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The Paleoepidemiology of Malaria in the Ancient Near East

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Abstract

The end of the Late Bronze Age in the Near East (1300 – 1200 BCE) saw the widespread collapse of several large cultural centers, the reasons for which are a subject of continued debate. Evidence from events leading up to this cultural collapse suggest epidemic disease may have factored into the eventual downfall of these early civilizations. Recent DNA analysis from Egyptian mummies who lived during the period leading up to the Late Bronze Age collapse identified malaria in several elite individuals, suggesting the widespread prevalence of this infectious disease in Egypt. However, the exact prevalence, antiquity, and dynamics of malaria in the Near East, including what role it may have played in the shifting cultural and political landscape of the Late Bronze Age, remain uncertain.

This dissertation delves into this question of malarial spread and impact in the Near East in a multidisciplinary approach. Existing evidence from ancient literary texts, biology and pathophysiology, theoretical models, entomology, paleoclimatology, and historical records of malaria epidemics are surveyed and incorporated into a paleoepidemiological reconstruction of malaria. This reconstruction relies heavily on methods from epidemiology to identify a previously undefined skeletal manifestation of malaria and form a set of diagnostic criteria for identifying the disease in ancient populations. The new diagnostic method is then applied to the tightly dated human skeletal remains recovered from the ancient city of Amarna, Egypt.

Results indicated five skeletal lesions effective in diagnosing malarial infection: cribra orbitalia, femoral cribra, humeral cribra, spinal lytic lesions, and periostitis. Although many of these lesions are not systematically reported by bioarchaeologists, high rates of cribra orbitalia over time and space in the Nile Valley suggest a malarial prevalence that remained substantial throughout dynastic Egypt. Furthermore, the application of the full diagnostic criteria to the

Amarna skeletons showed a high prevalence of malaria within that population, with around 50% of individuals showing signs of recent infection. This prevalence rate, combined with demographic features and patterns of abnormal burial practices within the cemetery at Amarna, strongly suggest that malaria featured in the epidemics that afflicted the Near East prior to the collapse.

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Dedication

This dissertation is dedicated to my mother, whose example as a strong, intelligent woman in science illuminated my way.

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Chapter 1 – Introduction

Malaria is an ancient disease, and continues to be one of the most deadly diseases affecting people around the globe today. Although it was successfully eradicated from the United States and several other countries through the Global Malaria Eradication Campaign, malaria continues to wreak havoc on the health of people living in tropical, developing nations (Nadjm and Behrens 2012). Even with increases in eradication efforts and availability of anti-malarial drugs, there were an estimated 198 million cases of malaria worldwide in the year 2013, resulting in 584,000 deaths, of which 78% were children under the age of five (World Health Organization 2014). Understanding the evolution, spread, and natural history of malaria, as well as the role humans have played in its history, will allow medical researchers to advance our defenses against this disease (Pinhasi and Turner 2008). Similarly, the presence and prevalence of malaria in past societies would have had major impacts on the health and success of the societies as a whole, and thus, is of great importance for researchers reconstructing the lives of ancient peoples.

The main goal of my dissertation is to investigate the prevalence of tropical malaria (caused by the deadly *Plasmodium falciparum* species) in the ancient Near East. Many theorists have suggested various routes and time spans for the spread of malaria out of Africa, but supporting evidence has been limited mainly to written documentation from the last three-thousand years (Bruce-Chwatt 1965; Sallares et al. 2004). Recently, methodological advances in bioarchaeology have shown promise with regard to cultural, spatial, and skeletal indicators of malaria. Furthermore, ancient deoxyribonucleic acid (aDNA) analyses have provided direct evidence of *P. falciparum* in ancient tissue. My research will seek to integrate this line of

evidence with new interdisciplinary approaches with toward the goal of elucidating the paleoepidemiology of malaria in the ancient Near East.

Unlike some other infectious diseases, malaria's skeletal manifestation is not well known. In order to identify the presence of malarial infection on ancient osteological remains from the Near East, this study uses data gathered on the manifestation of the disease on skeletons from a modern reference sample in Uganda where malaria is known to be holoendemic (i.e. continuous transmission year-round, affecting virtually everyone), and compares them to a similar modern sample in a malaria-free area. By documenting and comparing the lesions from modern samples, diagnostic criteria will be established to allow the identification of malaria in unknown skeletal remains. This type of study, involving a large sample from a clinical setting with known individual medical histories, is a common method for establishing diagnostic characteristics of disease in paleopathology, but has never before been attempted with regard to malaria (Setzer 2014).

Detecting the past presence of malaria in a region requires a multidisciplinary approach, applying methods from anthropology, climatology, epidemiology, microbiology, and entomology (Pinhasi and Turner 2008; Herring and Swedlund 2010). My research will take a holistic approach, incorporating evidence from each of these disciplines to form a comprehensive temporal and geographic frame for the spread of malaria out of Africa into the Near East. I will focus primarily on the anthropological perspective by compiling reports of physical indicators of malaria left on ancient human skeletons to create a timeline of malaria spread in this region. Subsequently, I will create similar timelines based on evidences from the other disciplines and compare to see how they match up.

1.1 Objectives

The goal of this dissertation project was to determine the skeletal manifestation of malaria in order to elucidate the prevalence and spread of the disease in the ancient Near East. This project had three primary objectives.

Objective 1: Testing models of malaria spread in the ancient Nile Valley – The various skeletal indicators that have been associated previously with malarial infection or anemia in general are tested for their ability to predict the spread of malaria up the Nile River Valley in the timeframe of Dynastic Egypt.

Objective 2: Identifying skeletal markers of malaria in a clinical case-control study approach – These existing purported skeletal indicators are then refined through a case-control study comparing skeletal lesions present on a collection of modern human skeletal remains of known medical history in Uganda to a similar sample from a non-endemic area for malaria. From this comparison, a group of diagnostic skeletal markers are identified and tested for their diagnostic power in an outcome algorithm (i.e. "if" condition) of weighted criteria.

Objective 3: Estimating the prevalence of malaria at Amarna, Egypt and its implications for the Near East – This diagnostic criteria is used to estimate the prevalence of malaria in the skeletal remains recovered from a non-elite cemetery at the ancient city of Amarna, Egypt and predict the prevalence and spread of the disease in the rest of the Near East.

1.2 Hypotheses

Hypothesis 1: Malarial individuals will have a higher rate of porous skeletal lesions due to hemolytic anemia (increased marrow space and bone resorption) caused by malaria infection.

Other skeletal markers of physiological stress (i.e. periostitis, enamel hypoplasias) will not follow the same trend as porosity.

Hypothesis 2: Stable malaria transmission in a population will be marked by higher rates of anemia in children and women of reproductive age, whereas both children and adults will be affected by anemia in areas of unstable transmission.

Hypothesis 3: Falciparum malaria was endemic throughout Egypt, but not yet established in the rest of the Near East and Mediterranean at the end of the Late Bronze Age. Isolated epidemics of falciparum malaria and endemic vivax malaria will be present at this time in some areas.

1.3 Significance

My project will establish diagnostic criteria by which other researchers performing paleopathological analyses on prehistoric and historic skeletal assemblages will be able to identify malarial infection of individuals. Through this diagnosis, researchers will not only be able to observe the presence of malaria in a population, but will be able to observe frequencies of infection within and across populations and geographic regions. Diseases play a part in every aspect of human experience, from social structure, to religion, to group interaction and warfare (McNeill 1977). Knowledge of the prevalence and impact of malaria on past societies will force the reexamination of current theories and perhaps reveal previously unknown aspects of human history.

With the results of this study, I will be able to address the question as to the plausibility of malaria as the cause of the Hittite plague and its relation to ancient Egypt at the end of the Late Bronze Age (c.a. 1300 BCE). I will also be able to select which Amarna skeletal individuals

were most likely to have died with a malaria infection, allowing for representative sampling for future DNA testing. Thus, my study will add evidence to speculations about ancient disease epidemics in both the ancient Hittite and Egyptian empires.

This potential for new information regarding past malaria prevalence will allow for the construction of new models portraying the history and spread of the disease throughout human existence. Current models for the spread of malaria out of Africa have been largely theoretical in nature, relying on surviving written accounts of fevers and estimations of temperature and mosquito presence. With my project's contribution, a more realistic picture of malaria's history based on multiple lines of evidence will be possible. It is also my intention that the results of my study will lead other researchers to incorporate similar modern reference samples to form diagnostic criteria for other diseases as well, leading to improved methods in paleopathological studies.

In a world of climate change and microbial resistance to drug treatments, knowledge of the evolution and history of human malarial infection is more important than ever. Anthropologists have the opportunity to provide new perspectives to the global effort to eradicate the disease by contributing to the understanding of the social and historical aspects of malaria (Magner 2009; Herring and Swedlund 2010). With a better understanding of the interaction between humans and malaria in the past, medical researchers will be able to produce a more effective response to these ancient microbial invaders.

1.4 Organization of Chapters

This dissertation is presented as a broad literature review and a series of three prepared articles. The first chapter introduces the topic, gives the goals and objectives, and outlines the

organization of the following chapters. The second chapter provides an in-depth literature review of key concepts and background information pertinent to the methods and interpretation of results presented in the later chapters. The subsequent three chapters encompass three articles that report the research undertaken to address the objectives of the dissertation. These three chapters are followed by a summary and conclusions chapter that makes broad observations about the big picture meaning of the research. The combined references are listed at the end of the dissertation.

The first article, presented in Chapter Three, is entitled "Cribra orbitalia in the ancient Nile Valley." This article presents the current hypotheses and models for malaria's origin and spread out of Africa, and attempts to test these models through the use of one of the current proposed skeletal markers of malaria: cribra orbitalia. Through a meta-analysis of published cribra orbitalia rates at various Nile River Valley archaeological sites, this study sought significant differences in the rates of the lesion over time and space. It is the first comprehensive meta-analysis of cribra orbitalia rates in the ancient Nile Valley, providing a more realistic view of how these rates relate to each other across the different sites and time periods. This paper has been accepted for publication in the *International Journal of Paleopathology*.

Chapter Four presents the second article, "The skeletal manifestation of malaria: a clinical case-control study." In this article, skeletal lesions present on a sample of skeletons from an endemic area for malaria are reported and compared with individuals from a non-endemic area. The endemic sample is split into two groups: anemic individuals (those whose cause of death included malaria or anemia) and non-anemic individuals. The samples are put through a series of statistical and epidemiological tests to determine which lesions are the best indicators of individuals infected with malaria. From these indicators and their relationship with each other,

diagnostic criteria are formed which can be used to diagnose unknown skeletal samples for malaria prevalence. This article has been submitted for publication in the *American Journal of Physical Anthropology*.

The last of the articles is presented in Chapter Five and is entitled, "The prevalence of malaria at Amarna, Egypt and its regional implications." This article takes the diagnostic algorithm produced in Chapter Four and applies it to the individuals recovered at the South Tombs Cemetery at Amarna, Egypt. The prevalence of malaria at ancient Amarna is estimated and discussed in reference to the larger interaction sphere and political turmoil in the Near East, especially in connection to the Hittite plague at the end of the Late Bronze Age (c.a. 1300 BCE). This article is in preparation for submission to the *American Journal of Physical Anthropology*.

Chapter 2 – Literature Review

2.1 Introduction

Paleopathology as a field of study has grown from something of a hobby of physicians in the 19th century, beginning with mummy unrollings and identification of chronic diseases of known skeletal manifestation, followed by collection of skulls to suggest biological affinity, to finally form a scientific field of research after 1945 with increased focus on standardized methods and systematic data collection (Aufderheide and Rodríguez-Martín 1998; Buikstra and Roberts 2012). This increase in scientific rigor came predominantly from the large Egyptian and Nubian archaeological salvage expeditions that came with the dam projects on the Nile River, in which archaeologists were removing whole populations of skeletons from the ground, not just mummies or skulls (Buikstra and Roberts 2012). This made a way for the study of overall trends in the health of populations over time.

The field of paleopathology, having developed out of physicians' diversions, has never truly had its own theory, but instead has made advances by borrowing and incorporating theoretical paradigms from other related fields of study (Grauer 2012). These fields include clinical medicine, microbiology, epidemiology, biochemistry, and ecology. Cutting-edge research in paleopathology today is that which incorporates all of the aforementioned paradigms to form a holistic model of the impact of specific diseases in past populations. Paleopathologists of past decades used non-specific markers of physiological stress to infer general health of populations; however, this practice is becoming less and less prominent in current research as questions of why and how are used to determine more specific afflictions of these groups (Pinhasi and Turner 2008).

More and more, paleopathologists are beginning to see the importance of the incorporation of scientific advances from other fields into the methods of paleopathological studies (Zuckerman et al. 2015). This study takes such an interdisciplinary approach to answer the question of malaria's impact on the health of past populations. In order to understand the complex nature of malaria paleoepidemiology, this chapter surveys current knowledge and evidence for ancient malaria from the following areas: ancient texts, malaria biology, vector ecology, paleoclimate reconstructions, theoretical models, and paleopathology.

2.2 Ancient Near Eastern texts

My research began with a question: could malaria have been responsible for the mysterious illness that plagued the Hittite empire at the end of the Late Bronze Age. In ancient Turkey (Anatolia), at the end of the 14th Century BCE, the Hittite King wrote a series of prayers pleading with the gods for relief from a widespread, 20-year epidemic that had already killed the two preceding kings, and continued to ravage his country. These ancient texts, known as the Plague Prayers of Mursili II, reveal that this deadly epidemic was brought by Egyptian prisoners of war taken to the Hittite capital city (Singer 2002). Mursili describes the plague as a divine punishment of his father, Suppiluliuma I, who attacked the Egyptian border in a breach of his oath of treaty with the Egyptians (Bryce 1998; Singer 2002). This attack occurred after another of Suppiluliuma's sons was murdered on his way to marry a prominent female figure in Egypt (perhaps King Tutankhamun's wife, Ankhesenamun), who had written to the Hittite king, pleading for the Hittite prince's hand in marriage (Schulman 1978).

Unfortunately, the disease responsible for the epidemic has never been identified definitively for two main reasons. First, the Hittites tended to cremate their dead; thus, no Hittite

cemeteries from this time period have yet been found (Emre 1991). Therefore, there is no direct evidence of the disease agent because there are no skeletons on which to identify this agent. Second, if the disease indeed came from Egypt, there is no textual evidence of this because Egyptians have been known to omit or change negative historical events in their writings. This tendency is best exemplified through the Battle of Kadesh (c.a. 1274 BCE), wherein Egyptian writings and reliefs depict a grand victory over the Hittites, but in actuality, historians and archaeologists consider the Hittite documents recording a victory on their side to be the more likely outcome (Hasel 1998). From the descriptions in Mursili's Plague Prayers, the disease seems to have spread rapidly after the prisoners entered the city, and infection was not dependent upon age or class (Singer 2002: 56–57). The texts do not mention specific symptoms, but Mursili emphasizes the deadliness of the disease and that it persisted for 20 years in Hittite lands. The prisoners must have showed no obvious symptoms until after entering the city, suggesting a long incubation period.

Some scholars have suggested that tumultuous events in Egypt leading up to this point in history indicate Egypt may have been stricken by the same epidemic disease as the Hittite empire (Kozloff 2012; Dodson 2014). The pharaoh Akhenaten suddenly changed the Egyptian religion and founded his new capital city of Amarna in a previously uninhabited area, which remained the capital for only 17 years (c. 1349–1332 BCE). Some scholars have attributed this abrupt religious and geographical shift to epidemic disease, perhaps even polio or bubonic plague (Nunn 1996; Kozloff 2006). However, even the abrupt movement of an entire capital city did not seem to prevent the spread of epidemic disease. Ancient texts and stelae found at Amarna point to a mysterious "Canaanite illness" and "hand of Nergal" that afflicted people even at the end of the Amarna period (Moran 1992: 107–109, letter EA 35; Assman 2003: 223–224).

Approximately two years before the estimated date of Suppiluliuma's death, Amarna was abandoned, scattering its occupants to the far reaches of the empire, and hence, possibly to the Egyptian-Hittite border. Along with the dispersion of people came a redeployment of military, such that any disease present at Amarna could also have spread to the borders of the Egyptian empire, potentially affecting those Egyptians subsequently taken to the Hittite capital. This coincidence of timing between a period of uncertainty and change in Egypt and a known epidemic in Anatolia makes it hard to imagine that disease that caused the Hittite epidemic was not present in Egypt at the same time (Assman 2003).

Two of the Hittite kings' deaths are attributed to this epidemic, and early deaths in the royal family at Amarna suggest a similar lack of immunity of the royalty, if indeed these were caused by the same disease agent. At Amarna, Akhenaten's second daughter, Meketaten died around age 12, and was commemorated by various depictions of the royal family mourning in her royal tomb (Redford 1984: 186; Tyldesley 2003). The early death of another of Akhenaten's children, the famous Tutankhamun, has long been the subject of serious debate as to the cause of death, with malaria recently implicated as such through ancient DNA (aDNA) evidence (Hawass et al. 2010). This aDNA study found positive genetic markers for two different strains of tropical malaria (*Plasmodium falciparum*) in Tutankhamun's mummified tissue, suggesting he had a double infection of malaria at his time of death. Two other members of the royal family were also tested and shown to be positive for malaria infection. The following sections in this chapter will outline evidence from various fields of study to address the unresolved question of malaria's role in the Near Eastern Late Bronze Age epidemics.

2.3 Biology, ecology, and pathophysiology of malaria

Malaria is a disease caused by protozoal parasites of the genus *Plasmodium* transmitted by the *Anopheles* mosquito vector. There are at least four *Plasmodium* species known to affect humans, all differing in their disease ecology (see Table 2.1). The two species of major global importance (i.e. have the greatest impact on human health) today are *P. falciparum* and *P. vivax* (Webb 2009). The main differences between these two species include their temperature requirements, severity of symptoms, and ability to cause relapses. *P. falciparum* malaria can only exist at temperatures above 19° Celsius (about 66°F), and is not known to have the ability to remain dormant in the liver like *P. vivax*, and thus, requires transmission all year round to keep a foothold within a population (Sherman 1998). It is for this reason falciparum malaria is also referred to as "tropical malaria," since it is most common in areas that maintain warm temperatures all year round. Falciparum malaria also distinguishes itself from vivax malaria in its severity, being more virulent in general and having a greater potential to cause death of its host (Webb 2009).

Table 2.1. Comparison chart of malaria species ecology

	P. falciparum	***P. vivax***	***P. ovale***	***P. malariae***
Global importance	Major	Major	Minor	Minor
Host	Humans	Humans	Humans	Humans + African apes
Incubation (on average)	12 days	15 days - 12 mo.	17 days	18-40 days
Relapse?	No	Yes	Yes	No
Fever wave freq.	Every 48 hours	Every 48 hours	Every 48 hours	Every 72 hours
Disease consequences	Severe anemia, cerebral malaria	Increasingly severe anemia		
In-utero infection?	Yes	Yes		
Post-partum antibodies?	Yes	No		
Required temp (°C)	>19	>15		

*Chart based on malaria species ecology from Webb (2009).

The identification of the protozoa responsible for malaria only occurred within the last 150 years, and its lifecycle was only recognized within the last 50 years (Sherman 1998). The modes of transmission begin when a gravid Anopheline mosquito takes a blood meal from a human infected with malaria. Once in the mosquito's stomach, the stomach acid activates malarial male and female gametocytes, which combine to produce zygotes called ookinetes. These ookinetes invade the midgut wall and develop into oocysts. The oocysts form on the outside of the mosquito stomach and rupture, releasing thousands of sporozoites which migrate to the mosquito's salivary glands.

As the mosquito takes its next blood meal, the sporozoites are injected with the saliva into the human's (or other warm-blooded animal's) bloodstream, where they are taken to the liver and therein invade hepatocytes. Within the hepatocytes, the sporozoites undergo asexual reproduction for a few days to form millions of merozoites, which eventually burst out of the hepatocytes into the bloodstream where they invade red blood cells. This is where the falciparum species differs from the others, causing the red blood cells it invades to adhere to the blood vessel walls and to other red blood cells; a phenomenon called rosetting (Gilles 1997). Researchers believe this rosetting has a major causative effect on the progression of the disease to cerebral malaria (Wahlgren et al. 1992).

Once inside the red blood cell, merozoites undergo further asexual reproduction, consume available hemoglobin as food, and simultaneously rupture out of the red blood cells at the same time all over the body, releasing toxins and millions more merozoites into the bloodstream, causing the high fevers in the host. This cycle in the blood continues until either the host dies or recovers, and some of the merozoites form into gametocytes. Meanwhile, the

next Anopheline mosquito to bite the infected individual picks up the gametocytes in its blood meal, thereby completing the life cycle (see Figure 2.1 for life cycle diagram).

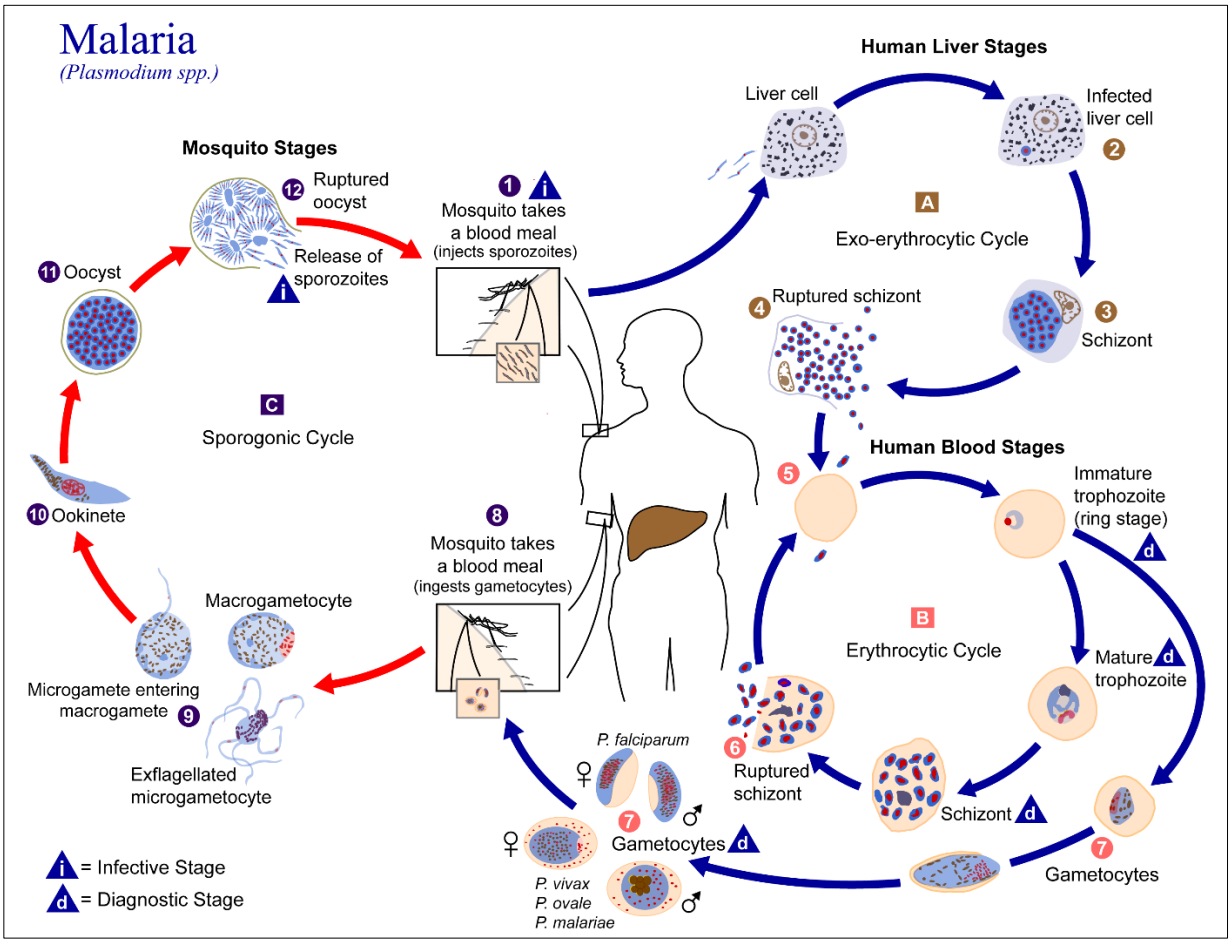


Figure 2.1. Malaria life cycle diagram (courtesy CDC – DPDx Alexander J. da Silva and Melanie Moser, content providers)

The clinical manifestation of malaria arises during the blood stage, with the release of toxins and pigments into the bloodstream as merozoites erupt from the red blood cells (Gilles 1997). Clinical symptoms include severe headaches, body aches, periodic extreme fever, enlarged spleen and liver, and hemolytic anemia. Additional deadly symptoms possible in severe falciparum infections include severe malarial anemia (most common in young children)

and cerebral malaria (most common in older children) (Reyburn et al. 2005; Roca-Feltrer et al. 2010; Billig et al. 2012; Botez and Doughty 2014).

The pathophysiology of malaria, especially regarding the chemical release and hormone activation of the blood stage, remain under research and debate. With regard to the skeleton, some recent experimental work with malaria in mice has suggested that the influx of free heme in the bloodstream leads to impaired bone formation and an imbalance favoring bone resorption (Moreau et al. 2012). This imbalance may also be affected by the increase of acid phosphatase, a known osteoclast stimulator, in the bloodstream during malaria infection (D'Souza et al. 2011). The hemolysis of red blood cells has also been shown to quickly affect bone marrow and bones in the malarial mice as compared with bled mice (Moreau et al. 2012). Thus, while skeletal changes involved in malarial infections are rarely the focus of research into the disease, malaria does appear to have a resorptive effect on the skeleton. More skeletal evidence from bioarchaeological contexts will be discussed below.

2.4 Models of malaria origin and spread

The origins of falciparum malaria are still the subject of debate among researchers today. Unlike many diseases, the origins of which can be back-calculated through changes to the genome, *P. falciparum* has a mosaic genome, complicating its hypothesized evolutionary history, as well as complicating any attempts at creating a vaccine (Zilversmit and Hartl 2005). Most biologists generally agree that this malaria species originated in Africa, probably in tropical West Africa (Sherman 1998). Livingstone (1958) was the first to consider malaria's evolution in a social, environmental, and genetic context. He suggested that falciparum malaria existed in tropical West Africa, but did not reach epidemic status until humans began practicing slash-and-

burn agriculture, which created pools of water suitable for higher populations of Anopheline mosquitoes to breed. According to Livingstone, it was during this period of agricultural innovation and larger population sizes, that falciparum malaria was able to get a foothold in human populations. In turn, humans with abnormal hemoglobin such as thalassemia and the sickle-cell trait had a greater chance of survival, leading to the increase in these genetic traits in human populations. This idea of genetic polymorphisms, the increased survival of heterozygous individuals for these deleterious genes due to their conference of malarial resistance, explained the retention of these genes in African, Asian, and Mediterranean populations today.

Many researchers have traditionally believed that due to its virulence, falciparum malaria must have evolved recently under the assumption that over time, diseases will find a balance with their host so that they can live together peacefully instead of killing off their hosts quickly (Livingstone 1958). However, this theoretical assumption has been called into question recently, especially when considering vector-borne and water-borne diseases, as well as those that can survive long periods of time in an external environment (Ewald 2003). Studies into the genome of the falciparum species have resulted in many far-reaching estimations for the age of this parasite, projecting its evolution anywhere from as recently as 5,000 years to as ancient as 3-4 million years (Hume et al. 2003; Rich et al. 2009; Datta and Chauhan 2010). Some have suggested that both of these age estimations are correct, and represent two separate regional expansions of the genome, with the most recent corresponding to the advent of agriculture in West Africa (Zilversmit and Hartl 2005). Vivax malaria is suggested to be 1-3 million years old, likely originating in Asia (Datta and Chauhan 2010).

Since Anopheline mosquitoes generally need clean, fresh water in which to breed, they tend to be found in marshy environments today. It is hypothesized that the seasonal flooding of

the Nile River and its utilization by ancient Egyptians through irrigation canals may have worsened an already prime niche for malaria to thrive (Scheidel 2001; Scheidel 2012). Considering this environmental advantage, coupled with the large cities of clustered potential hosts, researchers have generally hypothesized that malaria spread out of Africa and into Europe through the Nile Valley pathway (Bruce-Chwatt 1965). However, the date and pathway of this disease spread continues to be debated in the literature.

