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The Skeletal Manifestation of Malaria: An Epidemiological Approach Using Documented Skeletal Collections

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KEY WORDS *Plasmodium falciparum*; epidemiology; anemia; diagnostic criteria

ABSTRACT

Objectives: Recent studies in paleopathology have shown promise in associating some skeletal lesions with malarial infection. However, malaria's skeletal manifestation has never been confirmed using a large clinical reference sample from an endemic area for malaria with known individual causes of death.

Materials and Methods: To pinpoint evidence of malaria infection on ancient skeletal remains, this study uses an epidemiological approach to compare skeletal lesions in a modern reference sample of 98 individuals from Uganda, where malaria is holoendemic, to a similar modern sample of 106 individuals from a malaria-free area.

Results: Five porous skeletal lesions are identified that appear more frequently in the endemic area population, especially in anemic individuals. These appear on the cranium, vertebral column, and humeral and femoral necks. Periostitis also associates strongly with individuals in the endemic population; however, linear enamel hypoplasias show an inverse association. The identified lesions are tested for their association with each other, and then tested individually for their diagnostic power through measures of sensitivity and specificity. A diagnostic outcome algorithm is formed from the remaining skeletal lesions and their inter-lesion associations.

Discussion: Several etiological explanations for the characteristic malarial skeletal lesions are explored, including severe malarial anemia, an imbalance in bone remodeling, and extramedullary erythropoiesis. The importance of careful differential diagnoses between other infectious and noninfectious causes of these lesions is discussed, including the potential for coinfection of malaria with other infectious diseases. The findings of this study are pivotal in establishing diagnostic criteria by which we can identify the prevalence and impact of malaria on past populations. Am J Phys Anthropol 158:624–635, 2015.

INTRODUCTION

Over the past several decades, paleopathological research into the ancient history of malaria and its impact on past human civilizations has increased with advances in diagnostic techniques identifying malaria on ancient human remains. Successful biomolecular detection of antigenetic signatures and ancient deoxyribonucleic acid (aDNA) of the *Plasmodium falciparum* malaria parasite within ancient human skeletal and mummified tissue have shown promise in pinpointing malaria's presence in the past (Miller et al., 1994; Sallares and Gomzi, 2001; Bianucci et al., 2008; Nerlich et al., 2008; Hawass et al., 2010). However, similar attempts to detect the less virulent *Plasmodium vivax* biomolecular signatures in ancient human tissue have been unsuccessful (Pinello, 2008; Gowland and Western, 2012), perhaps owing to the lower parasitic load associated with this species (Brown and Brown, 2011). Furthermore, biomolecular techniques are costly and destructive, and therefore, are not usually performed on all individuals present in a skeletal series. Thus, biomolecular techniques can detect presence, but not prevalence of the disease in a past population.

To estimate the prevalence of malaria in an archaeological skeletal series, anthropologists and biochemists have advanced methods for potential recognition of malaria on human skeletal remains. Through immunological assay and skeletal lesion comparisons, Rabino Massa et al. (2000) found skeletal indicators of anemia (porotic hyperostosis and cribra orbitalia) present in 92% of individuals testing positive for *P. falciparum* malaria. Although their study provided a link between direct evidence for malaria and skeletal lesions associated with anemia, the prevalence of these skeletal lesions and number of positive individuals for malaria in this series was subsequently shown to be overstated (Raffaella Bianucci, pers. comm. 2015). Nevertheless, the association between skeletal indicators of anemia and positive biochemical markers of malaria was also noted in an aDNA study by Nerlich et al. (2008), in which both individuals testing positive for *P. falciparum* aDNA showed skeletal signs of chronic anemia.

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Paleopathologists have long reasoned that porotic hyperostosis and cribra orbitalia develop as a response to iron-deficiency anemia (Hengen, 1971; Carlson et al., 1974; El-Najjar et al., 1976; Lallo et al., 1977; Mensforth et al., 1978; Stuart-Macadam, 1987). However, a recent etiological reassessment by Walker et al. (2009) argued from a pathophysiological perspective that megaloblastic and hemolytic anemia are far more likely than iron-deficiency anemia to spawn these skeletal lesions. Megaloblastic anemia arises in individuals with a nutritional deficiency in vitamin B_{12}, and hemolytic anemia 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). Although Walker et al.'s (2009) article is still being debated in the literature (Oxenham and Cavill, 2010; Rothschild, 2012; McIlvaine, 2013), a recent article by Gowland and Western (2012) found evidence to support the connection between malaria and skeletal lesions of anemia. They 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. The same spatial connection was not shown for linear enamel hypoplasias (Gowland and Western, 2012).

