clay” of moderate compaction (GCCAS, 2000). The skeleton was subsequently analysed and identified as a 12 to 14 year old female, the bones being in good condition (Roberts, 2000b).

Figure 4-9 Original site photograph of Skeleton 1 from Pit 76 (Bourton-on-the-water Primary School) (GCCAS, 2000)

4.2.5 Eyebury, Peterborough

4.2.5.1 Site Information and Skeletal Recovery:

Name: Eyebury, Peterborough
Code: Unknown
Period (from which Sk. originates): Early Bronze Age

The excavation of this tumulus, designated Tumulus A by its excavator, Mr Leeds, took place during the first decade of the 20th Century and whilst a first, brief account was presented to the Society of Antiquaries (Leeds, 1912), a fuller account was later presented in 1915 (Leeds, 1915) after further excavation in 1911. The round barrow, known locally as Oliver Cromwell's Hill, lies just south of the village of Eye and is one tumulus of four to which Leeds refers, three of which lie very closely together on a North South Axis, Tumulus A being the most northerly and the largest; the fourth is known to have been destroyed through gravel digging in previous years and lying around one and a half miles south west of the Eyebury tumuli (Leeds, 1912: 82).

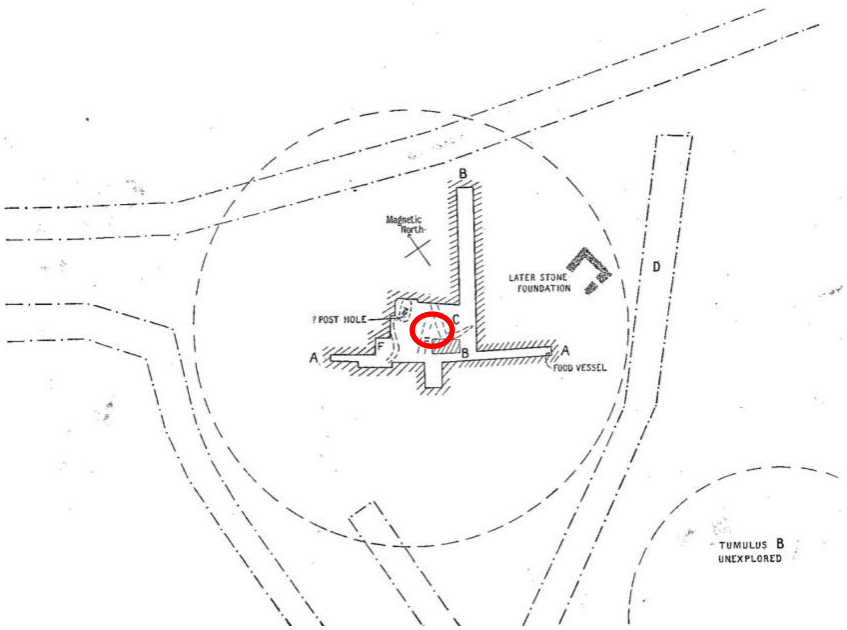

Figure 4-10 Plan of Tumulus A (also known as Oliver Cromwell's Hill) from Eyebury showing the areas excavated, the position of previous boundary ditches and the position of individual 223 (circled). (Leeds, 1912: 84, Fig. 3)

Tumulus A (Figure 4-10) was originally excavated in order to ascertain whether or not it actually was a barrow and, shortly after a trench was placed through the centre of the mound, areas of charcoal were noted and a small pot was unearthed (Leeds, 1912: 85-86). In addition to a number of animal bones, flint and pottery sherds, eventually the excavators located a burial, the grave being around 6ft long and around 2ft wide (Leeds, 1912: 90). The burial was reported to be lying on a layer of white sand, in a contracted position (on the right side), on a south west axis and with their right hand placed up towards the face (Leeds, 1912: 90) (Figure 4-11).

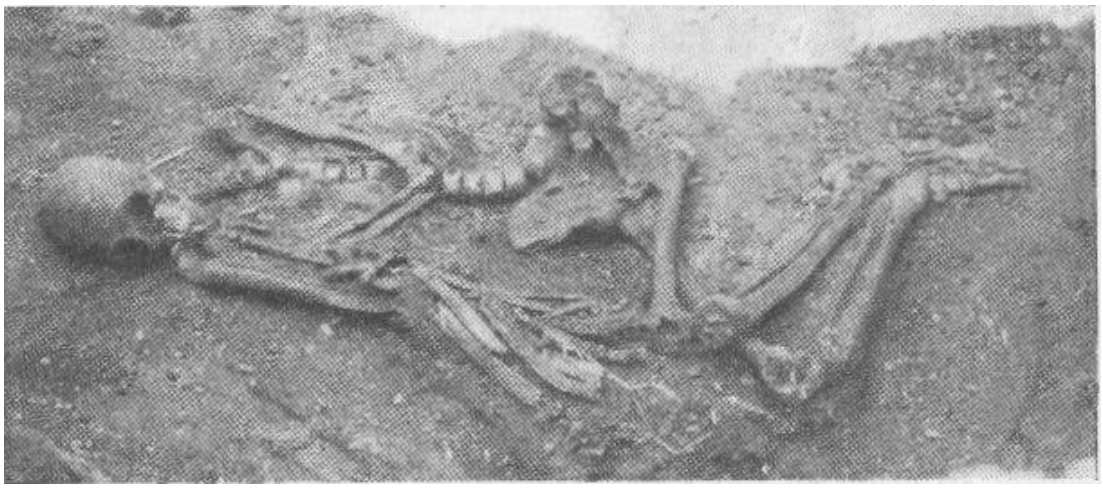

Figure 4-11 Photograph of individual 223 from Eyebury, in situ. (Leeds, 1912: Fig.9)

4.2.6 Methwold Severalls, Norfolk

4.2.6.1 Site Information and Skeletal Recovery:

Name:	Methwold Severalls, Norfolk
Code:	2542/c1 / NWHCM 1969.93
Period (from which Sk. originates):	Early Bronze Age (OxA-2862: 3840 +- 80BP)

The site of Methwold is one of many occupation sites in the area dating to the Bronze Age and is part of "a near continuous belt…some 50km long" (Healy and Housley, 1992: 949). Methwold, along with many of the other sites, would have benefited from the resources of both fen and upland, but wetter conditions seemed to have reduced the density of this occupation around c1300BC (Healy and Housley, 1992: 949). A number of skeletons were subjected to stable isotope analysis which demonstrated a strong reliance on terrestrial food sources (Lennon, 1996: 171). Cultivated cereals with a small amount of meat have been suggested as the content of the diet; the pattern of dental pathology supports the assumption of stone-ground cereal, with much tooth wear present (Lennon, 1996: 171).

Radiocarbon dating was used to establish more precise ages for the skeletons, the results of which placed the individuals, including Burial 1, firmly in the BA period (Healy and Housley, 1996: 39). Burial one is one of several partly articulated skeletons found in peat during 1968 (Figure 4-12). Discovered by Frank Curtis, the bodies showed evidence of disturbance both soon after original deposition and later by ploughing (Curtis, 1968). A full account of the details regarding the distribution and recovery of the bones can be found in the original report written by Curtis in which he states:

> *The skulls, in all cases, had either been sliced off by the plough, or crushed in by the weight of tractors…One body was in a contracted position although parts were missing (which may be among the disturbed bones in the topsoil).*
>
> *(1968: np)*

Although there appears to be some confusion over the 'ownership' of some skeletal elements, it is believed that the human remains examined here relate only to burial one, or record number 40/3. A number of additional skeletal elements, primarily loose teeth were stored with these remains but, due to the method of storage, it is not clear if they belong with this skeleton and for that reason they have not been recorded as such here.

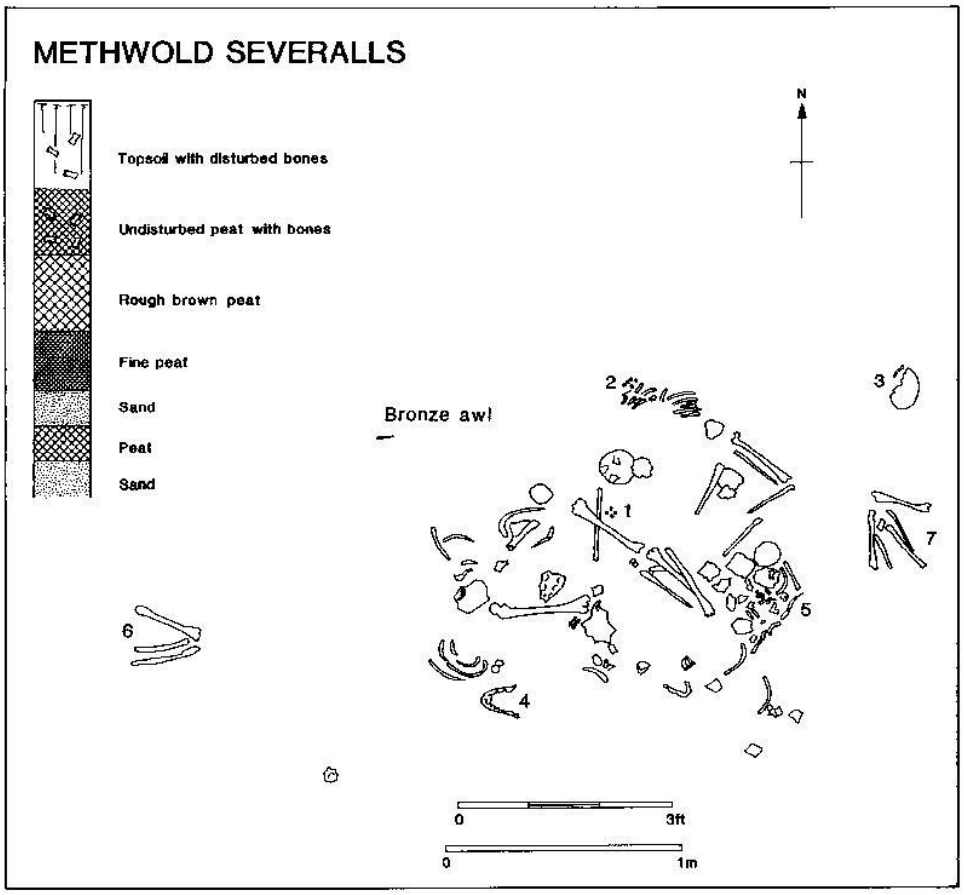

Figure 4-12 Plan of the 1968 excavation including Burial 1, based on the original drawing by Curtis (Healy, 1996: 38 Fig 21)

A published skeletal report states that the individual is represented by almost the entire skeleton (McKinley, 1988: 164) and, whilst the skeleton examined for this project is represented by much of the body, there are some notable absences which may indicate further confusion of skeletal elements with other burials excavated at this time. This is compounded by the fact that some previously reported pathology has not been noted here, most notably with regard to the upper incisors, suggesting further confusion during subsequent storage.

4.2.7 Twyford Down, Hampshire

4.2.7.1 Site Information and Skeletal Recovery:

Name:	Twyford Down
Code:	W437
Period (from which Sk. originates):	Bronze Age

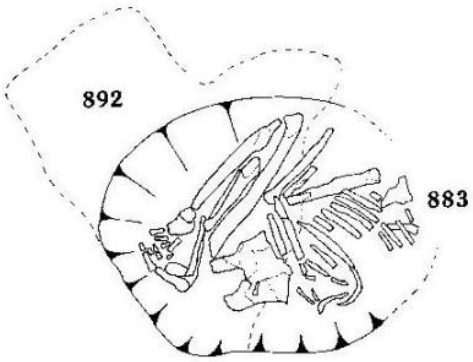

Figure 4-13 Plan of Individual 883 from Twyford Down (Adapted from McKinley, 2000:91 Fig.36)

Between 1990 and 1993, prior to a proposed extension of the M3 motorway, a series of archaeological excavations by Wessex Archaeology identified two areas of significant archaeological value which were chosen to be excavated (Areas A and B) (Walker and Farwell, 2000: 1) (Figure 4-14). However, many local sites of archaeological interest which were close to the proposed extension had been identified previously such as the barrow on Twyford Down (included in Area A), from which the human remains discussed here originate. Evidence showing a long history of human "exploitation and / or occupation" of the area surrounding Winchester included Lower Palaeolithic hand-axes right through to a medieval moated site, burial mounds in "Plague Pit Valley" and one of the earliest English Canals (Walker and Farwell, 2000: 3).

The Burial Mound in Area A, dating to the Early Middle Bronze Age (c.1700-1400BC) had previously been identified through aerial photography and was fully excavated during the course of this project. Not only were both inhumations and cremations found within the

barrow but also charcoal, burnt flint and faunal remains (Walker and Farwell, 2000: 7). Environmental samples also produced land snails and plant remains (Allen, 2000: 121), the most common of which included onion couch grass tubers and rootlets although, perhaps surprisingly, only a very small quantity of cereal plant remains were recovered; implied is that the surrounding area was mainly grassland (Allen, 2000: 128). Animal remains, some of which were directly associated with human remains from the barrow included sheep, goat, cattle, horse, pig, roe-deer, fox badger and wild cat as well as some bird, fish and microfauna identified through sieving (Powell *et al*., 2000: 132). A damp and "probably scrubby grassland or glade" is implied by the microfauna (Powell *et al*., 2000: 138).

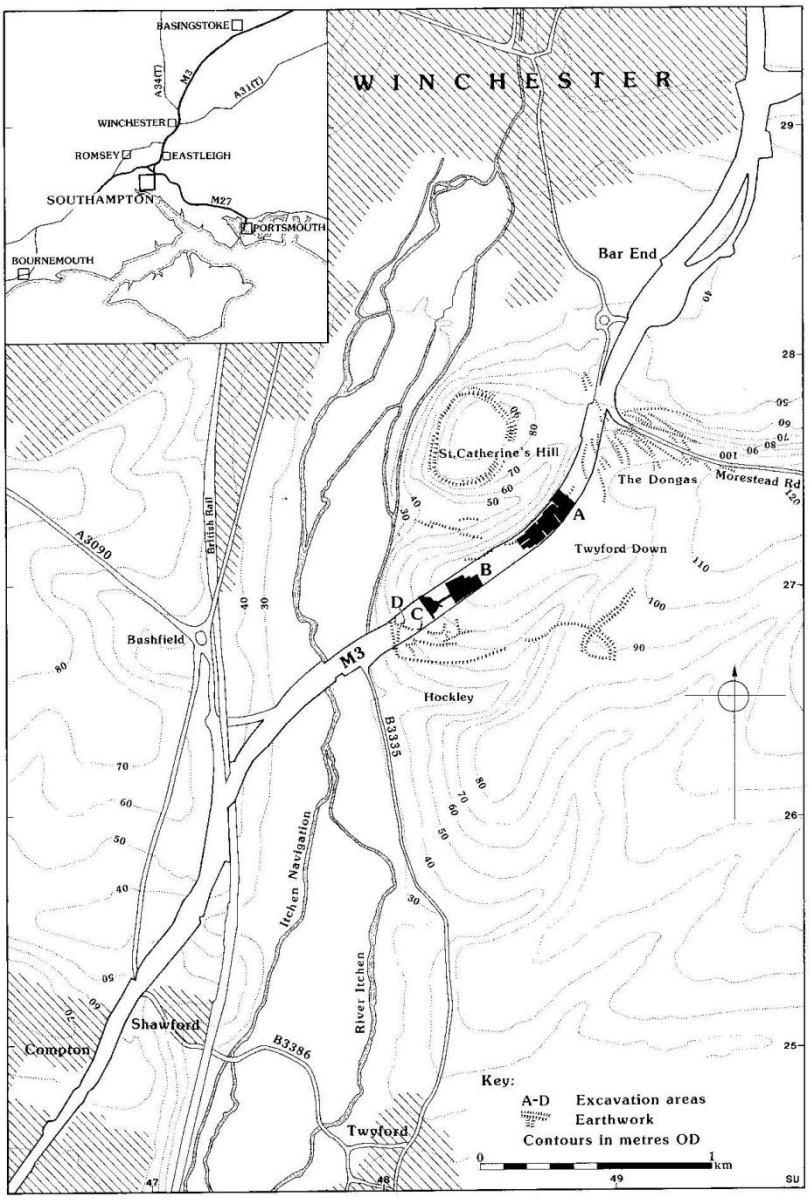

Figure 4-14 Plan of Twyford Down (M3 and excavation areas A-D) and surrounding area. (Walker and Farwell, 2000: 2 Fig.1)

At least 38 individuals were identified in the barrow, 19 of which were inhumation burials (Figure 4-15). Some of these inhumations showed evidence of disturbance, some through ploughing. The skeleton identified for discussion here is number 883 (Figure 4-13). Skeleton 883 was found within the barrow in Grave 858. The skeleton was crouched on the right hand side, but in poor condition due to plough damage, and orientated NNE-SSW (McKinley, 2000: 85).

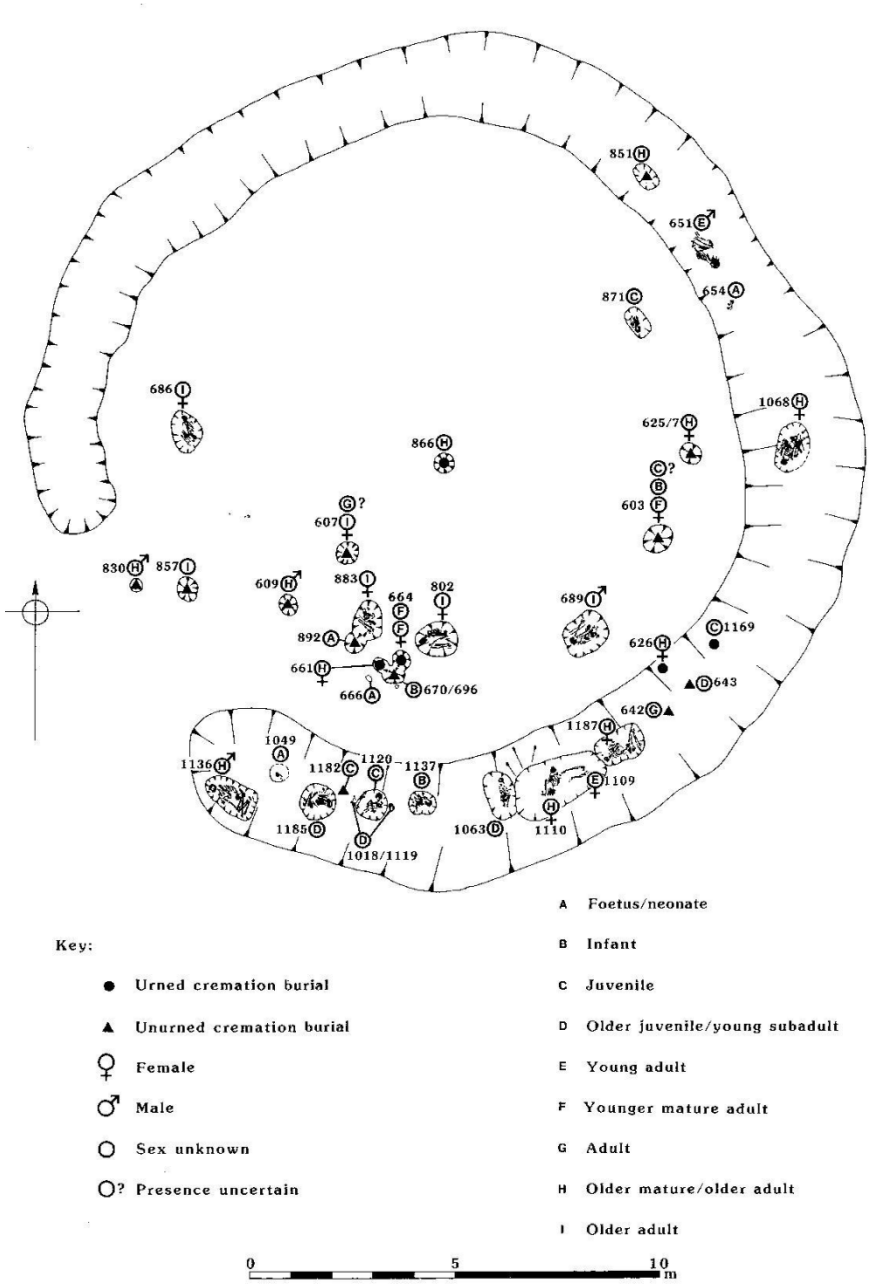

Figure 4-15 Plan of the Barrow including location of skeletal remains. (McKinley, 2000: 86 Fig. 32)

4.2.8 Waterhall Farm, Chippenham, Cambridgeshire

4.2.8.1 Site Information and Skeletal Recovery:

Name: Waterhall Farm, Chippenham
Code: Barrow A
Period (from which Sk. originates): Bronze Age

This barrow, (Barrow A), is one of a number of tumuli to have been excavated in the Chippenham area. Many of these were excavated by C.S. Leaf who published at least three papers in *Proceedings of the Cambridge Antiquarian Society*, the first in 1934-35 reporting the excavation of two barrows (1&2) in the North West area of the parish (Leaf, 1935). The second (1938-39) reported the further excavation of two barrows (3&4) in the south, plus partial excavation of a third in the same area as 1 & 2 (5), the latter containing a number of cremations and inhumations (Leaf, 1940). These barrows are in close proximity to a number of other notable tumuli excavated in the early to mid 20th Century (Figure 4-16) including those in Snailwell (Lethbridge, 1949), Beacon Hill (Cawdor and Fox, 1923), Swail's Tumulus to the north east (Briscoe,1956) and a Barrow at Pin Farm, Gazeley (Peterson, 1973).

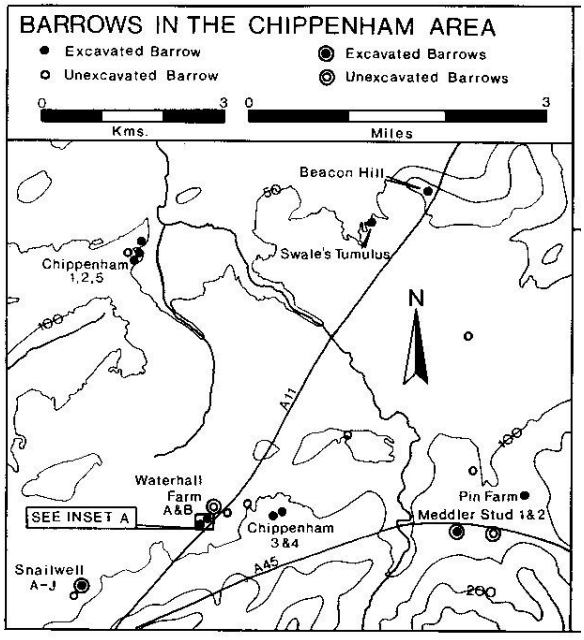

Figure 4-16 Map of Waterhall Farm (including Barrow A) and surrounding barrows. (Adapted from Martin, 1976: Fig. 1)

A 1973 excavation performed by Edward A. Martin during which Barrow A was excavated (and a second barrow, B), took place in advance of road-works for the Newmarket bypass and was located just off the A11 (Martin, 1976: 1) (Figure 4-16). The barrow material consisted of natural chalk, surrounded by blown sand, and it contained a number of burials (Figure 4-17) in what was considered a 'flat' cemetery, but it was cut into the natural curve of the mound and thus deposition chronology could be established (Martin, 1976: 4-5). Grave IV from which the skeletal remains to be discussed here were located, cut partly through Grave V and, as such, the fill of the Grave IV contained some skeletal elements thought to originate from the skeleton in Grave V. The rest of the fill covering the skeleton consisted of chalk and sand, and there was a layer of fine brown sand directly overlaying the skeleton; it was noted that the bones were somewhat soft and there were no accompanying grave goods (Martin, 1976: 8).

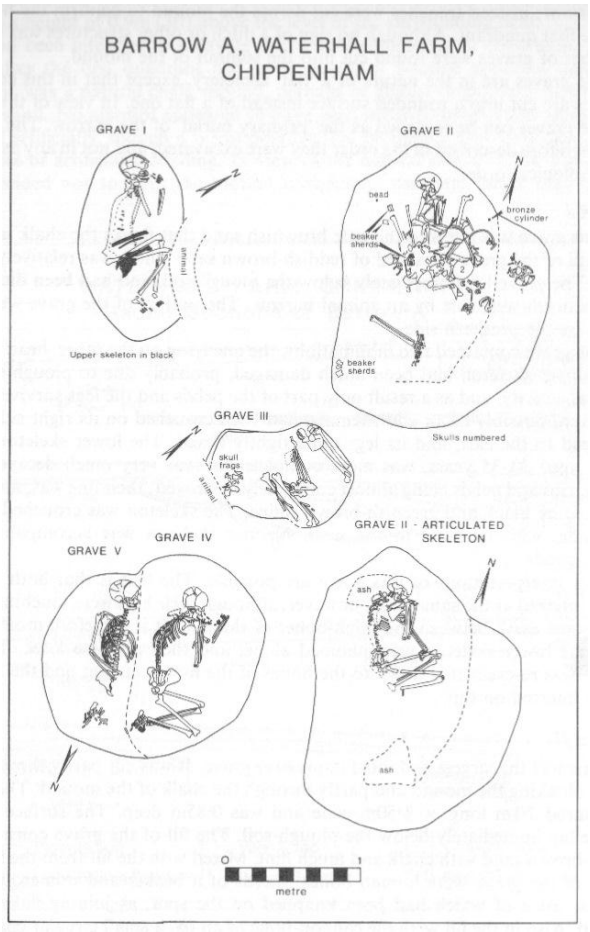

Figure 4-17 Plan of the skeletons contained within Barrow A (Waterhall Farm, Chippenham) including Grave IV (Martin, 1976: 6 Fig. 4).

The skeletal remains from this barrow were considered to form three 'groups' due to their positioning and thus suggested inter-relationships, the two inhumations in Grave I being the first, the multiple inhumations in Grave II forming the second, and Graves III, IV and V, with the addition of the cremation, being the third and final group (Martin, 1976: 12).

An additional report on the skeletal remains from this excavation was provided by C.B. Denston in the same volume and his observations closely match those of this study (Denston, 1976).

4.2.9 West Tump Long Barrow, Gloucestershire

4.2.9.1 Site Information and Skeletal Recovery:

Name: West Tump Long Barrow
Code: Unknown
Period (from which skeleton originates): Neolithic

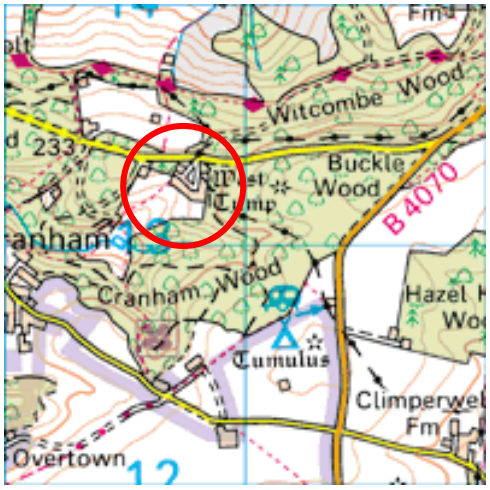

Figure 4-18 Map to show position of West Tump Long Barrow

The West Tump Long Barrow belongs to the Cotswold-Severn Group (Corcoran, 1969), a type of Megalithic chamber tomb found in Wales and South West England. Neolithic in date, this barrow is found in the parish of Brimpsfield, Gloucestershire (Figure 4-18). The barrow was originally found, by chance, in July 1880 by Mr. G.B. Witts, who was on an archaeological field trip with the Bristol and Gloucestershire Archaeological Society to visit a local round barrow. Mr. G.B. Witts subsequently excavated West Tump later that year with the assistance of Professor G. Rolleston, an anatomist (Witts, 1881: 201).

The site itself is located in the middle of a wood, and this was one of the factors which led the excavator to believe '…it had never been meddled with since it was constructed…' (Witts, 1881: 201). Whilst Witts refers to a total of around 21 skeletons having been located (1881: 207), later study of the material (apparently the first since the material was originally deposited) describes an MNI of 14 adults and eight subadults, with 3,100 human bone fragments present in the total assemblage (Smith and Brickley, 2004: 20).

The human remains found in West Tump were located in various parts of the barrow, some being present in the chamber and passage whilst others were located inside or outside of an encircling wall (Figure 4-19). The skeletons were comprised of both males and females and of old and young alike. In addition, some animal bone was also found within the barrow, including sheep, goat and red deer (Witts, 1881: 206).

Skeleton 7, the skeleton which demonstrated pathology relevant for this study, was located just outside of the south west wall, 68 feet from the southern "horn" and, due to it not being laid in a contracted position, was thought to have possibly been a later internment (Witts, 1881: 205). Witts describes in detail, the positioning of the skeletal elements as follows:

> *The skull and the feet were 48 inches apart, lying against the wall, and the space between them contained the skeleton, thus giving the idea at first that it was lying*

nearly at full length, but such was not the case. Next to the skull came the ossa coccygis, then twelve of the vertebrae in place, then the femora, tibias &c.

(1881: 205)

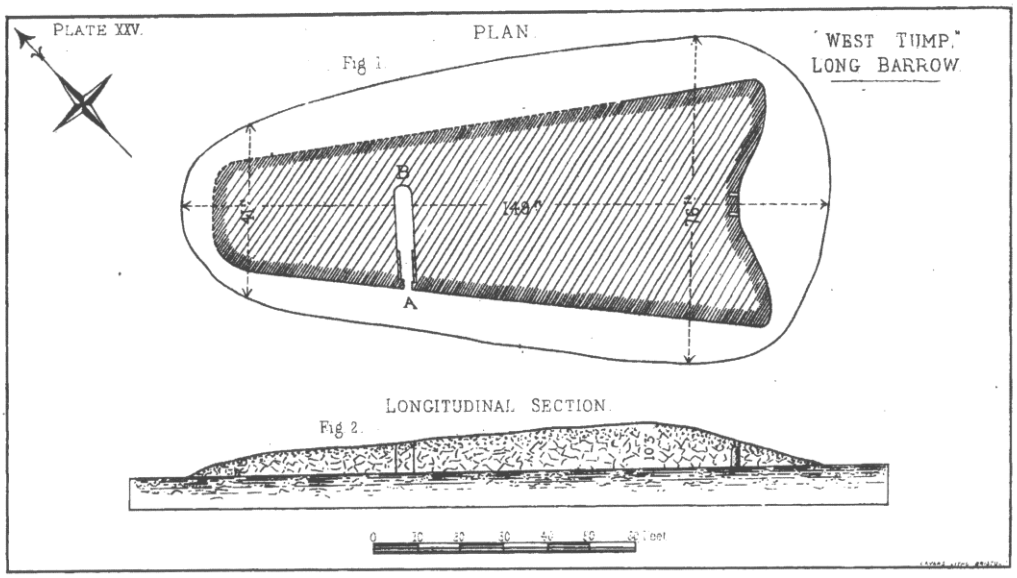

Figure 4-19 Plan of West Tump Long Barrow (Witts, 1881: Plate XXV)

4.2.10 Wetwang Slack, Yorkshire

4.2.10.1 Site Information and Skeletal Recovery:

Name:	Wetwang Slack
Code:	Unknown
Period (from which skeleton originates):	Iron Age

Figure 4-20 Location of Wetwang Slack (pink dot) (King, 2010)

Wetwang Slack is an Iron Age cemetery on the Yorkshire Wolds (Figure 4-20), from which the largest group of Iron Age burials in Britain were excavated. Original excavations were performed between 1975 and 1979 by Dent, the results of which formed the basis of his masters thesis (1984) and subsequent PhD thesis, (1985), amongst other publications (1982, 1979). A number of high profile burials were identified, some with carts or chariots (Selkirk and Selkirk, 1984, Dent, 1985, Hill, 2001, Hill, 2002), and human remains from the cemeteries have more recently been subject to various isotopic analyses (Jay, 2008, Jay and Richards, 2006) and re-analysis (King, 2010). Ancient DNA analysis has also been applied to pathological faunal remains from the site (Wooding, pers. comm).

The cemetery is linear, extending for 400m along a series of linear earthworks (Dent, 1995) (Figure 4-21).

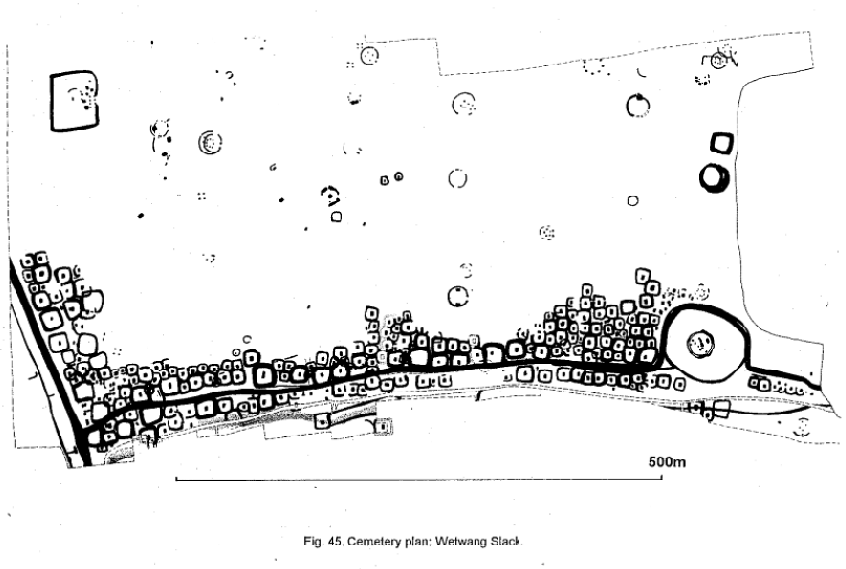

Figure 4-21 Plan of Wetwang Slack cemetery (Dent, 1995).

4.2.11 Wilsford Barrow G1, Normanton Down, Wiltshire

4.2.11.1 Site Information and Skeletal Recovery:

Name: Wilsford Barrow G1
Code: (SU/11114163)
Period (from which skeleton originates): Early Bronze Age

The Normanton Group of barrows has been described as being '…probably originally the finest barrow group in the vicinity of Stonehenge, and perhaps in Southern Britain.' (Grinsell, 1941: 30) This group of barrows lies in one of the richest archaeological areas in the world with a great number of other local barrows and groups of barrows (of many different types) such as the 'Lake Gorup', the 'Cursus Group' etc being nearby.

The first bowl barrow of this group was excavated in the 19th century but there is apparently no record of the 1805 excavations by William Cunningham except for there being a skeleton with an accompanying drinking cup and stag antlers in the central grave (Colt-Hoare, 1810). Further 'emergency' excavations in 1960 on behalf of the Ministry of Works (WAM, 1961: 30) resulted in the re-opening of what was then referred to as Grinsell's Barrow No.1 which actually demonstrated that there had been at least two inhumations and one cremation in the central grave. This later excavation located a further eleven burials in the north side of the barrow:

> *…Burials 5-10 were infant inhumations, and contained beakers; Burial 4 was also an inhumed infant, but contained a small vessel of urn type. Grave 11 contained the crouched skeleton of a young adult lying on its right side; behind the skull was a fragment of slate, which may be a copy of an early Irish flat bronze axe. Beaker sherds were also found in the grave filling.*
>
> (WAM, 1961:30)

Although this is essentially the extent of the documentation on the excavation of this barrow, and there was very little accompanying documentation with the skeleton analysed here, it is believed that the first skeleton analysed as part of this study is the skeleton from grave 11.

The second skeleton analysed was labelled with or had the accompanying information available: 'Burial VII with baby/foetus in causeway grave…NW Quad…Wilsford G1…"Beaker Body"'. Being burial 7, it appeared that this skeleton may be one of the five noted above, particularly as it was noted as a "Beaker Body" and was found with other numbered skeletons from this barrow (such as skeleton 5 from which the control sample was taken). However, no mention of the baby/foetus is made in the literature and the skeleton analysed was not an infant but a juvenile, which may demonstrate either a lack of osteological knowledge on behalf of the excavators or could suggest this skeleton (or label) has been confused with another. As the labelling clearly states 'G1' which matches the Wiltshire Heritage Museum listings, perhaps the former is the case.

4.3 Introduction to Macroscopic and Biomolecular Methods

There are two main elements to this study; macroscopic analysis of skeletal remains and pathogen aDNA analysis of samples of bone from selected skeletons. Skeletons were selected for aDNA analysis based on the outcome of the macroscopic analysis; those exhibiting possible or probable evidence of tuberculosis.

4.3.1 Methods: Macroscopic

In preparation for the research visits, skeletal recording forms were created using guidance from both Buikstra and Ubelaker (1994) and Brickley and McKinley (2004) (Appendix 3). A multi method approach for both sex and age estimation was employed in order to increase accuracy.

Upon arrival at a museum, databases were cross referenced with any catalogues available or, when not necessary, skeletons were immediately located from the appropriate stores and analysed. It should be noted that all skeletons that met the original criteria (see 4.1.1) were examined during the research visits but only those that demonstrated pathology of interest were subject to a full, recorded, skeletal analysis. Sometimes this would involve the study of many tens of skeletons within a narrow time frame. Where records suggested that some skeletons from a particular site may have met the necessary criteria, but not others, or where information was relatively vague, all skeletons or skeletal elements were sought where possible and subject to basic examination. This also resulted in skeletal material from many different sites being accessed, particularly where the majority of records were not available electronically and couldn't be considered in advance of the visit, or were vague as to exact skeletal holdings. This was time consuming but time was used as effectively as possible.

Firstly, it was necessary to establish that individual skeletal elements in any one box belonged to one skeleton as differing storage conditions can result in some human skeletal remains becoming mixed with others. In some cases, it is easy to establish when this has occurred but, at other times, where skeletal remains are extensively mixed, perhaps without any indicating markers on the individual bones, it can be extremely difficult if not impossible to separate the skeletal elements into their individual skeletons. If it was clear that this had occurred, these mixed remains were not used, to avoid confusion. It was only overlooked if there was some very convincing evidence of tuberculosis amongst mixed skeletal elements.

In order to analyse skeletons, a laboratory coat and gloves were worn in order to avoid unnecessary contamination during handling. Gloves were then changed between each different skeleton and disposed of. Skeletal elements present were inventoried as recommended by Brickley and McKinley (2004) and Buikstra and Ubelaker (1994) using a present / absent system for cranial bones, vertebrae and the hand and foot bones. All other elements were recorded according to a percentage of the bone present; for example the patella was recorded as being more than 75% present, 50 to 75% present, 15 to 25% present or less than 25%. Identification of fragments was aided by the use of White and Folkens (2005) and Bass (1995).

4.3.1.1 Age and Sex Estimation:

Sex and age estimations of the skeletons subject to analysis are particularly important when considering the possible presence of TB. This is because both factors could impact on an individual's susceptibility to the disease. It is possible that an X chromosome susceptibility gene may contribute to the excess of males with TB (Bellamy *et al*., 1999) seen, for example, in WHO report statistics (Diwan and Thorson, 1999), although other studies demonstrate that women are at more risk of extra-pulmonary TB infection (Yang *et al*., 2004). Perceived sex differences therefore, can be a highly complex area of study, influenced by many other factors, such as social factors leading to under-reporting of disease by women (e.g. Butalia, 1992). It is also important to clarify that sex and gender are different although are often confused as such, especially in bioarchaeology. Gender is related to a person's social identity as opposed to biological identity (White and Folkens, 2005: 385); the societal categories of 'male' and 'female' overlap (Oakley, 1985). Skeletal analysis enables the true biological sex of a person to be established where preservation and sexually distinct morphology allows. It is therefore important to collect this data from archaeological skeletons, wherever possible, to enable a greater insight into the distribution of TB which can then be considered within the context of any given population, a task more challenging for prehistoric studies.

Age estimation is equally important as age related susceptibility has also been demonstrated (Orme, 1987) and certain factors, including underlying illnesses and age-related diminution in immune function, can complicate both the diagnosis and treatment of the elderly (Rajagopalan, 2001). There is, however, evidence to suggest that infection with M. *tuberculosis* increases with age but actually declines in older adults (Andersen and Geser, 1960, Narain *et al*., 1966) and that children under five are at high risk of developing the disease after initial primary infection (Fine, 1994). It is of use to collect such data for archaeological populations in order to attempt to identify potential patterns of susceptibility in all age ranges in previous periods. It is also known that skeletal TB can manifest itself in different ways between adults and children (for example, tuberculous foci in children are

much more likely to present themselves in areas rich in haematopoietic marrow, such as the ribs, fingers and toes, and long bones) which may aid with the recognition of the disease and allow a more confident diagnosis based on the pattern and appearance of the pathology.

4.3.1.2 Sex Estimation

In general, assigning sex to a skeleton can be a fairly accurate process but it is possible for some young men to display very gracile characteristics and also for some older women to develop more masculine traits (Walker, 1995). For these reasons confusion can occur but, if numerous skeletal elements are considered before assigning sex, accuracy will be increased. If it is not possible to consider a skeleton male or female with any certainty, it should be recorded as such.

Additionally, it is vital to note is that assigning sex to a skeleton is only truly possible once an individual has reached maturity (White and Folkens, 2005: 386); before this time many bones in both males and females are similar in their growth patterns and do not really begin to change in a morphologically distinct manner. For this reason, sex was not assigned to subadults in this study, although it is generally accepted that sub-adult skulls displaying more male characteristics and sub-adult pelves displaying more female characteristics could be fairly reliable indicators of sex (Buikstra and Ubelaker, 1994: 16). This is because young males often demonstrate more gracile, female like characteristics in the skull before they begin to develop more mature 'male' morphology, acquired with age; thus, skulls from sub-adults which already demonstrate male elements are thought to be fairly reliable indicators of a male individual. The reverse is true for pelves; young females may often demonstrate relatively male like morphology as they have yet to develop the wider morphology associated with a female adult. Despite this, it was decided not to assign sex to sub-adult remains. Research into age and sex bias has demonstrated that subadult remains tend to be more poorly preserved than adult remains based on intrinsic as well as extrinsic factors (Bello *et al*., 2006), and thus the possibility or reliably estimating age and sex of individuals who have not yet reached maturity is further disadvantaged. However, the preservation and

completeness of skeletal remains is clearly also a factor for adult remains (Meindl *et al*., 1985); where only the cranium is present it is estimated that accuracy of sex determination is 90%, 95% using only the pelvis and 98% combined (Krogman, 1962).

There are two primary areas of the body used for sex estimation, the pelvis and the skull, both of which have numerous elements that can be examined for sex differences and which prove to be generally quite accurate. However, the pelvis is considered the most accurate of the skeletal elements because of sexually dimorphic characteristics seen in males and females. Using cranial features can be a less accurate exercise as morphology can vary immensely between populations, meaning that this method can prove reliable for some groups and not for others (Buikstra and Ubelaker, 1994: 19).

4.3.1.2.1 Using the Skull

Buikstra and Ubelaker (1994: 20) emphasise five areas of the adult skull which can be used in conjunction as sex indicators, after Acsadi and Nemeskeri (1970). These include, the nuchal crest which is generally minimally expressed in females but can be very large and pronounced in males; the mastoid process follows the same pattern of expression; the supraorbital margins tend to be thinner and sharper in females and more rounded and thicker in males; the supraorbital ridge or glabella, again is less pronounced in females as is the final area, the mental eminence (Figure 4-22). In addition, the shape of the mandible as seen from above was also considered; a wider, squarer shaped mandible tends to indicate male, and a smaller, more pointed shape, female. It should be noted that the drawings of the typical male and female mandibles provided in Buikstra and Ubelaker (1994) are not considered of great value for British skeletal material and have been substituted here for those provided in Brickley and McKinley (2004), along with more detailed consideration of features: overall size, width of ascending ramus, flaring of gonial angle, shape of chin (2004: 23). Whilst the elements above are suggested by Buikstra and Ubelaker (1994) as sexing criteria to be used in conjunction with each other, given the fragmentary nature of much of the material under study, the elements chosen are also easily recognizable amongst a

fragmentary cranium and thus damage would not necessarily preclude an assignment of a score.

Each element outlined above was scored on a scale of one to five, with female traits scoring lower than males. Once each surviving element was assigned a score, an overall sex was assigned. For this project, this system was then combined with the pelvis.

There are additional elements of the skeleton which may also be included when assessing biological sex, including metrical assessment. However, it was decided not to include such measurements in this study as, due to the age and type of material under study, it was unlikely that many of the bones would be complete enough to collect accurate data. Additionally, the ancestry of the reference collection should be the same as the population being studied (Cox and Mays, 2000: 119) and, again, due to the nature of the collections under study (often single individuals with no comparative material) it was impossible to collate such data.

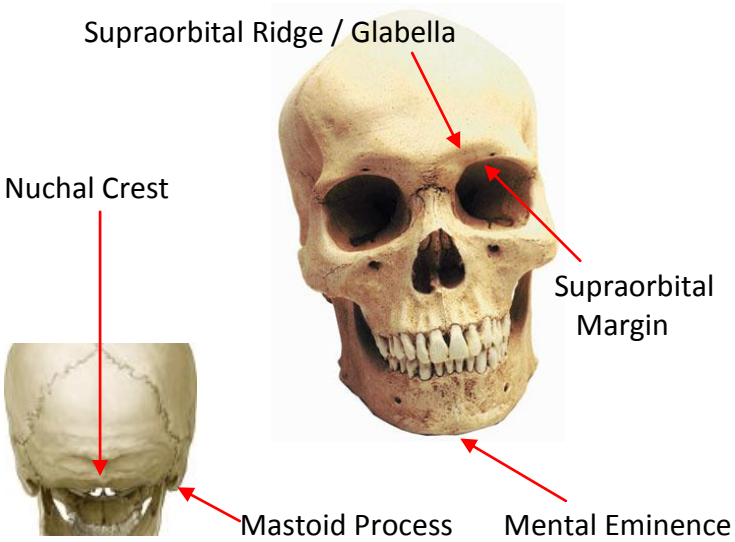

Figure 4-22 Markers on the Skull used to Estimate Biological Sex (http://skullduggery.com/skulls.htm)

4.3.1.2.2 Using the Pelvis

As expected, sexual dimorphism is most noted in the pelvis as male and female pelves fulfill different roles. Most obvious is the need for a female to be able to carry, protect and deliver a child, resulting in a wider pelvis overall. As emphasized in Buikstra and Ubelaker (1994),

the following elements were considered and scored, beginning with “The Phenice Method” (1969), focusing on the subpubic region, which incorporates the ventral arc, subpubic concavity and ischiopubic ramus (Figure 4-23). The ventral arc, which is an elevated ridge of bone, is only present in females, and its dimorphism related to “muscular origin and…differential growth patterns” (Anderson, 1990). The subpubic concavity which, again, in males is not present, and the ischiopubic ramus medial ridge is thinner and sharper in females. These areas were scored from one to three with one being female, two, ambiguous and three, male. Unobservable scores were recorded as blank.

The greater sciatic notch is also an element advocated by Buikstra and Ubelaker (1994: 18); this tends to be much wider and shallow in females and narrower in males (Hager, 1996) but is not considered to be as accurate as the subpubic region (Buikstra and Ubelaker, 1994: 18). This element was scored from one to five, with one indicating the most typically female morphology and 5, the most male.

Whilst the preauricular sulcus is an element also suggested for use by Buikstra and Ubelaker, it should be noted that ‘parturition scars’ are no longer recommended for or use as an indicator of sex as the presence or absence of the preauricular sulcus may not indicate a female as once thought, but rather be associated with pelvic shape and size (Cox and Mays, 2000: 118). For that reason, this element was not recorded during the course of this project.

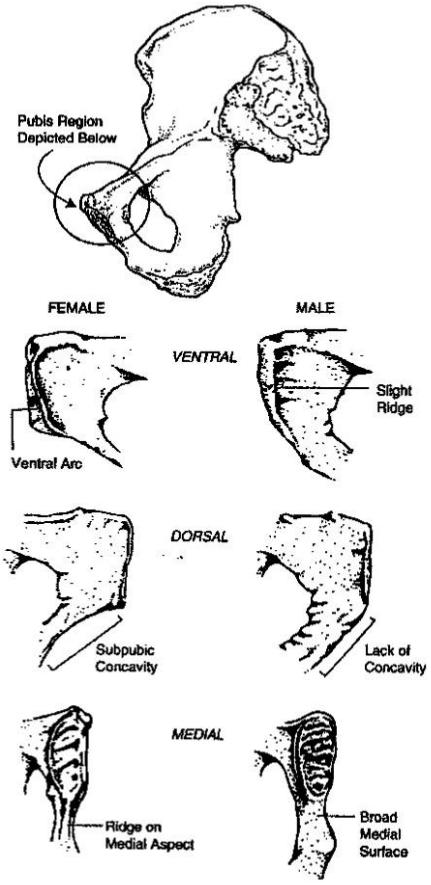

Figure 4-23 Sex differences in the subpubic region as outlined by Phenice. (Drawing by Zbigniew Jastrzebski in Buikstra and Ubelaker, 1994: Figure 1, after Buikstra and Mielke, 1885; Phenice, 1969).

4.3.1.3 Age Estimation

Aging an adult skeleton can be a complex task. A great deal of variation can be seen between populations, making the use of standardised age at death data gathered from more recent populations and applying them to historic or prehistoric populations, particularly challenging. The need to use population specific standards where possible is advocated (Schmitt *et al*., 2002) but this is often extremely difficult or impossible in studies of this nature, where often only a few, or even single, skeletons are available to represent an entire population, making comparative and population specific data impossible to determine. Significant differences even occur between individuals in any given population and this can be due to a range of factors, including quality of diet and nutritional intake, genetics or (and of critical note for this project), exposure to infectious disease (Roberts, 2009: 127).

> *Estimation of age-at-death involves observing morphological features in the skeletal remains, comparing the information with changes recorded for recent population of known age, and then estimating any source of variability likely to exist between the prehistoric and the recent population furnishing the documented data. This third step is seldom recognized or discussed in osteological studies, but it represents a significant element.*
>
> (Ubelaker and Grant, 1989: 63)

For this project a combination of age estimation techniques focused on areas throughout the skeleton in order to increase accuracy and, again, to increase the possibility of being able to employ at least some of these techniques, given the fragmentary nature of so many of the remains. The multi method aging method (Lovejoy *et al*., 1985) is considered to be a reliable method for ageing compared with single age indicators, supported by studies using skeletons with known age-at-death information (Bedford *et al*., 1993), although some studies have not demonstrated as much success (Saunders *et al*., 1992). However, using a wide range of techniques is useful as this '…helps control for variation in the changes that occur with age in any single morphological indicator' (Bedford *et al*., 1993: 287). As with sex estimation techniques, all the methods used here were recommended by both Brickley and McKinley (2004) and Buikstra and Ubelaker (1994).

4.3.1.3.1 The Clavicle

The medial clavicular epiphysis has been studied not only in the bioarchaeology literature but also in clinical and forensic work using plain film radiographs (Schulz *et. al.*, 2005) and computed tomography (Schulz *et. al.*, 2008). The clavicle is considered a useful bone for estimating age at death, particularly in subadult remains because it displays "the longest period of growth related activity" (Black and Scheuer, 1996). The detail for this method was taken from Scheuer and Black (2004).

4.3.1.3.2 The Sacrum

The sacrum allows fairly broad estimates to be made using the categories 'less than 20 years old', 'less than 25 years old' and 'older than 25'. These estimates are based on the fusion of sacral segments: between all for the first category, between the first and second for the second category, and complete union, usually seen after the age of 25 years. Again, the detail for this method was taken from Scheuer and Black (2004).

4.3.1.3.3 Pubic Symphysis

The method employed here was that of Suchey Brooks using replica casts of known age individuals for the development of the pubic symphysis (Brooks and Suchey, 1990). Although the Todd system described the first criteria for ageing the pubic symphysis (Todd 1920), and found that over-ageing was a problem, the system was revised numerous times and the Suchey Brooks method is now the most widely used. The system is based on six phases of development (each with an early and late stage), which are different for males and females. These phases allow the observer to place the individual into fairly narrow age ranges, although the breadth of the categories increases with age. There is a significant amount of published literature on age estimation from the pubic symphysis (Brooks and Suchey, 1990, Meindl *et al*., 1985, Overbury *et al*., 2009, Kimmerle *et al*., 2008)(Todd 1920), including work on whether bilateral asymmetry of the os pubis (in this case specifically in the pubic symphyses) has implications for this method; it was discovered that it did not if the older of the two is used for age estimation (Overbury *et al*., 2009). There is, however, a wide range of inter-observer variation for many of the aging methods, including the pubic symphysis (Kimmerle *et al*., 2008).

4.3.1.3.4 Auricular Surface

The auricular surface technique was first outlined by Lovejoy *et al.* in 1985 based on chronological age changes that could place an individual into a particular age range. The technique was also considered useful because it "carries the advantages of a higher preservation rate for the auricular surface in archaeological populations and continued age-

related change beyond the fifth decade." (Lovejoy *et al*., 1985: 15). Since its establishment as a usable method, tests of its accuracy have shown the changes to be too variable to be used as a method on its own, and the range of error to large for forensic purposes, but it is employed successfully in bioarchaeology again as one of many methods (Murray and Murray, 1991). A revised method was suggested more recently (Buckberry and Chamberlain, 2002) which was considered easier to apply, has lower levels of error and actually is slightly more accurate than the Suchey Brooks method using the pubic symphysis. However, the technique was later shown not to be as reliable as hoped (Falys *et al*., 2006) and the original Lovejoy descriptions are used here in order to estimate age. It should be noted that this method merges males and females together rather than, for example, the pubic symphysis method which requires different considerations and different reference casts based on biological sex (Brooks and Suchey, 1990). The age ranges seen for this method are also narrower than those seen in the pubic symphysis method.

4.3.1.3.5 Sternal Ends of Ribs

Casts of known age individuals were used to assess degeneration of the sternal end of the right 4th rib and assign a phase and age range (Işcan *et al*., 1985, Işcan *et al*., 1984). This method is often hindered when used with archaeological skeletons such as those encountered in this study, as the sex of the skeleton must be known in order to compare ribs to the casts, which are separated by sex, and the skeletal material is often highly fragmented. This fragmentation can often obscure the positive identification of the right 4th rib, if it is present at all. For this reason, there have also been studies to investigate whether or not the method can be applied to other ribs, rather than just the 4th , with some positive results (Aktas *et al*., 2004) although Dudar (1993) states that the standards can be applied "cautiously" to others. The original method has however, been supported by later independent tests to see whether it is a useful additional method which can reliably be included in multifactoral age estimations (Russell *et al*., 1993) where the correct rib can be identified. More recently, excellent results have been reported from the field of forensic science which demonstrated that this method could be applied to two and three dimensional multislice computed tomography reconstructions as well as to dry bone, with

no significant difference in reliability, showing that it is a versatile ageing method considered reliable enough for forensic investigation (Dedouit *et al*., 2008). A new method of ageing using the first rib has recently been suggested (DiGangi *et al*., 2009) but further research will be necessary to confirm its reliability. In fact, the original Iscan method has more recently been assessed and it was found that, despite some problems in reproducibility, the variables did provide objective information on age at death, but it was suggested that the method could be improved by using better descriptions of the above variables combined with multivariate statistical analysis (Fanton *et al*., 2010). It is clear that the ribs can play a major part in the ageing of adult skeletons.

4.3.1.3.6 Dental

Brothwell's dental attrition method of ageing (1981: Fig. 3.9) (Figure 4-24) was used, where possible, to support age estimation and, where a subadult skeleton was being recorded, Ubelaker's (2008:47) dental development and eruption data were employed. Although dental attrition will vary between populations, it serves as a useful guide where other methods are also being employed and Brothwell's dental attrition chart is an appropriate choice for this study as it shows a "tentative classification of age in Neolithic to Medieval British skulls" (1981: 72).

Dental development will also vary between populations and Ubelaker's chart of dental development (2008: 47) was based on the teeth of Native Americans but, as Scheuer and Black (2004: 176) explain, this method has been widely used and with many different samples. It is also recommended by the WEA (Workshop of European Anthropologists) (Ferembach *et al.*, 1980). Although there are some difficulties in this method of using estimated ages from the general developmental stage of the entire dentition, it is also quick and there is a range of age variation for each stage.

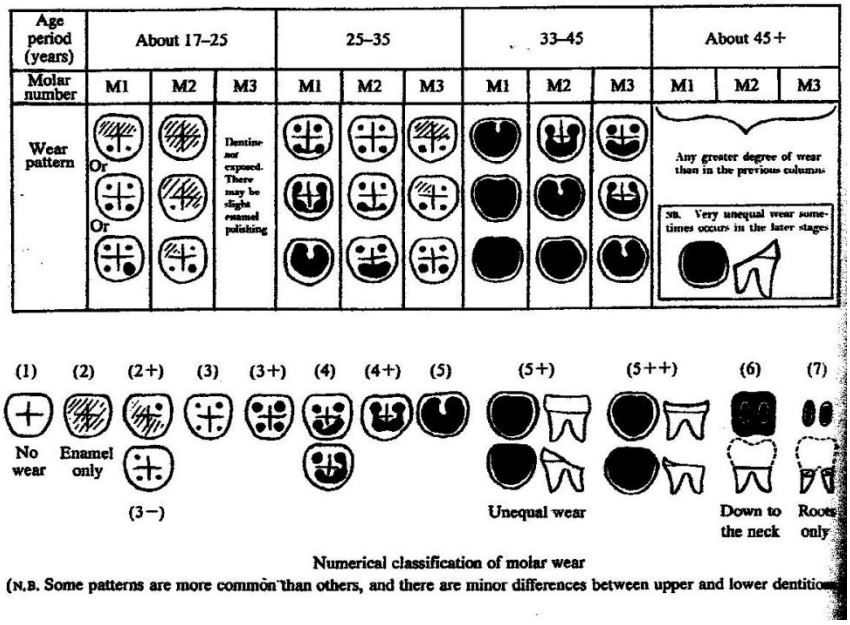

Figure 4-24Table of Dental Attrition used to Estimate Age at Death (based on Neolithic to Medieval data) (Brothwell, 1981:Figure 3.9)

4.3.1.4 Age Estimation for Sub-adults

In addition to dental eruption, mentioned above, a number of other methods were used in order to help accurately estimate the age at death of subadult skeletons studied. It is generally easier to attribute much more specific ages to subadults because, before maturity, the skeleton is continually developing in a known sequence of events although, again, this is dependent on both intrinsic and extrinsic factors.

> *Subsistence strategies, diet, disease experience, weaning patterns, and inherited conditions often influence maturing teeth and bones in a manner that reflects ancient lifeways*.
>
> *(Buikstra and Ubelaker, 1994: 39)*

For these reasons, using modern data to estimate age based on dental development, epiphyseal fusion and long bone length in archaeological populations is fraught with problems as there will be numerous differences between the population used to collect

these control data and the population to which the data is being applied. Without knowing the full details of the environment and conditions to which any given archaeological population were subject (which is in itself impossible), it is not possible to compensate for these differences. It is usually advocated that researchers therefore attempt to collate data from within the entire population under study (Schmitt *et al*., 2002), and therefore individuals can be compared with others who were likely subject to the same conditions as themselves. This of course, cannot account for individual differences but this is also true of modern populations also.

Epiphyseal fusion information was recorded wherever possible from all available skeletal elements using Scheuer and Black, (2004) as a guide, as advocated by Buikstra and Ubelaker (1994: Attachment 12) but, again, much of the data are derived from modern populations and it is possible that development in earlier populations differed (Nyati *et al.*, 2006). Long bone length was also measured where possible with reference to Scheuer and Black, (2004) and compared with data in Ubelaker (1989) in the absence of reference data from the population under study, as advocated by White and Folkens (2005: 373). This method is not as accurate as other methods and, again, data should ideally be compared with 'the same or a closely related skeletal collection' (White and Folkens, 2005: 373).