Bruce-Chwatt and de Zulueta (1980) theorize that falciparum malaria only arrived and began to spread in Europe during the age of the Roman Empire. They discount the textual and physical evidence of falciparum malaria, and insist that falciparum malaria could not have existed mainly due to lack of proper mosquito vectors. Even in ancient Egypt where the climate was undoubtably well-suited for malaria, de Zulueta (1987) claims that only a low prevalence of malaria would have existed due to lack of efficient vector. They also suggest that the ancient Mediterranean nautical warfare would have been markedly one-sided if falciparum malaria was at play (De Zulueta 1987).

Sallares and coworkers (2004) argue for a slightly earlier spread of malaria, possibly extending back to 700 BCE from Tunesia to Sicily, Sardinia, and Italy. They speculate that epidemics of falciparum malaria spread simultaneously with the migration of the mosquito vector *An. sacharovi* into Greece and Italy. This hypothesis seems to suggest that malaria spread gradually in Europe as mosquitoes slowly migrated, but does not consider that mosquitoes can (and often do in modern times) hitch rides on seafaring vessels to spread to new areas at the same time as their human transporters.

Thus, much is still unknown about the origins and spread of malaria in the past. For this reason, anthropology can be of use in identifying malaria's presence, impact, and

paleoepidemiology through the physical remains of past humans. By reconstructing disease dynamics of the past, anthropologists can contribute to overall understanding about how best to respond to this disease in the future (Brown et al. 1997).

2.5 Mosquito evidence

The successful spread of malaria to new locations depends on the presence and substantial population size of the correct species of mosquito (genus Anopheles), and at least one human who is infected or is an asymptomatic carrier of malarial *Plasmodium*. Climate, elevation, and breeding grounds are the most important factors determining the plausibility of large mosquito population sizes. Each species of Anopheles mosquito has its own preference as to the temperature and altitude range it likes to inhabit. For this reason, different geographic locations tend to have different dominant malaria vector species.

Furthermore, each of these species has its own behavioral differences as to what elevations it prefers to inhabit, where it prefers to breed, which animal it prefers to bite, and how it prefers to hibernate. Some species do not enter man-made structures, whereas others who prefer to enter structures, especially at night, making the latter a much more effective malaria vector than the former (Sherman 1998). Another factor in mosquito malaria transmission is whether or not the mosquito prefers to bite humans or other animals. Mosquitoes that prefer to bite humans over animals, known as anthropophilic mosquitoes, are much more likely to transmit malaria than those which prefer to bite other animals.

In the Near East, the dominant malaria vector species include *An. sacharovi*, *An. sergentii*, and *An. superpictus* (Sinka et al. 2010). *An. sacharovi* is the most important malaria vector species in modern Turkey, and its current habitat ranges from coastal areas bordering the

Mediterranean Sea in Greece, widespread in the whole of Turkey and the Fertile Crecent, and coastal areas bordering the Black Sea (see Figure 2.2). *An. sacharovi* has several behavioral advantages for successful malaria transmission. It will breed in stagnant fresh water or brine, is found in elevations up to 1720m, and has an incomplete hibernation in winter; thus, is able to cause new cases of malaria all year round (Alten et al. 2000). The most important malaria vector in modern Egypt is *An. sergentii*, known for its adaptability in desert climates and ability to overwinter, but of which host preferences are still under debate (Sinka et al. 2010; Manguin 2013). Less is known about *An. superpictus*, but it does seem to be particularly dangerous in open country and high altitudes where other mosquito species may be absent (Sinka et al. 2010).

For the reasons listed above, in order for malaria to spread in the Middle East region, a substantial mosquito vector must be present, and its population size must be maintained, including optimal temperatures, elevation, breeding grounds, and anthropophilic behavior patterns of the mosquito species present. Once the conditions are met for a plausible malaria vector population, transmission is possible during human migrations to new areas.

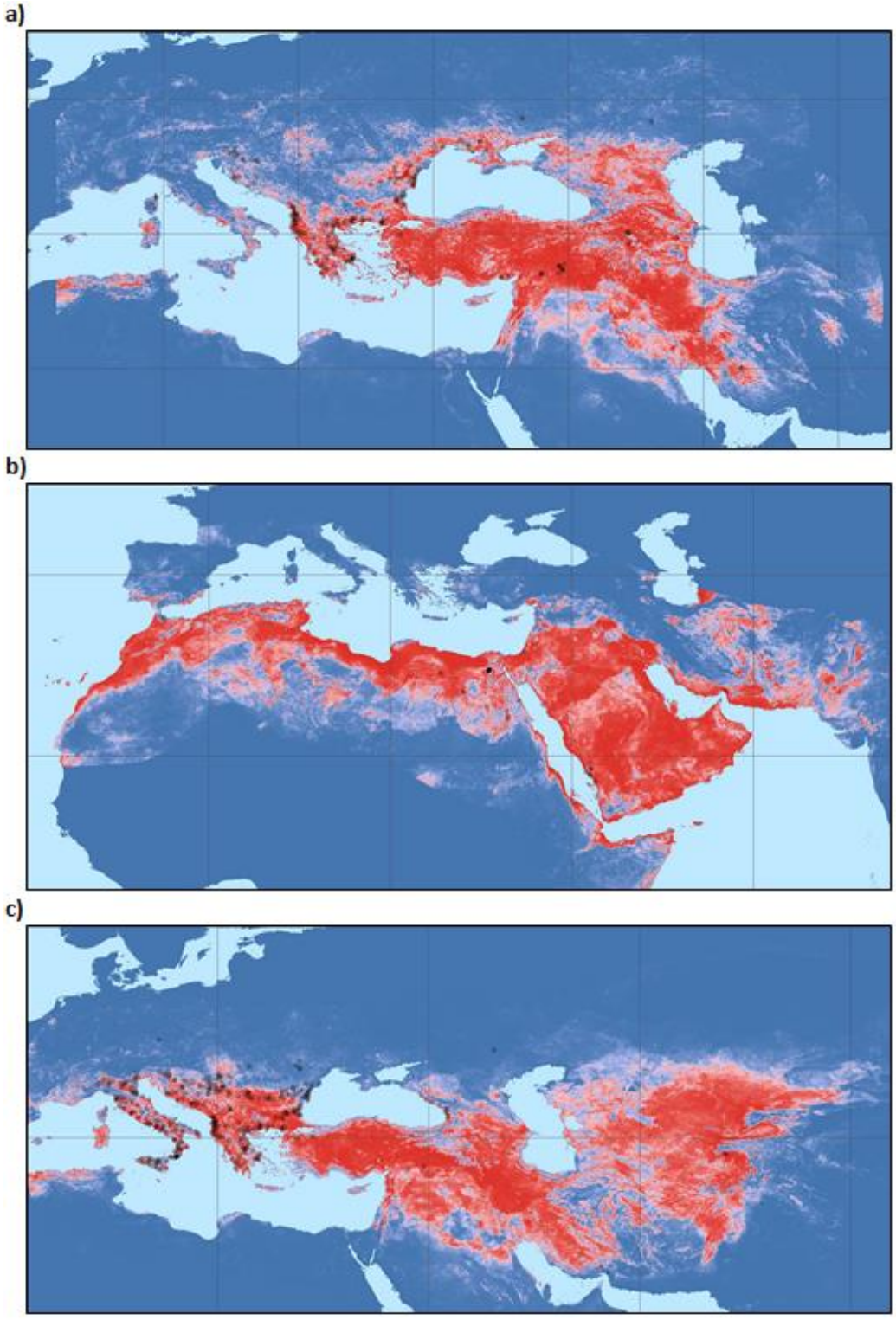


Figure 2.2. Sinka et al's (2010) predictive distribution maps of modern dominant Anopheles vector species of human malaria in Europe and the Middle East: a) *An. sacharovi*, b) *An. sergentii*, and c) *An. superpictus*. Red areas mark the distribution range.

2.6 Paleoclimate evidence

Climate is incredibly important to understanding the plausibility of past malaria epidemics because it controls the potential for large populations of malaria vector mosquitoes. As evidenced from fossilized pollen and charcoal analyses from a lagoon in the Nile Delta region, the climate in the ancient Middle East leading up to the expansion of city-state societies was characterized by a very moist and humid period from about 6000 – 3500 BCE (Bernhardt et al. 2012). After about a 500 year period of fluctuating rainfall, the climate shifted to a much drier state around 2800 BCE, likely involving droughts that impacted ancient peoples negatively (Kaniewski et al. 2013). In regard to mosquito populations, this drier climate meant highly decreased populations, probably only surviving in areas of remaining moisture, like the Nile Valley and Delta region.

This drought period was alleviated briefly by periods of increased rainfall between 1500 – 1100 BCE and a small spike around 500 BCE (Bernhardt et al. 2012). These periods are important because they represent time periods of increased mosquito population size and range in the Middle East, leading to higher malaria parasitemia in endemic regions, as well as higher possibility of malaria spread to new regions with human migration. From these reconstructions, it seems that the spread of falciparum malaria epidemics would have been possible in the Near East during the time of the Hittite plague (i.e. 1320 – 1300 BCE).

2.7 History of malaria epidemics

To reconstruct past disease spread and epidemics of malaria, historical documents of past epidemics can be used to suggest ancient disease dynamics. In dealing with falciparum malaria

spread in the Near East, historical malaria epidemics suggest generally devastating health effects in non-immune populations experiencing the parasite for the first time. One such epidemic occurred on the Northwest coast of the United States in the 1830's. From the detailed record keeping of the European settlers, anthropologist Robert Boyd was able to implicate malaria as the disease agent, which he proposes to have been brought in with a migrant from an endemic area on a ship that came into Portland, Oregon in September 1830 (Boyd 1975; Boyd 1999). The subsequent spread of the epidemic into California which decimated Native American villages there has also been tracked by historians to a group of fur-traders in John Work's Buenaventura brigade in August 1833 (Ahrens 2011).

After studying the accounts and reconstructing epidemiology of various other epidemics on the Northwest coast at this time (e.g. smallpox, measles, and syphilis among others), Boyd calls this malaria epidemic "the single most important epidemiological event in the recorded history" of this area (Boyd 1999: 84). Although Europeans were hard-hit during these yearly epidemics, the indigenous populations who did not have access to the typical cinchona bark (quinine) treatment were ravaged by this disease (Boyd 1999; Ahrens 2011). Historical accounts told of traditional healing techniques involving sweat baths followed by a plunge into cold river water, which seemed tragically to increase the deadly outcomes of the disease, as noted by the Europeans who told of whole villages disappearing during these epidemic years (Boyd 1999; Ahrens 2011).

Due to the lack of knowledge about the etiology of malaria at the time, it can only be speculated as to the species of malaria parasite that caused the 1830s Northwest coast epidemic. In recent times, multi-year malaria epidemics caused by *Plasmodium falciparum* entering into a naïve population have been documented in areas of increased human migration like the isthmus

of Panama (Calzada et al. 2008). During such an epidemic in 2005, nearly half of the indigenous Kuna community of Chepo became infected before the Panamanian health officials could intervene (Shah 2010). Unlike the temperate climate of the aforementioned Northwest coast epidemics, Panama's tropical climate provided the possibility of year-round transmission, which extended the duration and increased morbidity and mortality rates of the epidemic.

From these historical disease dynamics, key information for finding evidence for past epidemics can be elucidated. Primarily, we can see that malaria can be spread by just one single infected person migrating to a new area, as long as that new area is inhabited by a substantial Anopheline mosquito population. Secondly, not only biological immunity, but also cultural response to disease plays a large part in the duration and virulence of the disease on the population. Finally, climate is an intrinsic factor in the ability of an epidemic of falciparum malaria to gain a foothold in a population.

2.8 Skeletal evidence

J. Lawrence Angel was among the first to suggest a skeletal manifestation involving malaria through his work on archaeological sites in the Near East (Angel 1966; Angel 1967; Angel 1972; Angel 1978; Buikstra and Roberts 2012). Angel focused on the widespread porous lesions of the cranium, which he termed porotic hyperostosis, and hypothesized about their etiological link to the hemolytic anemia brought on by genetic conditions conferring resistance to malaria (i.e. thalassemia and sickle cell disorder) (Angel 1964a; Angel 1966). The lesions were thought to develop as a result of the expansion of the hemopoietic diploe of the cranium (seen as the characteristic "hair on end" appearance radiographically), to allow greater red blood cell generation to compensate for the severe anemia (Zaino 1964). He suggested that the appearance

of these lesions in ancient populations suggested the presence of falciparum malaria within these populations (Angel 1964a; Angel 1966). However, these genetic disorders are maintained at low levels in endemic populations, and so could not explain the high rates of these skeletal lesions within ancient populations (Hengen 1971). Moreover, since falciparum malaria is not thought to have existed in the New World prior to European contact, genetic hemolytic anemia cannot explain the porotic hyperostosis found at pre-Columbian American sites (Angel 1966; El-Najjar et al. 1976).

Thereafter, etiological theories shifted toward iron-deficiency anemia as the main causative agent for porotic hyperostosis. Hengen (1971) observed rates of the porous orbital lesions only (generally thought to be of similar etiology to porotic hyperostosis, but termed cribra orbitalia), noting their gradation in frequency rates in relation to the equator, with higher rates tending to appear at lower latitude sites. He suggested a possible connection between cribra orbitalia and greater iron-deficiency anemia due to parasitic worms, which are more common in tropical environments.

Mahmoud El-Najjar was among the first to make this connection with iron-deficiency anemia mainstream in his work on prehistoric sites in the Southwest of the United States (El-Najjar et al. 1975; El-Najjar et al. 1976). El-Najjar pointed to a lack of iron in the diet causing higher rates of porotic hyperostosis in populations dependent on maize agriculture as compared with nearby populations more dependent on meat for subsistence (El-Najjar et al. 1976). From this point on, paleopathologists began to shift their attention from potential presence of malaria in the past to dietary stress associated with agriculture (Carlson et al. 1974; Lallo et al. 1977; Mensforth et al. 1978; Stuart-Macadam 1987).

However, many anthropologists have pointed out flaws in the iron-deficiency anemia hypothesis. Many discredit the attribution of only dietary lack of iron to the formation of the cranial lesions, and have instead suggested a multi-factorial etiology including diet and other factors such as parasitic and diarrheal disease (Hengen 1971; Lallo et al. 1977; Mensforth et al. 1978; Walker 1986; Holland and O'Brien 1997; Wapler et al. 2004). Gleń-Haduch and coworkers (1997) found no significant correlations between the levels of iron in teeth and presence of cribra orbitalia, suggesting other etiological factors are more important than lack of iron in the development of this lesion. Wapler and coworkers (2004) suggest the lesions have been over-estimated in the bioarchaeological record due to misdiagnosis. He suggests microscopic examination and histology in order to differentiate between expansion of marrow space and other causes, such as inflammation and atrophy due to increase pressure or tissue hemorrhages caused by vitamin C deficiency (Wapler et al. 2004). Further, McClure and coworkers (2011) found high rates of cribra orbitalia in a population in Spain which they say could not have been caused by iron-deficiency anemia due to the high isotopic levels of animal protein in their diet. They suggest a combination of weaning-related vitamin B_{12} deficiency and malaria-induced hemolytic anemia as the cause.

The biggest criticism to the iron-deficiency anemia hypothesis came in an article by Walker and coworkers (2009). They reasoned that iron-deficiency anemia could not in fact induce the bone marrow hypertrophy responsible for producing these lesions because this type of anemia depresses red blood cell production. Instead, they pointed to megaloblastic and hemolytic anemia as the main factors triggering the formation of these skeletal lesions. The former type of anemia arises in individuals with a nutritional deficiency in B_{12}, and the latter

arises in individuals with genetic disorders conferring protection from malaria (thalassemia and sickle-cell anemia), as well as in individuals with a malaria infection (Walker et al. 2009).

Walker's article is still a matter of debate currently, with some suggesting that iron deficiency could still contribute to marrow space expansion because it causes ineffective erythropoiesis rather than a complete dyserythropoiesis (Oxenham and Cavill 2010). However, others refute this ineffective erythropoiesis claim, and instead insist that iron deficiency anemia is a side effect, not the cause, of porotic hyperostosis (Rothschild 2012). Further, McIlvaine (2013) suggests that if we refute the iron-deficiency anemia hypothesis, we should also refute the B_{12} deficiency explanation because the mechanisms behind both types of anemia are the same. At this point, bioarchaeologists have not come to a consensus on the etiology of porotic hyperostosis and cribra orbitalia, but there seems to be evidence for multiple factors at play (McIlvaine 2013).

Malaria is often dismissed in differential diagnoses by paleopathologists, many of whom hold that the disease does not manifest itself upon the skeleton (Nunn and Tapp 2000; Roberts 2000). However, recent research has shown evidence to the contrary. Rabino Massa and coworkers (2000) provided a link between direct evidence for malaria and skeletal lesions of anemia. They tested ancient Egyptian mummies for immunological evidence of malarial antigens, and of those testing positive for falciparum malaria, 92% had porotic hyperostosis and cribra orbitalia. This link was corroborated by Nerlich and coworkers (2008) who found concurrent positive malarial aDNA detection and skeletal markers of chronic anemia. Similarly, Gowland and Western (2012) mapped and associated cribra orbitalia with the distribution of large populations of *Anopheline* mosquitoes, lower altitude and marshy environments, and higher incidence of historic "fever and ague" (an archaic term for malaria) across Great Britain. Their

study found a correlation between vivax malarial infection and cribra orbitalia, which gives additional support to the hypothesis that malaria does indeed manifest itself in the skeleton.

2.9 Summary

Multiple lines of evidence must be used in describing malarial prevalence in the past, including textual, biological, historical, and climatological studies. However, none of these represent *direct* evidence of ancient malaria prevalence. This shortage of direct evidence can be improved through a greater understanding and refined methods for identifying the physical evidence for malaria on human skeletal remains. Although several studies have linked porotic hyperostosis and cribra orbitalia to malaria and genetic disorders conferring protection from malaria, much is still unknown about the etiology of these skeletal lesions. At the very least, there appear to be multiple factors leading to their manifestation, such as nutrition and parasitic infection (Holland and O'Brien 1997; Wapler et al. 2004; Walker et al. 2009). The research undertaken in this dissertation will test models and build on the previous studies by providing an *a priori* means by which to diagnose malarial infection in ancient remains through macroscopic skeletal examination, which will provide empirical evidence in considering malaria's involvement in the Hittite plague of 1320 BCE.

Chapter 3 – Cribra orbitalia in the ancient Nile Valley and its connection to malaria

3.1 Introduction

Cribra orbitalia is one of the most common skeletal lesions noted in ancient human skeletal remains excavated from the Nile Valley (Hillson 1980). Researchers have long explained this porous lesion of the eye orbits, along with the similar porous cranial vault lesions (porotic hyperostosis), as an expansion of the marrow space in the cranial vault caused by iron-deficiency anemia (Carlson et al. 1974; El-Najjar et al. 1976, 1975; Lallo et al. 1977; Mensforth et al. 1978; among others). This iron-deficiency anemia hypothesis has recently been called into question by a number of researchers, including Walker and coworkers (2009), who maintain the depression of red blood cell production in iron-deficiency anemia excludes the possibility of its participation in the stimulation of increased marrow space involved in porotic hyperostosis and cribra orbitalia formation.

While Walker and coworker's (2009) etiological reappraisal is still being debated in the literature (Oxenham and Cavill, 2010; Rothschild, 2012; McIlvaine, 2014), other researchers have shown an association between cribra orbitalia and malaria infection (Rabino Massa et al. 2000; Nerlich et al. 2008; Gowland and Western 2012). Malaria has been identified in the mummified tissue of ancient Egyptians of various time periods, dating back to as early as 3200 BCE using ancient DNA (aDNA) sequencing and antigen evidence (Miller et al. 1994; Bianucci et al. 2008; Nerlich et al. 2008; Hawass et al. 2010). This direct genetic and immunological evidence verifies the presence of malaria in antiquity, but leaves the prevalence and spread of the disease unknown.

Although there are many factors that could have potentially contributed to the overall anemia seen in the human skeletal remains of ancient Egypt, malaria infection has been shown to have a major synergistic effect with other factors to increase overall anemia levels, and thus, would have arguably raised the overall frequencies of cribra orbitalia (Nájera and Hempel 1996; Gilles 1997; Lusingu et al. 2004; Shanks et al. 2008). The present study surveys the temporospatial variability in rates of cribra orbitalia reported at archaeological sites along the Nile Valley in order to suggest ancient prevalence and distribution of malaria in this region. Tracking changes in cribra orbitalia in this region provides not only a more holistic picture of ancient Egyptian anemia, but also a potential way to test theoretical models of malaria's spread out of Africa.

3.2 Porotic hyperostosis, cribra orbitalia, and anemia

Genetic conditions conferring resistance from malaria were hypothesized to cause skeletal lesions such as porotic hyperostosis and cribra orbitalia in the ancient Mediterranean and Near East (Angel 1964a; Zaino 1964; Angel 1966; Angel 1967; Angel 1972). However, there is a discrepancy between low rates of these genetic disorders in modern endemic populations and the high rates of these skeletal lesions within ancient populations (Hengen 1971). Consequently, paleopathologists turned to iron-deficiency anemia, a main contributor to anemia in modern populations as the main causative agent implicated for these lesions (Hengen 1971; Carlson et al. 1974; El-Najjar et al. 1976; Lallo et al. 1977; Mensforth et al. 1978; Stuart-Macadam 1987). This hypothesis has been linked with agriculture through studies showing higher rates of porotic hyperostosis and cribra orbitalia in maize agriculturalists as compared with populations whose diets included meat (El-Najjar et al. 1976).

The porous lesions of the vault (porotic hyperostosis) and those of the orbits (cribra orbitalia) tend to show a connection, but also variability, in etiology (Stuart-Macadam 1989; Walker et al. 2009). Some consider cribra orbitalia as an early indicator of anemia, and porotic hyperostosis as an indicator of a more chronic, long term anemic state (Hrdlička 1914; Caffey 1937). Only children tend to display active lesions, leading to the widely-held explanation that these lesions form during childhood and are only maintained due to lack of bone turnover in adults (Stuart-Macadam 1985; Mittler and Van Gerven 1994).

However, many anthropologists have pointed out flaws in the iron-deficiency anemia hypothesis. Many discredit the attribution of dietary lack of iron as the main causative factor of the cranial lesions, and have instead suggested a multi-factorial etiology including diet and other factors such as parasitic and diarrheal disease (Hengen 1971; Lallo et al. 1977; Mensforth et al. 1978; Walker 1986; Holland and O'Brien 1997; Wapler et al. 2004). However, the role of parasites in the etiology of cribra orbitalia has also been disputed (DeGusta 2009). Gleń-Haduch and coworkers (1997) found no significant correlations between the levels of iron in teeth and presence of cribra orbitalia, suggesting other etiological factors are more important than lack of iron in the development of this lesion. Further, McClure and coworkers (2011) found high rates of cribra orbitalia with concurrent high isotopic levels of dietary animal protein in a population in Spain, precluding the possibility of iron-deficiency.

The biggest criticism to the iron-deficiency anemia hypothesis came in an article by Walker and coworkers (2009). They reasoned that iron-deficiency anemia could not in fact induce the bone marrow hypertrophy responsible for producing these lesions because this type of anemia depresses red blood cell production. Instead, they pointed to megaloblastic and hemolytic anemia as the main factors triggering the formation of these skeletal lesions. The

former type of anemia arises in individuals with a nutritional deficiency in B_{12}, and the latter arises in individuals with genetic disorders conferring protection from malaria (thalassemia and sickle-cell anemia), as well as in individuals with a malarial infection (Walker et al. 2009).

Walker's article is still a matter of debate currently, with some suggesting that iron deficiency could still contribute to marrow space expansion because it causes ineffective erythropoiesis rather than a complete dyserythropoiesis (Oxenham and Cavill 2010). However, others refute this ineffective erythropoiesis claim, and instead insist that iron deficiency anemia is a side effect, not the cause, of porotic hyperostosis (Rothschild 2012). Further, McIlvaine (2013) suggests that if the iron-deficiency anemia hypothesis is refuted, the B_{12} deficiency explanation should also be refuted because the mechanisms behind both types of anemia are the same. At this point, the exact etiology of porotic hyperostosis and cribra orbitalia remains uncertain, but appears to a combination of many factors (McIlvaine 2013).

3.3 Differential diagnosis of anemia in the Nile Valley

To explain the high frequencies of cribra orbitalia in the Nile Valley, many causes have been suggested, including schistosomiasis, intestinal worms, dietary deficiencies, brucellosis, and malaria. Schistosomiasis (a blood fluke infection) in ancient Egypt has been evidenced directly from mummified tissues and indirectly from ancient texts (Brier 2004). However, antigenic evidence of schistosoma infection was not shown to associate with skeletal lesions of anemia in non-adults at the Nubian site of Semna South (Alvrus 2006: 167), indicating other etiological factors are more important than schistosomiasis in the formation of these lesions in the Nile Valley.

Hookworms, common in modern tropical areas, are a notorious cause of iron-deficiency anemia, and have been implicated in causing higher rates of cribra orbitalia in equatorial areas (Hengen 1971). If Walker's (2009) position is correct that iron-deficiency anemia is unable to cause porotic hyperostosis and cribra orbitalia, then this type of anemia would be unlikely to generate these lesions. Vitamin B_{12} deficiency caused by hookworm infestation could be a factor, as the malabsorption of nutrients due to chronic diarrhea is attributed to megaloblastic anemia, which has been implicated as a cause of marrow hypertrophy (Walker et al. 2009; but see McIlvaine 2013 for critique). Nevertheless, Vitamin B_{12} deficiency-induced megaloblastic anemia is not a major contributor to total anemia worldwide, even in tropical, developing nations (Kassebaum et al. 2014). Therefore, neither hookworms nor other sources of Vitamin B12 deficiency were likely responsible for high skeletal anemia rates in the Nile Valley.

Brucellosis is a disease underestimated by paleopathologists in the past, consisting of a bacterial zoonotic infection passed from domestic cattle to humans usually through ingestion of raw milk (D'Anastasio et al., 2011). Brucellosis causes undulating fevers and hemolytic anemia similar to malaria, potentially inducing skeletal lesions of anemia like cribra orbitalia. However, brucellosis maintains a low prevalence in humans, even in high-risk occupational groups like dairy farmers (Lopes et al., 2010). Due to this low prevalence, and even lower chance of anemia severe enough to make a mark on the skeleton, it is not likely that high rates of skeletal anemia in the Nile Valley were caused by this disease.

Malaria is a disease caused by parasites of the genus *Plasmodium* and transmitted by the *Anopheles* mosquito vector. There are at least four species of the parasite known to infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. It has also been implicated for causing the chronic anemia pattern in the ancient Nile Valley. In ancient Egyptian medical texts,

the annual plague described in the Edwin Smith Surgical Papyrus has been attributed to seasonal epidemics of malaria during periods of annual Nile River flooding (Brier 2004). This disease does not discriminate against age or class, although does contribute to higher rates of anemia in women and children (World Health Organization 2014). Malaria is known to cause hemolytic anemia, which is capable of producing marrow hyperplasia (Walker et al. 2009). In addition to the classic model of skeletal anemia by expansion of marrow space, recent research has suggested that the hemolysis during the schizogony phase of malaria infection may contribute to porous skeletal lesion formation due to the release of acid phosphate, free heme, and the malarial pigment hemozoin into the bloodstream. This leads to an imbalance in bone remodeling by stimulating osteoclasts while simultaneously impairing osteoblasts (D'Souza et al. 2011; Moreau et al. 2012). Furthermore, severe malarial anemia may induce extramedullary erythropoiesis, which is known to cause cortical thinning and coarse trabeculation (Al-Aabassi and Murad 2005).

