CHAPTER 15

Beyond the differential diagnosis: new approaches to the bioarchaeology of the Hittite plague

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Introduction

Investigating the cause of an ancient epidemic requires gathering evidence from various disciplines to reconstruct the disease dynamics and fit clues from both ancient texts and human skeletons into population-based diagnostic models. In paleopathology, which is the study of ancient disease, differential diagnosis is generally applied to ancient skeletal individuals or samples to make inferences about bony lesions. This is accomplished by tallying all of the potential disease agents and eliminating them systematically based on similarities and differences to the study sample. In this chapter, we advocate expanding and incorporating the long established methods for differential diagnosis into population-based diagnostic modeling in order to investigate larger concepts of epidemic disease spread.

Specifically, we suggest an 11-step approach to investigating ancient epidemics wherein all possible causes are reduced to a single agent.

First, use historical texts from the specific time period of the epidemic(s) of interest to assemble evidence about the suspected disease's dynamics and symptoms.

Second, use population-based diagnostic modeling to reduce the number of possible diseases that fit the description of the past epidemic(s) and use modern disease epidemiology specific to the given region to guide reconstruction of ancient disease dynamics in order to identify the most plausible disease.

Third, use theoretical models of ancient evolution of the disease, such as existing historical and molecular clock evidence, and the timeframe of the disease spread, to confirm the possibility that the selected disease could have been present in the region at the time.

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Fourth, use historical descriptions of epidemics of the disease when it first appeared and of the disease's impact on affected populations to confirm the capability of the hypothesized agent to cause the past epidemic(s) in question.

Fifth, use knowledge of the suspected disease vector's behavior and ecology to confirm the past presence and behavior of the vector in question in the region.

Sixth, use reconstructions of ancient climate in the region to envisage how environments would impact the spread of the suspected disease.

Seventh, survey the published literature for established bioarchaeological methods that have been used to identify the suspected disease on skeletons from archaeological sites that are known to have been affected by the suspected disease, such as the analysis of ancient deoxyribonucleic acid (aDNA).

Eighth, compile data on frequencies of skeletal lesions from all published bioarchaeological reports in the region in order to estimate the disease's spatiotemporal prevalence, or the proportion of the population that was infected over space and time, in the region.

Ninth, use established methods of paleopathological differential diagnosis in order to consider other potential diseases that could have caused the skeletal lesions in skeletal samples recovered from the region.

Tenth, analyze bone lesions present in individuals with antemortem, clinical diagnoses of the suspected disease curated in modern clinical skeletal samples from endemic areas, as well as skeletons from non-endemic areas, to identify skeletal indicators diagnostic of the disease.

And lastly, apply these refined indicators to the analysis of ancient skeletons recovered from archaeological sites in the region dating to the time of the ancient epidemic under consideration in order to determine the presence and prevalence of the suspected disease.

Using these 11 steps in the following sections, we present a case study of our investigation into the causes of the Hittite plague. A devastating epidemic hit the Hittite empire in 1322 BCE, which seemingly was carried by Egyptian prisoners of war captured along the Hittite–Egyptian border entering the Hittite capital (Bryce 1998; Singer 2002). The Hittites were an ancient civilization whose empire extended across Anatolia, or modern-day Turkey, between the seventeenth and twelfth centuries BCE. Many scholars have speculated about the disease responsible for this mysterious plague, but they have had no evidence with which to pinpoint the infectious agent. For example, a recent theory that the epidemic was tularemia, a severe infectious bacterial disease of animals (e.g., rabbits, hares, rodents) caused by *Francisella tularensis*, was subsequently refuted for lack of evidence (Martin-Serradilla and Guerrero-Peral 2008; Trevisanato 2007). In this chapter, we present a population-based diagnostic model approach embedded within a biocultural framework, which incorporates and considers both biological and cultural aspects of a given phenomenon, for solving this mystery. The paucity of Hittite burials from this time period (Emre 1991) has forced us to use Egyptian burials from the same time period recovered from the site of

Amarna, Egypt, as a proxy or a representative sample that serves as a substitute for the absent sample, to identify the disease agent responsible for the Hittite plague.

Case study: investigating the cause of the Hittite plague

Step 1: Ancient Near Eastern texts

In the first step, historical texts from the time period of the epidemic are used to assemble evidence about the suspected disease's dynamics and symptoms. The Hittite King Mursili II wrote a series of prayers in 1300 BCE pleading with the gods for relief from a widespread, 20-year epidemic that had already killed the two preceding kings and ravaged his subjects. The "Plague Prayers of Mursili II" reveal that this deadly epidemic was brought by Egyptian prisoners of war as they marched through the Hittite capital city (Singer 2002). However, for paleopathologists, the lack of recovered Hittite burials and the ancient Egyptian tendency to omit negative historical events in their records has made it difficult to identify the responsible disease.

Tumultuous events in Egypt leading up to 1322 BCE suggest that Egypt may have been stricken by the same epidemic disease as the Hittites. Specifically, Pharaoh Akhenaten suddenly changed the Egyptian religion and founded his new capital city of Amarna in a previously uninhabited area. Some scholars have attributed this abrupt religious and geographical shift to epidemic disease, perhaps even polio or bubonic plague (Kozloff 2006). Further dramatic changes followed in approximately 1332 BCE when Amarna was abandoned, scattering its occupants to the far reaches of the empire and, hence, possibly to the Egyptian–Hittite border. Along with the dispersal of people, any disease present at Amarna could also have been spread throughout the Egyptian empire, potentially affecting the Egyptian prisoners of war who were implicated as the source of the Hittite plague.