4.3.1.5 Stature

Maximum tibial (TiL1) and femoral (FeL1) lengths were recorded, if possible, for adults using the standard practice of taking three measurements and using the average to work out an estimated stature. Measurements were then compared against the stature tables as outlined in Trotter (1970). Stature is an important element to record, not only to document possible changes in height over time, but also because stature is related to nutritional status and environmental stress (Steckel, 1995) which are two factors also related to susceptibility of disease which is a key focus of this study. For that reason, where possible, it was considered useful to record this information to see if any links could be found with those skeletons displaying pathology potentially related to infectious disease, and perhaps smaller stature than may have been expected. Without population specific data, however, this

would be a very difficult task and, again, it was expected that much of the skeletal material encountered would be too fragmentary to allow stature to be recorded. It was considered important enough, however, because of the reasons above, to record this information if at all possible.

4.3.1.6 Pathology

Systematic analysis of the skeleton was undertaken and any pathological lesions recorded. All areas of the skeleton present were examined and, for the purpose of this project, areas of the skeleton most likely to demonstrate specific or non -specific, infection due to TB were prioritised. These included, the vertebrae, hip and knee joints, ribs and endocranium, but the entire skeleton was examined where preservation allowed. Ortner (2003) and Aufderheide and Rodríguez-Martín (1998) were used to assist in the identification of relevant pathological conditions, in addition to other texts (Roberts and Manchester, 2005, Roberts and Buikstra, 2003).

4.3.1.6.1 The Spine

The most reliable identifiable lesion of TB in skeletal remains can usually be found in the spine (Potts Disease) as TB lesions involve the spine between 25 and 50% of the time (Resnick and Niwayama, 1995: 2462), and the often collapsed vertebra and resulting angular kyphosis is easily recognisable. TB of the spine is also identifiable by the areas of the vertebrae it usually involves, primarily the lumbar and thoracic regions, with the cervical and sacral regions being rarely affected (Resnick and Niwayama, 1995: 2463), although recent clinical studies demonstrate that there can be cervical involvement (Prasad *et al*., 2007, Kapoor *et al*., 2007). The number of vertebrae affected is a question that has been addressed many times (Nathanson and Cohen, 1941, Morse, 1961, Ortner and Putschar, 1981, Ghormley and Bradley, 1928) but studies such as that by Kastert and Uehlinger (1964: 486 cited in Ortner, 2003: 231) clearly demonstrate the extensive range of vertebrae that can be affected (Figure 4-25) and show that at least two adjacent vertebrae are involved in at least 80% of cases. For these reasons, the entirety of the spine was examined wherever

possible, with a focus on the thoracic and lumbar regions and, in particular, lesions which affected adjacent vertebrae were studied extremely closely. In addition, it is the anterior portion of the vertebral body which is most commonly affected, (Resnick and Niwayama 1995: 2464) with the tuberculous focus most commonly spreading to the adjacent vertebra through the area of the 'nucleus pulposus of the intervertebral disc'. (Ortner, 2003: 231).

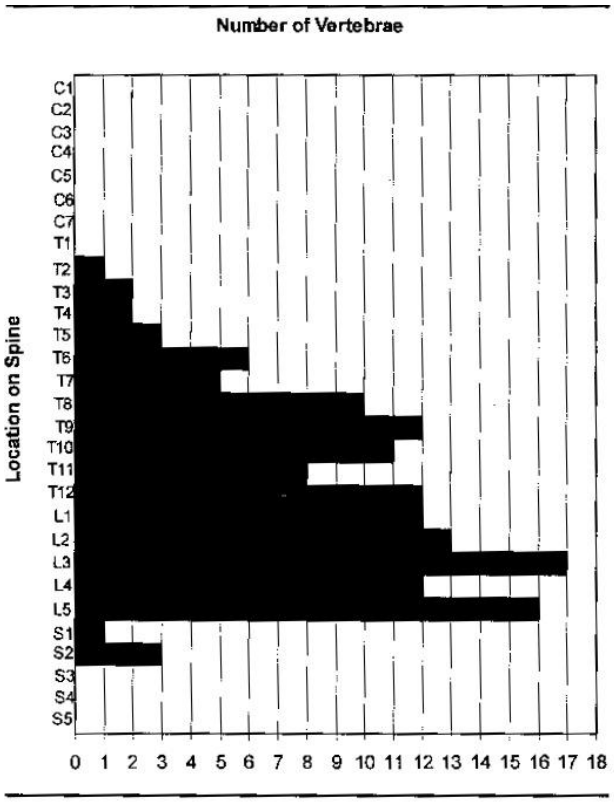

Figure 4-25: Distribution of lesions in 62 Autopsied Cases of Vertebral Tuberculosis (in Ortner, 2003: Table 10-3 after Uehlinger in Kastert and Uehlinger 1964) (C = Cervical, T = Thoracic, L = Lumbar, S = Sacrum).

4.3.1.6.2 The Hip

The hip comprises 20 percent of skeletal involvement (Aufderheide and Rodriguez Martin, 1998: 139), being the most frequent skeletal lesion after spinal involvement (Ortner, 2003: 235). Modern clinical figures demonstrate tuberculosis of the hip constitutes around 15% of all cases of osteoarticular tuberculosis (Babhulkar and Pande, 2002). A tuberculous focus of the hip usually starts in childhood, around four to six years of age with another smaller 'peak' occurring around the time of puberty (Sorrel *et al*., 1932: 309). Research has taken

place into the treatment effects and clinical outcomes of children presenting with tuberculosis of the hip (Campbell and Hoffman, 1995) and of the radiographic appearance of early stage bone and joint TB (including the hip), with suggestions for improved diagnostic techniques (Versfeld and Solomon, 1982). Tuberculosis of the hip is usually very destructive with foci in the femoral head and neck and the occurrence of lytic lesions on the acetabulum also possible. In some cases, where dislocation of the femoral head occurs, a 'neoacetabulum' forms and, in cases where the infection heals, the joint can become ankylosed (Ortner, 2003: 237). Bony destruction was apparent in all 17 children studied by Versfeld and Solomon, (1982) and all demonstrated demineralisation of the affected region, including all seven suffering from TB of the hip which is something that may be observed in archaeological skeletons if the spongy bone is visible. The primary differential diagnosis to consider when looking at archaeological remains is that of septic arthritis although there are some key diagnostic differences, partly based on the age of the individual; for example, generally, only infants would demonstrate complete destruction of the femoral head if suffering from septic arthritis (Ortner, 2003: 237).

4.3.1.6.3 The Knee

The knee joint is also an area which can suffer a great deal of destruction if infected with TB. According to Ortner, TB of this joint occurs about as often as that of the hip, if not more so (2003: 240) and, as with the hip, is often prone to onset during childhood (including infancy and adolescence - Ortner and Putschar, 1985). Others, however, argue that it is most frequently seen in adults (Turek, 1982 cited in Aufderheide and Rodriguez-Martin, 1998: 139). A study by Teklali *et al.* (2003) which reviewed 106 cases of paediatric peripheral osteoarticular TB (vertebral TB excluded) demonstrated 63% of osteoarticular foci was the result of hip and knee TB combined. A number of additional papers have also reviewed, retrospectively, the diagnostic and treatment progress of affected children (Lee *et al*., 1995, Hoffman *et al*., 2002; Kerri and Martini, 1985) in addition to specific case studies (Uzel *et al*., 2004). The destructive nature of the infection in the knee joint appears to be less destructive in adults (Sorrell and Sorrell-Dejerine cited in Ortner, 2003: 241). The infection generally begins as synovial TB and primary or simultaneous haematogenous osseous foci

can cause destruction or deformity of the femoral condyles and/or tibial plateau (Ortner and Putschar, 1985: 154), but rarely in the patella or fibula (Ortner, 2003: 240). It is generally not possible in archaeological individuals to know the origin of the infection, synovial or metaphysic (Aufderheide and Rodriguez-Martin, 1998: 139). During healing, ankylosis can occur and, according to Ortner, attempting to differentiate between end stage rheumatoid arthritis and septic arthritis can be impossible if there is limited (or no) bone destruction (2003: 241).

4.3.1.6.4 Ribs

Pathological changes on the ribs cannot, in themselves be considered pathognomonic for TB. This is despite strong correlations found between rib pathology and deaths known to be caused by TB (Roberts *et al*., 1994, Santos, 2000, Kelley and Micozzi, 1984) Santos and Roberts, 2001) and studies such as these suggest that rib lesions are most closely correlated with TB infection (Roberts and Buikstra, 2003: 105). Destructive or bone forming lesions in the ribs could be expected to occur in the rib heads attaching to any affected vertebra (Roberts and Buikstra, 2003: 103), and lytic lesions with fusiform enlargement of the involved area are often the result of haematogenous infection (Ortner, 2003: 246), although it is possible for the infection to occur from direct extension of adjacent tuberculous foci (Kremer and Wiese, 1930: 283 cited in Ortner, 2003: 246). Rib lesions may be caused by an extension of pulmonary TB, spread to the visceral surface of the ribs from the lungs and via the pleura (Roberts and Buikstra, 2003: 103).

TB in the ribs has been described as rare, at least in clinical literature (Johnson and Rothstein, 1952, Rechtman, 1929, Leader, 1950, Sinoff and Segal, 1975), although this is based on the appearance of destructive lesions identified by radiography (such as those described above), and it is possible that rib involvement may actually be as high as nine percent in patients with TB (Kelley and Micozzi, 1984, Roberts *et al*., 1994). Pfeiffer (1991) describes three categories of rib lesions; Plaque (periosteal new bone formation), expansion (porous cortical expansion extending from the head or neck), and resorption (erosive lesions). New bone formation on the visceral surfaces on the ribs may well be suggestive of

pulmonary TB (Roberts, 1999) but, in isolation, these changes could also be the result of other pulmonary infections and a number of studies have highlighted the associated problems and differential diagnoses possible due to the non-specific nature of the manifestation in archaeological material (Roberts *et al*., 1998, Roberts *et al*., 1994, Lambert, 2002, Santos and Roberts, 2006, Pfeiffer, 1991, Mays *et al*., 2002). Rib lesions may be found in skeletons who also display other diagnostic features of TB (Roberts, 1999)(Kelley and El-Najjar, 1980) which may add weight to the argument for ribs being affected in TB. It is clear that ribs should always be thoroughly analysed to contribute to the discussion surrounding their diagnostic potential and, for this study, pathological changes as outlined by Pfeiffer (1991) were used as a guide for lesion identification:

1. **Plaque:** layers of dense periosteal bone are deposited on the visceral surface of the bone as a discreet plaque which can become detached.
2. **Expansion:** a puffy cortical expansion with a lack of distinct edges and differentiated from plaque by its porosity.
3. **Resorption:** Erosive lesions of the rib head and neck.

4.3.1.6.5 The Endocranium

The skull has to be considered as three areas potentially affected by TB, as advocated by Ortner (2003: 247), the cranial vault (the most common site of cranial TB), cranial base, and facial bones. Tuberculosis of the skull is by all accounts rare, although there have been numerous clinical studies highlighting the appearance and treatment outcomes of cranial TB, including case reports (Le Roux *et al.*, 1990; Gupta *et al.*, 1989; Kang *et al.*, 2008; Shameem *et al.*, 2009) and wider studies (Barton, 1961; Mohanty *et al.*, 1981; Patankar *et al.*, 2000; Ramdurg *et al.*, 2010; Diyora *et al.*, 2009). TB affects the skull in young adults more frequently than in adults and is almost exclusively secondary to haematogenous dissemination (Aufderheide and Rodriguez-Martin, 1998: 140). Tuberculosis in all three areas of the skull outlined above appear to be more common in children, and the

haematopoietic marrow and growth activity at this age may be a determining factor as to the frequency of cranial involvement (Ortner, 2003: 248).

The general pattern of TB infection in the cranial vault is 'a round lytic focus of not more than 2 cm in diameter, with or without a "moth-eaten" central sequestrum, terminating in complete perforation of the inner and outer tables.' (Ortner, 2003: 248). The lesion can also cross cranial suture lines (Ortner and Putschar, 1985: 163) and this is one of the differentiating factors between TB and Langerhans cell histiocytosis (which also does not usually include a central sequestrum -Ortner, 2003: 248). It is also possible for new bone formation to occur on the endocranium as a response to infection in the adjacent soft tissue, possibly as a complication of tuberculous meningitis (Roberts and Buikstra, 2003: 100). Lesions on the endocranium can often appear as a cluster of vascular lines and it is important to note that destructive lesions usually result in a greater defect of the inner table than the outer, which is a key difference between TB and tertiary syphilis (Ortner, 2003: 248).

TB can, of course, affect any part of the body, with the exceptions of only teeth and hair (Fluck; 2004: 4) and, for that reason, all skeletal elements present from any single individual skeleton were analysed. This process also allowed any particular patterning of pathology to be noted and to ensure that all possible differential diagnoses be considered. When looking at affected vertebrae, it was also hoped that the number of affected vertebrae could be recorded to enable percentage frequencies to be calculated. Whilst all of the areas above were selected for study primarily because of their particular diagnostic features or frequency of occurrence in TB infection, the individual elements also form substantial areas of the skeleton that may have a higher chance of survival and recovery in archaeological deposits, as opposed to perhaps phalanges of the hands and feet or the small bones of the skull.

4.3.1.7 Other Areas of the Body which May be Affected

There are of course, a great deal more areas of the skeleton which may demonstrate features suggestive of TB which should be observed and a summary of these is included below:

- **Facial Bones:** The maxillary area of the facial bones are especially likely to be affected and may be caused by the spread of a secondary infection, or lupus vulgaris (TB of the soft tissue) or the nasal cavity secondarily affected by mucosal TB (Ortner, 2003: 250). According to Ortner (2003: 250), TB of the inferior, lateral, orbital margin of children is not uncommon which can involve the maxilla, and involvement of the zygoma is seen frequently, although most of the time multiple skeletal foci are also found elsewhere.

- **Shoulder:** TB infection in the shoulder is more common in adults than children but is not common in general (Auderheide and Rodriguez-Martin, 1998: 140). The infection in this area favours males, and in both males and females the right side is three times more affected than the left (Kremer and Wiese, 1930: 291 cited in Ortner, 2003: 242). Osseous foci are more frequently found in the head or proximal metaphysis of the humerus than the scapula and, certainly in adults, extensive destruction of both the humeral head and glenoid fossa can occur (Ortner, 2003: 242). The scapula itself is rarely involved except for this extension from the joint into either the glenoid fossa or the acromion (Ortner, 2003: 247).

- **Sternum:** Infection is usually lytic, seen in the manubrium, and can extend to the sternoclavicular joint, affecting the medial aspect of the clavicle (Ortner, 2003: 247).

- **Elbow:** According to Ganguli (1963), TB of the elbow is common, especially in those aged 20-30. The lesion usually begins in the humerus and can lead to very severe bone destruction of the joint which can cause the head of the radius to dislocate posteriorly (Aufderheide and Rodriguez-Martin, 1998: 140).

- **Wrist:** The wrist consists of the radiocarpal joint, the intercarpal joint and the carpo-metacarpal joint; according to Ortner (2003: 243) any or all may become involved in a tuberculous infection, although the location and manifestation depends on the age group into which the individual fits. In children, the cause is usually from extension from a carpal infection (Aufderheide and Rodriguez-Martin, 1998: 140), whereas in adults it is a result of adjacent soft tissue focus in the tendons of the wrist (Turek, 1982 cited in Aufderheide and Rodriguez-Martin, 1998: 140).

- **Hands and Feet:** Also known as *spina ventosa*, the affected bones form a bony shell giving a balloon like appearance and usually occur with other skeletal foci (Ortner, 2003: 242). This occurs most frequently in childhood due to the hemopoietic marrow found throughout the shaft (Ortner, 2003: 242).

- **Sacroiliac Joint:** occurs in up to 2% of cases of skeletal tuberculosis, usually bilaterally and is almost always associated with spinal involvement (Aufderheide and Rodriguez-Martin, 1998: 139).

- **Ilium:** Rare, although extension to the ilium from a psoas abscess can occur (Ortner, 2003: 239).

- **Ischium and Pubic Symphysis:** This is rare in both the ischium and pubic symphysis (Ortner and Putschar, 1981: 49-50) but, where it does occur, lesions are usually close to the symphysis and are usually lytic and destructive (Ortner, 2003: 239).

- **Shafts of the Long Bones:** This is not common, is seen more frequently in children, and is similar in appearance to the aforementioned spina ventosa; it is also usually seen in concurrence with other skeletal foci (Ortner, 2003: 245). In adults, the lesion is rare with minimal periosteal bone formation (Ortner, 2003:245). The bones most commonly affected (in descending order) are: tibia, ulna, radius, humerus, femur and fibula (Konschegg 1934: 422 cited in Ortner, 2003: 245).

- **Ankle and Foot:** Usually occurs by expansion from an adjacent tubercular soft tissue focus or via haematogenous dissemination and, whilst the tibiotalar joint is usually the focus in children, the talus and other tarsal bones are most affected in adults (Aufderheide and Rodriguez-Martin, 1998: 139). If the tibia is the origin of infection, a great deal of destruction can occur with healing always leading to ankylosis of the tibiotalar joint (Ortner, 2003: 241).

The presence or absence and any pathology relating to the teeth were also recorded as certain morphology, such as enamel hypoplasia, could suggest periods of malnutrition having been experienced by an individual. Malnutrition should be considered in the context of weakened immune systems and increased susceptibility to infectious disease such as TB. It is therefore very important to consider all aspects of pathology in any given skeleton as certain conditions may offer insights into aspects of social, environmental or economic conditions which could directly, or indirectly, impact on an individuals likelihood of contracting TB.

Notes and photographs were also included with the recording sheets.

4.3.2 Methods: Biomolecular

4.3.2.1 Sampling Selected Skeletal Material:

Where it was necessary to take a bone sample from skeletons examined macroscopically, further discussion with the curators of the skeletal collection took place, and appropriate paperwork completed.

A large enough sample was required so that part of the sample, if appropriate, could be independently analysed in the collaborating laboratory for the larger project, in Arizona, USA. It is essential for two laboratories to independently verify the results of pathogen aDNA analysis, for reasons discussed previously. It was made clear that the value of the

skeletal collection was appreciated and that, because the analysis was destructive, the sample location could be discussed.

A 3g bone sample from the tuberculosis or possibly tuberculosis affected skeleton (this would be equivalent to about a 3-4cm length of rib) was required and, ideally, this was from the tuberculous lesion or next to the lesion or, if not allowed, then from the long bone cortex. Three grams were needed to limit problems of contamination because the outer 2-3mm are removed before sample preparation. It was also necessary (wherever possible) to obtain a tooth from the same skeleton to act as a control because, theoretically, any pathogen aDNA present in the body should also be found in aDNA extracted from the tooth, and the tooth potentially offers a source of aDNA less prone to contamination. A same size bone sample from a non-tuberculous skeleton from the same site was also collected, where possible, to see if this was clear of any pathogen aDNA. If so, it would suggest that the original target skeleton (if it tested positive), was not necessarily contaminated with pathogen aDNA from other skeletons found close by. A positive control sample from another skeleton, however, could occur because that individual also had TB but showed no skeletal manifestations of the disease. This method is therefore not foolproof, but is certainly the best option available when working with so many variables. Ideally, it was requested that this control sample should be from the same bone as the tuberculous lesion on the tuberculous skeleton, a rib fragment or, where not possible, long bone cortex. If a skeleton was fragmentary it was often possible to use fragments of the correct weight rather than damage complete bones.

It was necessary to sample the number of non-tuberculous skeletons equivalent to 50% of the skeletons with TB.

4.3.2.1.1 Sampling Method

To ensure that there was no contamination during sampling (from the sampler), it was necessary to wear a laboratory coat, hair net, face mask and double layered gloves (latex and nitril) which were changed between each sample. When fragments of bone were not

available, samples were taken using a small electric hand held circular saw, with the blade cleaned between each sample, or a junior hacksaw.

Teeth were taken to the University of Manchester and then the sample removed via the root. Removing the aDNA sample via the root offers the greatest protection from contamination as the roots are generally long and relatively closed, with the exception of a very small hole at the tip. A small hand held drill bit is then inserted up through the root and powder from deep inside the tooth, well away from any areas previously exposed to the air (and thus likely contamination), is collected and used to perform the analysis. The teeth were then returned to the curating institution. Each individual sample was placed in a labelled airtight bag, and groups of samples placed in an outer airtight bag. Samples were stored in a refrigerator at the University of Manchester prior to preparation and analysis, and following analysis.

Details regarding the name of the sampler, university and project details were left in the box from wherever a sample was removed.

Thirteen samples were taken from skeletons dating to the Neolithic, Bronze Age and Iron Age, with an additional 11 samples being provided from skeletons that are part of the wider European project to which this study is attached. These additional samples were all dated to the British Iron Age although did not all originate from the geographic area previously established.

Preparation and analysis of these samples (24 in total) took place at the Manchester Interdisciplinary Biocentre (MIB) under the supervision of Professor Terry Brown and Dr Abigail Bouwman. All preparation of the samples took place in controlled environments, the extraction laboratory and PCR laboratory being exclusively used for aDNA. The samples themselves had been stored in clearly labelled bags and were refrigerated at Manchester MIB prior to, and following, analysis.

Controls to prevent contamination were based on Yang *et al.* (2005). Prior to any preparatory work, the laboratories used were kept under UV radiation when not in use. The

areas to be used were then cleaned with both bleach and DNAway solution (Molecular BioProducts) in order to reduce the possibility of nuclease and nucleic acid contamination. All equipment used was subjected to UV irradiation in order to sterilise it, and any person present was required to wear the following protective kit: a full, hooded forensic suit, two hairnets, one facemask, shoe covers (shoes themselves being removed in the antechamber), eye goggles and two sets of gloves. The outer layer gloves were regularly discarded and replaced. Additional steps taken included strict protocols on not entering either the extraction or PCR room on the same day as having been in the main laboratory, and a complete change of clothes and a shower were required before re-entering, even if this was during the following day.

4.4 Sampled Material

4.4.1 Abingdon Pipeline, Oxfordshire

4.4.1.1 Sample Removed for aDNA Analysis:

Skeleton Number: 4197

Sample Code: OxABS1

Date of Removal: 4th June 2008

Method of Removal: Removed already broken fragment.

Reason for Removal: Evidence of non-specific infection.

Bone Removed: Fibula fragment.

Description: A broken, individual piece of fibula from the right hand side of the body measuring 640mm in length displayed active, woven bone formation on the mid anterior shaft of the bone, bordering the distal third of a resorptive bone lesion on its lateral side and approximately 50mm on its distal, medial margin. The lesion measures 250mm in height and 60mm at its widest and demonstrates a shallow, concave shape (Figure 4-26).

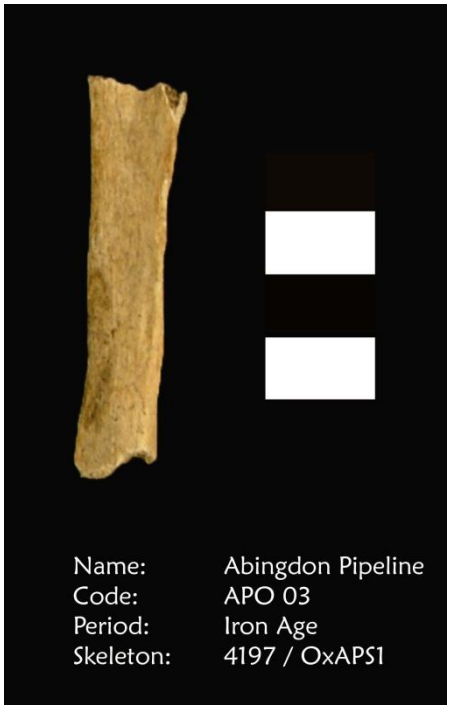

Figure 4-26 Right Fibula showing resorptive lesion in Sk. 4197

4.4.1.1.1 Control Sample (from alternative skeleton):

None Available

4.4.2 Ascott-under-Wychwood, Oxfordshire

4.4.2.1 Sample Removed for aDNA Analysis:

Skeleton Number:	A3 / Chamber 3 (A) (391/68)
Sample Code:	NHMA3
Bone Removed:	Half of L2 vertebral body (Figure 4-27).
Date of Removal:	23rd July 2009
Method of Removal:	Removed via a small electric dremel (drill).
Reason for Removal:	Possible evidence of inter-vertebral infection.

Description: The area removed for aDNA sampling was done so because it formed part of the affected vertebrae but was the least affected part of the pathological element, leaving the remainder available for future study. The section of vertebra removed was the right half of the second lumbar vertebra which was fused to the third lumbar vertebra. A small part of the smooth exterior wall of additional bone formation which starts just above the fusion of the two vertebrae on the right side, was included in the sample. In total, just less than half of this vertebra was removed weighing approximately 3g, as required for the aDNA analysis.

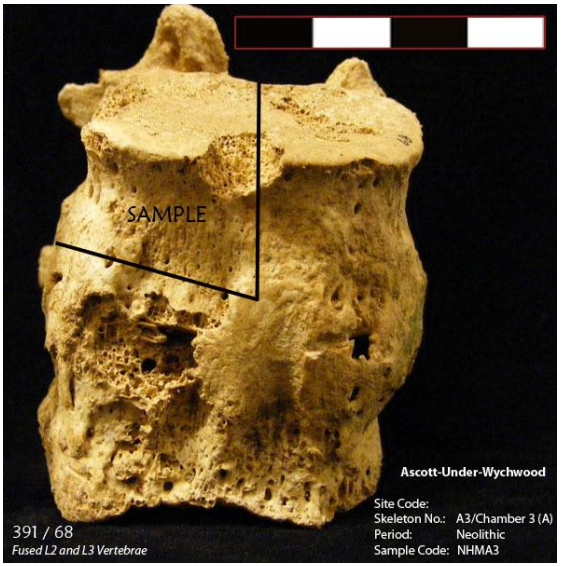

Figure 4-27 Photograph of the fused lumbar vertebrae from Individual A3 and the area removed for aDNA sampling.

4.4.2.1.1 Control Sample (from alternative skeleton):

Skeleton Number:	A2 **/** Chamber 3 (A) (391/112)
Sample Code:	NHMA2Cc
Bone Removed:	Half of L2 vertebral body (Figure 4-28).
Date of Removal:	23rd July 2009
Method of Removal:	Removed via a small electric dremel (drill).
Reason for Removal:	Control Sample

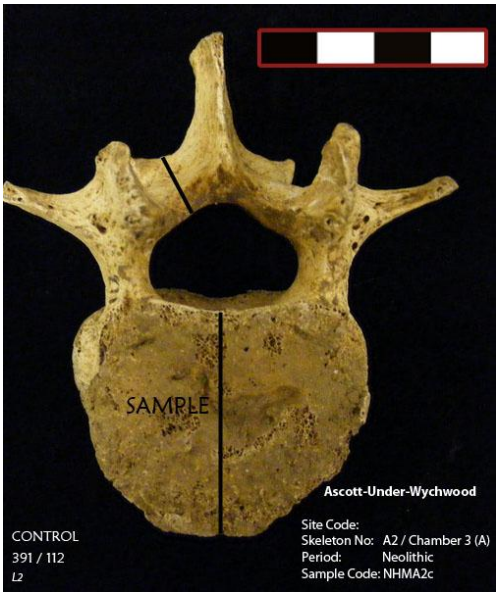

Figure 4-28 Photograph of area removed for aDNA sampling from Individual A2 as a control.

4.4.3 Boscombe Airfield Fire Hydrant, Wiltshire

4.4.3.1 Sample Removed for aDNA Analysis:

Skeleton Number:	(Unknown but Context 39)
Sample Code:	WxFHS1
Bone Removed:	Rib Fragments
Date of Removal:	9th December 2008
Method of Removal:	Removed Broken Fragments
Reason for Removal:	Evidence of Infection

Description: Three small pieces of broken left rib fragments were removed for sampling (Figure 4-29). All demonstrated evidence of nonspecific infection in the form of woven and / or lamellar bone formation on their visceral surfaces. The first fragment measured no more than 2.5cms in length and 1.5cm in width. The additional bone formation on this fragment was primarily a thin layer of woven bone which covered the majority of the available visceral surface. This piece of broken rib originated from the mid shaft area due to its more flattened appearance although, due to the fragmentary nature of the ribs, it is not possible to identify which rib specifically. The second piece of rib selected for sampling formed part of the rib angle and measured approximately 3cms at its longest part and 1cm in width. The

majority of pathology on this piece was healed, lamellar bone. The third piece of rib removed was from the mid shaft and was long but extremely thin as it was a fragment formed of only one side of the rib, i.e. cortical bone only.

Figure 4-29 Photograph of the three rib fragments removed for aDNA sampling from the Boscombe Down individual.

4.4.3.1.1 Control Sample (from alternative skeleton):

None Available

4.4.4 Bourton-on-the-Water, Gloucestershire

4.4.4.1 Sample Removed for aDNA Analysis:

Skeleton Number:	1
Sample Code:	BoWS1
Bone Removed:	Rib Fragments (Figure 4-29)
Date of Removal:	2nd May 2008
Method of Removal:	Removed Broken Fragments
Reason for Removal:	Evidence of Infection

Description: Two rib fragments were removed, one from the left and one from the right, both with the rib head and rib angle intact. Both displayed rib lesions on the visceral surface.

Figure 4-30 Photograph of the two rib fragments removed for aDNA sampling from Skeleton 1, Bourton-on-the-water.

4.4.4.1.1 Control Sample (from alternative skeleton):

Skeleton Number:	1161
Sample Code:	BoWS2c
Bone Removed:	Fragments of right femur (Figure 4-31)
Date of Removal:	2nd May 2008
Method of Removal:	Removed loose fragments
Reason for Removal:	Control Sample

Figure 4-31 Photograph of femur fragment removed for aDNA sampling (Bourton-on-the-Water, BoWS2c)

4.4.5 Eyebury, Peterborough

4.4.5.1 Sample Removed for aDNA Analysis:

Skeleton Number:	E.II.3.223
Sample Code:	NHMEye
Bone Removed:	Piece of ulna
Date of Removal:	23rd July 2009
Method of Removal:	Removed via a small electric dremel (drill).
Reason for Removal:	Possible evidence of infection due to destruction of joint area.

Description: A small piece of bone from the antero-medial proximal third of the ulna shaft was removed for sampling. This piece of bone displayed no pathology itself but was situated extremely close to the pathological joint of interest. By removing this piece of bone, the affected joint was left intact for future research potential (Figure 4-32).

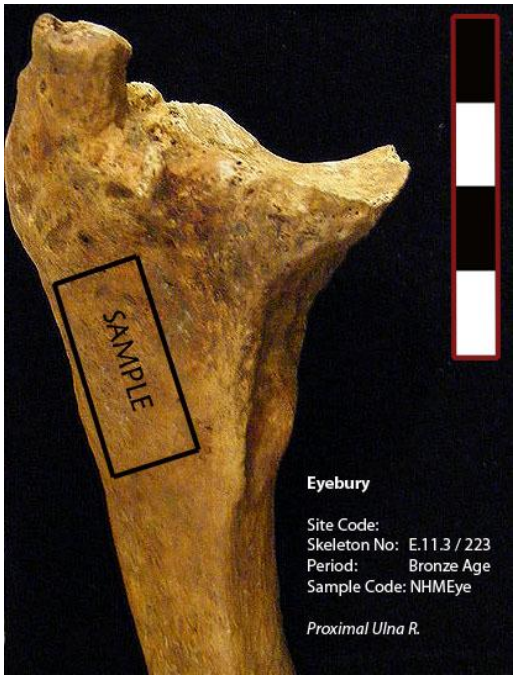

Figure 4-32 Photograph of affected ulna from individual 223 (Eyebury) and the area removed for aDNA analysis.

4.4.5.1.1 Control Sample (from alternative skeleton):

None Available

4.4.6 Methwold Severalls, Norfolk

4.4.6.1 Sample Removed for aDNA Analysis:

Skeleton Number:	Burial 1
Sample Code:	NkMS1
Bone Removed:	Half of L1 vertebra
Date of Removal:	9th June 2008
Method of Removal:	Small hand saw
Reason for Removal:	Evidence of infection.

Description: The piece of bone removed for aDNA sampling was the left half of the 1st lumbar vertebra, the vertebra which demonstrated relevant pathology. This vertebra displayed a destructive lesion on the L1 vertebral body, consisting of a deep depression present on the inferior aspect of the vertebral body. The lesion affected only the anterior portion of the vertebral body and was significantly deeper on the right side. Only the left half of the vertebral body was removed for sampling as opposed to any of the transverse process, articular facets or spinous process; due to this being a lumbar vertebra, this had sufficient weight to meet the sampling requirements. Removing only the left half of the body also allowed for the more extensive area of the lesion, on the right side, to remain intact for further research purposes but still allowed some of the affected pathological bone to be included in the sample. Using a small hand held hack saw, the vertebral body was cut directly through the centre (Figure 4-33).

Figure 4-33 Photograph of the remaining half of L1 once the sample NkMS1 was removed from individual 1, Methwold Severalls.

4.4.6.1.1 Control Sample (from alternative skeleton):

Skeleton Number: 883

Sample Code: NkMS2c

Bone Removed: Right Fifth Metatarsal Fragment (Figure 4-34)

Date of Removal: 9th June 2008

Method of Removal: Removed broken fragment

Reason for Removal: Control sample

Figure 4-34 Control sample from Burial 8, right fifth metatarsal fragment

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4.4.7 Twyford Down, Hampshire

4.4.7.1 Sample Removed for aDNA Analysis:

Skeleton Number: 883

Sample Code: WnTDS1

Bone Removed: Rib Fragments

Date of Removal: 10th October 2008

Method of Removal: Removed Broken Fragments

Reason for Removal: Evidence of infection.

Description: Four rib fragments were removed (Figure 4-35). All four demonstrated additional woven bone formation indicating active infection at the time of death. All woven bone on the two largest fragments is on the lateral edge of the fragments on the visceral surface, with only a very small quantity of additional bone evident on the opposing surface

of the rib. For this reason, it is unclear as to whether or not the lesions are a result of rib fracture or a disease process. However, the smallest of the fragments included the rib angle, on which a small amount of woven bone is present on the visceral surface in the centre of the fragment; this measures 50mm wide and is thus not directly associated with the site of a fracture or break.

Figure 4-35 Three of the four rib fragments removed for sampling from Sk. 883

4.4.7.1.1 Control Sample (from alternative skeleton):

Skeleton Number:	1136
Sample Code:	WnTDS2c
Bone Removed:	Rib Fragment (Figure 4-36)
Date of Removal:	10th October 2008
Method of Removal:	Removed broken fragment
Reason for Removal:	Control sample

Figure 4-36 Rib fragment removed for sampling from control skeleton 1136 – Twyford Down.

4.4.8 Waterhall Farm, Chippenham, Cambridgeshire

4.4.8.1 Sample Removed for aDNA Analysis:

Skeleton Number:	4
Sample Code:	DkChS1
Bone Removed:	Cervical Vertebra
Date of Removal:	31st October 2008
Method of Removal:	Removed Broken Fragments.
Reason for Removal:	Evidence of Infection.

Description: The 7th cervical vertebra (C7) was removed for sampling. This bone was selected because it was the neighbouring vertebra to the fused 1st and 2nd thoracic vertebrae which were of interest for this study. By selecting this vertebra, however, the fused T1 and T2 vertebrae were left intact for future research; what was present of C7 was also of the correct weight for the sampling requirements. Whilst the body was complete, there was no other bone attached on the right side and on the left side their was very little

spinous process remaining, only one small damaged area of the transverse process present, and finally the superior articular facet was intact (Figure 4-37).

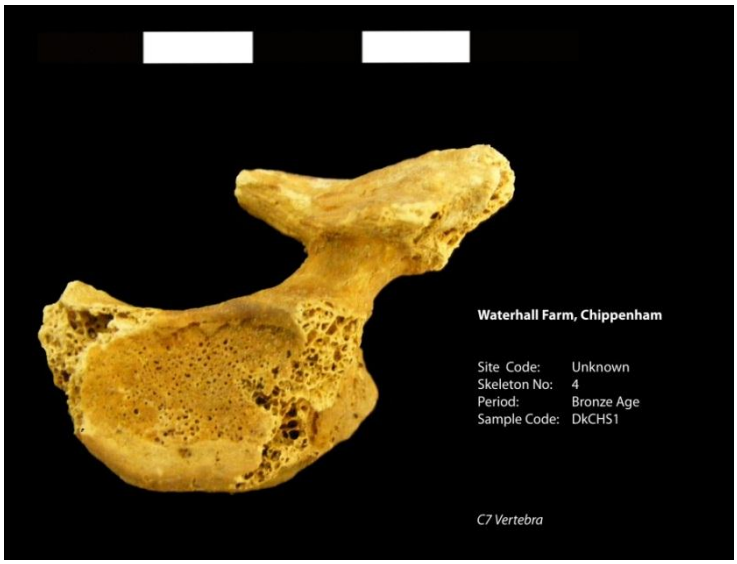


Figure 4-37 Photograph of 7th cervical vertebra removed for sampling (Waterhall Farm, Chippenham)

4.4.8.1.1 Control Sample (from alternative skeleton):

Skeleton Number:	5
Sample Code:	DkCHS2c (Figure 4-38)
Bone Removed:	Rib Fragment
Date of Removal:	31st October 2008
Method of Removal:	Removed broken fragment
Reason for Removal:	Control Sample

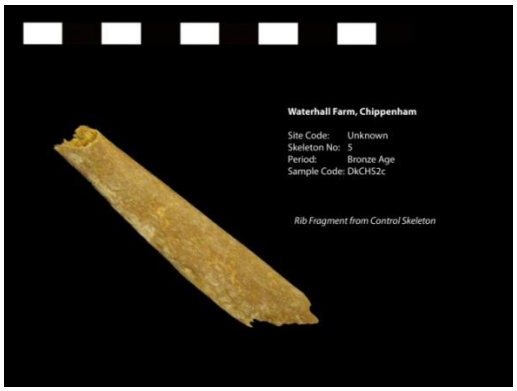


Figure 4-38 Photograph of rib fragment from control Skeleton 5 (Waterhall Farm, Chippenham)

4.4.9 West Tump Long Barrow, Gloucestershire

4.4.9.1 Sample Removed for aDNA Analysis

Skeleton Number: 7

Sample Code: ChWTS1

Bone Removed: Rib Fragment

Date of Removal: 13th August 2008

Method of Removal: Removed Broken Fragment.

Reason for Removal: Evidence of Infection.

Description: Pathology was noted on the visceral surface of a rib fragment in the form of a thin layer of periosteal new bone formation (Figure 4-39). The lesion measured a maximum of 20mm in length, the primary area being approximately 15mm in length by 7.5mm wide. The entire rib fragment measured a maximum of 45mm in length. The depth of the additional bone formation was less than 1mm.

Figure 4-39 Photograph of rib fragment removed from individual 7, West Tump.

4.4.9.1.1 Control Sample (from alternative skeleton):

Skeleton Number: 4

Sample Code: ChWTS1

Bone Removed: Rib Fragment

Date of Removal: 13th August 2008

Method of Removal: Removed broken fragment

Reason for Removal: Control sample

4.4.10 Wetwang Slack, North Yorkshire

4.4.10.1 Wetwang Slack (a)

4.4.10.1.1 Sample Removed for aDNA Analysis:

Skeleton Number: 13 (F9(i))

Sample Code: WWSS1 (Figure 4-40)

Bone Removed: Rib Fragment

Date of Removal: 22nd August 2008

Method of Removal: Removed Broken Fragment.

Reason for Removal: Evidence of Infection on Ribs

4.4.10.2 Wetwang Slack (b)

4.4.10.2.1 Sample Removed for aDNA Analysis:

Skeleton Number: 16 (F41 BW)

Sample Code: WWSS2 (Figure 4-41)

Bone Removed: Rib Fragment

Date of Removal: 22nd August 2008

Method of Removal: Removed Broken Fragment.

Reason for Removal: Evidence of Infection on Left Hip

4.4.10.3 Wetwang Slack (c)

4.4.10.3.1 Sample Removed for aDNA Analysis:

Skeleton Number: 47 (F192 DW)

Sample Code: WWSS5 (Figure 4-42)

Bone Removed: Rib Fragment

Date of Removal: 22nd August 2008

Method of Removal: Removed Broken Fragment.

Reason for Removal: Evidence of Infection on Vertebrae

4.4.10.4 Wetwang Slack (d)

4.4.10.4.1 Sample Removed for aDNA Analysis:

Skeleton Number: 94 (F291 JZ)

Sample Code: WWSS6 (Figure 4-43)

Bone Removed: Rib Fragment

Date of Removal: 22nd August 2008

Method of Removal: Removed Broken Fragment.

Reason for Removal: Evidence of Infection.

4.4.10.5 Wetwang Slack (e)

4.4.10.5.1 Sample Removed for aDNA Analysis:

Skeleton Number: 220 (WT56)

Sample Code: WWSS8 (Figure 4-44)

Bone Removed: Rib Fragment

Date of Removal: 22nd August 2008

Method of Removal: Removed Broken Fragment.

Reason for Removal: Evidence of Infection.

4.4.10.6 Wetwang Slack (f)

4.4.10.6.1 Sample Removed for aDNA Analysis:

Skeleton Number: 307 (WW 104)

Sample Code: WWSS9 (Figure 4-45)

Bone Removed: Rib Fragments

Date of Removal: 22nd August 2008

Method of Removal: Removed Broken Fragments.

Reason for Removal: Evidence of Infection.

4.4.11 Wetwang Slack Control Samples

4.4.11.1 Sample Removed for aDNA Analysis:

Skeleton Number: 36 (F238 HC)

Sample Code: WWSS3c

Description: Rib Fragment

4.4.11.2 Sample Removed for aDNA Analysis:

Skeleton Number: 37 (F229 GL)

Sample Code: WWSS4c

Description: Rib Fragment

4.4.11.3 Sample Removed for aDNA Analysis:

Skeleton Number: 163 (WE 223 ACS)

Sample Code: WWSS7c

Description: Rib Fragment

Figure 4-40 Photograph of rib fragment removed for aDNA sampling (Wetwang Slack, WWSS1)

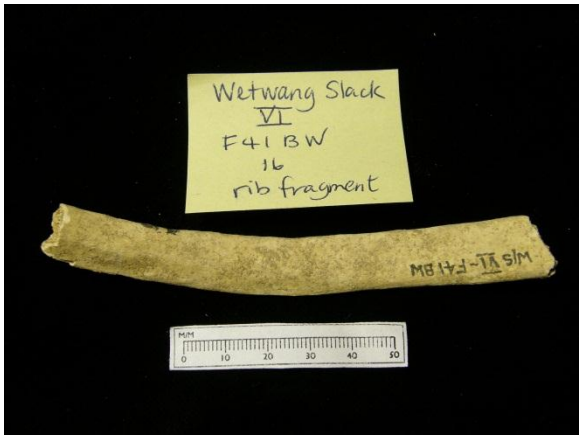


Figure 4-41 Photograph of rib fragment removed for aDNA sampling (Wetwang Slack, WWSS2)

Figure 4-42 Photograph of rib fragment removed for aDNA sampling (Wetwang Slack, WWSS5)

Figure 4-43 Photograph of rib fragment removed for aDNA sampling (Wetwang Slack, WWSS6)

Figure 4-44 Photograph of rib fragment removed for aDNA sampling (Wetwang Slack, WWSS8)

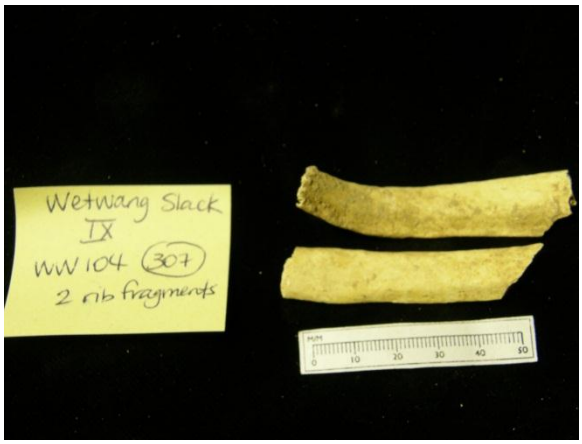


Figure 4-45 Photograph of rib fragment removed for aDNA sampling (Wetwang Slack, WWSS9)

4.4.12 Wilsford Barrow, Normanton, Wiltshire

4.4.12.1 Wilsford Barrow (a)

4.4.12.1.1 Sample Removed for aDNA Analysis:

Skeleton Number:	11
Sample Code:	DkWBS2
Bone Removed:	Cranial Fragments
Date of Removal:	31st October 2008
Method of Removal:	Removed broken fragments (Figure 4-46)
Reason for Removal:	Close to evidence of infection

Description: Two loose skull fragments were removed for sampling as the right mastoid process demonstrated evidence of infection, as did a number of cervical vertebrae. It was not deemed necessary or appropriate to remove the mastoid process itself. The first skull fragment measured approx. 2.5cm by 2.5cm at its widest / longest points and the second, approx. 3.5cm by 2cM. Neither piece demonstrated pathology and both originated from the occipital bone.

Figure 4-46 Photograph of cranial fragments removed for aDNA sampling (Wilsford Barrow DkWBS2)

4.4.12.2 Wilsford Barrow (b)

4.4.12.2.1 Sample Removed for aDNA Analysis:

Skeleton Number: 7

Sample Code: DkWBS1

Bone Removed: Long Bone Fragments

Date of Removal: 31st October 2008

Method of Removal: Removed broken fragment

Reason for Removal: Evidence of infection

Description: Two small fragments of affected long bone were removed for sampling. These demonstrated extensive woven bone formation overlying the cortical surface. The first fragment was 5cm in length and showed patchy put extensive active bone formation with clear porosity. The second fragment was slightly smaller in length (approx. 4.5cm) but slightly wider and demonstrating slightly less periostitis. It was not clear exactly which long bones these fragments represented (Figure 4-47).

Figure 4-47 Photograph of long bone fragments removed for aDNA sampling (Wilsford Barrow DkWBS1)

4.4.12.2.1.1 Control Sample (from alternative skeleton):

Skeleton Number: Sk. 5

Sample Code: DkWBS3c

Description: Rib Fragment

4.5 Biomolecular Laboratory Work

4.5.1 Preparation

The first stage of sample preparation required the outer 2-3mm of bone surface to be removed. Due to the extensive handling of some bones curated in museums and taphonomic processes, this procedure limits the amount of contaminated bone that will enter the extraction solution initially. This was performed using a scalpel blade in a clean area and on a layer of foil; once an initial layer had been removed, a second layer was scraped away in order to ensure all traces of the outer layer were removed. This took place in the area closest to the door in the extraction room, which has positive pressure, thus any traces of bone powder could not move to the far end of the room where the next stage of the extraction took place. The bone powder removed from the bone was collected in a sample tube should it be of use for further studies. The now scraped bone was placed in a clean sample bag, labelled and placed in the Stratalinker and exposed to UV radiation at 120,000µ joules/cm^2 for five to ten minutes either side, along with the scalpel blade used, also labelled appropriately. This process was repeated for every sample with the area being cleaned after each. The foil on which the scraping of each sample took place was disposed of once the sample was scraped, bagged and placed under UV radiation.

Following this, the bone was crushed in order to create a powder. The bag containing the sample was placed in a second bag in order to contain the resulting powder. Using a specific hammer wrapped in foil (changed after each sample) and designated area, the bone was crushed until it was a relatively uniform fine bone powder.

The now crushed bone was emptied carefully onto sterilised foil and, using a scalpel blade, the larger pieces of bone were separated. The finer bone powder which has a greater surface area was measured to a weight of 0.5g and placed in a 1.5ml centrifuge tube. This was repeated so that a total of three tubes contained 0.5g of the powder were available, and an additional tube was used to collect the excess. Two of these tubes were for use at the MIB and one was kept for use in an external lab for independent verification of results, with the excess being available as spare sample if necessary.

An extraction solution was then mixed in order to release any DNA present from the bone. The solution was comprised of 10ml EDTA (Ethylenediamine tetracetic acid), concentration 0.5M, which served as a chelating agent that targets and binds to the magnesium and de-calcifies the bone, encouraging DNA to be released. Additionally, 0.05g SDS (Sodium Dodecyl Sulphate), a biological detergent, was used to undermine the stability of the cell membrane by emulsifying the lipids and altering the polarity of the membrane due to its hydrophobic properties. Finally, the enzyme Proteinase K was used to digest proteins in the solution which could degrade the integrity of the DNA. Proteinase K is of particular use in this application as it is not inhibited by EDTA.

The solution was made up in a 15ml falcon tube and the quantities described are suitable for up to nine samples. The EDTA was added to the tube and the SDS weighed and also added. This mixture was then placed in a water bath for ten minutes (in order to dissolve the SDS) before the addition of 50µl Proteinase K (20mg/ml). The solution was then irradiated for five minutes and 1ml subsequently added to each sample, plus one extraction blank. The samples were then placed in a shaking incubator at a temperature of 55^{o}C, a speed of 650rpm and a length of 22-24hrs.

4.5.2 Extraction

The samples were removed from the shaking incubator and placed into a centrifuge for 5 minutes at a speed of 2Krpm to cause the bone powder to sink and the solution containing any DNA to sit on top. The following procedure was then completed in a category II safety cabinet to further minimise the risk of contamination. Using a pipette, 0.5ml of the supernatant was transferred to a 15ml Falcon tube, being careful not to transfer any of the visible bone debris. This was then added to 2.5ml PB buffer supplied by Quiagen which served to bind the DNA to the silica contained in the centrifuge column, which can only be achieved at the correct pH. Once 700µl of this solution was added to labelled spin column tubes, each tube was placed in the centrifuge and spun for one minute at maximum speed.

This caused the rest of the solution to pass through the silica whilst catching any existing DNA in the silica mesh.

Once the cycle was complete, the solution which had been passed through and subsequently collected in the tube below the level of the silica, was disposed of. Another 700µl of the PB buffer / sample solution was added to the spin column and spun at full speed. This process was repeated until all the solution had been utilised.

When finished, 0.75ml of PE buffer was added to the spin column which contained ethanol and served to cleanse the column and silica of any remaining PB buffer and contaminants. The spin column was centrifuged for a further minute at full speed, after which, the solution that had passed through was disposed of, and then the tube was centrifuged for a further minute to ensure all traces of PE buffer were removed. At this point the collection tubes were disposed of and the inner silica column placed into a new sterilised tube.

EB buffer was then added to the spin column but in such a way that the liquid was placed directly on to the centre of the silica mesh without piercing or damaging it. The column was left to sit for one minute in order to allow the PE buffer to effectively break the bonds tying the DNA to the silica mesh. In this way, the DNA was released into the collection tube once the column had been centrifuged again at full speed for a total of one minute. The process was again repeated in order to ensure that all of the DNA was released before the inner silica column was disposed of.

The final step of this process was to transfer 22µl of the solution now present in the collection tube, to a smaller labelled tube which was then placed in the freezer until the PCR process.

4.5.3 Polymerase Chain Reaction (PCR)

The PCR stage took place in a room specifically and exclusively designed and used for the purpose of aDNA and within a Laminar flow cabinet with UV irradiation. Firstly a PCR was

performed using IS*6110*, (Taylor *et al*., 1996) targeting 123bp (base pairs) using a forward primer F1 (dilution 25:206.5) and reverse primer R1 (dilution 25.224.2). This was followed by a nested PCR targeting 92bp using the primers F2 (dilution 25:154.2) and R2 (dilution 25:193.6). IS*6110* is a repetitive insertion sequence which is usually present in high numbers throughout *Mycobacterium tuberculosis* complex (MTB) strains (Spigelman *et al*., 2002). The specific primers for target region IS*6110* are described in the table below (Table 4-1).

Target	Locus	Amplicon Size (bp)	Oligonucleotide Primer	Anneal Temperature (^{o}C)
M.***tb***	IS*6110*	123bp	F1 $^{5'}$CCTGCGAGCGTAGGCGTCGG'^{3}	**58**
M.***tb***	IS*6110*	123bp	R1 $^{5'}$CTCGTCCAGCGCCGCTTCGG'^{3}	**58**
M.***tb***	IS*6110*	92bp	F2 $^{5'}$TCGGTGACAAAGGCCACGTA'^{3}	**58**
M.***tb***	IS*6110*	92bp	R2 $^{5'}$TTCGGACCACCAGCACCT'^{3}	**58**

Table 4-1 Details of specific locus IS*6110* including base pair (bp) length, primer sequences (forward and reverse) used to identify the locus and specific anneal temperatures of each primer.

This process was repeated using IS*1081*, an insertion sequence found only in MTB species and which has been shown to reliably differentiate between M. *bovis* and other strains present in the MTB complex (van Soolingen *et al*., 1992). The outer primers, F2 (dilution 25:218.1) and R2 (dilution 25:169) targeted 135bp, and the inner primers for a hemi-nested PCR, F2 and R1 (dilution 25:154.1), 113bp. Specific details of the primers are given in the table below (Table 4-2)

Target	Locus	Amplicon Size (bp)	Oligonucleotide Primer	Anneal Temperature (^{o}C)
M.***bovis***	IS*1081*	135	R2 $^{5'}$CTGCTCTCGACGTTCATCGCCG'^{3}	**58**
M.***bovis***	IS*1081*	135	R2 $^{5'}$GGCACGGGTGTCGAAATCACG'^{3}	**58**
M.***bovis***	IS*1081*	113	R1 $^{5'}$TGGCGGTAGCCGTTGCGC'^{3}	**55**
M.***bovis***	IS*1081*	113	F2 $^{5'}$CTGCTCTCGACGTTCATCGCCG'^{3}	**55**

Table 4-2 Details of specific locus IS*1081* including base pair (bp) length, primer sequences (forward and reverse) used to identify the locus and specific anneal temperatures of each primer.

Finally, the same procedure was followed to specifically test for the presence of *Brucella* DNA (which manifest as the disease brucellosis), after five samples from the repeat MTB assays, returned sequences demonstrating similarity with a number of known *Brucella* species sequences. Element IS*711*, formerly referred to as IS6501 (Ouahrani *et al*., 1993) was targeted as this is known to be present in all *Brucella* species and has previously been included in other aDNA studies (Bendrey *et al*., 2008). Only outer primers were used, to target 141bp, the details of which are given below (Table 4-3).

Target	Locus	Amplicon Size (bp)	Oligonucleotide Primer	Anneal Temperature (^{o}C)
***Brucella* species**	IS*711*	141	F1 $^{5'}$CTGCTCTCGACGTTCATCGCCG'^{3}	**58**
***Brucella* species**	IS*711*	135	R1 $^{5'}$GGCACGGGTGTCGAAATCACG'^{3}	**58**

Table 4-3 Details of specific locus IS711 including base pair (bp) length, primer sequences (forward and reverse) used to identify the locus and specific anneal temperatures of each primer.

All areas in use were cleaned with a bleach solution to reduce the possibility of contamination and then all solutions to be used were removed from the freezer in which they are kept with the exception of the Taq polymerase. All pipettes, tips and tubes were placed in the stratalinker and irradiated for ten minutes. In addition to the equipment, the deoxynucleoside triphosphates (dNTPs), dATP, dCTP, dGTP and dTTP (Fermentas Life

Sciences), buffer and magnesium chloride solutions were also placed in the stratalinker for a total of 10 minutes prior to their use. Once complete, the appropriate number of 1.5ml collection tubes were labelled, with 3 additional blanks added. A larger collection tube was used to create a master mix which would be transferred into each of the samples. The quantities given here are for 10 samples only.

Firstly 388.25µl of sterilised water was added to the collection tube to form the base of the master mix. This was followed by 55µl of AmpliTaq Gold Buffer from Applied Biosystems, which provided a suitable chemical environment in which the Taq polymerase could function, and 44µl of dNTPs (200mM). Magnesium chloride (1.5mM/2mM), also a vital cofactor for the Taq polymerase, was added at a volume of 33µl and then both the forward and reverse primers were added at 22µl each (400µM). Bovine Serum Albumin (BSA) was then added to the mix at a volume of 5.5µl, concentration 1%, in order to bind inhibitors, aiding amplification (Weissensteiner *et al.*, 2003:7), before the tube was closed and subject to 5 minutes UV irradiation. Once complete, 2.75µl (1.25 units) of Taq polymerase (AmpliTaq Gold® DNA Polymerase), an enzyme which is used for hot start PCR (Taq polymerase is heat stable), was added making the master mix complete and ready to transfer into the 1.5ml tubes at a volume of 47.5µl per tube. The Taq polymerase served to enzymatically build the copied DNA strands from the bases provided by the dNTPs. The final stage of this preparatory process was to add the correct sample to each of the labelled tubes. For the additional PCR blanks added at the beginning of the PCR stage, the sterile water was used in place of a sample, with one blank being placed first in the sample row and two at the end. All samples and blanks were added at a volume of 2.5µl making each tube up to a total volume of 50µl.

To mix the sample and master mix efficiently, the pipette tip used to add the sample was used to draw the mixture in and out about 3 times, being careful not to create any bubbles. Once each tube was closed and this part of the process finished, the samples were carefully and quickly taken up to the main laboratory where they were placed in a PCR machine used exclusively for aDNA.

The amplification cycling conditions for IS*6110* outer PCRs were as follows: a period of denaturation at 95 ^{o}C for seven minutes, followed by 35 cycles of annealing at 68 ^{o}C, extension at 72 ^{o}C and denaturation at 95^{o}C. This was followed by 1 further cycle of 68^{o}C for 1 minute, 72^{o}C for 10 minutes and finally 15^{o}C for 15 minutes. All cycling parameters were identical for the outer primers of IS*1081* with the exception of the annealing temperature being 58^{o}C. The cycling conditions varied slightly for the inner PCRs of both IS*6110* and IS*1081*, with 24 cycles for IS*1081* being employed and 29 cycles for IS*6110*. For the inner IS*6110* and inner IS*1081* primers, the annealing temperature was 55^{o}C rather than 58^{o}C and, for IS*6110* unlike the outer PCRs, the final stage involved 1 cycle of 58 ^{o}C for 2 minutes, 72 ^{o}C for 10 minutes, 15 ^{o}C for 10 minutes and finally 21 ^{o}C for 30 seconds. The *Brucella* PCR involved a period of denaturation at 95 ^{o}C for seven minutes, followed by 35 cycles of 60 ^{o}C for 1 minute, 72 ^{o}C for 1 minute and 94 ^{o}C for 1 minute, with a final cycle of 60 ^{o}C for 1 minute, 72 ^{o}C for 1 minute and 15 ^{o}C for 15 minutes.

4.5.4 Gel Electrophoresis

Firstly 50ml of 1xTBE buffer (diluted from10xTBE buffer: 54g Tris; 27.5g Boric Acid and 20 ml 0.5M EDTA, pH8) was measured and added to 1g of agarose gel powder in a clean beaker. A piece of Saranwrap was placed over the top of the beaker and pierced a small number of times. The beaker was then placed in a microwave for 2 minutes on a medium setting in order to melt the agarose powder into the buffer to create the gel solution. Once complete, the solution was checked to ensure that all the powder had melted and the solution was completely mixed. A total of 0.5µl of Gel Red (a sensitive, fluorescent nucleic acid dye which now replaces the use of ethidium bromide (EB)) was added to the gel (supplied by Cambridge Biosciences) which enables the gel to be seen under UV irradiation. The gel was left to cool to the point where it remained liquefied in order to take the shape of the loading trays, but not so hot that it caused damage to the plastic.