3.4 Models of malaria origin and spread

In his classic paper published in 1958, Livingstone was the first to consider malaria's evolution in a social, environmental, and genetic context. He suggested that malaria caused by the *P. falciparum* species (referred to as falciparum malaria) existed in tropical West Africa, but did not reach epidemic status until the advent of slash-and-burn agriculture in the region (approximately 2,000 – 4,000 years ago), which created pools of water suitable for higher populations of *Anopheles* mosquitoes to breed. This connection between tropical forest deforestation and increased malaria risk has been widely intimated in modern populations (Yasuoka and Levins 2007; Afrane et al. 2008; Hahn et al. 2014). According to Livingstone, it

was during this period of agricultural innovation and larger population sizes, that falciparum malaria was able to get a foothold on human populations. In turn, humans with abnormal hemoglobin such as thalassemia and the sickle-cell trait had a greater chance of survival, leading to the increase in these genetic traits in human populations (Livingstone 1958; Livingstone 1971). This idea of balanced polymorphisms, the increased survival of heterozygous individuals for these deleterious genes due to their conference of malarial resistance, explained the presence of these genes in African, Asian, and Mediterranean populations today (Livingstone 1958; Livingstone 1971).

The origins of *P. falciparum* malaria are still the subject of debate today due to its mosaic genome, which complicates its hypothesized evolutionary history (Zilversmit and Hartl 2005). There is a general consensus that this malaria species originated in Africa, most likely in the tropical West African region (Sherman 1998). Attempts at dating the genome of the falciparum species have resulted in many far-reaching estimations for the age of this parasite, projecting its evolution anywhere from as recently as 5,000 years to as ancient as 3-4 million years (Hume et al. 2003; Datta and Chauhan 2010). Some have suggested that both of these age estimations are correct, and represent two separate regional expansions of the genome, with the most recent corresponding to the advent of agriculture in West Africa (Zilversmit and Hartl 2005).

As the most virulent of the malarial species, researchers assumed that falciparum malaria evolved recently under the assumption that over time parasites will evolve a more symbiotic relationship with their hosts in order to propagate their offspring rather than killing off their hosts quickly (Livingstone 1958; Capasso 1998; Baum and Bar-Gal 2003). However, this theoretical assumption has been modified recently to consider the evolutionary advantage of virulence in immobilizing hosts for more effective spread of vector-borne diseases (Ewald 2003).

Nevertheless, genetic polymorphisms that confer resistance or immunity to malaria appear to have arisen within the last 5,000 years, giving support to theories of recent evolution of the more virulent falciparum malaria (Hedrick 2012).

Since *Anopheles* mosquitoes generally need clean, fresh water in which to breed, they tend to be found in marshy environments today. It is hypothesized that the seasonal flooding of the Nile River and its utilization by ancient Egyptians through irrigation canals may have worsened an already prime niche for malaria to thrive (Scheidel 2001; Scheidel 2012). Evidence for the use of irrigation in the Nile Valley dates back to 3200 BCE, but it is likely that the practice arose in earlier predynastic times (Nicholson and Shaw 2000). Considering this environmental advantage, coupled with the large cities of clustered potential hosts, researchers have generally hypothesized that malaria spread out of Africa and into Europe through the Nile Valley pathway (Bruce-Chwatt 1965; Schlagenhauf 2004). Some have theorized that the spread of malaria out of Africa occurred recently, as late as 2,000 years ago (Bruce-Chwatt and de Zulueta 1980; De Zulueta 1987). Bruce-Chwatt and de Zulueta based their reasoning of this late malarial diaspora on the assumption that efficient mosquito vectors had not yet migrated into the Mediterranean areas between the last glacial period (c.a. 12,000 years ago) and the Roman era (Bruce-Chwatt and de Zulueta 1980: 11–13). However, this assumption is difficult to prove due to the scarcity of *Anopheles* mosquitoes in the fossil record (Capasso 1998).

3.5 Paleopathology and malaria

Advances in aDNA extraction and immunological assays from skeletal and mummified tissues have revealed direct evidence for malaria's presence in the ancient Nile Valley through genetic markers of the *P. falciparum* malaria parasite in ancient mummified tissue (Miller et al.

1994; Bianucci et al. 2008; Nerlich et al. 2008; Hawass et al. 2010). However, these aDNA studies are limited to providing evidence for presence, but not prevalence, of the disease in the past.

Malaria is often dismissed in differential diagnoses by paleopathologists, many of whom hold that the disease does not manifest itself upon the skeleton (Nunn and Tapp 2000; Roberts 2000). However, recent research has provided evidence to the contrary. Rabino Massa and coworkers (2000) provided a link between direct evidence for malaria and skeletal lesions of anemia. They tested ancient Egyptian mummies for malarial antigens, and of those testing positive for falciparum malaria, 92% had porotic hyperostosis and cribra orbitalia. This link was corroborated through a similar aDNA study by Nerlich and coworkers (2008). Similarly, Gowland and Western (2012) mapped and associated cribra orbitalia with the distribution of large populations of *Anopheles* mosquitoes, lower altitude and marshy environments, and higher incidence of historic "fever and ague" (an archaic term synonymous with malaria) across Great Britain. Their study found a correlation between vivax malarial infection and cribra orbitalia, which gives additional support to the hypothesis that malaria does indeed manifest itself in the skeleton.

3.6 Materials and methods

The present study tests a theoretical Dynastic Egyptian time frame for the spread of malaria up the Nile Valley and out of Africa by using the variability of cribra orbitalia frequencies among ancient Egyptian and Nubian remains as a proxy for malarial infection. If malaria did spread into Egypt during the Dynastic period, an increasing trend in cribra orbitalia frequency over time from South to North in the Nile Valley was predicted.

Reports from 29 ancient Nile Valley sites were surveyed (see Appendix A), representing 4,760 individuals ranging from prehistoric to Christian periods (4400 BCE – 1500 CE) and situated between upper Nubia and the Nile delta (see Table 3.1 and Figure 3.1). Data collection was conservative, with several restrictions for unbiased comparison. If a report recorded less than 15 individuals containing observable orbits for scoring cribra orbitalia, then it was not included in the statistical analysis. If no number of observable individuals was mentioned in the report, the site was excluded. Similarly, sites reporting poor skeletal preservation were excluded.

Additional data on proportions of adult females (out of the total number of adults assigned a sex) and nonadults under 17 years of age (out of total individuals assigned an age) in each sample population was collected when available. The reason for collecting this demographical data was to ascertain any biases in the sample that would affect the total frequency of cribra orbitalia at the site. For example, since cribra orbitalia has been noted at higher rates in children, a skeletal sample containing only children may contain higher rates of cribra (Stuart-Macadam 1985). Similarly, since women and children are at higher risk for malarial infections and bear a greater anemia burden than adult males in endemic areas, higher proportions of either of these groups may influence the total cribra orbitalia rate of the sample population (Gilles et al. 1969; World Health Organization 2007; Billig et al. 2012). Table 3.2 lists the raw frequencies of the variables collected from the site reports.

Spatial comparison between sites was analyzed by latitudinal coordinates of site location in order to visualize changes in anemia along the Nile River. Temporal comparison between sites was accomplished by taking the mean of the occupation dates for the site. Analysis of the data consisted of comparison of overall distribution of the data to other existing cribra orbitalia meta-analyses, comparison of means through Student's *t*-test, and determination of associations

through Spearman's rank and Kendall's tau correlations. Statistical analyses were carried out using IBM SPSS 22.01. Statistical significance was set at $p \leq 0.05$.

Few meta-analyses have been published for comparison of cribra orbitalia rates across wide areas and time periods. The range of Nile Valley cribra orbitalia rates compiled in this study will be compared with other existing cribra orbitalia meta-analyses compiled from New World (Steckel and Rose 2002) and English samples (Gowland and Western 2012).

Table 3.1. Chronology of Ancient Egypt and Nubia (after Baines and Malek 1983)

Date	Egyptian	Nubian
4400 – 2600 BCE	Late Pre-Dynastic/Early Dynastic	A-Group
2600 – 2134 BCE	Old Kingdom	-
2134 – 2040 BCE	1st Intermediate Period	C-Group
2040 – 1640 BCE	Middle Kingdom	(Egyptian occupation)
1640 – 1550 BCE	2nd Intermediate Period	(Egyptian occupation)
1550 – 1070 BCE	New Kingdom	(Egyptian occupation)
1070 – 332 BCE	3rd Intermediate/Late Period	-
332 BCE – 1500 AD	Greco/Roman/Christian	Meroitic/X-Group/Christian

Figure 3.1. Map of the location of the sites used for this study. Map created using ESRI ArcGIS 10.0. Satellite imagery © CNES/Airbus DS, Earthstar Graphics. Source: Esri, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community | Esri, HERE, DeLorme.

Table 3.2. Frequencies of cribra orbitalia, females, and nonadults under 17 years by site

Site	Time Period	Region	*n*[*]	Cribra orbitalia (%)	Females[†] (%)	Nonadults[‡] (%)
Abusir (Mastaba of Ptahshepses)	Late Period	Lower Egypt	142	26.8	44.7	46.3
Abydos	Early Dynastic	N. Upper Egypt	106	49.1	-	-
Abydos	Old Kingdom	N. Upper Egypt	28	78.6	-	-
Abydos	Middle Kingdom	N. Upper Egypt	41	68.3	-	-
Abydos ('Tombs of the Courtiers')	Early Dynastic	N. Upper Egypt	30	40.0	-	-
Adaïma	Late Predynastic	S. Upper Egypt	272	26.5	-	100.0
Amarna (S. tombs)	New Kingdom	Middle Egypt	103	42.7	-	-
Aswan	Old Kingdom	S. Upper Egypt	18	61.1	-	-
Aswan	Middle Kingdom	S. Upper Egypt	47	63.8	-	-
Dendara	1st Intermediate	N. Upper Egypt	76	53.9	-	-
Dishasha	Old Kingdom	Middle Egypt	21	42.9	-	-
El-Badari (Badarian graves)	Late Predynastic	N. Upper Egypt	30	63.3	-	-
Elephantine	1st Intermediate	S. Upper Egypt	32	75.0	68.3	26.7
el-Raqaqna	Old Kingdom	N. Upper Egypt	17	52.9	-	-
el-Tarif	Middle Kingdom	S. Upper Egypt	54	55.6	-	-
Gebelein	Old Kingdom	S. Upper Egypt	23	73.9	43.3	8.6
Gebelein	1st Intermediate	S. Upper Egypt	47	78.7	43.6	20.8
Gebelein	Late Period	S. Upper Egypt	17	52.9	-	-
Hierakonpolis (HK27C)	1st Intermediate, Middle Kingdom	S. Upper Egypt	21	28.6	65.4	29.7
Hierakonpolis (HK43)	Late Predynastic	S. Upper Egypt	145	13.1	59.5	20.9
Hierakonpolis (Prehistoric &	Late Predynastic	S. Upper Egypt	39	71.8	-	-

Site	Time Period	Region	*n*[*]	Cribra orbitalia (%)	Females[†] (%)	Nonadults[‡] (%)
'Fort' cemeteries)						
Kerma	2nd Intermediate	Upper Nubia	306	13.7	61.5	4.2
Kulubnarti (21-R-2)	Christian	Upper Nubia	164	39.0	-	-
Kulubnarti (21-S-46)	Christian	Upper Nubia	170	51.8	-	-
Memphis	New Kingdom	Lower Egypt	306	24.8	44.3	3.9
Missiminia	Meroitic - Christian	Upper Nubia	333	27.9	48.3	-
Naqada (Great, B, and T cemeteries)	Late Predynastic	N. Upper Egypt	97	40.2	35.7	0.0
Naqada B cemetery	Late Predynastic	N. Upper Egypt	20	60.0	-	-
Naqada T cemetery	Late Predynastic	N. Upper Egypt	23	43.5	-	-
Qaw el-Kebir	Old Kingdom	N. Upper Egypt	27	70.4	-	-
Qaw el-Kebir	1st Intermediate	N. Upper Egypt	69	63.8	-	-
Qubbet el Hawa	Old Kingdom	S. Upper Egypt	156	48.7	39.4	19.2
Qubbet el Hawa	1st Intermediate	S. Upper Egypt	32	34.4	27.8	18.2
Qubbet el Hawa	Middle Kingdom	S. Upper Egypt	18	50.0	46.7	28.6
Qubbet el Hawa	2nd Intermediate	S. Upper Egypt	60	63.3	45.0	31.0
Qubbet el Hawa	Late Period	S. Upper Egypt	146	36.3	45.4	17.0
Qurneh	New Kingdom	S. Upper Egypt	172	16.3	52.0	7.5
Shellal	New Kingdom	Lower Nubia	154	20.1	47.7	3.8
Sidmant	1st Intermediate	Middle Egypt	55	67.3	-	-
Sidmant	Middle Kingdom	Middle Egypt	15	53.3	-	-
SJE (C-Group)	Middle Kingdom	Lower Nubia	205	14.1	64.8	12.9
SJE (Pharaonic)	New Kingdom	Lower Nubia	73	23.3	55.1	15.2
Tarkhan	Late Predynastic – Early Dynastic	Middle Egypt	29	72.4	-	-

Site	Time Period	Region	*n*[*]	Cribra orbitalia (%)	Females[†] (%)	Nonadults[‡] (%)
Tarkhan	Early Dynastic	Middle Egypt	26	34.6	-	-
Tell el-Dab'a	2nd Intermediate	Lower Egypt	41	26.8	40.8	48.1
Thebes-West	New Kingdom - Late Period	S. Upper Egypt	168	29.2	45.5	20.2
Thebes-West (Valley of the Queens)	Roman	S. Upper Egypt	212	18.4	48.0	19.2
Tombos	New Kingdom	Upper Nubia	83	10.8	59.5	15.0
Wadi Halfa (24I3)	X-Group	Upper Nubia	45	26.7	50.0	29.6
Wadi Halfa (6B13)	Christian	Upper Nubia	28	14.3	-	32.4
Wadi Halfa (6B16)	Meroitic	Upper Nubia	62	11.3	58.3	17.1
Wadi Halfa (6G8)	Christian	Upper Nubia	29	13.8	-	39.4
Wadi Halfa (NAX)	X-Group	Upper Nubia	127	26.7	56.6	14.1

[*] *n* = number of individuals with observable orbits.
[†] Proportion of adult females versus adult males reported at the site.
[‡] Proportion of nonadults under 17 years of age versus adults reported at the site.

3.7 Results

Generally high cribra orbitalia rates between 10.8% and 78.7% existed within each of the sites, with an overall mean of 42.8%. Figure 3.2 shows the greater overall rates of cribra orbitalia in the Nile Valley sample compared with other global cribra orbitalia meta-analyses. The comparisons with Steckel and Rose's (2002) meta-analyses, however, must be viewed with caution due to the small sample sizes of sites. Interestingly, the Nile Valley cribra orbitalia distribution only overlaps slightly the results from the English sample that purportedly contained *P. vivax* malaria infections, with a significant difference in means of the two distributions (t=7.898 (58), p=0.000).

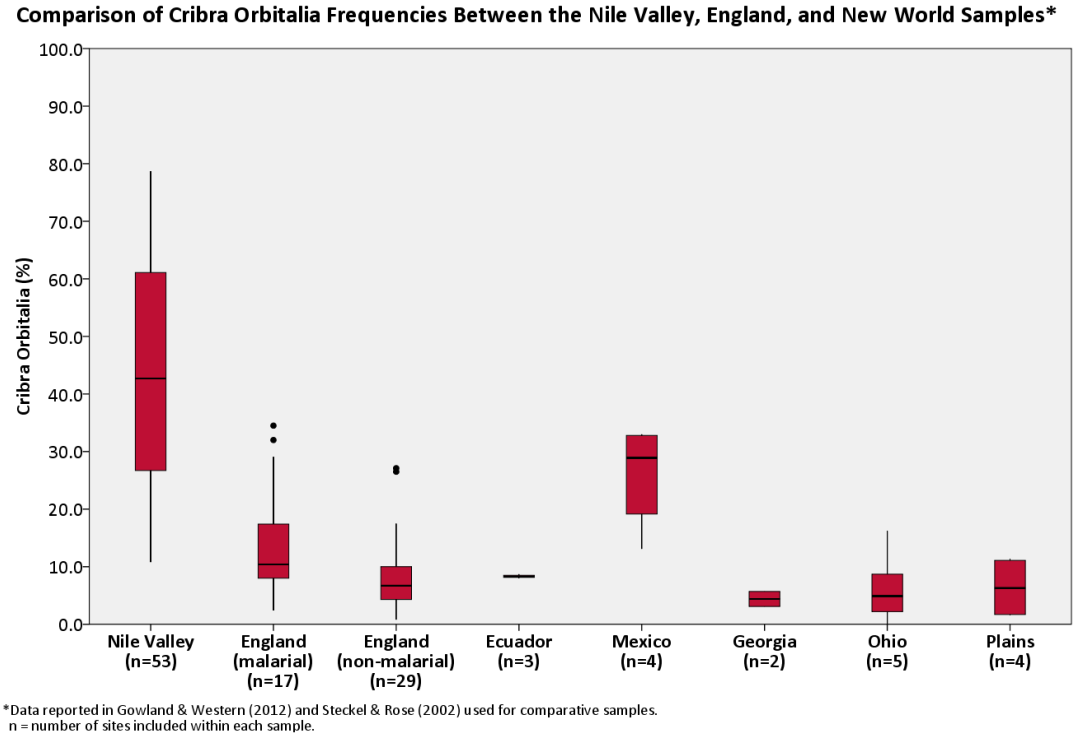


Figure 3.2. Boxplot showing cribra orbitalia frequency distributions for each location, with the horizontal line representing the median, box representing 50% of the data, and vertical lines extending to 95% confidence interval limits. Dots represent outliers.

Scatterplots of the total cribra orbitalia frequency against latitude and date showed three geographical clusters: Nubian sites, Upper and Middle Egyptian sites, and Lower Egyptian sites (see

Figure 3.3 and Figure 3.4). Thus, the data was analyzed separately within each of these groups and tested for correlations between cribra orbitalia and latitude, date of occupation, date of report publication, proportion of adults at the site classified as female, and proportion of individuals at the site classified as nonadults. There was no significant correlation for any of these variables (see graphs in Figure 3.5, Figure 3.6, and Figure 3.7; and Table 3.3 for statistical results).

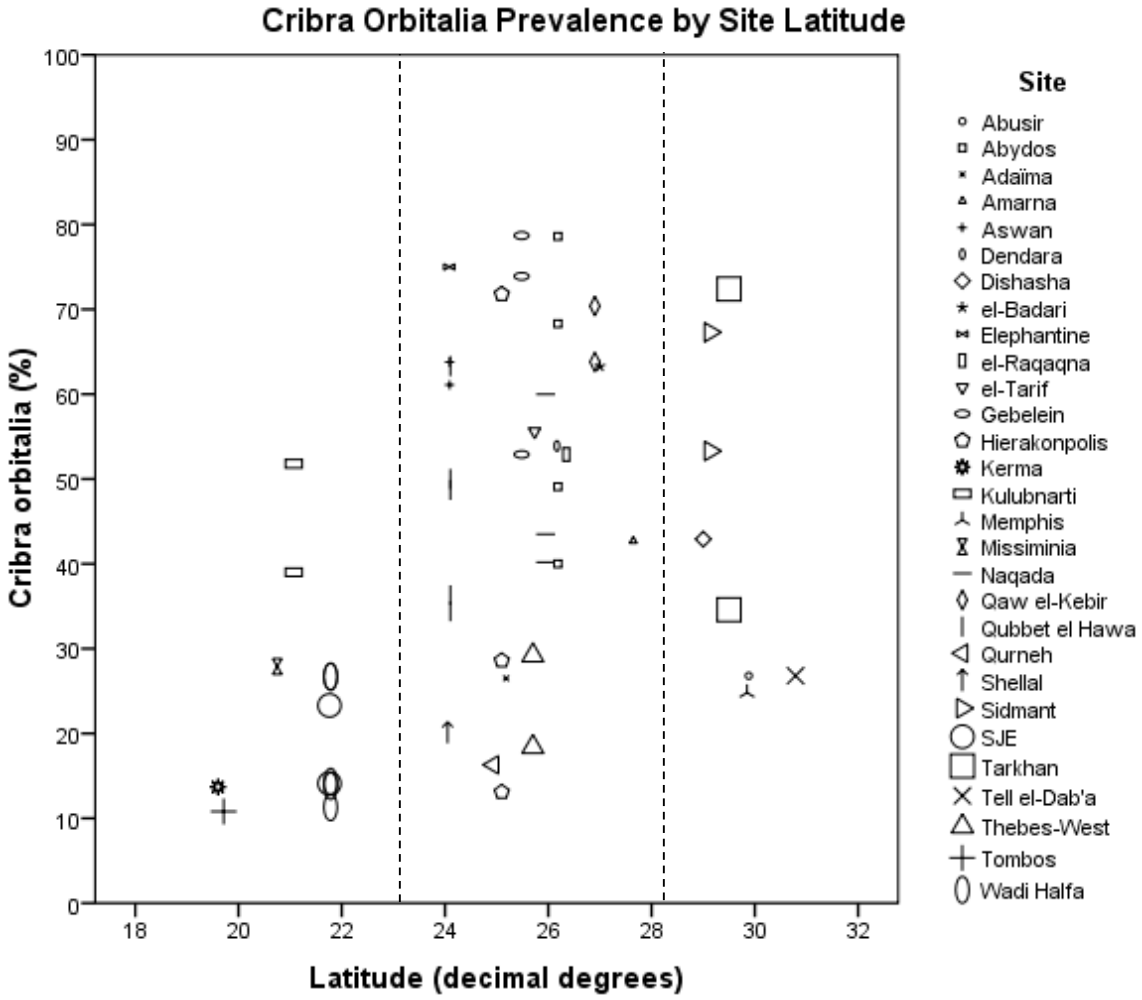


Figure 3.3. Scatterplot showing no trend in cribra orbitalia rate over location in the Nile Valley. Dotted lines delineate the three geographical clusters (Nubia, left; Upper/Middle Egypt, center; Lower Egypt, right).

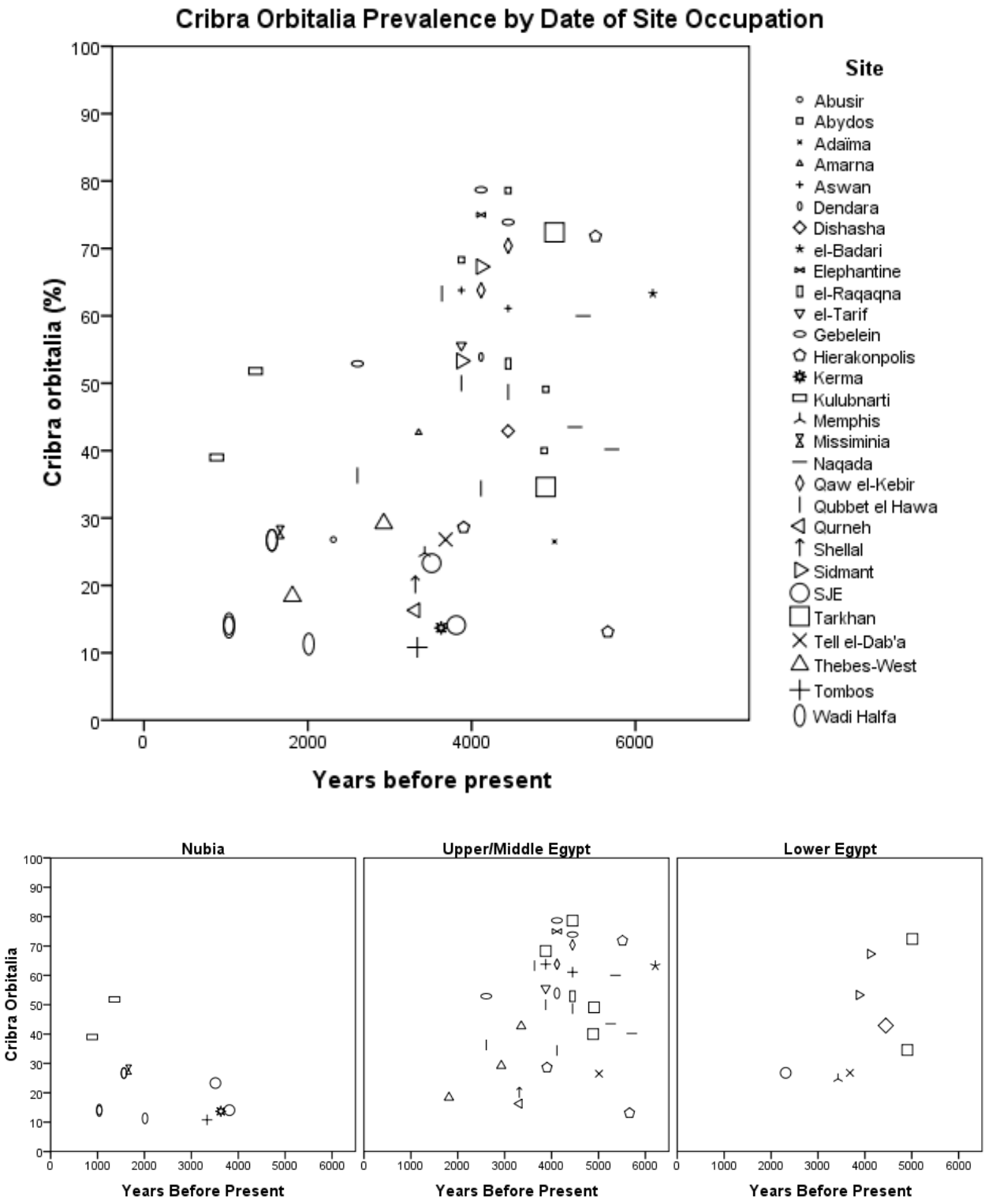


Figure 3.4. Scatterplots showing no trend in cribra orbitalia rate over time in the Nile Valley. Total sample (top) and geographic clusters (bottom).

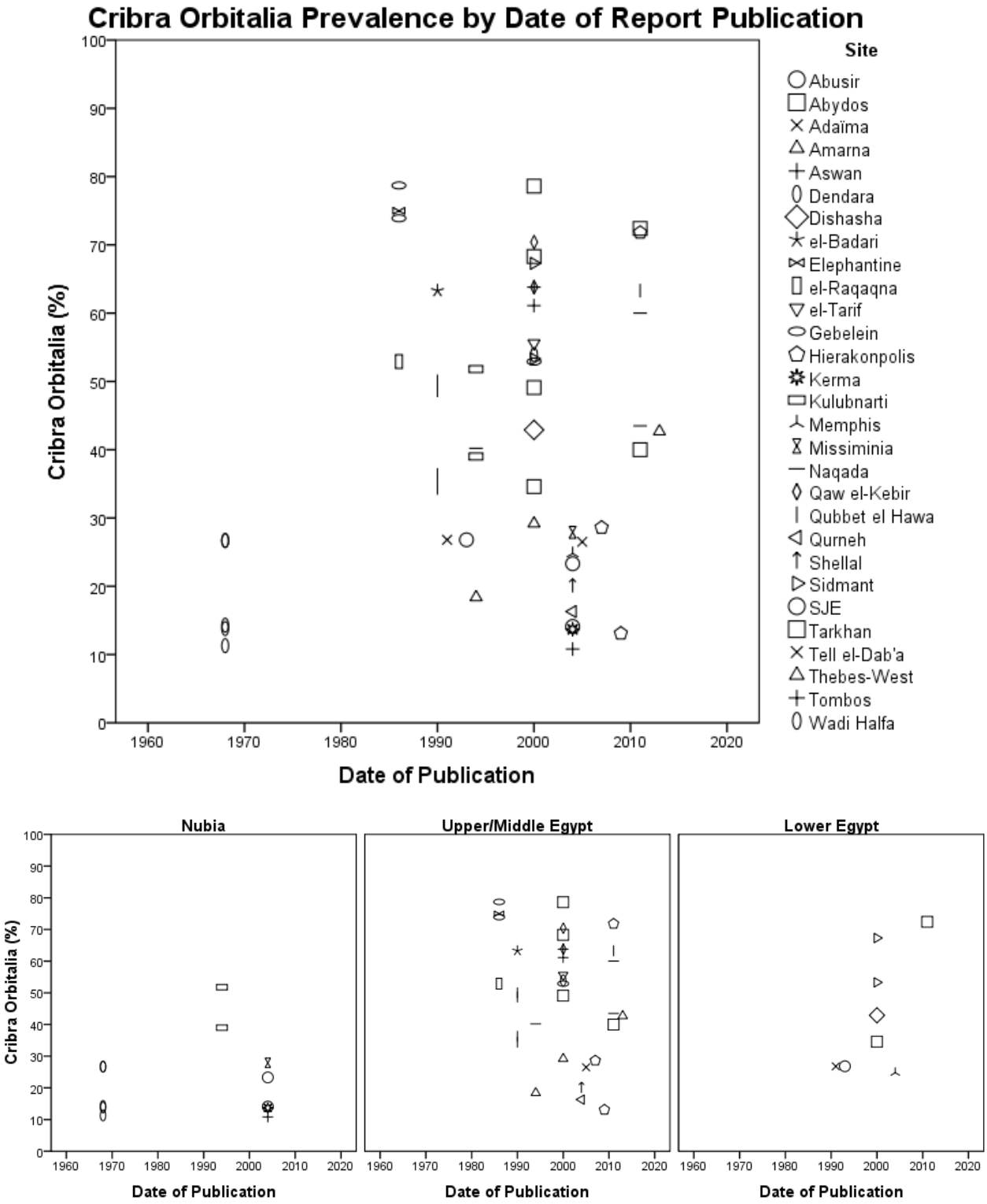


Figure 3.5. Scatterplots showing no trend in cribra orbitalia rate over date of report publication. Total sample (top) and geographic clusters (bottom).

