



Two Probable Cases of Infection with *Treponema pallidum* during the Neolithic Period in Northern Vietnam (~2000-1500B.C.)

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Title

- Two Probable Cases of Infection with *Treponema pallidum* during the Neolithic Period in Northern Vietnam (~2000- 1500B.C.)

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Running Title

- Treponematosi in Neolithic Northern Vietnam

KEY WORDS: AGRICULTURAL TRANSITION, SOUTHEAST ASIA, YAWS

Abstract

Skeletal evidence of two probable cases of treponematosi s, caused by infection with the bacterium *Treponema pallidum*, from the Northern Vietnamese Early Neolithic site of Man Bac (1906- 1523 cal B.C.) is described. The presence of nodes of subperiosteal new bone directly associated with superficial focal cavitations in a young-adult male (M20) and a 7-year-old child (M29) are strongly diagnostic for treponemal disease. Climatic and epidemiological contexts suggest yaws (*Treponema pallidum pertenue*) as the most likely causative treponeme. This evidence is the oldest discovered in the Asia-Pacific region and is the first well established pre-Columbian example in this region in terms of diagnosis and secure dating. The coastal ecology, sedentary settlement, and high fertility at the site of Man Bac all provided a biosocial context conducive to the spread of treponemal disease among inhabitants of the site. Co-morbidity with scurvy in both individuals demonstrates that malnutrition during the agricultural transition may have exacerbated the expression of treponematosi s in this community. Man Bac is a site of great regional importance as it temporally situated during the Neolithic transition of Mainland Southeast Asia. During this transition, approximately 4000 years ago, farmers migrating from Southern China into Southeast Asia influenced a number of changes in subsistence and demography and potentially introduced new infectious diseases such as treponematosi s to indigenous forager communities. The findings presented here will hopefully initiate re-evaluation of existing Southeast Asian skeletal samples and demonstrate the importance of using weighted diagnostic criteria for future reporting of treponematosi s cases.

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4 In recent years, intensive archaeological and bioarchaeological research in Mainland
5 Southeast Asia (MSEA) has described a unique agricultural transition that had
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7 significant impact on the genetics, demographics, social organization and subsistence
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9 base of the region (Lipson et al. 2018; Matsumura and Oxenham 2014; Oxenham and
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11 Buckley 2016). Strong morphological evidence for the admixture of local indigenous
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13 populations and migrants from southern China (Matsumura et al. 2019; Matsumura
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15 and Oxenham 2013a, 2013b, 2014; Matsumura et al. 2008) has been supported by
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17 recent genome-wide ancient DNA analyses (Lipson et al. 2018; McColl et al. 2018).
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19 The evidence for both co-habitation and genetic admixture of agriculturalists and
20
21 local foragers resulted in considerable social and demographic change at this time in
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23 MSEA (Lipson et al. 2018; McColl et al. 2018; Oxenham et al. 2011). While there
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25 remains a considerable focus on subsistence transitions and its impact on general
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27 health worldwide (Armelagos and Cohen 1984; Cohen and Crane-Kramer 2007;
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29 Snoddy et al. 2017; Temple 2010), less attention has been given to the mechanisms of
30
31 infectious disease transmission from one population to another where substantive
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33 levels of mobility (including migration) have been demonstrated. In these
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35 circumstances, interaction between two or more populations in so-called friction
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37 zones transpire (Bellwood and Oxenham 2008). Here genetic admixture and social
38
39 transition occurs between foragers and farmers. Epidemiological transitions may
40
41 follow population interactions, as subsistence transitions can cause micronutrient
42
43 deficiencies, and contact between populations (as evidenced in MSEA) encourages
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45 the spread of infectious disease from one group to another. The identification of
46
47 specific infectious diseases (such as leprosy, tuberculosis [TB], and treponemal
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49 disease) and the consequent epidemiological impact within the chronological, social,
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51 and environmental context of friction zones offers important information on human
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3 and pathogen co-evolution in the changing biosocial contexts of Southeast Asia over
4
5 time. An example of the possible introduction of infectious disease with increased
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7 population interaction has been suggested in the case of leprosy and TB at a late Iron
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9 age site in Northeast Thailand (Tayles and Buckley 2004), but has not been explored
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11 in other regions or during periods of agricultural transitions in this region.
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17 This article describes two cases of specific infectious and nutritional disease from the
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19 site of Man Bac in Northern Vietnam and explores the social, biological and
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21 ecological contexts which may have encouraged the spread of infectious disease
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23 during the Neolithic demographic transition in MSEA.
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28 **Materials and Methods**