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 its associated genetic disorders (Angel, 1966; Tayles, 1996; Rabino Massa et al., 2000; Buckley, 2006; Gowland and Western, 2012), much is still unknown about the etiology of these skeletal lesions. At the very least, multiple etiological factors appear to contribute to their formation, including nutrition and parasitic infection (Holland and O'Brien, 1997; Wapler et al., 2004; Walker et al., 2009).

No study to date has explicitly examined potential indicators of malaria on a modern documented sample which includes individuals known to have suffered from malarial infections, and the pattern in which these indicators co-occur. This study takes an epidemiological approach to examine lesions present within a modern, documented Ugandan skeletal collection in comparison to a similar skeletal collection from the United States as a first step in identifying diagnostic criteria for malaria on unknown human skeletal remains.

MALARIA DISEASE DYNAMICS AND PATHOPHYSIOLOGY

The identification of the protozoa responsible for malaria occurred within the last 150 years, and its life-cycle was recognized only within the last 50 years (Sherman 1998). Malaria is a blood-borne infection involving human hosts and *Anopheles* mosquito vectors. The five species of parasites which cause human malaria today are as follows: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*. The two species of major global importance (i.e., having the greatest impact on human health) today are *P. falciparum* and *P. vivax* (Webb, 2009). Falciparum malaria distinguishes itself from vivax malaria in its severity, being more virulent in general and having a greater potential to cause the death of its host (Webb, 2009); however, vivax malaria has also been implicated in major health complications and death in antiquity (Hume et al., 2003).

The pathophysiology of the illness caused by malarial infection can affect the skeleton in several ways. One of the primary health impacts caused by all forms of malaria is hemolytic anemia (i.e., severe malarial anemia) 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 expansion of the marrow space (Walker et al., 2009). Moreover, recent biomedical research has suggested that malarial hemolysis may impact the skeleton through acid phosphatase, free heme, and hemozoin release into the bloodstream (D'Souza et al., 2011; Moreau et al., 2012). The chemical release is higher in falciparum and mixed malarial infections (D'Souza et al., 2011), and causes an imbalance in bone remodeling through simultaneous osteoclast stimulation and osteoblast impairment (Moreau et al., 2012). Moreover, severe malarial anemia can induce extramedullary erythropoiesis, which is known to cause cortical thinning and coarse trabeculation in the skeleton (Al-Aabassi and Murad, 2005).

In general, the main defense against severe health complications during malarial infections is an acquired immunity in individuals who have survived malarial infections in the past. Thus, travelers coming from a nonendemic area into an endemic area are at an increased the risk for severe malarial infections since they lack this acquired immunity. However, some genetic protections against the *P. vivax* species of malaria result in more severe parasitemia in falciparum malaria. For example, in patients negative for the Duffy antigen (a genetic protection against *P. vivax* malaria), platelet-mediated destruction of falciparum malaria is ineffective (McMorran et al., 2012). Therefore, populations with increased survival for vivax malarial infection may experience higher mortality rates in falciparum malaria epidemics.

Even in endemic areas, malaria is known to be especially dangerous for children and pregnant women (Gilles et al., 1969; Lusingu et al., 2004; World Health Organization, 2007; Billig et al., 2012). Children make up 78% of current global malaria deaths, with severe malarial 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; World Health Organization, 2014). Lack of acquired immunity to malaria and small body size contribute to the increased severe malarial health impacts on children (Billig et al., 2012). In areas of high malarial transmission, falciparum malaria is the leading cause of parasitic anemia in children (Lusingu et al., 2004). For women, acquired immunity to the parasite is suppressed during pregnancy, leading to life-threatening anemia and increased risk of miscarriage or low fetal birth weight (Gilles et al., 1969; Brabin, 1983; Nájera and Hempel, 1996; World Health Organization, 2007).