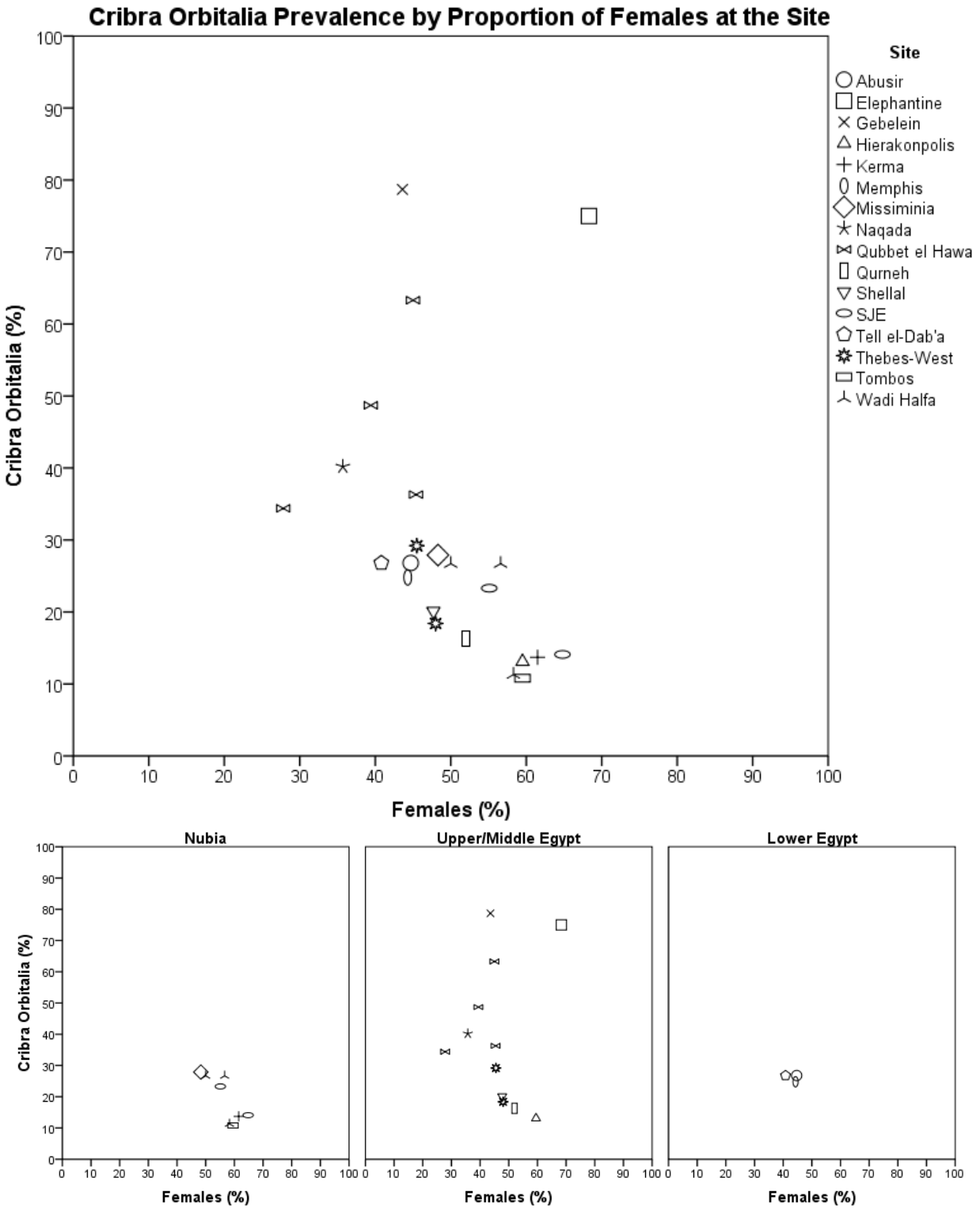


Figure 3.6. Scatterplot showing no trend in cribra orbitalia rate over proportion of females at the site. Total sample (top) and geographic clusters (bottom).

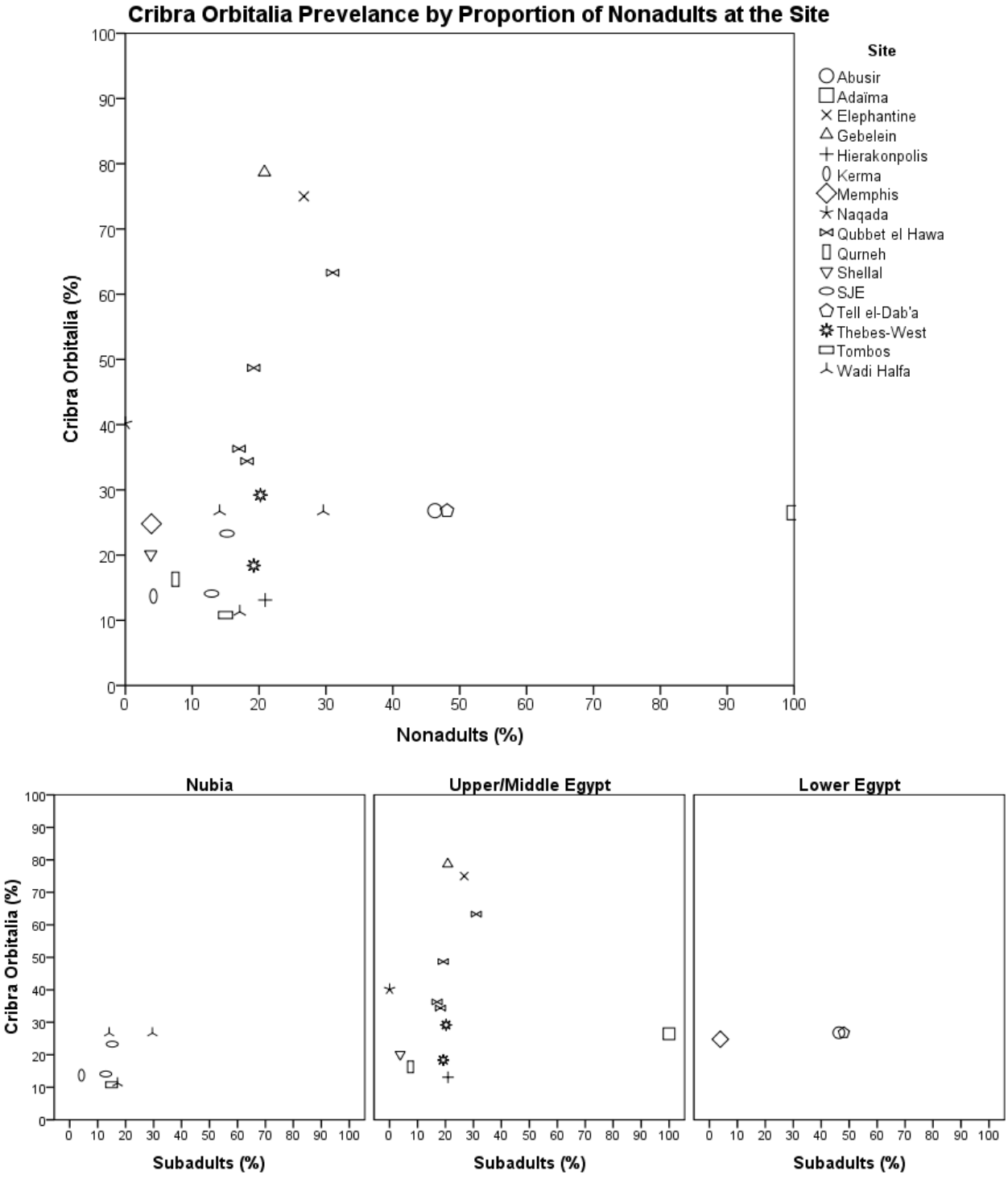


Figure 3.7. Scatterplot showing no trend in cribra orbitalia rate over proportion of nonadults at the site. Total sample (top) and geographic clusters (bottom).

Table 3.3. P-values showing insignificant correlations between cribra orbitalia and other variables within three regions

	Nubia	Upper/Middle Egypt	Lower Egypt
Latitude	0.886	0.327	0.164
Date of occupation	0.129	0.261	0.061
Date of publication	0.822	0.131	0.229
Females	0.061	0.075	-
Nonadults	0.543	0.582	0.221

3.8 Discussion

Although most theoretical models of falciparum malaria's spread out of Africa take place before or during Dynastic Egypt, the physical evidence goes against this model of disease spread. Genetic and immunological studies have provided direct evidence of malaria's presence in numerous Egyptian mummy studies dating as far back as 3200 BCE (Miller et al. 1994; Bianucci et al. 2008; Nerlich et al. 2008; Hawass et al. 2010). This genetic evidence suggests high prevalence of malaria in the ancient Nile Valley, but does not provide information on prevalence rates.

Since cribra orbitalia has been linked to malaria infection in recent literature, an increasing frequency trend in these lesions in the Nile Valley was expected when compared by time and space in accordance with Bruce-Chwatt's (1965) theoretical model. Though cribra orbitalia is likely caused by multiple factors, malaria's synergistic role with other diseases and major impact on modern anemia rates in endemic areas indicate that the presence of this disease in the Nile Valley would have caused a general increase in overall cribra orbitalia rates (Nájera and Hempel 1996; Gilles 1997; Lusingu et al. 2004; Shanks et al. 2008). However, Nile Valley cribra orbitalia rates showed no trend throughout time and space, and were generally high when

compared with New World samples. There was no evidence to suggest malaria, or any other source of increased skeletal anemia, arrived suddenly in the Nile Valley during Late Predynastic through Christian periods. Moreover, there was no association of cribra orbitalia with location, estimated date, proportion of females versus males at the site, proportion of nonadults versus adults at the site, or date of report publication. The lack of any significant trend in cribra orbitalia over space or time highlights the importance of considering holistic trends rather than comparing only the skeletal assemblages of a few sites. The lack of association with publication dates indicated importantly that researchers recognized the lesion during early excavations to the same extent as in present studies.

This study has three implications for interpreting the etiology of cribra orbitalia and health in the Nile Valley. First, the failure to correlate cribra orbitalia frequency with age proportion at the site suggests that the main cause of the high cribra orbitalia rates is not age-specific. Cribra orbitalia is generally considered a lesion formed in childhood, due to the principal location of erythropoiesis in the cranium, thinner cranial bones, weaning stresses, and perhaps inadequate vitamin intake necessary for their growing bodies (Mittler and Van Gerven 1994; Walker et al. 2009). The results of this study go against this assumption, as the amount of nonadults at the site did not affect cribra orbitalia rates. This lack of age-controlled prevalence suggests childhood factors such as diet, exposure to parasitic worms, or nutritional stress caused by weaning did not have a large effect on the formation of this lesion in the Nile Valley. Instead, the main contributing factor seems to be an infectious cause that affects all age groups indiscriminately.

Second, assuming that cribra orbitalia is indeed indicative of malaria infection (as suggested by Rabino Massa and coworkers (2000) and Gowland and Western (2012)), the ubiquity of high cribra orbitalia rates shown in this study suggest this disease had a general high

prevalence in the Nile Valley long before Dynastic Egypt. This implication is supported by the aDNA evidence, and supports earlier theoretical timelines for malaria's spread out of Africa. From the differential diagnosis of the potential causes of anemia in the Nile Valley, it seems reasonable to assume that malaria would have had a great impact on the frequencies of cribra orbitalia in the region. Thus, if the high cribra orbitalia rates in the Nile Valley are tantamount to high malaria rates, malaria must have spread up the Nile Valley and out of Africa before the Badarian period (4400 – 4000 BCE), which is the earliest date used in this study. Alternatively, this higher anemia burden in the Nile Valley sites could simply reflect the multitude of factors combining to cause and aggregate anemia in this region.

Third, Gowland and Western (2012) showed an association of cribra orbitalia with *P. vivax* malaria infection in their meta-analysis of English sites, while the sites used in this study would have included individuals infected with the *P. falciparum* malaria species. The mean rates of the cribra orbitalia frequencies found in the English study and this Nile Valley study differed significantly, perhaps reflecting the higher levels of severe malarial anemia generally associated with *P. falciparum* infections (Billig et al. 2012; Botez and Doughty 2014). This finding is important because it suggests that although *P. vivax* infections tend to involve a chronic, but less severe anemia than *P. falciparum* infections, the latter species is associated with higher rates of skeletal responses to infection.

One of the main limitations of this study involved the clustering of many dates and locations of sites, leading to a greater variability of cribra orbitalia frequencies in these clusters simply because of the greater number of sites. This limitation forced the statistical analysis to follow the clustering by separation into three groups by regional position. This study was also limited by the many sites that had to be excluded because they reported the presence of cribra

orbitalia and porotic hyperostosis together, combined under the name porotic hyperostosis. Nevertheless, the great variation in cribra orbitalia rates of sites included in this study is such that including more sites will not change the absence of a significant association between cribra orbitalia rates and date or latitude.

3.9 Conclusion

This study tested a method of identifying malaria in the Near East, and shed new light on the patterns of health in the ancient Nile Valley by providing a holistic view of anemia present throughout time and space. In compiling cribra orbitalia rates from sites along the Nile Valley from various time periods, no significant association was shown between cribra orbitalia rates and date or latitude. Furthermore, cribra orbitalia rates were not affected by the proportion of females or nonadults in the sample, or by the date of site report publication. These results support the notion of a major infectious causative factor for cribra orbitalia in the ancient Nile Valley, and add credence to previous studies associating cribra orbitalia with malaria. With Gowland and Western's (2012) English malarial sample, this study provided the first interspecific (*P. vivax* versus *P. falciparum*) malaria comparison through large-scale cribra orbitalia frequency comparisons across many sites.

The interpretations of this study rely on the assumption that the hemolytic anemia caused by malaria is responsible for high cribra orbitalia rates, but do not account for additional skeletal lesions that may also be caused by malarial infection. To identify these potential additional skeletal lesions of malaria, future studies are planned involving a clinical comparison in a modern skeletal collection from an endemic malarial area, which will provide better diagnostic criteria for malaria.

Chapter 4 – The skeletal manifestation of malaria: a clinical case-control study

4.1 Introduction

Attention to global climate change and its environmental effects have increased over the last decade, highlighting the importance of understanding tropical vector-borne diseases and their impact on past populations. One of the most ancient of these diseases is malaria, which continues to be a major global health problem today (Nadjm and Behrens 2012). In investigating the impact of malaria on ancient peoples, anthropologists must be able to determine the prevalence of the disease in archaeological populations through its skeletal manifestation. Currently, anthropologists can only suggest malaria prevalence from the presence of porotic hyperostosis and cribra orbitalia on ancient remains under the assumption that these lesions are indicators of hemolytic anemia (Setzer 2014). However, this assumption has never been confirmed and the reliability of these markers never tested for sensitivity (i.e. how well the presence of the lesions correctly identifies malarial individuals) and specificity (i.e. how well the absence of the lesions correctly identifies non-malarial individuals).

This paper develops more reliable diagnostic criteria for identifying malaria on human skeletal remains through the combination of epidemiological and anthropological methods in a case-control model using clinical skeletal collections of known malarial status. The results of this study will be of use to other anthropologists in discerning the prevalence, spread, and impact of malaria, providing more rigorous methods for reconstructing malaria disease patterns and effects on human societies throughout our existence.

4.2 Background

Malaria is often dismissed as a differential diagnosis by paleopathologists, many of whom hold that the disease does not manifest itself upon the skeleton (Nunn and Tapp 2000; Roberts 2000). Recent research on skeletal indicators of anemia, however, has shed new light on malaria's effect on bones. With advances in ancient deoxyribonucleic acid (aDNA) extraction and immunological assay from skeletal and mummified tissues, many researchers have been successful in isolating the antigenetic signatures and aDNA of one species of malaria parasite: *Plasmodium falciparum* (Miller et al. 1994; Bianucci et al. 2008; Nerlich et al. 2008; Hawass et al. 2010). This method, although able to determine the presence of malaria in a population, is costly and destructive, and therefore is not usually performed on all individuals present in a skeletal assemblage. Thus, aDNA can detect presence, but not prevalence of the disease in a past population.

To overcome this problem, anthropologists and biochemists have advanced methods for potential recognition of malaria on human skeletal remains. For example, through DNA and skeletal lesion comparisons, Rabino Massa and coworkers (2000) examined the remains of ancient mummified Egyptians of known positive malarial antigen status for macroscopic indicators of anemia, porotic hyperostosis and cribra orbitalia, and found these skeletal lesions present in 92% of malarial individuals. Their study provided a link between direct evidence for malaria and skeletal lesions previously associated with iron-deficiency anemia. This link was later corroborated through an aDNA study by Nerlich and coworkers (2008).

Further, Walker and coworkers (2009) reasoned that iron-deficiency anemia, long held to be the main cause of porotic hyperostosis and cribra orbitalia in cranial bones, could not in fact produce the bone marrow hypertrophy responsible for producing these lesions. Instead, they

pointed to megaloblastic and hemolytic anemia as the main factors triggering the formation of these skeletal lesions. The former type of anemia arises in individuals with a nutritional deficiency in B12, and the latter arises in individuals with genetic disorders conferring protection from malaria (thalassemia and sickle-cell anemia), as well as in individuals with a malaria infection. Walker's article is still being debated in the literature (Oxenham and Cavill 2010; Rothschild 2012; McIlvaine 2013), but seems to be gaining general acceptance within the field of paleopathology.

Building on the previous two studies, Gowland and Western (2012) showed through a spatial epidemiological approach that the presence of cribra orbitalia lesions in skeletal remains across Great Britain matched with higher *Anopheles* mosquito vector presence, lower altitude and marshy environments, and higher incidences of historically recorded undulating fevers consistent with malarial infection. Their study found a correlation between non-tropical malarial infection and cribra orbitalia, which gives additional support to the hypothesis that malaria manifests itself in the skeleton.

This paper will build on the previous studies by providing an *a priori* means by which to diagnose malarial infection in ancient remains through macroscopic skeletal examination. As mentioned above, genetic studies can provide researchers with direct evidence showing the infection of a skeletal individual with falciparum malaria, but is limited by the costly and destructive nature of the assay, as well as the factors of genetic preservation and disease latency. Therefore, genetic and immunological studies cannot indicate the prevalence of a disease in the entire population, limiting their utility for paleoepidemiologic approaches to the broad reconstruction of disease in the past.

Multiple lines of evidence must be used in describing malarial prevalence in the past, including physical evidence from skeletal remains. Although several studies have linked porotic hyperostosis and cribra orbitalia to malaria and genetic disorders conferring protection from malaria, much is still unknown about the etiology of these skeletal lesions. At the very least, there appear to be multiple factors leading to their manifestation, such as nutrition and parasitic infection (Holland and O'Brien 1997; Wapler et al. 2004; Walker et al. 2009).

4.2.1 Malaria disease dynamics and pathophysiology

Understanding the disease dynamics and pathophysiology of malaria through modern and historical epidemiological studies is paramount to understanding the manifestation of the disease in the past. The identification of the protozoa responsible for malaria only occurred within the last 150 years, and its lifecycle was only recognized within the last 50 years (Sherman 1998). The modes of transmission begin when a gravid *Anopheles* mosquito takes a blood meal from a human infected with malaria. Once in the mosquito's stomach, the stomach acid activates malarial male and female gametocytes, which combine to create an oocyst which forms on the outside of the mosquito stomach and ruptures, releasing thousands of sporozoites which migrate to the mosquito's salivary glands. As the mosquito takes its next blood meal, the sporozoites are injected with the saliva into the human's bloodstream, where they are taken to the liver and therein invade hepatocytes.

Within the hepatocytes, the sporozoites undergo asexual reproduction for a few days, eventually bursting out millions of merozoites into the bloodstream where they invade red blood cells. This is where the falciparum species differs from the others, causing the red blood cells it invades to adhere to the blood vessel walls and to other red blood cells (called rosetting). Researchers believe this rosetting has a major causative effect on the progression of the disease

to cerebral malaria. Once inside the red blood cell, merozoites undergo further asexual reproduction, consume available hemoglobin as food, and simultaneously rupture out of the red blood cells at the same time all over the body, releasing toxins and millions more merozoites, as well as some gametocytes into the bloodstream, causing the high fevers in the host. This cycle in the blood continues until either the host dies or recovers; meanwhile, the next Anopheline mosquito picks up the gametocyctes in its blood meal to complete the life cycle.

Key features of this disease cycle are important in understanding the pathophysiology of the illness caused by malarial infection, and its relation to the skeleton. One of the primary health impacts caused by malaria is hemolytic anemia, often referred to as severe malarial anemia or SMA, caused by the massive, simultaneous destruction of parasitized and unparasitized red blood cells (Sherman 1998). Such hemolytic anemia in malaria has been implicated in skeletal lesions of expanded marrow space (Walker et al. 2009). Moreover, recent research has suggested that the hemolysis during the schizogony phase of malaria infection may contribute to porous skeletal lesion formation due to the release of acid phosphate, free heme, and the malarial pigment hemozoin into the bloodstream. This leads to an imbalance in bone remodeling by stimulating osteoclasts while simultaneously impairing osteoblasts (D'Souza et al. 2011; Moreau et al. 2012). Furthermore, severe malarial anemia may induce extramedullary erythropoiesis, which is known to cause cortical thinning and coarse trabeculation (Al-Aabassi and Murad 2005).

Several risk factors and at-risk members of society have been identified (see Figure 4.1). In general, the main defense against malarial infection is an acquired immunity in individuals whose bodies have experienced such infections in the past. The lack of acquired immunity puts an individual at an increased risk for infection. This is especially relevant for travelers coming

from a non-endemic area into an endemic area, since they lack the immunity acquired during childhood by individuals of the endemic area.

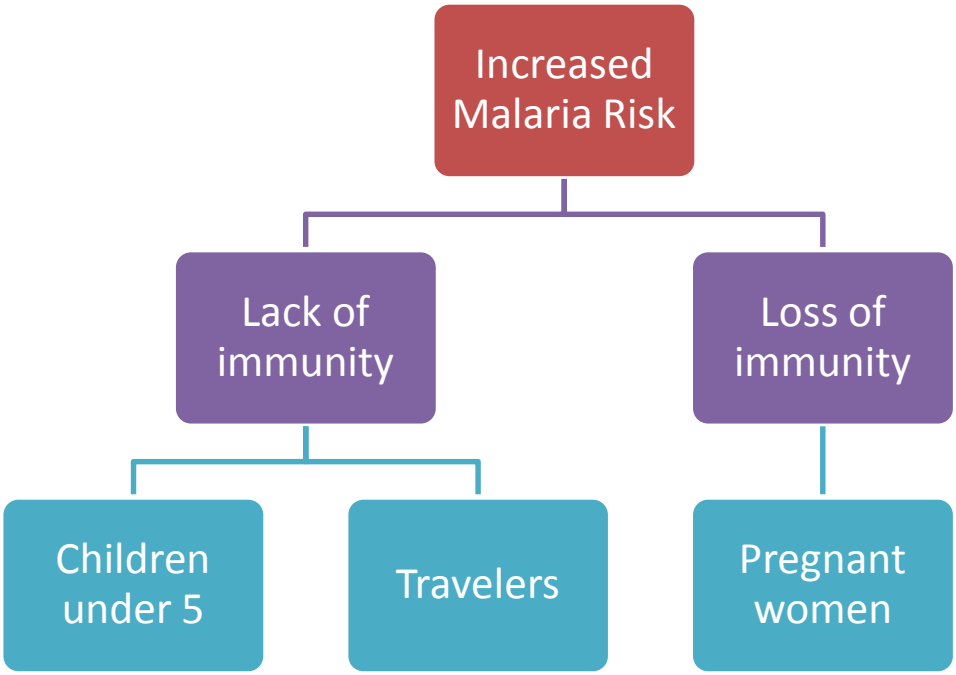


Figure 4.1. Web of risk factors and at-risk groups for malaria infection

Malaria is known to be especially dangerous for pregnant women and children up to age five (Gilles et al. 1969). For women, any acquired immunity to the parasite disappears during pregnancy, leading to life-threatening anemia and increased chances of miscarriage or low fetal birth weight. In order to study this phenomenon in sub-Saharan Africa, Gilles and coworkers (1969) set up a cohort-based longitudinal epidemiological study of pregnant women in which they gave half of the women anti-malarial treatments, and periodically tested all of the patients for anemia and parasitemia. To their surprise, they could not complete the study because all of the women who had not been taking anti-malarial medicine contracted the disease, with some even having to be hospitalized for severe malarial anemia.

Children make up 80% of current global malaria cases, with severe malaria anemia being more common in children up to age five, and cerebral malaria more common in children older than age four (Billig et al. 2012). It has been suggested that not only does lack of acquired immunity factor into the increased burden on children, but that there is also a factor of allometry, with small body sizes and allometric developmental changes in red blood cell production, liver, spleen, and blood flow throughout the body playing a significant part in malaria infection in children (Billig et al. 2012). Lusingu and coworkers (Lusingu et al. 2004) studied malaria parasitemia and anemia rates in children of Tanzanian villages with different endemic malaria statuses. They found that in the highly endemic villages, malaria was the most important cause of parasitic anemia in children.

Adult men are also at high risk during malaria epidemics, especially due to malaria's tendency to combine with other diseases to create a more deadly health outcome. In their analysis of historical medical records from the penal colony on the Andaman Islands in the South Pacific, Shanks and coworkers (Shanks et al. 2008) found that malaria was the most important factor in all-case mortality for men, including tuberculosis and dysentery cases especially. In other words, malaria has a strong indirect effect on mortality due to its synergy and co-infection with other diseases. Similarly, historical accounts from the colonial Carolinas note that the two most deadly diseases of the southern colonies were malaria and dysentery, for which cinchona bark or going "out to sea" were the usual treatments (Duffy 1952). This phenomenon has been noted by many independent researchers, although the cause of this increased mortality has not been identified. The current consensus is that perhaps malarial anemia increases susceptibility for other infectious diseases and lowers immune response generally (Shanks et al. 2008).

From these historical and modern epidemiological studies, it can be hypothesized that ancient populations affected by malaria would show high rates of maternal, fetal, and infant mortality that was directly caused by malaria anemia and cerebral malaria. This preferential mortality dynamic has been argued to have been the case at an archaeological site in Italy (Soren 2003). The men would have also suffered with this disease, likely causing higher rates of respiratory and intestinal disease which combined to cause deadly health consequences. The greatest chance of survival would have come from the presence of abnormal hemoglobins conferring malarial resistance in the blood of some members of the population (Sherman 1998). However, some genetic protectors against vivax malaria result in more severe parasitemia in falciparum malaria. Recent studies of patients negative for the Duffy antigen (used by the *P. vivax* parasite to invade red blood cells), have shown that platelet-mediated destruction of falciparum malaria is ineffective without the Duffy antigen (McMorran et al. 2012). Therefore, members of a population that had increased survival for vivax malaria infection may have experienced a higher mortality rate in a falciparum malaria epidemic.

This paper combines epidemiological and anthropological methods to develop more reliable diagnostic criteria for identifying the disease on human skeletal remains. Two skeletal samples are used in a case-control study format: 98 individuals of known malarial exposure from Uganda (Galloway Osteological Collection sample) and 352 individuals of known non-exposure to the disease from Louisiana, USA (LSU FACES lab sample).