The loading trays were prepared by placing the well templates into the correct position, at a uniform height, ready for the gel to be poured into them, Whilst the gel continued to cool, 1.5ml tubes were prepared for each of the samples and blanks, in addition to two more, the contents of which would serve as ladders once the gel was complete. A total of 0.5µl of loading dye (consisting of 0.025g xylene cyanol; 0.025g Bromophenol Blue; 1.25ml of 10%

SDS; 12.5ml glycerol and 6.25ml H_2O) was added to each of the tubes by which time the agarose gel had cooled enough to now be poured into the trays and left to set for around 20 minutes.

The preparation of the tubes continued by adding 4µl of ladder solution to those tubes which served as benchmarks on either end of the gel, which would allow easier identification of DNA of the correct length. The ladder solutions PstIλ and HinDIIIλ consisted of 66µl of 0.3mg/µl Lambda DNA; 4µl of enzymes; 10µl of 1x buffer; 10U/ml of *Pst*Iλ and *HinD*IIIλ, respectively, and made up to 100µl with 20µl H20, resulting in a solution of 20µg of DNA digested by 40U of enzymes per 100µl of solution. It was then necessary to add 16µl of distilled water to these tubes in order to make the solution up to the same quantity as those which contained blanks and samples. The blanks and samples were then added to their respective tubes and mixed using the pipette tip. The pipette tip was then left in the sample tube in preparation for filling the wells of the gel. By this time, the agarose gel had set and was ready to be placed into a bath of TBE buffer (just enough to cover it) once the well templates had been carefully removed, leaving one well empty on either side, the solutions were pipetted carefully, and in order, into each of the wells.

The order started with a ladder (*Pst*Iλ), followed by blanks, samples, blanks and then the other ladder (*HinD*IIIλ). Once complete, the power supply was set to 84 volts and left to run for around and hour and a half. When this process was finished, the gel was removed from the bath and placed in a machine (GENE Flash) which emits UV radiation, enabling any banding of aDNA to be seen and then printed out. The machine was turned on, set to 'live' and the 'UV' switched on. The machine is only handled without gloves, and the UV radiation is used only long enough to be able to get a clear picture of the bands in the gel before printing a photograph, as the UV is destructive to the integrity of the aDNA. If unsuccessful, the gel was disposed of, but if bands were visible in the target region then the bands were cut out and purified.

4.5.5 Cutting Out Bands

Gels which demonstrated banding in the target areas were taken to a specially prepared darkrooM. Whilst wearing the appropriate safety equipment, including a visor and gloves which covered the wrists to prevent UV burns, the gels were placed onto light-boxes which emit strong UV irradiation, enabling greater visibility of the bands. Printouts of the gel were also taken to the darkroom to ensure accuracy of identification as it was this printout which enabled original identification of any aDNA present.

Using a sterile razor blade (one for each different sample), the bands demonstrating aDNA of the correct base pair length (or extremely close) were carefully cut out. This was done individually, making small incisions around the band, taking care not to cut into a neighbouring sample. The small pieces of gel were then placed into pre-prepared, correctly labelled tubes and taken back to the main laboratory for purification. The gels from which the aDNA bands had been cut were disposed of in the appropriate waste, and the area cleaned in preparation for its next use.

4.5.6 Purification

Using the QIAquick Gel Extraction kit, the aDNA extracted from the gel had to be purified in preparation for sequencing. The first step was to add Buffer QG to the gel square at a ratio of 6:1 (e.g. 600ml Buffer QG to 100ml sample) because the agarose gel is less than the 2% required for a ratio of 3:1. In order to accurately achieve this ratio, the gel slice was weighed with any excess gel having been removed in the previous stage. In order for the aDNA to be released from the gel into the buffer solution, the tube was placed in a variable temperature water bath at a setting of 50^{o}C for 10 minutes, or until the gel was fully dissolved. To encourage the gel to dissolve, the tube was removed every 2-3 minutes during the incubation period and placed in a vortex machine. Once completely dissolved into the buffer, the colour of the mixture should and did remain yellow, as opposed to orange or violet. (If the latter occurs, 10ml of 3 M sodium acetate pH 5.0 needed to be added and mixed with the solution in order to regain the correct pH as the DNA can only be efficiently absorbed into the QIAquick membrane at a pH of less than 7.5).

Isopropanol was then added at a ratio of 1:1 to the gel slice which should have served to encourage a greater yield of DNA fragments of less than 500bp. A spin column was then placed in a 2ml collection tube in preparation for centrifuging. The sample was then eluted into the spin column and centrifuged at full speed for one minute. This process causes the aDNA to bind to the silica membrane as in the extraction process.

Once the cycle was complete, the flow though, collected in the collection tube, was discarded and the spin column replaced in the collection tube. At this point, 0.5ml of Buffer QG was added to the spin column and again, centrifuged for one minute on full speed. This additional step was to remove all traces of agarose, necessary when the sample was subsequently to be subject to direct sequencing.

Buffer PE, a wash buffer, was then added at a volume of 0.75ml and the column centrifuged at full speed for a further minute. Again, because the sample would be subject to direct sequencing, the wash buffer was allowed to stand for 2 to 5 minutes prior to centrifuging which would ensure maximum efficiency of this step. The flow thorough in the collection tube was discarded and the spin tube centrifuged for a further minute. Once this was complete, the collection tube and its contents were discarded and replaced with a clean 1.5ml microcentrifuge tube.

To release the aDNA from the silica mesh, 30µl of Buffer EB was applied directly onto the mesh. After leaving the column to stand for one minute, it was centrifuged at full speed for one further minute. The flow through of eluted aDNA was then ready to be sequenced.

4.5.7 Direct Sequencing

Once purified, samples were sent to an external laboratory for direct sequencing. Direct sequencing determines the order of the nucleotide bases in a given sample. The sequences returned, that were readable, were edited in software called ‘Bioedit’. This enabled the base pairs to be checked against their corresponding chromatogram to ensure bases had been read correctly; bases can missed out or additional ones added which obscures the true sequence. Sequences were then run through an online programme called BLAST, a

programme supported by the National Centre for Biotechnology Information (NCBI), in order to identify their origin against the database of all known and named sequences.

4.5.8 Real-Time PCR

Real-time PCR was used three times during the course of the study. It is “powerful, simple and rapid”, (Wittwer and Kusukawa, 2004: 71) allowing data to be collected and analysed throughout the PCR cycling process. This method also reduces the possibility of contamination as the samples are sealed in their wells during the set up process. Real-time PCR was employed as an additional method to the traditional PCR technique in order to verify any positive or negative results for tuberculosis. It was also then used to identify any human DNA present.

4.5.9 TB DNA:

The set up process for real-time PCR mirrors the standard PCR in many ways. Firstly, a master mix is created. For the *Mycobacterium tuberculosis* Complex PCR, which looks to identify a multi-copy sequence 63 bp long, six master mixes were created in accordance with the six groups of samples. Each master mix, which was created separately using clean pipette tips and 1.5ml tube, contained 250µl of 50% TaqMan (Applied Biosciences), 7.5µl forward primer (20umol), 22.5µl reverse primer (20umol) (see Table 4-4 for primer details), and 12.5µl of FAM-MGB Probe (10 umol), with the final addition of 157.5µl of H_2O. Each master mix was distributed between the 10 wells corresponding to the 10 samples per group (including one PCR blank) at a quantity of 45µl per well. A sample of 5µl was then added to each well to make a total of 50µl per well. The first group, having only six samples in total, required a proportionately lesser quantity of master mix to be created.

The wells, in which the master mix and samples were eluted, were part of a MicroAmp® Optical 96-Well Reaction Plate (labelled in a grid format, e.g. A1 and thus each sample had a unique well ID), which was then covered directly with an adhesive filM. The plate was then transferred to the main laboratory and placed in the Techne real-time PCR machine where

the correct programme from the Quansoft software was selected. For this real-time PCR, the following cycling parameters were selected: 95°C for 10 minutes; 55 cycles of 95°C for 15 seconds and 60°C for 1 minute.

4.5.10 Human DNA:

Real-time PCR was then used to identify human DNA as opposed to pathogen DNA in order to support any positive results for TB. This is a process which is by some, considered unnecessary, difficult and even complicating for pathogen aDNA studies (Taylor *et al*, 2010), but was a process included for thoroughness as it is strongly advocated by others (Wilbur *et al*, 2009). The first of the two PCRs to do this focussed on mitochondrial DNA (mtDNA), which is maternally inherited and not specific to one individual. As it is a multi-copy genetic element, it is present in greater numbers per cell and is thus suited to aDNA studies as there is an increased chance of identifying fragments.

The master mixes for this PCR included the same components as that for the TB, with the addition of BSA at 5µl and different quantities of reverse primer at 7.5µl and H_2O at 167.5µl. Details of the primers which were designed in the laboratory and the probe used are included below (Table 4-4). The cycling conditions in the Techne real-time PCR machine were one cycle at 50 °C for 2 minutes followed by 95 °C for 10 minutes. This was then followed by 55 cycles at 95 °C for 15 seconds and 60 °C for 1 minute.

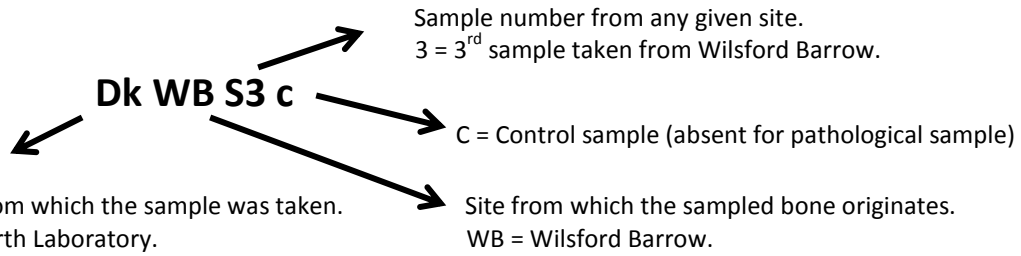
The second of the two real-time PCRs used to identify human DNA was aimed at evidence of the LCT gene, which controls lactase in humans, the enzyme responsible for the efficient digestion of lactose found in milk (Kuokkanen *et al*, 2006). The majority of humans produce this enzyme, resulting in them being lactase persistent as opposed to lactose deficient which results in lactose intolerance. This is a single copy element and thus not as extensive as the mtDNA PCR. The components of the master mix were, as before, with the addition of 5µl BSA, and this time 152.5µl H_2O. The primers were again designed in the laboratory specifically for this PCR, the details of which can be found in the table below. Cycling conditions were exactly the same as for the mtDNA PCR.

Target	Amplicon Size (bp)	Oligonucleotide Primer	Probe	Anneal Temperature (^{o}C)
M.***tb***	63	$^{5'}$GGGTAGCAGACCTCACCTATGTG'^{3} $^{5'}$GCCACTGTTTCCGGTGCAT'^{3}	ACCTGGGCAGGGTT	**60**
MtDNA	57	$^{5'}$AAACCCCCTCCCCATGCTTACAA'^{3} AACTACACACTATCAACTC'^{3}	CAAGTACAGCAATCAA	**60**
LCT	80	$^{5'}$CAAACATTATACAAATGCAACCTAAGG'^{3} $^{5'}$GCGACCGTTATGTCTATTCTATTACATC'^{3}	AGAGTTCCTTTGAGGCCAG	**60**

Table 4-4 Details of primers and probes used for real-time PCR: MTB, MtDNA and LCT.

5 Results

Example and Explanation of Sample Code:



5.1 Introduction

The skeletons referred to in this chapter are those that were selected for inclusion in the project based on their displaying relevant pathology, from the total skeletons examined during research visits to examine skeletal collections, which met the original criteria, and which were able to be accessed. The skeletons included here represent a very small proportion of those examined overall; only those skeletons included however, were subject to full, recorded, macroscopic analyses including the collection of age and sex data.

The first part of this chapter provides an outline of the characteristics of the individual skeletons and their pathological lesions that were ultimately analysed using biomolecular techniques. Basic data regarding the individuals is presented in Table 5-1 summarises estimated biological sex, age at death and the primary possible tuberculous pathology. Each individual is then explored in more depth with descriptions of overall skeletal preservation and pathology noted. This is followed by collation of all the basic data regarding distribution of age, sex and period from which the samples originate and pathological distribution in relation to sex, age and period of the individuals included in the study. Seventeen individuals in total were included in the project and subject to both osteological and aDNA analyses, with a further ten control samples from associated skeletons at some sites, where available and possible.

Site	Period	Sk. No.	Sex	Age	Primary Pathology	Project Code
Abingdon Pipeline, Oxfordshire **APO 03**	IA	4197	F	35+	Extensive periostitis on long bones	**OxABS1**
Methwold Severalls, Norfolk **NWHCM: 1969.93.bulk:A**	EBA (OxA-2862: 3840 + - 80BP)	1	F	25-45	Destructive lesions in L1	**NkMS1**
West Tump Long Barrow, Gloucestershire **1913.33.280 (+461)**	NEO	7	U	Young Adult	Rib periostitis	**ChWTS1**
Wilsford Barrow, Normanton, Wiltshire (a)	EBA	7	U	9-12	Long bone periostitis AND cranial lesions (new bone formation and destruction)	**DkWBS1**
Wilsford Barrow**, Normanton, Wiltshire (b)**	EBA	11	F	17-34	Infected mastoid process AND vertebral destruction C2 / C3 / C5 / C7	DkWBS2
Twyford Down, Hampshire W437	BA	883	F?	Adult	Rib lesions	**WnTDS1**
Waterhall Farm, Chippenham, Cambridgeshire	BA	4	F	25-35	Fused vertebrae T1 / T2	**DkCHS1**
Fire Hydrant: Boscombe Airfield, Wiltshire, 62260	EBA (cal. 1747 – 1607BC)	39	M	35-44	Rib periostitis	**WxFHS1**
Ascott-under-Wychwood, Oxfordshire	NEO	A3	M	25-35	Fused vertebrae L2 / L3	**NHMA3**
Eyebury, Peterborough	EBA	223	M	Approx. 30	Destroyed Proximal Ulna	**NHMEye**
Bourton-on-theWater, Gloucestershire BPS00	MIA	1	U	12-16	Rib periostitis	**BoWS1**

Wetwang Slack, Yorkshire (a)	IA	13(F9(i))	M	13-17	Rib periostitis	**WWSS1**
Wetwang Slack, Yorkshire (b)	IA	16(F41 BW)	M	36-45	Destruction of left hip	**WWSS2**
Wetwang Slack, Yorkshire (c)	IA	47(f192 DW)	M	36-45	Lytic lesions on T12, L2	**WWSS5**
Wetwang Slack, Yorkshire (d)	IA	94 (F291 JZ)	M	36-45	Lytic lesions on upper thoracic vertebrae	**WWSS6**
Wetwang Slack, Yorkshire (e)	IA	220 (WT56)	F	18-25	Lytic lesion on right ilium	**WWSS8**
Wetwang Slack, Yorkshire (f)	IA	307 (WW104)	M	26-30	Psoas abscess evidence on left femur	**WWSS9**

Table 5-1 Summary of all individuals included in the study including estimated sex, estimated age at death and the period from which they originate. 'Primary Pathology' refers to the potential tuberculous pathological lesions which determined inclusion of the individual in the study but may not represent all pathological lesions noted in an individual skeleton. (Neo=Neolithic, BA=Bronze Age, EBA=Early Bronze Age, IA=Iron Age, MIA=Middle Iron Age, F=Female, M=Male, U=Unknown, L=Lumbar vertebra, C=Cervical vertebra, T=Thoracic vertebra).

5.2 The Results in Detail

5.2.1 Abingdon Pipeline, Oxfordshire

5.2.1.1 Skeleton Information:

Skeleton No: 4197 *(Sample Code: OxABS1)*

Condition: Surface integrity good – some fragmentation of bones

Period: Iron Age

Age: 35+

Sex: Female

5.2.1.2 Preservation:

All limb bones were represented, although only the left radius was complete. The remainder of the arm bones were for the most part present, although the right radius was missing both distal thirds of their shaft and the distal joint surface; both humeri were missing the proximal thirds of their shafts, and both ulnae were missing their distal joint surfaces. The leg bones were, however, less well represented with the greatest loss occurring on the left side of the body where the femur was represented, by only the proximal third and distal thirds of the shaft. The left tibia and fibula were both also missing the proximal and distal thirds of the shafts with the fibula also devoid of the proximal joint surface. The right tibia and fibula were much more complete with only their proximal joint surfaces lost and the distal joint surface also lost for the fibula. The cranium was well represented with only the nasal, lacrimal, palatine and vomer missing; the hyoid was not present. Neither the left or right ischia nor pubic symphyses were present or identifiable although the ilium was represented equally on both sides (50-75%). Small parts of the scapula and clavicle from the left side were present, although much better represented on the right (with 50-75% and >75%, respectively) and only the left patella was present. Neither the sternum or coccyx was identifiable and less than 25% of the sacrum was present. Most of the thoracic vertebral bodies were missing although all were represented to some degree, as were those for the lumbar region. Of the cervical vertebrae, only C5 and C7 were present. No metatarsals were present. Ten left and eight right ribs were believed to be represented by the rib fragments present, although this may be a slight underestimate as there were more rib fragments which could not be attributed to either the left or right side. There were a minimum of three right metacarpals present but it was not possible to accurately attribute them to a specific metacarpal. A number of hand and foot phalanges were present but were in no way entirely represented. A number of carpals were present (more from the right than left side) but, again, were not complete and no tarsals could be accounted for. Overall, a greater presence of upper body elements than lower was demonstrated.

5.2.1.3 Dentition:

Three teeth were missing, lost ante mortem (lower right first and second molars and lower left second premolar.

5.2.1.4 Age/Sex Estimation:

Age estimation was derived from the appearance of the auricular surface (Lovejoy *et al*., 1985) and dental attrition (Brothwell, 1981). Sex estimation was based primarily on cranial features as advocated by Buikstra and Ubelaker (1994), excluding the nuchal crest which was unavailable for study, but including the mandible (Brickley and McKinley, 2004). The only area of the pelvis available for sex estimation was the greater sciatic notch (Buikstra and Ubelaker, 1994) which gave a clear female score.

5.2.1.5 Pathology:

Distribution of overall pathology is shown in Figure 5-1.

Numerous hand phalanges show additional bone formation on their palmar aspect from both the left and right sides. Both the left and right ulnae demonstrated an additional strip of lamellar bone formation on their proximal articular surfaces, whilst the left radius showed evidence of a mid-shaft fracture; poorly healed and misaligned. Two of the heavily fragmented ribs showed additional bone formation although not on their visceral surfaces. These areas of bone were circular, seen midshaft and were small, each measuring less than 1cm in diameter. The first lumbar vertebra shows a depression on the superior body, a Schmorl's Node, whilst the superior articular facets on L2 are asymmetrical; one retains a normal curvature but the other is much flatter in shape, thus creating an articular surface at a right angle to the centre line of the vertebral body. L3 demonstrated distinct evidence of degenerative joint disease (DJD) in the form of osteophyte formation surrounding the vertebral body. The left fibula, now broken showed possible additional bone formation at the break; it is not easy to determine whether the break was ante, peri or even possibly post-mortem.

5.2.1.6 Primary Pathology

The right femur showed evidence of active periostitis in the form of woven bone formation on the lateral aspect of the posterior shaft (Figure 5-2). A strip of this additional bone formation runs vertically, mainly constrained to the mid and upper third of the shaft. The left fibula has both active woven bone on the anterior midshaft and a resorptive bone lesion on the anterior, distal third of the bone shaft. Active, woven bone formation on the mid part of the anterior shaft of the bone borders the distal third of a resorptive bone lesion on its lateral side, and approximately 50mm on its distal, medial margin. The lesion measures 250mm in height and 60mm at its widest and demonstrates a shallow, concave shape. The resorptive lesion has a rough, fairly porous surface and demonstrates some evidence of longer term healing, but it was clearly still active at the time of death as suggested by the surrounding woven bone formation.

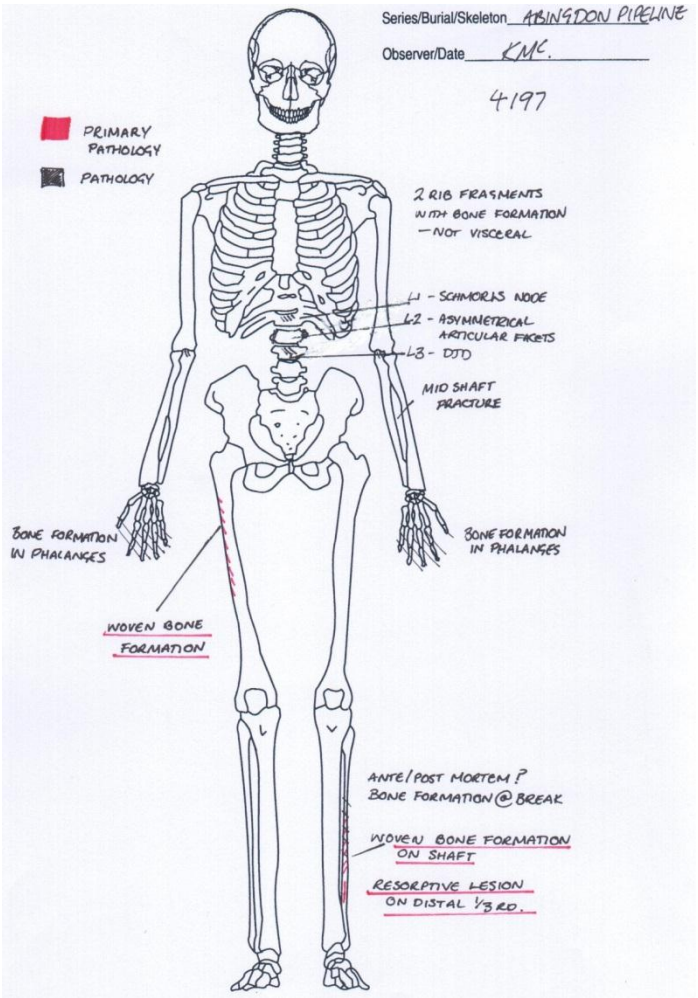


Figure 5-1 Distribution of pathological lesions in Sk. 4197 from Abingdon Pipeline.

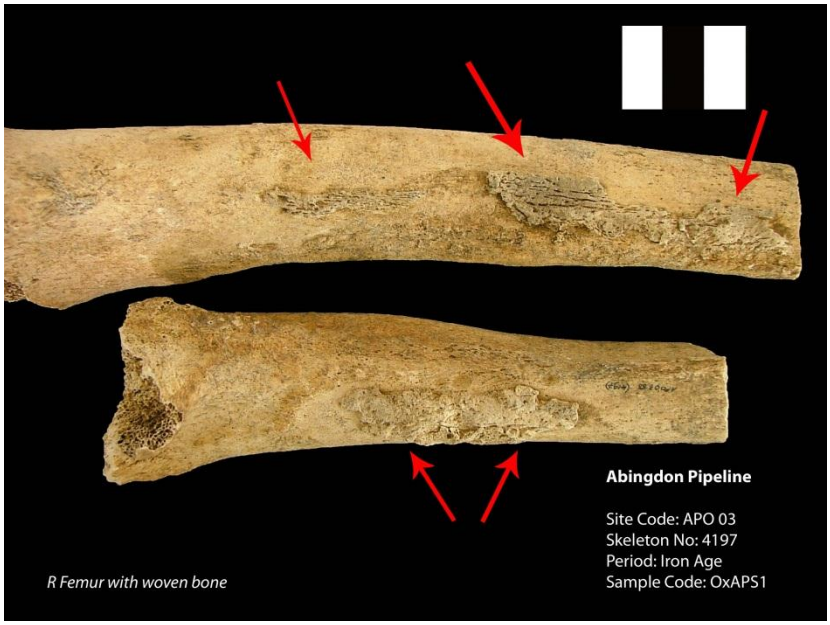


Figure 5-2 Right Femur showing additional bone formation in Sk. 4197 (Abingdon Pipeline)

5.2.1.7 Dental:

The majority of teeth were very badly worn, particularly the incisors, both mandibular and maxillary. Two teeth demonstrated caries, including the upper left second molar and the lower right third molar. The upper left first molar displayed evidence of an abscess with elliptical bone resorption in the maxilla.

5.2.2 Ascott-under-Wychwood, Oxfordshire

5.2.2.1 Skeleton Information:

During initial study of these remains, no skeletal report was available to the researcher for consultation. Each of the bones from within the burial chamber was individually bagged and stored in various boxes. There seemed no way by which to assign or group the single bones into individuals at that time. For this reason, no skeletal analysis was attempted and

therefore no estimations of age or sex were derived. However, an analysis of individual A3 is available from the literature (Galer, 2007). For the purpose of allowing a more comprehensive interpretation and discussion to be formed, it is from this report that the information below derives (in abbreviated form), with the exception of the pathology description.

Skeleton No: A3 *(Sample Code: NHMA3)*
Condition: Many elements complete and in excellent condition.
Period: Neolithic
Age: 25-35
Sex: Male

5.2.2.2 Preservation:

Several vertebrae, the sacrum, left Os coxae, left and right tibiae, left fibula, scapula and humerus, left and right ulnae and right radius.

5.2.2.3 Age/Sex Estimation:

Sex estimation was based on pelvic and cranial morphology and robusticity of the long bones, whist age estimation was derived from ossification of sacral elements and dental development (Galer, 2007: 196).

5.2.2.4 Primary Pathology:

The pathology noted for this study is also the only pathology described in the skeletal report (Galer, 2007). All skeletal elements mentioned here were available for study. The second and third lumbar vertebrae are fused together around the entirety of the vertebral body margins (Figure 5-4). A layer of bone, impossible to differentiate from the rest of the vertebrae, has formed over the line of fusion resulting in a smooth exterior wall of bone with a convex central third. Osteophyte formation is evident on the inferior margin of L3 extending to the

superior margin of L4 which, in itself, has also developed osteophytes; the morphological change has created a wave like appearance. L4 demonstrates a Schmorl's node on the superior surface of the vertebral body.

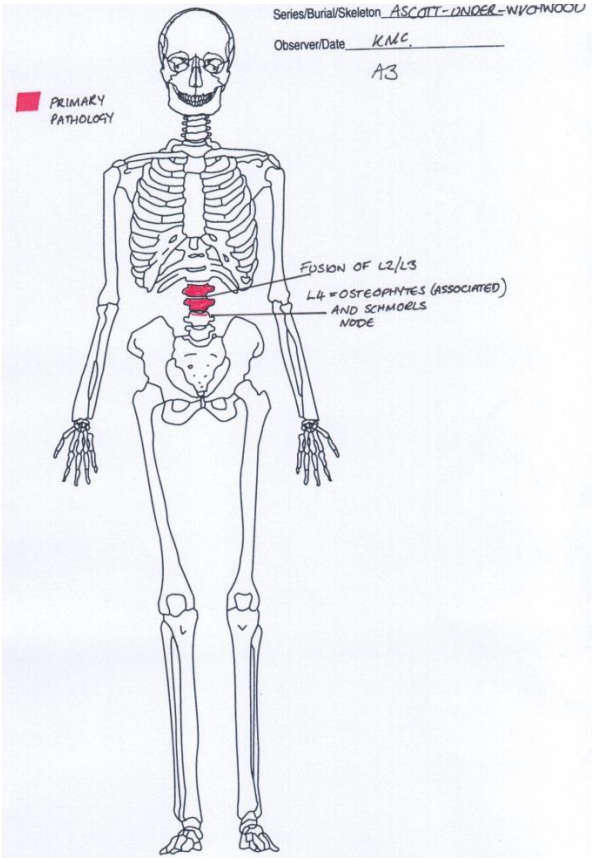


Figure 5-3 Distribution of pathological lesions in Sk. A3 from Ascott-under-Wychwood.

Figure 5-4 Fused L2 and L3 vertebrae with L4 from Individual A3 from Chamber 3(A), Ascott-Under-Wychwood.

5.2.2.5 Dental:

The report refers to hypoplastic bands on the lower left and right canines, and caries of the lower right first molar.

5.2.3 Boscombe Airfield Fire Hydrant, Wiltshire

5.2.3.1 Skeleton Information:

Skeleton No:	39 *(Sample Code: WxFHS1)*
Condition:	Fairly poor – highly fragmented and surfaces moderately eroded.
Period:	Early Bronze Age (post-Beaker period) (cal. 1747 – 1607BC)
Age:	35-44
Sex:	Male

5.2.3.2 Age/Sex Estimation:

Estimation of age was based solely on the appearance of the auricular surface (Lovejoy *et al*., 1985) which crossed two categories, giving a more broad age range. Sex estimation was based on the greater sciatic notch which returned a score of 4, suggesting the skeleton was likely to be that of a male.

5.2.3.3 Preservation:

The skeleton was highly fragmented and, as a result, a number of smaller fragments could not be positively identified. The cranium in particular was poorly represented with only minimal fragments representing the frontal bone and right parietal. All cervical vertebrae were missing but fragments of a minimum of two thoracic vertebrae between T4 and T7

were identified, and a minimum of three vertebrae between T8 and T12. All lumbar vertebrae were represented as were a minimum of ten ribs from the left side, although these were highly fragmented. The left humerus, radius and fibula were present but missing their proximal and distal joint surfaces whilst the ulna, femur and tibia, also from the left side, had their proximal ends present to some degree, in addition to the mid third of their shafts but they all lacked either the distal or proximal third. There were no arm bones from the right side, but the femur, tibia and fibula were present to varying degrees. The ilium and ischium were present but were less than 25% complete, whereas the left ilium was 25 – 50% preserved. All other pelvic bones were not present, nor were the scapula, clavicle and patella from either side, with the exception of a very small fragment of left scapula. Less than 25% of the sacrum was evident. Three left metacarpals (2^{nd}, 4^{th} and 5^{th}) were evident, one proximal and one distal phalanx from a hand, and three proximal and three middle phalanges from the feet. There were many small fragments of bone which could not be confidently identified and thus the true representation of presence/absence of bones for the entire skeleton should be considered conservative.

5.2.3.4 Dentition:

No teeth were present.

5.2.3.5 Pathology:

The distribution of pathological lesions is shown in Figure 5-5.

The right iliac crest displayed a small area of periosteal new bone formation on the adjacent, supero-lateral aspect. The lesion area measured no more than 20mmx8mm (although original report suggests 23x8mm), but also as described in the original report, this area is damaged and therefore the full extent of the lesion cannot be known (McKinley, nd.) (see 6.2.1.3). Some minor destructive lesions were evident on the superior body of the 1^{st} sacral vertebra, no L5 vertebra was available to observe for adjacent lesions, but the lesions appeared degenerative. Due to the presence of enthesophytes on three metacarpal shafts

from the left hand noted in the original report, subsequent radiography confirmed an oblique but well healed fracture of the 5th metacarpal (Manning *et al*., 2010).

5.2.3.6 Primary Pathology:

Periosteal new bone formation was also noted on the visceral surfaces of at least ten rib fragments (Figure 5-6), consisted of both lamellar and woven bone (sometimes woven overlying lamellar), indicating both healed and active infection, respectively, and primarily confined to the mid shaft areas. The longest length of active bone formation was 26mm in length and healed lamellar, 84mm.

5.2.3.7 Dental: *N/A*

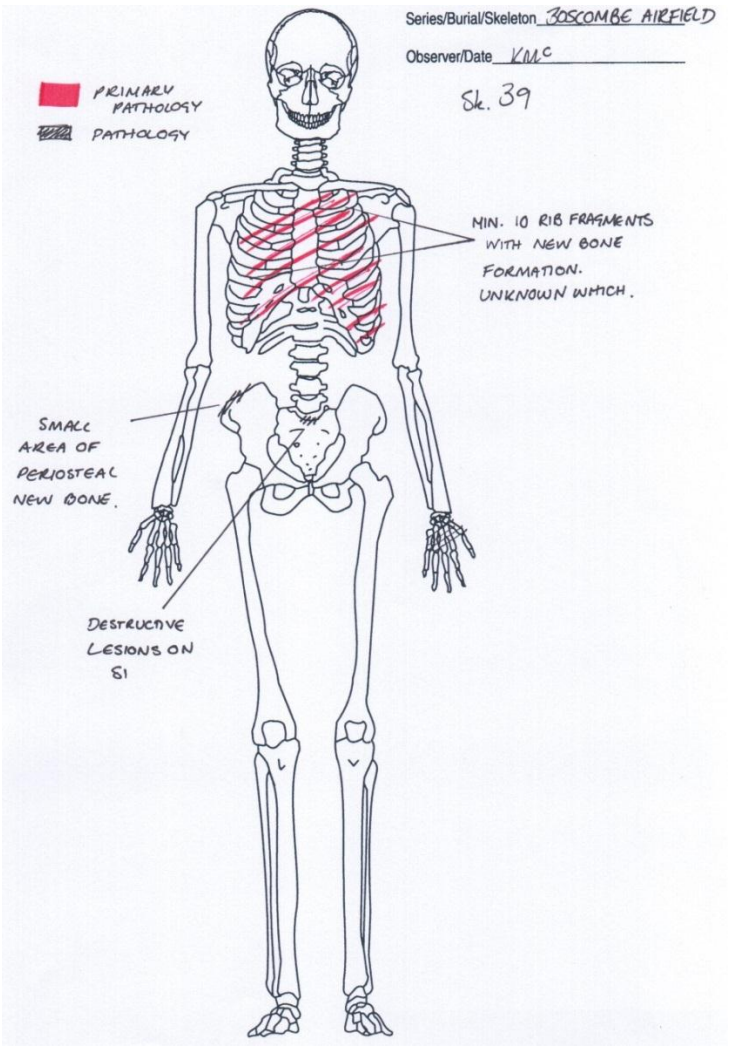


Figure 5-5 Distribution of pathological lesions in Sk. 39 from Boscombe Down Fire Hydrant.

Figure 5-6 Mixed rib fragments from the Boscombe Down skeleton demonstrating woven bone formation. The most obvious areas of this are highlighted by the red circles.

5.2.4 Bourton-on-the-Water Primary School, Gloucestershire

5.2.4.1 Skeleton Information:

This skeleton forms part of the group of skeletons selected for sampling by a member of the research team for the wider project, incorporating European skeletons across all time periods. For this reason, there is not the same level of detail available for the individual skeleton regarding further analysis, as recorded by the author, but basic data and the pathology noted which led to the decision to take a bone sample for aDNA analysis. However, a skeletal report compiled for Gloucestershire County Council (Roberts, 2000b) does provide details as to the pathology noted in the individual in addition to the condition of the material and age and sex confirmation.

Skeleton Code: 1
Condition: Good
Period: Middle Iron Age
Age: 12-16 years
Sex: Unknown

5.2.4.2 Preservation:

Most of the skeletal elements were in good condition, including the long bones; many hand and feet bones were present and, in general, little fragmentation was seen (Roberts, 2000b: 1). The skull was present but its integrity could not be maintained during cleaning (Roberts, 2000b: 1) (although it has since been reconstructed by Caroline Wilkinson at Manchester (Roberts, 2000b: Appendix 1).

5.2.4.3 Age/Sex Estimation:

Age estimation was based on both dental eruption (Van Beek, 1983) and bone development (Scheuer and Black, 2000) which put the age between 12 and 14 years (Roberts, 2000b: 1). The above age of between 12 and 16 years is given, as this was based on the notes of the member of the research team who ultimately took the sample for aDNA testing. It was decided that the largest age range should be presented. Sex estimation was more difficult given the age of the skeleton and thus lack of maturity of the skeleton, but it was suggested that the sciatic notch was wide and the skull also demonstrated feminine features (Buikstra and Ubelaker, 1994) along with a femoral head measurement falling within the female range (Roberts, 2000b: 2)(Bass, 1987). However, the author of the skeletal report made it clear that young males can demonstrate feminine features (Walker, 1994) and therefore this estimation is only probable, not definitive (Roberts, 2000b: 2).

5.2.4.4 Dentition:

All teeth present except unerupted or absent 3rd molars (Roberts, 2000b).

5.2.4.5 Pathology:

Very little pathology was noted on this individual during its original analysis (Roberts, 2000b) except fused mid and distal phalanges of the foot (Figure 5-8).

5.2.4.6 Primary Pathology:

The presence of new bone formation on the visceral surface of a left and right rib was also noted (Roberts, 2000b: 3) (Figure 5-7) and was the reason for this individuals selection for subsequent sampling and analysis.

Figure 5-7 New bone formation on ribs from Sk. 1, Bourton-on-the-Water.

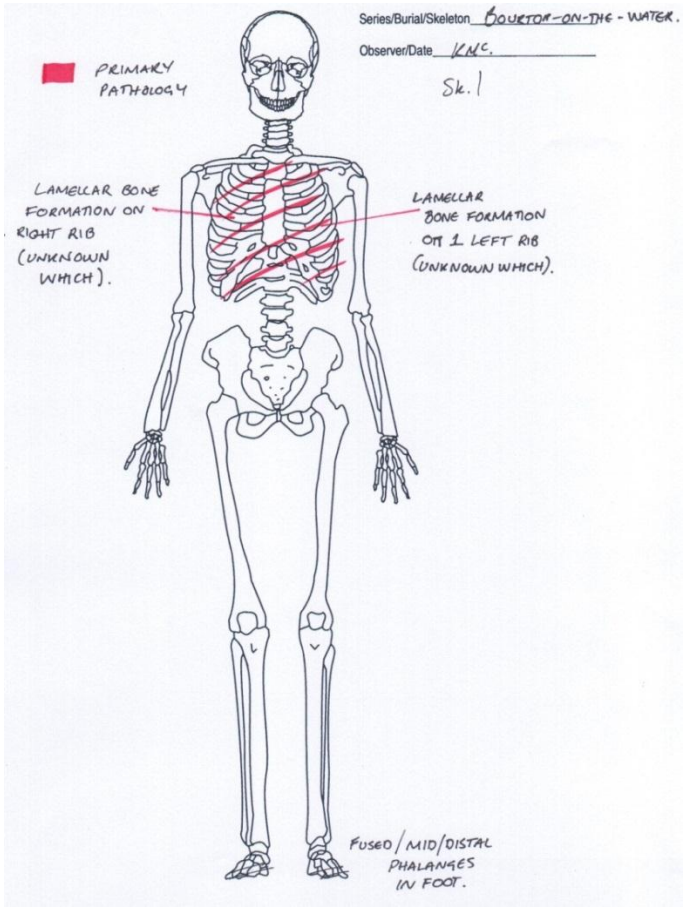


Figure 5-8 Distribution of pathological lesions in Sk. 1 from Bourton-on-the-Water.

5.2.4.7 Dental:

Some dental defects were noted on the upper incisors plus some calculus (Roberts, 2000b).

5.2.5 Eyebury, Peterborough, Cambridgeshire

5.2.5.1 Skeleton Information:

Unfortunately, at some point the remains of this skeleton had been mixed with other remains. Four bones (1 tibia, 1 ulna, 1 distal femur, right innominate) are labelled as belonging to E.II.3.223 but the association between these bones themselves appears to be questionable. It seems as though, from reading the later report regarding tumuli B and C, that some remains from the excavation of B have been added to this box (and at some point mislabelled) as there are some which show evidence of burning. There is certainly an MNI (minimum number of individuals) of 2 individuals contained within the box due to the presence of 3 innominate bones. For this reason, to prevent confusion and inaccurate analysis, no attempt to record presence/absence, age or sex was attempted. However, the original report presented by Leeds does offer an insight into the general preservation of the skeleton (1912: 90). The age and sex of the skeleton noted below is derived from this same publication but an accurate description of the pathological ulna selected for sampling is described below after having been examined for this study.

Skeleton No: E.II.3.223 *(Sample Code: NHMEye)*
Condition: Poor
Date: Early Bronze Age
Age: Adult, approx. 30 years.
Sex: Male

5.2.5.2 Preservation/Dentition:

"The bones were much broken and many of them in a somewhat rotten condition, but it was possible to remove them all…The teeth are in beautiful preservation but much worn down, while one of the wisdom teeth has been unable to force its way out normally and lies parallel to the jaw with its crown against the side of the adjacent second molar." (Leeds, 1912: 90).

5.2.5.3 Age/Sex Estimation:

Leeds does not explain the methods used to identify either the age or sex of the individual but simply states "[the skeleton]…has since proved to be an adult man of about thirty years of age…" (1912: 90).

5.2.5.4 Primary Pathology:

The primary pathology noted in this skeleton was at the proximal end of the left ulna (Figure 5-9 and Figure 5-10). The olecranon process is absent as is the trochlear notch, although there is a spur of bone reaching in a superior direction on the medial aspect of the ulna head. This spur of bone extends 10mm above the highest point on the posterior area of the joint surface. There is also a second, smaller spur of bone, also extending in a superior direction but more laterally placed to that of the first. This spur of bone measures approximately 10mm at its widest point but does not extend above the level of the remaining joint surface.

Figure 5-9 Proximal Ulna from Skeleton E.11.3/223 (Eyebury) showing unusual pathology affecting the joint surface.

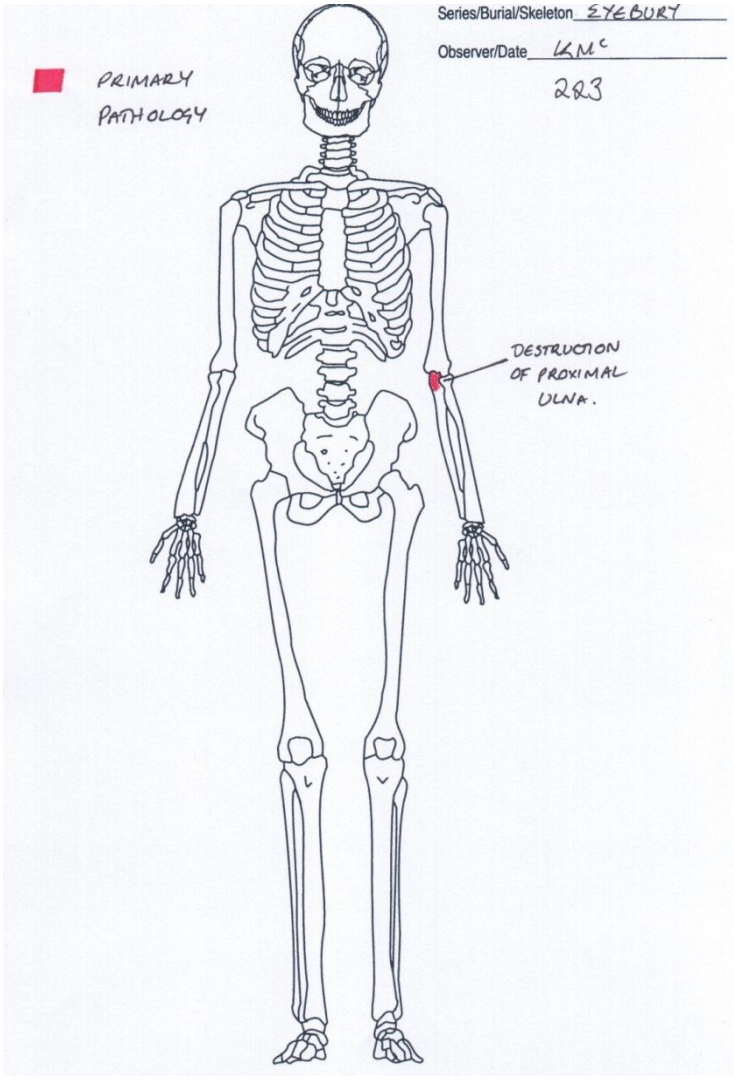


Figure 5-10 Location of pathological lesion in Sk. 223 from Eyebury.

5.2.5.5 Dental:

See above.

5.2.6 Methwold Severalls, Norfolk, Norwich

5.2.6.1 Skeleton Information:

Skeleton No:	Burial 1 *(Sample Code: NkMS1)*
Condition:	Fair – quite fragmented but the structural integrity of most fragments was maintained though heavily peat stained.
Date:	Bronze Age (OxA-2862 / 3840+-80BP)
Age:	Adult (25 – 45)
Sex:	Female

5.2.6.2 Preservation:

The majority of the skeleton was represented although much fragmented. The majority of the thoracic vertebrae were missing with only T1 positively identified, although three more thoracic bodies were present. All lumbar vertebrae, and the majority of cervical, were present, with just C2 and C3 missing. Most arm bones from the right side of the body were present with only the distal joint surface of the ulna missing. The left arm bones, however, were quite poorly represented with only the ulna being almost entirely present (missing only the distal joint surface); both the humerus and radius were missing with the exception of the mid-third of the radial shaft. The leg bones were for the most part present, although there was a notable absence of the entire right femur and left tibia. The major cranial bones were present but the lacrimals, sphenoid, vomer and ethmoid were missing; the hyoid was not present. The pelvic area was moderately represented with the left and right ilium over 75% present, as well as the left pubis. Unfortunately the ischium and pubis were entirely

missing on the right side and the ischium also missing on the left. The clavicles and patellae were present on both sides, although the scapulae were both represented by 50-75%. No sternum or coccyx could be identified although the sacrum was almost entirely present; four left and five right ribs could be accounted for. No hand bones were present but six middle foot phalanges, four right and four left metatarsals were present (plus one broken post-mortem which may have originated from either side). There were also a number of tarsals from both the left and right feet including the talus, calcaneus, 1st cuneiform, navicular and the left cuboid.

5.2.6.3 Dentition:

There were a number of teeth present (Fig.4.6), many still in situ, but a number of additional loose teeth were also found with this skeleton and it was unclear if they were associated with this skeleton or not.

5.2.6.4 Age/Sex Estimation:

Sex of this skeleton was inferred from a number of cranial and pelvic criteria. The morphology of the greater sciatic notch suggested female (Buikstra and Ubelaker, 1994). Cranial scores (Buikstra and Ubelaker, 1994, Brickley and McKinley, 2004) also strongly implied the skeleton was female. The estimated age range was broad and based on sacral fusion (Scheuer and Black, 2004), auricular surface morphology (Lovejoy *et al*., 1985), pubic symphysis (Brooks and Suchey, 1990) and dental attrition (Brothwell, 1981). The greatest age range was derived from the latter, but this was complicated by peat damage; this damage obscured many of the observations hence the resulting broad age range.

5.2.6.5 Pathology

A minor exostosis was noted on the anterior aspect of the patella; on the superior margin of the quadriceps tendon insertion. Extensive osteophytes were seen around the margins of the vertebral body of L5. The distribution of this pathology can be seen in Figure 5-11.

5.2.6.6 Primary pathology:

A destructive lesion on the L1 vertebral body (Figure 5-12); this was deep and present on the inferior aspect of the vertebral body. The lesion measured 34mm in width, and penetrated the bone to a maximum depth of 40mm at its deepest point. The interior surface of the lesion, or that below the level of the superior face of the body, was both uneven and rough. The lesion was significantly deeper on the right side of the body although did not penetrate the lateral margin of the vertebra. However, the supero-anterior margin was distorted in shape as the lesion had penetrated through from the main body. The lesion only affects the anterior half of the vertebra.

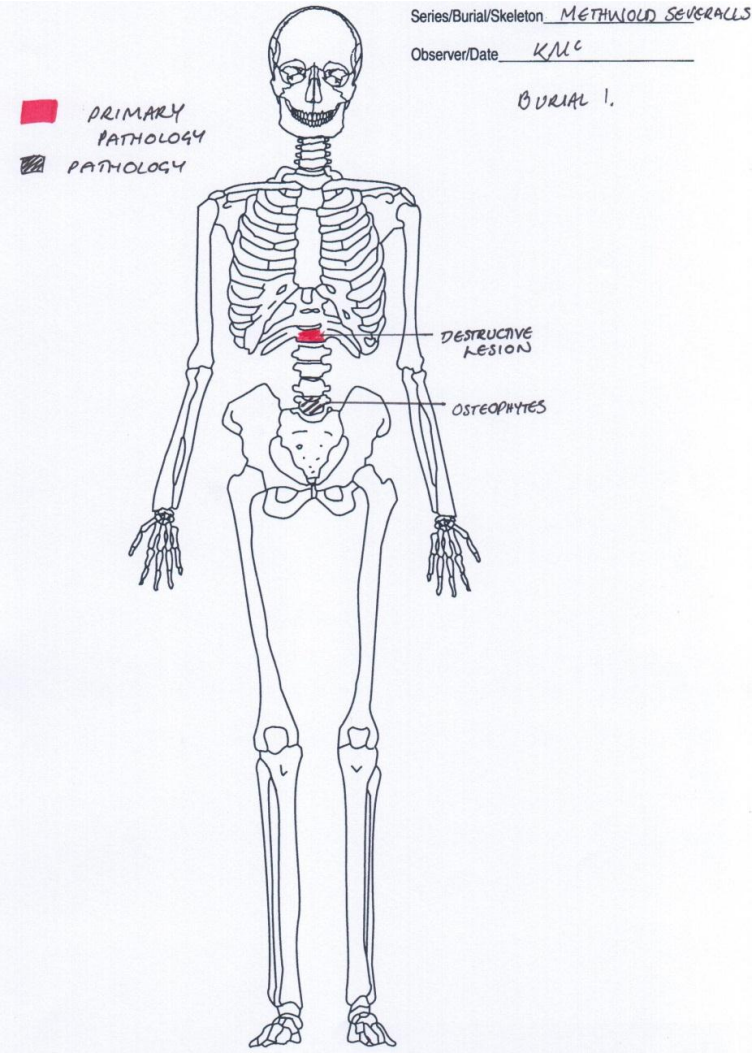


Figure 5-11 Distribution of pathological lesions in Sk. 1, Methwold Severalls.

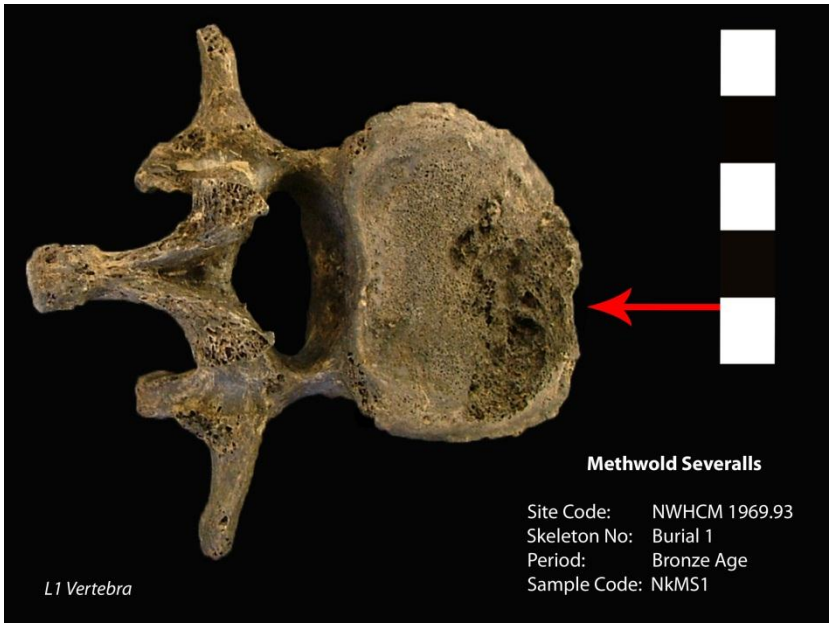


Figure 5-12 Large area of destruction on the L1 vertebral body from Burial

5.2.6.7 Dental:

Dental pathology consisted of some dental hypoplasia on both incisors and molars; caries was also present in some teeth including a large lesion visible on the mandibular right third molar (Figure 5-13).

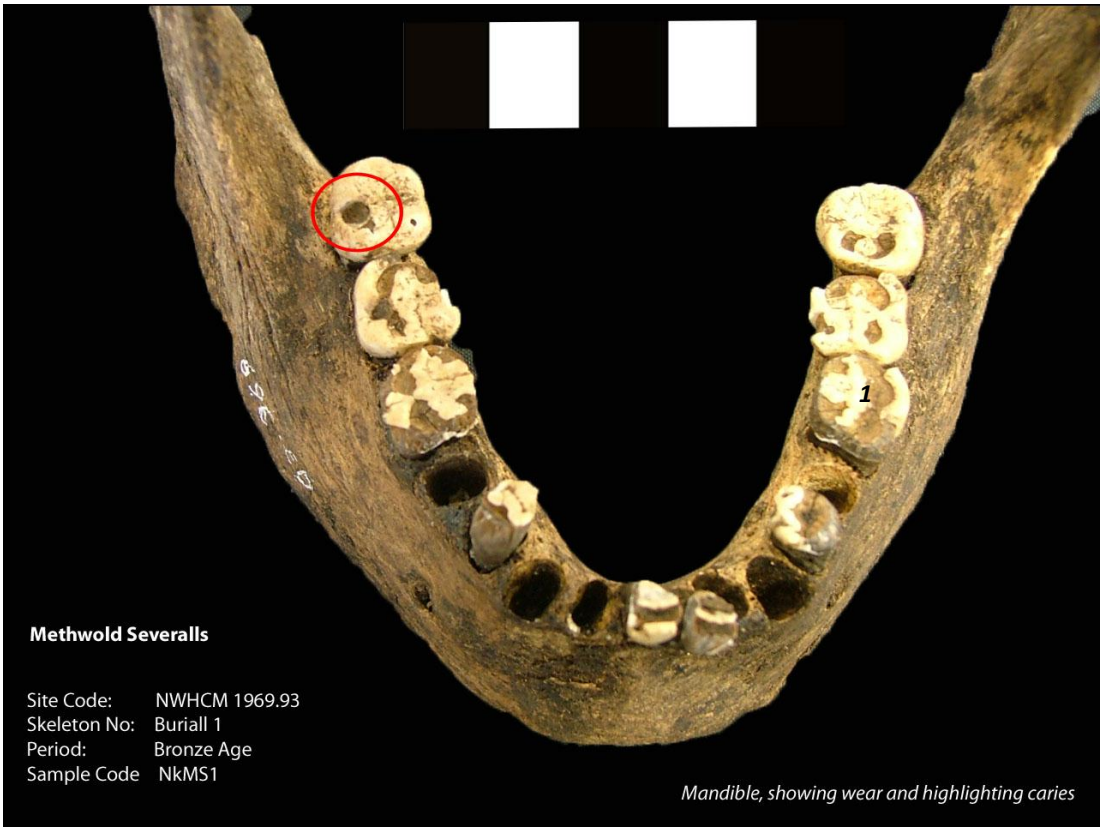


Figure 5-13 Mandible of Burial 1 showing dental wear, erosion and caries.

5.2.7 Twyford Down, Gloucestershire

5.2.7.1 Skeleton Information:

Skeleton No: 883 *(Sample Code: WnTDS1)*

Condition: Very Fragmented

Date: Bronze Age

Age: Adult

Sex: Female?

5.2.7.2 Preservation:

Very little of the skeleton remained. The majority of the skeletal material observed originated from arm and leg bones, but with notable exceptions such as the right humerus and left femur and fibula. Only L5 of the vertebrae was present and, although represented, the ilium consisted of only half of the right acetabulum and anterior iliac spine whilst, also on the right hand side, the Ischium consisted purely of half of the ischial tuberosity. The only other skeletal elements (other than the hands and feet) to be represented included the left patella, less than 25% of the sacrum and an assortment of rib fragments (23 in total) which were so fragmented they could not, with any certainty, be attributed to the right or left side of the body. No bones of the skull were present. Of the hands and feet, six metatarsals in total were present, although fragmented, no metacarpals and a minimal number of phalanges from both hands and feet. Although no carpals were identifiable, there were some fragmented tarsals from both feet were. Some pieces of bone were so heavily fragmented they could not be identified as any specific skeletal element, resulting in the above description being a slight understatement of true preservation.

5.2.7.3 Dentition:

N/A

5.2.7.4 Age/Sex Estimation:

There were no skeletal elements available for study which allowed an accurate age range to be suggested; the most that could be inferred from what remained was that the skeleton was that of an adult. Sex estimation was based on one criterion alone, that of the greater sciatic notch on the left innominate (Buikstra and Ubelaker, 1994). The score was firmly female but, with no other elements present to assess sex, the sex estimation of the skeleton can only be considered as possibly or probably female.

5.2.7.5 Pathology:

An exostosis was noted on the right, first distal foot phalanx at its proximal end. Evidence of degenerative disc disease (ddd) was noted on the lumbar vertebrae and signs of osteoarthritis on both the L5 and S1 bodies. The lateral condyle of the remaining right humerus displayed some new bone formation around its margins although this was minimal. The patella also showed an exostosis. The distribution of these lesions can be seen in Figure 5-14.

5.2.7.6 Primary Pathology:

The most significant pathology noted for this individual was new bone formation (woven bone) recorded on four individual rib fragments, all affected on their visceral surface (Figure 5-15).

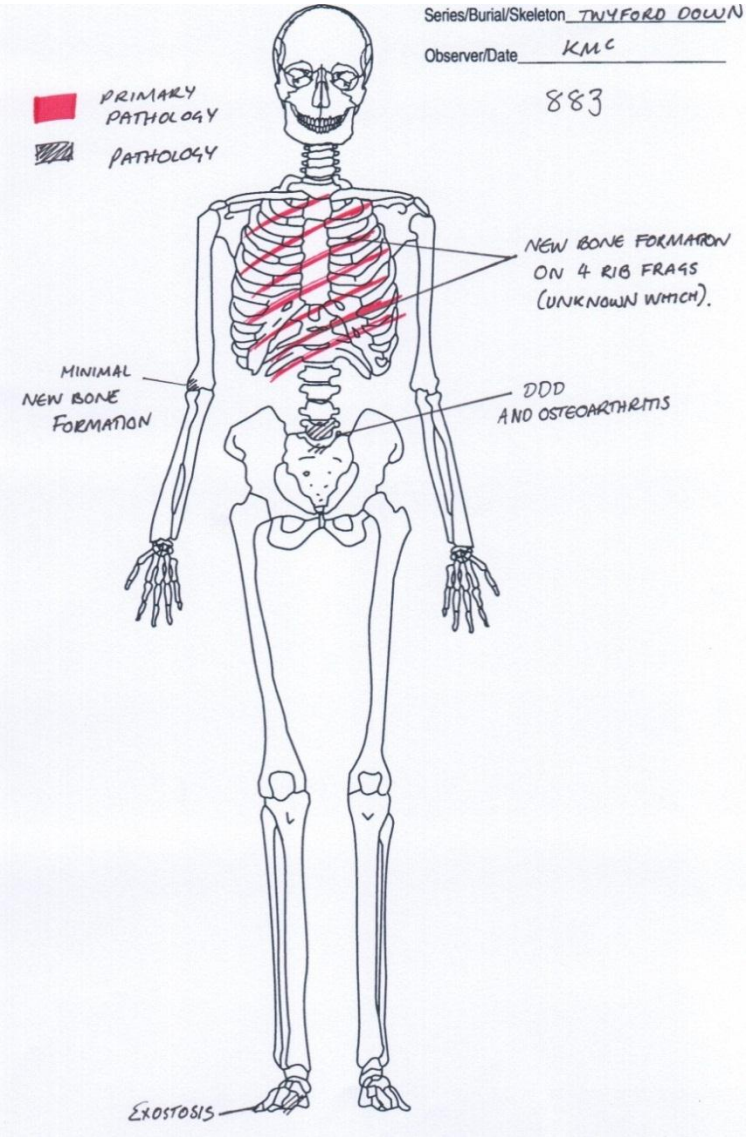


Figure 5-14 Distribution of pathological lesions in Sk. 883, Twyford Down.