Step 2: Population-based diagnostic model of the Hittite plague

In the second step, population-based diagnostic modeling is used to reduce the number of possible diseases that fit the description of the past epidemic and use modern disease epidemiology for the region to guide reconstruction of ancient disease dynamics. A population-based diagnostic model of this epidemic must consider diseases thought to have plagued ancient Egyptians in addition to those diseases previously suggested by scholars to have caused the Hittite plague. Ancient Egyptian medical texts and well-preserved ancient Egyptian human remains are excellent sources for reconstructing ancient disease in the Near East. Paleopathologists have been working in Egypt for over a century, and

Table 15.1 Diseases potentially responsible for the Hittite plague.

Disease	Mortality*	Demography*	Incubation*	Persistence*
Anthrax	High	All	1 day–2 months	Non-epidemic
Ascariasis	Low	All	None, often no symptoms	Non-epidemic
Bubonic plague	High	Higher risk in ages 12–45	7–10 days	Epidemic, or endemic with epidemic waves
Cholera	High	All	<1–5 days	Epidemic/endemic
Hemorrhagic fever	High	All	2–21 days	Short-lived epidemics
Influenza	Low	Higher risk in infants, elderly	2 days	Seasonal epidemics
Malaria	High	All (on virgin ground)	10–15 days	Epidemic/endemic
Measles	High	Young children	10–12 days	Epidemic/endemic
Poliomyelitis	Low	Young children	7–10 days	Epidemic/endemic
Schistosomiasis	Low	Aquatic occupational risk	14–84 days	Endemic
Smallpox	Can be high	All	12–14 days	Epidemic
Tuberculosis	Low	Young adults	Weeks–years	Endemic
Tularemia	Low	Domestic animal herding occupational risk	3–5 days	Non-epidemic
Typhoid fever	Can be high	All	1–3 weeks	Epidemic/endemic
Typhus	High	All	5–14 days	Epidemic/endemic

*General information on mortality, demography, incubation period, and persistence of the diseases sourced from the World Health Organization and Centers for Disease Control.

have established the existence of specific diseases at particular points in time. Similarly, solving the mystery of the Hittite plague has been a popular pastime of scholars who have championed a wide range of hypothetical diseases (see Table 15.1).

We use evidence of disease dynamics suggested by ancient texts to reduce the list of potential causes of the Hittite epidemic. Several details are key. For one, Mursili II blames the Egyptians for causing the plague, which means that the prisoners of war must have exhibited symptoms soon after they were brought into the city. The disease also spread quickly, infecting and killing the king, Suppiluliuma I, and his first son and successor, Arnuwanda II, within one year. Additionally, Mursili II identifies the age groups affected by the plague in his third plague prayer (Singer 2002:58):

> For twenty years now people have been dying [in great numbers] in Hatti ... Hatti has been very much oppressed by the plague. [If someone] produces a child, [the ...] of the plague [snatches (?)] it from him. Should he reach adulthood, he will not attain old age. [And even if old age (?)] will be left for someone, he [will be oppressed (?) by] the plague. He will not [return] to his previous condition. When he reaches old age, [he will ...], but he will not keep warm.

We know that the disease spread rapidly after the prisoners entered the city, and that infection with the disease was not dependent upon age or class. The texts do not mention specific symptoms, but we know the disease was deadly and persisted for 20 years. The prisoners showed no obvious symptoms until after entering the city, suggesting a long incubation period.

Next, we removed the diseases from Table 15.1 that do not fit the dynamic described by Mursili II. It is important to make uniformitarian assumptions about the nature of disease, specifically assuming that the disease-causing organisms have not changed in their behavior or virulence, even though we know that diseases can and do in modern times evolve in response to human-induced changes to their environment (Cohen and Crane-Kramer 2003). It is also possible that the disease responsible for the Hittite epidemic no longer exists or at least no longer infects humans. Nevertheless, we must make these assumptions because we simply do not have evidence of how each of these pathogens may have behaved differently in the past.

We were able to remove several diseases. Naturally acquired anthrax, most commonly the cutaneous form, tuberculosis, poliomyelitis, ascariasis, which is a parasitic roundworm infection, and schistosomiasis, which is a parasitic blood fluke infection, are unlikely to have been responsible for the massive death toll among the Hittites. This is because today they do not cause fast-spreading, deadly epidemics (CDC 2014; Hamborsky *et al.* 2015; WHO Expert Committee 2002; World Health Organization 2008). Conversely, viral hemorrhagic fevers, such as Ebola, are transmitted too easily and kill too many too quickly to ravage Hittites for 20 years (CDC 2015).

We also removed tularemia from our list. While it has been suggested as the cause of the Hittite plague (Trevisanato 2007), it was later discounted because of inconsistencies between known characteristics of the Hittite plague and modern epidemics of tularemia (Martin-Serradilla and Guerrero-Peral 2008). We add here that tularemia's incubation period of 3–5 days and tendency to be occupation specific (e.g., shepherds) make it incongruous with transport by the Egyptian captives.