4.2.2 Skeletal samples

4.2.2.1 Galloway Osteological Collection

The Galloway Osteological Collection, housed at the Makerere University Medical School in Kampala, Uganda, is a large medical collection of unclaimed and donated Mulago Hospital patients who died between 1947 and 1980. This collection consists of 592 individuals native to Uganda and neighboring East African countries, many of whom were refugees due to the political turmoil in their home countries (Musoke 1961). Since East Africa is known to be highly endemic for tropical malaria, all of the individuals present in the collection likely experienced multiple infections of malaria during their lifetimes. Additionally, records associated with the collection provide demographic information (i.e. age, sex, tribe) and cause of death for each individual.

Epidemiological studies published in the years during which the collection was being formed give the prevalence and types of malaria and malaria-related disorders seen at Mulago Hospital during that time. One such study that gathered data on 570 pregnant women giving birth at the hospital in 1964-65 reported that 16.1% of placentae tested positive for malaria (Jelliffe 1968). Of those testing positive, 54.3% were infected with *P. falciparum* malaria, 20.7% *P. malariae*, and 4.3% mixed *P. falciparum* and *P. malariae*. The remaining 20.7% were non-diagnostic for parasites. Another study gathered data on children (aged zero to six years) admitted to the pediatric ward of Mulago Hospital in 1950-51 (Musoke 1961). Routine blood slides for malarial parasite identification was performed in 85% of the 1,380 cases registered, and of these, most were identified as *P. falciparum*. There were 181 children admitted for clinical malaria, and an additional 55 cases were identified by blood slides in children admitted for other reasons. Therefore, approximately 20% of children seen at the pediatric ward were

infected with malaria. Sickle cell anemia was identified in 45 of the 66 children positive for sickling (5.6% of the total analyzed). Other studies confirmed the dominance of *P. falciparum* species of malarial parasites in Uganda, with a minor presence of *P. malariae*, and near absence of the other two species (Onori 1967; World Health Organization 2012).

The Galloway collection began as a teaching collection for the anatomy department of the university medical school. Mulago Hospital patients whose bodies were unclaimed were required by law to be buried by the hospital; however, the medical school requested a few bodies to be used for soft-tissue dissection and the remaining skeletal material to be cleaned and used to osteological instruction (William Buwembo, pers. comm. 2013). In addition to its use for teaching purposes, the Galloway collection has been used as a research collection, mostly for osteometric studies.

Unfortunately, handling and use by students over the past seven decades, as well as the change of hands in the administration leading to less-than-ideal storage of the skeletal material, has impacted the preservation of this collection. Many individuals are missing various skeletal elements, most notably cranial elements. A number of individuals listed in the register are missing entirely. Still some others have elements from other individuals commingled in the wrong crate, or consist of elements too greasy or fragmented to be easily observed. All of these preservation and storage pitfalls limit the number of individuals available to be used in unbiased research projects.

4.2.2.2 LSU FACES Lab Collection

The Forensic Anthropology and Computer Enhancement Services (FACES) laboratory at Louisiana State University (LSU) houses upwards of 300 unidentified and donated skeletons

from forensic cases in the state beginning in 1980 and continuing presently. The United States has eradicated malaria and only sees a few cases of malaria per year imported by travelers. Therefore, the individuals present in the collection at the FACES lab are not likely to have ever experienced an infection of malaria in life, and can be used as a control sample for comparison with the Ugandan sample.

By definition, a forensic collection will be less complete in general than a medical collection. The skeletons may have many absent or unobservable elements due to trauma or taphonomic processes from the environment. The preservation of the remains is variable depending on the context in which they were found (i.e. charring from a fire or extreme weathering from exposure to external environmental conditions). Additionally, demographic information and life history of the individuals are not known and must be inferred by the forensic anthropologists based on whatever evidence is obtainable. However, once recovered and placed in the lab, the skeletons are maintained in optimal storage conditions within a climate-controlled lab, in cardboard boxes with careful packing procedures and small elements preserved in cloth bags. To combat mold growth in the humid Louisiana climate, the skeletons are periodically cleaned and re-boxed.

4.3 Materials and methods

The Galloway collection skeletons were analyzed for visible pathologies, with analysis focused especially on porous lesions of the cranial and postcranial skeleton due to their hypothesized association with anemia (Rabino Massa et al. 2000; Djuric et al. 2008; Nerlich et al. 2008; Gowland and Western 2012), but also on other markers of specific or nonspecific infection: periosteal reactions, linear enamel hypoplasias, periodontal disease. The collection of

data proceeded in three phases: (1) individuals whose cause of death was malaria or anemia, (2) matched cases of individuals of the same age, sex, and tribe as malarial/anemic individuals, and (3) all remaining individuals with skulls present. This third phase was unplanned, but deemed necessary due to the paucity of cranial elements in the first two phases. Using this phased approach, the data is comparable to epidemiological matched case-control studies. The total number of skeletons analyzed was 98, with each phase making up approximately one-third of the total sample.

The FACES lab skeletons were analyzed for all visible pathologies, including x-ray analysis for Harris lines on the tibiae. The good preservation of the majority of the collection allowed for complete description of the pathologies present on the skeletons. For comparison with the Ugandan material for this research, more analyses had to be conducted to obtain frequencies of non-mainstream skeletal markers (i.e. spinal porosity, humeral cribra, and femoral cribra). Through correspondences with LSU graduate student Nicole Klein, I coordinated data entry and further investigative probes into this collection to obtain a completely comparable sample. All digitization of the LSU data was gratefully provided by the FACES lab staff for this research.

In order to create a method for identifying malaria prevalence on ancient skeletal material, multiple stages of data analysis were undertaken. First, contingency tables and tests of independence were used to verify significant osteological markers in malarial and anemic individuals versus non-malarial and non-anemic individuals within the Galloway sample. Markers with a higher prevalence in the anemic group were then tested against each other to determine associations between each marker. Next, the Galloway sample was compared with the control sample from the LSU FACES lab for significant differences in the frequencies of

markers found to be prevalent in the above tests. Based on the assumption that the Galloway sample contains individuals who have at some point been infected with malaria and that the LSU sample contains individuals who have never been infected, each skeletal lesion was evaluated for its diagnostic power by substituting the skeletal lesions for symptoms in epidemiological properties of diagnostic power, following Boldsen's (2001) example. These properties are defined as:

1. $\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive+False negative}}$
2. $\text{Specificity} = \frac{\text{True negative}}{\text{True negative+False positive}}$
3. $\text{Positive Predictive Value} = \frac{\text{True positive}}{\text{True positive+False positive}}$
4. $\text{Negative Predictive Value} = \frac{\text{True negative}}{\text{True negative+False negative}}$
5. $\text{Positive Likelihood Ratio} = \frac{\text{Sensitivity}}{1-\text{Specificity}}$
6. $\text{Negative Likelihood Ratio} = \frac{1-\text{Sensitivity}}{\text{Specificity}}$
7. $\text{Diagnostic Odds Ratio} = \frac{\text{Positive Likelihood Ratio}}{\text{Negative Likelihood Ratio}}$

To form diagnostic criteria for identifying malaria prevalence in past populations, methods followed those described by Pinhasi and Turner (2008), which incorporate an epidemiological outcome algorithm with the type of data with which paleopathologists work. This method calculates prevalence rates based on differentially weighted criteria; applying an "if" condition comparing skeletal manifestations of malaria and their relationship to each other to diagnose the disease. The algorithm formulated for malaria was then tested using a case-control study format to determine how well it identifies people with the disease and those without.

4.4 Results

4.4.1 Anemic versus non-anemic within endemic sample

The data collected from the Galloway collection skeletons was divided into two samples: an anemic sample (those whose reported cause of death included malaria or anemia; *n*=27), and a non-anemic sample (those who died of other causes; *n*=71). Malarial and anemic individuals were grouped together due to the fact that malaria is one of the most significant causes of anemia in Sub-Saharan East Africa (Kassebaum et al. 2014), and the other two major causes (i.e. hookworms and iron-deficiency) would be categorized separately as having "malnutrition" or "hookworm anemia" as the cause of death. The anemic and non-anemic samples were not significantly different in demography (age group and sex). Five porous skeletal lesions were identified that appear at high frequencies, and especially in the anemic sample. These appear on the cranium (cribra orbitalia and porotic hyperostosis), vertebral column (including vertebral and sacral elements, see Figure 4.2), and humeral and femoral necks (see Figure 4.3 and Figure 4.4). Included among these are all of the features of Djuric's (2008) "cribrous syndrome" for anemia (cribra orbitalia, humeral cribra, and femoral cribra). The frequencies of these porous lesions, along with frequencies of other non-specific inflammatory lesions – periostitis, alveolar resorption (periodontitis), and linear enamel hypoplasias (LEHs) – are shown in Figure 4.5. None of the frequency differences was found to be significant when tested for association with chi-square and Fisher's exact tests.

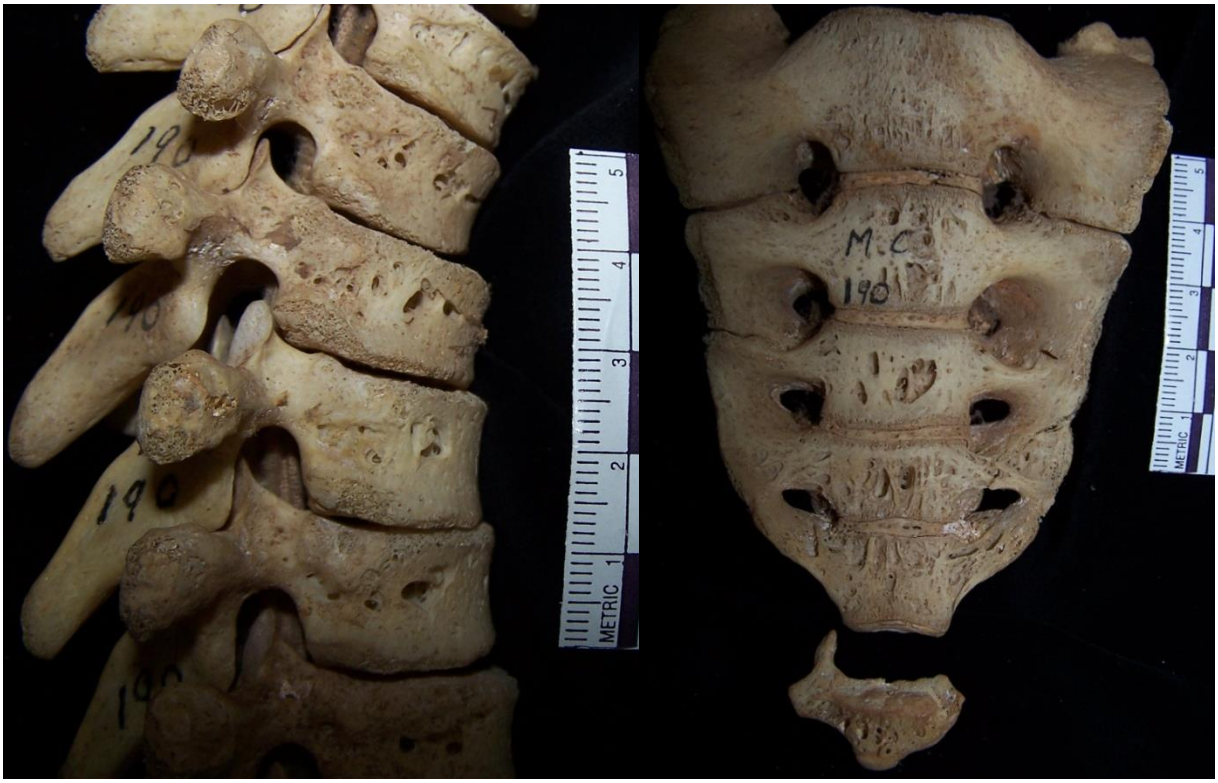


Figure 4.2. "Spinal porosity" on the vertebral (left) and sacral (right) bodies of individual MC190. Photos by Nicole E. Smith.

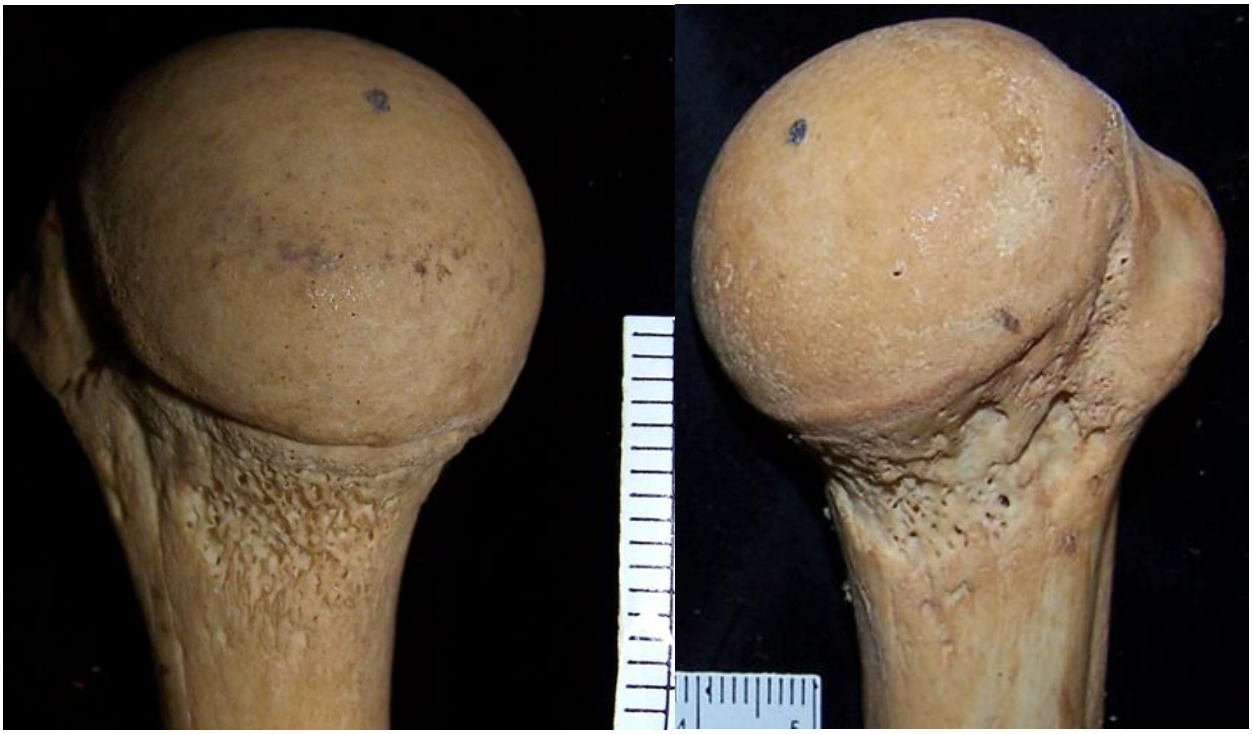


Figure 4.3. “Humeral cribra” on individual MC100 (left) and individual MC53 (right). Photos by Nicole E. Smith.

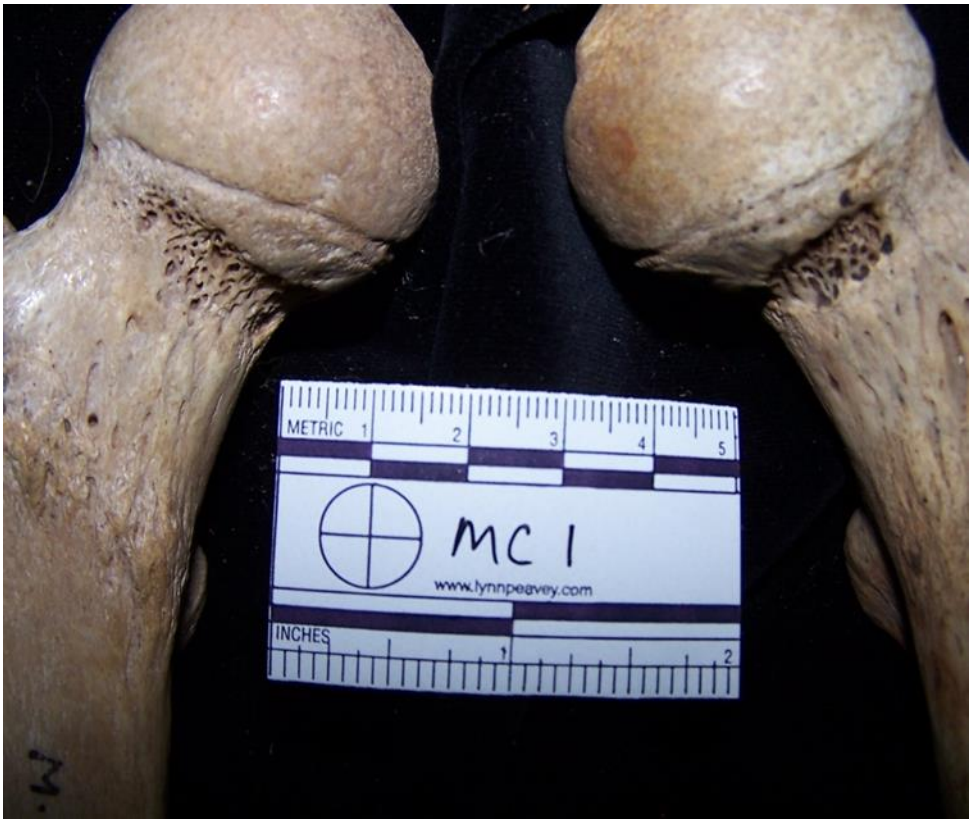


Figure 4.4. "Femoral cribra" on individual MC1 (bilateral). Photo by Nicole E. Smith.

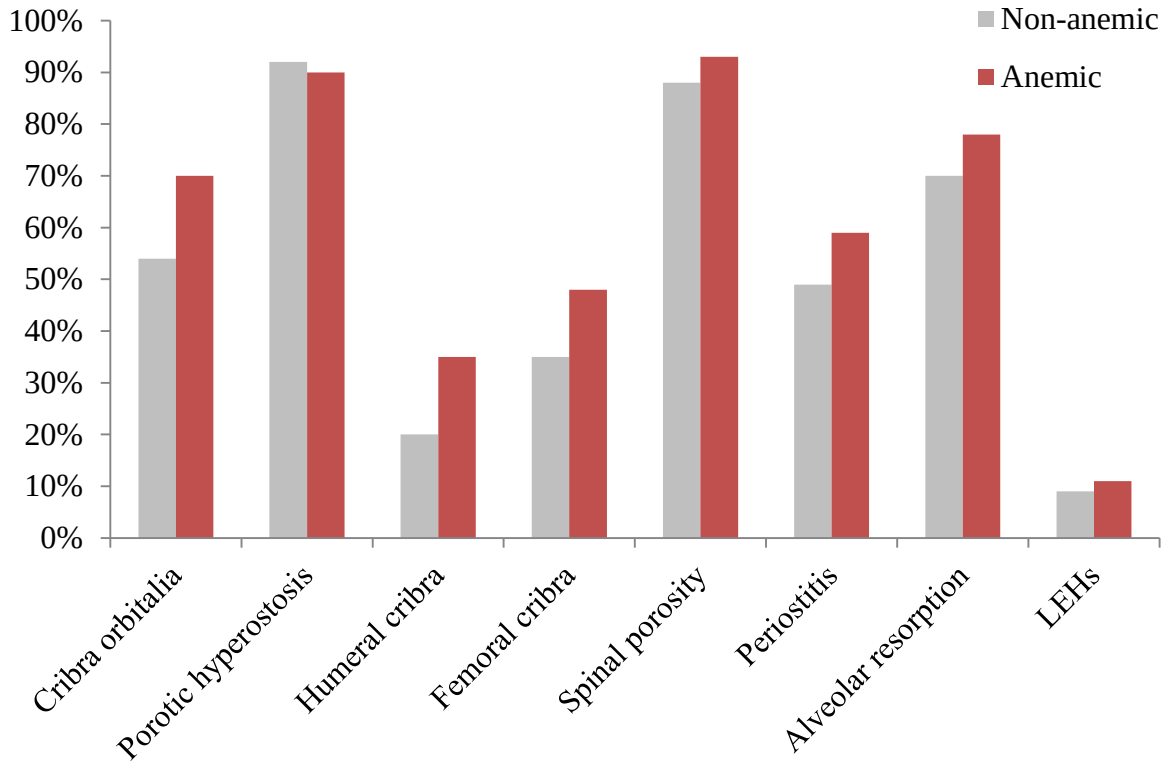


Figure 4.5. Comparison of skeletal lesions in anemic vs non-anemic within the Galloway sample

Only humeral cribra and femoral cribra were associated with age at death, and both showed a greater prevalence of the lesion in younger individuals (Student's t-test, p=0.000 and p=0.003, respectively). None of the lesions were significantly linked with sex.

To determine associations between the skeletal lesions, each lesion was tested for association with each other lesion (Table 4.1). Cribra orbitalia presence was significantly associated with the presence of cranial vault porosity (porotic hyperostosis), and the odds of the linked presence of both lesions was also significant. Although humeral and femoral cribra were highly associated (Fisher's exact, p<0.000), neither feature was found to be associated with cribra orbitalia, perhaps signifying that multiple factors contribute to the development of cribra orbitalia in this population. The presence of periostitis was associated with spinal porosity and

femoral cribra, perhaps suggesting that all three of these lesions are part of the same inflammatory response.

Table 4.1. Relationship of prevalent lesions to each other within the Galloway sample

	CO[1]	PH	HC	FC	SP	P	AR	LEH
CO								
PH	FET, p=0.012* OR 9.7 (CI 1.1-86.4)*							
HC	FET, p=0.199 OR 2.9 (CI 0.7-12.0)	FET, p=1 OR 1.8 (CI 0.2-17.2)						
FC	FET, p=0.051 OR 3.5 (CI 1.1-11.7)	FET, p=0.405 OR 3.8 (CI 0.4-34.0)	FET, p=0.000** OR 8.3 (CI 2.7-25.7)					
SP	FET, p=0.686 OR 0.5 (CI 0.1-2.8)	FET, p=1 OR 0.9 (CI 0.1-8.9)	FET, p=0.673 OR 2.4 (CI 0.3-20.9)	FET, p=0.143 OR 5.0 (CI 0.6-42.5)				
P	χ^2, p=0.520 OR 1.4 (CI 0.5-4.0)	FET, p=0.648 OR 0.5 (CI 0.1-3.3)	χ^2, p=0.545 OR 1.4 (0.5-3.7)	χ^2, p=0.050* OR 0.4 (CI 0.2-1.0)	FET, p=0.042* OR 5.3 (CI 1.1-26.7)			
AR	FET, p=0.755 OR 0.7 (CI 0.2-2.5)	FET, p=0.624 OR 1.7 (CI 0.3-11.3)	FET, p=0.710 OR 0.7 (CI 0.2-2.8)	χ^2, p=0.176 OR 0.4 (CI 0.1-1.5)	FET, p=0.657 OR 0.4 (CI 0.0-3.5)	χ^2, p=0.962 OR 1.0 (CI 0.3-3.2)		
LEH	FET, p=0.636 OR 2.8 (CI 0.3-27.2)	FET, p=1 OR 0.9 (CI 0.1-8.4)	FET, p=1 OR 0.7 (CI 0.1-7.2)	FET, p=1 OR 1.3 (CI 0.2-8.3)	FET, p=1 OR 1.2 (CI 0.1-11.7)	FET, p=0.358) OR 0.3 (CI 0.0-2.5)	FET, p=0.137 OR 0.2 (CI 0.0-1.5)	

[1]Abbreviations in headers: CO=cribra orbitalia; PH=porotic hyperostosis; HC=humeral cribra; FC=femoral cribra; SP=spinal porosity; P=periostitis; AR=alveolar resorption; LEH=linear enamel hypoplasias
* Significant at the 95% confidence interval
**Significant at the 99% confidence interval

4.4.2 Endemic versus non-endemic sample

The second stage of testing involved the comparison of the skeletons from the Galloway collection (endemic sample of individuals with malaria exposure; *n*=98) to the LSU FACES lab

skeletons (non-endemic sample of individuals unexposed to the disease). The non-endemic sample was limited to only those individuals with African-American ancestry (n=106) in order to minimize the potential for a confounding effect of the sickle cell trait on the results. The demography between the two samples was significantly different (age at death: χ^2, df=5, p=0.017; sex: χ^2, df=1, p=0.014), so the results of their comparison could be impacted by this difference. All of the non-dental lesions were found at much higher frequencies in the endemic sample than the non-endemic sample (Figure 4.6).

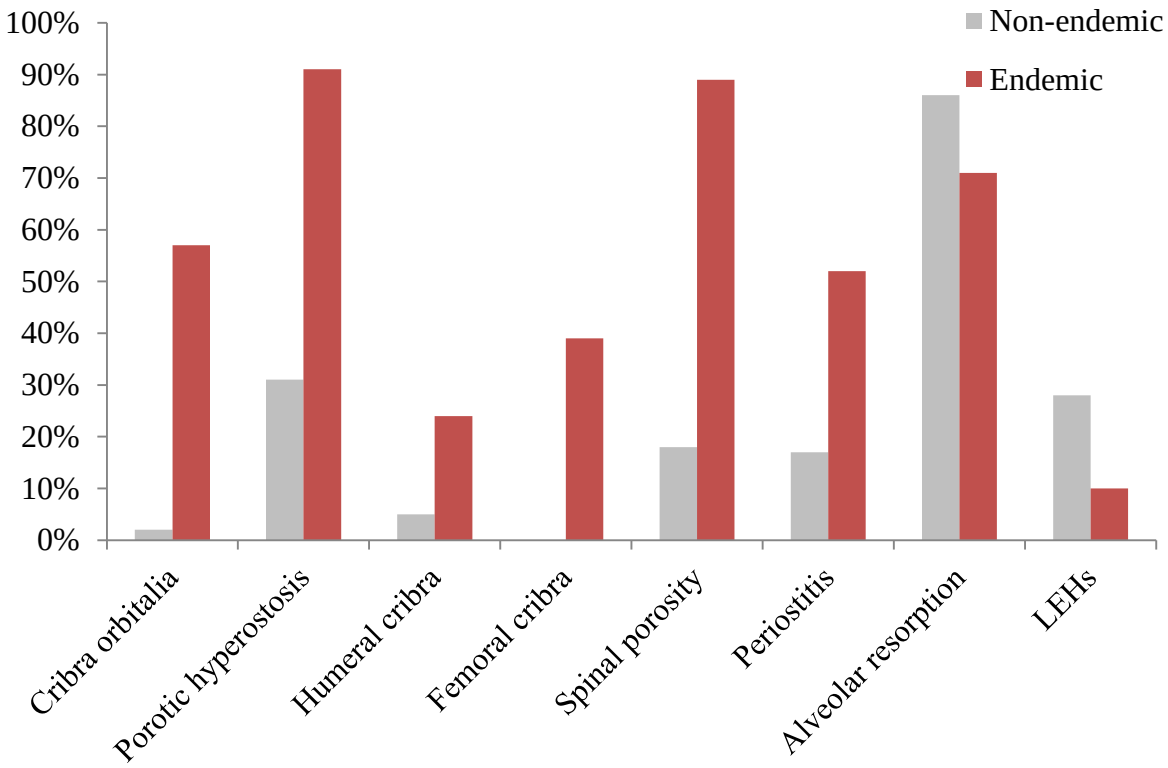


Figure 4.6. Comparison of skeletal lesions in endemic vs non-endemic populations

Highly significant associations were found for endemicity with cribra orbitalia, porotic hyperostosis, spinal porosity, femoral cribra, and periostitis (Fisher's exact, p<0.000), and humeral cribra (Fisher's exact, p=0.006). Alveolar resorption was more common in the non-

endemic sample, but not significantly so. Linear enamel hypoplasias associated significantly with the non-endemic sample (χ^2, df=1, p=0.017). Frequencies and Odds Ratios for all lesions by sample are recorded in Table 4.2.