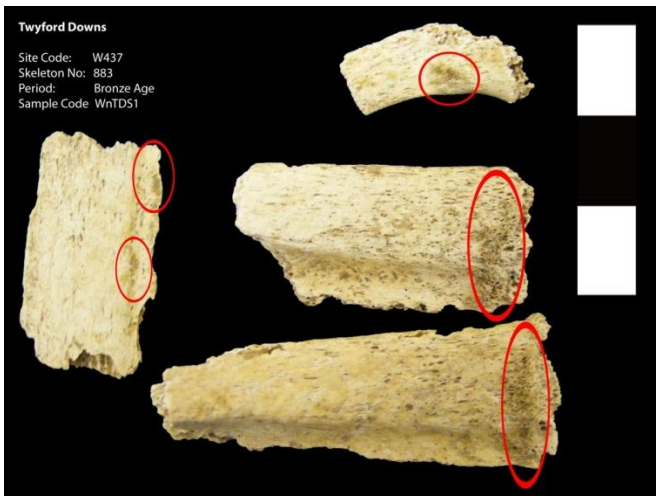


Figure 5-15 Four rib fragments from Skeleton 883 (Twyford Down) showing woven bone formation on visceral surfaces.

5.2.7.7 Dental:

N/A

5.2.8 Waterhall Farm, Chippenham, Cambridgeshire

5.2.8.1 Skeleton Information:

Skeleton No:	Burial IV *(Sample Code: DkCHS1)*
Condition:	Poor - Fragmentary
Date:	Bronze Age
Age:	25 – 35
Sex:	Female

5.2.8.2 Preservation:

The majority of skeletal elements for this skeleton were present, although it was noted that the bones were 'soft' during excavation which has since resulted in very fragmentary remains. Whilst the parietal bones were present, along with partial frontal and occipital bones, along with the right half of the mandible, the remaining cranial bones were too fragmentary to positively identify. The majority of the vertebrae were present: all thoracic and lumbar, and cervical C3, C4 and C7. There were more rib fragments evident from the right side of the body (approx.10) than the left (approx. two) and, again, these were extremely fragmentary. All long bones were represented with the exception of the left fibula, but a number of these were missing their proximal joint surfaces (right humerus, left humerus, radius and ulna) and/or their distal joint surface (both ulnae). There was a small fragment of mid fibula shaft but this could not be positively assigned to either left or right

sides. The right ilium was almost entirely represented, the left less so (50-25%), and missing the auricular surface, and for the ischia less than 25% was present for both sides. The pubis was missing for both sides. Less than half of the left and right scapulae were present, and the left clavicle being marginally better represented than the right. No patellae were present. The sternum was absent, the coccyx preserved less than 25%, and the sacrum being almost entirely present. There were many fragmentary metacarpals and metatarsals present, and the only ones which were positively assigned were the first and third right metatarsals and the second, third and fourth left metatarsals. One proximal foot phalanx was identified. No carpals were present, but the right talus, calcaneus, 3rd cuneiform, navicular and cuboid were preserved. Left tarsals included the talus, 3rd cuneiform, cuboid and half the calcaneus.

5.2.8.3 Dentition:

Dental preservation matched exactly that of the original report; fourteen from the maxilla and fifteen in the mandible, totalling 29 teeth.

5.2.8.4 Age/Sex Estimation:

Age at death was inferred from fusion of the sacrum (Scheuer and Black, 2004), the appearance of the right auricular surface (Lovejoy *et al*., 1985) and dental attrition (Brothwell, 1981). Sex was inferred cranially by the female appearance of the mastoid process, supraorbital margin and glabella (Buikstra and Ubelaker, 1994). The pelvis produced a number of female scores (although poor preservation made this difficult), primarily in the wide angles of both the left and right greater sciatic notches (Buikstra and Ubelaker, 1994).

5.2.8.5 Pathology:

The only pathological lesions observed for this skeleton were found in the spine (Figure 5-16). Minor evidence of osteoarthritis was present on one of the lumbar vertebra which

had also been noted in the original skeletal report as 'a spur of bone spreading outwards from the superior rim, very suspicious of osteo-arthritis' (Denston, 1976:21). However, the vertebral body when examined here was extremely fragmentary and so this suggestion is tentative but not unfounded.

5.2.8.6 Primary Pathology:

The most significant pathology noted was fusion of two thoracic vertebrae, T1 and T2. These two vertebrae were entirely fused via both their bodies and articular processes (Figure 5-17).

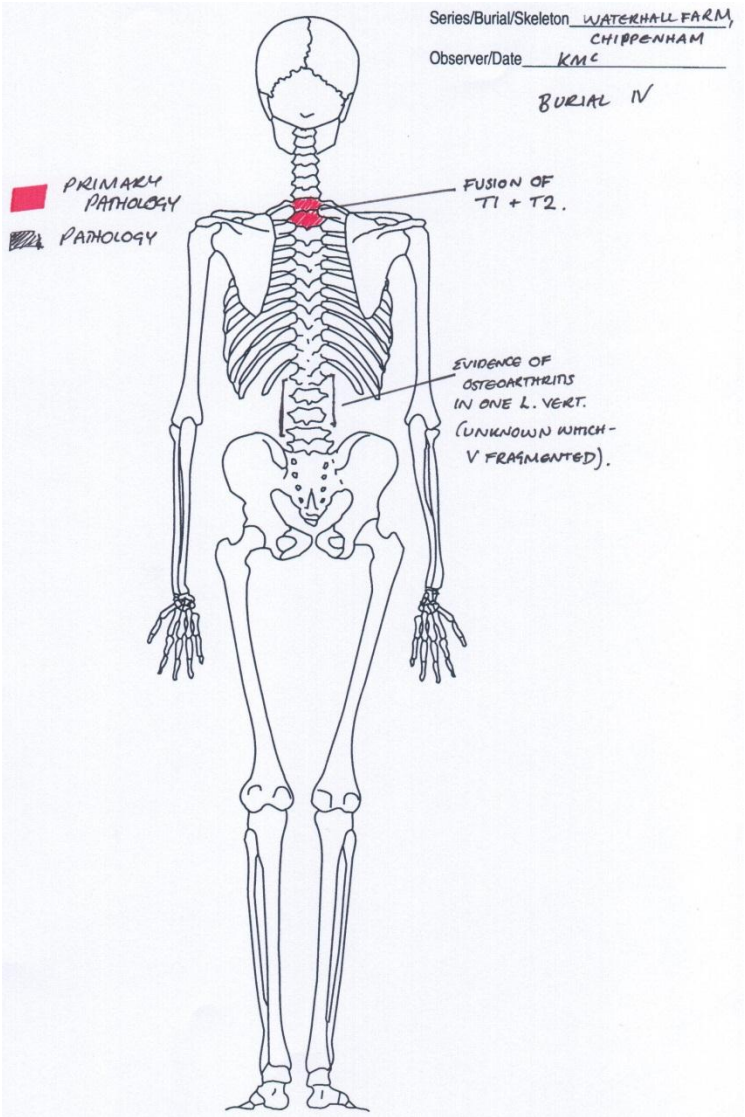


Figure 5-16 Location of pathological lesions in Burial 4, Waterhall Farm, Chippenham.

Figure 5-17 Fused thoracic vertebrae from Skeleton 4 (Waterhall Farm, Chippenham)

5.2.8.7 Dental:

None of the dentition displayed caries or significant wear although there was minimal evidence of dental hypoplasia on a small number of teeth.

5.2.9 West Tump Long Barrow, Cheltenham, Gloucestershire

5.2.9.1 Skeleton Information:

Skeleton No: 7 (*Record No.* 1001963 / 1001964 *Object No.* 1913.33.280 / 1913.33.461) *(Sample Code: ChWTS1)*
Condition: Very fragmented
Date: Neolithic
Age: Adult
Sex: Unknown

5.2.9.2 Preservation:

What remained of this skeleton was highly fragmented although the majority of fragments themselves were in a reasonably good state of preservation. This corresponds with other work that describes the cortical surfaces of the bones as being intact, with preservation of features such as root marks, suggesting little, if any surface exposure (Smith and Brickley, 2004: 20).

In terms of total preservation of skeletal elements, the skull was very poorly represented with only small fragments attributable to the left parietal bone, the occipital bone, the maxilla and nasal, both sides of the mandible (including both foramen and three tooth sockets), and the right orbit. There were around six fragments too small or too damaged to be able to identify other than being attributable to the cranium. The vertebrae were all present between T10 and L5, and C6, T4 and T6 were also identifiable, and one more cervical vertebra, being either C3 or C4. There were at least seven right ribs and one left rib present, but there was a great many small unidentifiable fragments, meaning that it is likely this minimum number is highly conservative. There were elements of all left and right limb bones present, although both the tibiae and fibulae were the most poorly represented of all; the ulnae were the most poorly represented arm bones. The right humerus, left radius and femora were the best preserved, being broken but almost entirely represented. In addition, there were many small, fragmented pieces of tibiae but they could not be assigned a side. The pelvic area was quite poorly represented, with only 50% or less of the ilium (left and right), and less than 25% of the right pubis. The ischium was present on both sides and preserved at 50 – 75%. Only two segments of the sacrum were present with no representation of the scapulae, clavicles, patellae, sternum or coccyx. Two metacarpals, three metatarsals and two phalanges (one hand, one foot) were present as well as four tarsals.

5.2.9.3 Dentition:

Three teeth were present including a right mandibular second molar and two maxillary teeth (a left canine and a right premolar). The exact identification of these last two teeth is tentative as both were broken.

5.2.9.4 Age/Sex Estimation:

No sex estimation criteria could be made because the pelvic and cranial features used in this process were either absent or not sufficiently well preserved. The same can be said for ageing, although parts of the sacrum still present suggested a younger adult (Scheuer and Black, 2004) and parts of the right pubic symphysis fell into the range of II-2 or III-1 for males and III-1 for females (Brooks and Suchey, 1990). The fragmentary nature of the skeletal elements made this task difficult and this was compounded by not knowing the sex of the individual. The age range suggested by the pubic symphysis was so broad that the decision to assign the skeleton as 'adult', as opposed to a specific age range, was taken.

5.2.9.5 Primary Pathology:

No specific pathology was evident in these skeletal remains other than that which was removed for sampling (Figure 4-39 and Figure 5-18). Pathology was noted on the visceral surface of a rib fragment in the form of a thin layer of periosteal new bone formation. The lesion measured a maximum of 20mm in length, the primary area being approximately 15mm in length by 7.5mm wide. The entire rib fragment measured a maximum of 45mm in length. The depth of the additional bone formation was less than 1mm.

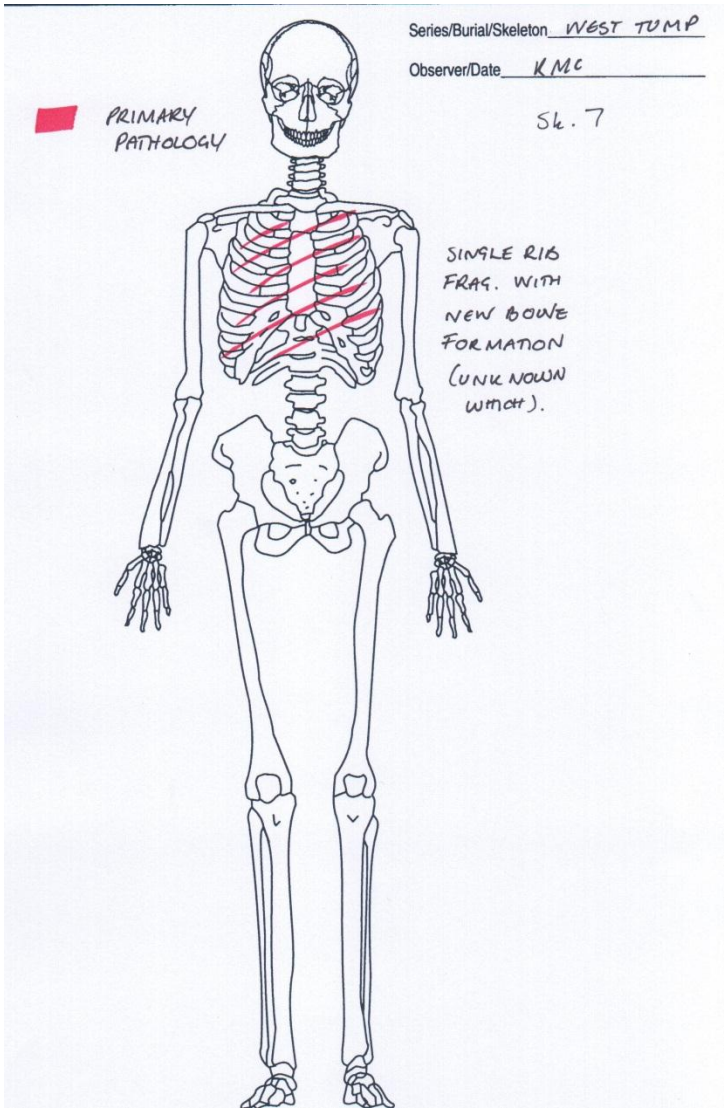


Figure 5-18 Location of pathology in Sk.7 from West Tump.

5.2.9.6 Dental:

N/A

5.2.10 Wetwang Slack, Yorkshire

***5.2.10.1** Skeleton Information:*

These skeletons form part of the group of skeletons selected for sampling by a member of the research team for the wider project, incorporating European skeletons across all time periods. For this reason, there is not the same level of detail available for the individual skeletons regarding further analysis; basic data and the pathology noted, led to the decision to take aDNA samples. Additional information from notes made during the initial skeletal recording after excavation (Dawes, n.d.) were also consulted, although via a transcribed copy supplied by Dr Mandy Jay (Jay, Pers. Comm.). The information contained within the original notes, does not always match those of the more recent, indicating the reason for sampling. For this reason, only comments pertaining to the pathology noted more recently and chosen for sampling, are included below, to retain clarity; original notes are currently held at Bradford University if required for further reference. For this reason, the pattern of full skeletal pathology cannot be recorded here, but diagrams relating to the pathology which led to sampling, are included for completeness. The estimated sex and ages of the skeletons mentioned below have also been modified through recent reanalysis (King, 2010), using more modern techniques than would have been available originally. The information included here is for the purpose of completeness and coherence as these samples are also included in the aDNA results section and were previously described in the materials section.

5.2.11 Wetwang Slack (a)

Skeleton Code: 13 (F9(i)) *(Sample Code: WWSS1)*
Condition: Unknown
Period: Iron Age
Age: 14 years
Sex: Unknown

5.2.11.1 Primary Pathology:

This skeleton was selected for sampling due to demonstrating lesions of new bone formation on multiple ribs (Figure 5-19 and Figure 5-20). The original report refers to the ribs as healthy (Dawes, n.d.).

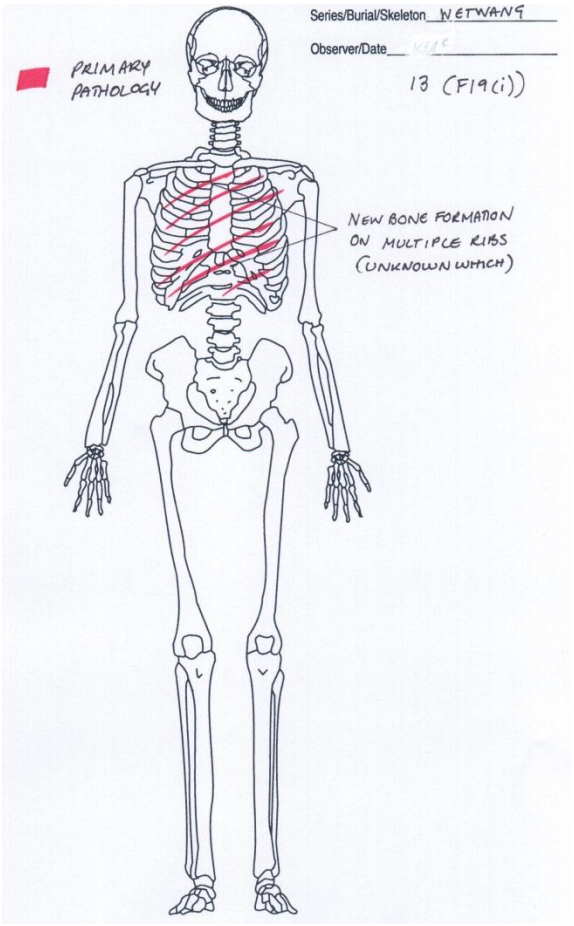


Figure 5-19 Location of primary pathology in Sk. 13 (F9(i)), Wetwang Slack.

Figure 5-20 Rib lesions in Sk, 13 (F9(i)), Wetwang Slack

5.2.12 Wetwang Slack (b)

Skeleton Code: 16 (F41 BW) *(Sample Code: WWSS2)*
Condition: Unknown
Period: Iron Age
Age: 36-45 years
Sex: Male

5.2.12.1 Primary Pathology:

This skeleton was selected for sampling due to demonstrating destruction of the left hip (Figure 5-21 and Figure 5-22). The original report refers to this as:

> *Gross enlargement & flattening of left femur head with associated lipping and increase of acetabulum…Left femur head – eburnation, surface roughening. Gross lipping especially on lower surface. Very large ulcer cavity on upper posterior side of head. Probably suppurating. Injury or infection of long standing, acetabulum grown forward & osteomyelitis. Possibly tuberculosis*
>
> *(Dawes, n.d.).*

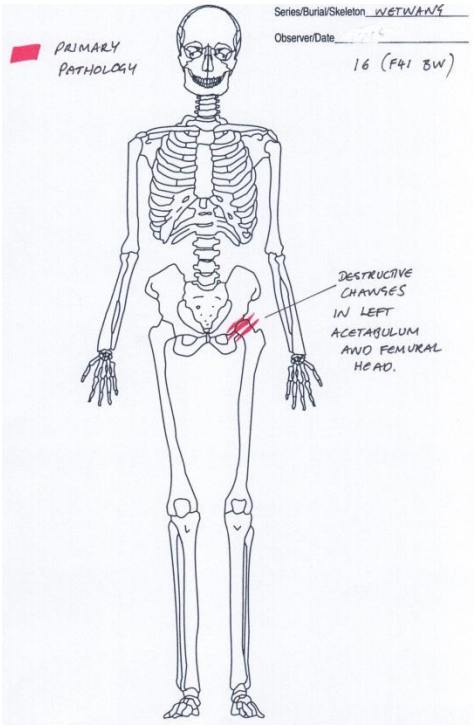


Figure 5-21 Location of pathological lesion in Sk. 16 (F41 BW), Wetwang Slack

Figure 5-22 Destruction on left hip from Sk. 16 (F41 BW), Wetwang Slack

5.2.13 Wetwang Slack (c)

Skeleton Code: 47 (F192 DW) *(Sample Code: WWSS5)*

Condition: Unknown
Period: Iron Age
Age: 26-35 years
Sex: Male

5.2.13.1 Primary Pathology:

This skeleton was selected for sampling due to lytic lesions on the 12th thoracic vertebra and the second lumbar vertebra (Figure 5-23 and Figure 5-24) – no further detail is available. The only vertebral pathology referred to in the original report is the anterior compression L2 (Dawes, n.d.), which is most likely the original reason for analysis of the skeleton during the course of this project.

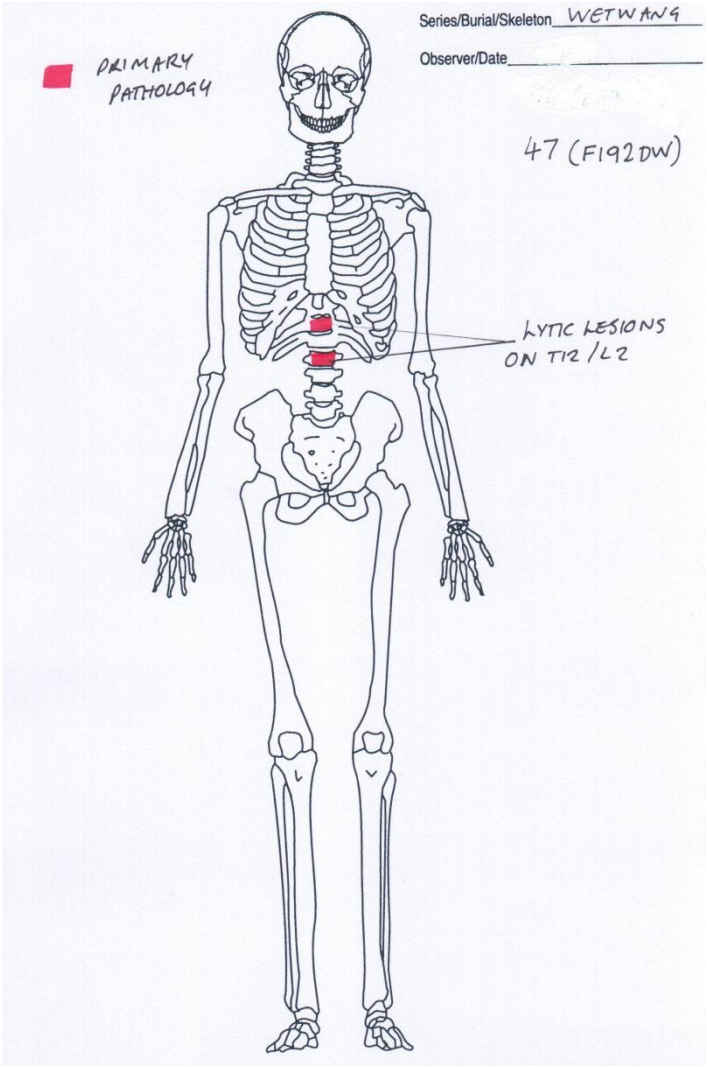


Figure 5-23 Location of pathology in Sk. 47 (F192DW), Wetwang Slack.

Figure 5-24 Lytic lesions on T12 and L2 in Sk.47 (F192DW), Wetwang Slack.

5.2.14 Wetwang Slack (d)

Skeleton Code: 94 (F291 JZ) *(Sample Code: WWSS6)*
Condition: Unknown
Period: Iron Age
Age: 26-35 years
Sex: Male

5.2.14.1 Primary Pathology:

This skeleton was selected for sampling due to lytic lesions on the upper thoracic vertebrae (Figure 5-25 and Figure 5-26). The original report refers to 'Collapse of C6 to thin sliver. C7 & T1 partial central collapse of vertebral body on adjacent faces – circular recession. ?TB' (Dawes, n.d.)

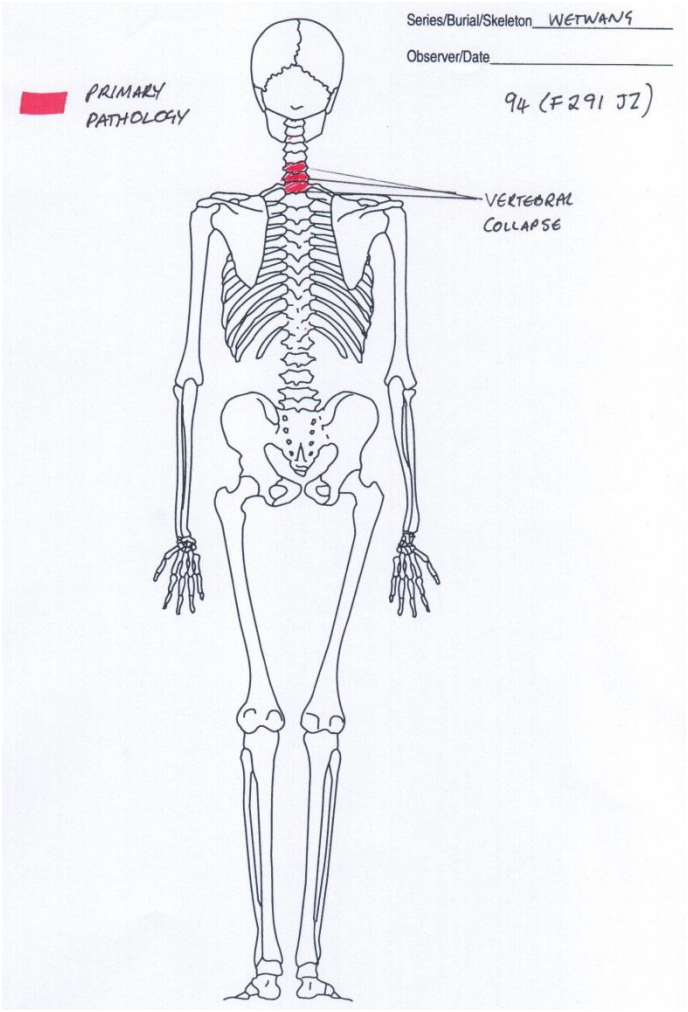


Figure 5-25 Location of pathological lesions in Sk. 94 (F291 JZ), Wetwang Slack.

Figure 5-26 Destructive lesions in C7 and T1 vertebrae in Sk. 94 (F291 JZ), Wetwang Slack.

5.2.15 Wetwang Slack (e)

Skeleton Code: 220 (WT56) *(Sample Code: WWSS8)*
Condition: Unknown
Period: Iron Age
Age: 18-25 years
Sex: Female

5.2.15.1 Primary Pathology:

This skeleton was selected for sampling due to a lytic lesion on the right ilium – no further information is available and the original report makes no reference to this lesion (Figure 5-27 and Figure 5-28).

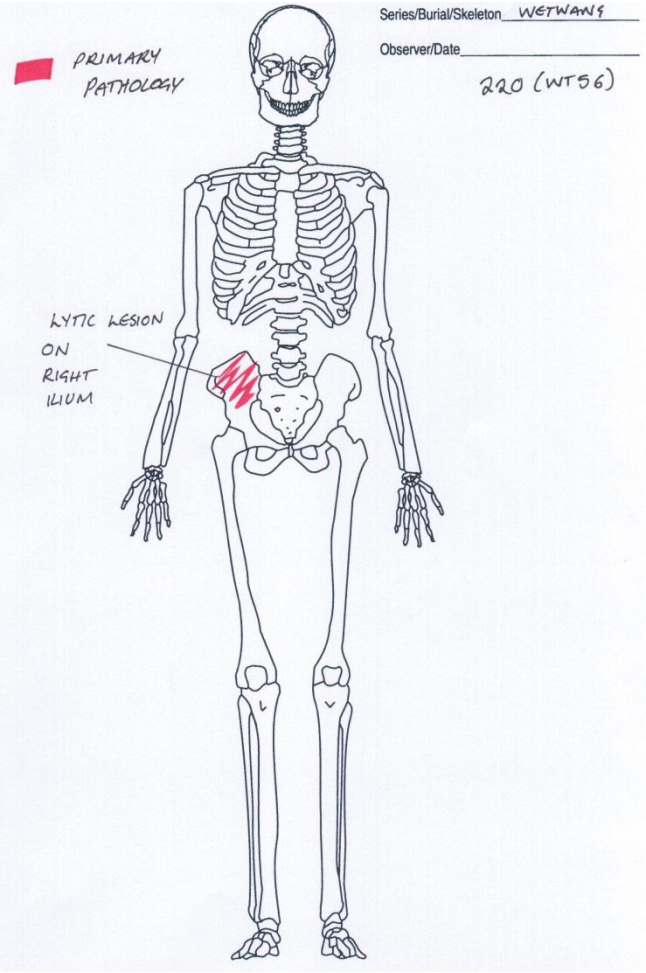


Figure 5-27 Location of pathological lesion in Sk. 220 (WT56), Wetwang Slack.

Figure 5-28 Lytic lesion on right ilium in Sk. 220 (WT56), Wetwang Slack.

5.2.16 Wetwang Slack (f)

Skeleton Code: 307 (ww104) *(Sample Code: WWSS9)*
Condition: Unknown
Period: Iron Age
Age: 25-30 years
Sex: Male

5.2.16.1 Primary Pathology:

This skeleton was selected for sampling due to demonstrating evidence of a psoas abscess on the left femur. Dawes' (n.d.) report refers to this is 'Gross osteomyelitis neck left femur with complete parting of head & neck from shaft & separate osseous plate. Head of femur &acetabulum considerably feathered & spread.' (Figure 5-29 and Figure 5-30).

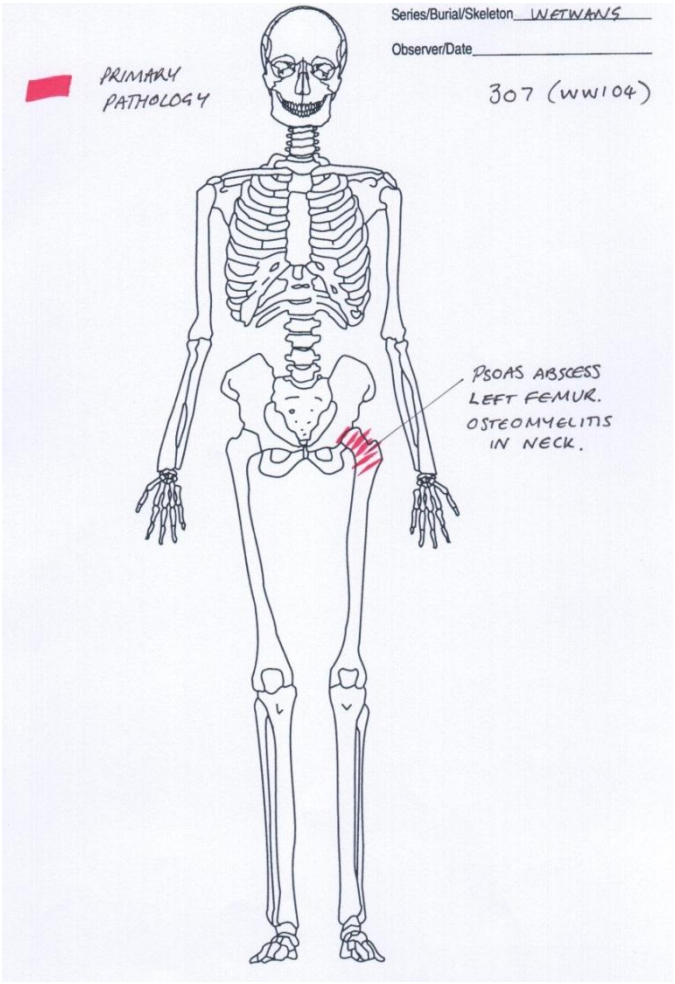


Figure 5-29 Location of pathological lesion in Sk. 307 (ww104), Wetwang Slack.

Figure 5-30 Evidence of psoas abscess in left hip of Sk, 307 (ww104), Wetwang Salck.

5.2.17 Wilsford Barrow, Normanton, Wiltshire (a)

5.2.17.1 Skeleton Information:

Skeleton No: 11 (Eu 1.4.73) *(Sample Code: DkWBS2)*
Condition: Good although some areas very fragmented.
Period: Bronze Age
Age: 17-34
Sex: Female

5.2.17.2 Preservation:

The vast majority of the skeleton was present and in generally good condition with some long bones being complete. The cranium, however, was rather fragmented making positive

identification of all elements difficult. Areas for which this was the case included the nasal, lacrimal, palatine, sphenoid, vomer and ethmoid bones but it is believed that most, if not all, of these elements were represented to a greater or lesser degree through the available fragments. The parietal, temporal, frontal and occipital bones were all present, in addition to the zygomatic bone and mandible. All ribs and vertebrae were accounted for, although C2 was only 50% present, and there were many small rib fragments. The long bones, with the exception of the left femur, were all accounted for, although the right humerus had sustained post-mortem damage at both the proximal and distal ends and the right tibia and the proximal. Whilst both ulnae were present, the right ulna was missing its distal joint surface, and both the distal third of the shafts and the distal joint surface of the left were absent. Both fibulae were entirely present but broken postmorteM. The left and right ilia and ischia were 75% and 50-75% preserved, respectively. The left and right pubic symphyses, clavicles and patellae were all at least 75%, and the scapulae on both sides between 50 and 75%, but this did include both the acromium and glenoid fossa on the left side. The sternum and sacrum were also at least 75% present but the coccyx was missing entirely. All metacarpals and metatarsals were accounted for, with the exception of the left 4^{th} metatarsal. No proximal hand phalanges were recorded but two and five were present for the middle and distal regions, respectively. For the foot, five proximal, ten middle and five distal phalanges were present. The right and left carpals were moderately represented although the left lunate and both hamates were missing; no pisiform or sesamoid bones were present for either side. The tarsals were slightly better represented with only the right 2^{nd} cuneiform bone missing.

5.2.17.3 Dentition:

All teeth were present except the left mandibular first premolar and second molar which were probably lost post mortem.

5.2.17.4 Age/Sex Estimation:

Although fragmented a number of cranial elements could be assessed to provide an estimation of sex. The mastoid process, supraorbital margin (Buikstra and Ubelaker, 1994) and mandible (Brickley and McKinley, 2004) provided firm female scores, whilst the glabella (Buikstra and Ubelaker, 1994) was somewhat more ambiguous. Pelvic elements, however (Buikstra and Ubelaker, 1994), all convincingly demonstrated female characteristics. Age estimation was also hindered slightly by fragmented skeletal elements such as the clavicle (Scheuer and Black, 2004) and pubic symphyses (Brooks and Suchey, 1990). Fusion of the sacrum (Scheuer and Black, 2004), appearance of the auricular surface (Lovejoy *et al*., 1985), morphology of the sternal end of the fourth rib (Işcan *et al*., 1984), in addition to dental attrition (Brothwell, 1981) and data from the clavicle and pubic symphyses already mentioned, provided a relatively broad but very convincing age of between 17 and 34 years.

5.2.17.5 Pathology:

The distribution pattern of all pathology (including primary), is shown in Figure 5-31 and Figure 5-32.

Pathology was noted on the proximal anterior surface of the left tibia. This consisted of a small hole in the centre of the anterior surface, approximately 1mm in diameter. It showed a small amount of bone remodelling around its edge. There was also a second hole, elliptical in shape, on the proximal anterior surface, to the left of centre and slightly below the first hole. This hole showed no bone remodelling and may possibly be post-mortem damage as opposed to pathology. Both radial heads showed a slight exaggerated lateral angle. The auricular surface on the left innominate showed lytic lesions.

5.2.17.6 Primary Pathology:

There were a number of pathological areas noted in the vertebrae (Figure 5-32). In cervical vertebra C3 destructive cavitation lesions were observed in the vertebral body, as in C5. C5 showed destruction on the right side of the inferior body, and the area leading to the right articular facet was also affected by destructive lesions. C7 also showed destructive lesions on the superior part of the vertebral body, but some of this may be due to post mortem damage. The axis suffered significant destruction extending to the right side and right articular facet (Figure 5-33).

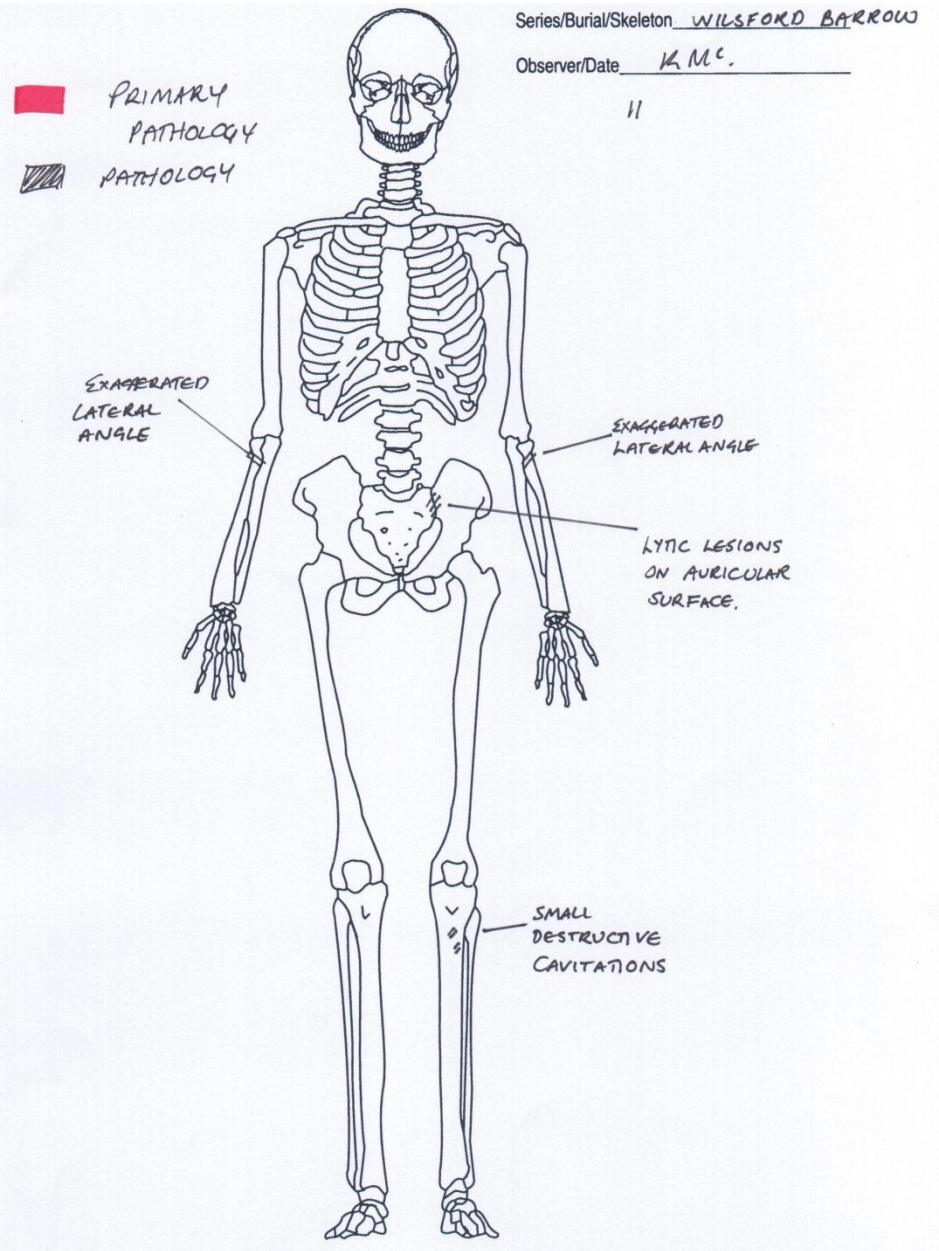


Figure 5-31 Distribution of pathological lesions in Sk. 11, Wilsford Barrow (Anterior View)

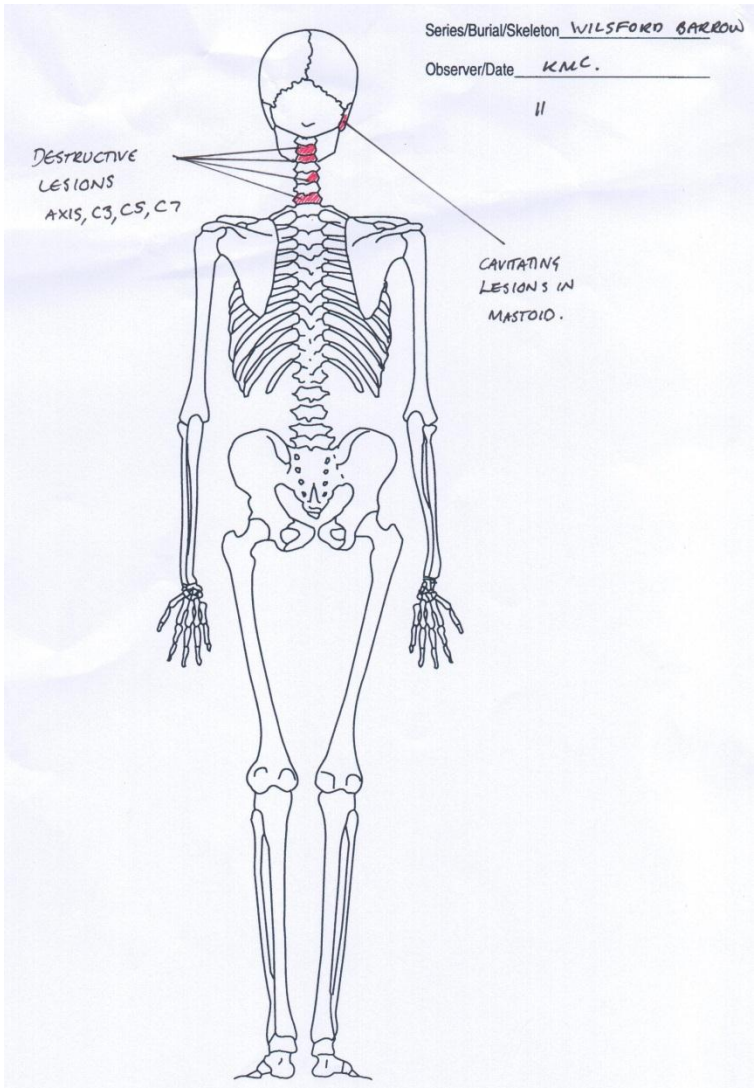


Figure 5-32 Distribution of pathological lesions in Sk. 11, Wilsford Barrow (Posterior View)

Figure 5-33 Axis vertebra with extensive destruction of the body, Sk. 11, Wilsford Barrow.

The most unusual pathological lesion seen on this individual is present in the right mastoid process (Figure 5-34). Numerous extensive cavitating lesions are present over the majority of the surface, although less on the anterior surface, with a greater prevalence on the tip of the mastoid process body. These are smooth edged and possibly examples of well healed and extensive bone remodeling. The largest of these holes is approximately 10mm at its widest, the smallest being approximately 1mM. The cavitations also extend beyond the body of the mastoid process itself, the largest reaching slightly above the body onto the more flattened area of the temporal bone. The holes, or cavitations resemble a 'swiss cheese' appearance and further holes are visible inside many of these cavitations.

Figure 5-34 Right mastoid process showing unusual cavitations throughout, Sk. 11, Wilsford Barrow.

Dental: Whilst all but two teeth are present, there is no pathology of which to speak although there is a calculus on the left and right maxillary 1st and 2nd molars in addition to some wear seen on all mandibular and maxillary incisors and canines. There was also some green staining on the crown on the 2nd left mandibular molar tooth.

5.2.18 Wilsford Barrow, Normanton, Wiltshire (b)

5.2.18.1 Skeleton Information:

Skeleton Code: Burial 7 (Eu.1.4.81) (Burial VII with baby/foetus in Causeway Grave) *(Sample Code: DkWBS1)*
Condition: Very Fragmentary

Period: Early Bronze Age
Age: 9-12
Sex: Unknown

5.2.18.2 Preservation:

The general state of the skeletal material was very fragmentary. Both left and right parietal, temporal and maxillary bones were present, with the frontal and occipital bones represented but not in their entirety. The only other cranial bones represented were the right zygomatic and the mandible. The vertebrae were also highly fragmentary but the cervical area was represented by three bodies, five right neural arches and four left, the thoracic by 11 bodies, 12 right arches and 11 left, whilst the lumbar spine preserved only half of one body and one left and one right neural arch. The sacrum preserved two bodies and one right and one left arch. The ribs were again fragmentary, but 12 left and six right were definitely present, although due to the quantity of fragments this is conservative.

There were many small fragments from the long bones, many of which could not be confidently identified. For example, there were many small tibial fragments but their preservation was such that they could not be assigned a side or definitively identified as belonging to the proximal, mid or distal thirds of the shaft. The right humerus was present apart from the distal epiphyses, whereas the left was only represented by fragments of the distal third. No evidence of the right radius was identified but the middle and lower portions for the left were present. The right ulna preserved parts of both the proximal and mid thirds of the shaft; the left preserved fragments from all shaft areas. The left femur was absent, although the right was represented by various fragments such as the proximal epiphyses, the mid third of the diaphysis, and half of the distal epiphyses. The tibiae, as already mentioned, could not be clearly identified, and the right fibula was only represented by the mid third of the shaft, whereas the left preserved the entire shaft but no epiphysis. The presence of parts of the innominate bones were negligible with nothing present for the left and only a small percentage (less than 25%) of the ilium for the right. There was a similar quantity of material for the scapulae but the clavicle from the right was better represented being almost complete, and the left showing at least 50-75% presences.

The hands and the feet demonstrated some presence with 10 metacarpals and 8 hand phalanges, in addition to possibly six carpal bones being present. These were damaged and relatively indistinguishable.

5.2.18.3 Dentition:

All teeth were present for this skeleton which was of great assistance for ageing the remains. The dentition was in a phase of change from deciduous to permanent.

5.2.18.4 Age/Sex Estimation:

No sex could be estimated for this individual as the remains were clearly sub-adult. Age estimation was derived from epiphyseal fusion and dental eruption (Ubelaker and Grant, 1989); no long bones were complete and thus no accurate measurements could be taken. Ageing was extremely difficult due to the extremely fragmentary nature of the skeleton. For this reason, although epiphyseal fusion was recorded where possible and as accurately as possible, the primary age estimation was derived from dental eruption. There was some ambiguity with the stage of dental eruption; eruption of the second molars (both the left mandibular and maxillary), suggested an age of 11 to 12 years. The remaining dentition, a mix of deciduous and permanent teeth, suggested an age of nine to 10 years. Whilst the majority of dentition suggested a lower age, for the purpose of clarity and accuracy, the age has been suggested as spanning both categories, thus nine to 12 years.

5.2.18.5 Primary Pathology:

On many of the long bone fragments, there were patches of periosteal new bone formation; the tibia or tibiae were definitely affected although it is not possible to be more precise. The cranium, however, showed clear areas of extensive, destructive pathology. This was specific to the left parietal bone; the bone had been broken into four relatively even parts, but subsequently glued back together. At the point of these four bones meeting – one crack

running from directly above the temporal bone, level with the posterior, protruding portion of the zygomatic arch and intersecting approximately half way between the top of the temporal bone and saggital suture, the other running at a right angle to this from the coronal to lambdoid sutures – a large, irregular shaped hole had formed (Figure 5-35). Both the endocranial and ectocranial surfaces are perforated, although it is suggested that some of this irregularity at the margin of the hole may have been caused by damage when these four parts of the parietal bone were independent of each other and not glued during post excavation work. Some of the glue used to perform this reconstruction now overlies some of the edges of the hole, obscuring the true nature of the surface. None of the edges looked freshly broken, based on colour of the bone, and on closer inspection it was not obvious that any recent breakage had occurred on at least the majority of the lesion edge. It is possible however, that any pathological focus in this area may have weakened the bone, allowing some damage post deposition rather than post excavation. Evidence to suggest that this is most likely to be a pathological perforation on the bone, however, rather than just damage, is visible on the endocranial surface. Here, a cluster of vascular lines are evident on the bone surface, most focussed around the perforation and further plaque like, active new bone radiates away (Figure 5-36). The perforation measures approximately 30mm at its longest (antero-posterior) and approximately 15mm at its widest.

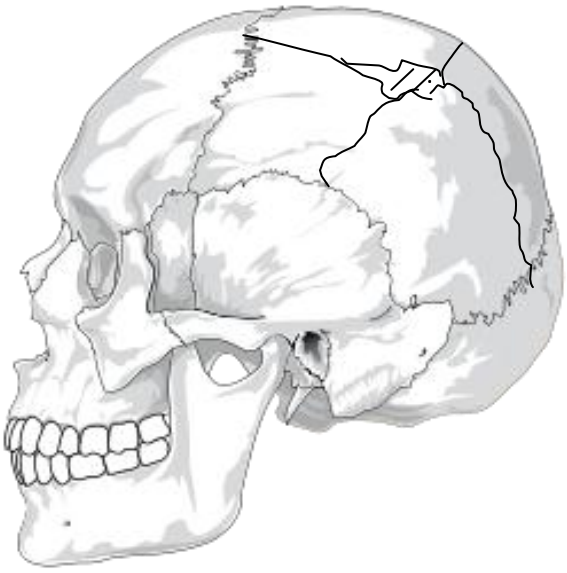


Figure 5-35 Diagram to clarify the position of parietal perforation.

Figure 5-36 Endocranial view of right parietal bone showing vascular lesions and additional plaque like bone on the inner table, Sk. 7, Wilsford Barrow.

5.2.18.6 Dentition:

None

5.3 Collated Data

Due to the relatively small numbers of sampled individuals, intra-population comparisons cannot be presented, including prevalence rates for the particular pathologies noted above. However, some basic data collation is described, which will be discussed in further detail in Chapter 6.

5.3.1 Number of Neolithic, Bonze Age and Iron Age Samples:

Seventeen non-control individuals were also included in the study from the same sites. These individuals spanned the three prehistoric periods discussed throughout, the Neolithic, Bronze Age and Iron Age (Figure 5-37). Only two non-control individuals from Neolithic

contexts were analysed, (from the sites of West Tump and Ascott-under-Wychwood), with an almost equal number included between Bronze and Iron Ages, eight and seven respectively. The number of sites represented for each period are two for the Neolithic (as described above), six from the Bronze Age (Methwold Severalls, Wilsford Barrow (two individuals), Twyford Down, Waterhall Farm – Chippenham, Boscombe Airfield and Eyebury) and three from the Iron Age (Abingdon (one individual) Bourton-on-the-Water (one individual) and Wetwang Slack). Wetwang Slack was most represented, with six individuals.

During the course of sampling, it was possible to obtain a control sample for most of the sites included in the study (Figure 5-38), with the exception of Boscombe, Eyebury and Abingdon for which there were no other associated individuals.

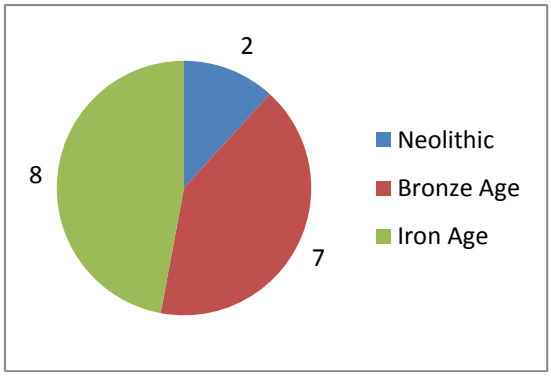


Figure 5-37 Distribution of all individuals included in the study, based on the prehistoric period from which they originate.

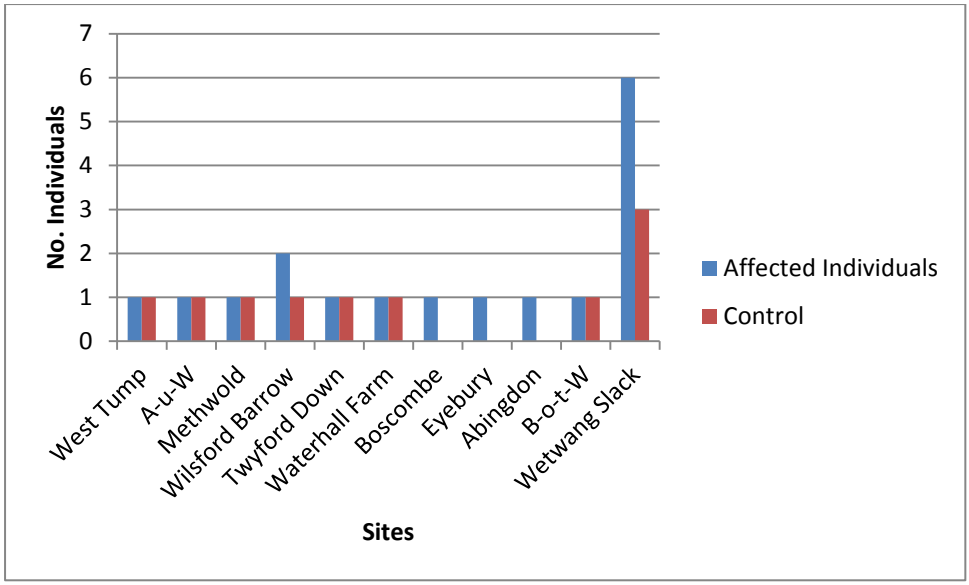


Figure 5-38 Distribution of all individuals included in the study, based on the site from which they originate and their associated control samples.

5.3.2 Biological Sex Distribution:

Figure 5-39 shows a higher number of males included in the study, eight in total, than females, which number five. There is one "possible" female, however, which will be considered "female" in the following collated data. This then results more even number of males and females selected for analyses but the number of males is still slightly higher. The "unknown" individuals in the table are composed of juveniles for which sex estimation cannot be offered.

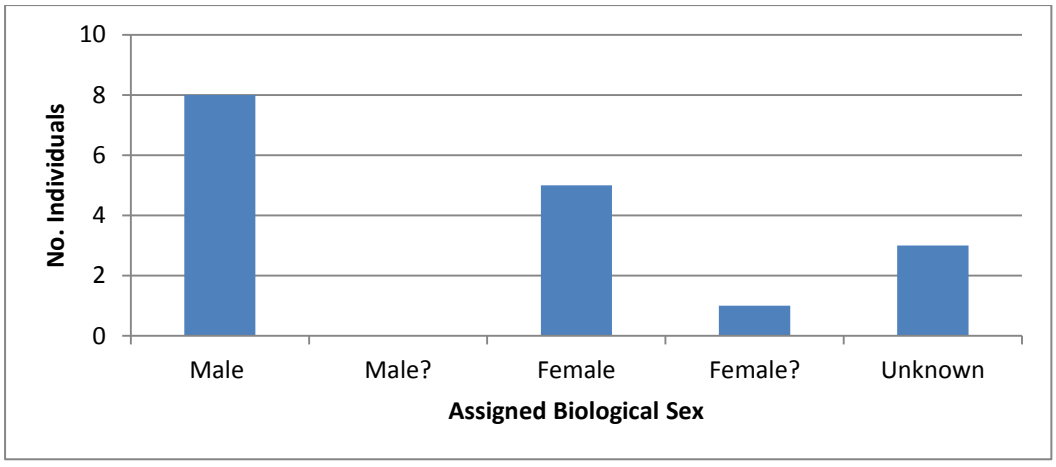


Figure 5-39 Collated data on estimated biological sex of all individuals across all sites and periods (excluding controls).

5.3.3 Estimated Age at Death Distribution:

Age at death estimations demonstrate that the vast majority of individuals included in the project were considered to be young adults aged 18-34 (Buikstra and Ubelaker 1994) (Figure 5-40). Only one adult could not be assigned an age category due to poor preservation and there were no old adults (aged 50 or above). Juveniles (any individual aged less than 18) accounted for three out of seventeen individuals, young adults (aged 18-34) accounted for eight, and middle adults (aged 35-49), five.

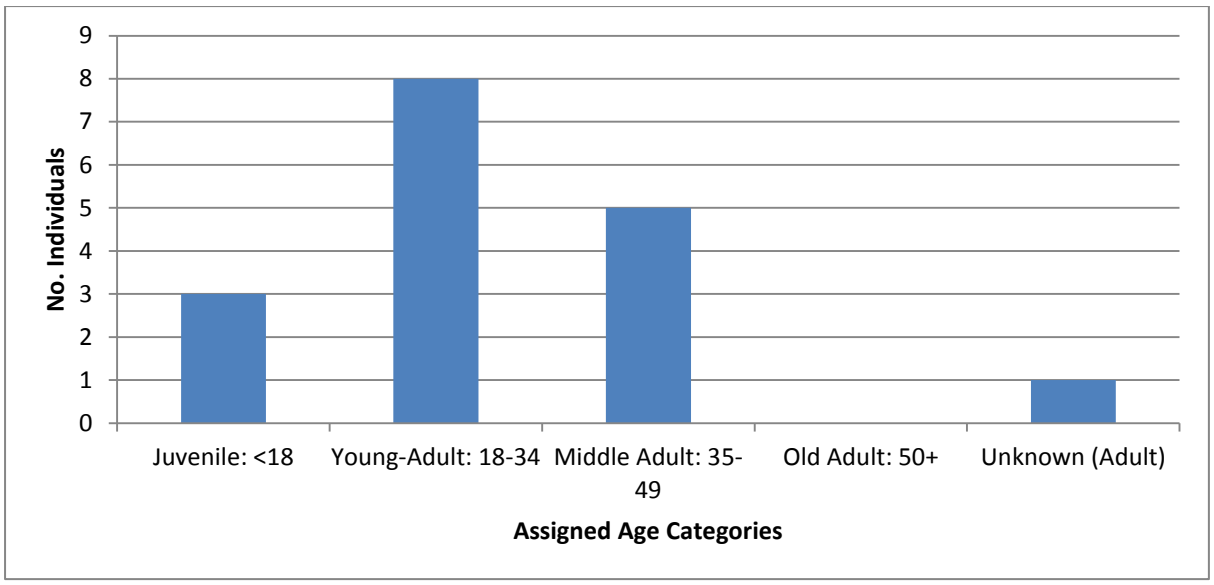


Figure 5-40 Collated data on estimated age of death of non-control individuals across all sites and periods.

5.3.4 Skeletal Distribution of Pathology:

Figure 5-41 demonstrates the range of skeletally distributed pathology between the individuals. It should be noted that this figure is not inclusive of *all* pathology recorded in *all* individuals but rather, includes the key pathology which prompted the decision to include an individual in the study. This was due to the pathology being potentially suggestive of TB infection despite it being classified nonspecific in nature. More in-depth discussion of the total pathological lesions relevant to the potential diagnosis of TB can be found in Chapter 4, and is also described above in this chapter. The appearance of incomplete individuals in Figure 5-41 (0.5 values) is due to two examples where the determining pathology was split between two skeletal areas. In these cases, the 1 unit value for the individual was split in

half, with each 0.5 unit value being assigned to one of the two skeletal locations of pathology.

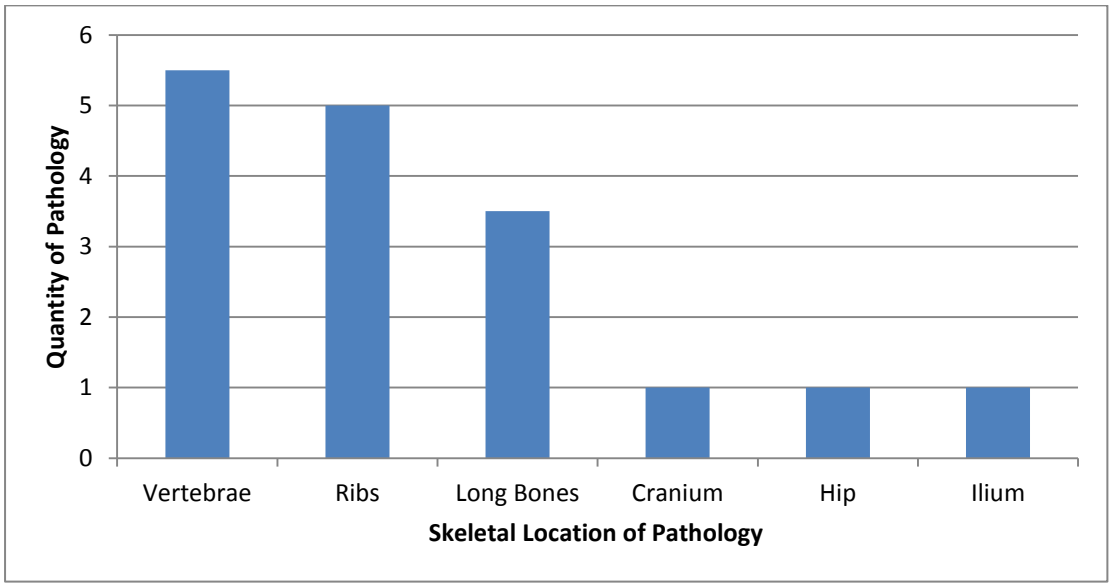


Figure 5-41 Skeletal Location of key pathology included in the study demonstrated by non-control individuals across all sites and periods.

5.3.5 Pathology Relative to Age at Death Estimation

Juveniles demonstrated pathology in the ribs, long bone and cranium. Young adults were primarily affected in the vertebrae but also in the long bones, ribs, ilium and cranium. Middle aged adults demonstrated pathology in the ribs, equal in number to that of young adults (one example), the long bones (one less example than that of young adults) and in the hip. The adult, to whom an age category could not be assigned, demonstrated rib lesions. These results are summarised in Figure 5-42.