Adult men are also at high risk during malaria epidemics due to malaria's tendency to combine with other diseases to create a more deadly health outcome. Malaria's strong indirect effect on mortality due to its synergy and coinfection with other diseases has been made clear by many independent researchers (Duffy, 1952; Shanks et al., 2008; Haldar and Mohandas, 2009;

Faure, 2014). Malarial anemia seems to increase susceptibility for other infectious diseases while lowering immune response generally (Shanks et al., 2008). However, parasitic immunosuppression and coinfection can have beneficial health effects as well [see Faure (2014) for a detailed summary of malaria's beneficial disease interactions and the use of malarial therapy throughout history].

From malaria's modern risk factor dynamics, it can be hypothesized that ancient populations affected by falciparum malaria would show high rates of maternal, fetal, and infant mortality that was directly caused by malarial anemia and cerebral malaria. This preferential mortality dynamic has been argued to have been the case at the archaeological site of Lugnano, Italy, in which a cemetery of 47 infant burials was associated with an epidemic of falciparum malaria (Soren, 2003). However, mortality for men in ancient populations affected by malaria would have also risen due to its synergistic effects with other diseases.

In investigating the impact of malaria on ancient populations, anthropologists must be able to determine the prevalence of the disease through its skeletal manifestation. Currently, anthropologists can only suggest malarial prevalence in an ancient population from the presence of porotic hyperostosis and cribra orbitalia under the assumption that these lesions are strongly impacted by the hemolytic anemia caused by malarial infections (Setzer, 2014; Smith-Guzmán, 2015). However, this assumption has never been confirmed clinically 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 nonmalarial individuals).

This paper represents the first effort to develop 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 in the past.

MATERIALS AND METHODS

Skeletal samples

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 holoendemic for falciparum malaria, all of the individuals present in the collection likely experienced multiple infections of malaria during their lifetimes. Additionally, medical records associated with the collection provide demographic information (i.e., age, sex, tribe) and cause of death for each individual.

Although the WHO sponsored Global Malaria Eradication Program was active in Southwestern Uganda between 1955 and 1969, the few attempts at mass antimalarial chemoprophylaxis failed due to noncompliance (Webb, 2014; Talisuna et al., 2015). It is likely that medical intervention with administration of antimalarial drugs would have taken place if the patients presented with symptoms of malaria when they were admitted to the hospital, no matter the determined cause of death. In the mid-20th century, chloroquine and pyrimethamine were the main antimalarial drugs used in Africa to ameliorate the symptoms of malarial infections, including severe anemia (Musoke, 1961; Webb, 2014; Talisuna et al., 2015). However, due to the rapid effects of hemolysis on the bone marrow and bone seen in murine models (Moreau et al., 2012), the bony changes resulting from chronic malarial anemia are not likely to have been affected by antimalarial drug administration because they would have already manifested by the time the patient felt sick enough to seek medical treatment. Thus, the bony changes seen in the Galloway collection individuals should be comparable to those of ancient populations lacking modern medical treatments for the disease.

Epidemiological studies published in the years during which the Galloway 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 from 1964 to 1965 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 nondiagnostic for parasites. Another study gathered data on children (aged $0-6$ years) admitted to the pediatric ward of Mulago Hospital from 1950 to 1951 (Musoke, 1961). Routine blood slides for malarial parasite identification were 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, $\sim$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 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 for 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. Handling and use by students over the past 7 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 and limited the number of individuals available to be used in this study.

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 sees very few cases of malaria per year imported by travelers. Therefore, the individuals present in the collection at the FACES lab have likely never 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. 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.

Skeletal lesion analysis

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 malarial anemia (Rabino Massa et al., 2000; Djuric et al., 2008; Nerlich et al., 2008; Gowland and Western, 2012), but also on other markers of nonspecific infection: periosteal reactions, linear enamel hypoplasias, periodontal disease. The porous lesions were scored as present when pores perforated completely through the cortical bone. These lesions tended to appear bilaterally; thus, they were recorded as present when they appeared on right, left, or both skeletal elements. Periosteal reactions were scored as present when raised longitudinal striations or unorganized woven bone overlay the cortical bone. Linear enamel hypoplasias were scored as present when at least one linear groove was observed macroscopically on at least one tooth in the dentition. Periodontal disease was scored as present when over half of the root was exposed on any tooth in the dentition.