Table 4.2. Frequencies of lesions in endemic and non-endemic samples

	Endemic	Non-endemic	Odds Ratio for Endemicity
Cribra orbitalia	33(56.9%)**	1(1.8%)	73.9 (95% CI = 9.6 – 571.1)*
Porotic hyperostosis	53(91.4%)**	20(30.8%)	23.9 (95% CI = 8.3 – 68.7)*
Spinal porosity	84(89.4%)**	9(18.4%)	37.3 (95% CI = 14.1 – 99.1)*
Humeral cribra	21(23.6%)**	2(4.5%)	6.5 (95% CI = 1.5 – 29.1)*
Femoral cribra	37(38.5%)**	0(0.0%)	29.5 (95% CI = 3.9 – 222.8)*
Periostitis	51(52.0%)**	9(17.3%)	5.2 (95% CI = 2.3 – 11.8)*
Alveolar resorption	37(71.2%)	43(86.0%)	0.4 (95% CI = 0.2 – 1.1)
Enamel hypoplasia	5(9.6%)	15(27.8%)*	0.3 (95% CI = 0.1 – 0.8)

* Significant at the 95% confidence interval
**Significant at the 99% confidence interval

From the frequencies of the lesions, we can eliminate alveolar resorption and linear enamel hypoplasias as effective diagnostic markers of malaria. Based on the assumption that the endemic sample contains individuals who have at some point been infected with malaria and that the non-endemic sample contains individuals who have never been infected, we can evaluate each skeletal lesion for its diagnostic power. Substituting the skeletal lesions for symptoms in epidemiological calculations of sensitivity and specificity following Boldsen's (2001) example, each marker is evaluated in Table 4.3. The absence of cribra orbitalia is a good indicator of the absence of malaria (false negative rate < 2%), but the presence of the lesion cannot be used alone

to diagnose malaria (false positive rate > 43%). Conversely, porotic hyperostosis presence is a good indicator of malaria (false positive rate < 9%), but it also yields false negatives in more than 30% of cases. Spinal porosity tested fairly well in both sensitivity and specificity, yielding false positives in about 10% of cases and false negatives in about 20%. Humeral and femoral cribra both tested similarly, with poor sensitivities (62 – 76% false positives) and excellent specificities (0 – 5% false negatives). Finally, periostitis presence produced 48% false positives and 18% false negatives.

Table 4.3. Epidemiological properties of diagnostic power for skeletal markers of malaria

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Positive Likelihood Ratio	Negative Likelihood Ratio	Diagnostic Odds Ratio
Cribra orbitalia	0.569	0.983	0.971	0.691	32.431	0.439	1.404
Porotic hyperostosis	0.914	0.692	0.726	0.900	2.970	0.125	0.807
Spinal porosity	0.894	0.816	0.903	0.800	4.865	0.130	1.129
Humeral cribra	0.236	0.955	0.913	0.382	5.191	0.800	2.391
Femoral cribra	0.385	1.000	1.000	0.443	37.113*	0.615	2.223*
Periostitis	0.520	0.827	0.850	0.478	3.001	0.580	1.779

* Positive likelihood ratio and diagnostic odds ratio are not solvable for femoral cribra as there were no false negatives. The ratios for this marker were estimated by adding 0.5 to all the counts of this marker, though it should be stated that this introduces a bias in the results.

4.4.3 Forming an Outcome Algorithm for Case Diagnosis

From Table 4.3, we see that the diagnostic markers perform fairly well, with the exception of porotic hyperostosis (diagnostic odds ratio below unity). Therefore, porotic hyperostosis was henceforth excluded from the diagnostic criteria formulated due to its poor

diagnostic performance. To successfully diagnose malaria on ancient skeletons, the remaining markers can be used to define a set of diagnostic criteria. By differentially weighting the appearance of the markers and their relationship to each other (see Table 4.1), such a refined criterion should be possible. Using Pinhasi and Turner's (2008) example equation, we arrive at the following logical expression for an "if" condition, or outcome algorithm, for malaria:

$$C_i = 1 \text{ if } \{(\text{CO or HC or FC} = 1) \text{ AND } (\text{SP or P} = 1)\}; \text{else } C_i = 0$$

where C_i is case i in a skeletal sample and the diagnosis is coded in binary classification: '1' denotes positive diagnosis for malaria, whereas a '0' value denotes a negative diagnosis. The skeletal markers are scored similarly (1 for presence of the lesion; 0 for absence of the lesion) and abbreviated as CO: cribra orbitalia; HC: humeral cribra; FC: femoral cribra; SP: spinal porosity; and P: periostitis.

This algorithm was tested using the total Galloway collection (*n*=98) and total LSU collection (*n*=352) samples. Of these samples, there were only 142 individuals observable for all of the lesions specified in the outcome algorithm: 75 Ugandans and 67 Americans. The model produced two false positives (3%) and 23 false negatives (30%). The diagnostic test characteristics are shown in Table 4.4.

The false negatives produced can be explained by the presence of individuals in the Galloway sample who were not infected with malaria at the time of death, and whose malarial markers have resorbed. This reasoning was confirmed by retesting the diagnostic power of the algorithm with only those individuals in the Ugandan sample whose cause of death included anemia or malaria (*n*=20) selected as the positive gold standard (see right columns in Table 4.4). Though the sample size was lower, the number of false negatives was cut in half, leading to only 15% of the anemic East Africans incorrectly diagnosed as not having malaria.

Table 4.4. Diagnostic test characteristics for skeletal malaria outcome algorithm with total Ugandan sample included (left) and with only anemic Ugandan sample included (right)

	Total Ugandan sample			Anemic Ugandan sample		
	Estimated Value	Lower CI	Upper CI	Estimated Value	Lower CI	Upper CI
Sensitivity	0.693	0.575	0.792	0.850	0.611	0.960
Specificity	0.970	0.887	0.995	0.970	0.887	0.995
Positive predictive value	0.963	0.862	0.994	0.895	0.655	0.982
Negative predictive value	0.739	0.632	0.824	0.956	0.868	0.989
Positive likelihood ratio	23.227	5.882	91.710	28.475	7.182	112.894
Negative likelihood ratio	0.316	0.225	0.445	0.155	0.054	0.439
Prevalence	0.528	0.443	0.612	0.230	0.149	0.335

4.5 Discussion

The results of this study have identified five skeletal lesions that were shown to be indicative of malarial infection: cribra orbitalia, spinal porosity, humeral cribra, femoral cribra, and periostitis. These lesions were all found to occur at high rates in the Galloway collection individuals, especially in those whose cause of death included malaria or anemia. Periostitis is described in general by paleopathologists as an inflammatory reaction of the periosteum, which is considered a non-specific stress indicator. This skeletal lesion is seen in most specific infectious diseases (Pinhasi and Mays 2008). In the case of malaria, periostitis likely arises due to the systemic infection and high fevers. Cribra orbitalia, humeral cribra, and femoral cribra have all been implicated previously as a joint trifecta of anemia indicators called "cribrous syndrome" (Miquel-Feucht et al. 1999; Djuric et al. 2008). From the associations in the Galloway collection, we see that all three of these features do indeed appear at higher

frequencies in anemic individuals, and that humeral and femoral cribra are strongly associated. However, cribra orbitalia shows no association with the other two lesions, suggesting that different etiological factors contribute to their development. Similarly, cribra orbitalia tended to affect people of all ages, whereas humeral and femoral cribra trended significantly toward younger individuals.

In bioarchaeological contexts, if cribrotic lesions, spinal lesions, and periosteal reactions are seen at high frequencies in a skeletal sample, it is likely that the overall population contained some cases of malaria. This is due to the fact that the specificities of all of these lesions are above 80%, with the cribrotic lesions all over 90%. To estimate overall prevalence of malaria at a site, the outcome algorithm should be used to score each skeleton individually for the combination of these lesions. Additional demographical profiles of the sample population can provide evidence of the endemicity of the disease. High proportions of women and children with skeletal markers of malaria at the site could reflect the higher malarial risk in these demographical groups within endemic areas. Conversely, if all age and sex groups are affected by malaria equally, this could reflect the dynamics of epidemic malaria where all members of the population were at risk for disease.

4.5.1 Etiological implications

Femoral cribra is a new name for an old feature (e.g. the "reaction area" or the "cervical fossa of Allen") that has long undergone discussion amongst physical anthropologists in the last century as to its etiology (Angel 1964; Finnegan 1978; Meyer 1924; Radi et al. 2013a). Under these names, femoral cribra tends to be viewed together with other features on the femoral neck (i.e. Poirier's facet), and described as an activity-related morphological variant. A wide range of specific activities have been suggested as to the causation of this feature (e.g. sleeping position,

walking downhill, squatting, etc.), but no consensus has ever been reached (Radi et al. 2013). Inconsistent naming schemes have led to confusion and miscommunication regarding what the feature entails. Even more confusing, those publishing about the anemia etiology of "femoral cribra" failed to acknowledge the existence of the other proposed activity-related etiology and vice versa.

Considering the etiology of femoral cribra as it appears in the Galloway sample, this lesion appears to be related to anemia and linked with humeral cribra, and it appears predominantly in younger individuals. From the positioning of lesions on the long bones at the region of epiphyseal fusion, it seems logical to assume that these two features arise during development, while the long bones are still growing at the metaphyses. In anemic individuals, humeral and femoral cribra could be explained as cortical defects that form as the epiphyses fuse at the growth plate due to the increased need for red blood cell production (erythropoiesis). Cribra orbitalia and porotic hyperostosis have been explained by similar processes, where the need for increased erythropoiesis forces expansion of the medullary cavity in the cranium.

These epiphyseal defects may also be related to the extramedullary erythropoiesis known to occur in hematological diseases (i.e. splenomegaly in malaria), where the increased need for erythropoiesis results in the formation of a red blood cell producing tissue mass located outside of the medullary cavity (Johns and Christopher 2012). This phenomenon sometimes appears in CT-scans of living individuals with thalassemia as variable, tumor-like tissue masses located just adjacent to the cortical surface of a bone, and feeding into it (Al-Aabassi and Murad 2005). This interpretation must be taken with caution, however, due to a lack of consensus and need for further understanding of extramedullary erythropoiesis and its etiology in the current clinical

medical literature. Nevertheless, this phenomenon could very well play a part in the skeletal markers that prevail in individuals from endemic areas for malaria.

The spinal porosity described in the Galloway collection was compared with, and determined to be similar to, lytic cavitation of vertebral bodies characteristic of brucellosis infection (Ortner 2003). When comparing the spinal porosities common in anemic individuals to the spinal lesions present in an individual with known brucellosis infection at the time of death, the anemic individual's spine porosity appears similar in morphology (i.e. sharp-edged cavitations with no associated reactive bone) but with smaller pores in general (see Figure 4.7). As brucellosis and malaria are similar in many attributes, including the induction of high, undulating fevers in their patients and hemolytic anemia, it is not surprising that they also share a similar skeletal manifestation. A differential diagnosis of this lesion is the erosive lesion characteristic of spinal tuberculosis ("Pott's disease"), however, this lesion tends to be focal, affecting only a few vertebrae as large cavitations of the vertebral bodies leading to eventual vertebral collapse (Mann and Hunt 2005). The spinal lesions present in the Galloway collection individual with brucellosis were on the anterior aspects of the vertebral bodies and quite large in diameter (up to 12mm on the vertebrae and 17.5mm on the sacrum). In contrast, the lesions seen on anemic individuals tended to be on the lateral aspects of the vertebral bodies and much smaller in diameter (up to 5.5mm).

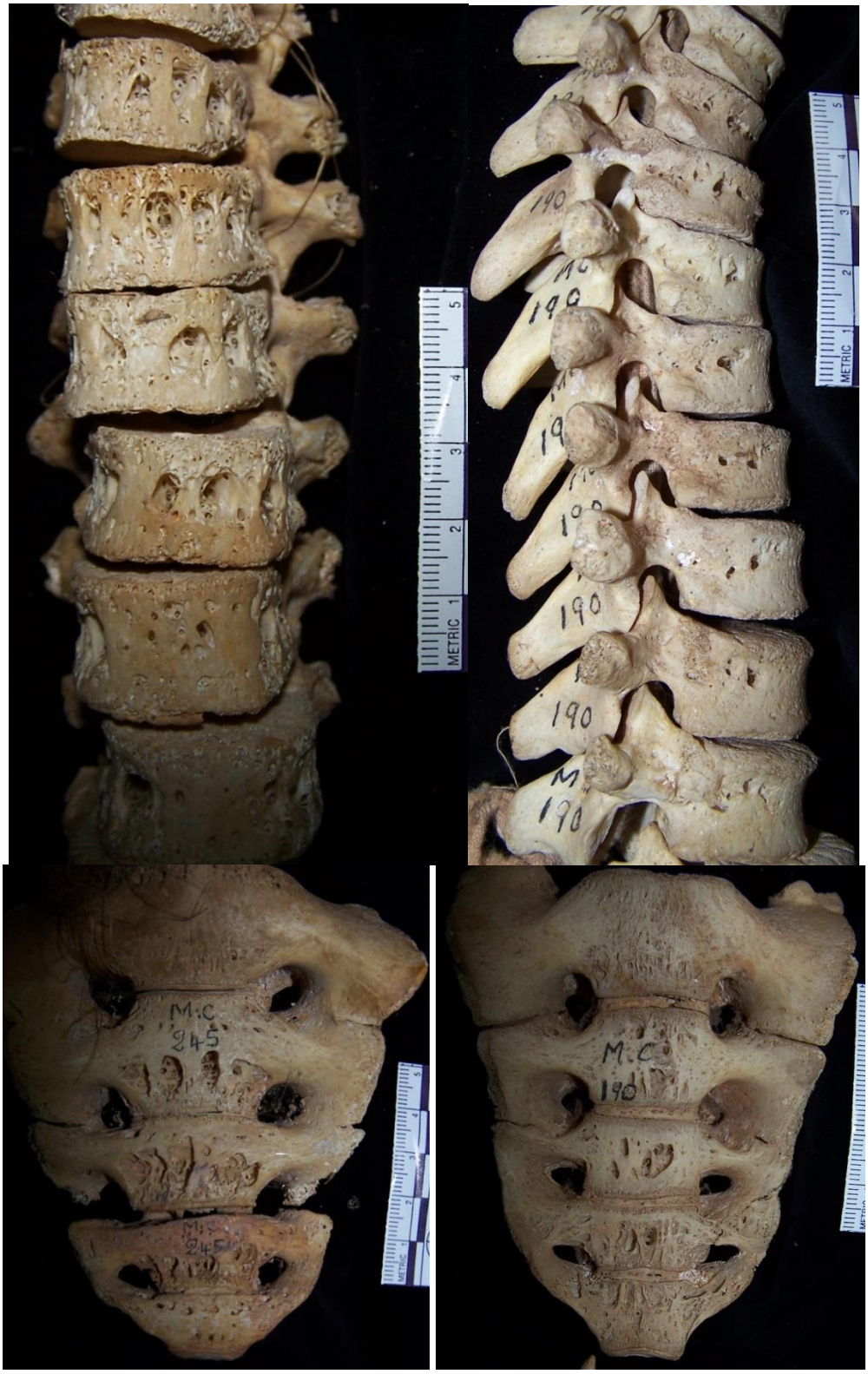


Figure 4.7. Comparison of porous spinal lesions between brucellosis patient (left) and anemia patient (right) from the Galloway collection. Photos by Nicole E. Smith.

4.5.2 Limitations

Paleopathological diagnoses are limited inherently by the inability to know the symptoms of the individuals by which to assign a particular disease. Here, skeletal lesions were used instead of symptoms and tested through associations in known clinical cases. The significant lesions were then used as a gold standard for testing the diagnostic power of the outcome algorithm, although they are not a true gold standard for many reasons. The Galloway collection includes many individuals with other known and unknown health afflictions, such as tuberculosis, malnutrition, and hookworm anemia. These conditions were not likely to be as prevalent in the LSU collection, and therefore, could have influenced the resulting lesion frequencies. Nevertheless, the majority of the lesions assessed in the Galloway collection had been associated with malaria or anemia previously (although indirectly), providing more confidence in the associations reported in this study. Further studies with different skeletal samples in endemic malarial areas are needed to provide more evidence toward the true gold standard of malaria's skeletal manifestations.

As mentioned earlier, there are many diseases that co-infect with malaria, including tuberculosis and dysentery. Recent aDNA studies have illuminated this subject by identifying tuberculosis and malaria co-infection from human mummified tissue in Egypt (Lalremruata et al. 2013). The presentation of these co-infections on the skeleton is unknown at the present, but may provide interesting avenues for future paleopathological studies of malaria.

4.6 Conclusion

This study identified five skeletal markers of malaria through an epidemiological case-control study approach using clinical samples of known cause of death or malaria exposure. The prevalent lesions were then tested for diagnostic power through measures of sensitivity and

specificity. An outcome algorithm was created from the associations of these markers that will provide a diagnostic tool for identifying malaria on unknown cases in archaeological contexts. Etiological interpretations of the causes for these skeletal lesions pointed to hemolytic anemia and general systemic stress as the main contributing factors leading to their manifestation in malarial individuals.

The use of this model for identifying malaria on human skeletal remains must be taken with caution until it has been repeated successfully with additional skeletal samples of known medical history. Nevertheless, the diagnostic power estimates of the skeletal lesions identified in this study provide paleopathologists with a means for suggesting the potential presence and prevalence of malaria in ancient populations. Future research will seek further validation of this diagnostic model through aDNA comparisons.

Chapter 5 – The prevalence of malaria at Amarna, Egypt and its regional implications

5.1 Introduction

One of the most popular, mysterious moments in ancient Egyptian history is the Amarna Period (1349–1332 BCE), during which substantial shifts in religion and location of the capital city took place. Some scholars have attributed this abrupt religious and geographical shift to epidemic disease occurring before, and perhaps continuing during, the reign of the pharaoh Akhenaten (Moran 1992; Nunn 1996; Assman 2003; Kozloff 2006). Several members of Akhenaten's royal family are known to have died early, and several had malarial infections at their time of death (Redford 1984; Tyldesley 2003; Hawass et al. 2010). Though malaria is known to have been present in ancient Egypt, the prevalence and dynamics of this disease in the region remain largely unknown.

This paper explores the potential prevalence of malaria at Amarna as evidenced by the human skeletal remains of the people that lived in the capital city. Skeletal manifestations of malarial infection are assessed by the presence of cribra orbitalia, humeral cribra, femoral cribra, spinal porosity, and periostitis. These data are integrated into a diagnostic outcome algorithm and contextualized through demographic data and archaeological evidence of burial ritual. This paleoepidemiological approach to malarial prevalence in the past broadens our understanding of factors leading to this distinctive period in history and malaria's role in broader sociopolitical events in the Near East at the end of the Late Bronze Age.

5.2 Background

The ancient site of Amarna, Egypt represents a unique window into the life of ancient Egyptians as it is the only site in the Nile River Valley that remained unoccupied until the Amarna Period (1349–1332 BCE) and abandoned thereafter. While other Egyptian locales tended to have multiple occupations at the same site, rebuilding upon the same structures repeatedly since predynastic times, Amarna is a single occupation site, decisively founded on unoccupied ground in a veritable wasteland for purification purposes with the newly founded cult of Aten (Kemp et al. 2013). From a bioarchaeological prospective, this precisely dated 17-year occupation period offers the opportunity to glean information about this exact time period in ancient Egypt, and study important aspects of individual and populational health trends that are not obfuscated by expansive time ranges or multiple generations in the same burial context.

Although the rock-cut tombs of the elite population at Amarna are immediately obvious in the cliffs nearby the city, no human remains were found inside, and no non-elite cemeteries were discovered before 2002 (Rose and Zabecki 2009). The newly discovered non-elite cemetery, named the South Tombs cemetery was excavated from 2006 to 2013, and produced 438 skeletal individuals. These skeletal remains were then analyzed by a team of researchers and students associated with the University of Arkansas Bioarchaeology Field School at Amarna each summer until completion in 2014.

5.2.1 Amarna and the Hittite Plague of 1320 – 1300 BCE

In ancient Turkey (Anatolia), at the end of the 14th Century BCE, the Hittite king wrote a series of prayers pleading with the gods for relief from a widespread, 20-year epidemic that had already killed the two preceding kings, and continued to ravage his country. These ancient texts, known as the Plague Prayers of Mursili II, reveal that this deadly epidemic was brought by

Egyptian prisoners of war taken to the Hittite capital city (Singer 2002). Amarna's part in this ordeal involved a series of letters written by a prominent female widow in Egypt (generally accepted to be King Tutankhamun's widow, Ankhesenamun) to the Mursili II's father, King Suppiluliuma, in which she begged for a Hittite prince to marry (Schulman 1978). The death of this prince on his way to marry the Egyptian queen instigated the battle in which the prisoners of war that caused the plague were taken. With the lack of Hittite burials and the tendency of ancient Egyptians to omit negative historical events in their writings, the disease responsible for the epidemic has never been identified definitively (Emre 1991).

Tumultuous events in Egypt leading up to this point in history, as well as other evidence of epidemic disease mentioned in the ancient Amarna Letters texts, suggest Egypt may have been stricken by the same epidemic disease as the Hittite empire (Moran 1992; Assman 2003; Kozloff 2012; Dodson 2014). The pharaoh Akhenaten suddenly changed the Egyptian religion and founded his new capital city of Amarna in a previously uninhabited area, which remained the capital for only 17 years (c. 1349–1332 BCE). Some scholars have attributed this abrupt religious and geographical shift to epidemic disease, perhaps even polio or bubonic plague (Nunn 1996; Kozloff 2006). Importantly, Akhenaten also defunded the Egyptian military, preferring diplomacy within the existing Egyptian borders than the widespread military campaigns in the borderlands that prevailed in previous dynasties.

The end of this period in Egyptian history signified the abandonment of Amarna, scattering its occupants to the far reaches of the empire. During Tutankhamun's reign, the military campaigns were reinstated, with troop redeployment under Horemheb to re-establish territories in the Near East, including the Egyptian-Hittite border. Along with the dispersion of

people, any epidemic disease present at Amarna could also have spread throughout the Egyptian empire, potentially affecting those Egyptian prisoners subsequently taken to the Hittite capital.

5.2.2 Malaria in the Near East

Evidence of malaria's presence in the Near East has been intimated both directly and indirectly. Direct evidence using ancient DNA (aDNA) and malarial antigen detection of the *Plasmodium falciparum* species has verified a malarial presence in Egypt as far back as 3200 BCE (Miller et al. 1994; Cerutti et al. 1999; Rabino Massa et al. 2000; Bianucci et al. 2008; Nerlich et al. 2008; Hawass et al. 2010; Lalremruata et al. 2013). Similarly, several independent researchers have found evidence of genetic polymorphisms conferring resistance to malaria in ancient Anatolia (Dogan Alakoc and Akar 2011). These molecular methods, although useful, are fraught with limitations, including contamination, molecular non-survival, specimen destruction, and expense, that make their application difficult especially in archaeological contexts (Nielsen 2001; Zink et al. 2002; Setzer 2014).

The indirect evidence of malaria in the Near East comes mainly from human skeletal remains with lesions characteristic of beta-thalassemia. Thalassemia is a disease caused by a genetic deficiency of the hemoglobin that confers resistance to malaria. Due to its prevalence in Mediterranean populations in modern times, thalassemia was originally thought to be responsible for the high frequencies of porotic lesions in crania from Near Eastern archaeological sites (Angel 1966; Angel 1972; Angel 1978). However, more specific evidence is required to separate the true cases of thalassemia from the other disorders that may cause porotic cranial lesions (Caffey 1937). Such confirmed cases with postcranial anomalies characteristic of thalassemia have been identified in a few Near Eastern sites dating as far back as approximately 6000 BCE off the coast of Israel (Hershkovitz and Edelson 1991). This indirect evidence suggests the

widespread presence of one of the malarial parasites in the Near East; however, it is unknown whether this malarial presence was caused by the *P. vivax* or *P. falciparum* species.

5.2.3 Malaria transmission dynamics

Teasing out the plausibility of malaria as a potential cause of the Hittite plague requires more information on the disease transmission dynamics in the region. Malaria is a complex disease that requires multiple factors for its continued success within a region. Primarily, a substantial population of the correct mosquito vector (genus *Anopheles*) must be present, with access to pools of standing water in which it can breed. Though mosquitoes are difficult to find in the archaeological record, the near continuous belt of three *Anopheles* mosquito species known to be effective malaria vectors in the region today suggests their presence in antiquity (Alten et al. 2000; Sinka et al. 2010; Manguin 2013). The paleoclimate data from the Late Bronze Age in the Near East suggests a warm and wet period during the time of the Hittite Plague, providing an even better niche for mosquito proliferation (Bernhardt et al. 2012; Kaniewski et al. 2013).

When the conditions are right, malaria can gain a foothold within a population. However, there are three types of malaria transmission which manifest differently in terms of at-risk groups, immunity, and periodicity (see Table 5.1). Areas of stable, or holoendemic, malaria transmission occur in tropical regions where temperature and rainfall levels are suitable for uninterrupted mosquitoes and malaria presence year-round. Here, the groups at highest risk for infection include young children who have yet to build up an acquired immunity to the disease, and pregnant women who lose their acquired immunity during their term of pregnancy (Carter and Mendis 2002; Botez and Doughty 2014). In areas of unstable (or hypo-endemic) malaria transmission, there are gaps in contact between mosquitoes and humans, leading to potential

partial losses of acquired immunity depending on the duration of the non-contact period. Thus, this transmission dynamic can have serious health repercussions for all age groups, and lead to periodic epidemics of malaria within the endemic area (Carter and Mendis 2002; Botez and Doughty 2014). Finally, in non-endemic areas where there is little or no malarial transmission under normal circumstances, an epidemic of malaria would have devastating health consequences for all members of the population due to the total lack of acquired immunity.

Table 5.1. Carter and Mendis' characteristics of malaria transmission chart (2002)

Type of malaria	**Geographical location(s)[a]**	**Malaria inoculation rates**	**Protective immunity in the population**	**Transmission characteristics**
Stable malaria	Sub-Saharan Africa	Regular, low to very high	High in older age groups; low in children under 5 years	Perennial or seasonal; regular contact between vectors and human hosts
Unstable malaria	(Europe) and Mediterranean, Asia and Western Pacific, (North), Central and South America and Caribbean	Irregular, low to medium	Unreliable in older age groups; absent in children under 5 year old	Perennial or seasonal; irregular contact between vectors and human hosts
Epidemic malaria	Highland areas of tropical Africa; Central Asia and Caucuses; Asia and Latin America	Rising suddenly, low to medium	Low or absent in all age groups	Very variable, subject to sudden and rapid change

[a] Malaria is not at present endemic in the geographical locations shown in parenthesis.

These transmission types are intrinsic to the trifecta of environmental conditions, mosquito population density, and human population density. From the paleoclimate reconstructions and large capital cities in Egypt and Anatolia at the end of the Late Bronze Age, it appears the conditions may have been right for endemic malaria. This endemicity would likely have been unstable due to the cool winter weather precluding mosquito activity in both of these

regions. This study will assess the human skeletal remains recovered at Amarna in order to clarify this theory of malarial endemicity in the region and shed light on the potential for malaria to have caused the Hittite Plague.

5.3 Materials and methods

To determine the probability of endemic malaria at ancient Amarna, Egypt, 405 skeletons from the South Tombs Cemetery were analyzed for the features found to be associated with modern cases of malaria (see previous chapter). The cemetery is located in a dry channel, or *wadi* next to the elite South Tombs carved into the cliffs, and was excavated in roughly four clusters of units to probe the extent of the cemetery and sample its differential burial practices during use (see Figure 5.1 for plan of the site). These sections of the cemetery seem to have been populated in chronological order as follows: the Wadi Mouth Site (the closest to the elite tombs and, therefore, assumed to be oldest section), Lower Site, Middle Site, Upper Site, and Wadi End site. As the Wadi Mouth Site abuts and appears similar to the Lower Site (no significant differences), these sections are combined and subsumed under "Lower Site" for the rest of this chapter. The Wadi End site had too few individuals to be representative (n=8) and was, therefore, excluded from statistical testing.