Lastly, we removed the diseases that do not fit the demographic profile; Mursili II wrote that the disease causing the Hittite plague did not discriminate against age or class. Therefore, we removed bubonic plague, influenza, and measles from our list (see demography column in Table 15.1) as they have a higher risk of infection for people between the ages of 12 and 45 years, infants and the elderly, and young children, respectively. Cholera certainly has the potential to cause a major, long-lasting epidemic, but it does not fit our model because it has

a very short incubation period of 1–5 days and its symptoms of severe diarrhea and dehydration would have been noticed as the Egyptians were marched from modern-day Syria to central Turkey. Smallpox is an excellent candidate as it affects all members of society, and the incubation period of approximately two weeks is long enough to allow travel to central Turkey before causing symptomatic illness or death. Nonetheless, smallpox was eliminated because we would expect to see its characteristic lesions – large fluid-filled blisters covering the entire body, which leave noticeable scars on survivors – mentioned in the Hittite texts. Typhoid fever, a bacterial infection, is another probable candidate as it was proposed as a cause of the Plague of Athens in 430–426 BCE (Papagrigorakis *et al.* 2006; but see critique by Shapiro *et al.* 2006). However, this disease is associated with poor sanitation, and the Hittites had strict rules regarding hygiene and cleanliness, especially concerning water, and most particularly for the king (Bryce 2004). This makes it unlikely for two kings to have died of typhoid. Lastly, we removed epidemic typhus, another bacterial disease, which is known to flourish under wartime conditions due to its spread by human lice. However, it is hard to imagine the king having been infected by lice directly from the prisoners of war. Typhus only survives within the living cells of lice or humans, so it is typically passed from bodily contact or from infested clothing (McQuiston 2016).

Finally, we come to malaria, the last on our list of suggested sources. Malaria is 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 15.2). The two species of major global importance which have the greatest impact on human health today are *Plasmodium falciparum* and *Plasmodium vivax* (Webb 2009). Malaria seems to fit all of the disease dynamic and population conditions of the Hittite plague, and was likely present in the ancient Near East. However, only the milder *P. vivax* species (Hershkovitz *et al.* 1991; Hume *et al.* 2003) has the ability to remain dormant in the liver of its host, and thus sustain itself during cold winter seasons, which are common in modern-day central Turkey, when its mosquito vector cannot survive. *P. falciparum*, the more deadly form of malaria, does not have this ability to relapse from the liver stage. Thus, warmer and wetter conditions than those found in modern-day central Turkey would be required for this species to have sustained the Hittite plague. Malaria does not discriminate against class, as demonstrated by recent ancient DNA studies of the Pharaoh Tutankhamun, who likely grew up at Amarna, Egypt, which revealed that he was co-infected with two strains of falciparum malaria at the time of his death (Hawass *et al.* 2010). Having eliminated all the other suggested causes of the Hittite plague, we probe further into the possibility of malaria being the culprit.

Step 3: Theoretical models of malaria origin and spread

In the third step, theoretical models of ancient evolution of the disease are used to confirm the possibility that the disease could have been present in the region

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Table 15.2 Comparison chart of malaria species ecology*.

Species	Global importance	Host	Incubation (avg.)	Relapse?	Fever wave freq.	Disease consequences	*In utero* infection?	Postpartum antibodies?	Required temp (°C)
P. falciparum	Major	Humans	12 days	No	Every 48 hours	Severe anemia, cerebral malaria	Yes	Yes	>19
P. vivax	Major	Humans	15 days to 6–12 mo.	Yes	Every 48 hours	Increasingly severe anemia	Yes	No	>15
P. ovale	Minor	Humans	17 days	Yes	Every 48 hours				(Tropical)
P. malariae	Minor	Humans + African apes	18–40 days	No	Every 72 hours				>15

*Chart based on malaria species ecology from Webb's (2009) *Humanity's Burden*.

at the time of the epidemic. Most literature discussing the history of malaria cite Bruce-Chwatt and de Zulueta's (1980) theory that falciparum malaria only arrived in Europe during the Roman Empire. They discount the textual and physical evidence of falciparum malaria, and insist that it could not have existed because they believed the *Anopheles* mosquito vectors were not present. Even in ancient Egypt where the warm climate and riverine urban landscape were undoubtedly well suited for malaria, de Zulueta (1987) claims that an effective mosquito vector – an *Anopheles* species whose biting behavior favors malaria transmission to humans – was absent.

Sallares and co-workers (2004) argue for a slightly earlier spread of malaria, possibly extending back to 700 BCE. They speculate that epidemics of falciparum malaria spread simultaneously with the migration of the mosquito, *Anopheles sacharovi*, into Greece and Italy from Tunisia. This hypothesis suggests that malaria spread gradually in Europe as these mosquitoes slowly migrated, but it does not consider that mosquitoes can, and often do in modern times, hitch rides on sea-faring vessels to arrive in new areas at the same time as their human hosts (Bataille *et al.* 2009; Guagliardo *et al.* 2015).

In contrast to historic reconstructions, genetic analyses indicate that falciparum malaria evolved in Africa anywhere between 5000 and 3 million years ago (Datta and Chauhan 2010), but the young age (less than 5000 years) of genetic variations that confer resistance or immunity to malaria suggests a more recent evolution of malaria, perhaps coinciding with the origins of agriculture between 10 000 and 5000 years ago (Hedrick 2012). This association with agriculture further suggests that malaria must have used the Nile River corridor with its early agricultural settlements to leave Africa for Europe and Asia.

Step 4: Modern recorded malaria epidemics

In the fourth step, historical descriptions of epidemics of the disease when it first appeared and its impacts in these early epidemics are used to confirm the capability of the hypothesized agent to cause the epidemic in question.

In reconstructing the past spread of malaria and modeling its population dynamics during past epidemics, we used historical records from well-documented epidemics. Historical malaria epidemics suggest generally devastating health effects in non-immune, naive populations experiencing the parasite for the first time. One such epidemic occurred on the north-west Coast of the United States in the 1830s. Detailed record keeping by the affected European settlers enabled historians to track down the exact person who carried the parasite on the specific ship that came into Portland, Oregon, in 1830 (Boyd 1999). With Oregon's already substantial population of *Anopheles* mosquitoes, this one infected traveler spawned a five-year seasonal epidemic of malaria. Although Europeans were hard-hit during these yearly epidemics, the indigenous populations, who did not have access to the standard cinchona bark, or quinine, treatment, were ravaged by the disease (Boyd 1999). There is

no information as to which species of malaria caused the 1830s epidemic but in recent times, multi-year malaria epidemics caused by *P. falciparum* entering a naive population have been well documented, such as in the Isthmus of Panama (Calzada *et al.* 2008). There, during an epidemic in 2005, nearly half of the indigenous Kuna community of Chepo became infected before the Panamanian health officials could intervene (Shah 2010).