The collection of data proceeded in three phases: (1) individuals whose cause of death was listed as "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 data collected from the Galloway collection skeletons were subsequently divided into two samples: an anemic sample (those whose reported cause of death included "malaria" or "anemia"; $n = 27$), and a nonanemic sample (those who died of other causes; $n = 71$). Individuals with a listed "malaria" or "anemia" cause of death were grouped together due to the fact that malaria is one of the most significant causes of anemia in Sub-Saharan Africa (Kassebaum et al., 2014), and most individuals in the collection had no cause of anemia listed. It should be noted that individuals with anemia caused by nutritional deficiency or parasitic worms, as well as those with an inherited hemolytic anemia, may be included within this category. Of the combined anemic sample, four individuals were noted specifically as having a malarial cause of death; however, many of the other individuals in the Galloway collection with other reported causes of death may have also had an active malarial infection at their time of death. Table 1 gives the age and sex distribution of the Galloway collection skeletal sample within the subsets used in this study.

The LSU FACES lab skeletons were analyzed similarly for all visible pathologies. 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, additional data were collected to obtain frequencies of nonmainstream skeletal lesions (i.e., spinal porosity, humeral cribra, and femoral cribra). All lesions were scored using the same criteria described above.

In comparisons with the Ugandan sample, the FACES lab sample was limited to only those individuals with African-American ancestry ($n = 106$) in order to minimize the potential confounding effect of the sickle cell trait on the results. Table 2 gives the age and sex distribution of the LSU skeletal sample within the subsets used in this study.

Statistical analyses

Statistical analyses were carried out using IBM SPSS 22.01. Statistical significance was set at $P \leq 0.05$. 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 test prevalent osteological markers in malarial and anemic individuals versus nonmalarial and nonanemic individuals within the Galloway sample. Similarly, contingency tables and tests of independence were used to determine significant associations between each osteological marker denoting marker concurrence. Next, the Galloway sample was compared with the control sample from the LSU FACES lab for significant differences in the frequencies of markers found at high frequencies in the Galloway sample. 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 (2001). 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. $\text{Positive Likelihood Ratio} = \frac{\text{Sensitivity}}{\text{1-Specificity}}$
6. $\text{Negative Likelihood Ratio} = \frac{\text{1-Sensitivity}}{\text{Specificity}}$
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

TABLE 1. Age and sex distribution of Galloway collection sample (N = 98)

	0–15		16–25		26–35		36–45		46–55		56+		Total	
	F[a]	M	F	M	F	M	F	M	F	M	F	M	F	M
Anemic	1	3	1	7	4	1	2	2	0	4	0	2	8	19
Non-anemic	2	4	4	15	9	14	4	12	0	5	0	2	19	52
Total	3	7	5	22	13	15	6	14	0	9	0	4	27	71

[a] F, female; M, male.

TABLE 2. Age and sex distribution of LSU FACES lab sample (N = 352)

	0–15			16–25			26–35			36–45			46–55			56+			Adult[a]			Total		
	F[b]	M	I	F	M	I	F	M	I	F	M	I	F	M	I	F	M	I	F	M	I	F	M	I
African-American	2	1	0	5	12	0	21	10	0	6	11	0	3	8	0	5	11	0	2	2	7	44	55	7
Other ancestry	0	2	3	14	16	1	12	34	3	13	23	1	5	20	0	2	11	0	4	16	66	50	122	74
Total	2	3	3	19	28	1	33	44	3	19	34	1	8	28	0	7	22	0	6	18	73	94	177	81

[a] Adults of indeterminate age.
[b] F, female; M, male; I, individual of indeterminate sex.

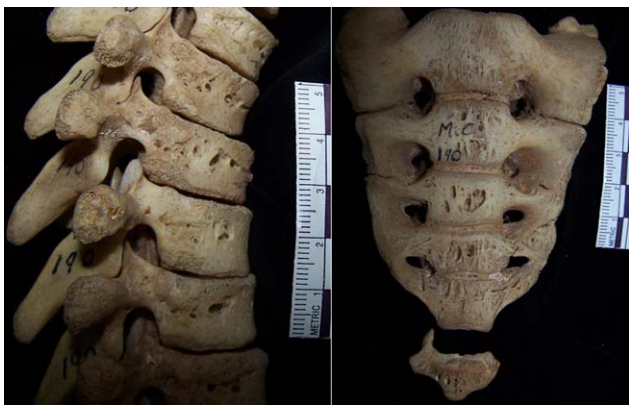

Fig. 1. "Spinal porosity" on the vertebral (left) and sacral (right) bodies of individual MC190. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