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30 The site

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33 Man Bac is a Phung Nguyen period early Neolithic habitation site with an associated
34
35 cemetery located in Ninh Binh province in Northern Vietnam, known for complex
36
37 incised designs on pottery vessels, intricate stone craftwork, and interaction with
38
39 Chinese Neolithic farming societies (Oxenham et al. 2011). Cultural material reflects
40
41 both foraging and agricultural subsistence (Oxenham et al. 2011). Excavated in 1999,
42
43 2001, 2004/5 and 2007, the cemetery component of the site consists of 101
44
45 individuals of exceptional skeletal preservation. Rib samples from four individuals
46
47 from Man Bac have been dated to 1906- 1523 cal B.C. (IntCal13, 95% CI, with
48
49 marine reservoir effect applied; **Text S1; Table S1-S4; Fig. S1**). While insufficient
50
51 collagen yield meant direct dates could not be derived from the two individuals of
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53 focus in this paper, all burials were found within a secure single stratigraphic layer
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55 (Oxenham et al. 2011). The burial layer was found below two occupation layers.
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3 Cultural material from all layers are contemporaneous, suggesting an association
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5 between the three layers. Charcoal from the occupation layers date to between 2016-
6
7 1524 cal B.C. (95% CI; Oxenham et al. 2011). As such, Man Bac is the only well-
8
9 documented example of an early Neolithic settlement in Southeast Asia where local
10
11 indigenous people co-existed with migrants from Southern China who introduced
12
13 agricultural practices to the region approximately 4500 to 3500 years ago (Bellwood
14
15 and Oxenham 2008; Matsumura 2011a, 2011b).
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21 It is not possible to estimate the population density of the Man Bac community
22
23 because the number of burials is not likely representative of the numbers in the living
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25 population and the full extent of the site has not been excavated. However, the Man
26
27 Bac cemetery sample is characterized by a high fertility ratio and high rate of natural
28
29 population growth with 47.5% of individuals under the age of 5 years (McFadden et
30
31 al. 2018). All burials, with the exception of three flexed inhumations, were in a supine
32
33 extended position (**Fig. 1**), which is the normative pattern of burial at this time
34
35 throughout Southeast Asia (Higham et al. 2011). Grave goods and ritual tooth
36
37 removal (ablation) suggest age-based social hierarchy and affinal relationships that
38
39 may have affected the distribution of resources within the community (Tilley and
40
41 Oxenham 2016). Archaeological remains reveal a mixed or transitional subsistence
42
43 based on foraging and agricultural practices, similar to other Phung Ngyuen period
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45 settlements. A diet of both terrestrial and aquatic protein resources is indicated by
46
47 stable isotope collagen values, with more than 50% from aquatic resources (Oxenham
48
49 et al. 2011). The presence of domesticated pig (*Sus scrofa*) dominates the
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51 zooarchaeological assemblage (Jones et al. 2019; Oxenham et al. 2011).
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3 Today, Man Bac is situated approximately 25km from the coast (Oxenham et al.
4
5 2011). However, given high sea levels in the mid-Holocene, Man Bac was likely
6
7 much closer to the coast within an estuarine zone (Tanabe et al. 2006). The variety of
8
9 faunal sources indicate that the inhabitants of Man Bac were exploiting a number of
10
11 ecological zones including forests, grasslands, lowlands, and marine and estuarine
12
13 environments (Sawada et al. 2011; Toizumi et al. 2011). The site forms part of the
14
15 Phung Nguyen complex, a series of sites found within the Ninh Binh region along the
16
17 Red River Delta. The presence of contemporaneous pottery suggests extensive
18
19 interaction between the sites farther inland along the river, as well as shared
20
21 archaeological material with agricultural sites in Southern China (Bellwood 2008;
22
23 Nguyen 2008). The type site of Phung Nguyen is approximately 160km from today's
24
25 coastline, which represents the possible extent of interaction of farming settlements
26
27 within northern Vietnam, although this site is dated to slightly later at around 1500
28
29 B.C. (Khoach 1980). Evidence of housing styles was not excavated at Man Bac.
30
31 However, they may have been similar to other Phung Nguyen habitation sites where
32
33 large houses on stilts (see Oxenham et al. (2015) for a discussion of built structures in
34
35 the slightly later site of Rach Nui in Southern Vietnam) appear to have been built,
36
37 possibly for similar reasons as constructed in mountainous regions of Vietnam today,
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39 to keep out snakes and small animals (Khoach 1980).
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49 Concerning the general health of people buried at Man Bac, previous research has
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51 demonstrated high levels of non-specific stress; 92.3% of males and 53.8% of females
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53 presented with cribra orbitalia and 64.9% of the total assemblage presented with
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55 linear enamel hypoplasia of the incisors or canines (Oxenham et al. 2011). Forty-two
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57 per cent of individuals had localized primary canine hypoplasia which may be related
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3 to calcium, Vitamins A, or D deficiencies during gestation (McDonell and Oxenham
4
5 2014). Therefore, stress either from parasitic, dietary or infectious origin was a
6
7 commonplace experience during childhood for many of the individuals from Man
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9 Bac. The detailed paleopathology analysis of the total sample will be presented in
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11 forthcoming publications.
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17 Methods for age and sex estimation