We employed the disease dynamics of these historical epidemics to identify the defining elements of past epidemics. Primarily, just one infected person migrating into a new area can spread malaria, as long as the area has a substantial anopheline mosquito population. Secondly, the absence of biological immunity plays a large part in the duration and virulence of the epidemic. Lastly, climate is an intrinsic factor in the ability of an epidemic of falciparum malaria to gain a foothold in a population, because it determines the dynamics of mosquito ecology.

Step 5: Entomology of the malaria vector

In the fifth step, knowledge of the suspected disease vector's behavior and ecology is used to confirm the past presence and behavior of the vector in the region in question. The successful spread of malaria to new locations depends upon the existence of a substantial population of *Anopheles* mosquito, and at least one human who is infected with malarial *Plasmodium* (Sherman 1998). Each species of *Anopheles* mosquito has its own preference for temperature and altitude range. Thus, different geographic locations tend to have different dominant malaria vector species. Some species do not enter man-made structures whereas others do, especially at night, making the latter a much more effective malaria vector than the former (Sherman 1998). Mosquitoes that prefer to bite humans instead of 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, throughout Turkey, to coastal areas bordering the Black Sea. *An. sacharovi* has several behavioral advantages for successful malaria transmission. It will breed in stagnant fresh water or brine, is found at elevations up to 1720 m, and has an incomplete hibernation during winter; thus, it is able to cause new cases of malaria all year round (Alten *et al.* 2000). This year-round transmission is especially important when considering the plausibility of *P. falciparum* malaria causing the Hittite plague because this parasite does not have the ability to remain dormant in the liver, unlike *P. vivax* (Sherman 1998).

In sum, in order for malaria to have spread throughout the Near East, a substantial anthropophilic mosquito vector population size must have been maintained by optimal temperatures, elevation, and breeding grounds. Once these

conditions are met, transmission spawning an epidemic is possible when even a single human with malaria enters a new area.

Step 6: Paleoclimate of the Near East

In the sixth step, reconstructions of ancient climate in the region are used to envisage how environments would impact the spread of the suspected disease. Fossilized pollen and charcoal analyses from a lagoon in the Nile Delta indicate that the climate in the ancient Near East was very moist and humid from 6000 to 3500 BCE during the expansion of city-state societies (Bernhardt *et al.* 2012). After approximately 500 years of fluctuating rainfall, the climate became drier around 2800 BCE, likely involving droughts that negatively impacted ancient peoples (Kaniewski *et al.* 2013). This drier climate meant greatly decreased mosquito populations, probably only surviving in the Nile River floodplain and delta region. However, this drought was alleviated briefly by periods of increased rainfall between 1500 and 1100 BCE (Bernhardt *et al.* 2012). Thus, an increased mosquito population size and range in the Near East were possible during this wetter period that also encompassed the Amarna period and the Hittite plague.

Step 7: Skeletal indicators of malaria

In the seventh step, the published literature is surveyed for bioarchaeological methods that have been used to identify the suspected disease on skeletons from archaeological sites where the condition was present. Malaria is often dismissed in the differential diagnosis of pathological skeletal lesions by bioarchaeologists and paleopathologists because many think that the disease does not manifest itself upon the skeleton (Nunn and Tapp 2000; Roberts 2000). Genetic conditions conferring resistance to malaria, such as thalassemia and sickle cell disorder, have been suggested as causes of skeletal lesions such as porotic hyperostosis (PH) and cribra orbitalia (CO), porous lesions found on the cranial vault and eye orbits, respectively (Angel 1966, 1972). However, these genetic disorders, which are maintained at low frequencies, cannot explain findings of uniformly high rates of these skeletal lesions within ancient skeletal samples. Consequently, etiological theories have shifted toward iron deficiency anemia as the main cause of these lesions, and paleopathologists shifted their attention from the potential presence of malaria to dietary stress associated with agriculture (Stuart-Macadam 1987).

Attention slowly came back to malaria with advances in aDNA extraction from skeletal and mummified human remains. Many researchers have been successful in isolating the aDNA of *P. falciparum* in Egyptian mummified tissue and determining the presence of malaria (Bianucci *et al.* 2008; Hawass *et al.* 2010; Miller *et al.* 1994; Nerlich *et al.* 2008). However, this method is costly and therefore not routinely performed on entire skeletal assemblages. Thus, aDNA can be used to detect presence but not prevalence of the disease in a past population.

Rabino Massa and co-workers (2000) tested ancient Egyptian mummies for malarial aDNA. Of those positive for falciparum malaria, 92% had PH and CO. Their study provides a link between direct evidence for malaria and these lesions, which were previously associated with iron deficiency anemia. Further, Walker and co-workers (2009) reasoned that iron deficiency anemia, long held to be the main cause of PH and CO, could not in fact produce the bone marrow hypertrophy responsible for these lesions. Instead, they pointed to megaloblastic and hemolytic anemia. Megaloblastic anemia arises in individuals with a nutritional deficiency in vitamin B12 or folic acid, and hemolytic anemia arises in individuals with some genetic disorders conferring protection from malaria, as well as in individuals with a malaria infection.