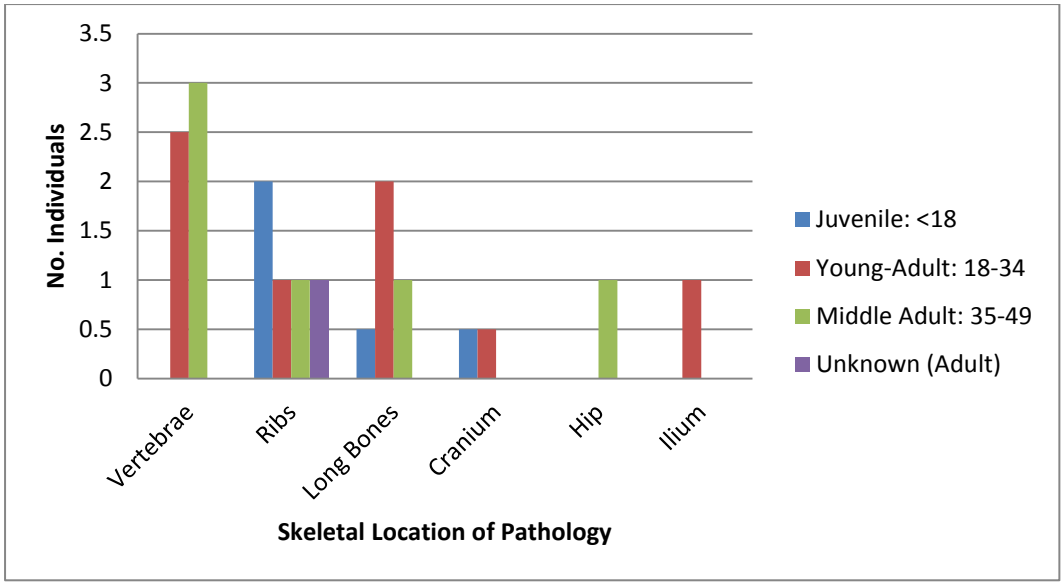


Figure 5-42 Location of pathology in relation to age at death in all non-control individuals across all periods and sites.

5.3.6 Pathology Relative to Estimated Biological Sex

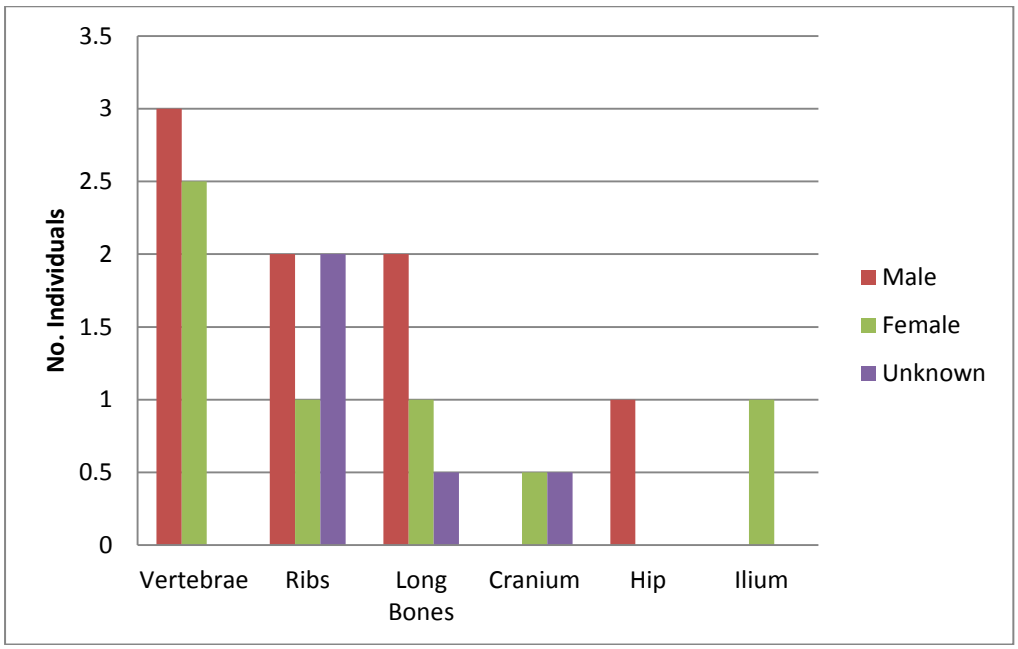


Figure 5-43 Location of pathology in relation to estimated biological sex in all non-control individuals across all periods and sites.

For the purpose of these results, the single "female?" (rather than "female") skeleton was considered as "female", for clarity. Identified males mostly demonstrated vertebral pathology, followed by equal examples of rib and long bone pathology; hip pathology was demonstrated by a single male. Females also mostly demonstrated vertebral pathology.

Long bones, ribs and the ilium were the second most common area for pathological lesions, followed by the cranium. As with the pathology in relation to estimated biological sex, half units values (0.5) are apparent in Figure 5-43 and this is again due to the two types of pathology present on two specific individuals which gave cause for inclusion in the study. The individual who could not be assigned a biological sex demonstrated pathology on the cranium and on a long bone.

5.3.7 Pathology Relative to Prehistoric Period Origin

Individuals from the Neolithic demonstrated two locations for skeletal pathology; in the vertebrae and the ribs. Pathologies in the Bronze Age individuals were spread across the vertebrae, ribs, long bones and cranium, in descending order. The 1.5 unit value of long bone pathology is again due to two pathological lesions being represented by one individual. The Iron Age shows the greatest distribution of pathology, represented by all areas, with the exception of the cranium. These results are presented in Figure 5-44.

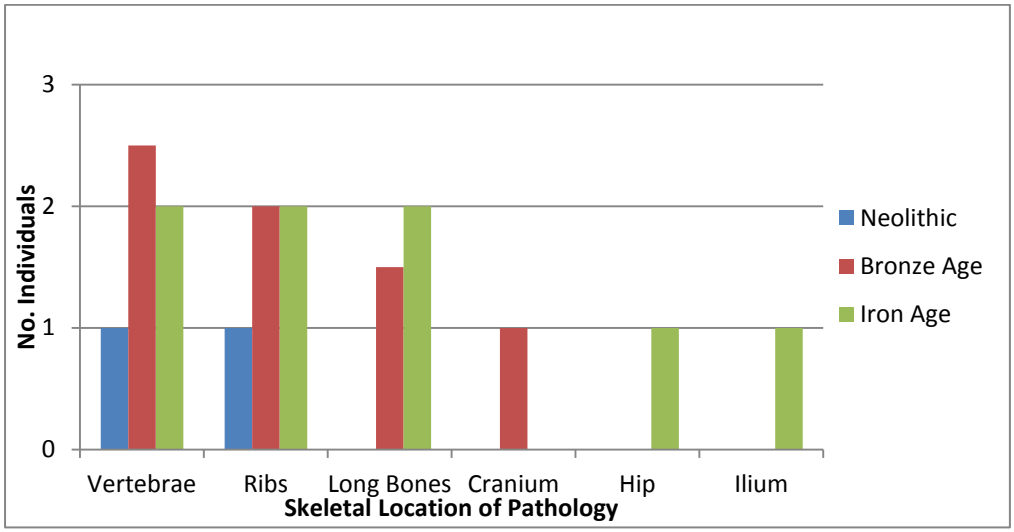


Figure 5-44 Location of Pathology in Relation to Prehistoric Period in All Non-control Individuals Across All Sites.

5.3.8 Distribution of Vertebral Pathology:

Figure 5-45 demonstrates the range and frequency of vertebrae identified as having pathological lesions as part of this study. Only vertebral lesions suspected of being potentitally involved in TB infection are included here, as opposed to any vertebrae mention in the text demonstrating pathology due to other causes such as DJD. The vertebrae

included are mostly C7, T1, and L2, with two examples each respectively. Other vertebrae affected in the upper lumbar and lower thoracic area are adjacent to one another (or L2) and include (with one example each) T12, L1 and L3. Those vertebrae affected in the upper spine, other than those already mentioned, are C2 and C3, adjacent to one another, C5 which stands alone in the Figure and T2 which lies adjacent to T1, one of the most affected vertebra.

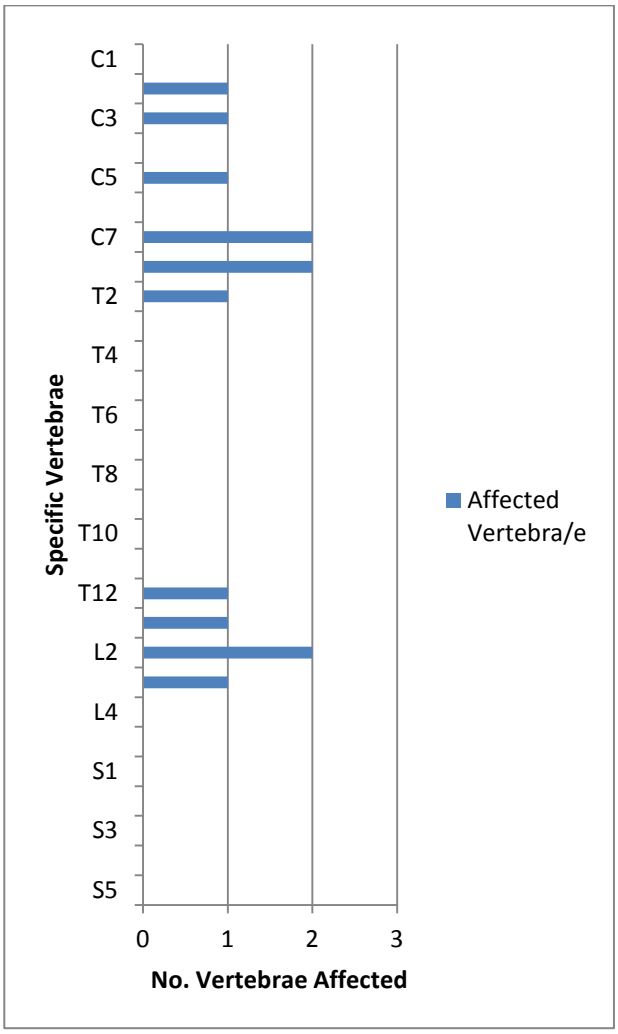


Figure 5-45 Vertebral pathology distribution across all non-control individuals sampled.

5.4 Biomolecular Results

Samples were divided in to six groups, with each including at least three extraction stage blanks and two PCR stage blanks (Table 5-2).

	Total Samples									
Group 1	G1-P1	G1-B1	DkCHS1	DkCHs2c	DkWBS1	DkWBS2	DkWBS3c	G1-B2	G1-B3	G1-P2
Group 2	G2-P1	G2-B1	WnTDS1	WnTDS2c	WxFHS1	ChWTS1	ChWTS2c	G2-B2	G2-B3	G2-P2
Group 3	G3-P1	G3-B1	BotWS1	BotWS2c	NkMS1	NkMS2c	OxABS1	G3-B2	G3-B3	G3-P2
Group 4	G4-P1	G4-B1	WWSS1	WWSS2	WWSS3c	WWSS4c	WWSS5	G4-B2	G4-B3	G4-P2
Group 5	G5-P1	G5-B1	WWSS6	WWSS7c	WWSS8	WWSS9	G5-B2	G5-B3	G5-B4	G5-P2
NHM Group	NHM-P1	NHM-B1	NHMA2Cc	NHMA3	NHMEYE223	NHM-B2	NHM-P2			

(Table 5-2: Samples divided into their groups as used in the PCR stage. P = PCR blank and B = extraction stage blank).

Of the six groups, consisting of 27 samples (ten of which were samples from control skeletons), no positive results were obtained for either IS element, IS*6110* or IS*1081*. This demonstrates a lack of positive identification for tuberculosis, targeting repetitive elements commonly found in M. *tuberculosis* (IS*6110*) and M. *bovis* (IS*1081*), the two most frequent strains that affect humans.

5.4.1 1st Round MTB *Assays*

The first round of results, which at first included 24 of the final 27 samples (the Natural History Museum (NHM) samples had not yet been collected at the time of analysis), produced a number of positive PCR products which were sent to Applied Biosystems for direct sequencing (Appendix 4). The NHM samples were analysed separately at a later date but are presented here, alongside the other five groups. Samples selected for purification and subsequent sequencing, displayed banding (identified by gel electrophoresis) at, or close to, their appropriate targeted base pair level (e.g. Figure 5-47). For the outer IS*6110* primers this was 123bp and for the nested or inner IS*6110* primers, 92bp. The outer IS*1081* primers targeted 135bp and the inner primers for the hemi-nested PCR, 113bp. These bands, or base pair levels, were identifiable by using the known base pair distribution numbers of ladders PstIλ and *Hin*dIII which were both included in each gel run (Figure 5-46).

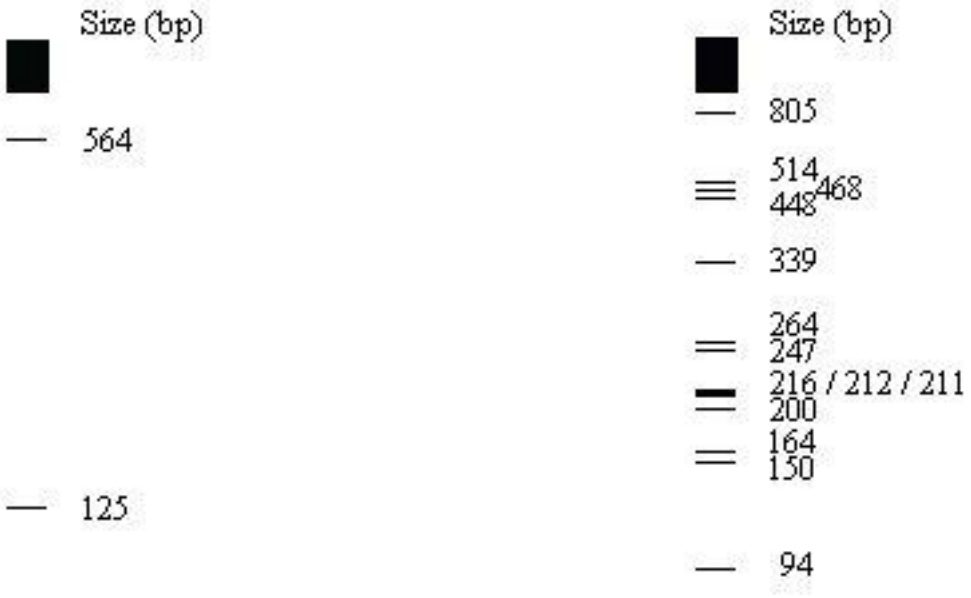


Figure 5-46 (Left) λ DNA restricted by *Hin*dIII on a 3% agarose gel. (Right) λ DNA restricted by *Pst*I on a 3% agarose gel. (Bouwman, 2004).

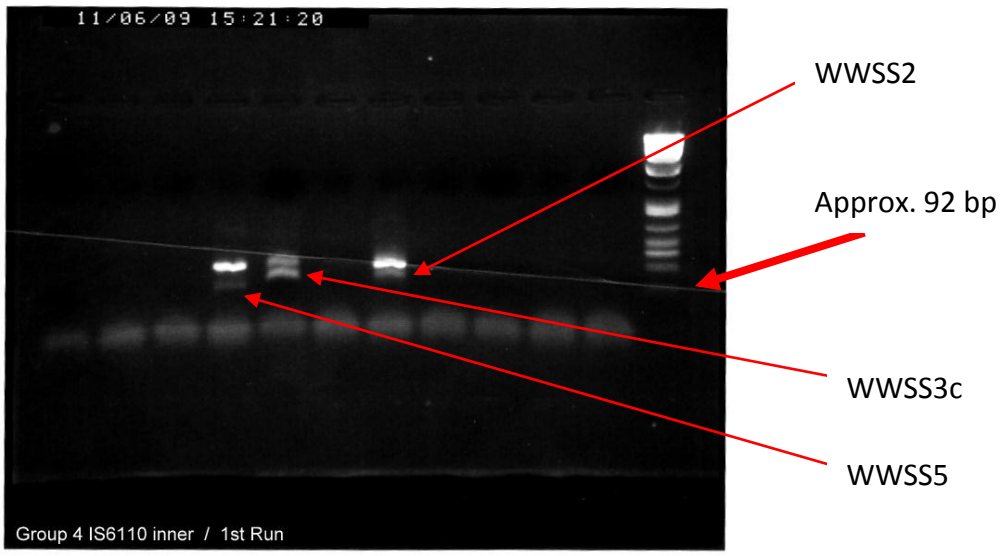


Figure 5-47 Example of PCR product appearance after gel electrophoresis. This example is from the 1st round MTB **assays and shows three products visible from group 4: WWSS2, WWSS3c, WWSS5.**

The specific bands representing PCR product, which were identified on the agarose gels, related to each specific IS element (IS*6110* and IS*1081*) using both outer, and inner (hemi-nested) primers. These are summarised below (Table 5-3).

	Sample	IS*6110* Outer	IS*6110* Inner	IS*1081* Outer	IS*1081* Inner
GROUP 1	G1-P1				
	G1-B1				
	DkCHS1			X	X
	DkCHs2c		**X**	X	X
	DkWBS1			X	X
	DkWBS2			X	X
	DkWBS3c	X	X	X	X
	G1-B2				
	G1-B3				
	G1-P2				
GROUP 2	G2-P1				
	G2-B1				
	WnTDS1			X	X
	WnTDS2c			X	X
	WxFHS1	X		X	X
	ChWTS1	X		X	
	ChWTS2c	X		X	
	G2-B2				X
	G2-B3				
	G2-P2				
GROUP 3	G3-P1				
	G3-B1				
	BotWS1			X	X
	BotWS2c			X	X
	NkMS1				
	NkMS2c				
	OxABS1			X	X
	G3-B2		**X**		
	G3-B3				
	G3-P2				
GROUP 4	G4-P1				
	G4-B1				
	WWSS1			X	X
	WWSS2		X	X	**X**
	WWSS3c		X		**X**
	WWSS4c			X	X
	WWSS5		X	X	X
	G4-B2				
	G4-B3				
	G4-P2				
	G5-P1				
	G5-B1				
	WWSS6		X		
	WWSS7c				X

GROUP 5	WWSS8				X
	WWSS9				
	G5-B2				
	G5-B3				
	G5-B4				
	G5-P2				
NHM	NHM-P1				
	NHM-B1				
	NHMA2C				
	NHMA3	X			
	NHMEYE223				
	NHM-B2				
	NHM-P2				

Table 5-3 Table demonstrates samples according to their group for 1st round MTB **assays. X = a sample where PCR product was identified, with banding appearing a or close to the targeted base pair region, all of which were cut out and sent for sequencing Those in bold represent sequences which were returned with readable sequences.**

Of the above samples which were returned from sequencing, seven returned readable sequences with a limited number of unreadable bases. Those which produced readable sequences consisted of four separate samples in total, one of which was an extraction blank (G3B2 / IS*6110* inner / forward and reverse primers) and two which were original control samples, DkCHS2c (IS*6110* / inner / forward and reverse) and WWSS3c (IS*1081*/ inner / forward and reverse). The fourth sample was WWSS2 (IS*1081* / inner / forward primer).

These sequences were subsequently run through BLAST, an online database of all known and named sequences. Sample DkCHS2c (both forward and reverse sequences), consisted of sequence lengths too long to identify with M.*tb* (175bp and 268bp, respectively), suggesting the possibility of human contamination, and confirmed by results returned through BLAST. The forward primer for G3B2 also returned results consistent with human contamination, having also demonstrated a sequence of increased size to that expected. The sequence length returned for sample G3B2 (reverse primer) was also too long to identify with MTB (244bp) but no results were returned through BLAST, demonstrating the possibility of random amplification of bases during the PCR. Sample WWSS2 showed a sequence too short for MTB, having only 64 base pairs, demonstrating what is likely to be random amplification of base pairs during the PCR stage. Sample WWSS3c (forward primer) had a

short sequence length of 76bp and could not be confidently matched to any known sequence. Sample WWSS3c, using the reverse primer sequence, although consisting of an even smaller sequence length (71bp), was partially identified with a number of TB specific sequences, including M. *bovis* insertion sequence IS*1081*. This match showed an 82% maximum identity, much of which could be accounted for by sequence matching of the primer, and thus the result is not significant.

5.4.2 Repeat MTB *Assays*

All samples were subject to a repeat of the PCR and gel electrophoresis stages. Many demonstrated bands which appeared to be of a similar size to the target sequences and were thus cut out, purified and sent for sequencing as with the first round of assays (Appendix 4). Those which demonstrated banding of interest and were sent for sequencing are summarised below (Table 5-4).

	Sample	**IS*6110* Outer**	**IS*6110* Inner**	**IS*1081* Outer**	**IS*1081* Inner**
GROUP 1	G1-P1				
	G1-B1				
	DkCHS1	X	**X**		
	DkCHs2c	X	X		
	DkWBS1	X	X		
	DkWBS2	X	**X**		
	DkWBS3c	X	X		
	G1-B2				
	G1-B3				
	G1-P2				
GROUP 2	G2-P1				
	G2-B1				
	WnTDS1		X		X
	WnTDS2c		X	X	X
	WxFHS1		X	**X**	X
	ChWTS1			X	**X**
	ChWTS2c			**X**	X
	G2-B2				
	G2-B3				
	G2-P2				
	G3-P1				

GROUP 3	G3-B1				
	BotWS1			X	
	BotWS2c			X	
	NkMS1				
	NkMS2c				
	OxABS1			**X**	
	G3-B2				
	G3-B3				
	G3-P2				
GROUP 4	G4-P1				
	G4-B1				
	WWSS1			X	
	WWSS2			**X**	
	WWSS3c				
	WWSS4c			X	
	WWSS5			**X**	
	G4-B2				
	G4-B3				
	G4-P2				
GROUP 5	G5-P1				
	G5-B1				
	WWSS6				
	WWSS7c			**X**	
	WWSS8			**X**	
	WWSS9				
	G5-B2				
	G5-B3				
	G5-B4				
	G5-P2				
NHM	NHM-P1				
	NHM-B1				
	NHMA2C	X	X	X	**X**
	NHMA3	X	X	X	X
	NHMEYE223				
	NHM-B2				
	NHM-P2				

Table 5-4 Table demonstrates samples according to their group for repeat MTB **assays. X = a sample where PCR product was identified, with banding appearing a or close to the targeted base pair region, all of which were cut out and sent for sequencing Those in bold represent sequences which were returned with readable sequences.**

A total of 16 sample sequences were returned with mostly readable base pairs, accounting for 11 individual samples in total. These samples were as follows:- DkWBS2 (IS*6110* / inner /

reverse primer); DkCHs1 (IS*6110* / inner / reverse); ChWtS1 (IS*1081* / inner / forward and reverse); ChWTS2c (IS*1081* / outer / forward and reverse); WxFHS1 (IS*1081* / outer / forward and reverse); OxABS1 (IS*1081* / outer / forward); WWSS2 (IS*1081* / outer / forward and reverse); WWSS5 (IS*1081* / outer / forward); WWSS7c (IS*1081* / outer / reverse); WWSS8 (IS*1081* / outer / forward); NHMA2C (IS*1081* / inner / forward and reverse).

The sequences returned included a combination of samples tested for IS*6110* and IS*1081* elements, both outer and inner (hemi-nested), and forward and reverse primers. Of the 16 sample sequences returned, seven of these all demonstrated one identical sequence, another group of five, also demonstrated a different but identical sequence to one another, leaving a total of four with completely individual sequences.

The seven identical sequences were from all those directly sequenced from forward primers and of element IS*1081* (all outer primers with the exception of one) were ChWtS1 (inner), ChWTS2c, WxFHS1, OxABS1, WWSS2, WWSS5, WWSS8. The repetitive sequence was as follows:-

GCGTCNGAACTGCAATGACGACCGCGACGGATGCAATCCCTGcaAAGCtGACCAGCCCGGCATAA AACTCAGCAAGCGTCACGGGGCGTGATTTCGACACCCGTGCCA

There were no identifiable differences between any of the sequences, although between 75bp and 90bp there were a few unidentifiable bases (Appendix 5), the pattern of which still matched the specific sequence outlined above. ChWTS2c was the only sequence which was 100% complete and it is that sequence which is shown above.

BLAST results identified an 80% maximum identity with *Pantoea ananatic* LMG 20103 which had a very low expectant value of 1.1, but this covered only 38% of the specified sequence. There was a maximum identity of 100% against *Mycobacterium tuberculosis*complex, including M. *bovis* insertion sequence IS*1081* specifically, covering 19% of the sequence, which demonstrates recognition of the original IS*1081* primer. Other than the primer, no confirmation of tuberculosis was evident in these results.

The second group of sequences to demonstrate a largely identical sequence consisted of ChWtS1 (inner), ChWTS2c, WxFHS1, WWSS2, WWSS7c, all of which were directly sequenced using the reverse primer and, again, the insertion element IS*1081*. These sequences demonstrated the following pattern of bases:

TCTGCTCTCGACGTTCATCGCCGTGCGCCAGCGGAAGATGTAGTGGCTGACGAAAAACCGCGTCA GAACTGCAATGACGACCGCGACGGATGCAatCCCTGCAAAGCtgA

Again, with the exception of a few missing, additional or unidentifiable bases, all the sequences identified above, were identical. BLAST results generated particularly interesting results, showing a maximum identity of 80% with a number of *Brucella* species and variations including *Brucella microti, melitensis, abortus, suis, canis, and ovis*. All results showed the 80% maximum identity, coverage of 85%, and a total/max scored of 89.7, which therefore demonstrated a sequence familiar to all *Brucella* species identified (Appendix 6), warranting further tests.

Those sequences which were returned but showed sequences not matched to any other, were DkCHS1 (IS*6110* / inner / reverse), NHMA2C (IS*1081* / inner / forward), NHMA2c (IS*1081* / inner / reverse), and DkWBS2 (IS*6110* / inner / reverse). None of these sequences were matched to tuberculosis using BLAST, but for sample NHMA2c both forward and reverse primers showed evidence of contamination.

5.4.3 Brucellosis:

Results for brucellosis were negative. One sample, DkCHS1, demonstrated evidence of PCR product with strong banding. The position of the band appeared to suggest product too long to be represent IS*711*, but was sent for sequencing. Sequencing did not produce a positive result as the sequence was unreadable.

5.5 Real Time PCR Results

5.5.1 Tuberculosis

Real time PCR results for TB did not demonstrate any amplification. There was a small increase in fluorescence, the reason for which is unknown, but the results are definitely devoid of TB aDNA.

5.5.2 Human

Results for mtDNA showed contamination across all groups, with only four blanks uncontaminated (Figure 5-48). Repeats could not be performed at this time.

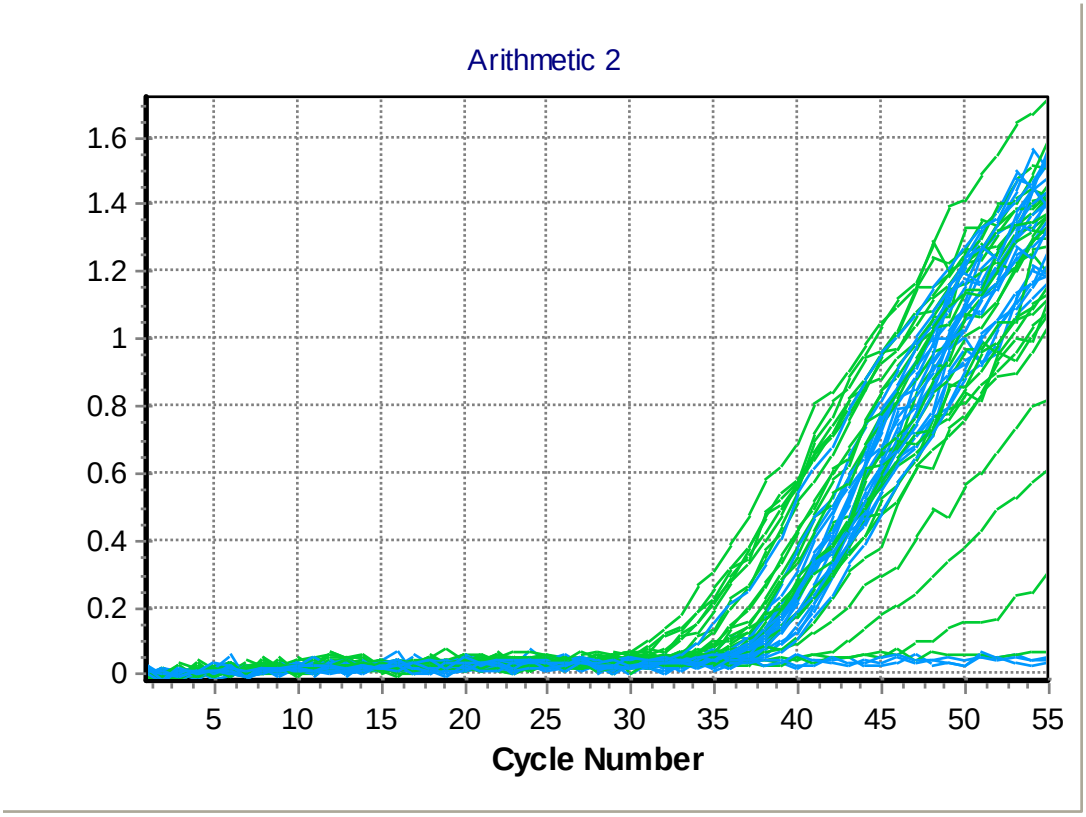


Figure 5-48 Graph showing results of real time PCR: relative fluorescence of all samples tested for mtDNA. Results demonstrate almost complete contamination across all five groups.

Results for LctDNA showed four positive samples across five of the six groups: NHMA2C (NHM: Figure 5-49), DkWbS1 (Group 1: Figure 5-50), WxFHS1 (Group 2: Figure 5-51), WWSS8 (Group 5: Figure 5-52). A fifth sample, from Group 3 also tested positive but this was a PCR blank (G3-P3) (Figure 5-53). No other PCR blanks showed contamination.

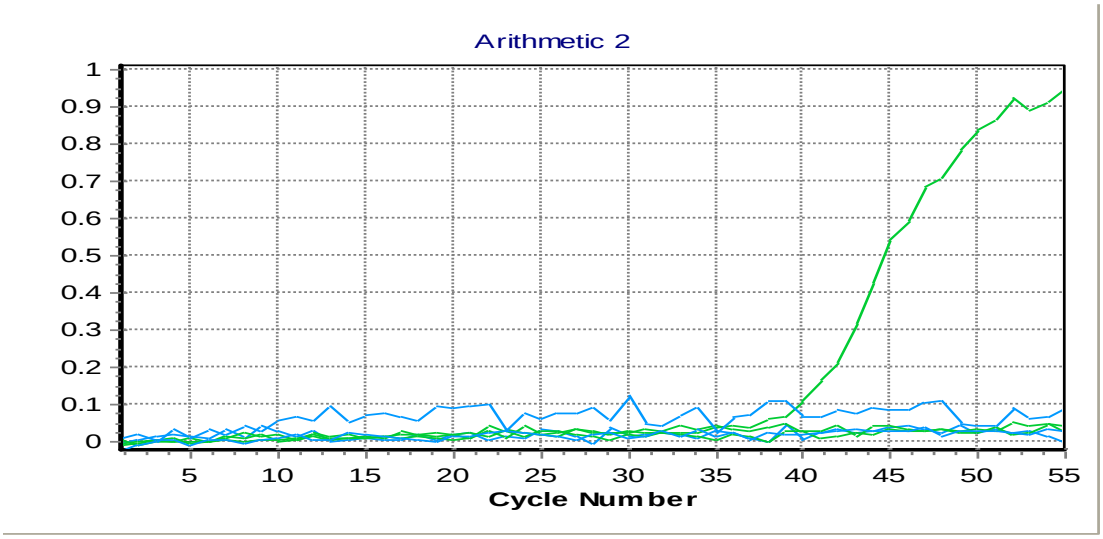


Figure 5-49 Graph showing results of real time PCR: relative fluorescence of all samples tested for LctDNA in NHM Group. Results demonstrate a positive result for sample NHMA2c.

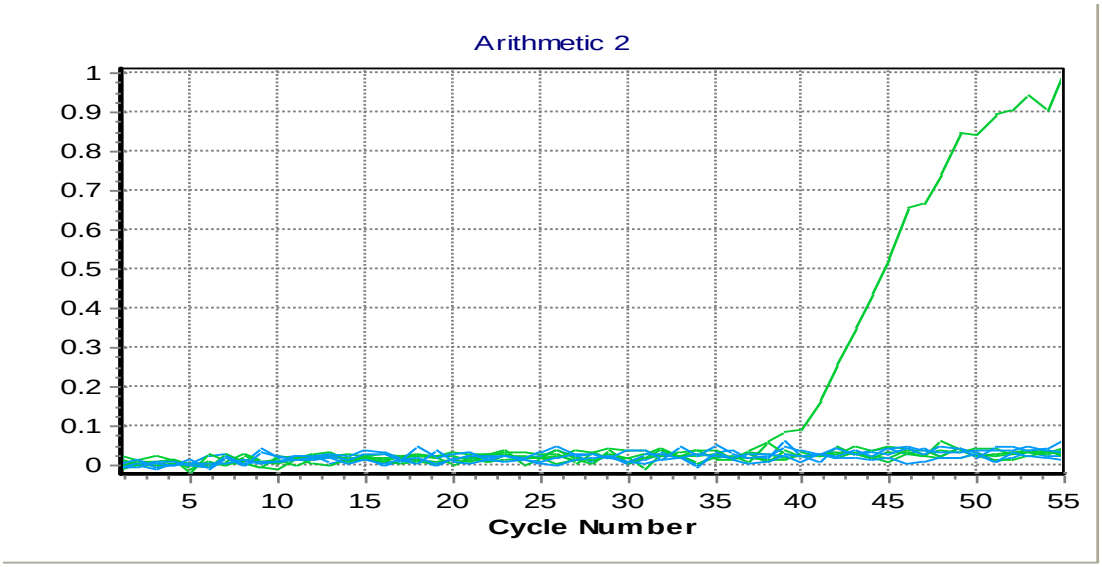


Figure 5-50 Graph showing results of real time PCR: relative fluorescence of all samples tested for LctDNA in Group 1. Results demonstrate a positive result for sample DkWbS1.

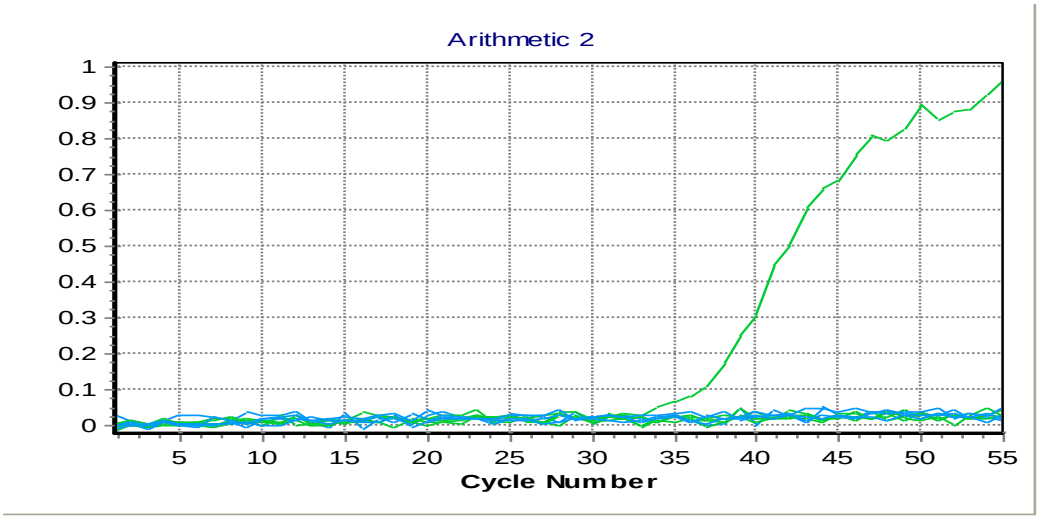


Figure 5-51 Graph showing results of real time PCR: relative fluorescence of all samples tested for LctDNA in Group 2. Results demonstrate a positive result for sample WxFHS1.

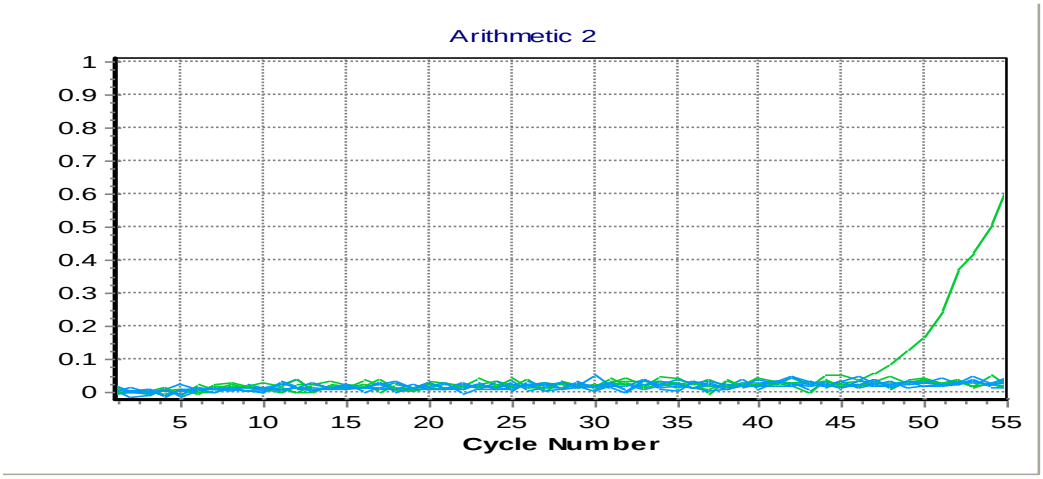


Figure 5-52 Graph showing results of real time PCR: relative fluorescence of all samples tested for LctDNA in Group 5. Results demonstrate a positive result for sample WWSS8

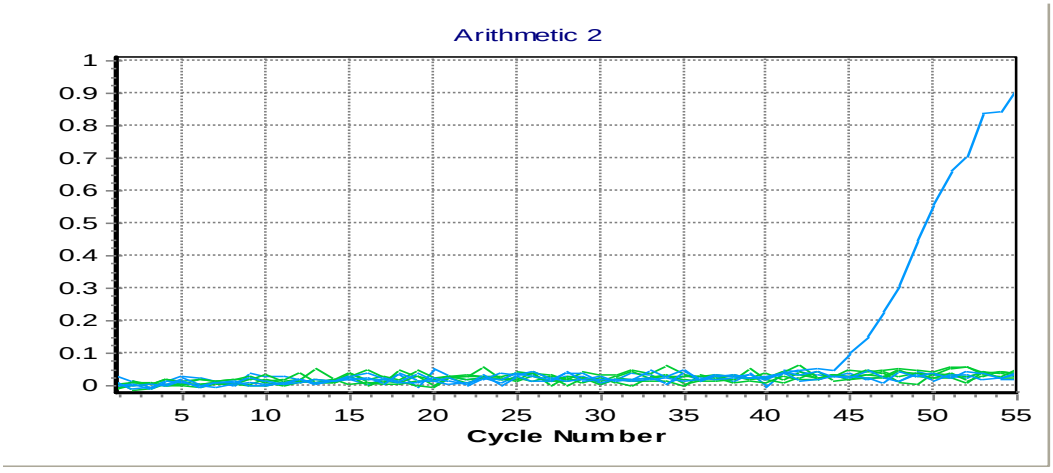


Figure 5-53 Graph showing results of real time PCR: relative fluorescence of all samples tested for LctDNA in Group 3. Results demonstrate a positive result for PCR blank: G3-P3.

6 DISCUSSION

6.1 Introduction:

This chapter brings together and discusses the information so far presented. This includes the nature of TB as a disease and the various social and environmental aspects of the Neolithic, Bronze and Iron Age that may have allowed, or inhibited, TB's ability to become established, if not already so. The results of the macroscopic analyses are discussed in more detail with interpretation of the various pathological lesions which are then considered within the context of both the general periods from which they originate, along with additional information about their specific sites of origin, where possible.

6.1.1 Aims and Objectives

- The **aim** of the project was to test the hypotheses that TB was present in British prehistory, prior to the first published evidence identified in the Iron Age (Mays and Taylor, 2003), and that the infection was caused by both *M. tuberculosis* and *M. bovis.*

- The **objective** of the project was to use ancient DNA (aDNA) from human skeletons to study the bacteria responsible for TB (*M. tuberculosis* complex) in order to then study the origin and evolution of the strains of the bacteria causing TB in prehistoric Britain.

Due to the non-specific nature of the pathology identified and the negative aDNA results returned, it has not been possible to support the hypotheses proposed. The hypotheses are not, however, unsupported by this data as there are many factors which play a part in the study of disease in archaeological populations which must be taken into consideration. The objective was, in part, met as aDNA analysis was performed on all skeletal samples selected. Due to the negative results obtained during the course of this analysis, it was not possible to meet the extended aim of the objective, to study the origin and evolution of the TB strains.

By following strict laboratory protocol however, it is hoped that the biomolecular procedure and issues encountered will contribute to the study of ancient pathogens, particularly that of TB. Overall, this work will contribute to future research in this area, both with respect to supplementing and enhancing the current literature, and acting as a springboard for further investigation into the negligible history of TB in Britain.

It must be highlighted that the skeletons examined during the course of this study (see 4.1.1) were those which were accessible for research purposes (for only one site, was permission denied due to the fragility of the bones), but it is possible that more lie undocumented, or with little documentation, and which are thus not easily visible to the researcher. Finding out the existence of, and location of, those sites which were included in the project, was in itself often time consuming, and on arriving at a museum (not limited to those from which material was actually sampled), there was often additional material which could be considered for examination, but for which information was not accessible prior to the visit, and therefore any examination of these skeletons had to be included in the time previously allotted for that material already selected based on the project criteria. The greatest time constraint however, came from those research visits where no electronic version of a skeletal database existed and therefore, with large collections, either a preliminary visit had to be scheduled or additional time allotted for an unknown quantity of skeletal material to be examined, and potentially, analysed and sampled. It was not known prior to the start of the project, just how time consuming this process would be, and it is suspected that it was compounded by the fact that the skeletal remains required, were of prehistoric date, some having not been analysed since their excavation. The overall process was extremely difficult, and as a result, only skeletal remains from the south of England were included in the project, whereas it was originally hoped that relevant Scottish material could also be included. Further discussion of limitations can be found in section 7.2.

It should also be noted, that the title of this thesis has retained the word ‘Britain’, as opposed to ‘England’ because of the nature of the discussion within this project; the author believes (within the context of this study), it would be difficult and unwise to place an arbitrary geographical limit to the island in the prehistoric periods under study, particularly

when increasing mobility plays a key role in any discussion of infectious disease, modern or ancient.

6.2 Interpretation of Results

6.2.1 Macroscopic analysis

6.2.1.1 Abingdon Pipeline

This skeleton demonstrated a significant amount of pathology distributed throughout the skeletal elements, including the spine, hands and long bones, but these lesions were not individually considered as representing TB related pathology. The pathology most relevant for this study was located in the right femur, where a significant amount of periosteal new bone formation, caused by inflammation of the bone's cortical surface, was apparent. There was also a small destructive lesion on the right fibula which may or may not have been related. This small section of bone, which was loose, was removed to form the aDNA sample due to its proximity to additional periostitis and its inclusion of the resorptive lesion.

It is 'highly probable' that the most common cause of periostitis is infection, although the percentage of periostitis seen in archaeological skeletons attributable to this is unknown (Ortner, 2003: 209) however, periostitis can have many causes, and is frequently attributable to trauma. Infection of the long bones, caused by TB, mostly occurs through haematogenous dissemination (Aufderheide and Rodríguez-Martín, 1998: 137) which may suggest that any periosteal reaction has the ability to be widely distributed because of the systemic nature of the disease. TB in the shafts of long bones is, however, uncommon in adults and usually demonstrates limited periosteal bone formation (Ortner, 2003: 245). In this case, it may be appropriate to refer to the periostitis seen in the femur and fibula as primary periostitis, although 'Designation of primary periostitis in palaeopathological skeletons may mean that only a more specific diagnosis is impossible and does not eliminate the possibility that the periosteal reaction is secondary to a specific disease process.' (Ortner, 2003: 208). Another alternative diagnosis is available as a study on the Coimbra

Identified Skeletal Collection (CISC) has demonstrated a link between TB and hypertrophic osteoarthropathy (HOA) (Santos and Roberts, 2001). The results of another study, combining aDNA analysis and macroscopic analysis have also strongly suggested TB to be the cause of HOA in a medieval skeleton from England (Mays and Taylor, 2002). In this condition, the tibiae, fibulae, radii and ulnae, in addition to some tubular bones of the hands and feet can be affected by a symmetrical diaphyseal periostitis (Ortner, 2003:354). Although there can be many causes of this condition (Aufderheide and Rodriguez Martin, 1998:91) TB is certainly a strong candidate. In this individual, although not considered on their own as primary pathology, the distribution of periostitic lesions is quite wide, with both ulnae showing additional bone formation (although not on the diaphyses), multiple bones of the hands showing additional bone formation, periostitis on the shaft of the right femur and the on the shaft of the left fibula. Preservation of the long bones in this skeleton was variable, with all long bones (except the left radius) broken post mortem and missing various elements and therefore additional evidence of periostitis could be missing, but this is conjecture. Based on this information, HOA should be considered as a possible diagnosis and as such, strongly linked with the possibility of respiratory infection such as TB.

Despite the non- specific nature of this pathology, this skeleton was considered a good candidate for aDNA analysis in order to isolate any pathogenic, causative bacteria if present. The type of lesions identified in the individual, their distribution between the two bones and the lack of any obvious direct trauma, suggest that infection may have been the likely cause of the periostitic reaction. It is impossible to be certain that this infection was TB, although it remains possible; by its very nature, this study hoped to identify TB pathogen aDNA in those skeletons exhibiting non-specific (possibly tuberculous) evidence of infection which, given the time from which these skeletons originate, may or may not reflect the skeletal pathology patterns recognised as typically TB related today.

6.2.1.2 Ascott-under-Wychwood

The fused vertebrae (L2 and L3) were of significant interest for this study. Although, as mentioned above, it was not possible to study the entire skeleton in detail, this pathology had been reported within a previous publication (Benson and Whittle, 2007). The conclusion

reached by previous work was that this was evidence of injury, or a bone-forming condition, due to the preservation of the intervertebral space, unfused articular facets and a Schmorl's node on the superior surface of L3 (Galer, 2007: 196). Both of these suggestions hold weight but some alternative suggestions are offered below.

The ankylosis of L2 and L3 are as a result of extensive osteophyte formation. A thin layer of bone, from the exterior, impossible to differentiate from the rest of the vertebrae, has formed over the line of fusion, resulting in a smooth exterior wall of bone with a convex central third. The convex nature of these fused vertebrae suggested the possibility of a paravertebral abscess, the skeletal response to which can result in "flaired, shell like bony extensions from the affected vertebra" (Ortner, 2003: 232). It was thought that this may be a well healed example of an abscess that was located around the intervertebral disk space, to account for the bony wall extending over this area. It is stated (Galer, 2007: 196) that, through the use of radiographs, the disk space itself is preserved; it is therefore assumed that no specific areas of demineralisation or extensive cavities were seen as none are discussed. It should be noted that fusion of vertebrae in a similar manner to that described, can also be as a result of DISH (diffuse idiopathic skeletal hyperostosis) but that DISH tends to manifest primarily on one side of the vertebral column, whereas in this example, the fusion affects both side, and also does not resemble the typical "dripping candle wax" appearance of DISH. This shell like bone formation fusing the two affected vertebrae is however, described elsewhere as part of a very different disease process, that of ankylosing spondylitis:

> *Calcification of the periphery of the annulus fibrosus on the intervertebral disk…resulting in the formation of marginal, symmetric syndesmophytes (bony bridges) between the vertebrae. It is the anterior projection of these excrescences (in the region of the intervertebral disk area) at regular intervals as the disease spreads up and down the spine that has given rise to the simile 'bamboo spine'.*
>
> (Aufderheide and Rodríguez-Martín, 1998: 103)

Although no other pathology was reported for Individual A3, as would be expected for ankylosing spondylitis, which is usually initiated in the sacroiliac joints, the description of the bony bridges is identical to the two fused vertebrae presented here. This individual is also reported as being male and between 25 – 35 years old; about 90% of cases of ankylosing spondylitis affect males and, according to Murray *et al*. (1990), the usual age of onset is between 15 and 35 years. Aufderheide and Rodriguez-Martin (1998: 102) define the disease 'a systemic, progressive, non infectious, inflammatory disorder of connective tissue calcification'.

Perhaps there is some merit in suggesting that an infective bacillus could have caused a similar inflammation of the connective tissue, resulting in the same type of pathology but with an alternative cause. Other than the description above relating to the "flaired, shell like bony extensions from the affected vertebra" (Ortner, 2003: 232), no other known description of skeletal TB could account for that seen in this individual, but the vertebrae make for an extremely interesting discussion as to their morphological cause. TB of the spine is identifiable by the areas of the vertebrae it usually involves, primarily the lumbar and thoracic regions, the former of which is demonstrated in this individual. The question as to the number of vertebrae affected has been addressed many times (Nathanson and Cohen, 1941, Morse, 1961, Ortner and Putschar, 1981, Ghormley and Bradley, 1928), but studies such as that by Kastert and Uehlinger (1964: 486 cited in Ortner, 2003: 231) clearly demonstrate that at least two adjacent vertebrae are involved in at least 80% of cases. The number of vertebrae involved in this individual, can be considered as three, which sits in the middle of most estimations, and all vertebrae are adjacent to one another. These factors certainly add some weight to the theory that TB could be the cause. It was hoped that biomolecular analysis could, at the very least, rule out an infectious agent (TB) as the cause, if not demonstrate a different skeletal reaction to that previously reported in the palaeopathological literature. Unfortunately, this was not possible, and the reasons why will be discussed later in the chapter.

6.2.1.3 Boscombe Airfield

The remains of this skeleton demonstrated multiple pathological lesions, including new bone formation on the iliac crest and some destructive lesions in the first sacral vertebra. The new bone formation on the iliac crest, should, in hindsight, have been classified as primary pathology; although rare (Ortner, 2003:239), lesions in the ilium can occur. Due to the post mortem damage suffered by the ilium, it is not known how large the original lesion was but given the small area of new bone formation, perhaps this was a result of extension from a soft tissue focus, in this case, perhaps as a result of gastrointestinal TB. The minimal destructive lesions noted on the first sacral vertebra appeared to represent a degenerative process (ddd) as opposed to an infectious one. The pathology noted during initial analysis as being most likely to represent TB infection however (and which is recorded as primary pathology in the collated data), was the periosteal new bone formation seen on the visceral surface of a number of rib fragments (at least ten). Pathological changes on the ribs cannot be considered pathognomonic for TB despite strong correlations found between rib periostitis and deaths known to have been caused by TB (Santos, 2000, Roberts *et al*., 1994, Kelley and Micozzi, 1984, Santos and Roberts, 2001). Rib lesions are, however, strongly *suggestive* of TB. The lesions in this individual consisted of both lamellar and woven bone, demonstrating both healed and active infection. This could be the result of different aetiological factors, including infecting organisms, causing respiratory infections over a period of time, or they could be a result of one infection being subject to re-activation or re-infection occurring, as can occur in TB. Whichever of these it may be, the evidence certainly suggests this individual suffered from a pulmonary infection, and the number of rib fragments affected suggest that several ribs were infected, as opposed to just one. Whilst it is not possible to precisely identify the ribs and therefore make comment as to whether several *adjacent* ribs were affected, it is quite possible. If so, the lesions are likely to be as a result of a subjacent infection of the pleura and lungs (Kelley and Micozzi, 1984) with clinical studies also demonstrating that the most common cause of pulmonary and pleural disease is TB (Eyler *et al*., 1996), although these primarily describe rib destruction as opposed to new bone formation (see 4.3.1.6.4) .

6.2.1.4 Bourton-on-the-Water

The skeleton from the site of Bourton-on-the-Water, likewise, displayed rib lesions, and they were interpreted as being consistent with a pulmonary infection. Whilst the skeleton was not analysed by the author, but was sampled, rather as part of the wider project, no other pathology of interest was noted other than the rib lesions. This was the only skeleton sampled from the collection. The individual from which the affected rib fragments were removed was 12-16 years of age. TB is often found in areas of high hemopoietic marrow content (Aufderheide and Rodríguez-Martín, 1998: 133) which includes the ribs, but at all ages, and therefore its manifestation, at least skeletally, is not limited to the young for this area. There is little doubt that this skeleton displayed evidence of non-specific infection in an area of the body often known to reflect a tuberculous infection in the pleura or lungs (see above).

6.2.1.5 Eyebury

The pathological elbow joint identified in this individual, specifically the head of the right ulna, was subject to some discussion in the original excavation report. The ulna is missing much of its olecranon process and semi-lunar notch, which articulates with the humerus. Whilst the original report suggests that the epiphysis of the ulna had 'broken off' (Leeds, 1912: 90), it is suggested here that this may not be an accurate diagnosis. Unfortunately, due to the confusion as to the contents of the box labelled as being that of Skeleton E.II.3.223, it was not possible to reconstruct all elements of the skeleton, nor definitively identify the humerus to which this ulna would have articulated. No pathology was noted on any humerus in the box but, as stated, a full analysis was not performed in order to prevent further confusion of these clearly mixed skeletal remains. The proximal ulna analysed here does not demonstrate clear evidence of the proximal area having been 'broken off'; if so it was certainly not a clean break and the remaining spurs of bone seem ill-placed as a regenerative bony reaction. Two additional theories seem plausible, the first being that this was perhaps some form of congenital abnormality as the bone present is obviously long established. The second suggestion, the basis of its selection for biomolecular study, is that the joint area has been extensively destroyed by an infectious process but had subsequently

remodelled. It is widely reported that the elbow is very frequently involved in skeletal joint TB, and often the most of all upper extremities (Ortner, 2003: 243). The joint commonly becomes completely deformed (Aufderheide and Rodriguez-Martin, 1999: 140) and much of the focus of the elbow destruction does seem to be associated with the ulna; one study found that the olecranon portion of the ulna was affected in only slight fewer cases than the distal humerus (Konig, 1906: 141-142 cited in Ortner, 2003: 243). The area is clearly remodelled and certainly not in the middle of healing as the bone spurs noted are smooth walled, with no sign of active, woven bone formation. Whilst it cannot be said for certain that this ulna was the focus of a destructive tuberculous process, it certainly seems reasonable to consider the possibility. It is unfortunate that the rest of the skeletal elements could not be confidently assigned in order to get a better picture of the overall health status of this individual.

6.2.1.6 Methwold Severalls

According to Ortner, the lower spine is the primary focus of skeletal tuberculosis, regardless of age (2003: 231). The pathology of relevance noted on this skeleton was considered potentially indicative of an isolated tuberculous focus in the anterior portion of the 1st lumbar vertebral body (the anterior body being the most commonly affected tuberculous area of the vertebrae (Resnick and Niwayama 1995: 2464). The cavitation is seen here as a large lytic lesions and no additional or reactive bone formation is present. The destruction also extends to the supero-anterior margin, causing a distortion of the normal morphology. This type of lesion, given its position in the vertebral body and in the spine as a whole, is very suggestive of spinal TB, or perhaps the early stages. However, the adjacent vertebra did not seem to be affected, as might be expected given the depth and level of destruction seen at this stage in the body of L1. Alternative suggestions as to the cause of this pathology include a particularly large Schmorls node, although the usual width of a Schmorl's node is up to 5mm with a depth of no more than 10 – 15mm (Aufderheide and Rodríguez Martín, 1998: 97), whereas this example exceeds these general limits, with a width of 34mm and a maximum depth of 40mM. For this reason, the suggestion of an extremely large Schmorls node is extremely tentative. A more likely suggestion would be intervertebral osteochondrosis given the slightly crescent shape of the lesion; this can often be confused

with TB (Kelley, 1982). The lesion is certainly destructive and its surface is rough and uneven, which may suggest active bone destruction at the time of death. This individual was considered to be important for sampling for further analyses, but the burial environment to which the skeletal remains had been subject not favourable for such analyses, as will be discussed later.

6.2.1.7 Twyford Down

The lesions present on the four rib fragments from Sk. 883 were very similar in character to those described by Pfeiffer (1991) as puffy and porous cortical expansions, entirely different to the "plaque" seen on other ribs described here. There is some question as to the true nature of the resulting pathology on at least two of the rib fragments. This is due to the position of the additional bone formation, on the lateral edge of the broken fragment, and the small quantity of woven bone seen on the opposing surface (also on the lateral edge) of each fragment. It is suggested that certainly, the lesions on these two ribs may not be attributable to an infectious disease process, but may in fact be the site of rib fractures that occurred close to the individuals' time of death and still be in the process of healing. The edges of the fragments did not provide a definitive answer based on colour or surface morphology, but this must be considered a possibility. However, one fragment, without doubt, shows additional woven bone formation on the rib angle which is not directly associated with any potential fracture. Destructive or bone forming lesions in the ribs would be expected to occur on the rib heads attaching to any affected vertebra (Roberts and Buikstra, 2003: 103). The preservation of this individual was poor and only one vertebra was present, meaning it was not possible to see any possible association between the affected rib and any possibly infected vertebrae.

6.2.1.8 Waterhall Farm

Fusion of two thoracic vertebrae (T1 and T2) may indicate an infectious process, potentially originating in the intervertebral disc space and causing it to narrow, the ankylosis resulting during subsequent healing. However, this is not a classic sign of spinal TB despite the

thoracic region being one of the most commonly affected areas of the spine. The fact that the spinous processes are also fused could indicate congenital fusion of these two vertebrae with no infectious involvement. An additional theory could include a compression fracture, but resulting fusion from such traumatic events are usually limited to small 'nodular' areas (Aufderheide and Rodriguez-Martin, 1998: 25), unlike the complete fusion seen here. Congenital scoliosis was also considered but little other skeletal pathology was noted, as would be expected, although the shape of the fused vertebrae did suggest another possibility. An influencing factor in the decision to sample this skeleton was due to the "tilted" appearance of the fused vertebrae, suggestive of a possible intervertebral lytic lesion or possibly psoas abscess, known to occur mostly in the posterior regions of the upper thoracic vertebrae in TB (Aufderheide and Rodriguez-Martin, 1998: 136) causing the slight reduction in height of T1 on its right side; this was most noticeable on its superior and posterior aspects where the vertebral body appears to have a slightly wedged shaped. Without radiographic assessment it was not possible to confirm if any areas of demineralised trabecular bone were present within the body of either vertebrae or emanating from the disc space. No exterior lytic lesions were present so if any infectious process was the cause of this pathology, it was isolated within the healed or fused bone and, although regeneration of bone is skeletal TB is uncommon, spontaneous fusion is known to infrequently occur (Aufderheide and Rodriguez-Martin, 1998: 137). The pathology noted here is only presented cautiously as possible evidence of infection, particularly given the fusion of the spinous processes; it is clear there are many other possible diagnoses other than infection such as TB. With the ability to analyse bone samples via biomolecular analysis however, it appeared logical to confirm or rule out the possibility of TB being the cause of this pathology as such fusion is not unknown.

6.2.1.9 West Tump

The pathology of interest from Skeleton 7, originating from the West Tump Long Barrow was a lesion on the visceral surface of a small rib fragment. The lesion is consistent with the 'plaque' described by Pfeiffer (1991). Although only one rib fragment showed any sign of this pathology, it was noted that the number of small rib fragments was extremely high and they were stored in a bag together. For these reasons, it may be quite possible that any

further pathology has been lost as the plaque easily detaches from the original bone surface. The pathology here is distinct and almost certainly indicates the presence of a non-specific infection, as described above, and thus this rib was removed for biomolecular analysis.