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19 The age and sex of individuals from Man Bac were previously reported by Domett
20
21 and Oxenham (Domett and Oxenham 2011). Adult sex estimations were based on
22
23 standard methods for qualitative morphological estimates from the skull and pelvis
24
25 (Acsádi et al. 1970; Buikstra and Ubelaker 1994; Phenice 1969). Non-adult age
26
27 estimates were based on standards for the timing of dental calcification and eruption
28
29 (Moorrees et al. 1963; Ubelaker 1999). Where teeth were not available for age
30
31 assessment in nonadults, long bone lengths were compared to nonadults with dental
32
33 estimates available, as well as to standards for long bone estimates in non-adults by
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35 Scheuer and Black (2000). Adult age estimation followed Suchey-Brooks standards
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37 for the pubic symphysis (Brooks and Suchey 1990).
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45 Recording methods for abnormal variation (pathological changes)

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47 Proliferative (lamellar and woven bone) and lytic lesions as well as bone deformities
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49 were recorded in the entire preserved skeleton. Lesions with the potential to
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51 contribute to diagnosis were also radiographed. A differential diagnosis was
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53 performed with the basis drawn from seminal literature in paleopathology (Brickley
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55 and Ives 2010; Lewis 2017; Ortner 2003; Snoddy et al. 2018; Weston 2012), as well
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57 as clinical literature concerning specific diseases (Hackett 1976; Jaffe 1972; Resnick
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3 1995a). Lesions were characterized as diagnostic and suggestive of specific disease
4 based on recommendations by Snoddy et al. (2018). A clinical basis for diagnosis of
5 specific disease has also been championed by Mays (2012), Ortner (2011), Snoddy et
6 al. (2020), and Zuckerman et al. (2016) and others (for treponemal disease, Powell
7 and Cook advocated such criteria in their 2005 book, as do Baker et al. (2020). In the
8 Snoddy et.al (2018) criteria, lesions are only considered to be diagnostic if there is
9 strong clinical basis or considerable body of paleopathological work supporting the
10 diagnostic weight of the lesion. Lesions where there is no consensus in the clinical or
11 paleopathological literature, but remain anatomically intuitive, were designated a
12 suggestive value. In addition, lesions that have been considered elsewhere as
13 pathognomonic for disease are here scored as strongly diagnostic (after Brickley and
14 Ives 2010). For a probable case of disease at least one strongly diagnostic and/or two
15 diagnostic lesions are required (Brickley and Ives 2010; Snoddy et al. 2018). A
16 possible case of disease requires a minimum of one diagnostic lesion and/or two
17 suggestive lesions (Snoddy et al. 2018).
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40 Clinically documented associated pathology was also considered in discussion of the
41 disease expression but was not a primary deciding factor in the differential diagnosis
42 due to their poor diagnostic strength in dry bone. These included generalized
43 proliferative bone changes which can be caused by multiple diseases (See **Table S5**
44 for summary of diagnostic strength of lesions).
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53 **Results**

54 The lesions at Man Bac were predominantly bilateral, symmetrical and discrete, and
55 affected both the postcranial and cranial remains. As the focus of this paper is
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concerned with the identification of specific infectious disease at Man Bac, the causes of the more generalized pattern of proliferative lesions at Mac Bac will be reported at a later date, but are likely a result of a number of etiologies.