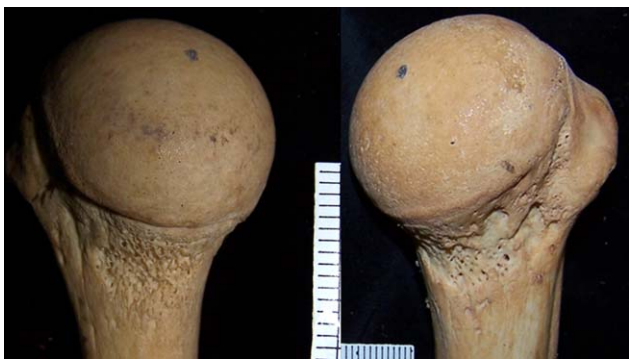

Fig. 2. "Humeral cribra" on individual MC100 (left) and individual MC53 (right). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

skeletal manifestations of malaria and their relationship to each other to diagnose the disease. The algorithm formulated for malarial diagnosis was then tested using the endemic vs. nonendemic samples to determine how well it identifies people with the disease and those without.

RESULTS

Anemic versus nonanemic within endemic sample

The anemic and nonanemic 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 Fig. 1), and humeral and femoral necks (see Figs. 2 and 3). 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 nonspecific inflammatory lesions—periostitis, alveolar resorption (periodontitis), and linear enamel hypoplasias (LEHs) —are shown in Figure 4. None of the lesion frequency differences between the anemic and nonanemic samples was found to be significant when tested for association with chi-square and Fisher's exact tests (see Supporting Information Table 1 for raw data). However, this result likely reflects the ubiquity of malarial infection in this holoendemic region. Although the subsample of individuals whose cause of death was listed as "malaria" was too small to be compared statistically with the other subsamples, these individuals presented no unique skeletal lesions that were not seen on individuals who died of other causes.

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 linked significantly with sex.

To determine associations between the skeletal lesions, each lesion was tested for association with each other lesion within the pooled Galloway collection sample (see Table 3). Cribra orbitalia presence was significantly

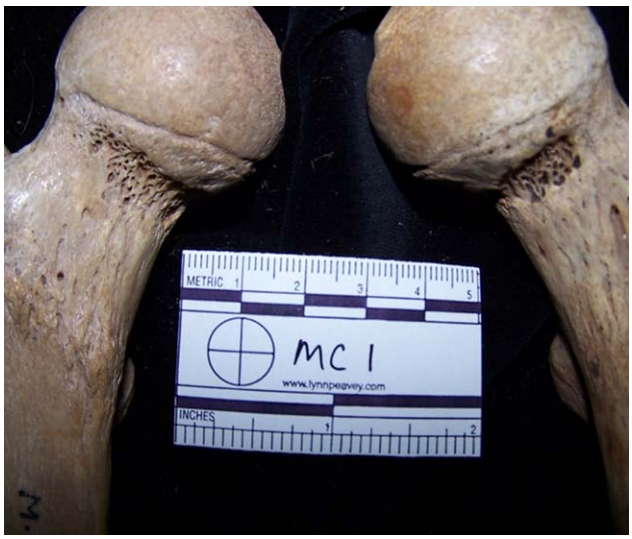

Fig. 3. "Femoral cribra" on individual MC1 (bilateral). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

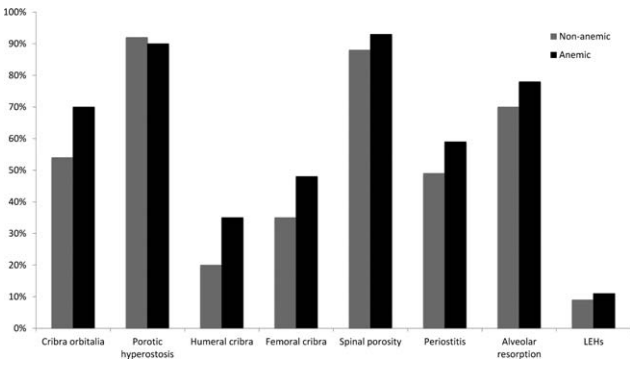

Fig. 4. Comparison of skeletal lesion frequencies in anemic vs non-anemic subsets within the Galloway sample. LEHs = linear enamel hypoplasias.