6.2.1.10 Wetwang Slack

As explained in Chapter four, these skeletons formed part of the group of skeletons selected for sampling by a member of the research team for the wider project. For this reason, there is not the same level of detail available for the individual skeletons regarding further analysis, just basic data and the pathology noted which led to the decision to take samples for biomolecular analysis. Additonal information was obtained from the original analysis notes made after excavation (Dawes, n.d.) but there were some discrepancies between these two sources of information. Without further details, it is difficult to offer any alternative diagnoses for the pathologies seen in the Wetwang Slack individuals, but they are presented below for completeness, with some additional comments where appropriate. All individuals included in the study and described below, were good candidates for aDNA analysis as each demonstrated non-specific evidence of infection, all of which, could be attributable to TB.

6.2.1.11 Wetwang Slack (a)

This skeleton displayed rib lesions, and the potential link between these and TB has already been explored in detail. Multiple ribs were involved and it is likely many of them constitute adjacent ribs.

6.2.1.12 Wetwang Slack (b)

Destruction of the left hip was the reason for this individual being selected for aDNA analysis. The hip comprises 20 percent of skeletal involvement in TB (Aufderheide and Rodriguez Martin, 1998: 139), being the most frequent skeletal element affected after the

spine (Ortner, 2003: 235). The description from the original notes (Dawes, n.d.) is very suggestive of those changes which can be found in hip involvement of TB including enlargement of the acetabulum and an ulcer cavity on the 'upper posterior side of [the] head'; the original notes themselves suggest TB as a possible diagnosis and based on the description offered (complete in section 5.2.12.1) it seems likely. Without seeing the bones involved in their entirety, it is hard to offer a more in depth discussion but bone destruction is expected at joints as seen here.

6.2.1.13 Wetwang Slack (c)

Lytic lesions on T12 and L2 of this individual were noted. The frequency, distribution and pattern of skeletal involvement of the various vertebrae have also been explored in detail.

6.2.1.14 Wetwang Slack (d)

This individual also demonstrated lytic lesions on an upper thoracic (T1) and lower cervical vertebrae (C6/7). Although spinal involvement of the various vertebrae in relation to TB has already been explored in detail, it is interesting to note here that the original notes (Dawes, n.d.) state 'collapse', or at least partial collapse, in relation to all three involved vertebrae. This is very suspicious of the process by which the vertebral body demineralises and can no long support the weight of the trunk, thus titling forward and often being reduced to wedge shaped vertebra and often suffering significant destruction. Again, the original report suggests TB as a possibility and although the cervical region is not the most common area of the spine for TB involvement, it does occur and thus this diagnosis seems well founded.

6.2.1.15 Wetwang Slack (e)

A lytic lesion on right ilium led to this individual being included in the study. Whilst involvement of the ilium is rare, extension to the ilium from a psoas abscess can occur (Ortner, 2003: 239); lesions on the ilium also tend to consist of round or oval cavities, and

the lesion on this skeleton follows this pattern perfectly (see Figure 5-28) making it highly suggestive of possible TB.

6.2.1.16 Wetwang Slack (f)

This individual, demonstrated evidence of a psoas abscess on the left femur, which appears to be close to the femoral head, which is also commonly affected in TB (Aufderheide and Rodriguez-Martin, 1998: 139). This may be due to the escape of bacilli from capillaries in the synovial membrane (Messner, 1987 cited in Aufderheide and Rodriguez-Martin, 1998: 139). Again, this skeleton appeared a good candidate for biomolecular analysis.

6.2.1.17 Wilsford Barrow (a)

The skeleton from which a sample was removed for biomolecular analysis was that of a 9-12 year old. Whilst lesions seen in the diaphyses of long bones are not that common in TB (Aufderheide and Rodriguez-Martin, 1998: 137), they are found more in children than adults. The bones affected most frequently (in descending order), are the tibia, ulna, radius, humerus, femur and fibula, (Konschegg, 1934: 422 cited in Ortner and Putschar, 1985: 161). The fragmentary bones that showed extensive periostitis and that were analysed include ulna, femur, tibia and fibula. Much additional bone formation was seen on fragments of shafts for all these bones, sometimes in patches, although this may be due to the loss of some of the lesions through handling and storage of the skeletal remains. The additional widely distributed bone formation seen here clearly manifested as a reaction to some form of infection, but is unlikely to be a result of trauma. It is however, quite possible that this represents HOA as referred to previously, particularly as both tibiae are affected, and the tibia is one of the most affected bones in this condition (Ortner, 2003:354). The fragmentary nature of this skeleton meant that during analysis, periostitis was thought to affect more long bones than just the tibiae but the fragments were too small to be able to confidently identify. To support this diagnosis, it is also possible for HOA to cause new bone deposition on the inner table of the skull (Jaffe, 1972: 290) as seen in this skeleton. There is certainly a strong argument here to support his diagnosis and for it to be potentially associated with TB

infection, again, supported by previous links between HOA and TB in a collection where cause of death is known (Santos and Roberts, 2001) and examples supported by aDNA confirmation of TB (Mays and Taylor, 2002).

Although the skull also remains a rare site of skeletal TB, it is affected more in young children (Ortner, 2003: 246) and is, in young adults, almost exclusively secondary to haematogenous dissemination of TB (Aufderheide and Rodriguez-Martin, 1998: 140). In this example, perforation of the cranial vault is visible on the left parietal bone which, initially, appears as possible post-mortem damage. On closer inspection, the majority of the margins of the lesion are not discoloured and are smooth. The cranium in this area had been previously reconstructed using glue and therefore the shape of the hole may be more "jagged" than in its original form, as the bones may be slightly out of place and small areas of the margins may be missing. That the skull was fractured (either during its interment or since), with four clear breaks emanating from the site of the lesion, is an indication that the hole may have preceded the fracture damage (and thus formed a weakened area). Once the interior of the cranial vault was inspected, it was clear that its origin lies in a disease process rather than trauma or later damage. The hole, even in its modified appearance (since the pieces have been glued together) barely exceeds the characteristic 2cm diameter identified by Murray *et al.* (1990) and does demonstrate poorly defined margins. Endocranially, the area surrounding the perforation appears "moth-eaten" and vascular lesions are readily apparent, which may indicate reaction to an adjacent soft tissue lesion (Ortner, 2003: 250). There is a great deal more reaction on the inner table than the outer which suggests the lesion is not syphilitic in nature (Erdheim, 1932: 355 cited in Ortner, 2003: 248) (Figure 6-1), nor is there extensive osteoblastic activity as would be expected of metatastic neuroblastoma (Ortner, 2003: 248). Numerous small areas of destruction may be expected on the skull (Murray *et al.*, 1990) whereas here there is only one perforated area. There is, however, an additional almost circular area to the anterior of this perforation, which may indicate an earlier stage of this same disease process. It may also be possible that this area demonstrates post mortem damage as the bone is slightly lighter in colour, and no lesions are visible on the endocranium in this area, other than the same vascular lesions that extend over a wide area of the parietal bone. A study by Sorrel-Dejerine demonstrated that of the three areas of the cranium to be considered (cranial vault, base and face), the cranial vault is

the most common location for cranial TB (1932: 78). The active bone formation seen on a number of the long bones, and the cranial pathology, noted do suggest that this individual was suffering from some infectious disease process, with TB being a strong candidate as the causative agent.

It should be noted that the new bone formation on the endocranium, is strikingly similar to an example from Raunds, Northamptonshire, where pathology may be indicative of tuberculosis-induced meningitis (Roberts and Buikstra, 2003: 100) (Figure 6-2).

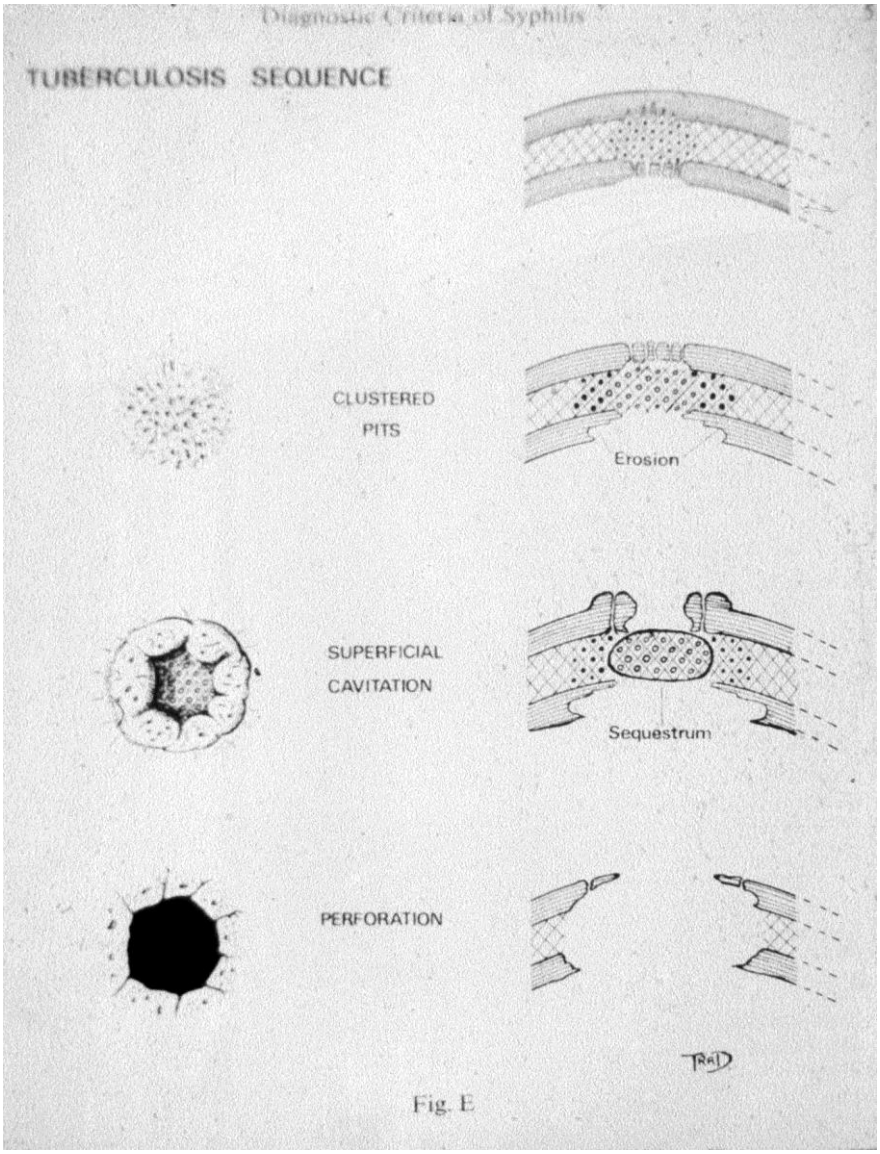


Figure 6-1 Sequence of TB infection, perforation of the cranium from the inner table through a process of clustered pits, superficial cavitation and finally, perforation (Hackett, 1976)

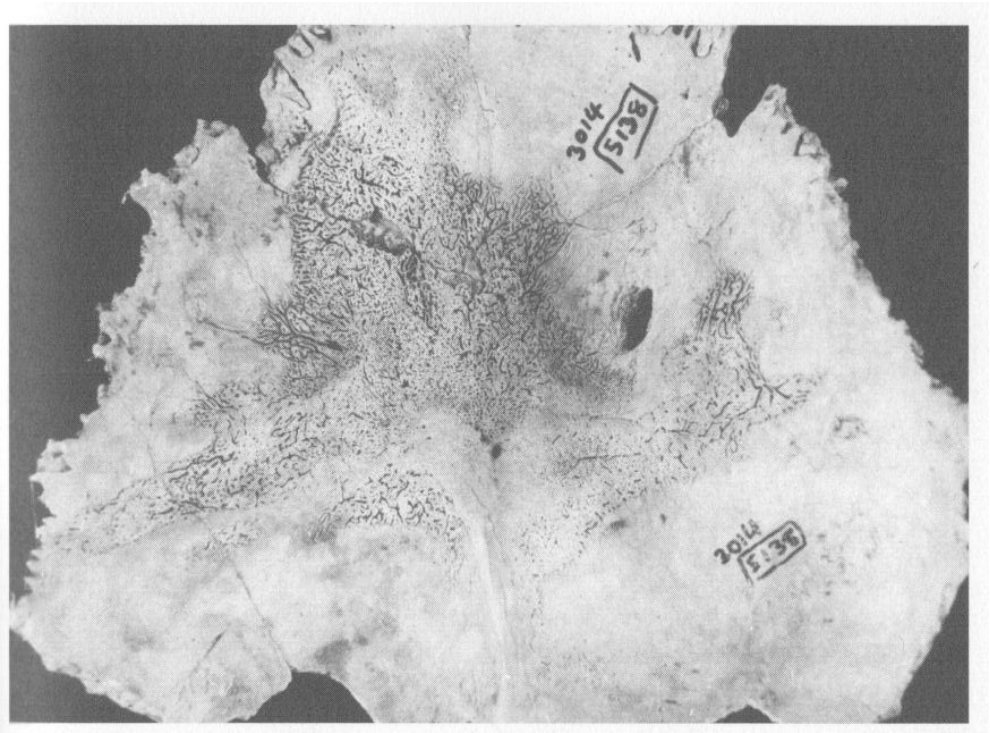


Figure 6-2 New bone formation on endocranial surface of the skull, Raunds, Northamptonshire. Photo by Jean Brown.

Whether the pathology distributed in this skeleton is considered representative of HOA it is still considered 'highly probable' that the most common cause of periostitis is infection (Ortner, 2003: 209) and infection of the long bones, caused by TB, mostly occurs through haematogenous dissemination (Aufderheide and Rodriguez-Martin, 1998: 137), which may suggest that any periosteal reaction has the ability to be widely distributed, as is demonstrated in this particular example.

6.2.1.18 Wilsford Barrow (b)

Skeleton II showed a significant amount of pathology distributed throughout the skeleton. Whilst the small lesion in the left tibia, and exaggerated lateral angle of both radial heads are not likely to be related to the possible presence of TB, there is much bone destruction seen in the spine, the auricular surface of the innominate bone, and destructive lesions in the mastoid process.

The spine, perhaps the most significant area affected, demonstrates destructive lesions throughout the cervical area, with four out of the seven vertebrae being affected to some degree. Clearly the vertebrae had been subject to some post-mortem damage which was difficult to differentiate from areas of early bone resorption. However, there was extensive pathological change in the vertebrae, the axis being the most clearly and severely affected. Deep destructive cavitations were present in the vertebral body, severely disfiguring the area. A result of this destruction is the almost complete loss of the right transverse process, although the laminae, spinous process and articular processes remained unaffected. The atlas was only partially present and what was left demonstrated no obvious pathology, although C3 also demonstrated some minor surface bone resorption, with the inner trabecular bone displayed on the superior aspect of the vertebral body. C5 demonstrated some minor bone resorption on the right lateral margin of the inferior part of the vertebral body, extending to the articular transverse process, which appeared to have been lost through bone resorption, as opposed to damage, as with many of the other vertebrae. Some bone resorption is suggested for C6 and T1, both on the vertebral body and, for the latter, also on the transverse process, but this remains tentative as it could be post-mortem damage, leaving the axis the most severely altered and most relevant pathological vertebra. However, it should also be noted that L5 showed compression on the left side of the vertebral body. The cervical spine was not the most common area affected by TB as the thoracic and lumbar regions are much more likely to exhibit evidence of the disease. However, cervical TB is certainly reported in the clinical literature (Fang *et al*., 1983) and therefore should not be overlooked in the archaeological record, despite its apparent rarity.

Other pathology of note in this skeleton includes possible evidence of an abscess in the centre of the left auricular surface visible as destructive lesions leading to a moderately shallow pit with an uneven bone surface and highly irregular margins. This irregular oval shaped pit measured more than 2cms at its longest and approximately 1cm at its widest. Isolated tuberculous foci in the ilium are rare, but when they do occur they are often round or oval in shape (Konschegg 1934: 409-410 cited in Ortner, 2003: 239) – it is suggested that this could be the early stages of such a focus. The final and perhaps most interesting pathology can be seen in the mastoid process where the cortical bone, which usually covers the internal air cells of the mastoid process, has in some areas perforated, creating the

appearance of small lytic lesions with smooth rounded margins and inner surfaces with deep cavities; this gives the mastoid process a look of “swiss cheese”. Whilst there are a cluster of these perforations at the very distal end of the mastoid process, they are also present on the majority of the surface, extending above the proximal part of the mastoid process and into the temporal bone itself. It is suggested that this is the result of infection in the mastoid process and is quite possibly, as an extension of otitis media. An infectious complication of otitis media can be chronic mastoiditis (Chole and Choo, 1998) if the bacteria extend posteriorly to the air cells of the mastoid process (Aufderheide and Rodriguez-Martin, 1998: 253). This extension can lead to a breakdown in the thin walls between the air cells and in some cases, the abscess can ‘perforate the cortical surface and drain exteriorly’ (Aufderheide and Rodriguez-Martin, 1998: 253). Coalescent mastoiditis, which is essentially the erosion of the walls within the mastoid process and subsequent development of an empyema - a collection of pus or fluid from infected tissue – can also lead to the thinning of the outer mastoid cortex and form a subperiosteal abscess (Cummings, 1986 cited in (Nemzek and Swartz, 2003). Necrosis of the mastoid tip can then allow the infection to spread deep into the neck (Nemzek and Swartz, 2003), so there is perhaps sufficient evidence to suggest that that the cervical lesions seen in this individual, could be associated with an extension of this disease process.

The perforations of the mastoid process could also be due to the formation of a cholesteatoma (which can occur as a complication of otitis media), which can ultimately result in destruction of the mastoid process. Cholesteatoma is the ‘presence of the stratified squamous epithelium within the middle ear space’ which can lead to secondary infection and bone erosion (Zarandy and Rutka, 2010: 9). Aufderheide and Rodriguez-Martin refer to this as ‘a localized accumulation of exfoliated, dead, benign squamous epithelium’ (1998: 253). While cholesteatoma can be congenital or acquired (Lesinskas *et al.*, 2002 cited in Zarandy and Rutka, 2010: 11), erosion into the ‘otic capsule of the lateral semicircular canal’, allowing secondary infection to result in disease spread, ultimately into the mastoid, is usually seen in the latter (Zarandy and Rutka, 2010: 11).

In this individual, the multiple perforations to the exterior cortical surface of the mastoid process may well be reflective of such a process but, regardless of its ultimate

manifestation, the key element here is that otitis media may well have been the primary focus of a bacterial infection, the cause of which may have been due to *Mycobacterium tuberculosis* and, for that reason, this individual was subject to aDNA analysis. TB of the middle ear is quite rare in modern clinical settings and is usually seen in association with (or secondary to) pulmonary TB (Mahajan *et al*., 1995). That the margins of these perforations are smooth walled, may suggest that the infection was experienced some time before the individual's death, as they suggest complete healing and bone remodelling. Otitis media is one of the most common diseases of childhood (Chole and Choo, 1998) and it may be, therefore, that this individual (who was a young adult) could have experienced the infection during childhood. With only dry bones to examine, the ability to see the soft tissue damage and perform clinical and laboratory tests, as employed in modern clinical setting (Arya *et al*., 2009) to test for the presence of *Mycobacterium tuberculosis* or *Mycobacterium*. *bovis*, the latter of which has also been identified as the causative bacterium in tuberculous mastoiditis (Smith and Anders, 1994), is not possible.

6.2.2 Biomolecular Results

The results of the aDNA analysis have been shown to be negative, the reasons for which are discussed further in section **Error! Reference source not found.**. Through gel lectrophoresis, there was some PCR product banding which appeared to demonstrate sequences similar in length to that which were sought but there was also regular indication of degraded aDNA, visible by the "staining" during the gel electrophoresis stage (see Appendix 4). The outer IS*1081* primers targeted 135bp, the inner primers for the hemi-nested PCR, 113bp and for IS*6110*, the outer IS*6110* primers targeted 123bp, and for the nested or inner IS*6110* primers, 92bp. However, after purification and sequencing, these sequences were found to be either too long, too short, or did not identify with any known MTBC sequences. Some may simply represent random amplified product whereas others were shown to represent sequences likely to be representative of contaminating bacterial sequences which can be very similar to pathogenic DNA, particularly those of TB (Pääbo *et al*., 2004, Wayne *et al*., 1999, Tsangaras and Greenwood, in press) and in this case, Brucellosis. Real time PCR performed for identification of TB yielded no positive results.

These negative results should not, however, be taken as a definitive lack of the disease as, amongst other factors, it may be possible that the target pathogen DNA did not survive, given the age of samples (see 2.2.2). Attempts to identify human DNA using real time PCR yielded some positive results, with four positive results for the LCT assay although mtDNA analysis demonstrated that the samples were subject to extensive contamination with almost all control blanks showing positive results; unfortunately it was not possible to repeat the real time PCR analysis process at that time. Further PCR and sequencing revealed no positive evidence of IS711, the chosen demonstrative IS element for brucellosis, a disease which carries many similar signs and symptoms in humans, as tuberculosis. The decision to test for brucellosis however, was based on the appearance of a number of samples (ChWtS1 (inner), ChWTS2c, WkPHS1, WWSs2, WWSS7c) demonstrating evidence of an identical sequence with a maximum identity of 80% with a number of *Brucella* species and variations including *Brucella microti, melitensis, abortus, suis, canis, and ovis*. This is therefore likely to be representative of a similar but un-sequenced contaminating, environmental organism.

Ultimately, neither TB or brucellosis were positively identified in any of the samples subject to aDNA analysis.

6.2.3 Results within the Context of Period and Site

6.2.3.1 Neolithic

The increasing application of scientific techniques to archaeological materials is, beginning to shed light on the social and economic transformation that began to take place around 6000 years ago. There are many aspects of changing lifestyles that will impact on an individual's health but, from the evidence so far discussed, it is suggested that the key socio-economic change of this period, was the adoption of agriculture and living in settled communities. It would be easy to list the numerous environmental, social and economic changes witnessed in the period as individual events, but the truth of the matter is more

complex; they are intimately linked, with a strong argument able to be made, that the adoption of agriculture was either the direct, or indirect catalyst for almost all.

The extent to which these changes (such as landscape manipulation, occupations practiced, and increased mobility and contact), their effects (increased population density, sedentary lifestyle and diet) and resulting impacts on health, are reflected in the skeletal evidence does, however, remain somewhat difficult to interpret. This is primarily the result of limited skeletal material being available for the period, which is discussed further in 3.6., but it should be noted that there is only a tiny quantity of British Mesolithic skeletal material available, meaning direct comparison of pre, and post agricultural transition populations cannot be made. This limiting factor has resulted in other health related studies having to compare British Neolithic material to Mesolithic material from northwestern Europe and southern Scandinavia (Chamberlain and Witkin, 2007); the comparison is justified by the authors by the probable high mobility of hunter gatherers at this time, and the terrestrial connection of Britain and Europe into the late Mesolithic (Coles, 1998). The use of mortuary monuments such as the Neolithic typical earthen long barrow, provide the primary source of skeletal material but it is unknown who, exactly, is represented in the skeletal assemblages and how reflective these are of a given population.

The two Neolithic sites included in this study are both chambered tombs of the Cotswold-Severn Group and there is a relatively high presence of skeletal remains in the region, although most have not been subject to adequate publishing (if at all) (Wysocki and Whittle, 2000: 591). West Tump was first excavated in 1880 (Witts, 1881) and, until recently, has had very little attention paid to its skeletal inhabitants. The recent re-evaluation of the skeletal material has demonstrated some interesting results but have mainly focussed on the post depositional taphonomic changes to which the bones have been subject (Smith, 2001), interpretation of funerary practice (Smith, 2005), and the presence of toolmarks (Smith and Brickley, 2004). In contrast, the Ascott-under-Wychwood long barrow is almost entirely excavated (one of only three of the Cotswold-Severn tombs to have been), and has had extensive analyses performed on every aspect of its creation, use and excavation which have been collated into a single volume (Benson and Whittle, 2007).

As a result, there is far more data for interpretation against which to discuss the single Ascott-under-Wychwood individual (A3), as opposed to Skeleton 7 from West Tump. This immediately demonstrates the difficulty in placing single or limited numbers of individuals into context, especially where this additional information is lacking. Despite this difficulty, it may be of use to note some of West Tump's original excavator comments. These must be viewed with caution given the relative lack of expertise of the excavators and the time in which they were made but, nevertheless, they may prove useful.

It may be expected, given the extensive literature on the decline in health seen with the adoption of agriculture (e.g. Starling and Stock 2007, Roberts and Cox, 2007, Cohen and Armelagos, 1984), that the two Neolithic individuals included in this study, would demonstrate typical pathology, such as dental disease and, potentially, evidence of periods of biological stress due to the population dependant nature on agriculture, and domesticated plants and animals. To some extent this is the case, particularly in the case of individual A3 from the Ascott-under-Wychwood Long Barrow.

Early Neolithic activity at the Ascott-under-Wychwood site (pre-barrow), demonstrates evidence of land clearance and numerous structures being built, which appear to represent settlement related activity (Evans *et al*., 2006, McFadyen *et al*., 2007: 27). Data suggest that cattle, sheep and pigs were the 'mainstay of the economy' but, with limited evidence of butchery and the varying ages of cattle, it has been suggested that dairying was their primary use (McFadyen *et al*., 2007: 33). This is supported by the large quantity of lipid residues of dairy fat origin identified on the site's pottery, the highest ever detected in a pottery assemblage (Copley and Evershed, 2007: 287), and is a feature noted of Neolithic farming from the middle of the 5th millennium onwards (Copley *et al*., 2003, Copley *et al*., 2005a). The animal bones recovered from the site are dominated by domesticates (Mulville and Grigson, 2007: 238), and isotopic values suggest a high level of animal protein consumption (Hedges *et al*., 2007: 262). Therefore, it is clear that animals played a major part in the economy, diet and general life of the Ascott-under-Wychwood Neolithic peoples. Witts reported the presence of sheep, goat and red deer in the West Tump barrow (1881), but the degree to which these constituted part of the diet is unknown.

The extensive use of animals at Ascott-under-Wychwood may have been a double edged sword in that it provided a diet rich in protein, but the practice of dairying would have placed people in danger of zoonotic diseases such as TB if, by this time, cattle harboured the disease. Under such conditions, M. *bovis* could have been contracted from infected cow's milk, via the respiratory tract, or even via the use of dung for various purposes, such as fuel (Roberts and Buikstra, 2003: 77).

This high level of protein in the diet of the Ascott-under-Wychwood population would have been nutritionally beneficial, and is not consistent with a diet of reduced animal protein consumption and reliance on foods lacking in nutritional variety, previously assumed with agriculture (Larsen, 2003). It does not, however, dispute this as a general trend but rather complements the regional variation in diet witnessed between the residents of four Cotswold-Severn tombs found by Richards (2000).

The picture that emerges from this dietary snapshot does not suggest poor nutrition and this is reflected in the skeletal material as a whole. Compared to other archaeological populations, a particularly low occurrence of enamel hypoplasia was recorded (only 3 out of 222 teeth) which '…suggests that these individuals are not likely to have been subject to high levels of stress during early life.' (Galer, 2007: 215). Individual A3, however, was one of the few skeletons to demonstrate enamel hypoplasia. When statures were calculated and compared against the average for the period (Roberts and Cox, 2003), A3 was also shorter than expected (155.9-161.6cm) compared to average data of Neolithic individuals derived from Roberts and Cox (2003), and in comparison to the other three skeletons found within the same cist. Unfortunately, no such information could be gained for Skeleton 7 from West Tump, in part due to the fragmentary nature of the bones and because only three teeth were present, two of which were broken. This difference in skeletal health of individual A3, in comparison to the other occupants of the cist, who are thought to perhaps be a familial group (Galer, 2007: 218), may suggest something of the individual's status within that group, but it is not possible, with any confidence, to support such assumptions based on one skeleton. Suggestions as to the possibly lower status of Skeleton 7 from West Tump was made, however, on the basis of their burial position, along with two other skeletons just outside the circumscribing wall of the burial chamber (Witts, 1881). This positioning may or

may not reflect status differentiation and the many possible health effects which so often can accompany it, but it should be remembered that it has been argued that status differentiation and agriculture occur at virtually the same time (Price and Bar-Yosef, 2010).

Evidence of a projectile caused wound has been identified in another individual from West Tump (Smith, 2001, Smith, 2005) which could either have been accidently or purposefully inflicted; an arrowhead is also seen embedded in a vertebra of individual B2. Evidence for interpersonal and collective violence for the period has previously been highlighted having been identified at a number of causewayed enclosure sites in the south of Britain (Figure 3-5). It is possible that this violence is linked with power struggles associated with the massive population increase between 6100 cal BP and 5400 cal BP (4150BC-3450BC) (Collard *et al*., 2010), evidence for which is complemented by the discovery of Britain's first "Building Boom" (Bayliss *et al*., 2011, Ghosh, 2011). These factors would likely have increased the pressure on resources and led to increasing reliance on agriculture. This would in turn, may have placed greater strain on the land (as discussed in 3.2), and ultimately had a negative impact on health.

Looking wider, into the Cotswolds and neighbouring Upper Thames Valley over the course of the Neolithic, the picture emerges of a dynamic landscape, probably reflective of shifting residencies every three to five generations (Whittle *et al*., 2007a: 337). It has already been suggested that the people of the Neolithic remained relatively mobile (Darvill and Thomas, 1996), and this may be reflective of such practice. It may also be important to note that the Thames Valley is home to extensive river systems which likely enabled trade and movement of goods/people via the Channel, diffusing inland, as is known to have happened in the Bronze and Iron Ages (Needham, 2009: 18, Cunliffe, 2009: 87). Movement of people over long distances within Britain during the Neolithic has already been convincingly demonstrated, (albeit with a small number of samples) (Montgomery *et al*., 2000), as has contact with the continent due to the presence and distribution of jadeite axes whose origins lie in the foothills of the Alps (Petrequin *et al*., 2002 in Bradley, 2007). If it is assumed that at some point, TB entered Britain with people from the continent, which seems the most likely scenario, it is clear that movement of people from as far back as the Neolithic could have been a facilitator.

The two Neolithic individuals included in this study, were chosen because they exhibited (non-specific) evidence of infectious disease. Individual A3 demonstrated possible infection of the vertebrae and Skeleton 7, pulmonary infection. The lack of positive aDNA results for either M. *tuberculosis* or M. *bovis* has not allowed these pathologies to be identified as being TB related (possible reasons as to why are further discussed in 6.2.2), although the macroscopic analyses maintain this is a possibility, though it is never possible to definitively attribute specific pathology to a disease, even on the basis of positive biomolecular results.

The prehistoric periods in Britain demonstrate minimal evidence of disease and this is probably due to their relatively small populations and the remains available for study (Roberts and Cox, 2007). However, from these two skeletons, it is clear that the individuals were likely subject to some environmental and social factors which could have encouraged communicable diseases, such as TB. This need not have a minimum population level to persist once an individual is infected due to its latent and chronic tendencies (McKeown, 1988: 38). It is the dense living conditions, however, which lead the infection to thrive and, despite a large increase in population at this time, perhaps the land was not so populated as to subject populations to 'overcrowding'. Some evidence for room partitions in Neolithic houses may suggest that there may have been separate sleeping, cooking and working areas (Darvill and Thomas, 1996: 57) thus reducing, to some degree, the transmission potential of infectious disease. The pulmonary infection indicated by rib lesions in Skeleton 7, however, may suggest that, even if living conditions were not as rudimentary as perhaps assumed, it was not enough to keep respiratory infections "at bay". If the rib lesions do represent infection, this individual was, at some point, exposed to conditions which enabled an infection to be passed from one individual to another, possibly encouraged by poor air quality due to the open hearths such as those identified in buildings at the Ascott-under-Wychwood site. This is of course, speculation, based on one individual with evidence of non-specific infection and, thus, does not lend much weight to the theory of TB compliant conditions in the Neolithic. It must, however, be catalogued with other pathologies noted in the Neolithic should further analytical methods be developed in the future.

It cannot be denied, however, that the trade and travel mentioned offered the opportunity for the introduction of the disease from the continent, from where there is evidence of TB from Neolithic Italy (Canci *et al*., 1996). Of particular relevance is the recent supporting evidence for agriculture having been introduced by migrant farmers (Collard *et al*., 2010). The subsequent movement of people within Britain would have enabled any infection to be spread between communities, and population pressure could have created stresses that left individuals more open to the contraction of such a disease.

There is little more that can be said about Skeleton 7 from West Tump, other to reiterate that rib lesions are convincing evidence of an adjacent pulmonary infection, that can be seen particularly with dense living conditions and with a generally poorer standard of living. Individual A3 from Ascott-under-Wychwood was interred with people who appeared to be relatively healthy. The diet at Ascott-under-Wychwood certainly does not seem to reflect one poor in protein, possibly associated with increased susceptibility to TB, but perhaps individual A3 did not have equal access to such food, or perhaps their childhood coincided with a particularly difficult time in respect of resources. This individual may not, perhaps, have been local.

6.2.3.2 Bronze Age

The individuals selected from the Bronze Age period, lived against a backdrop of increasing agricultural intensity, social hierarchical development and a dynamic relationship with the continent (e.g. Needham, 2009). The span of the Bronze Age also saw great climatic change, with a move towards a wetter and colder climate towards the end of the period (Roberts, 1998: 168). The individuals represented here display further evidence of non-specific infection, and it is interesting to note that there are more Bronze Age than Neolithic samples and that they represent a greater number of sites than those from the Iron Age; no greater comment can be made given the small number of individuals represented overall as it is likely that it was the small quantity of skeletal material available which, at least partly, led to this division.

The pathologies noted include destructive vertebral and joint lesions, periostitis on ribs and long bones, and more unusual cranial lesions. Looking more closely at the specific sites from which the individuals lived may shed some light on the reasons why such infections occurred. As with the Neolithic samples, however, this is hindered by a lack of analysis and interpretation for some sites and skeletal assemblages.

The skeletons identified as specifically originating from the Early Bronze Age (through radiocarbon dating) include the Boscombe Airfield and the Methwold Severalls individuals. Both skeletons had been subject to previous macroscopic analysis (Manning *et al*., 2010, McKinley, 1996) and, whilst Skeleton 39 from Boscombe had no accompanying teeth, Skeleton 1 from Methwold showed evidence of both caries and enamel hypoplasia. Some of the skeletons recovered from the Methwold site were subsequently subject to stable isotope analysis in order to gain an insight into their diet. From this, a strong reliance on terrestrial food sources was in evidence, 'to the virtual exclusion of seafood' which may be reflective of the local Early Bronze Age environment which saw the restoration of freshwater conditions (Lennon, 1996: 171). It is suggested the diet consisted of cultivated cereals and a small amount of meat. A general pattern noted was extensive dental wear which further supports the assumption of stone-ground cereal inclusion in the diet (Lennon, 1996: 171).

Skeleton 1 from Methwold also demonstrated Harris Lines at the proximal ends of the tibiae which were identified using radiography (McKinley, 1996). In the original report, the skeletal material from Methwold Severalls was examined alongside that of Hill Close, Feltwell and other sites in the Methwold Fen. The general pattern that emerged was one of increased stress for females over males, based on the presence of enamel hypoplasia and Harris lines, which led the conclusion that 'this may be interpreted as showing that female children were considered of less importance than males and were therefore at the end of the food queue in times of shortage' (McKinley, 1996: 169). This may, in one respect, be supported by isotopic evidence which demonstrates little evidence of difference between male and female diets or between adults and children (Lennon, 1996). Thus, perhaps it was a case of lesser quantities afforded to female children, rather than lesser quality.

Individual 223 from Eyebury may have been subject to similar environmental influences, given the relative proximity to the Fens and that they may too have lived during the Early Bronze Age, if not the Neolithic/Bronze Age transition (Leeds, 1912). The excavator of the tumulus thought it formed part of the 'fairly large community thought to exist on the western edge of the marshy gulf of the Fens.' (Leeds, 1912: 82), and that the presence of this skeleton demonstrated the use of The Wash route 'by the invaders who introduced the knowledge of metal into Britain' (Leeds, 1912: 92). It has previously been discussed that there may be some merit in the analysis of skull shape to identify migration (Brothwell, 2000, Mays, 2000) and according to Thomas and Knowles of the Department of Human Anatomy at Oxford, the height and cephalic index of Skeleton 1 shows more 'affinities' with the known Bronze Age types than the Neolithic, although it lacks the 'pronounced brachycephalic characteristics met with in many of the skulls of the immigrant race' (Leeds, 1912). Whether this individual was local to the area or had travelled during their lifetime, perhaps from the continent, is not currently known, although it would be interesting to apply stable isotope analysis to answer the question. It should also be noted that skeleton one was buried with two black flint scrapers, the flint, according to Leeds, having been imported (1912).

There is of course great precedent for long distance movement of people during the Bronze Age, with the Amesbury Archer (Fitzpatrick, 2002: 177) and Boscombe Bowmen being two of the most famous Early Bronze Age examples (Evans *et al*., 2006) and there is little doubt that sea travel between the Britain and the Continent fully enabled this movement, given the discovery of the first known Bronze Age boat (Clark, 2009). Skeleton 39 from Boscombe Airfield, of course, lies close to both of these examples and, as previously mentioned, has been dated to the Early Bronze Age. It has already been noted that the airfield and Boscombe Down sites lie 'within a particularly rich archaeological landscape' and that this burial was a significant discovery in terms of its potential relationship with other known burials and features in the locale; the evidence suggests there to have been a substantial Late Neolithic/Early Bronze Age mortuary landscape (Manning *et al*., 2010). This burial has, however, been identified as post-Beaker, towards the end of the Early Bronze Age, and possibly contemporary with evidence of settlement structures close by (Lawson, 2007). It

could be 'the first firmly dated element of a significant group of post-Beaker burials and cremations within the Boscombe Down landscape.' (Manning *et al*., 2010).

The picture that emerges here is that of a rich and dynamic landscape, travelled to or settled in by people from deep within the continent from at least the Early Bronze Age period. This individual, therefore, lived in a landscape where these early 'migrants' or visitors left such an impression as to be afforded rich burials such as that of the Amesbury Archer and were, therefore, presumably, integrated with the local population. Whether or not these people brought with them diseases from the continent, such as TB, either previously unknown in Britain, or perhaps reactivating infections lying dormant within previously infected individuals is still unknown, but it seems increasingly likely. Skeleton 39 displayed rib lesions that were not just indicative of infection (healed and woven bone formation), but of chronic, long term, or recurring infection, no doubt from a respiratory condition.

Close to Skeleton 39 from Boscombe Airfield, lies the Normanton Down group of barrows, in which the Bush Barrow is considered one of the most impressive from the Wessex Culture (Parker Pearson, 1999: 89). The Normanton Group of barrows has been described as being '…probably originally the finest barrow group in the vicinity of Stonehenge, and perhaps in Southern Britain." (Grinsell, 1975: 30). It is in the Early Bronze Age that archaeologists have postulated the rise of an elite who controlled 'chiefdoms' (Darvill, 1987: 75) and the skeletons found within some of the Normanton Down group of barrows (such as the Bush Barrow, G7, G8, and G23) are certainly reflective of influential figures within society (Needham *et al*., 2010). It has also been suggested, however, that the occupants of the other barrows, containing less "spectacular goods" may also have been ranked just as highly and that changing burial practice and attitudes are but two of the factors which led to this apparent difference in burial accompaniments (Needham *et al*., 2010). As previously discussed, the interpretation of grave goods, especially in relation to status, is exceptionally difficult (see Parker Pearson, 2003).

Assuming the occupants of the barrows were of greater status than the general population, it may be assumed that less evidence of biological stress would be evident, and it is true

that no evidence of enamel hypoplasia was noticed on either of these two skeletons, although it was not possible to record stature, nor use radiography to identify Harris Lines. Both Skeleton 7 and Skeleton 11, however, show different but extensive evidence of infection and, as has been discussed previously, with the potential to have been caused by TB. Again, as with the many other individuals included in this study from the Bronze Age, both of these people lived in a time of extensive continental contact, increasing population and clearly in a busy landscape, the cause of which could perhaps have been, at least in part, the proximity of Stonehenge, and perhaps, therefore, this area more than others demonstrated a very diverse population at any given time.

There is relatively little that can be said about Skeleton 4 from Waterhall Farm in Chippenham, other than they, too, originate from an area rich in funerary monuments and, presumably, associated settlement. The presence of hypoplasia on some teeth may again suggest a previous illness or childhood period of malnutrition, which perhaps left them weakened and more susceptible to infection, although a number of differential diagnoses for the pathology seen in this individual have been offered above. No directly associated settlement evidence is visible for Twyford Down, from which Skeleton 883 originates, but archaeological evidence has demonstrated a long history of exploitation or occupation of the surrounding area (Healy, 1996: 1). There is, however, limited evidence for agriculture based on the small amount of evidence for cereal plant remains found, but looking at the two episodes of burials, the numbers of buried skeletons during each period suggest a small domestic farmstead sized group spanning several generations (Healy, 1996: 17).

The limited evidence of agriculture makes it impossible to suggest the kind of diet consumed by the occupants of this tomb, including Skeleton 883 but, amongst the individuals analysed in an earlier study, evidence of enamel hypoplasia and cribra orbitalia were apparent (McKinley, 1996: 103), as has been the case with a number of Bronze Age skeletons examined here. Despite the intensification of agriculture, seen particularly in the later Bronze Age (Champion, 1999: 103) there were some positive developments in terms of dietary inclusion. Oats began to be cultivated in this period, which are a good source of energy, providing high quality protein, minerals, essential fatty acids and high concentration of soluble fibre (Welch, 1995, Peterson, 1992).

In general, it might be fair to suggest that the Bronze Age of Britain was a “busy” time, with visitors or migrants from the continent, particularly demonstrated in the area surrounding the resting place of both the Boscombe Airfield individual and those located in Wilsford barrow G1 at Normanton, and possibly also at Eyebury. It was also a period of increased agricultural intensity which, on the evidence available cannot be confirmed at sites such as Twyford Down, Waterhall Farm or Eyebury, but which is clearly evident at Methwold. Palynological evidence and the identification of cereal processing artefacts, such as saddle querns, demonstrates that the post fen clay period, from which Skeleton 1 originates, was a time of increased cultivation, processing and consumption of cereals (Healy and Housley, 1992). The faunal evidence from Methwold is not considered satisfactory for interpretation due to its quantity and poor preservation, but does contain evidence of cattle, sheep and possibly pig, with an even greater range of animals identified at Eyebury (Witts, 1881). The increasing dependence on cultivated crops and domestic animals, rather than wild (Maltby, 2001: 20), and the no doubt increased sedentary nature of life, would have led to reduction in health so often witnessed as a product of agriculture. The changing climate to a colder and wetter environment towards the end of the Bronze Age, affecting the growth of crops (Cunliffe, 1995: 16) and cultivatable land, would also no doubt have compounded the problem, and presented additional direct and indirect effects on health and susceptibility to TB (See 2.4.2).

The pathology presented here from skeletons primarily originating from the Early Bronze Age is consistent with this but, as previously stated, these are a limited number of examples from which to draw broad conclusions. Given the relatively few skeletal remains for the period however, and the limitations of this study in terms of what could be analysed, seven possible examples of infection are important data.

6.2.3.3 Iron Age

Three sites from the Iron Age are represented in the study, with a total of eight individuals. Two sites are from within the primary geographical target of this study, Bourton-on-the-Water and Abingdon Pipeline. The other site is that of Wetwang Slack, a well-known Iron

Age cemetery in North Yorkshire, considered “exceptional” due to the large number on inhumation burials discovered at the site (Dent, 1984). This site was originally sampled as part of the wider project, but the individuals selected for inclusion and consequently sampled by Dr. Darlene Weston, were subject to aDNA analysis here because of the Iron Age date. Of course, TB is already documented for the British Iron Age, although the discovery at Tarrant Hinton in Dorset remains the only confirmed case for the period. With the exception of large cemeteries like Wetwang, the researcher is, however, dealing with the “dearth” of skeletal information for the period (Mays, 2000: 283).

Skeleton 4197, which demonstrated periostitis on long bones, was found in the excavated area for a new pipeline in Abingdon (Cotswold Archaeology, 2003). The area has demonstrated numerous Iron Age farming settlements and in the Vale of the White Horse nearby (Hearne, 2001). The same is true for the site of Bourton-on-the-Water, where evidence of settlement is evident from the Neolithic onwards and the large site of Salisbury Camp lies only 400m to the east (Cotswold Archaeology, 2010). This intensive settlement evidence is no doubt reflective of the need to support an ever growing population, and with this would presumably come the detrimental health defects seen with a more sedentary lifestyle. Due to the size of the Wetwang Slack cemetery, some inferences about the size of the population the cemetery serviced at any given time have been made, with a figure of around 35-84 individuals, roughly the size of a hamlet or village containing a number of families (Dent, 1982: 452).

A full discussion of the settlement evidence can be found in Dent (1995) but it may be of use to select some key elements demonstrated by the Wetwang site to provide background to the skeletal interpretation. Firstly, the burials in the cemetery are of the Arras tradition, which is seen only in East Yorkshire but which is closely associated with continental parallels (Cunliffe, 2004). As such, it has been assumed that, much like the “Beaker Folk” of the Bronze Age, this burial tradition is reflective of immigrants to the area (Hawkes, 1959, Hodson, 1964), although this has been questioned (Stead, 1991). Even if the Arras burials are not those of immigrants, it does however provide evidence of continental influence and contact. This is further demonstrated by the presence of certain artefacts, such as brooches, which are heavily influenced by continental design (Dent, 1995: 56).

‘From this cultural evidence can be discerned a stratified, perhaps feudal society, with wealthy leaders who enjoyed long distance exchange links’ (Dent, 1995: 7). It is clear that a hierarchical society, probably partly as a consequence of agriculture, was continually developing from the Neolithic onwards. This is particularly evident by the monumental architecture seen in the Neolithic, the development of fortifications towards the end of the Bronze Age and in the strong regional grouping in the Iron Age, to name but a few. As previously discussed, with hierarchy often comes consequences for health due to social inequalities, and this was no doubt true for these Iron Age populations.

On average, it has been noted that the people of Wetwang Slack (at least those represented through the burials), were somewhat shorter than average (Dent, 1984), although there was no evidence of enamel hypoplasia and radiographic analysis had not been done to detect Harris lines (see 3.4.2 for further discussion of Harris Lines). It is interesting to also note that a higher proportion of children found were males (although overall there were reportedly more females present in the cemetery). This has been interpreted as suggesting females had a better chance of survival, which may be due to better immune responses to disease as discussed by Ortner (2003:115); there was apparently no evidence for detrimental treatment of female children (Dent, 1984). It must be noted that the skeletal remains of children are generally not sexed as sex specific morphological features do not develop until after puberty, therefore these interpretations must be viewed with caution.

It was also calculated that eighteen per cent of the population died between the ages of 14 and 25 to which Dent states “…this may have been due to diseases such as tuberculosis, the dangers of which tend to have been forgotten since the disease was effectively eradicated earlier this century.” (Dent, 1984: 87). Unless biomolecular analyses were applied to a greater proportion of skeletons from the cemetery, not only those demonstrating skeletal changes, it will not be possible to prove this statement to be true or false. Pathogen aDNA analysis has, however, been applied to iron age faunal remains (including the primary domesticates: cattle, sheep/ goat, horse and pig, but additionally, examples of dog, modern badgers and a deer) from this cemetery, in additional to iron age and roman faunal bones from the site of Tarrant Hinton, Dorset, and iron age faunal remains from Danebury,

Hampshire. Wooding (2010), selected bones based on non-specific evidence of TB, but unfortunately no positive results were obtained for these sites. Mycolic acid testing was also employed but did not yield any positive results; research is on-going (Wooding, pers. comm).

The Iron Age demonstrates a mixed and varied economy and a general expansion of agriculture (Huntley and Birks, 1983). Faunal assemblages are dominated by the primary domesticated species including cattle, sheep, pigs, horses and dogs, although there is evidence for much intra-site variability (Maltby, 2001: 20). However, the beginning of the Iron Age was also subject to the same climatic deterioration as that witnessed at the end of the British Iron Age (Darvill, 1987: 133), and this combination of reliance on agriculture and conditions perhaps unfavourable to growing stability, could have resulted in a further decline in health, leaving the Iron Age inhabitants of Britain susceptible to diseases from the continent. Evidence of TB has already been confirmed as being present by the middle of the Iron Age. Whether or not this is related to Tarrant Hinton's geographical location, along the southern coast of the country, and therefore truly represents the first origins of TB being brought across the Channel to Britain is unknown. It would seem unlikely however, to have identified the truly first case of TB ever seen in Britain, given the limitations of the skeletal material as discussed below. What is certainly evident is that, by the Iron Age, conditions were clearly favourable to the presence of TB and it would not have taken long, given the mobility of Iron Age peoples, to have spread further inland.

7 CONCLUSION

This chapter very briefly summarises the results for clarity before presenting a critique of the methodological approaches applied during the course of the research to establish if these may have affected the final results, both macroscopic and biomolecular. Limitations are further explored with respect to the skeletal material analysed and the positive outcomes of the project are highlighted with suggestions for further work. The chapter ends with a final overall summary.

7.1 Summary of Results

The macroscopic and biomolecular data from this study do not support the original hypotheses proposed, that TB was present in prehistoric Britain. However, they do not disprove it. The much repeated truism, ”absence of evidence is not evidence of absence” is certainly appropriate in this case. All samples, with the exception of those from Wetwang Slack, originated from sites in the south of Britain and thus are not representative of prehistory in the entirety of Britain and, whilst every effort was made to identify all skeletal collections in the south of Britain that fulfilled the criteria, as identified in the osteological methods, it is unlikely that all skeletal collections were located (7.2.3.1).

What the results do indicate is evidence of infection across all periods, similar to that which could manifest as a result of TB. The skeletons that demonstrated rib lesions on the visceral surface of the ribs, including those from Twyford Down, Boscombe Airfield and those from Wetwang Slack, are certainly candidates for respiratory infection, and possibly TB, while all the other examples of pathology identified could equally be the skeletal manifestation of a tuberculous infection, perhaps in an early stage, of little of which is currently known, or in the case of a number of Wetwang Slack skeletons, more advanced, but the pathology not able to be considered diagnostic on its own, just highly suggestive. The possible presence of HOA in more than one skeleton makes an interesting discussion point, unfortunately, poor survival of skeletal elements thwart any attempt at a more confident diagnosis. The aDNA analysis could not confirm a link with TB in any skeleton, but the osteological analyses still

demonstrate that infections occurred from at least the Neolithic onwards, which complements previously reported data (Roberts and Cox, 2003, Roberts and Cox, 2007).

7.2 Limitations

7.2.1 The Skeletal Material

7.2.1.1 Quantity and Preservation

The quantity of skeletal material discovered and excavated for prehistoric Britain is likely to be highly disproportionate to the actual level of population (Waldron, 1994). An inherent problem throughout all archaeological disciplines is that only a small percentage of archaeological material is available for study, compared to what was once present. A number of factors, both intrinsic and extrinsic, determine the quantity of skeletal material that will be recovered from a buried population, and each of these factors further reduce the final number of individuals that are excavated and thus analysed (Figure 7-1).

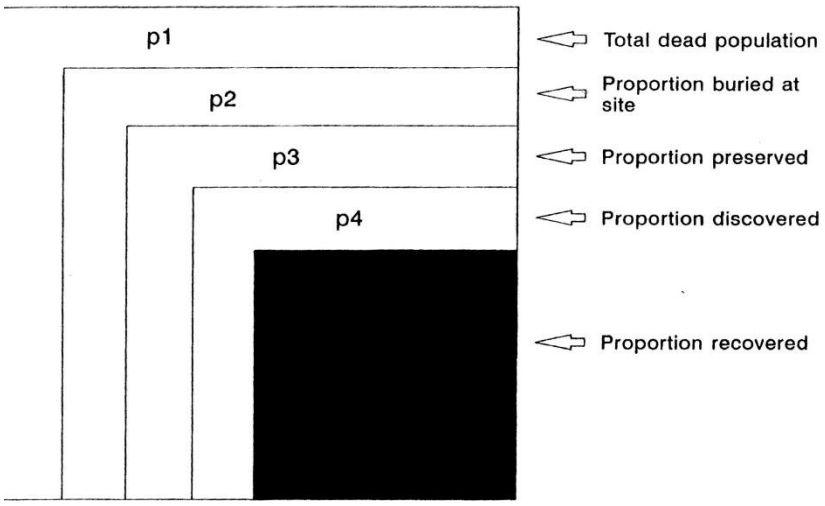


Figure 7-1 Diagram demonstrating the limiting factors of archaeological recovery of skeletal populations (Waldron 1994: 13)

The quantity of skeletal material available for study has previously been referred to in the context of the individual prehistoric periods under study. Skeletal remains excavated from

the Neolithic, for example, are generally confined to those associated with funerary monuments or causewayed enclosures. The situation is much the same in the Bronze Age, with funerary monuments changing in shape to round barrows, with many of the different forms visible in the Wessex landscape (Grinsell, 1941) and with more varied forms of deposition, such as in caves or watery places (Parker Pearson, 1999: 87). During this period, cremation also comes in and out of fashion, sometimes examples of both cremation and inhumation appearing in the same funerary context (Bradley, 2007: 162); information from cremated bones is harder to extract as it depends on level of fragmentation, level of recovery and specialist interpretative skills (McKinley, 2000). Despite a larger population during the Iron Age, human remains are primarily confined to the large cemeteries such as Wetwang Slack which, as with the other periods may provide a rather biased representation of the populations at this time, depending on the criteria for burial within these places. It would be beneficial to also look to the context from which much of the oldest material (in terms of time elapsed since excavation) was excavated, particularly with reference to the Neolithic and Bronze Age. Antiquarian excavations in Britain were more often than not concerned with the prehistoric mounds scattered across the countryside, many of which remain at least in some degree, intact and visible today. Whilst these intriguing ancient earthworks are known to have attracted even Roman interest, it is the antiquarian interest in the eighteenth and nineteenth centuries which has had most direct impact upon our ability to continue to study the human remains found within long barrows and similar earthen structures. William Stukeley (1687-1765), an example of an early antiquarian, was responsible for the excavation of a number of barrows but, despite his training as a medical doctor, showed little interest in any human remains encountered (Smith and Brickley, 2009: 19).

The result was that early barrow diggers often returned excavated remains to the ground, unaware at that time of the information which could be extracted from them. Occasionally, only crania were deemed important enough for recovery, which proved useful in later calculating cranial shape and size. This was seen as an important facet in differentiating between indigenous Bronze Age people and “invading” Beaker people (Crawford, 1912: 188). Whilst these measurements, observations and subsequent conclusions may hold some weight (Brothwell, 2000: 3), by ignoring the post cranial remains the bigger picture of these

past populations including, crucially, any possibility of assessing their relative health, is now lost. An example of this interest in, and bias towards, the retention of skulls, to the detriment of other skeletal elements, is suggested by Witts' original report from West Tump (1881). It is implied that perhaps not all fragments of skeletal remains were accessioned to, first Gloucester Museum and subsequently Cheltenham, or were retained first for further study, stating "All the skulls found in the "West Tump"…have been properly cared for, and…will shortly find a resting place in the Gloucester Museum, with other typical bones from the same barrow." (Witts, 1881: 208).

Whilst human remains are now thoroughly recorded, whether excavated or for some reason, left in situ, there can still be problems with both identification and retrieval of remains, with the small bones of the hands and feet often lost, as with the very small epiphyses of unfused bones from neonates and infants. Most archaeologists are not trained osteologists, which means some bones can be mistaken for those of animal bones or, more often, vice versa. An example of this in the early days of excavation comes again from Witt who originally identified a human baby at the site of West Tump (Witts 1881: 207), which later proved to be an immature dog skeleton (Brickley and Thomas, 2004). Most human remains are now submitted to specialists for analysis but this was not always the case, and it must not be forgotten that analytical techniques are continually improving, meaning that skeletal collections analysed in the past, even a few decades ago, may not have yielded the same level of information as could be extracted now.

Bones will also be subject to taphonomic processes which can result in lost skeletal elements, perhaps through disintegration, or even by scavenging. Burials can be disturbed, such as Skeleton 39 from Boscombe Airfield, and modern activities such as ploughing are frequently responsible for this disturbance, as was the case of Skeleton 1 at Methwold Severalls – this skeleton was also subject to disturbance soon after deposition (Curtis, 1968). Burials are frequently truncated, mixed or lost through various processes, further compounding the problem of the limited quantity of material available for the prehistoric periods and, in many cases, limited quality.

Problems with recovery, particularly from prehistoric contexts, are not just due to the limitations of excavation practice or post-depositional disturbance and taphonomy. The Neolithic is an excellent example of where pre-depositional and depositional practice, can greatly impact on the success of, particularly, identification of discreet burials. Burial practices of the Neolithic frequently resulted in commingled remains, sometimes with the loss of particular skeletal elements, discussed further in Mays (1998) and Brickley and Smith (2009). Individual A3 from Ascott-under-Wychwood is a key example of this, being disarticulated with two other individuals (Whittle *et al*., 2007b: 139). It was not possible to confidently assign *some* skeletal elements to A3 specifically, given the distribution within the cist (Figure 7-2 and Figure 7-3). This type of practice, the mixing of skeletal remains resulting in less than complete skeletons, can make it exceptionally hard to study patterns of pathology throughout the body. This was the case for a study of this nature, where articulated burials would of course be preferable in order to more confidently offer diagnoses.