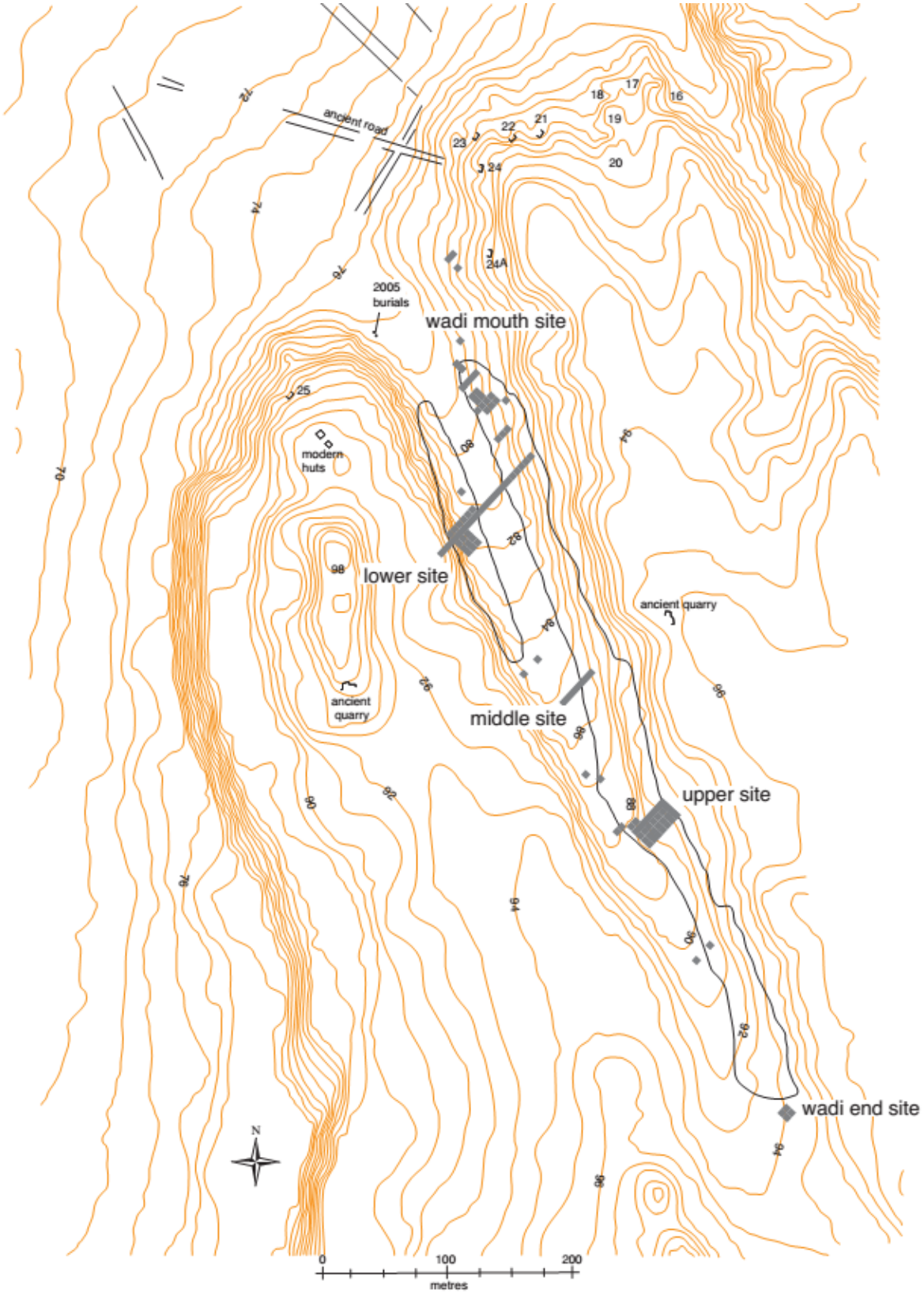


Figure 5.1. Plan of the Amarna South Tombs Cemetery

Demographic and stature data calculated by the Amarna bioarchaeology team, as well as burial characteristics noted by the excavation team, were assessed for differences by cemetery section in order to determine potential changes over the time during which the cemetery was used. Early patterns at the Wadi Mouth and Lower cemetery sites probably reflect health during the time just prior to the Amarna period because the people buried here would not have spent many years at Amarna before their time of death. However, later patterns at the Middle and Upper sites should reflect health of people who spent many years of their lives at Amarna.

Each individual was assessed for the presence of cribra orbitalia and periosteal reactions by the bioarchaeological field school team. Heidi Davis gratefully undertook the assessment of 38 individuals for humeral cribra, femoral cribra, and spinal porosity during the 2014 field season. Further femoral cribra data was obtained through photographs and datasheets of individuals from previous seasons in which the antero-inferior neck was visible or noted as having a "reaction area." The lack of data for the individuals other than the 38 scored by Davis forced conservative statistical analysis. Prevalence of malaria was determined by inputting the individuals into an outcome algorithm shown to be effective in diagnosing malaria from the skeleton:

$$C_i = 1 \text{ if } \{(\text{CO or HC or FC} = 1) \text{ AND } (\text{SP or P} = 1)\}; \text{else } C_i = 0$$

where C_i is case i in a skeletal sample and the diagnosis is coded in binary classification: '1' denotes positive diagnosis for malaria, whereas a '0' value denotes a negative diagnosis. The skeletal markers are scored similarly (1 for presence of the lesion; 0 for absence of the lesion) and abbreviated as CO: cribra orbitalia; HC: humeral cribra; FC: femoral cribra; SP: spinal porosity; and P: periostitis. This diagnostic estimate was performed twice; once with all individuals included, and once with just the 38 individuals observed by Davis for all of the

lesions. The crude prevalence rate (CPR) was determined by the proportion of positive diagnoses versus (observable) negative diagnoses, and the confidence intervals determined according to Brown and coworkers (2001). The true prevalence rate (TPR) for each sample was estimated according to Rogan and Gladen's (1978) methods.

All statistical analysis was performed in IBM SPSS 22.01. It is important to note that when confronted with missing variables in logical expressions, SPSS can determine true values, but never false values. In other words, if enough markers are present that the individual would be determined to be positive anyway, the missing values are irrelevant. However, in the case that the observable markers fail to determine a positive outcome and there are missing variables that *could* have led to a positive diagnosis, the individual is not diagnosed. This method is conservative, but may lead to an overestimation of true prevalence.

5.4 Results

As a way to evaluate the possible existence of endemic or epidemic malaria at Amarna, the demographic variables, stature, burial patterns, and skeletal lesions indicative of malaria are assessed here. Assessment of demography consisted of 353 individuals grouped into ten-year ranges. The largest age group (42%) contains children under the age of 16 (Figure 5.2), and of this age group, childhood mortality peaks around birth and two years of age (Figure 5.3). These trends did not differ significantly across the three cemetery sections (χ^2, df=10, p=0.063).

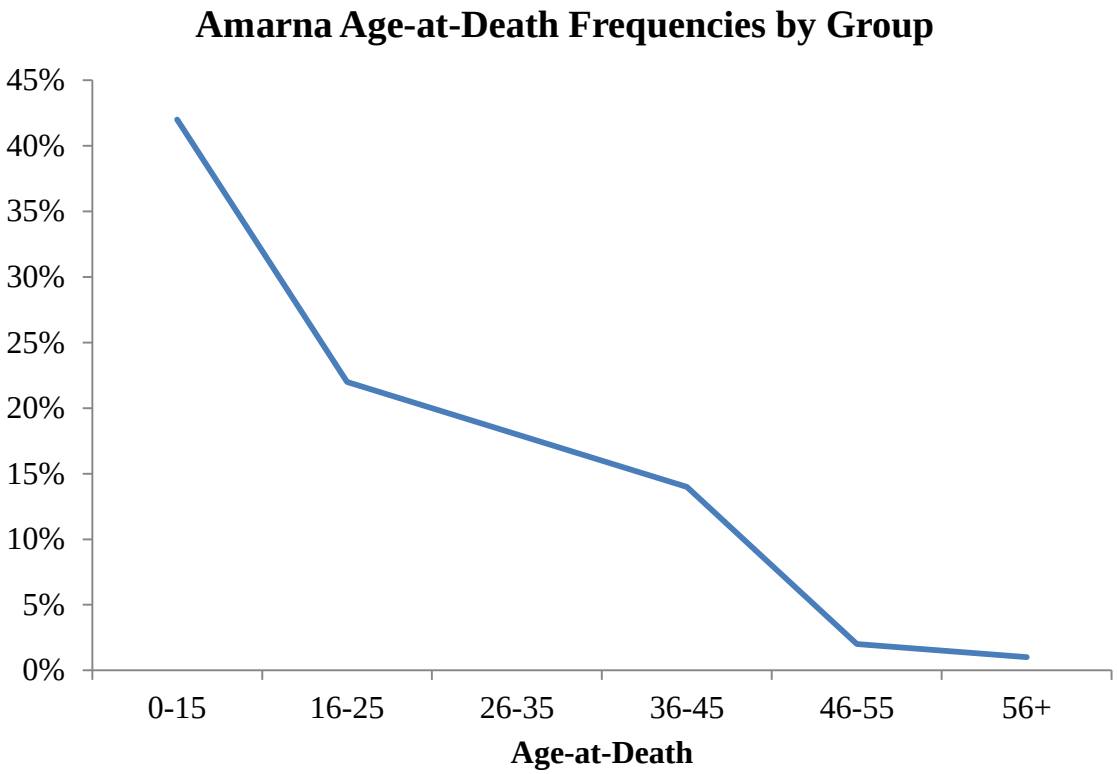


Figure 5.2. Amarna age-at-death frequencies by group

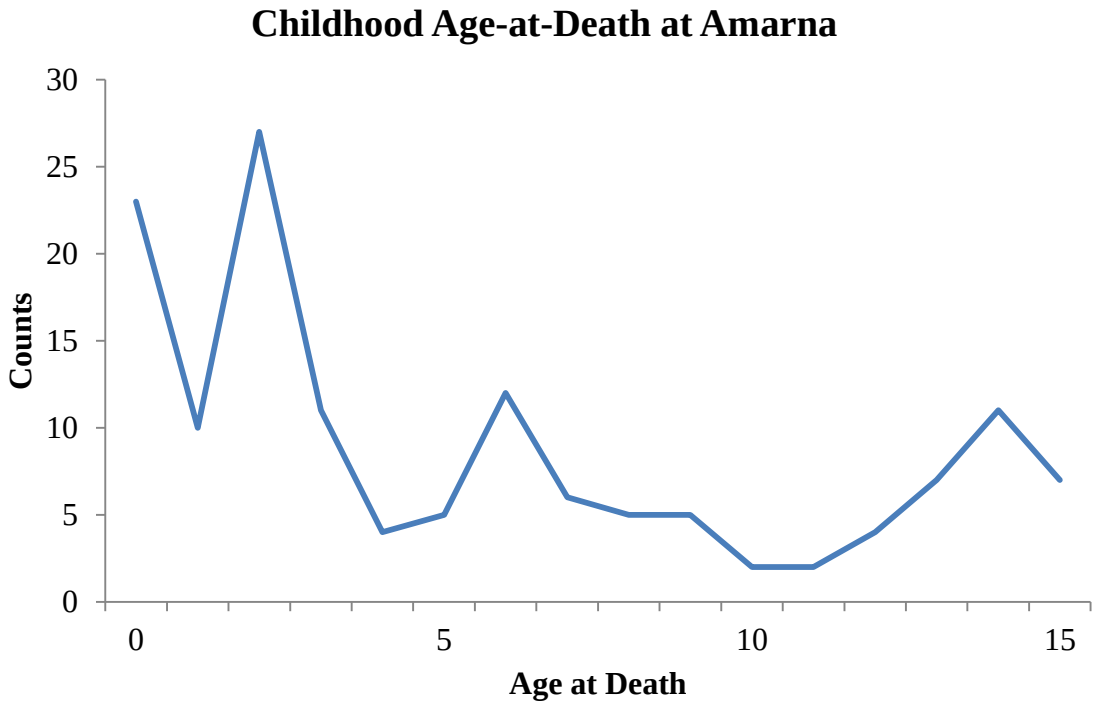


Figure 5.3. Childhood age at death at Amarna

In adults of determined sex, a total of 124 females and 87 males were assessed. Adults of indeterminate sex were excluded from the statistical assessment. Females were more common (59%) than males at the site in general (Figure 5.4), but this difference was not statistically significant.

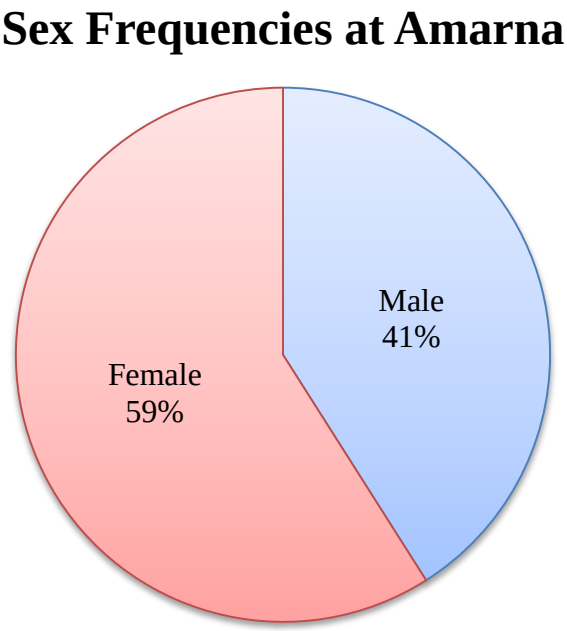


Figure 5.4. Sex frequencies at Amarna's South Tombs Cemetery

Multiple burials, in which more than one person was found buried in the same burial pit, increased incrementally over time of the cemetery's use (Figure 5.5; χ^2, df=2, p<0.000). Multiple burials were also more likely to contain females than males (χ^2, df=1, p=0.025). There was no significant difference observed for the age group frequencies associated with single versus multiple burials. Interestingly, there was also no significant difference in burial treatment

between single and multiple burials, with similar frequencies of people buried in wood or mud coffins and plant material matting in single and multiple burials.

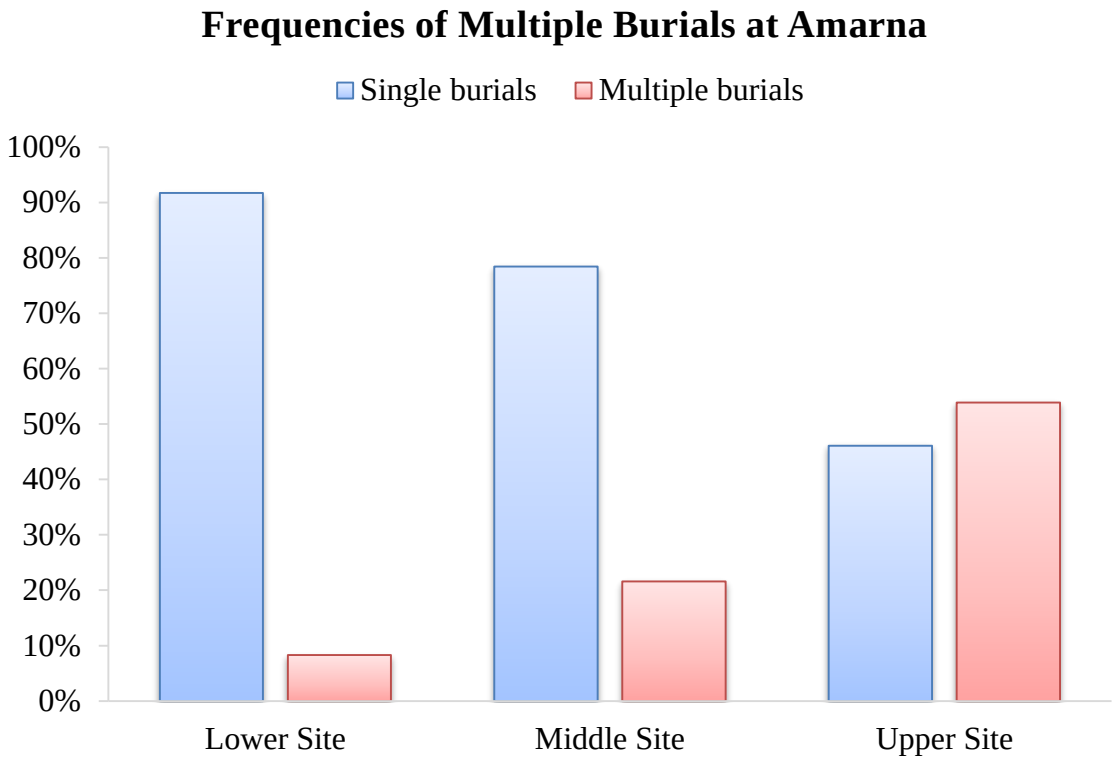


Figure 5.5. Frequencies of multiple burials by section of the South Tombs Cemetery at Amarna

Table 5.2. Frequencies of multiple burials by sex at Amarna

	Females	Males	Total
Single burial	85(54%)	72(46%)	157
Multiple burial	38(72%)	15(28%)	53
Total	123	87	210

Stature was assessed as a proxy for overall health (Table 5.3). Female stature decreased over time incrementally (Figure 5.6), whereas male stature was more variable across the

cemetery. This stature difference was not significant statistically; however, unequal sizes of the three samples constrain accurate statistical assessment.

Table 5.3. Average stature (cm) at Amarna

	Females			Males		
	Length	sd	n	Length	sd	n
Lower Site	153.70	5.30	46	163.05	5.71	47
Middle Site	153.54	4.54	14	162.60	2.03	7
Upper Site	153.42	5.11	54	163.83	4.66	25

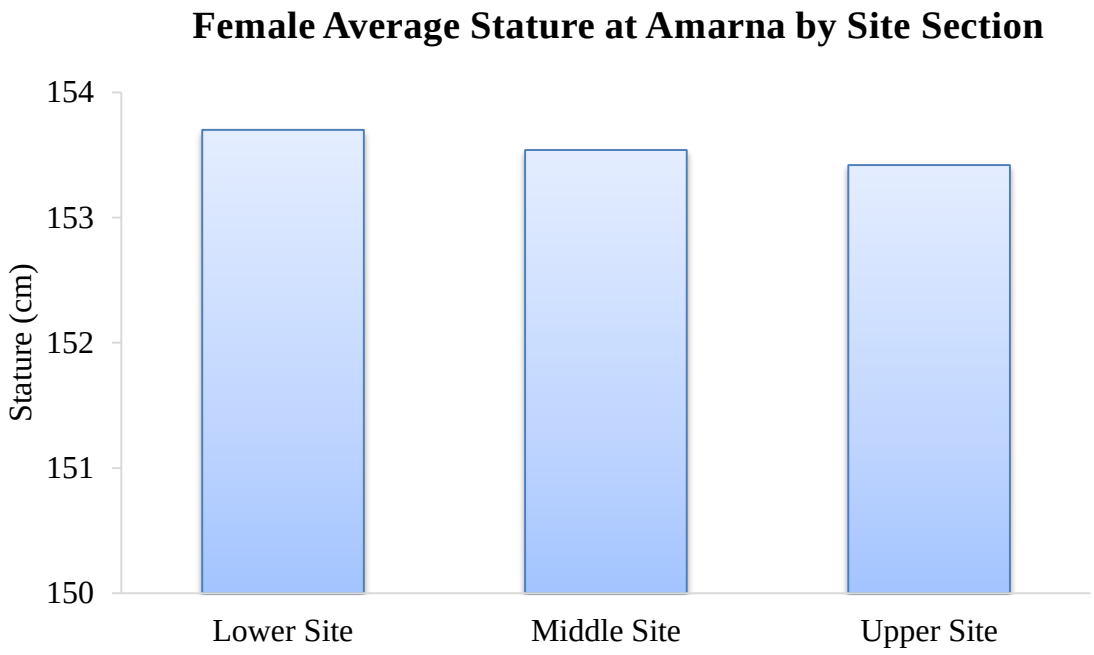
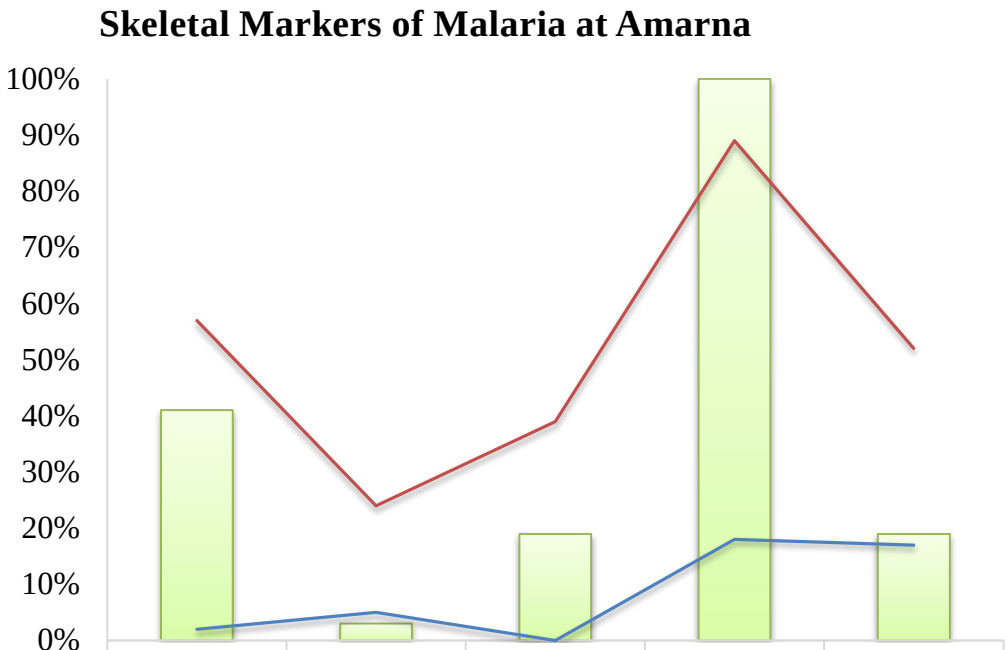


Figure 5.6. Female differences in average stature at Amarna by site section

Frequencies of cribra orbitalia, humeral cribra, femoral cribra, spinal porosity, and periostitis at Amarna tended to fall between those of the malarial and the non-malarial

frequencies, as shown in Figure 5.7. When the lesions for the total sample were entered into the diagnostic outcome algorithm (*n*=31), 68% of observable individuals were diagnosed as having malaria. However, when considering a conservative sample of only the 38 individuals scored for all lesions by Davis (*n*=20), this prevalence dropped to 50%. The data for diagnosing malaria per each individual appears in Table 5.4. The calculated prevalence rates and their confidence intervals for each sample are shown in Figure 5.8 and listed in Table 5.5. Confidence intervals took into consideration the sample size for sensitivity and specificity of the gold standard population (Rogan and Gladen 1978). Individuals diagnosed as positive for malaria did not differ significantly from those diagnosed as negative in any demographical category or burial custom.



	Cribra orbitalia	Humeral cribra	Femoral cribra	Spinal porosity	Periostitis
Amarna	41%	3%	19%	100%	19%
Non-malarial	2%	5%	0%	18%	17%
Malarial	57%	24%	39%	89%	52%

Figure 5.7. Frequencies of skeletal markers of malaria at Amarna compared with known modern malarial/non-malarial samples

Table 5.4. Diagnostic data by individual for determination of malaria prevalence at Amarna

	CO*	HC	FC	SP	P	$C_{i\ (total)}$	$C_{i\ (conserv)}$
Ind 9	0	-	1	-	1	1	-
Ind 28	1	-	0	-	1	1	-
Ind 153	1	-	-	-	1	1	-
Ind 154	0	-	1	-	1	1	-
Ind 180	1	-	0	-	1	1	-
Ind 206	1	-	0	-	1	1	-
Ind 218	1	-	-	-	1	1	-
Ind 222	1	-	0	-	1	1	-
Ind 240	0	1	0	1	0	1	1
Ind 243	0	0	0	1	1	0	0
Ind 248	0	0	0	1	0	0	0
Ind 256	1	0	0	1	0	1	1
Ind 284	0	0	0	1	0	0	0
Ind 288	0	0	0	1	0	0	0
Ind 297	1	0	-	1	0	1	1
Ind 299	-	0	1	1	0	1	1
Ind 317	1	-	0	-	1	1	-
Ind 321	1	-	0	-	1	1	-
Ind 328	0	0	0	1	0	0	0
Ind 336	-	0	1	1	0	1	1
Ind 357	1	0	0	1	1	1	1
Ind 358	0	0	1	1	0	1	1
Ind 359	0	0	0	1	0	0	0
Ind 360	-	0	1	1	0	1	1
Ind 361	1	-	0	-	1	1	-
Ind 362	0	0	1	1	0	1	1
Ind 363	0	0	0	1	1	0	0
Ind 364	0	0	1	1	0	1	1
Ind 375	0	0	0	1	0	0	0
Ind 384	0	0	0	1	0	0	0
Ind 386	0	0	0	1	0	0	0

* Skeletal markers are scored as 0: not present, 1: present, or - : unobservable, and abbreviated as follows: CO: cribra orbitalia; HC: humeral cribra; FC: femoral cribra, SP: spinal porosity; P: periosteal reaction. $C_{i\ (total)}$ and $C_{i\ (conserv)}$ indicate the diagnosis of the total sample and conservative sample, respectively, where 0 denotes negative and 1 denotes positive for malaria.

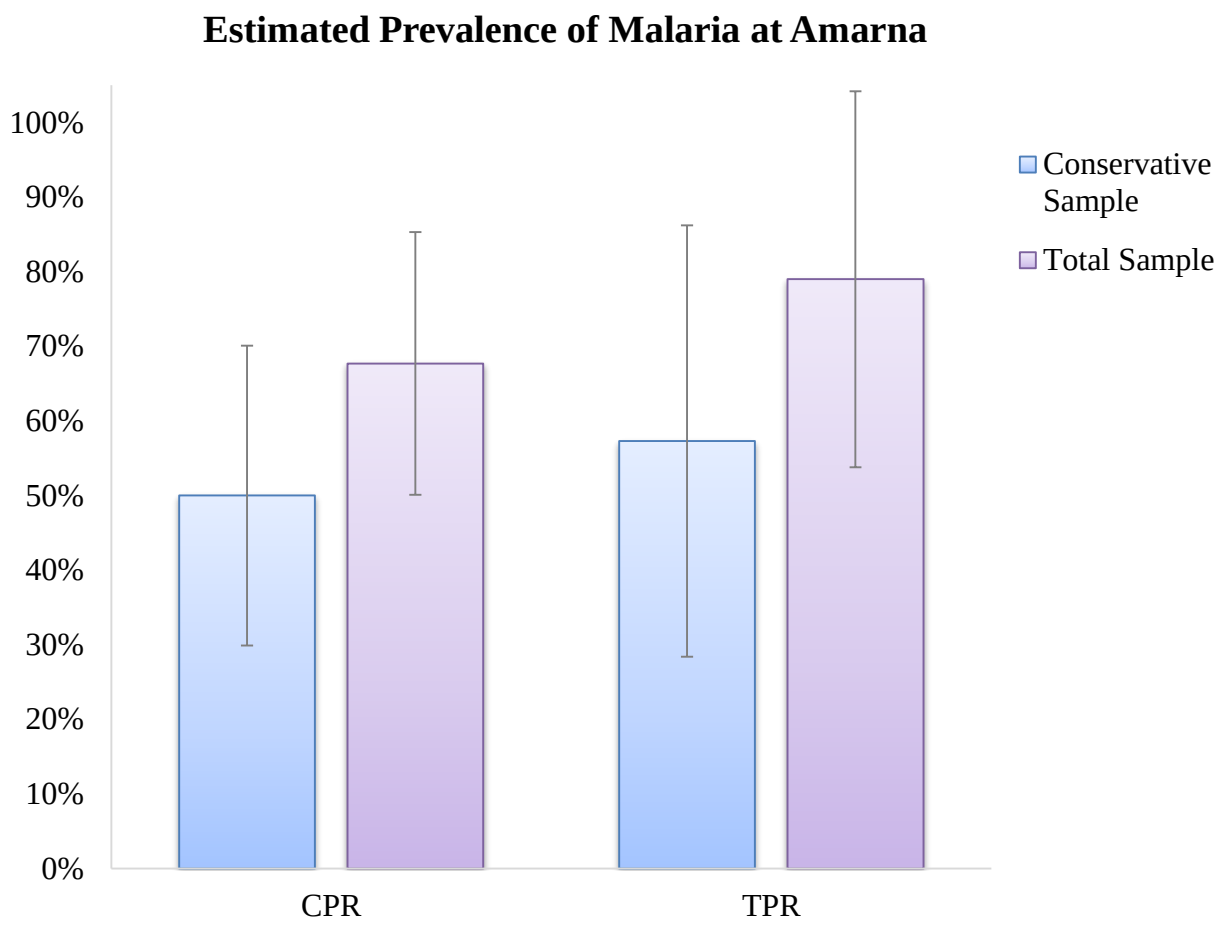


Figure 5.8. Estimated prevalence of malaria at Amarna's South Tombs Cemetery: crude prevalence rate (CPR) and calculated true prevalence rate (TPR) with 95% CI error bars.