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.

Endemic versus nonendemic 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 (nonendemic sample of individuals unexposed to the disease; $n = 106$). The demography between the endemic and nonendemic 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.

TABLE 3. Relationship of prevalent lesions to each other within the Galloway sample [Significant cells are highlighted in yellow in the online issue, which is available at wileyonlinelibrary.com.]

	CO[a]	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)	

[a] 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.

All of the nondental lesions were found at considerably higher frequencies in the endemic sample than the nonendemic sample (see Fig. 5).

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 nonendemic sample, but not significantly so. Linear enamel hypoplasias associated significantly with the nonendemic sample (χ^2, df = 1, $P = 0.017$). Frequencies and Odds Ratios for all lesions by sample are recorded in Table 4.

From the frequencies of the lesions, alveolar resorption and linear enamel hypoplasias were eliminated 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 nonendemic sample contains individuals who have never been infected, each skeletal lesion was evaluated for its diagnostic power. Substituting the skeletal lesions for symptoms in epidemiological calculations of sensitivity and specificity, following Boldsen's (2001) model for skeletal diagnostic criteria of leprosy, each marker is evaluated in Table 5. 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.

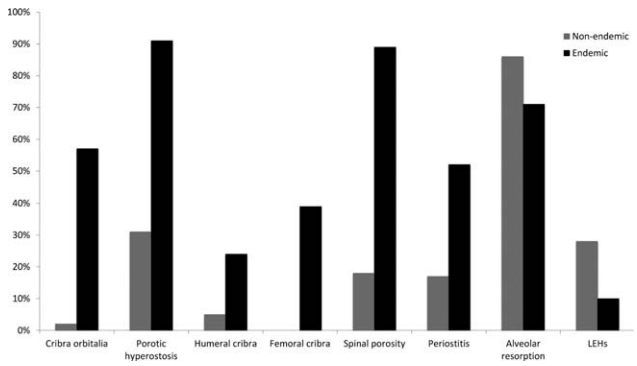

Fig. 5. Comparison of skeletal lesion frequencies in endemic vs nonendemic populations.

Forming an outcome algorithm for case diagnosis

From Table 5, 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. As a first step toward a diagnosis of malaria on ancient skeletons, the remaining markers were used to define a set of diagnostic criteria. By differentially weighting the appearance of the markers and their relationship to each other (see Table 3), the most effective diagnostic combination of multiple diagnostically sound lesions based on this study population was formulated. Using Pinhasi and Turner's (2008) example equation, the following logical expression for an "if" condition, or outcome algorithm, was devised 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 for efficacy in assigning individuals to the correct endemic and nonendemic populations using the total Galloway collection ($n = 98$) and total LSU collection ($n = 352$) samples. Of these samples, there were only 142 individuals that contained observable elements from which a positive or negative outcome could be determined 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 6.

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 any bony indication of previous malarial infection has resorbed. This reasoning was confirmed by retesting the diagnostic power of the algorithm with only those

TABLE 4. Frequencies of lesions in endemic and nonendemic samples

	Endemic	Nonendemic	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.

TABLE 5. 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[a]	0.615	2.223[a]
Periostitis	0.520	0.827	0.850	0.478	3.001	0.580	1.779

[a] 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.

TABLE 6. 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

individuals in the Ugandan sample with observable elements whose cause of death included anemia or malaria ($n = 20$) selected as the gold standard (see right columns in Table 6). Though the sample size was lower, the number of false negatives was cut in half, leading to 15% of the anemic East Africans incorrectly diagnosed as not having malaria.

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 generally described by paleopathologists as an inflammatory reaction of the periosteum, which occurs as a result of trauma or infection (Ortner, 2003). This skeletal lesion is seen in most specific infectious diseases (Pinhasi and Mays, 2008) as a result of sub-periosteal pressure and pus build-up due to chronic inflammatory response (Klaus, 2014). In the case of malaria, this inflammatory response 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. The tendency for cribra orbitalia to affect all age groups in this study counters the widespread assumption that cribra orbitalia is a skeletal lesion formed in childhood but retained in adulthood (Stuart-Macadam, 1985; Mittler and Van Gerven, 1994; Walker et al., 2009). This difference in affected age groups of the lesions may represent differences in etiological factors leading to their development, or may reflect differential remodeling rates of different areas of the skeleton. The etiological implications are discussed in further detail in the paragraphs to follow.