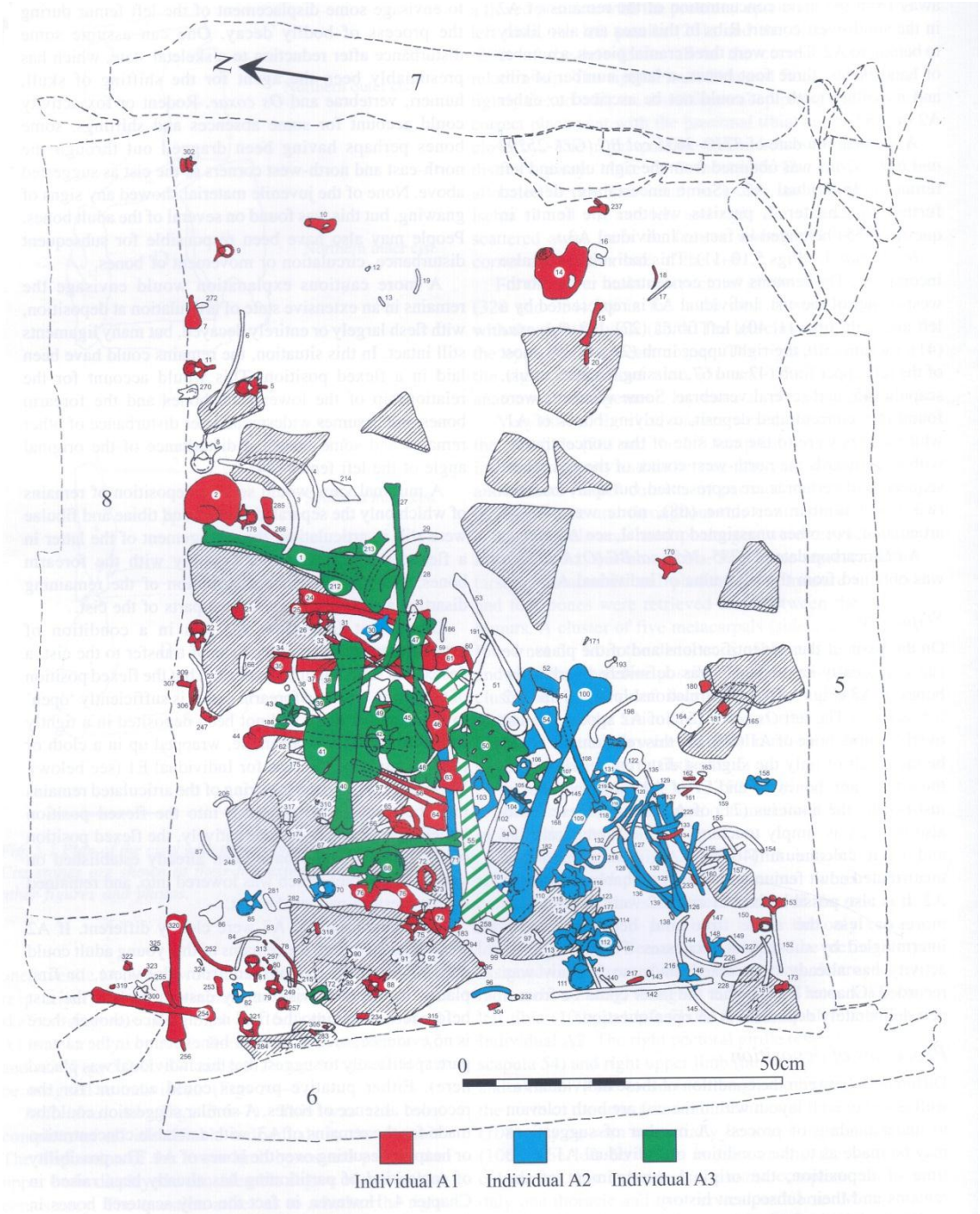


Figure 7-2 Distribution of skeletal elements of Individuals A1, A2, A3 in Deposit A, the southern inner cist – upper part of the main deposit. (Whittle et al, 2007: Fig.5.2)

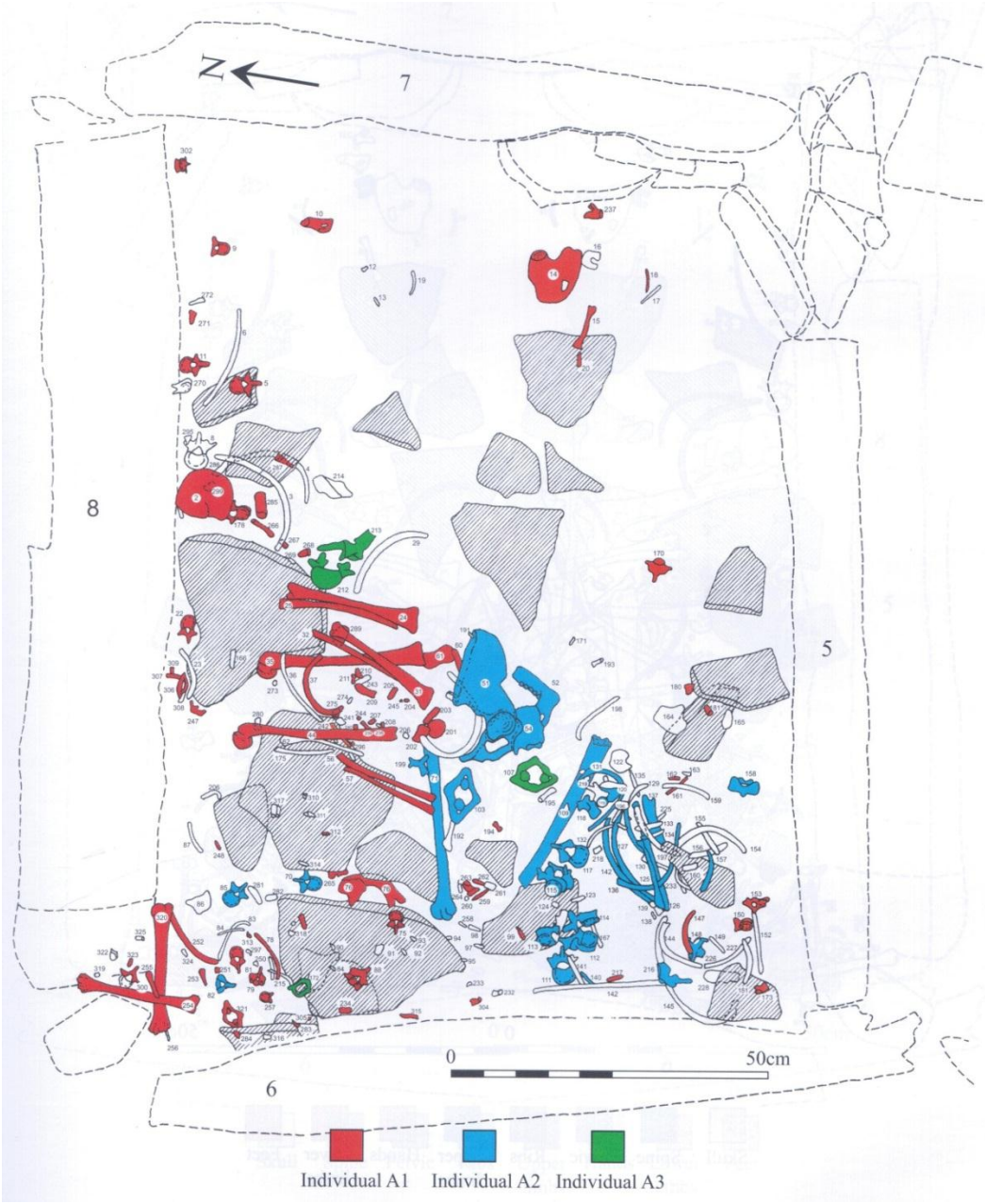


Figure 7-3 Distribution of skeletal elements of Individuals A1, A2, A3 in Deposit A, the southern inner cist – lower part of the main deposit. (Whittle et al, 2007: Fig.5.3.)

Preservation of the skeletal material in terms of its structural integrity is also of importance here:

> *The fully preserved, gleaming white skeleton is a thing which survives only in the minds of writers of fiction; the reality is quite likely to be something which resembles well chewed digestive biscuit and which may be about as easy to deal with.*
>
> (Waldron, 1994: 14)

Whilst some post depositional activities can result in the movement of skeletal remains, sometimes towards the ground surface, resulting in greater exposure to the elements and the likelihood of scavenging, the nature of the environment in which the burials are placed is key to their survival. Degradation of bone is affected by many factors, including biological age and, importantly, soil pH, 'as soil pH decreases, the destruction of osseous materials increases.' (Gordon and Buikstra, 1981). Skeleton 1, recovered from Methwold Severalls was found in a peat environment (Fenland peat is neutral), and deposited during relatively wet conditions. As such, the bones were deeply stained but in relatively good condition. Many of the individuals included in this study were found in chalk soils, such as Skeleton 4 from Waterhall Farm, Skeletons 7 and 11 from Wilsford Barrow, the multiple Skeletons from Wetwang Slack and Skeleton 883 from Twyford Down. This kind of environment is usually preferable to bone preservation but, because Skeleton 883 was disturbed, it was poorly preserved, due, in part, no doubt to the very shallow nature of the grave cut and possible truncation (McKinley, 2000: 100). The skeleton from Waterhall farm demonstrated good preservation although was described as 'quite soft' (Leaf, 1940: 8), which is an observation echoed by Witts with reference to Skeleton 7 at West Tump (1881). The original report for the excavation at Eyebury notes that 'The bones were much broken and many of them in a somewhat rotten condition' (Leeds, 1912: 90), after having been lying on a bed of white sand. Good preservation was seen in the Skeleton A3 from Ascott-under-Wychwood, given the protective environment of the chambered long barrow and also of the skeleton found at Bourton-on-the-Water where the deposit was primarily composed of 'silty clay' of moderate compaction (GCCAS, 2000), and the skeleton found at Abingdon, again was covered by a dark sandy silt resulting in good surface integrity.

It should not go unmentioned that the treatment of human remains whilst in storage can also impact on their continual preservation. It has been clearly demonstrated that the more skeletons are handled, the greater chance there is that they will suffer loss of bone elements and damage (Caffell *et al*., 2001). Collections which have not been recently studied, or not at all since their excavation, are often found to be in poor condition and, whilst they may not have been subject to the levels of handling with regularly studied material, environmental factors and storage methods will impact on their continual

preservation (Janaway *et al*., 2001). Poor packaging can result in further fragmentation of already fragile material; boxes which are both over packed and under packed risk damage to the individual bones and, importantly for the study of ancient disease, the loss of important pathological data. This is particularly true of discreet pathology such as rib lesions where the plaque like lesions described by Pfeiffer (1991) can easily be disturbed during inadequate storage and poor handling. There is no doubt that this will have resulted in some pathologies having been lost (Figure 7-4), and therefore gone undocumented. This study, which required the identification of such discreet pathology, may have been severely disadvantaged by the repeatedly poor storage conditions of the skeletons examined. It is often found that a box is not big enough to comfortably accommodate the longest element, usually the femur, which is very often found to be "wedged" in boxes diagonally (Caffell *et al.*, 2001), and this was certainly noted during the course of this study, although was only directly a problem when pathology was noted at the proximal or distal ends of a femur and the storage conditions have compromised the integrity of the bone and/or pathology.

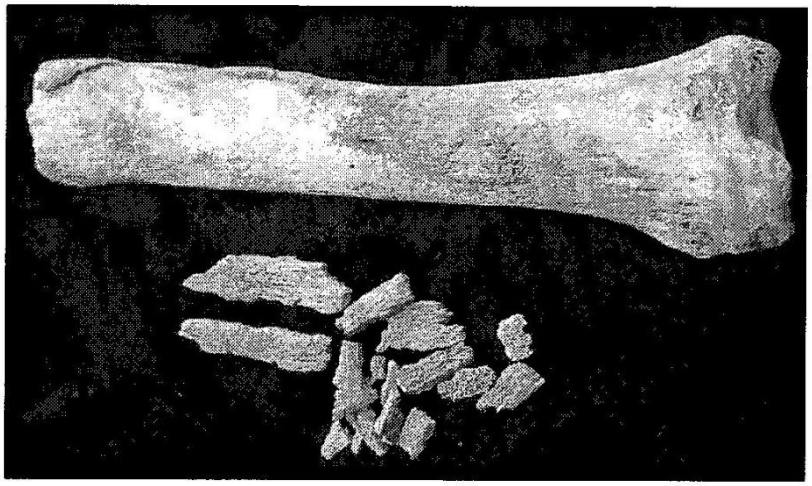


Figure 7-4 Loss of new bone formation from bone surface through poor handling. (Caffell *et al.* 2001)

7.2.2 Locating Material and Associated Documentation

Identification of skeletal collections matching the following criteria was required:

- Originating from sites in the south of England.

- Individual skeletons, or those that could be identified as such, rather than mixed remains (meaning mixed skeletal elements originating from two or more different individuals and which could not positively be attributed to one specific individual).
- Of prehistoric date (Neolithic, Bronze Age, Iron Age).
- Of reasonable preservation both in quantity and quality (although this was in most cases based on individual judgment).
- Inhumations as opposed to cremations.

Many of the problems associated with fulfilling these criteria are discussed above. The experience of actually finding relevant collections is presented here. The WGHR *Scoping Survey of Historic Human Remains In English Museums* (2003) provided some useful information as to the location of prehistoric material (although it was borne in mind that the data was possibly not up to date) and, to a lesser degree, so did Culture24 (then 24 Hour Museum), which was used wherever records existed, although at that time the extent of this resource was limited. Other individual researchers or researcher groups were also kind enough to provide some further details such staff from The Beaker Project (Sheffield University), Dr. Martin Smith (Bournemouth University) and Dr. Jacqueline McKinley (Wessex Archaeology). Ultimately, the search strategy was reduced to scouring the wide literature on excavations from the prehistoric period and emails and phone calls to speak directly with curators from museums to ascertain their current holdings of interest. This was, of course, time consuming and relied on the availability and willingness of museum staff to search their own records and provide the information. No national database exists, from which to devise a systematic plan of research.

Even once possible collections of interest were identified and located, problems particularly arose when trying to identify specific skeletons for study and plan efficient research visits. This was due to a number of museums and institutions (not all) having one of the following key problems with regard to matching skeletons to the desired criteria:

a) An internal electronic database did not exist for the skeletal collections.
b) An internal electronic database did exist but specific details could not be sent electronically in advance of the research visits.

c) An electronic database did exist and suggested possibly useful collections for study, but did not contain enough detail to confidently narrow the search to particular skeletons prior to a research visit.

This resulted in a great many hours lost at the museums or research institutions by time spent finding collections which, upon initial inspection, could not be utilised. It was not expected that the researcher would concentrate solely on material already analysed, focussing on those with non-specific infection already having been identified. By the very nature of the project, it was necessary to look wider to those collections not subject to previous analysis, that had been analysed a long time ago, or whose discreet pathology may previously have been overlooked. It was, however, an inefficient use of time to source skeletons which did not fit into the original criteria, mostly because of poor records. Potential solutions to this problem are discussed further in 7.3.2, but it must be emphasised that these solutions do not lie solely at the feet of museum curators and collection managers, but rather the problem must be addressed by all who curate and utilise the skeletal collections for research.

If better records of the location and content of skeletal collections had been available, the efficiency of the project would certainly have been improved, allowing identification of specific skeletons matching the project criteria to be confidently identified prior to a research visit, (also taking into consideration issues such as condition and previously identified pathology). Due to the time saved a more extensive data set could have been located and studied, perhaps increasing the geographical coverage of the project.

A great many of the skeletons analysed as part of this study had been excavated over a century ago. For this reason, it was often difficult to trace the original reports relating to their excavation, although the vast majority had ultimately been published in periodicals which, when the reference was found, were relatively easy to acquire. Some documents were not so easy to locate, although the search was aided through the recent digitisation process to which a great deal of theses held in British Institutions are now subject and, through communication with other members of the field.

Their content, however, (primarily of the older reports) was not always of great use as, very rarely, did the text provide much detail about the skeleton or skeletons themselves. Problems with documentation available for consultation stem from the earliest days of excavation onwards. For example, the well-known 19th century antiquarians, William Colt Hoare and William Cunnington, who together were responsible for the excavation of at least 465 barrows, made very slight progress in comparison to their predecessors. They followed a more systematic investigation process but still any reference to skeletal remains remained minimal (Smith and Brickley, 2009: 20). With reference to Colt Hoare, Grinsell states '[he]… almost invariably omitted to give any information concerning the bones' but does describe an exception from Whitesheet Hill, Wiltshire, where a skull 'grinned horribly a ghastly smile'. Pitt Rivers is known to have commented 'no doubt the skeleton must have been laughing at him for his unscientific method of dealing with it' (see Smith and Brickley, 2009). Although Colt Hoare recorded numbers of individuals present, he did not record the methods for presenting these data, and Cunnington reburied the human remains and thus the numbers cannot be verified (Smith and Brickley 2009).

Limited documentation is often the result of poorly analysed skeletal material, which, in part, can be due to the actions of early excavators, as noted above, but even when early reports are present they can be misleading. One such example is the misidentification of the baby, now known to be an immature dog skeleton from West Tump, as mentioned previously. If these bones had not been subject to more recent skeletal analyses, the 'fact' would have remained undisputed in the literature. On occasion, there would be more detailed discussion as to the position and condition of the skeleton(s), but not necessarily in what would now be considered a scientific manner. On occasion, discussion of pathology included assumptions that would not be seen in more recent reports so as not to cloud the diagnostic judgement of later professionals or, in one particular case, simply because it seems now to be a rather ridiculous assumption (although it must be read bearing in mind its date). This example can be found during the discussion of the skeleton found at Eyebury where the author, when referring to the pathological elbow noted in the skeletal report here, offered a theory, the first part of which appears to be perfectly valid:

> *There were signs of the deceased having been in the wars, as the epiphysis of the left ulna had been broken off, and, though there is considerable evidence of osseous growth on the main part of the bone, the broken part had never anchylosed. A medical friend, after examining the bones, has told me that every movement of the elbow would thereafter have caused the most exquisite pain, if they took any notice of such trifles in those prehistoric times.*
>
> (Leeds, 1912:90)

Other problems with the associated documentation were simply a mismatch of what was stated and what was present in a given box. Comparing the skeletal elements available for skeleton 883 from Twyford Down with the published report, it seemed likely that some elements were missing, either through loss, separation in storage or mislabelling. This will now have created disparities between the original skeletal report and that presented here, and this was the same for a number of skeletons. On occasion, it was not in fact possible to locate particular skeletons requested, presumably due to them having been misplaced or moved without records having been updated. The lack of particular skeletal elements may, on occasion, be the result of destructive testing having taken place or other analyses where return of material has not occurred, and which is not documented. Sometimes the presence of additional skeletal material in a box labelled as one individual, demonstrating a clear MNI of at least two, gave rise to suspicions over bones belonging to the individual intended; this was the case for the Eyebury material, whose condition was questionable enough to prevent a full skeletal analysis in order to prevent further confusion. Many of the problems noted above could be the result of actions taken long ago or more recently, but *all* create difficulties in (a) trying to identify skeletons, (b) locating them and (c) analysing them.

7.2.3 Methods:

7.2.3.1 Macroscopic

The macroscopic methods of analysis employed here was based on published recommendations and standards within the field of osteoarchaeology (Brickley and McKinley, 2004, Buikstra and Ubelaker, 1994). Establishing the age and sex of individuals analysed was considered of great importance for the study of a disease that can choose its hosts, and often the type of TB and the bones affected are determined by age and sex. For example, children often suffer with the disease in bones with the greatest quantities of red marrow such as the vertebrae, ribs and sternum, due to the high circulatory and metabolic rate of these areas (Ortner; 2003: 228-9). From a social perspective age and sex can also play a key role in a person's susceptibility to disease due to status variations and potentially weakened immune systems through undernutrition. To record the age and sex of skeletons possibly demonstrating TB was a key element of the process wherever possible.

Based on the limitations of the materials discussed above, however, it is clear that applying these methods were hindered by missing skeletal elements and poor bone preservation. Mostly, sex estimation could be determined, with the exception of Skeleton 7 from West Tump due to its fragmentary nature, and age at death was recorded for all individuals, except skeleton 7, also for this reason. Age at death estimations were, however, limited to relatively broad categories, except for younger individuals. The methods employed for assessing age at death, biological sex and stature, and their various advantages and disadvantages, have been discussed (4.3.1.1) but it is the limitations in the ability to record pathology which must be addressed here.

This study aimed to explore the evidence for TB in prehistoric Britain, prior to that already identified in the Iron Age. The reasons as to why TB had not been previously found can include the following:

1. It was not actually present in the Neolithic or Bronze Age.
2. It was present, but skeletons demonstrating skeletal evidence have not been excavated.
3. It was present, but the pathology is discreet/subtle and/or untrained eyes did not in the past record or identify any evidence.

4. It was present, but affected the bones in a way not recognised as typically TB associated pathology now.
5. It was present but people did not live long enough for the bones to be affected i.e. their immune response was low.
6. preservation was poor

It is 3 and 4 which limit the effectiveness of the macroscopic methods employed. Firstly, if TB did affect the bones in a way not recognised now, it will be exceptionally difficult to create any form of criteria for its identification, now or in the future. This is, however, where the use of combined macroscopic and biomolecular analyses are most useful, although aDNA analysis is itself subject to limitations (see 2.2.2).

The manifestation of TB in the skeleton can result in pathological lesions that are highly specific and is thus easily diagnostic. The most identifiable feature of skeletal TB is usually that of Potts Disease but, in the absence of this, the researcher looks to the general pattern of pathologies in the skeleton. Even when '"typical" TB related pathology is noted, differential diagnoses should be explored. As TB can affect any part of the body, often causing severe destruction of joint surfaces or, in some cases, additional bone formation, it can often be confused with a number of other diseases, or even in some cases trauma. Some examples of these include brucella osteomyelitis, septic arthritis, ankylosing spondylitis or even healed vertebral fractures (Aufderheide and Rodriguez-Martin, 1998: 140). In the absence of any "classic" TB related pathologies, the researcher is unable to make more than general comments regarding the possibility of the disease. The example of rib lesions is a key example as despite a strong correlation between rib lesions and TB having been found, (Kelley and Micozzi, 1984, Roberts *et al*., 1994, Santos and Roberts, 2006), the pathology itself cannot be considered pathognomonic of the disease, particularly in isolation. It may be that TB in earlier populations did not affect individuals to the degree that it has since, in which case the pathology may not show on the skeleton or be very mild, i.e. appear non-specific in nature. This study was, in part, an attempt to identify possible early stages of TB related pathology or the skeletal reaction to an early form of the disease – potentially only possible in combination with aDNA analysis, but the choice of material to be included in the project was inherently limited for this very reason. In addition, bone itself is

predictable in its processes, in that it can either be destroyed, or form new bone, but no two humans will have identical immune responses to disease and, although TB can produce a recognised pattern of pathology in the human skeleton, it is not always so predictable as to be confidently diagnosed. As a disease that can travel freely through the body via the bloodstream and lymphatic system, thus able to affect any part of the body including bone and soft tissue (with the exception teeth and hair), its manifestations can vary significantly in both location and appearance, often resembling other disease processes.

Secondly, that people may not have lived long enough to display any skeletal form of the disease is yet again the result of the 'osteological paradox'; those who appear in good health, with no or limited pathology may, in fact, have died during the early stage of disease, and those who lived long enough with a disease to display skeletal pathology, were the ones with better immune systems and thus, may be considered healthier (Wood *et al*., 1992). Again, because of this, the macroscopic methods employed were limited; it is not possible to diagnose what is not visible and, due the destructive nature of aDNA analysis and the costs involved, it is not yet feasible to subject all excavated skeletons to biomolecular testing.

7.2.3.2 Biomolecular

The use of pathogen aDNA for diagnosis is a valuable analytical technique. For example, by extracting pathogen aDNA it is possible to confirm the presence of disease (although cannot be used directly confirm the cause of skeletal pathology), such as the first case of TB from Britain (Mays and Taylor, 2003) and, in some cases, it may even be possible to identify the causative strain of an infectious disease (Taylor *et al*., 2007). The technique is however, subject to a number of problems, and the most relevant to this study can be considered to be:

- Limited experience of the researcher
- Poor survival of aDNA
- Contamination

- Limits of interpretation

aDNA analysis is a complex process and to be performed correctly and with the greatest possibility of success, the investigating researcher must be familiar with correct laboratory procedure and protocol. All possible preparation was made by this researcher before undertaking both the sampling and laboratory work, however it must be noted that this area of research and practice required 'on the job' training having had no prior experience. Despite this, all work was at first supervised and correct procedure learned and followed rigorously. The primary disadvantage however, was the time taken for the analysis, which was undoubtedly slower at the beginning of the project than it was by the end once the researcher was confident with procedure. Although extensive care was taken when performing mtDNA real time PCR analysis, contamination was demonstrated, which could be down to the researchers lack of expertise in this area, although Taylor *et al* specifically refer to mtDNA work as being 'extremely difficult to maintain contamination-free due to the ubiquity of human DNA sequences' (2010: 748).

Poor survival of aDNA no doubt played a part in the negative aDNA results produced in this study. There was, however, evidence in at least four samples of surviving human aDNA (Lct) suggesting that the burial conditions to which these particular individuals were subject, may not have destroyed any surviving pathogen aDNA if present. PCR, because of its sensitivity (Brown, 2000), was used in order to try to identify and amplify even very small quantities of surviving pathogen aDNA, but it cannot, of course, amplify what is not there; any remaining pathogen aDNA could also have been too heavily degraded to have been amplified in any recognisable form. TB is, however, thought to demonstrate increased likelihood of survival of because of a resistant mycolic acid component of the cell wall (Zink *et al*., 2002, Donoghue *et al*., 2004), and evidence of the disease has been identified in archaeological skeletons previously (see 2.3).

Other reasons for poor retrieval of aDNA may include:

- Type of bone selected for sampling: A wide variety of bones were sampled for this project and none demonstrated any TB specific sequences, however, it has been

suggested that bone condition may influence the chances of survival of aDNA, with bones that are hard, heavy and compact, are more likely to yield aDNA such as the diaphyses of long bones rather than more porous bones such as the vertebrae (Bollongino *et al*., 2008). Priority was given to sampling at the site of pathology or as close to the lesion as possible, because of the increased likelihood of pathogen aDNA presence, although condition of the bone was given some consideration with bones demonstrating less exposure to external contaminants (e.g. the Ascott-under-Wychwood vertebrae) chosen wherever possible. Teeth were originally collected as part of the study in order to act as additional control samples for results on the assumption that teeth are generally less exposed to contaminants than bone (Rudbeck *et al*., 2005), although they too are open to contamination (Richards *et al*, 2005), but are often used as a number of studies have reported greater aDNA yields in teeth than bone (Oota *et al*., 1995, Kurosaki *et al*., 1993). The teeth collected for this study were ultimately not analysed, partly because of time constraints and the time consuming nature of retrieving dentine as previously reported (Spencer, 2008), partly because teeth were only collected for a few samples overall due to differing rates of skeletal element survival between skeletons analysed, and partly because although teeth were sought that were likely to have been most protected from environmental contaminants (found in situ with minimal damage), because of the different levels of preservation between skeletons, a number were still in poor condition. For this reason, teeth were returned to the curating institution.

- There is evidence to suggest that skeletal samples lose aDNA integrity rapidly after excavation, this was highlighted in one study, regarding auroch bones: during 57 years of storage 'at least as much amplifiable DNA was lost as during the previous 3,200 years of burial' (Pruvost *et al*., 2007: 739). This is of great significance for this study, as the majority of skeletal material obtained from the study was from skeletal remains that were excavated, in some cases, over a century ago (Witts, 1881) meaning that it is possible that the amount of aDNA in the bones (if present at all), may be a fraction of what it was at the time of excavation.

- A recent paper also noted that some methodological aspects of sampling bone for aDNA studies could be detrimental to DNA survival. Adler *et al*, (2011) demonstrated that high drill speeds (used to reduce the damage to skeletal remains), has a detrimental effect on the survival of mtDNA (and probably nuclear aDNA) as the high temperatures generated by the drill can decrease mtDNA yields by up to 30 times potentially creating false negative results. This is presumably also applicable to pathogen aDNA and although the use of a drill to sample bones was limited in this study (with small, broken bones from at, or close to, the site of pathology chosen wherever possible, to reduce damage to the original skeletal material), it was employed during the sampling of the NHM material. This may therefore, have compromised the survival of any aDNA and created a false negative result.

Richards *et al* have previously argued that contamination, as opposed to aDNA survival, is however, the greatest problem in aDNA studies (Richards *et al*., 1995). Contamination is a constant problem for those working with aDNA, with even gelatine-based bone glue, which has often been used over the last two centuries to preserve hard tissues, including bone in museum collections, can be a major source of contamination which has been overlooked (Nicholson *et al*., 2002). Despite adhering to strict sampling and laboratory protocols including performing all analyses in specialised laboratories (Bouwman and Brown, 2005, Bramanti *et al*., 2003) without analytical instruments ever being exchanged between them (Yang *et al*., 2003, Herrmann and Hummel, 1994), the wearing of protective clothing in the aDNA work area (Yang *et al*., 2003, Bramanti *et al*., 2003) and the use of control samples (where possible; Stoneking, 1995, Yang *et al*., 2003), it was not possible, on all occasions, to prevent contamination. Contamination is possible from within the burial environment, (Gilbert *et al*., 2004, Gilbert *et al*., 2005a) and through human contact from the time of excavation through to curation and research, although some argue that this is not as relevant for pathogen aDNA studies as for human (Taylor *et al*, 2010). Certainly, in the past, gloves were rarely worn on site for the excavation of human remains, as advocated by Brown (2000), and in the experience of this researcher, are only recently (though still sporadically), being used more frequently in museums and other curating institutions, no

doubt due to the increase in requests for destructive sampling received, and as greater understanding of contamination controls are recognised across different disciplines. The researcher was very careful to avoid contamination with the laboratory environment, but, despite this, occasional contamination did occur. On one occasion, a PCR product which was in fact too long, was selected for sequencing but this demonstrated contamination with human DNA. With the exception of the real time TB aDNA analysis, which demonstrated contamination across almost all samples (most likely due to modern human DNA), it appears that the vast majority of contamination was due to the penetration of soil bacteria. Soil may contain bacterial sequences which are very similar to pathogenic DNA, particularly that of TB (Pääbo *et al*., 2004, Wayne *et al*., 1999). This was noted in some sequences obtained for this study (See 5.4), where sequences similar to brucellosis strains were identified but on testing specifically for *Brucella* species, it was discovered that the sequences were most likely representative of unidentified soil bacteria. The extensive decontamination procedures employed during the laboratory clearly demonstrate the difficulties in working with archaeological bone which has been buried in a soil environment for, in this case, thousands of years – particularly when searching for pathogens which have many similar sequenced cousins found in the local soil environment.

In light of the negative results, the third point to address was not ultimately applicable to this study but, as a limitation for studies of this nature, it should be reiterated. Using a combined macroscopic and biomolecular approach of the study of ancient disease should allow for a more thorough understanding of the pathology related to a particular disease. This is not however, entirely straight forward which is, again, best illustrated with reference to the presence of rib lesions. There is a strong correlation between rib lesions and TB occurrence, noted in both historical (Roberts *et al*., 1994) and modern clinical populations (Brown, 1980). However, because rib lesions can be caused by other pulmonary infections, not just TB, the identification of TB aDNA cannot confirm that the rib lesions were as a result of pulmonary TB and, therefore, could still not be considered wholly diagnostic. In this case, it could perhaps be considered probable but not definite. Even if additional TB evidence is present on the skeleton, alongside rib lesions, it must be remembered that it is quite possible for a person to suffer from more than one disease at a time, and that presence of

pathogen specific aDNA cannot prove a link between lesions identified macroscopically, and disease identified via biomolecular methods.

The application of aDNA studies is indeed a useful development of the last three decades, but it will require continued application and development to further improve, particularly in terms of limiting the impact of contamination. It is unfortunate that data generated from this study did not identify TB in prehistoric Britain and thus allow for the study of specific strains, the question as to the incomplete history of TB in Britain therefore remains unanswered.

7.3 Further Work

7.3.1 Extension of the Project

7.3.1.1 Geographical Range

With the exception of the site of Wetwang Slack, this project focussed specifically on the south of Britain, the reasons for this, (which are discussed in more detail in 4.1.1) included the need to limit the geographical area due to time constraints and, based on evidence that one of the primary areas of contact with the Continent was across the Channel. However, the study would benefit from extension of this geographical area, particularly along the West Coast and Scotland, given the evidence for travel and contact along the western seaways in prehistory (Garrow and Sturt, 2011). The mobility witnessed throughout British prehistory, however, with migrants arriving from the continent and travelling through the country (Fitzpatrick, 2002: 177) and extensive movement of indigenous individuals themselves within Britain, (Montgomery *et al*., 2000), suggests that any incoming disease would have had the opportunity to penetrate a much wider geographical area than was studied here. Ideally, future studies would systematically review all prehistoric skeletal material throughout Britain.

7.3.1.2 Methods

As both macroscopic and aDNA techniques continually improve, these methods can be applied to future work. However, extension of this project would benefit from the application of additional biomolecular techniques, using various chemical and chromatographic methods, which would complement the aDNA work (Thi-Nguyen-Ny *et al*., 2011, Minnikin *et al*., 2010). Mycolic acids, which are contained within the waxy coat of mycobacteria such as TB, have the potential for long term survival (Gernaey *et al*., 1999). Using additional techniques, which are not all subject to many of the problems of aDNA analysis, would improve the interpretative abilities of future projects. The more techniques that can be used in conjunction with one another, the greater the reliability of results, each techniques perhaps contributing to the continual improvement of the other. In addition, the application of next-generation sequencing platforms may make future PCR analyses faster, cheaper and bypass some of the major contamination issues previous PCR techniques faced. It would be advisable in future to also limit the use of high speed drills for the extraction of bone samples, given the degree to which they can destroy aDNA and if necessary to use drills, then to use lower speeds (Adler *et al.,* 2011). If possible, freshly excavated bones should also be studied in preference to those which have been stored for long periods in museums (Pruvost *et al.,* 2007), although for the limited quantity of Neolithic, Bronze Age and Iron Age remains available, this will be difficult.

7.3.2 The Curation of Skeletal Remains

This study has clearly highlighted some of the problems associated with the study of archaeological skeletal remains. Many of the limitations outlined previously, regarding small samples, taphonomic processes or disturbance to remains prior to excavation, cannot be improved – that is the nature of archaeological work. However, the methods with which skeletal remains are dealt, from excavation through to analysis, can be addressed. As biomolecular analyses become more common, the need to limit human contamination during the excavation process is a necessity and as Brown (2000) advocates, the use of gloves for this process (amongst other precautions) must be employed by all future excavators of human remains. Although not all agree with the need for such precautions, or

at least the feasibility of them, on the off chance excavated remains will be subject to biomolecular analyses (Taylor *et al.*, 2010), in light of the growing numbers of aDNA studies, the apparent benefits of analysing freshly excavated remains and continual developments in biomolecular techniques, it would seem wise to establish at least this simple precaution on site and during subsequent handling, on the assumption that the remains will or may well be analysed in the future.

The second improvement which must be emphasised is the need for better storage of human remains. There are now specific boxes used, such as that designed by Cassman (2008: 110) or the Bowron Box (Bowron, 2003). These aim to limit further damage to archaeological bone, whilst accommodating even larger bones more comfortably, such as the femur, which is so often found wedged into inappropriately sized boxes (Caffell *et al*., 2001). It is noted, however, that these boxes are large and that many museums and archaeological services are exceptionally limited in space. If these larger boxes are not an option, there must at least be a reassessment of skeletal remains and up to date condition reports produced, as advocated by Janaway *et al.* (2001); repackaging in newer boxes and appropriate packing would, in many cases, limit further damage. These boxes must also display clear labelling.

Obviously, handling of these remains must take place in order to facilitate research but it is imperative that both curators and researchers are appropriately trained in the most appropriate handling methods of delicate bones. This includes the use of gloves at all times and handling only when necessary; the more skeletons are handled, the greater chance there is that they will suffer loss of elements and damage (Caffell *et al.*, 2001). Most curators and researchers do exercise caution with the bones they handle, but the point cannot be emphasised enough to students rising in all related fields in order to preserve these skeletal collections for future work.

Finally, the opportunity must be taken to yet again call for the creation of a centralised database of all skeletal collections in the UK (Roberts, 2009, Roberts and Cox, 2003, Mays, 2010a). Guidance issued by the Department for Culture, Media and Sport (2005) recommended that inventories of skeletal holdings be made public and freely available

(Swain, 2005). As no additional resources were provided to museums for this purpose, little progress has been made (Mays and Roberts, 2010), although The Wellcome Osteological Research Database (WORD) at the Museum of London (White, 2006) is a testament to what can be achieved when the funding is available. The creation of accessible databases by individual museums is perhaps the first step towards the aim of collated information and forming of a national database. The advantages of both are clear, although a centralised database would further enhance many of the benefits offered here:

- The creation of databases, or more detailed ones than already exist for public release, would, in many cases, require a **reassessment of collections**, the preservational benefits of which are described above.

- Research potential of collections is often inadvertently obscured through lack of detailed associated documentation, rendering some little known and poorly studied. Nationwide documentation, including information about smaller, lesser known collections may **reduce the geographical bias of collections use and the physical "use load"** on large, already well-studied collections, such as Christ Church, Spitalfields, London curated at The Natural History Museum, London and Wharram Percy, North Yorkshire, curated at English Heritage in Portsmouth, which are the two most repeatedly studied collections in the country (Mays and Roberts, 2010).

- This would also ensure that **knowledge is not limited** to just a few of the many sites available for study (Mays and Roberts, 2010), would enable more detailed information to be fed back to the curating institution, and more varied data sets being available in the academic and public spheres.

- By making information about previously unknown or little studied collections easily accessible, the result is also one of **increased value** of those collections. If remains are not being utilised for research and education, questions must be asked as to the value of retention (Roberts, 2009: 28) and this retention is under close scrutiny by interested parties (Mays, 2010b).

- Both museum specific databases and a centralised database (primarily the latter) would **Increase the efficiency** of research projects:

  Currently we are in a position where students and established staff begin a project and have to start by trying to identify where the relevant skeletal collections are (or whether they are still above the ground); this is a very time-consuming process.

  (Roberts and Cox, 2003: 402).

  Time saved by reduced queries and subsequent response requirements regarding skeletal collections to, and by, curators, respectively, would also no doubt be a welcome by-product of this system.

- A centralised database, accessible online would most certainly **encourage international researchers** to also make better use of the wide range of skeletal collections held in the UK (Roberts and Cox, 2003: 402, Mays, 2010a).

As mentioned previously, improving the future curation and research of skeletal collections lies with all of those involved in their care and use. Researchers must ensure that any information gained from collections is fed back to the curating institution and/or published, or they are further contributing to the lack of associated documentation available for many collections. However, I it is suggested that the majority of research done on skeletal remains may not, for various reasons, reach publication (Mays and Roberts, 2010). It is vital that collections of human skeletal remains, and the research carried out on them is transparent, as advocated by the DCMS guidance (Swain, 2005). This will hopefully ensure that the public and other interested parties are aware of the many benefits of research on human remains and the relevance of information that can be gained; a centralised, accessible database is the first step toward this transparency and towards better interaction and education of the public with their own history, in addition to the many benefits to researchers and the material itself, as outline above.

Whilst most agree that a centralised database would be useful, perhaps the supposed enormity of the task has at this point, dissuaded funding being granted to produce one. A

database such as this would require a great deal of time and, most importantly, consistency in its entries in order to become a useful tool. Broken into small research projects, perhaps forming part of postgraduate research topics, in which the completion of database entries became standard dissemination practice, the task may not, however, be as complicated or as arduous once thought.

7.4 Concluding Summary

The aims of this study were twofold, to both test the hypotheses that TB was present in British prehistory, prior to that previously identified in the Iron Age (Mays and Taylor, 2003) and that the infection was caused by both *M. tuberculosis* and *M. bovis*. The objective of the project was to use ancient DNA (aDNA) from human skeletons to study the bacteria responsible for TB (M. *tuberculosis* complex) in order to then study the origin and evolution of the strains of the bacteria causing TB in prehistoric Britain. A combined analytical approach, employing both macroscopic analysis of human skeletal material, and biomolecular techniques (aDNA), hoped to add a piece to this prehistoric puzzle, the benefits of such knowledge having the potential to be useful to a vast spectrum of end users (see 1.5). There is a surprising lack of evidence regarding TB in prehistoric Britain, especially given the very early evidence of TB on the continent, to which Britain has enjoyed long and extensive connections. The results of the study did not support either hypothesis, but it could not refute them either; “absence of evidence is not evidence of absence”.

The individual skeletons selected for study were done so primarily on the basis of their preservation, period, location of the site of origin and, finally, the presence of pathology possibly attributable to TB infection. With regard to preservation, consideration had to be made with respect to both ability to complete a macroscopic assessment of the material, and the potential for aDNA pathogen survival, although identifying material fulfilling the latter criterion is no easy task, as has been evident within this study. Better survival of MTBC has previously been demonstrated over that of other pathogens, but this is thought to be attributable to the bacteria’s thick, waxy cell wall (Zink *et al*., 2002, Donoghue *et al*., 2004).

The macroscopic analysis itself yielded some promising results, with convincing evidence of infection from the Neolithic periods onwards, although this was universally, non-specific in nature; this was to be expected, as no "specific" pathology had been previously reported. TB does not progress to active infection in the majority of infected individuals and, of those it *does*, only 3-5% display skeletal involvement. Therefore, the chances of identifying TB infected skeletons from the relatively few prehistoric remains excavated is limited, but not impossible. In addition, one of the primary limitations of any bioarchaeological work, is, and will continue to be, the effect of the osteological paradox, as outlined by Wood *et al* (1992); that those displaying skeletal pathology may represent individuals healthier than those without, as their immune system would have been stronger in order to survive long enough to exhibit skeletal change at death. These two factors, the limited progression to skeletal involvement, and varying immune responses of individuals, mean that skeletons not selected for macroscopic and biomolecular analysis (based on a lack of pathology known to be associated with TB, or general infection), hold virtually the same potential for harboring the TB pathogen as those that are selected. In effect, this is an "inverse selection potential" and should be borne in mind when selecting "control" skeletons; positive results from these may not be representative of contamination, but of genuine infection. This situation is inevitable and unavoidable.

Conscious of the literature calling for better technical application of DNA analysis, such as Cooper and Poinar (2000), in which they stated: 'Do it right or Not At All' and of Roberts and Ingham's (2008) survey of aDNA literature which highlighted some of the problems with previously published papers (regarding the lack of methodological information provided), laboratory conditions and protocol were followed in this study as far as the material allowed, and step by step methods were included, as advocated by best practice studies. That these were not enough to remove all contamination (or identify any evidence of TB) is, arguably, more a reflection of the preservation of the material than of the methods employed.

The biocultural approach employed in this study enabled a better understanding of the social, economic and environmental conditions to which the individuals in the study would

have been subject. An in-depth study of the Neolithic, Bronze and Iron Ages, which, together, constitute around 4000 years of British history, was simply not possible within the confines of this study, but an overview of factors pertaining to TB susceptibility and transmission was offered, with a view to assessing the likelihood of TB being able to establish itself in Britain, if introduced. The review clearly demonstrates a dynamic British landscape from at least the Neolithic onwards, with the advent of agriculture, population growth and contact with the continent already occurring. Population growth, contact, mobility and hierarchy all continued to develop over time, with an increasing intensity of agriculture, despite some rather detrimental climatic changes, certainly towards the end of the Bronze Age. These factors have been demonstrably associated with reduced health in living and archaeological populations and, as a consequence, increased susceptibility and/or exposure to TB can occur. That evidence of TB has been found in the Iron Age, despite all the methodological factors weighted against it, is surely suggestive of an earlier occurrence of the disease, unless the very first case of TB in Britain is really that which has been excavated, which does of course remain a possibility.

The question as to whether TB has a history (in Britain) spanning back to the Neolithic or Bronze Ages is yet to be settled; it is hoped this project will encourage further research into this question and others. Gaps in current knowledge have been highlighted, some skeletal material not examined since its excavation has been included, and aDNA analysis has been applied using advised standards as far as possible, with the negative results and methodology available for consultation by future researchers, in an effort to continually improve the technique. This study would certainly benefit from wider application, to incorporate the areas of Britain not included in this study, due to time constraints. Additional biomolecular methods should also be used in conjunction with those applied here, to maximise the efficiency of techniques which require destructive sampling, and to aid interpretation of results within the project.

As an additional element, this study has also highlighted the continual problems faced by researchers, in terms of inefficient location of skeletal remains due to the lack of a national database, and of current curation standards of human skeletal material. These are issues which must be rectified both to improve efficiency, and encourage better preservation of

skeletal material, both its structural integrity and preserving access to it, as an educational and research resource.

TB may or may not have been present in prehistoric Britain, either in latent or active form, prior to the Iron Age. On the basis of the information explored and presented here, the researcher feels that an argument can be made for it being “quite possible” in the Neolithic, especially in light of the population increase and increasing sedentary lifestyle, and “highly probable” during the Bronze Age, when well-travelled individuals from deep within the continent, were making use of the extensive trade routes and settling in Britain. If not present in these periods, the author would still suggest that the Iron Age example of TB so far identified, is unlikely to be the only case in Britain at that time, but that archaeological and methodological limitations have, to this point, caused the evidence to remain elusive, and that it is only a matter of time before the evidence is identified.

This project, focussing on ancient Britain, aimed to add to what is currently a sparsely documented history of the disease in this country. Whilst TB is currently a global pandemic, has it always been so geographically extensive? Other questions which may have benefited from positive aDNA data include TB’s effect on the body; has it always manifested itself in the same way, caused the same symptoms and looked the same at a molecular level? While a great deal of work has been undertaken in the last few years to answer the last question, both proving an evolution of the disease geographically and disproving some commonly held theories as to which strains of the disease developed first, questions as to its origins in Britain, remain unanswered.

Despite promising data of falling incidence from the most recent WHO reports, TB remains a global health crisis. The need, therefore, to add to what is known of the pathogens evolution, is critical. It can only be hoped that more data will allow further insight to the future evolutionary paths the disease may take and, in doing so, will give humans an advantage in the global fight against the disease, with the ultimate aim of complete eradication. Perhaps Mercer’s optimism will finally be applicable in a few generations time: “But at long last the disease is being wiped out…to see the enemy in retreat is one of the rare and most exhilarating experiences reserved for our generation.” (Mercer, 1964: 253).

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APPENDIX

APPENDIX 1:

Last Dying Speech and Confession of
BACILLUS TUBERCULOSIS, Esq.,
Late of Belfast,

With a Full History of his many Crimes and Murders, and his most Edifying End.

'Twas a weariful bacillus, old and faded, worn and gray,
Who, drying on the coverslip, spake thin and far away:
"Proud mortal, ere my form you steep in carbol fuchsin stain,
Ere I bathe in acid alcohol again and yet again,
I would fain recount my story to your sympathetic ear;
But wet my lips with saline, for the Bunsen flame's too near.
I was once a gay young microbe, and I floated round the town,
Wrapped up in well-dried mucus, light as the thistle down.
My race was old and mighty; Koch made us known to fame,
For the Tubercle Bacillus is my far-renowned name.
In the heyday of my vigour, when the world and I were young,
My aims were high — I sought and found the apex of the lung.
With a chemotactic longing leucocytes came flocking round,
We dallied fondly till we changed th' expiratory sound,
So Professor Lindsay spotted me, and ordered me to quit —
I find fresh air unhealthy, I thought it best to flit.
More cautious now, I sought to rest embraced by giant cells,
Within a deep cervical gland that near the phrenic dwells.
Unhappy choice! for Surgeon Kirk removed me all complete,
With half a foot of jugular and half a pound of meat.
My bonds with man, so rudely torn, gave all my faiths a shock,
I doubted — 'Am I human? p'raps I ought to try the flock;
I may be bovine after all; since man evicts me still
I'll look for compensation under Mr. Birrell's Bill'.
So I found a country dairy, just back of Grosvenor Street;
A friendly stripper took me in and lodged me in her teat.
Here, amid rustic sights and smells, I ruralised a space,
Then borne upon a stream of milk rejoined the human race.
Snug in a mesenteric nook I soon addressed my mind,
By fission's simple easy arts, to propagate my kind.
Over the serous surfaces quick spread my hardy brood;
Ascitic fluid came in floods — we found it very good.
But Thomas, prince of opsonists, by fell mischance came nigh:
He took the index of our host, and found it very high.
Treatment on scientific lines we heard him then discuss —
Tuberculin, one miligramme, soon decimated us!
Fleeing the slaughter of my tribe, my powers now rather weak,
With hearty zest I made a nest upon a damask cheek.
There, in an apple-jelly speck, I'd hoped to end my life,
But X-Rays pierced me to the quick — I left th' unequal strife.

Since then I've wandered round Belfast, but find the world grown hard,
Man's bowels yearn no more for me, and bovine breasts are barred.
A band, with demonstrating ways and eloquence profound,
'Gainst me the people's passions raise, and loud the tocsin sound.
Chief instigator of the fray, Sir John — 'No quarter' — cries,
When knights were bold they fought with things — well, nearer their own size!
A surgeon, too, a vet. as well, physicians add their breath,
And gents from sanatoria, where we are fed to death,
And several more who show my crimes, while all the people stare;
Though many a fee they've got for me — they'll get no more, I swear!
For now those oft-respired airs, in which a microbe blooms,
Are blown to Hades by the breeze denouncing 'Stuffy Rooms'.
They've cleared away the dust, in which I used to lurk and hope;
They hear 'What other nations do' — the Dutch are fond of soap.
I lived with darling children once, in tissues soft as silk;
I simply can't get near them now — they sterilise the milk!
Aye, worse than that — excuse the tear of pity in my eye —
The poor milch cows that harbour us, for that offence must die.
The very things I most detest I strive in vain to flee,
All round it's sunlight, food, fresh air, to kill 'the Scourge' — that's me.
Why, many good, hard-drinking souls are sorely put about —
They're going to dock the beer, because I like men fond of stout.
At peril oft before I scoffed, I've managed to outpace
The dreaded phagocyte's pursuit, his fatal slow embrace;
I've laughed to scorn iodoform, and once — 'twas rather warm —
Passed through a disinfector, hid in blankets, without harm!
But at the fate we've met of late imagination swoons —
Frizzling to death by millions in combustible spittoons!
Well, when assailed by these alarms I had begun to quake,
Professor Symmers welcomed me for old acquaintance sake.
Said he — 'Can I believe my eyes, and have we met at last,
Sole Tubercle Bacillus left alive in all Belfast?
Nay, come; I'll gladly take thee in, and gladly give thee place
Upon this spacious agar slope, last scion of thy race.
With glucose will I nourish thee, and human serum too,
And thou shalt grow apace, and I will put thee oft on view —
The parasite that lived and throve — believe it now who can —
In pre-Exhibition ages on pre-Exhibition man'.'

The murmur ceased, the microbe passed; the relics are on view —
A crimson speck, in balsam, on a ground of methyl blue.

For many years the author of these lines was unknown, but the text was found with the papers of Professor Sir Robert Johnstone after his death, and there is good evidence that he was the writer.

Ulster Med J. 1989; 58(Suppl): 52–56.

APPENDIX 2:

Curatorial / Source Information for Sites Included in the Study:

SITE:	CURRENTLY CURATED AT:	PRIMARY REFERENCE(S) FOR SKELETAL MATERIAL:
Abingdon Pipeline, Oxfordshire	Oxford Museums Resource Centre	Cotswold Archaeology 2003
Methwold Severalls, Norfolk	Norwich City Museum	Curtis 1968 McKinley 1988
West Tump Long Barrow, Gloucestershire	Cheltenham Art Gallery and Museum	Witts 1881 Smith and Brickley 2004
Wilsford Barrow, Normanton, Wiltshire	The Duckworth Laboratory, Cambridge	WAM, 1961
Twyford Down, Hampshire	Hampshire Archaeology	McKinley, 2000
Waterhall Farm, Chippenham, Cambridgeshire	The Duckworth Laboratory, Cambridge	Martin, 1976 Denston 1976
Fire Hydrant: Boscombe Airfield, Wiltshire	Wessex Archaeology	Manning *et al*. 2010
Ascott-under-Wychwood, Oxfordshire	Natural History Museum	Galer, 2007
Eyebury, Peterborough	Natural History Museum	Leeds 1912
Bourton-on-the-Water, Gloucestershire	Gloucester County Archaeology Service	Roberts 2000
Wetwang Slack, North Yorkshire	Div. of Archaeological Sciences, University of Bradford	Dent, 1985 King 2010

APPENDIX 3:

Recording Forms Used for Macroscopic Analysis

ADULT:

Code:

SKELETAL RECORDING FORM (Adult)

Institution:

Observer: ..... Date: .....

Site Name: ..... Site Code: .....

Skeleton Number:..... General Condition: .....

SEX ESTIMATION:

Pelvis: *Buikstra and Ubelaker 1994*	**L**	**R**	**Skull:** *Buikstra and Ubelaker 1994*	**L**	**R**
Ventral Arc (1-3)	.....	.....	Nuchal Crest (1-5)	.....	.....
Subpubic Concavity (1-3)	.....	.....	Mastoid Process (1-5)	.....	.....
Ischiopubic Ramus Ridge (1-3)	.....	.....	Supraorbital Margin (1-5)	.....	.....
Greater Sciatic Notch (1-5)	.....	.....	Glabella (1-5)	.....	.....
			Mandible (1-5)	.....	.....
			(Brickley and McKinley 2004)		
Estimated Sex, Pelvis (0-5)	.....		Estimated Sex, Skull (0-5)	.....	

AGE ESTIMATION:

Skeletal Region	Observations	Phase/Stage	Inference
Medial Clavicle *Scheuer and Black 2004*			
Sacrum *Scheuer and Black 2004*			
Pubic Symphysis *Suchey-Brooks 1990*			
Auricular Surface *Lovejoy et al 1985*			
Sternal End of Ribs *Iscan and Loth 1984*			
Dental Attrition *Brothwell 1981*			

Overall Estimated Age:

Stature:

TiL1 (mm) :

FeL1 (mm) :

COMMENTS:

.....

.....

.....

Code:

PATHOLOGY:

Note: Shape / Size / Bone Loss / Bone Formation etc.

No.	Bone	Position / Side	Notes
1			
2			
3			
4			
5			
6			
7			
8			

GENERAL COMMENTS:

.....

.....

.....

CORRESPONDING PHOTOGRAPH CODES:

1.	2.	3.	4.	5.	6.
7.	8.	+	+	+	+

ADULT:

Code:

PRESENCE / ABSENCE:

Cranial Bones and Joint Surfaces								
	L	**R**		**P/A**	**Vertebra**	**P/A**	**Vertebra**	**P/A**
Parietal			Frontal		C1		T6	
Temporal			Occipital		C2		T7	
Maxilla			Spenoid		C3		T8	
Nasal			Vomer		C4		T9	
Zygomatic			Ethmoid		C5		T10	
Lacrimal			Hyoid		C6		T11	
Palatine			Cricold		C7		T12	
Mandible			Thyroid		T1		L1	
Orbit					T2		L2	
					T3		L3	
RIBS: **Left:** ☐ **Right:** ☐					T4		L4	
					T5		L5	

RIGHT						**LEFT**					
	P J.S.	**P 1/3**	**Mid 1/3**	**D 1/3**	**D J.S.**		**P J.S.**	**P 1/3**	**Mid 1/3**	**D 1/3**	**D J.S.**
Humerus						Humerus					
Radius						Radius					
Ulna						Ulna					
Femur						Femur					
Tibia						Tibia					
Fibula						Fibula					

RIGHT						**LEFT**					
	> 75%	**75-50**	**50-25**	**<25%**			**> 75%**	**75-50**	**50-25**	**<25%**	
Ilium						Ilium					
Ischium						Ischium					
Pubis						Pubis					
Scapula						Scapula					
Clavicle						Clavicle					
Patella						Patella					

Bone	**> 75%**	**75-50**	**50-25**	**<25%**	**NOTES:**
Sternum					
Coccyx					
Sacrum					

RIGHT	1	2	3	4	5	LEFT	1	2	3	4	5
Metacarpals						Metacarpals					
Metatarsals						Metatarsals					

Hand:	Proximal phalanges ☐	Middle phalanges ☐	Distal phalanges ☐
Foot:	Proximal phalanges ☐	Middle phalanges ☐	Distal phalanges ☐

	Scaphoid	Lunate	Triquetral	Pisiform	Trapezium	Trapezoid	Capitate	Hamate	Sesmoid
Right									
Left									
	Talus	Calcaneus	1st Cun	2nd Cun	3rd Cun	Navicular	Cuboid		Sesmoid
Right									
Left									

Dental Notes:

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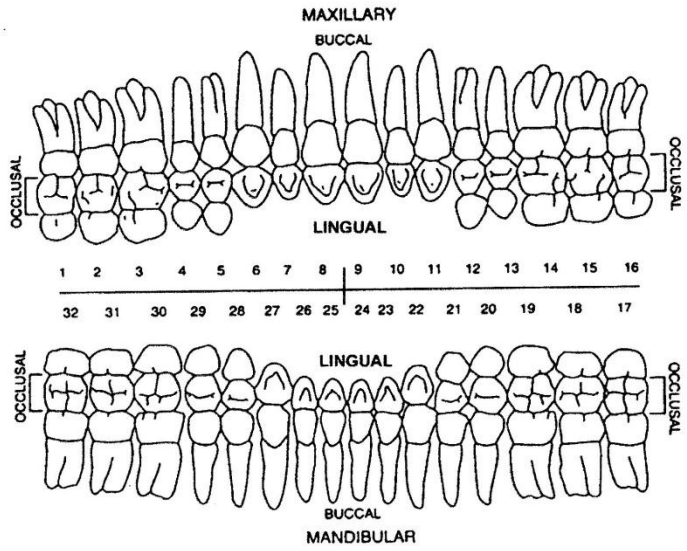

Code:

SKELETAL RECORDING FORM (Immature)

Institution:

Observer: …… Date: ……..

Site Name: ……..…….. Site Code: ……

Skeleton Number:…….. General Condition: ……..……

AGE ESTIMATION **(Foetal / b – 5 / 5 – 10 / 10 – 15 / 15 – 20 / 20 +)**

Stage of Union: blank = unobservable; 0 = open; 1 = partial union; 2 = complete union

Epiphyseal Fusion

Bone	Epiphysis	S.O.U.
Cervical Vertebrae	Superior	….....
	Inferior	……..
Thoracic Vertebrae	Superior	……..
	Inferior	……..
Lumbar Vertebrae	Superior	……..
	Inferior	……..

		L	R
Scapula	Coracoid	.....	.....
	Acromion	.....	.....
Clavicle	Sternal	.....	.....
Humerus	Head	.....	.....
	Distal	.....	.....
	M. epicondyle	.....	.....
Radius	Proximal	.....	.....
	Distal	.....	.....
Ulna	Proximal	.....	.....
	Distal	.....	.....
Os Coxae	Iliac Crest	.....	.....
	Ischial tub.	.....	.....
Femur	Head	.....	.....
	G. Troch.	.....	.....
	L. Troch.	.....	.....
	Distal	.....	.....
Tibia	Proximal	.....	.....
	Distal	.....	.....

Primary Ossification Centres

Bone	A.O.U	Extent
Os Coxae	Ilium-pubis	..……
	Ischium-pubis	..……
	Ischium-ilium	..……
Sacral Segments	1-2	..……
	2-3	..……
	3-4	..……
	4-5	..……

Cervical Vertebrae	
neural arches to each other	…….
neural arches to centrum	…….
Thoracic Vertebrae	
neural arches to each other	……
neural arches to centrum	……
Lumbar Vertebrae	
neural arches to each other	……
neural arches to centrum	……
Cranium	
spheno-occipital synchondrosis	…..
Occipital	
lateral part to squama	…..
basilar part to lateral part	…..

Fibula			
	Proximal	…..	…..
	Distal	…..	…..

IMMATURE:

Long Bone Length (mm):
Ubelaker, 1989

Bone	Length	Estimated Age	Bone	Length	Estimated Age
Humerus: (HuL1)	.....	.....	Tibia: (TiL1)	.....	.....
Radius: (RaL1)	.....	.....	Fibula: (FiL1)	.....	.....
Ulna: (UlL1)	.....	.....	Ilium:	.....	.....
Femur: (FeL1)	.....	.....			