Building on the previous two studies, Gowland and Western (2012) mapped and associated CO with the distribution of large populations of *Anopheles* mosquitoes, lower altitude, marshy environments, and higher incidence of historic "fever and ague," an archaic term for malaria, across Great Britain. They found a correlation between malarial infection and CO, supporting the hypothesis that malaria manifests itself in the skeleton. Despite these results, much is still unknown about the etiology of these skeletal lesions. At the very least, multiple factors apparently lead to their manifestation, such as nutrition and parasitic infection, which could include malaria (Holland and O'Brien 1997; Walker *et al.* 2009; Wapler *et al.* 2004). Multiple lines of evidence must be employed to establish the skeletal manifestations of malaria for the purposes of differential diagnosis, including postcranial evidence of severe anemia and a demographic bias towards women and young juveniles in a given skeletal sample – malaria produces highest mortality in pregnant women, who lose acquired immunity during pregnancy, and children under the age of five (Gilles *et al.* 1969; World Health Organization 2007).

Step 8: Spatial epidemiology of cribra orbitalia at Nile Valley archaeological sites

Methods

In the eighth step, data on frequencies of skeletal lesions from all published bioarchaeological reports in the region are compiled in order to estimate the disease's spatiotemporal prevalence in the region. The connection between CO and malaria suggested by previous studies (Gowland and Western, 2012; Rabino Massa *et al.* 2000) prompted the first author to survey CO frequencies in the ancient Nile Valley using published reports (Smith-Guzmán 2015a). This study tested Sallares and co-workers' (2004) theoretical model for the spread of malaria up the Nile Valley and out of Africa using the variability of CO frequencies. This analysis surveyed reports from 29 ancient Nile Valley sites, representing 4760 individuals ranging from prehistoric to Christian periods (4400 BCE–1500 CE) and situated between upper Nubia and the Nile delta.

Results

Generally, high rates of CO (between 10.8% and 78.7%) existed at each of the sites, with an overall mean of 42.8%. The Nile Valley samples had greater overall rates of CO compared with CO meta-analyses from other regions. There was no significant correlation with geographical location or time, suggesting that high levels of hemolytic or megaloblastic anemia affected individuals in the Nile Valley equally from predynastic to Christian times. No association was found between the frequency of CO and the proportion of individuals under 18 years of age or the proportion of females versus males.

The gradual increase in CO over space and time that was hypothesized was not confirmed and the following interpretations were made. First, contrary to small-scale comparisons between Nile Valley sites, CO did not increase or decrease in frequency but stayed prominent over time. Second, the failure to associate the high CO rates with age suggests that the main cause was not age specific, like diet, exposure to parasites, or nutritional stress caused by weaning. Lastly, assuming that CO is indicative of malaria infection, an assumption derived from Gowland and Western (2012), then it must have been endemic long before the unification of Egypt. This interpretation is supported by aDNA evidence (Hawass *et al.* 2010; Nerlich *et al.* 2008; Rabino Massa *et al.* 2000).

These results rely on the assumption that the hemolytic anemia caused by malaria is responsible for high CO rates; the other causes of CO cannot be eliminated. A clinical comparison using a modern skeletal collection from an endemic malarial area is necessary to identify specific skeletal lesions associated with malaria using the differential diagnosis approach.

Step 9: Differential diagnosis for anemia in Egypt

In the ninth step, established methods of paleopathological differential diagnosis are used to consider other potential diseases that could have caused the skeletal lesions in samples recovered from the region. The high frequencies of CO indicating severe anemia (Hillson 1980) have prompted scholars to suggest a number of causes, including schistosomiasis, intestinal parasites, dietary deficiencies, brucellosis, and malaria. The presence of schistosomiasis in ancient Egyptians is well evidenced from preserved eggs in mummies, as well as from ancient textual references reporting characteristic symptoms such as bloody urine (Brier 2004). To test relationships between CO, PH, and schistosomiasis in the past, Alvrus (2006) compared CO and PH rates with tissue samples positive for an antigen indicating schistosome infection in ancient Nubians. She found a lack of association between these lesions and schistosome infection. This was particularly true for juveniles, who exhibited the greatest percentage of crania with these lesions. Alvrus interprets this disparity as evidence that other factors were more important causative agents of anemia than schistosomiasis. However, as others have noted, individuals can be co-infected with

schistosomiasis and other diseases, increasing infection intensity and general anemia (Campbell Hibbs *et al.* 2011).

Hookworms, parasites which are common in modern tropical areas, cause blood loss, chronic diarrhea, and vitamin deficiencies, and are notorious causes of iron deficiency anemia (Hengen 1971). This type of anemia reduces the production of red blood cells, however, making it unable to cause the expansion of marrow space seen in PH and CO (Walker *et al.* 2009). The malabsorption of nutrients, particularly vitamin B12, due to chronic diarrhea can cause megaloblastic anemia and osseous expansion of marrow spaces. This prompted Walker and co-workers (2009) to suggest that megaloblastic anemia was the cause of PH and CO in some ancient contexts, such as the ancient North American south west. However, in the modern era, megaloblastic anemia due to vitamin B12 deficiency comprises a miniscule proportion of the total anemia worldwide (Kassebaum *et al.* 2014).

Brucellosis is a bacterial zoonotic infection commonly passed from domestic cattle to humans through ingestion of raw milk, and causes undulating fevers, as well as hemolytic anemia in some cases (d'Anastasio *et al.* 2011). Importantly, brucellosis existed in the ancient Mediterranean and Near East. Therefore, we postulate that anemia from brucellosis could induce marrow space expansion. However, modern epidemiological studies of regions where brucellosis is endemic indicate that its prevalence in humans tends to be low, affecting less than half as many humans as cattle, even among high-risk occupational groups such as dairy farmers (Lopes *et al.* 2010). Considering that not all brucellosis infections will result in anemia severe enough to cause skeletal lesions, it is unlikely that high rates of skeletal anemia can be attributed to brucellosis. Having excluded the above, the major cause of anemias in the Near East producing skeletal changes is most likely malaria.