Table 5.5. Crude and true prevalence rates of malaria at Amarna's South Tombs Cemetery

	n	CPR	95% CI	TPR	95% CI
Conservative sample	20	50.0%	29.9% – 70.1%	57.3%	28.4% - 86.3%
Total sample	31	67.7%	50.1% - 81.4%	79.0%	53.8% - 104.1%

5.5 Discussion

Though the presence of multiple strains of falciparum malaria aDNA in individuals known to have lived at Amarna suggested the widespread presence of malaria during the Amarna Period, the prevalence of the disease at Amarna has remained unknown (Hawass et al. 2010: 646). This study supports this theory of widespread malaria. The skeletal evidence at Amarna's South Tombs Cemetery revealed high frequencies of lesions associated with malaria, which fell into a range between known holoendemic and non-endemic areas for malaria. When entered into an outcome algorithm that combined these lesions to make a diagnosis (see Methods and Materials), approximately 50% of individuals were diagnosed as having had a recent malarial infection.

Does this high prevalence of malaria at Amarna represent endemic or epidemic malaria? The frequencies of malarial indicators fell generally below those of individuals known to have lived in an area holoendemic for malaria. One explanation for this lower rate could be that an epidemic of malaria hit late in the occupation of the site, thereby affecting only a subset of the population buried in the cemetery. Alternatively, this discrepancy could represent the difference between endemic versus epidemic malaria; the latter of which would tend to kill off its victims before their bodies began to show skeletal signs of the disease. In order to elucidate the nature of malaria at Amarna, we must consider the demography of the site as it relates to risk factors and disease patterns in modern endemic and epidemic malaria.

Several interpretations can be made from the observed differences in demography at Amarna. Since females tended to be buried in multiple burials more frequently than males, and they also declined in stature across the cemetery, it seems reasonable to infer that the health of women at Amarna was impacted to a higher degree than that of men. Additionally, children

under the age of 16 are abundant at the site in general, with a greater proportion of children under the age of five. Both of these demographic patterns at Amarna seem to indicate a health-related preferential mortality burden for women and children. Such a preferential disease burden exists in areas where malaria is endemic due to the lack or loss of acquired immunity and associated increased malaria risk for these two demographic groups, specifically pregnant women who lose their immunity and children under five years (Gilles et al. 1969; Lusingu et al. 2004; World Health Organization 2007; Billig et al. 2012). The spike in child mortality around age two is very similar to what we would expect in cases of severe malarial anemia and cerebral malaria in children who lose maternal immunity after weaning (Botez and Doughty 2014).

The pattern of progressively more multiple burials along the cemetery suggests a gradual increase in the number of people dying simultaneously during the Amarna Period. This could reflect an increase in mortality from infectious cause, perhaps including malaria. Although the malarial individuals did not associate with any burial type or section of the cemetery, malaria is known to co-infect with many other infectious diseases, such as tuberculosis and dysentery, to increase all-cause mortality (Shanks et al. 2008). If malaria was endemic at Amarna, it could have easily opened the door for such high-mortality *syndemics* (i.e. epidemics of increased disease burden due to the synergistic association between multiple disease co-infections, as defined by Barrett (2010: 86)). One of the ways to test this hypothesis in the future may be to identify other specific diseases present at Amarna. This process is ongoing but has indicated evidence of scurvy in several individuals thus far. The presence of scurvy and malaria at Amarna is intriguing in light of the purported anti-malarial effect of Vitamin C (Sabbatani et al. 2010).

Considering the impact of malaria on Amarna, the data assessed in this study match more closely to a situation of hypo-endemic malaria, with skeletal signs of malaria present and greater morbidity and mortality falling on non-immune children and women who lose their acquired immunity during pregnancy. This interpretation is reinforced by the spatial comparison of cribra orbitalia in Chapter 3, which showed ancient Egypt to have had high rates of anemia throughout time and space, and by Tutankhamun's multiple-strain malaria infection at the time of his death. Future aDNA testing of Amarna skeletons for *P. falciparum* DNA will seek to further strengthen the malarial diagnoses made in this study.

At this time, the cause of the Hittite Plague of 1320 BCE cannot be pointed to with certainty. However, this study has shown that the malaria present at Amarna, Egypt in the years leading up to this plague was quite prevalent, and aligns with hypo-endemic models of transmission. This finding agrees with theoretical models that suggested malaria would have thrived in the environment of natural seasonal flooding and man-made irrigation technology along the Nile River in ancient Egypt (Scheidel 2001; Scheidel 2012). This study also noted a pattern suggesting higher mortality for women over time of the cemetery's use at Amarna, which arguably could represent an epidemic of malaria co-infecting with another infection, or multiple strains of malaria piggybacking on one another in the immunocompromised host, to create a heightened mortality event (Scheidel 2001; Shanks et al. 2008; Engelkirk et al. 2011).

Such an epidemic at Amarna could very well have spread to the Hittite empire at the end of the Amarna Period due to the increased diplomatic and military interactions between the two kingdoms (Cohen and Vestbrook 2000; Assman 2003). Therefore, it is reasonable to conclude that malaria at the very least played a part in the Hittite Plague. More concrete evidence could be obtained through continued assessment of malarial prevalence at other sites in the Near East

with similar occupation dates. For now, this study has shown the considerable plausibility of malaria's role in the epidemics noted in ancient texts at the end of the Late Bronze Age.

5.6 Conclusion

This study has examined the skeletal remains from the South Tombs Cemetery at Amarna for patterns of malarial prevalence at the precisely dated Egyptian capital city between 1349 and 1332 BCE. Skeletal lesions evidenced the recent impact of malarial infection on around half of the population. Demographic, burial pattern, and stature analyses showed a greater mortality risk for women and children, with declining health and abnormal burial patterns especially for women over time. These patterns align with models of unstable endemic malaria, which likely co-infected with other diseases present at Amarna to increase total mortality. This malarial disease state at Amarna undoubtedly affected other densely populated Near Eastern regions, contributing to the epidemics noted in ancient texts, predominantly the Hittite Plague of 1320 BCE. Additional analyses of human skeletal remains in other Near Eastern sites dating to this time may lead to additional evidence of malaria's role in the region at the end of the Late Bronze Age.

Chapter 6 – Summary and Future Directions

The ancient Near East has an important place in the history of the origins of ancient agriculture and complex societies, which go hand in hand with the history of ancient disease spread (McNeill 1977). This environment of increased human population density, decreased sanitation, and increased standing water in agricultural fields provided a launching pad for some of the most deadly diseases that continue to wreak havoc on human populations today. Malaria is one of these diseases, representing a severe global health problem and evading repeated attempts at prevention and eradication (World Health Organization 2014). Anthropology can contribute to the efforts to combat malaria by reconstructing malarial disease dynamics in the past so that we can make inferences into how to best respond to the disease in the future (Brown et al. 1997).

Previous theoretical studies have linked the spread of malaria out of Africa to the marshy corridor of the Nile River Valley, exacerbated by human irrigation activities and population density of Pharaonic Egypt to provide an excellent niche for the mosquito malaria vector (Bruce-Chwatt 1965; Bruce-Chwatt and de Zulueta 1980; De Zulueta 1987; Scheidel 2001; Hawass et al. 2010; Scheidel 2012; Sallares 2013). Though there has been ample direct and indirect evidence to suggest a malarial *presence* in ancient Egypt (Hershkovitz and Edelson 1991; Miller et al. 1994; Bianucci et al. 2008; Hawass et al. 2010; Lalremruata et al. 2013), much about the antiquity, prevalence, and behavior of malaria in the region remained unknown.

Malaria is a complex disease, the impacts of which are difficult to assess even in modern populations, which may indicate why paleopathologists have tended to ignore its impacts on past populations until very recently (Webb 2009; Setzer 2014). Though malaria was originally

believed to leave no trace on the skeleton by which to identify it in ancient human remains (Nunn and Tapp 2000; Roberts 2000), recent research has provided evidence to the contrary (Rabino Massa et al. 2000; Gowland and Western 2012). Unfortunately, the cranial lesions shown to associate with malaria are believed to have a complex etiology, with many factors other than malaria also leading to their appearance (Walker et al. 2009; Oxenham and Cavill 2010; McIlvaine 2013).

This dissertation takes a multidisciplinary approach to these issues in past malarial impact in the Near East by applying methods from anthropology, climatology, epidemiology, microbiology, and entomology. In this way, this study tests the plausibility of theoretical models of malaria's origin and spread with existing associated skeletal indicators of the cranium. New information on the postcranial manifestation of malaria on the skeleton are gleaned from clinical skeletons with known malarial histories, and the power of these skeletal indicators tested in correctly diagnosing individuals with malarial infections. To test the hypothesized impact of malaria's involvement at the tempestuous end of the Late Bronze Age, the new skeletal diagnostic criteria is applied to skeletal remains of a precisely dated cemetery at Amarna, Egypt; the population from which a massive epidemic in ancient Anatolia purportedly spawned.

6.1 Project Objectives

This dissertation project had three primary objectives toward its goal of determining the skeletal manifestation of malaria and its impact on the ancient Near East. First, theoretical models of malaria's spread up the Nile River Valley during Dynastic Egypt were tested through a meta-analysis of cribra orbitalia (one of the skeletal indicators previously associated with malarial infection). The meta-analysis used cribra orbitalia frequencies at 29 ancient Nile Valley

sites, representing 4,760 individuals ranging from prehistoric to Christian periods and situated between the 3rd Cataract and Nile Delta. This study represents the largest inter-site comparison of cribra orbitalia in the Near East to date.

The second objective was to refine the known skeletal indicators of malaria by conducting a case-control study of modern individuals from malarial and non-malarial areas. This study analyzed skeletal lesions present on 98 East Africans of known age, sex, tribe, and cause of death. As a control sample, 352 North Americans from forensic contexts were analyzed for similar lesions. This study represents the only large-scale clinical assessment of malaria's skeletal manifestation, and one of very few studies in paleopathology in which epidemiological tests of diagnostic power are reported.

The third objective was to use the verified skeletal manifestation of malaria to assess the disease's impact at Amarna, Egypt and its inferred impact on the broader Near East. This study used skeletal lesion data from 405 skeletons from the South Tombs Cemetery at Amarna Egypt to diagnose malaria presence and calculate prevalence from these diagnoses. It also examined data on demography, stature, and burial patterns at the different sections of the cemetery to assess patterns of disease dynamics. This study is the first to use direct evidence from human skeletal remains to suggest a disease contributing to the Hittite Plague of 1320 BCE.

Brief summaries and concluding remarks for each objective are presented in the sub-sections below.

6.1.1 Objective 1: Testing models of malaria spread in the ancient Nile Valley

Cribra orbitalia is one of the most common skeletal lesions noted in ancient human skeletal remains excavated from the Nile Valley. Long thought to be a sign of iron-deficiency anemia, understanding of cribra orbitalia etiology has shifted recently to cast doubt on this iron-

deficiency hypothesis, instead indicating megaloblastic and hemolytic anemia to be the more likely factors leading to the appearance of this lesion (Walker et al. 2009). One of the main causes of acquired hemolytic anemia is malaria infection. Recent research has provided evidence supporting the link between cribra orbitalia and malaria infection. One study found cribra orbitalia to be present in 92% of skeletons testing positive for *Plasmodium falciparum* antigens (Rabino Massa et al. 2000). Another study found higher rates of cribra orbitalia lesions on skeletons buried near locations of greater Anopheles mosquito distribution and known malaria infection from historical records (Gowland and Western 2012).

Some theoretical models have pointed to the Nile Valley as the pathway of malaria from Africa to Europe within the time frame of Dynastic Egypt (Bruce-Chwatt and de Zulueta 1980). Through ancient DNA and antigenic analysis of Egyptian mummies, direct evidence of malaria's presence in the Nile Valley dates as far back as 3200 BC (Cerutti et al. 1999); however, the prevalence, spread, or endemic/epidemic status of the disease in this region in antiquity remains unknown.

The objectives of the present study were as follows: (1) to test the theoretical Dynastic Egyptian time frame for the spread of malaria up the Nile Valley and out of Africa; (2) to use variability in levels of cribra orbitalia present on ancient Egyptian and Nubian remains to track the spread of malaria; (3) to test a hypothesized increasing trend in cribra orbitalia frequency over time from South to North in the Nile Valley.

This study surveyed cribra orbitalia frequencies tallied in site reports from 29 ancient Nile Valley sites, representing 4,760 individuals ranging from prehistoric to Christian periods (4400 BC – 1500 AD) and situated between upper Nubia and the Nile delta. See Appendix A for the sources of data used in this meta-analysis. To avoid potential sources of statistical error,

samples of fewer than 15 individuals were excluded from analysis, as well as sites reporting poor skeletal preservation. Analysis of the data consisted of comparison of overall distribution of the data to other existing cribra orbitalia meta-analyses, and determination of associations through Spearman's rank and Kendall's tau correlations.

Results showed generally high cribra orbitalia rates between 10.8% and 78.7% of the total population affected, with an overall mean of 42.8%. There were greater overall rates of cribra orbitalia in the Nile Valley sample compared with other global cribra orbitalia meta-analyses. Interestingly, the Nile Valley cribra orbitalia distribution only overlaps slightly with the English sample associated with *P. vivax* malaria infection. The data showed no significant correlation over time and geographical location, suggesting that high levels of hemolytic anemia affected individuals in the Nile Valley equally from pre-dynastic to Christian periods. No association was found between the frequency of cribra orbitalia present at the sites and proportion of females or non-adults in the sample, or year of report publication.

The gradual increase in cribra orbitalia over space and time that was hypothesized for this study was not confirmed by the results. Rates of cribra orbitalia in the Nile Valley were generally high throughout time and space when compared with New World samples, and showed no association with location, estimated date, proportion of females or non-adults, or date of report publication. From these results, the following interpretations can be made. First, contrary to small-scale comparisons between sites, cribra orbitalia did not increase or decrease in frequency, but stayed prominent over time throughout the Nile Valley. Second, the failure to associate cribra orbitalia frequency with sex or age suggests that the main cause of the high cribra orbitalia rates is not sex-specific or age-specific (like diet, exposure to parasitic worms, or nutritional stress caused by weaning).

Assuming that cribra orbitalia is strongly influenced by malarial infection, this study suggests the disease was already endemic in the Nile Valley during the Neolithic period (c. 6000 BCE). This interpretation is supported by ancient DNA evidence, and pushes back the date for theoretical models of malaria's spread out of Africa. This study has shed new light on the patterns of health in the ancient Nile Valley by providing a more holistic view of anemia present throughout time and space. Through comparison with Gowland and Western's (2012) English malarial sample, this study has also potentially provided the first interspecific malarial comparison through large-scale cribra orbitalia frequencies.

6.1.2 Objective 2: Identifying skeletal markers of malaria in a clinical case-control study approach

Unlike some other infectious diseases, malaria's skeletal manifestation has never been confirmed using a large sample from a clinical setting with known individual medical histories. Currently, paleopathologists can only suggest malarial infection in gross analyses of human skeletal remains from the presence of cribra orbitalia and porotic hyperostosis under the assumption that these lesions are indicators of the hemolytic anemia caused by malaria (Setzer 2014). This study sought to refine these existing skeletal indicators of malaria through clinical comparisons and epidemiological diagnostic testing.

To pinpoint evidence of malaria infection on ancient skeletal remains, this study compared skeletal lesions in a modern reference sample from Uganda where malaria is holoendemic to a similar modern sample from a malaria-free area. The malarial sample consisted of 98 East Africans, separated by those who died of malaria or anemia and matched cases for age and sex. The non-malarial sample consisted of 106 African Americans with estimated frequencies of sickle cell trait that are similar to those of Ugandans.

Five porous skeletal lesions were identified that appear more frequently in the malarial sample ($p<0.01$), especially in anemic individuals. These appeared on the cranium, vertebral column, and humeral and femoral necks. Periostitis also associated strongly with individuals in the malarial sample ($p<0.01$); however, linear enamel hypoplasias showed the converse association ($p=0.017$). The identified lesions were tested for their association with each other, and then tested individually for their diagnostic power through measures of sensitivity and specificity. At this point, porotic hyperostosis was excluded as a useful indicator of malaria due to its low specificity and diagnostic odds ratio below unity. From the remaining skeletal lesions (i.e. cribra orbitalia, humeral cribra, femoral cribra, spinal porosity, and periostitis) and their inter-lesion associations, an outcome algorithm was formed to diagnose individuals for malarial infections. If an individual had at least one of the cribrous lesions and either spinal porosity or periostitis, the individual was diagnosed as positive for malaria. Otherwise, the individual was diagnosed as negative.

Several etiological explanations for the characteristic malarial skeletal lesions were explored. High rates of porous lesions in malarial individuals were reasoned to be attributed to three potential causes from clinical observations: (1) severe malarial anemia causing expansion of marrow space; (2) an imbalance in bone remodeling due to chemical release during hemolysis; or (3) extramedullary erythropoiesis, which is known to cause cortical thinning and coarse trabeculation in clinical cases of genetic hemolytic anemia. The importance of careful differential diagnoses between other infectious and non-infectious causes of these lesions was discussed, including the potential for co-infection of malaria with other infectious diseases. These findings are pivotal in establishing diagnostic criteria by which we can identify the prevalence and impact of malaria on past populations.

6.1.3 Objective 3: Estimating the prevalence of malaria at Amarna, Egypt and its implications for the Near East

The Amarna Period in ancient Egypt represents a unique time in which major social and political changes occurred suddenly and with little explanation. From the ancient literary texts recovered at various Near Eastern sites during this period, there is ample mention of epidemic disease (Moran 1992; Singer 2002; Assman 2003), which some believe may have contributed to these extreme shifts (Kozloff 2006; Dodson 2009). Ancient DNA evidence lends to the hypothesis that malaria may have contributed to these ancient epidemics and could have had a substantial impact on the politics, interactions, and rise and fall of empires in the Near East at the end of the Late Bronze Age (Hawass et al. 2010).

To consider the impact of malaria on the Near East during this tumultuous time, his study assessed 405 skeletons buried at Amarna for malarial prevalence and patterns of endemicity. Pathology data indicating skeletal lesions of malaria were tallied and entered into an outcome algorithm for diagnosis of malaria by individual. From these diagnoses, prevalence of malaria at the site was calculated. Data on demography, stature, and burial types were used to infer transmission type for malaria endemicity.

Results of this study revealed high rates of skeletal indicators of malaria, which were positioned between those of populations in known non-endemic and holoendemic areas for malaria. The diagnostic individuals for malarial lesions were prevalent in around half of the population tested. Demographic trends showed higher proportions of women and children than men, suggesting a higher mortality rate for these demographic groups. Similarly, women were significantly more likely to be buried in multiple burials than men, and the average stature measurements in women had a decreasing trend throughout the duration of the cemetery use.

This differential mortality burden that was higher in women and children aligned with the higher risk of infection and mortality due to issues of acquired immunity in endemic areas for malaria. The rates of skeletal indicators of malaria that were lower than the hypo-endemic area for malaria suggests an unstable transmission type of endemicity, where individuals partially lose their acquired immunity periodically, leading to small epidemic cycles. The increasing rates of multiple burials and decreasing stature in women suggests an event of lowered general health states for women, which arguably could represent the co-infection of malaria with other diseases at Amarna.

In sum, this study showed malaria to have had a high prevalence and wide impact on the population at Amarna, which bolsters Hawass and coworkers' (2010) suggestions based on their ancient DNA analyses. Thus, it would be difficult to believe that malaria did not play a part in the epidemics and rise and fall of empires in the Near East at the end of the Late Bronze Age, predominantly including the Hittite Plague of 1320 BCE. More research into the prevalence of malaria at other Late Bronze Age sites in the Near East is needed to further explore the impact of malaria on these ancient civilizations.

6.2 Future Goals

My future goals related to this project are manifold. Primarily, I intend to refine the techniques for diagnosing malaria developed in this dissertation through the inclusion of more clinical samples of malaria's skeletal manifestation. I am aware of many other reference collections available for study in malarial areas, including some that are already being studied for this exact purpose. There is a small but growing group of researchers in the field of paleopathology interested specifically in the detection of malaria in ancient human remains.

Plans are already underway for a symposium focused on this subject, which will incite communication between researchers and hopefully lead to more collaboration. Through this collaboration, a deeper knowledge of the ways in which this complex disease affects bone can be achieved.

One of the major ways in which this research can be tested further is through a combination of gross skeletal lesion analysis and ancient DNA testing to verify the lesion's association with malaria. Such testing is planned and has been given governmental approval for the skeletons at Amarna. It is hoped that this future study will be able to identify the pathogen DNA; however, this will rely on the preservation of the DNA within bone more than 3,000 years old. Ancient pathogen DNA testing has been successful in Egyptian mummies, but may prove challenging on dry bone recovered from a dry, desert environment.

One of the interests sparked by this project regards the interspecific differences in malarial infection on human bone. From Gowland and Western's (2012) study, it seems likely that vivax malaria manifests itself in similar ways to falciparum malaria on the skeleton, but perhaps to a lesser extent of severity. This hypothesis seems reasonable given the pattern of symptomatic severity between the two *Plasmodium* species, but requires more conclusive evidence. Such conclusions could be made possible if the diagnosis of malaria and reporting of its disease prevalence becomes more widespread in future bioarchaeological research.

Finally, much is still unknown about the origin and spread of the four human malarial parasite species within the Old and New World. Malaria is assumed to never have entered the New World prior to European contact based on the lack of genetic polymorphisms conferring resistance to malaria in indigenous populations. However, this assumption fails to address the traditional ethnobotanical knowledge of one of the preeminent antimalarial plants known to exist

in the world: cinchona bark, or quinine. The fact that this plant remedy was used by ancient South Americans to treat fevers suggests the presence of at least one of the malarial species in the Pre-Columbian New World. The identification of malaria on archaeological skeletons from New World sites would address this question of malaria diaspora more definitively.

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Appendix A: Cribra Orbitalia Data Compiled for Nile Valley Meta-Analysis

Site	x̄ Years BP	Latitude (DD)	n	CO (%)	Females (%)	Nonadults (%)	Source
Abusir (Mastaba of Ptahshepses)	2312 (± 400)	29.9	142	26.8	44.7	46.3	Strouhal & Bares 1993
Abydos	4905 (± 207)	26.2	106	49.1	-	-	Duhig 2000
Abydos	4446 (± 253)	26.2	28	78.6	-	-	Duhig 2000
Abydos	3875 (± 163)	26.2	41	68.3	-	-	Duhig 2000
Abydos ('Tombs of the Courtiers')	4887 (± 125)	26.2	30	40.0	-	-	Musselwhite 2011
Adaïma	5012	25.3	272	26.5	-	100.0	Dabernat et al. 2005
Amarna (STC)	3340 (± 8)	27.7	103	42.7	-	-	Kemp et al 2013
Aswan	4446 (± 253)	24.1	18	61.1	-	-	Duhig 2000
Aswan	3875 (± 163)	24.1	47	63.8	-	-	Duhig 2000
Dendara	4115 (± 78)	26.2	76	53.9	-	-	Duhig 2000
Dishasha	4446 (± 253)	29.0	21	42.9	-	-	Duhig 2000
El-Badari (Badarian graves)	6212 (± 200)	27.0	30	63.3	-	-	Musselwhite 2011
Elephantine	4115 (± 78)	24.1	32	75.0	68.3	26.7	Pecotte 1986
el-Raqaqna	4446 (± 253)	26.3	17	52.9	-	-	Duhig 2000
el-Tarif	3875 (± 163)	25.7	54	55.6	-	-	Duhig 2000
Gebelein	4446 (± 253)	25.5	23	73.9	43.3	8.6	Pecotte 1986
Gebelein	4115 (± 78)	25.5	47	78.7	43.6	20.8	Pecotte 1986
Gebelein	2607 (± 70)	25.5	17	52.9	-	-	Pecotte 1986
Hierakonpolis (HK27C)	3902 (± 190)	25.1	21	28.6	65.4	29.7	Judd 2007
Hierakonpolis (HK43)	5662 (± 150)	25.1	145	13.1	59.5	20.9	Kumar 2009; Larsen 2009

Site	x̄ Years BP	Latitude (DD)	n	CO (%)	Females (%)	Nonadults (%)	Source
Hierakonpolis (Prehistoric & 'Fort' cemeteries)	5512 (± 500)	25.1	39	71.8	-	-	Musselwhite 2011
Kerma	3627 (± 65)	19.6	306	13.7	61.5	4.2	Buzon 2004
Kulubnarti (21-R-2)	887 (± 375)	21.1	164	39.0	-	-	Mittler & Van Gerven 1994
Kulubnarti (21-S-46)	1362 (± 100)	21.1	170	51.8	-	-	Mittler & Van Gerven 1994
Memphis	3428 (± 124)	29.9	306	24.8	44.3	3.9	Buzon 2004
Missiminia	1662 (± 250)	20.5	333	27.9	48.3	-	Wapler et al 2004
Naqada (Great, B, and T cemeteries)	5712 (± 700)	25.9	97	40.2	35.7	0.0	Bartell 1994; Kumar 2009
Naqada B cemetery	5362 (± 150)	25.9	20	60.0	-	-	Musselwhite 2011
Naqada T cemetery	5262 (± 250)	25.9	23	43.5	-	-	Musselwhite 2011
Qaw el-Kebir	4446 (± 253)	26.9	27	70.4	-	-	Duhig 2000
Qaw el-Kebir	4115 (± 78)	26.9	69	63.8	-	-	Duhig 2000
Qubbet el Hawa	4446 (± 253)	24.1	156	48.7	39.4	19.2	Rosing 1990
Qubbet el Hawa	4115 (± 78)	24.1	32	34.4	27.8	18.2	Rosing 1990
Qubbet el Hawa	3875 (± 163)	24.1	18	50.0	46.7	28.6	Rosing 1990
Qubbet el Hawa	3637 (± 75)	24.1	60	63.3	45.0	31.0	Rosing 1990
Qubbet el Hawa	2607 (± 70)	24.1	146	36.3	45.4	17.0	Rosing 1990
Qurneh	3312 (± 250)	24.9	172	16.3	52.0	7.5	Buzon 2004
Shellal	3312 (± 250)	24.1	154	20.1	47.7	3.8	Buzon 2004
Sidmant	4115 (± 78)	29.1	55	67.3	-	-	Duhig 2000
Sidmant	3875 (± 163)	29.1	15	53.3	-	-	Duhig 2000
SJE (C-Group)	3812 (±	24.0	205	14.1	64.8	12.9	Buzon 2004

Site	x̄ Years BP	Latitude (DD)	n	CO (%)	Females (%)	Nonadults (%)	Source
	200)						
SJE (Pharaonic)	3512 (± 150)	24.0	73	23.3	55.1	15.2	Buzon 2004
Tarkhan	5012	29.5	29	72.4	-	-	Musselwhite 2011
Tarkhan	4905 (± 207)	29.5	26	34.6	-	-	Duhig 2000
Tell el-Dab'a	3682 (± 130)	30.8	41	26.8	40.8	48.1	Winkler & Wilfing 1991
Thebes-West	2927 (± 585)	25.7	168	29.2	45.5	20.2	Nerlich et al 2000
Thebes-West (Valley of the Queens)	1812 (± 200)	25.7	212	18.4	48.0	19.2	Macke & Macke-Ribet 1994
Tombos	3337 (± 75)	19.4	83	10.8	59.5	15.0	Buzon 2004
Wadi Halfa (24I3)	1562 (± 100)	21.8	45	26.7	50.0	29.6	Armelagos 1968
Wadi Halfa (6B13)	1037 (± 425)	21.8	28	14.3	-	32.4	Armelagos 1968
Wadi Halfa (6B16)	2012 (± 350)	21.8	62	11.3	58.3	17.1	Armelagos 1968
Wadi Halfa (6G8)	1037 (± 425)	21.8	29	13.8	-	39.4	Armelagos 1968
Wadi Halfa (NAX)	1562 (± 100)	21.8	127	26.7	56.6	14.1	Armelagos 1968