Dental Eruption .....
Ubelaker, 1989

Overall Age Estimation: [ ]

Notes:

IMMATURE:

Code:

PRESENCE / ABSENCE:								
Cranial Bones and Joint Surfaces								
	L	**R**		**P/A**		**# Bodies**	**# R Arches**	**# L Arches**
Parietal			Frontal		Cervical			
Temporal			Occipital		Thoracic			
Maxilla			P. Basillaris		Lumbar			
Nasal			Ethmoid		Sacrum			
Zygomatic			Spenoid					
Lacrimal			Fontanelle		Rib			
Palatine			Hyoid		Sternum	# Sternebrae =		
Mandible			Atlas					
P. Lateralis			Axis					

RIGHT						**LEFT**					
	P Epip.	**P 1/3**	**Mid 1/3**	**D 1/3**	**D Epip.**		**P Epip.**	**P 1/3**	**Mid 1/3**	**D 1/3**	**D Epip.**
Humerus						Humerus					
Radius						Radius					
Ulna						Ulna					
Femur						Femur					
Tibia						Tibia					
Fibula						Fibula					

RIGHT						**LEFT**					
	> 75%	**75-50**	**50-25**	**<25%**			**> 75%**	**75-50**	**50-25**	**<25%**	
Ilium						Ilium					
Ischium						Ischium					
Pubis						Pubis					
Scapula						Scapula					
Clavicle						Clavicle					
Patella						Patella					

	# Present		**# Present**
Metacarpals		Carpals	
Metatarsals		Tarsals	
Hand Phalanges		Foot Phalanges	

Other Unfused Bone Elements Present:

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Dental Notes:

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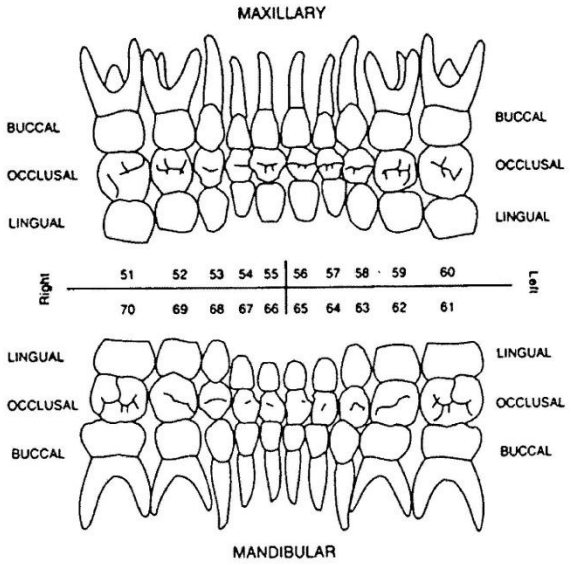


PATHOLOGY:

Note: Shape / Size / Bone Loss / Bone Formation etc.

No.	Bone	Position / Side	Notes
1			
2			
3			
4			

CORRESPONDING PHOTOGRAPH CODES:

1.	2.	3.	4.	5.	6.
+	+	+	+	+	+

APPENDIX 4:

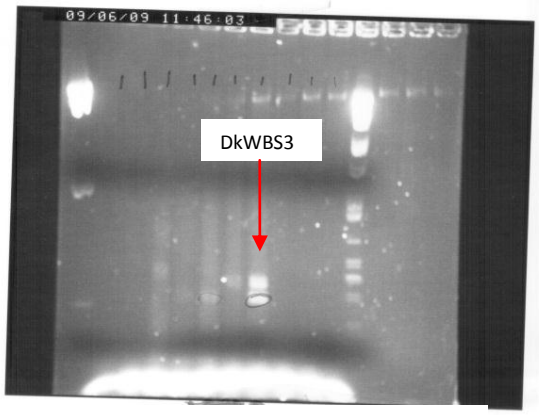
Gel Electrophoresis Runs:

Tuberculosis – 1st Round:
IS*6110* Outer
IS*6110* Inner
IS*1081* Outer
IS*1081* Inner
NHM: IS*6110* Outer / IS*6110* Inner / IS*1081* Outer / IS*1081* Inner

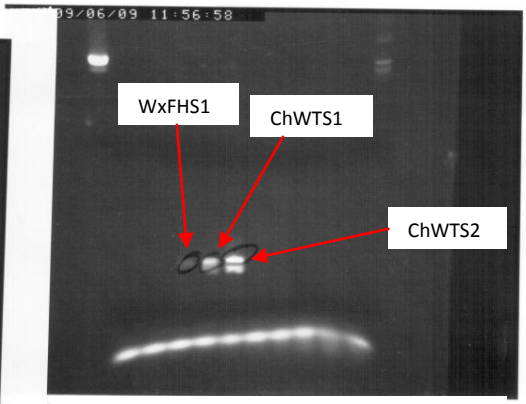
Tuberculosis - Repeats:
IS*6110* Outer
IS*6110* Inner
IS*1081* Outer
IS*1081* Inner

Brucellosis:
IS*711* Outer

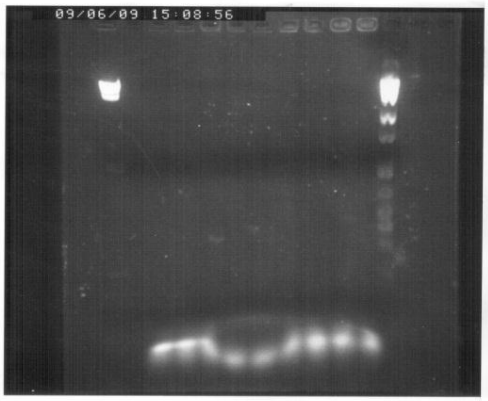
IS*6110* Outer – 1st Round



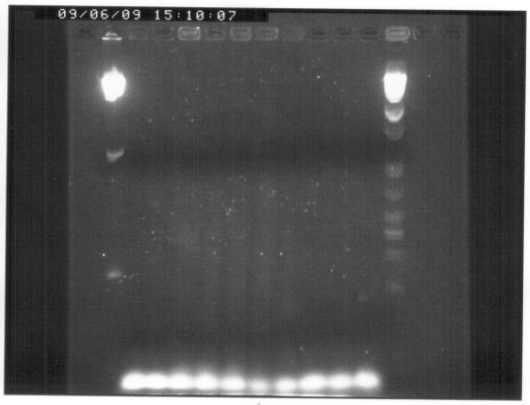
Group 1



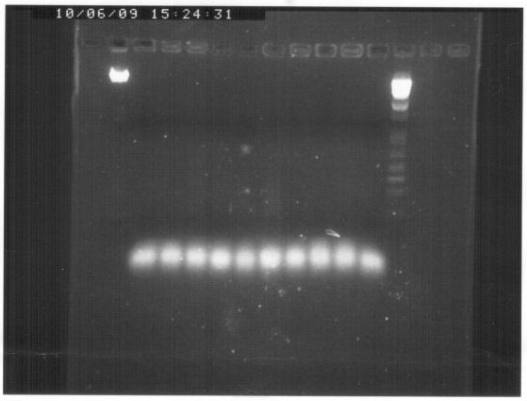
Group 2



Group 3



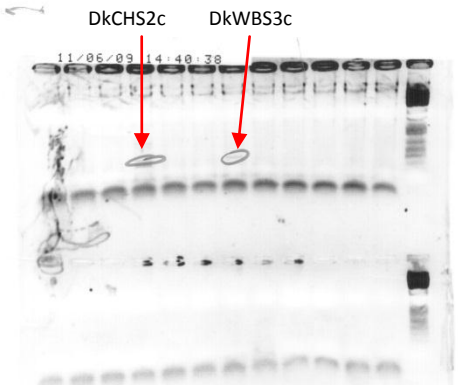
Group 4



1st Round

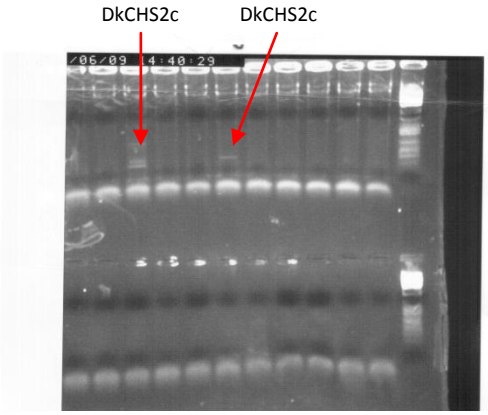
Group 5

IS*6110* Inner – 1st Round



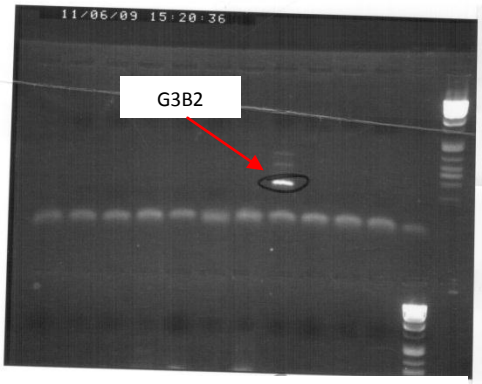
Group 1

Group 2



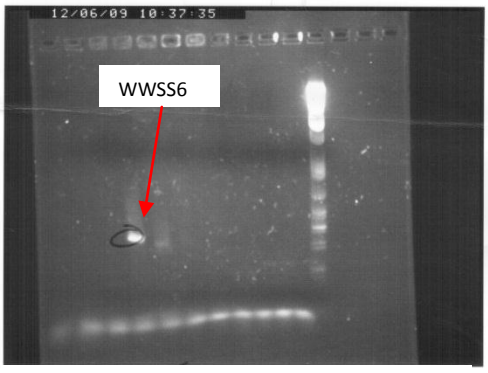
Group 1

Group 2



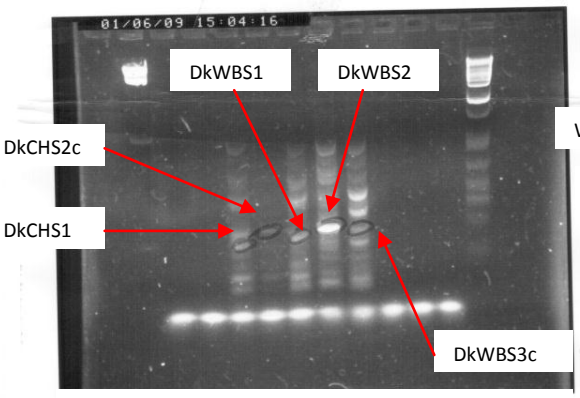
Group 3

Group 4

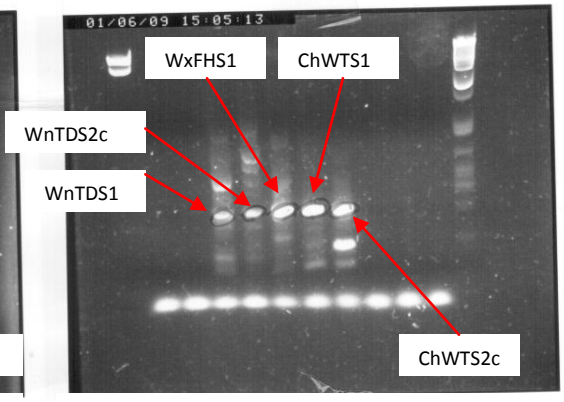


Group 5

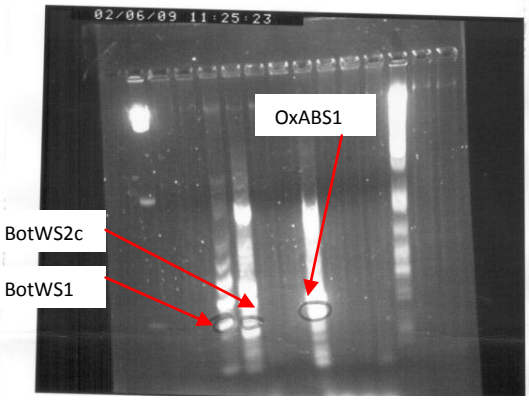
IS*1081* Outer – 1st Round



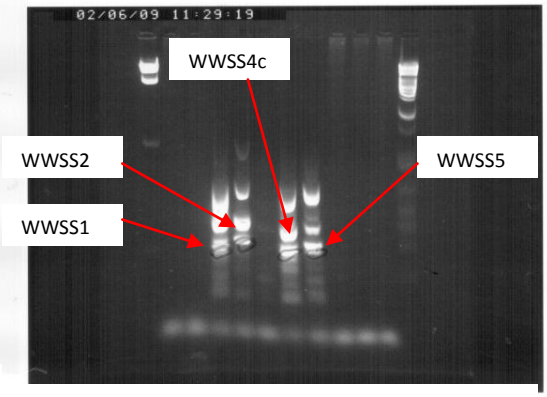
Group 1



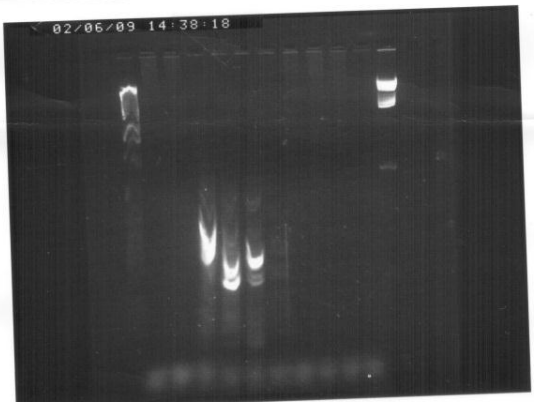
Group 2



Group 3

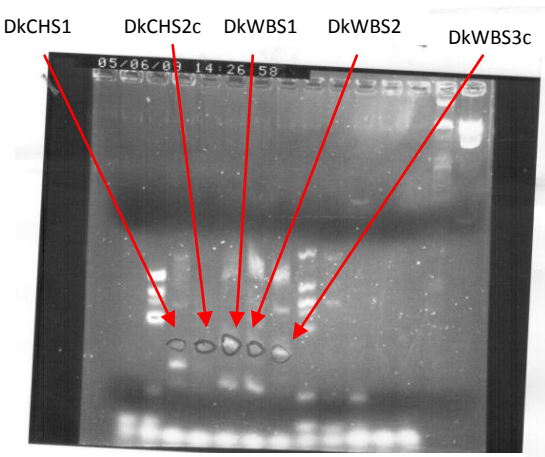


Group 4

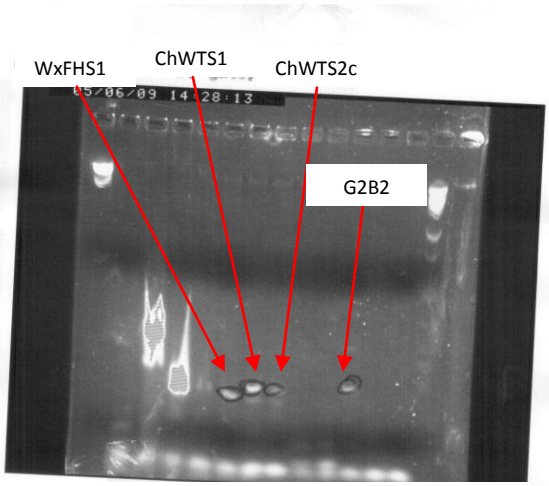


Group 5

IS*1081* Inner – 1st Round



Group 1



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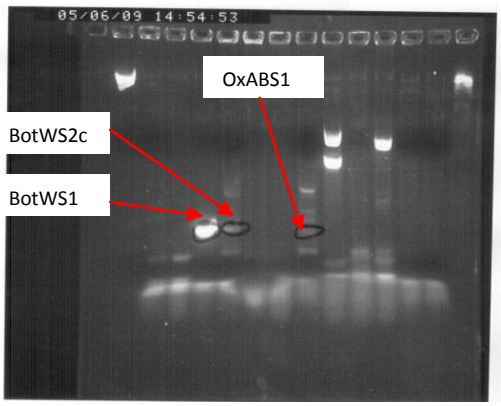
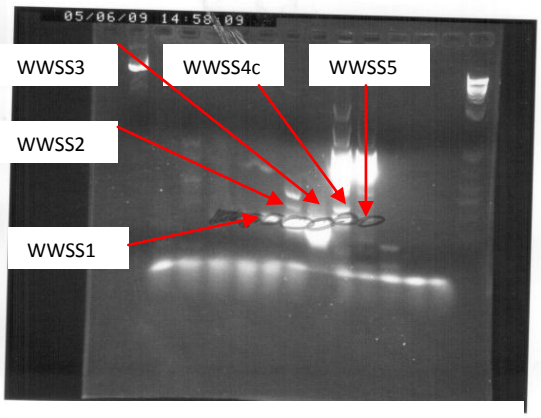
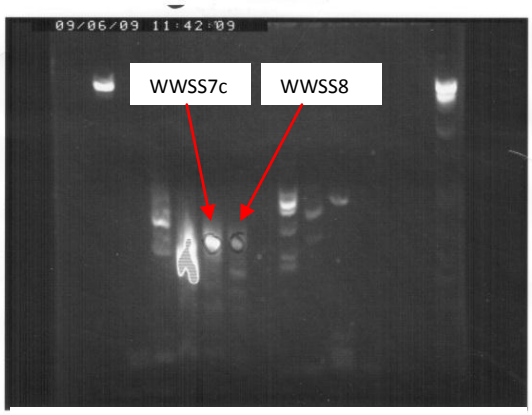


Group 3

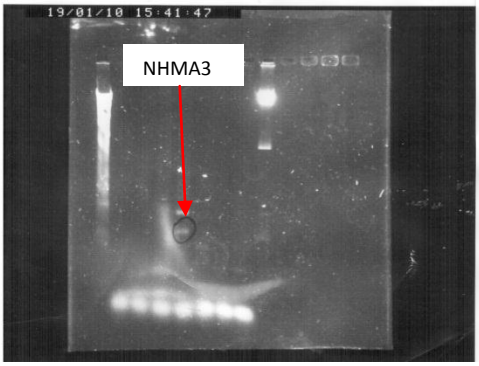


Group 4

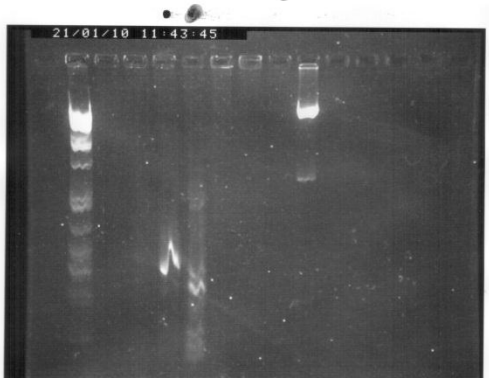


Group 5

NHM: IS*6110* Outer / IS*6110* Inner / IS*1081* Outer / IS*1081* Inner (1st Round)



NHM IS*6110* Outer

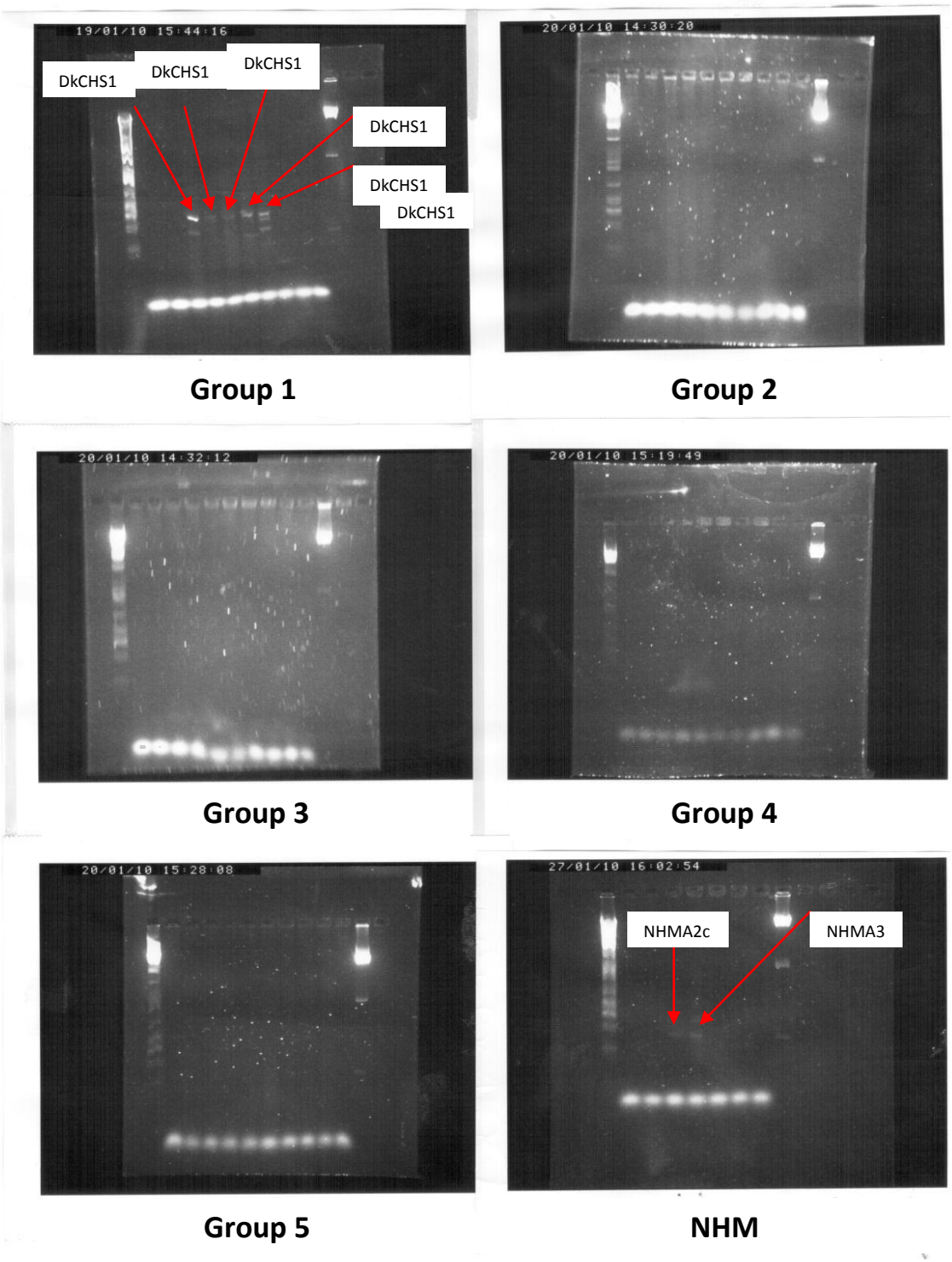


NHM IS*1081* Outer

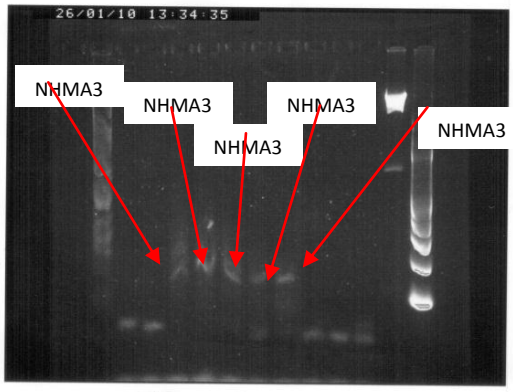
NHM IS*6110* Inner

NHM IS*1081* Inner

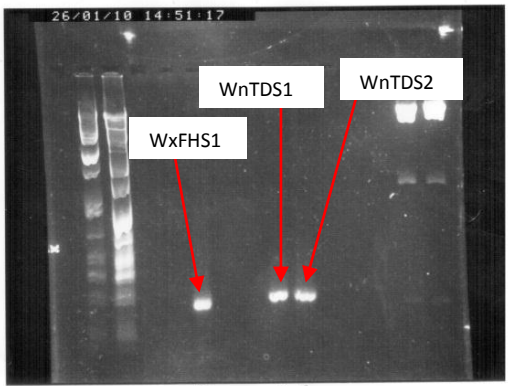
IS*6110* Outer - Repeats



IS*6110* Inner – Repeats



Group 1

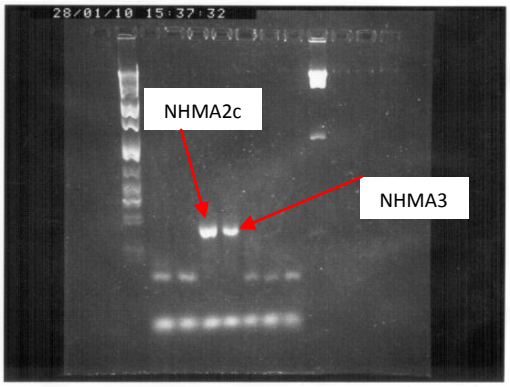


Group 2

Group 3

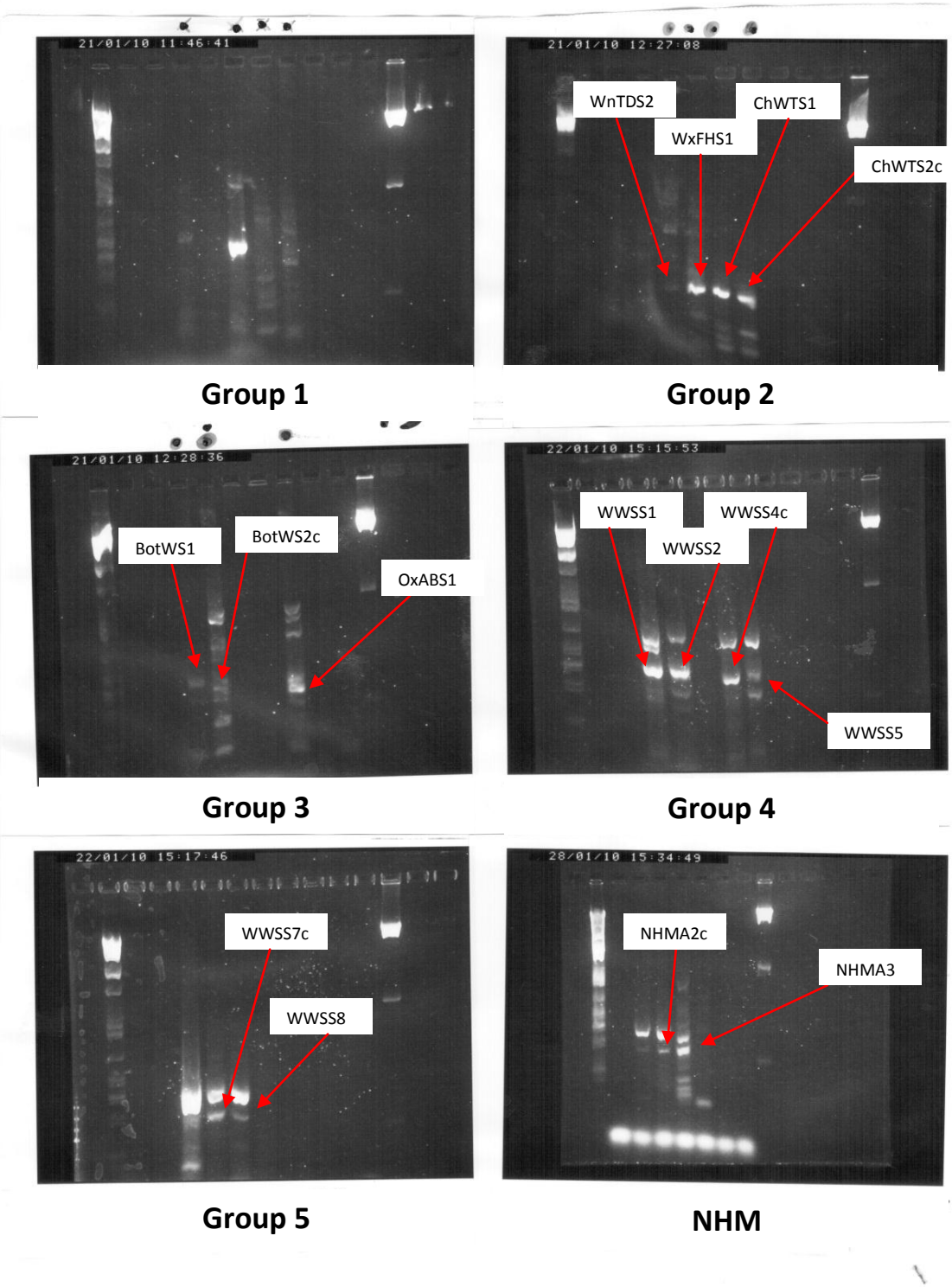
Group 4

Group 5



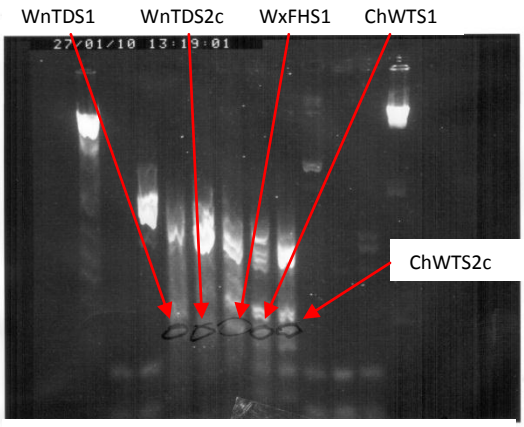
NHM

IS*1081* Outer – Repeats



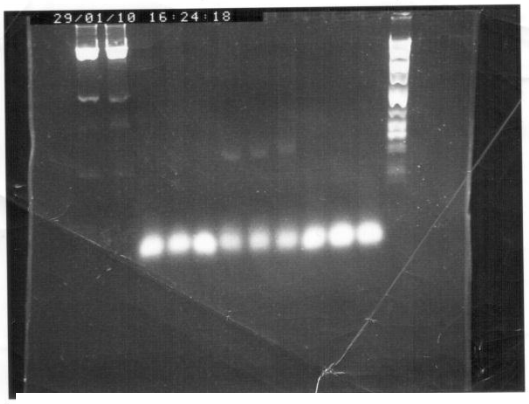
IS*1081* Inner - Repeats

Group 1

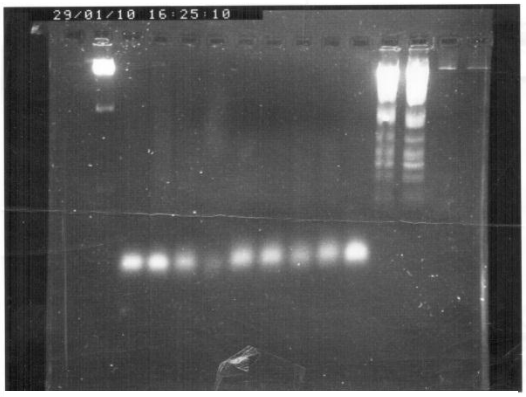


Group 2

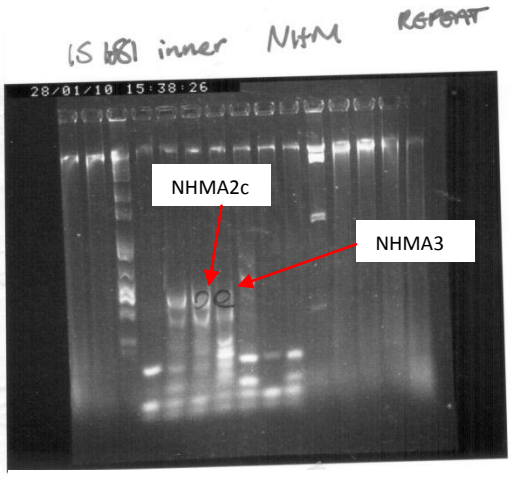
Group 3



Group 4

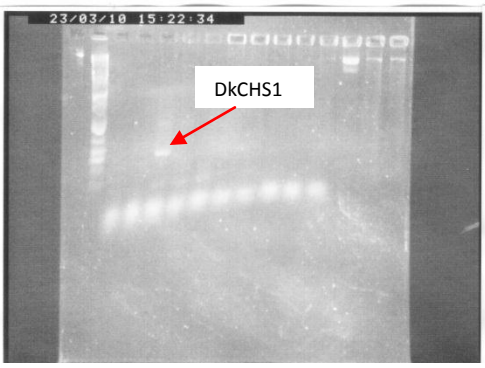


Group 5



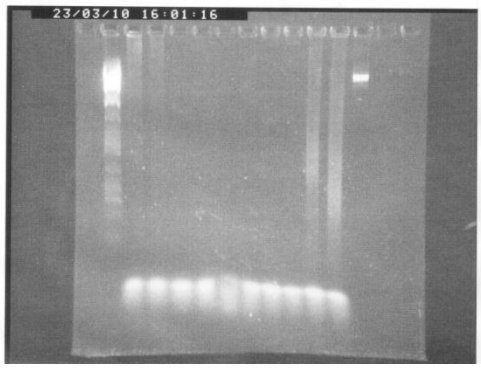
NHM

IS*711* Outer

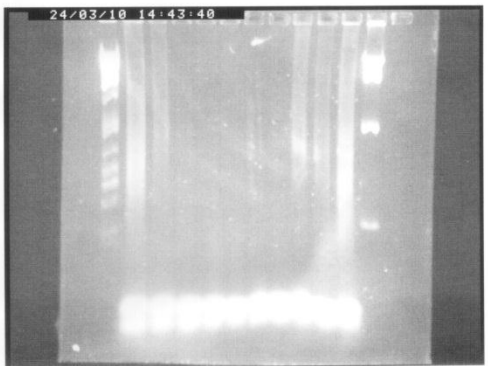


Group 1

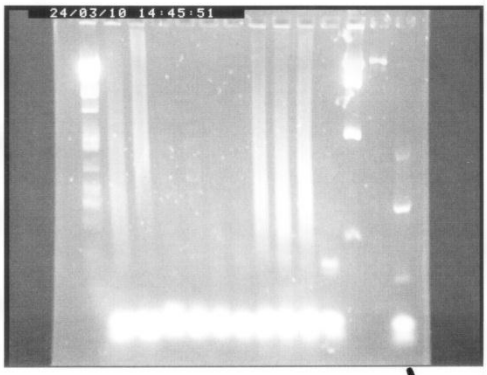
Group 2



Group 3



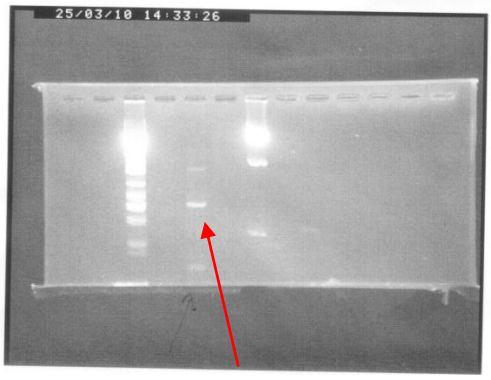
Group 4



Group 5

NHM

G1 DkCHS



Group 1: DkCHS1 (Repeat)

APPENDIX 5:

SEQUENCE DATA

Corrected Sequences for 1st Round MTB Assays

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50
IS6110          cctgcgagcg taggcgTCGG tgacaaaggc cacgtaGGCG AACCCT~GCC
CHS2c I/F       CTTCTGCT~c tGGCTGGnGG CCCCCAnCAA nTTCACCnGT CCCACn~GCT
CHs2c I/R       NGGAGGAATC CTGGGCNGAC TNCNNNTCAG CANTTCCAAN TNNCNN~NCN
G3B2 I/F        CTTCTGCTCc tGGCTGGTGG cccccaccaA GTTCacCTGT CccAcG~GCT
G3B2 I/R        nCAnCnGGNG AAAnGGnnAT nTGnCnCAAn nCGATtnAnA nGGGnA~AnG
IS1081          ctgctctcga cgttcagcgc cgCCTTGATG GGGGCTGAAG CCGACG~CCC
WWSs2 I/F       ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~cN
WWSs3 I/F       ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~cg agcacgGCCG
WWSs3 I/R       ctnctctcgg aCGTTCATCG CCGCGTTGAT GTCGGCCGAG GCCGACGCCG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                         60         70         80         90        100
IS6110          CAGGTCGACA CATAGGTGAG GTCTGCTACC CACAGCCGGT Taggtgctgg
CHs2c I/F       TCCAnacann nnnnnnnAnG AGAAnAAGAA nAAGCTCCCA ACACCAGcTG
CHs2c I/R       NNNNAGGCGA NCTCTATTGG CGGGGACAAT AGNCACGGAA GATTCNGGGG
G3B2  I/F       TCcAGAcaNg aNNNNNNAGA AGAAGAAnAA n~~~CTCCCA ACACCAGCTG
G3B2  I/R       nCAGGGTnTT CCCAGnCAnG nnnTTGTnAn nnGAnnnCAc attnaaCACn
IS1081          TGTGCGGGGC GGGCTACCGC GAACGCAGCG ATGAGCGGTC CAATCAgcgc
WWSs2 I/F       tcNGcNGCGC GCCCTA~CGG AGTGGcAGCC CGGATCGGGT GAACGTGcGc
WWSs3 I/F       TCTGCGGCGC GCCCTACGGA GTGGCCAGCC CGGATCGGGT GAACGTGCgC
WWSs3 I/R       TCTGCGGCGC GCCcTacGga g......... .......... ..........

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        110        120        130        140        150
IS6110          tggtCCGAAg cggcgctgga cgag...... .......... ..........
CHs2c I/F       GAnnTCCCGT GGnAACGnAG AAAGCTGCCn CTGnGATCAA GCCCAGTGTC
CHs2c I/R       GGCACCNGCC CGNNNNTATT TTNTGAGGNN GANTCGCGAG GTGCNANANA
G3B2  I/F       GAnNTCcCgN GGGAACGGAG AAAGCTGCCC CTGGGATCAA GCCCANTGTC
G3B2  I/R       nnnAnGAnTC AnnAnnGGGA GAATnCnGnn CCGGGnnCnC CCCCnnnGTC
IS1081          aacggctacc gccaCcgtga tttcgacacc cgtgccgcaa c.........
WWSs2 I/F       A~NNNNtAcN GcCA...... .......... .......... ..........
WWSs3 I/F       aaCgGCTACC GCCa...... .......... .......... ..........
WWSs3 I/R       .......... .......... .......... .......... ..........

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        160        170        180        190        200
IS6110          .......... .......... .......... .......... ..........
CHs2c I/F       CGAAAGCCnA TTCaNATCAA TANnTCC... .......... ..........
CHs2c I/R       GGTGCNTNGT NCAGTNTCTA AAGGAATTNC GCAGCCCCTG GCGCAGGTGC
G3B2  I/F       CGAAAGCCCA TNCnnATCAn nAAgnC.... .......... ..........
G3B2  I/R       GACGGAaAnA AtAANGnGnn CAcgAAnTCC tGCnGCCCnn nnNNTACNNN
IS1081          .......... .......... .......... .......... ..........
WWSs2 I/F       .......... .......... .......... .......... ..........
WWSs3 I/F       .......... .......... .......... .......... ..........
WWSs3 I/R       .......... .......... .......... .......... ..........

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        210        220        230        240        250
```

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IS6110        .......... .......... .......... .......... ..........
CHs2c I/F     .......... .......... .......... .......... ..........
CHs2c I/R     ANGCCCTACC GCACCTGCCA CNCCANCCCT CCTTATANAT GACTTNNGGT
G3B2  I/F     .......... .......... .......... .......... ..........
G3B2  I/R     NnTCnnAnCG GCCGCCnCCn CnGNGGNNNN CnAGNCNTNT TCCCT.....
IS1081        .......... .......... .......... .......... ..........
WWSs2 I/F     .......... .......... .......... .......... ..........
WWSs3 I/F     .......... .......... .......... .......... ..........
WWSs3 I/R     .......... .......... .......... .......... ..........


              ....|....| ....|....
                     260
IS6110        .......... .........
CHs2c I/F     .......... .........
CHs2c I/R     CAGCCTGCCA GNAAGGAAN
G3B2  I/F      .......... .........
G3B2  I/R      .......... .........
IS1081        .......... .........
WWSs2 I/F     .......... .........
WWSs3 I/F     .......... .........
WWSs3 I/R     .......... .........
```

Corrected Sequences for Repeat MTB Assays:

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                ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50
IS6110          cctgcgagcg taggcgTCGG tgacaaaggc cacgtaGGCG AACCCT~GCC
DkWBS2 I/R      CCCANCATTC GAGCTCGGTA CCCGGGGATC CCANTGATGC NCATCAAggG
DkCHs1 I/R      CCGTCCAGGc TCCAGAAAGt TTCCTGCAGG ATATTGTGGA TCCAGAATTC
IS1081          ~ctgctctcg acgttcagcg ccgCCTTGAT GGGGGCTGAA GCCGACG~CC
ChWtS1 I/F      ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~GCGTCAGAA CTGCAATGAC
ChWTS1 I/R      TCTGCTCTCG ACGTTCATCG CCGTGCGCCA GCGGAAGATG TAGTGGCTGA
ChWTS2c O/F     ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~GCGTCNGAA CTGCAATGAC
ChWTS2c O/R     TCTGCTCTCG ACGTTCATCG CCGTGCGCCA GCGGAAGATG TAGTGGCTGA
WxFHS1 O/F      ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~GCGTCAGAA CTGCAATGAC
WxFHS1 O/R      TCTGCTCTCG ACGTTCATCG CCGTGCGCCA GCGGAAGATG TAGTGGCTGA
OxABS1 O/F      ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~C CGCGTCAGAC TGCAATGACG
WWSs2  O/F      ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~GCGTCAGAc TGCAATGACG
WWSS2  O/R      ~CTGCTCTCG ACGTTCATCG CCGTGCGCCA GCGGAAGATG TAGTGGCTGa
WWSS5  O/F      ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ TGGCTGANGa AaAcCGCGTC
WWSS7c O/R      TCTGCTCTCG ACGTTCAtcG CCGTGCGCCA GCGGAAGATG TAGTGGCTGA
WWSS8  O/F      ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~GNC TGAcGAAACC GCNgaCNGAA
NHMA2C I/R      AAAACnCCCC CCCCCATGCT TACAAGCAAG TACAGCAATC AACCCTCAAG
NHMA2C I/F      ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ GaCgAGAAGG GAtTTCGACT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                         60         70         80         90        100
IS6110          CAGGTCGACA CATAGGTGAG GTCTGCTACC CACAGCCGGT Taggtgctgg
DkWBS2 I/R      AATTCCGACT ATGCGTTGGA ATGTGNCCAG GTGGCGGCGC AGTCGGGTGC
DkCHs1 I/R      GAGCTCGGTA CCCGGGGATC CCACTGGTGG NNATCAAGCG AATCCCGACT
IS1081          CTGTGCGGGG CGGGCTACCG CGAACGCAGC GATGAGCGGT CCAATCAgcg
ChWtS1 I/F      GACCGCGACG GATGCAATCC CTGCAAagNN tgaNNagccc GCATAAAACT
ChWTS1 I/R      CGAAAAACCG CGTCNAGAAC TGCAATGACG ACCGCGACGG ATGCAatCCC
ChWTS2c O/F     GACCGCGACG GATGCAATCC CTGcaAAGCN GACCAGCCCG GCATAAAACT
ChWTS2c O/R     CGaaaaaccG cGTCAGAACT GCAATGACGA CCGCGACGGA TGCAatCCCT
WxFHS1 O/F      GACCGCGACG GATGCAATCC CTGCAAagCt gaccagcCCG GCATAAAACT
WxFHS1 O/R      CGAAAAACCG CGTCAGAACT GCAATGACGA CCGCGACGGA TGCAatCCCT
OxABS1 O/F      ACCGCGACGG ATGCAATCCC TGCaAagNNg NNNNNNNNgG CATAAAACTC
WWSs2  O/F      ACCGCGACGG ATGCAATCCC TGcaaagNgN ccNNcccNgC ATAAAACTCA
WWSS2  O/R      cgaaaaNNNN NtcAGAACTG CAATGACGAC CGCGACGGAT GCAATCCCTG
WWSS5  O/F      AGAACTGCAA TGACGACCGC GACGGATGCA ATCCCTGCNN agNgNNNNNc
WWSS7c O/R      cgaaaaNccc tCAGAACTGC AATGACGACC GCGACGGATG CAATCCcTGC
WWSS8  O/F      CTGCAATGAC GACCGCGACG GaTGCAATCC CTGCaaAgNg NNcNNNNcNc
NHMA2C I/R      TATCACACAT CAACTGCAAC TCCAAAGTCA CCCCTCACCC ATTAGGATAC
NHMA2C I/F      GTAATGTGCT ATGTACGGTA AATGGCTTTA TGTaCtATGT ACTGGTAAGG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        110        120        130        140        150
IS6110          tggtCCGAAg cggcgctgga cgag...... .......... ..........
DkWBS2 I/R      CGATGTNTTA GTGcTCTGCG ATaCGCGCGG CGGCCGCCTG TCGCAcGAAA
DkCHs1 I/R      ATGCGTTGGA ATGTCTGCAG GTGGCGGCGC AGTCGGGAGC CGATGTCTTA
IS1081          caacggctac cgccaCcgtg atttcgacac ccgtgccgca ac........
ChWtS1 I/F      CAGCAAGCGT CACGGGGCGT GATTTCGACA CCCGTGCCA. ..........
ChWTS1 I/R      TGCAAAGCtg A~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~.........
ChWTS2c O/F     CAGCAAGCGT CACGGGGCGT GATTTCGACA CCCGTGCCA. ..........
ChWTS2c O/R     GCAAAGCNGA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ..........
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WxFHS1 O/F   CAGCAAGCGT CACGGGGCGT GATTTCGACA CCCGTGCCA. .........
WxFHS1 O/R   GCAAAGCtgA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~.. .........
OxABS1 O/F   AGCAAGCGTC ACGGGGCGTG ATTTCGACAC CCGTGCCA.. .........
WWSs2  O/F   GCAAGCGTCA CGGGGCgTga TTTCGACACC CGTGCCA... .........
WWSS2  O/R   CAAAGCTGAC CAG~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ .........
WWSS5  O/F   ccNgCATAAA ACTCAGCAAG CGTCACGGGG CGTGNTTTCG ACACCCGTGC
WWSS7c O/R   AAAGCTGACC AGCCC~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~......
WWSS8  O/F   cggCATAAAA CTCAGCAAGC GTCACGGGGC GTGaTTTCGA CACCCGTGCC
NHMA2C I/R   CAACAAACCT ACCCACCCTT AACAGTACAT AGTACATAAA GCCATTTACC
NHMA2C I/F   GTGGGTAGGT TTGTTGGTAT CCTAATGGGT GAGGGGTGAC TTTGGAGTTG


                ....|....| ....|....| ....|....| ....|....| ....|....|
                       160        170        180        190        200
IS6110       .......... .......... .......... .......... ..........
DkWBS2 I/R   TTCaGGCGGc GATGGnTGGT GCACGAAAAG TGGTGGCGAC TCCCTTTGGG
DkCHs1 I/R   GTGcTcTGCG ATACGCGCGG CGGCCGCCTG TCGCACGAAA TTCAGGCGGC
IS1081       .......... .......... .......... .......... ..........
ChWtS1 I/F   .......... .......... .......... .......... ..........
ChWTS1 I/R   .......... .......... .......... .......... ..........
ChWTS2c O/F  .......... .......... .......... .......... ..........
ChWTS2c O/R  .......... .......... .......... .......... ..........
WxFHS1 O/F   .......... .......... .......... .......... ..........
WxFHS1 O/R   .......... .......... .......... .......... ..........
OxABS1 O/F   .......... .......... .......... .......... ..........
WWSs2  O/F   .......... .......... .......... .......... ..........
WWSS2  O/R   .......... .......... .......... .......... ..........
WWSS5  O/F   CA........ .......... .......... .......... ..........
WWSS7c O/R   .......... .......... .......... .......... ..........
WWSS8  O/F   A......... .......... .......... .......... ..........
NHMA2C I/R   GTACATAGCA CATTACAGTC AAATCCCTTC TCGtCcCCAT GGAGA.....
NHMA2C I/F   CAGTTGATGT GTGATACTTG AGGGTTGATT GCTGTACTTG CTTGTAAGCA


                ....|....| ....|....| ....|....| ....|....| ....|....|
                       210        220        230        240        250
IS6110       .......... .......... .......... .......... ..........
DkWBS2 I/R   ATCCAtTGTC ACAACGAtTG aGAATTGGCG GTCGCGAaTT CGcTAGCACG
DkCHs1 I/R   GATGGCTGCT GCACGAAAAG TGGTGGCGAC TCCCTTTGGG ATCCCTTGTC
IS1081       .......... .......... .......... .......... ..........
ChWtS1 I/F   .......... .......... .......... .......... ..........
ChWTS1 I/R   .......... .......... .......... .......... ..........
ChWTS2c O/F  .......... .......... .......... .......... ..........
ChWTS2c O/R  .......... .......... .......... .......... ..........
WxFHS1 O/F   .......... .......... .......... .......... ..........
WxFHS1 O/R   .......... .......... .......... .......... ..........
OxABS1 O/F   .......... .......... .......... .......... ..........
WWSs2  O/F   .......... .......... .......... .......... ..........
WWSS2  O/R   .......... .......... .......... .......... ..........
WWSS5  O/F   .......... .......... .......... .......... ..........
WWSS7c O/R   .......... .......... .......... .......... ..........
WWSS8  O/F   .......... .......... .......... .......... ..........
NHMA2C I/R   .......... .......... .......... .......... ..........
NHMA2C I/F   TGGGGGGGGG GGTTTTATNG GTATTT.... .......... ..........


                ....|....| ....|....| ....|....| ....|....| ....|....|
                       260        270        280        290        300
IS6110       .......... .......... .......... .......... ..........
DkWBS2 I/R   GCCGCACGCc TCTTCCGGAG TGTACAGGCA TGCCgTcg~~ ~~~~~~~~~~
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DkCHs1 I/R   ACAACGATTC TAAATTGGCG GtCgaTAATN NNCTaTCACG GCCGAACGCG IS1081
.......... .......... .......... .......... ..........
ChWtS1 I/F   .......... .......... .......... .......... ..........
ChWTS1 I/R   .......... .......... .......... .......... ..........
ChWTS2c O/F  .......... .......... .......... .......... ..........
ChWTS2c O/R  .......... .......... .......... .......... ..........
WxFHS1 O/F   .......... .......... .......... .......... ..........
WxFHS1 O/R   .......... .......... .......... .......... ..........
OxABS1 O/F   .......... .......... .......... .......... ..........
WWSs2  O/F   .......... .......... .......... .......... ..........
WWSS2  O/R   .......... .......... .......... .......... ..........
WWSS5  O/F   .......... .......... .......... .......... ..........
WWSS7c O/R   .......... .......... .......... .......... ..........
WWSS8  O/F   .......... .......... .......... .......... ..........
NHMA2C I/R   .......... .......... .......... .......... ..........
NHMA2C I/F   .......... .......... .......... .......... ..........


             ....|....| ....|....| ....|....| ..
                     310        320        330
IS6110       .......... .......... .......... ..
DkWBS2 I/R   ~~~~~~~~~~ ~~~~~~~~.. .......... ..
DkCHs1 I/R   TCTTCCGGAG TGTACAggCA TgCGGTcGac cC
IS1081       .......... .......... .......... ..
ChWtS1 I/F   .......... .......... .......... ..
ChWTS1 I/R   .......... .......... .......... ..
ChWTS2c O/F  .......... .......... .......... ..
ChWTS2c O/F  .......... .......... .......... ..
WxFHS1 O/F   .......... .......... .......... ..
WxFHS1 O/R   .......... .......... .......... ..
OxABS1 O/F   .......... .......... .......... ..
WWSs2  O/F   .......... .......... .......... ..
WWSS2  O/R   .......... .......... .......... ..
WWSS5  O/F   .......... .......... .......... ..
WWSS7c O/R   .......... .......... .......... ..
WWSS8  O/F   .......... .......... .......... ..
NHMA2C I/R   .......... .......... .......... ..
NHMA2C I/F   .......... .......... .......... ..
```

Repeat MTB Assays – Samples showing similar sequences (Forward):

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50
IS1081          ~ctgctctcg acgttcagcg ccgCCTTGAT GGGGGCTGAA GCCGACG~CC
ChWtS1  I       ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~GCGTCAGAA CTGCAATGAC
ChWTS2c O       ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~GCGTCNGAA CTGCAATGAC
WxFHS1  O       ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~GCGTCAGAA CTGCAATGAC
OxABS1  O       ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~C CGCGTCAGA~ CTGCAATGAC
WWSs2   O       ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~GCGTCAGA~ cTGCAATGAC
WWSS5   O       ~~~~~~~~~~ ~~~~~~~~GC TGANGaAaAc CGCGTCAGAA CTGCAATGAC
WWSS8   O       ~~~~~~~~~~ ~~~~~~~GNC TGAcGAAACC GCNgaCNGAA CTGCAATGAC


                ....|....| ....|....| ....|....| ....|....| ....|....|
                        60         70         80         90        100
IS1081          CTGTGCGGGG CGGGCTACCG CGAACGCAGC GATGAGCGGT CCAATCAgcg
ChWtS1  I       GACCGCGACG GATGCAATCC CTGCAAagNt gaNNagcccG ~CATAAAACT
ChWTS2c O       GACCGCGACG GATGCAATCC CTGcaAAGCt GACCAGCCCG GCATAAAACT
WxFHS1  O       GACCGCGACG GATGCAATCC CTGCAAagCt gaccagcCCG GCATAAAACT
OxABS1  O       GACCGCGACG GATGCAATCC CTGCaAagNN gNNNNNNNNg GCATAAAACT
WWSs2   O       GACCGCGACG GATGCAATCC CTGcaaagN~ gNccNNcccN gCATAAAACT
WWSS5   O       GACCGCGACG GATGCAATCC CTGCNNagN~ gNNNNNcccN gCATAAAACT
WWSS8   O       GACCGCGACG GaTGCAATCC CTGCaaAgN~ gNNcNNcccg gCATAAAACT


                ....|....| ....|....| ....|....| ....|....| ...
                       110        120        130        140
IS1081          caacggctac cgccaCcg~t gatttcgaca cccgtgccgc aac
ChWtS1  I       CAGCAAGCGT CACGGGGCGT GATTTCGACA CCCGTGCCA. ...
ChWTS2c O       CAGCAAGCGT CACGGGGCGT GATTTCGACA CCCGTGCCA. ...
WxFHS1  O       CAGCAAGCGT CACGGGGCGT GATTTCGACA CCCGTGCCA. ...
OxABS1  O       CAGCAAGCGT CACGGGGCGT GATTTCGACA CCCGTGCCA. ...
WWSs2   O       CAGCAAGCGT CACGGGGCgT gaTTTCGACA CCCGTGCCA. ...
WWSS5   O       CAGCAAGCGT CACGGGGCGT GNTTTCGACA CCCGTGCCA. ...
WWSS8   O       CAGCAAGCGT CACGGGGCGT GaTTTCGACA CCCGTGCCA. ...
```

Repeat MTB Assays – Samples showing similar sequences (Reverse):

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50
IS1081          ~ctgctctcg acgttcagcg ccgCCTTGAT GGGGGCTGAA GCCGACG~CC
ChWTS1  I       TCTGCTCTCG ACGTTCATCG CCGTGCGCCA GCGGAAGATG TAGTGGCTGA
ChWTS2c O       TCTGCTCTCG ACGTTCATCG CCGTGCGCCA GCGGAAGATG TAGTGGCTGA
WxFHS1  O       TCTGCTCTCG ACGTTCATCG CCGTGCGCCA GCGGAAGATG TAGTGGCTGA
WWSS2   O       ~CTGCTCTCG ACGTTCATCG CCGTGCGCCA GCGGAAGATG TAGTGGCTGa
WWSS7c  O       TCTGCTCTCG ACGTTCAtcG CCGTGCGCCA GCGGAAGATG TAGTGGCTGA


                ....|....| ....|....| ....|....| ....|....| ....|....|
                         60         70         80         90        100
IS1081          CTGTGCGGGG CGGGCTACCG CGAACGCAGC GATGAGCGGT CCAATCAgcg
ChWTS1  I       CGAAAAACCG CGTCAGAACT GCAATGACGA CCGCGACGGA TGCAatCCCT
ChWTS2c O       CGaaaaaccG cGTCAGAACT GCAATGACGA CCGCGACGGA TGCAatCCCT
WxFHS1  O       CGAAAAACCG CGTCAGAACT GCAATGACGA CCGCGACGGA TGCAatCCCT
WWSS2   O       cgaaaa~NNN NNtcAGAACT GCAATGACGA CCGCGACGGA TGCAATCCCT
WWSS7c  O       cgaaaa~~Nc cctCAGAACT GCAATGACGA CCGCGACGGA TGCAATCCcT


                ....|....| ....|....| ....|....| ....|....| ....|.
                        110        120        130        140
IS1081          caacggctac cgccaCcgtg atttcgacac ccgtgccgca ac....
ChWTS1  I       GCAAAGCtgA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ......
ChWTS2c O       GCAAAGCNGA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ......
WxFHS1  O       GCAAAGCtgA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~.. ......
WWSS2   O       GCAAAGCTGA CCAG~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~.....
WWSS7c  O       GCAAAGCTGA CCAGCCC~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
```

APPENDIX 6:

Results returned from BLAST showing a maximum identity of 80% with a number of *Brucella* species.

Descriptions

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
CP001578.1	Brucella microti CCM 4915 chromosome 1, complete sequence	89.7	89.7	85%	2e-15	80%	
CP001488.1	Brucella melitensis ATCC 23457 chromosome I, complete sequence	89.7	89.7	85%	2e-15	80%	
AE008917.1	Brucella melitensis bv. 1 str. 16M chromosome I, complete sequence	89.7	89.7	85%	2e-15	80%	
CP000887.1	Brucella abortus S19 chromosome 1, complete sequence	89.7	89.7	85%	2e-15	80%	
CP000911.1	Brucella suis ATCC 23445 chromosome I, complete sequence	89.7	89.7	85%	2e-15	80%	
CP000872.1	Brucella canis ATCC 23365 chromosome I, complete sequence	89.7	89.7	85%	2e-15	80%	
CP000708.1	Brucella ovis ATCC 25840 chromosome I, complete sequence	89.7	89.7	85%	2e-15	80%	
AE014291.4	Brucella suis 1330 chromosome I, complete sequence	89.7	89.7	85%	2e-15	80%	
AF244996.1	Brucella abortus strain 2308 BacA (bacA) gene, complete cds	89.7	89.7	85%	2e-15	80%	
AM040264.1	Brucella melitensis biovar Abortus 2308 chromosome I, complete sequence, strain 2308	89.7	89.7	85%	2e-15	80%	
AE017223.1	Brucella abortus biovar 1 str. 9-941 chromosome I, complete sequence	89.7	89.7	85%	2e-15	80%	
BA000040.2	Bradyrhizobium japonicum USDA 110 DNA, complete genome	86.0	86.0	85%	3e-14	80%	
AF322012.1	Bradyrhizobium japonicum symbiotic gene region, part 1 of 2	86.0	86.0	85%	3e-14	80%	
CP001891.1	Klebsiella variicola At-22, complete genome	77.0	77.0	68%	2e-11	82%	
CP000964.1	Klebsiella pneumoniae 342, complete genome	77.0	77.0	68%	2e-11	82%	
AP006725.1	Klebsiella pneumoniae NTUH-K2044 DNA, complete genome	75.2	75.2	41%	5e-11	95%	
CP000758.1	Ochrobactrum anthropi ATCC 49188 chromosome 1, complete sequence	75.2	75.2	50%	5e-11	89%	
CP000647.1	Klebsiella pneumoniae subsp. pneumoniae MGH 78578, complete sequence	75.2	75.2	41%	5e-11	95%	
AL591985.1	Sinorhizobium meliloti 1021 plasmid pSymB	75.2	75.2	41%	5e-11	95%	
X73522.1	R.meliloti bacA gene	75.2	75.2	41%	5e-11	95%	
CP001191.1	Rhizobium leguminosarum bv. trifolii WSM2304, complete genome	73.4	73.4	40%	2e-10	95%	
EU927393.1	Mesorhizobium huakuii strain 7653R BacA (bacA) gene, complete cds	68.0	68.0	40%	8e-09	93%	
CP001918.1	Enterobacter cloacae subsp. cloacae ATCC 13047, complete genome	66.2	66.2	41%	3e-08	91%	
CP000783.1	Enterobacter sakazakii ATCC BAA-894, complete genome	66.2	66.2	41%	3e-08	91%	
CP000739.1	Sinorhizobium medicae WSM419 plasmid pSMED01, complete genome	66.2	66.2	90%	3e-08	74%	
CP001622.1	Rhizobium leguminosarum bv. trifolii WSM1325, complete genome	64.4	64.4	40%	1e-07	91%	
AM236080.1	Rhizobium leguminosarum bv. viciae chromosome complete genome, strain 3841	64.4	64.4	40%	1e-07	91%	
BA000012.4	Mesorhizobium loti MAFF303099 DNA,	64.4	64.4	40%	1e-07	91%	