Malaria is an unbiased infection – one that affects all members of a population – and causes hemolytic anemia, which has been linked with PH and CO. Malarial anemia is more severe in individuals without acquired immunity, including young children, pregnant women, and recent immigrants to a given region where malaria is endemic who have no acquired immunity. In addition to the classic model of skeletal anemia by expansion of marrow space, recent research has suggested that hemolysis during the schizogony phase of malaria infection – wherein parasites burst out of and destroy host red blood cells – may contribute to widespread porous skeletal lesion formation. This may occur due to release of acid phosphate, free heme, and the malarial pigment hemozoin from the red blood cells into the host's bloodstream. This may lead to an imbalance in bone remodeling by stimulating osteoclasts, preventing bone resorption, while simultaneously impairing osteoblasts, preventing bone formation (d'Souza *et al.* 2011; Moreau *et al.* 2012). Furthermore, severe malarial anemia may induce extramedullary erythropoiesis, the production of red blood cells outside the bone marrow, which is known to cause thinning of bone cortex and coarsening of the spongy, or trabecular, bone (Al-Aabassi and Murad 2005).

Step 10: Epidemiological approach to skeletal lesions through clinical samples

Methods

In the penultimate step, bone lesions in skeletons with antemortem, clinical diagnoses of the disease from endemic regions and those from non-endemic regions are evaluated to identify diagnostic indicators with a high degree of certainty.

The first author compared skeletal lesions in a modern reference sample from Uganda, where malaria is holoendemic, or ubiquitous, to a similar modern sample from a malaria-free area (Smith-Guzmán 2015b). The goal was to record all porous lesions of the cranial and postcranial skeleton that might be associated with anemia (Djuric *et al.* 2008; Gowland and Western 2012; Rabino Massa *et al.* 2000), but also other markers of specific or non-specific infection, such as periosteal reactions, which are areas of subperiosteal bone deposition; linear enamel hypoplasias, which are linear defects in the dental enamel caused by growth arrest from physiological stress during dental development in childhood; and periodontal disease, resorption of the bone tissue surrounding the teeth due to gum disease (gingivitis). The data collection proceeded in three phases: (1) individuals whose known cause of death was malaria or anemia, (2) individuals with other causes of death with the same age, sex, and tribal group as the malarial/anemic individuals, and (3) all remaining individuals with crania present. Because of this phased approach, the resulting data are comparable to modern epidemiological matched case/control studies and were analyzed as such.

Results

Five porous skeletal lesions were identified that appear more frequently in the Ugandan sample, especially in anemic individuals (Figure 15.1). These included CO, PH, and spinal porosity, consisting of porous lesions on the vertebral and sacral bodies; and cribra (porous lesions) on the humeri and femora. Frequencies of periosteal reactions were also higher in the malarial sample; however, linear enamel hypoplasias were conversely associated in this sample. Next, interlesion associations were examined, and epidemiological methods for determining the diagnostic certainty of a given lesion to a given disease condition (Boldsen 2001; Pinhasi and Turner 2008) were applied. Several lesions were confirmed as useful indicators of malaria: CO, humeral cribra, femoral cribra, spinal porosity, and periosteal reactions. These were combined into an outcome algorithm in order to diagnose individual skeletons for which their malarial status – negative or positive – is unknown. Based on this algorithm, if an individual has at least one of the cribrous lesions and either spinal porosity or periosteal reactions, the individual is diagnosed as positive for malaria. Without them, the individual is diagnosed as negative.

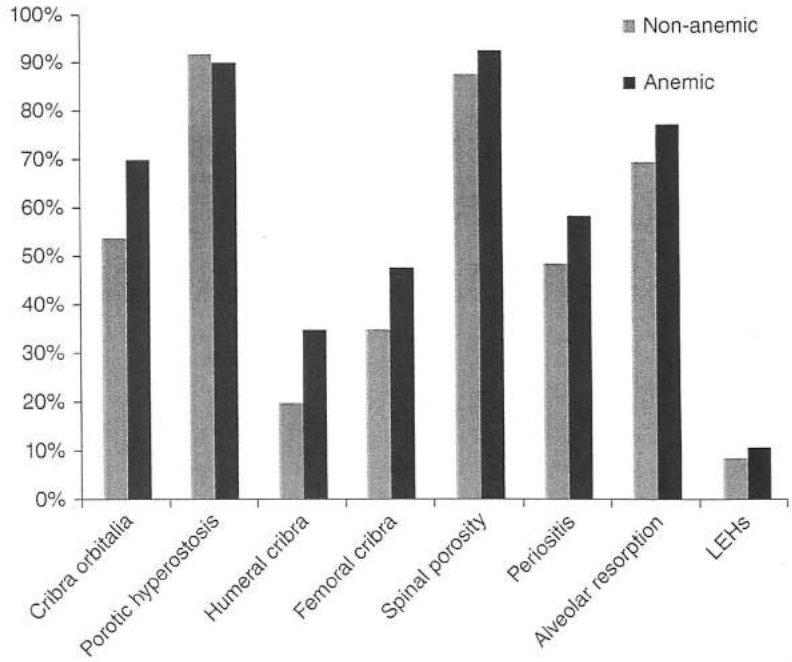

Figure 15.1 Comparison of skeletal lesions in Ugandans who died of anemia and those who died of other causes.