In bioarchaeological contexts, the results of this study suggest that malaria should be considered in paleopathological differential diagnoses within areas of known or suspected malarial presence. 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 diagnostic outcome algorithm should be used to score each skeleton individually for the combination of these lesions, keeping in mind that this algorithm was developed from a population affected primarily by falciparum malaria. The measures of sensitivity and specificity for the outcome algorithm may be used in combination with the gold standard population sample sizes to estimate true prevalence of malaria within the unknown skeletal population, following the methods of Rogan and Gladen (1978).

Malaria is a complex disease that is affected by multiple societal and environmental factors which must be taken into account when interpreting skeletal lesions in an archaeological context (Soren, 2003). In an

archaeological skeletal series with features suggestive of malaria, 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.

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., 2013). 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. Adding to the confusion, those publishing on the association of anemia with "femoral cribra" failed to acknowledge the existence of the other proposed activity-related etiology and vice versa (Miquel-Feucht et al., 1999; Djuric et al., 2008; Radi et al., 2013).

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 indeed 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 in the same collection 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 Fig. 6). 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 may 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 appeared on the anterior aspects of the vertebral bodies and were quite large in diameter (up to 12 mm on the vertebrae and 17.5 mm on the sacrum). In contrast, the lesions seen on anemic individuals tended to

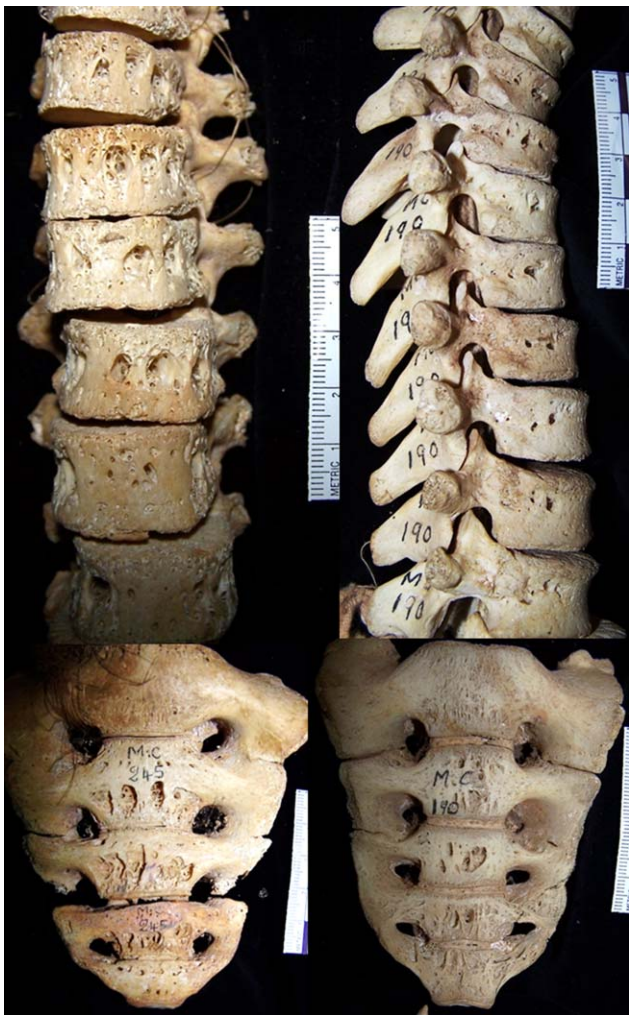

Fig. 6. Comparison of porous spinal lesions between brucellosis patient (left) and anemia patient (right) from the Galloway collection. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

be on the lateral aspects of the vertebral bodies and much smaller in diameter (up to 5.5 mm).

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 within this sample population, 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. Awaiting these future studies, caution must be taken in using the results of this study and the outcome algorithm to diagnose unknown remains.

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

CONCLUSIONS

This study identified five skeletal lesions suggestive 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 inflammation 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.

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