Step 11: Application of refined criteria to Amarna skeletons

Methods

In the final, 11th step, these refined indicators are assessed in skeletons recovered from archaeological sites in the region dating to the time of the ancient epidemic in order to determine the presence and prevalence of the suspected disease.

We analyzed 417 skeletons from the South Tombs Cemetery, which was utilized during the Amarna Period, 1349–1332 BCE, for the skeletal indicators of malaria identified above in order to determine the probability that malaria might have caused the Hittite plague. The cemetery is located in a dry channel, or wadi, next to the elite South Tombs carved into the cliffs. It was excavated in roughly four sections to sample the possible differential burial practices. These sections of the cemetery seem to have been populated in chronological order as follows: the oldest, the Wadi Mouth Site, Lower Site, Middle Site, and the youngest Upper Site. Excavations merged the Wadi Mouth and Lower Sites and they are considered as a single unit.

Results

Frequencies of skeletal indicators of malaria at Amarna tended to fall between those of the malarial and the non-malarial frequencies, as shown in Table 15.3.

Table 15.3 Frequencies of skeletal anemia at Amarna compared with modern malarial/non-malarial reference samples.

	Amarna	Malarial	Non-malarial
Cribra orbitalia	81 (40%)	33 (57%)	1 (2%)
Humeral cribra	5 (4%)	21 (24%)	2 (5%)
Femoral cribra	47 (17%)	37 (39%)	0 (0%)
Spinal porosity	32 (100%)	84 (89%)	9 (18%)
Periostitis	60 (19%)	51 (52%)	9 (17%)

Applications of the diagnostic outcome algorithm for skeletal indicators of malaria predicted a high prevalence of malaria amongst the Amarna skeletons, with around 50% of individuals showing signs of recent infection.

The frequency of malarial indicators at Amarna could mean several things. First, it could indicate an epidemic of malaria late in the site's occupation, thereby affecting only a subset of the population in the cemetery. Alternatively, this could represent the difference between endemic and epidemic malaria. Epidemic malaria would tend to kill its victims before their bodies began to evidence severe malarial anemia, while endemic malaria would produce more chronic anemia. In order to elucidate the nature of malaria at Amarna, the demography of the site as it relates to disease patterns in modern endemic and epidemic malaria must be considered.

In the demography of the Amarna South Tombs Cemetery, the largest age group (160 skeletons or 44% of the sample) contains juveniles under the age of 16 (Figure 15.2). Further, 59% of the 206 adults of determined sex are female

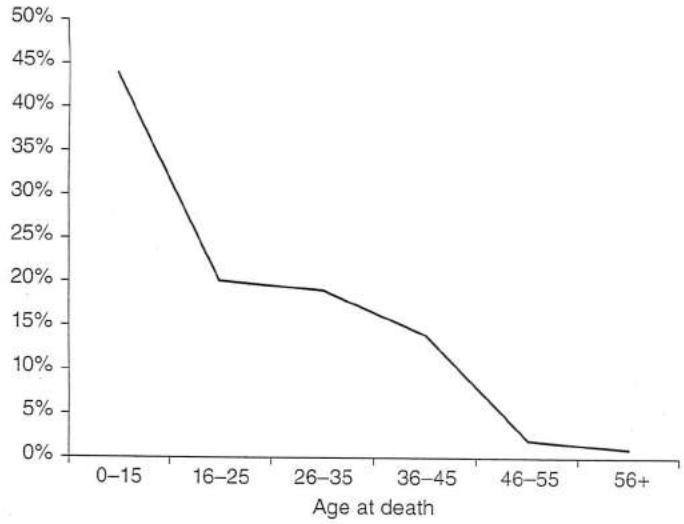

Figure 15.2 Amarna age-at-death frequencies by group.

(Figure 15.3). Multiple burials, which increased incrementally over time during the cemetery's use (Figure 15.4), were more likely to contain females than males. Stature for females also decreased incrementally over time (Figure 15.5), whereas male stature was more variable across the cemetery.

Several interpretations derive from these differences. First, the pattern of progressively more multiple burials suggests a gradual increase in the number of

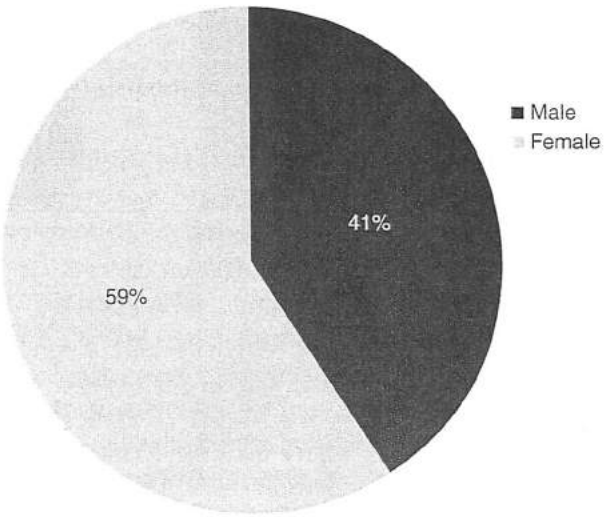

Figure 15.3 Sex frequencies at Amarnâ's South Tombs Cemetery.

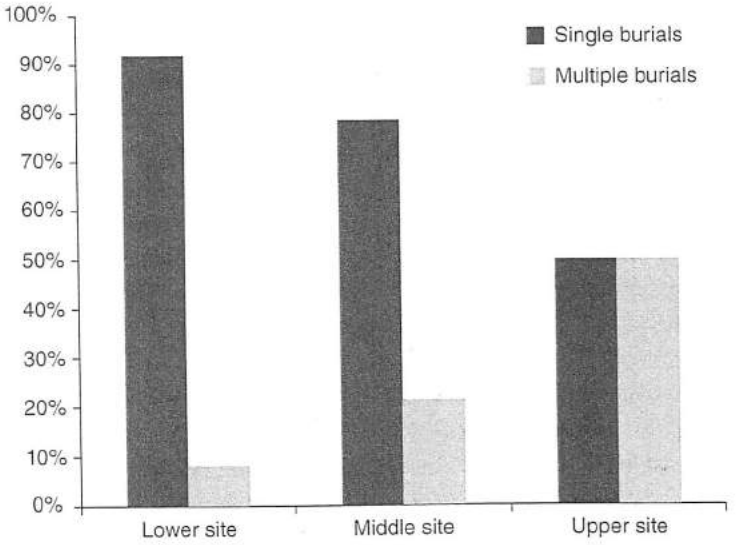

Figure 15.4 Frequencies of multiple burials by section of the South Tombs Cemetery at Amarna.

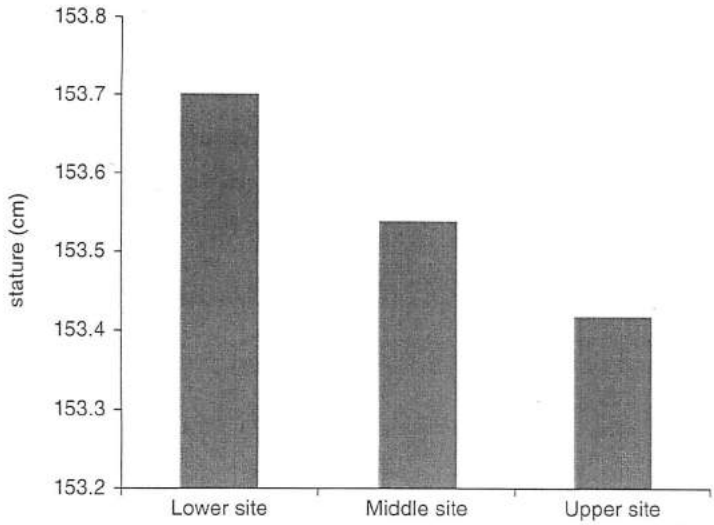

Figure 15.5 Female stature differences at Amarna by site.

people dying simultaneously during the Amarna period. Second, since females tended to be buried in multiple burials more frequently than males, and they also declined in stature across the cemetery, we infer that the health of women at Amarna was impacted to a greater degree than that of men. Additionally, juveniles under the age of 16 are abundant at the site in general, with a greater proportion of those under the age of five (Figure 15.6). In sum, the demographic patterns at Amarna seem to indicate a health-related preferential mortality burden for women and children.

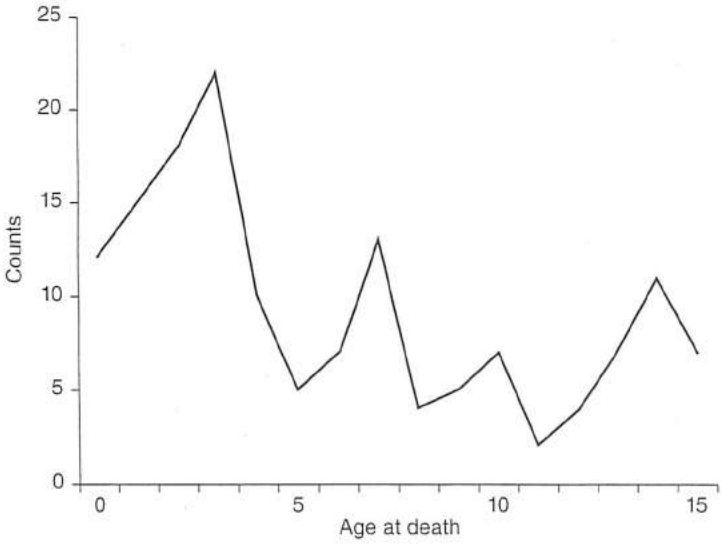

Figure 15.6 Childhood age at death at Amarna.

Our Amarna data match most closely with endemic malaria, with a 50% estimated prevalence based on skeletal indicators of malaria and greater morbidity and mortality falling on non-immune juveniles and reproductive-age females. This interpretation is reinforced by our spatial comparison of CO, which shows that ancient Egypt yielded high rates of anemia throughout time and space, and by Tutankhamun's multiple strain malaria infection. We plan to further confirm these findings of endemic malaria at Amarna by testing some of the anemic skeletons from the site for *P. falciparum* DNA.

Discussion and conclusion

Through our case study, we have demonstrated that the climate, parasite and vector ecology, and historic data all suggest that at least one Egyptian prisoner of war taken to the Hittite capital could have harbored malaria in his veins, spawning the 20-year epidemic. Further, through spatial epidemiology and clinical evidence, combined with skeletal analysis of a sample from Amarna, we have presented evidence that malaria had a high prevalence and wide impact on the population living in the Egyptian capital city during the Amarna period and that the abandonment of Amarna and the scattering of its population could easily have supplied the infected individuals who initiated the Hittite plague.

We have shown that investigating an ancient epidemic must go far beyond the differential diagnosis of individual skeletons and employ population-based diagnostic modeling. Consideration must be given to the intrinsic intertwining of relationships between pathogen, vector, and host, as viewed within a biocultural framework. Evidence from many other disciplines including epidemiology, climatology, and history demonstrates that both biology and culture impact the way humans experience epidemic disease. Bioarchaeologists can say much more about ancient health and disease when we break outside of our discipline to incorporate knowledge and methods from related fields of study.

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