A total of seven individuals (10%) from Man Bac share a similar skeletal pattern of diffuse proliferative new bone lesions consistent with systemic disease (two adults, two adolescents, and three children). Two of these individuals presented with widespread subperiosteal new bone, endosteal enlargements in the long bone shafts *and* focal superficial lytic lesions within nodes of subperiosteal new bone (localized enlargements of subperiosteal new bone). These two individuals are the focus here due to the diagnostic potential of their lesions, with a brief discussion of a further six cases with lower diagnostic strength. A differential diagnosis was conducted on MB05M20 and MB07H2M29, which includes systemic conditions known to produce lesions similar to those observed here: osteomyelitis (non-specific bone infection), leprosy, tuberculosis, brucellosis, mycosis, treponematosi s, ricketts/ osteomalacia, and scurvy. A description of the bone pathology in these two individuals and a full differential diagnosis are presented. Detailed descriptions of lesions in all seven individuals are found in **Table S5**. Descriptions of skeletal lesions consistent with possible and probable treponemal disease in all affected Man Bac individuals can be found in **Table S6**.

M20

MB05M20 (known henceforth as M20) is a young adult male (15-29 years old). The individual was buried east to west in a supine position with three ceramic pots characteristic of the Phung Ngyuen period (**Fig. 1**). The skeleton is excellently

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3 represented with all limbs, skull (and teeth), hands and feet complete, and 60%
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5 representation of the ribs and vertebrae. The surfaces of the bones are in excellent
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7 condition. M20 presented with diffuse thick and remodeled new bone with endosteal
8
9 enlargement of the entire shafts of the right humerus, tibiae, and fibulae (**Fig. 2**).
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11 Discrete nodes of new bone are present on the medial midshaft of the right tibia and
12
13 on the distal third of both fibulae symmetrically. The new bone is present on the
14
15 metaphyses and shafts of the tibiae and fibulae and the distal metaphysis of the right
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17 humerus. There are pathological pseudobowing deformities in the tibiae similar to
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19 bowing in a saber shin. While new bone is macroscopically only visible on the distal
20
21 metaphysis of the right humerus, radiographs reveal endosteal enlargement of the
22
23 entire shaft, suggesting prior osteoblastic activity that has since remodeled. Further
24
25 discrete deposits of new bone can also be observed on the medial proximal left femur
26
27 and medial and lateral proximal tibiae. Multiple superficial focal lytic lesions are
28
29 present on the distal fibulae shafts, proximal right fibula, lateral metaphysis of the left
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31 humerus, and the lateral aspect of the left calcaneus. The focal lytic lesions of the
32
33 fibulae are associated with discrete nodes of new bone and active new bone is
34
35 observed around the margins of the lytic lesions. Two focal lesions of the right distal
36
37 fibula have been damaged postmortem, so it is not possible to determine whether the
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39 lesions are superficial or extend into the medulla. However, a small focal superficial
40
41 lesion is present adjacent to the larger two lesions (Fig. 2f), supporting the
42
43 interpretation that the lesions with postmortem damage do not extend beyond the
44
45 external cortical bone surface. The two focal lesions of the left distal fibula are clearly
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47 superficial, within a node of new bone and with smooth sclerotic margins (Fig. 2g).
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3 No lytic or diffuse proliferative lesions were present on the cranial vault. Bilateral and
4
5 symmetrical discrete deposits of remodeled new bone and abnormal cortical porosity
6
7 are present on the external greater wings of the sphenoid bones, temporal bone
8
9 squama, and posterior and anterior zygomatic bones. The lesions are clearly discrete
10
11 and do not represent diffuse inflammatory changes in the skull. There are also porotic
12
13 lesions across the ectocranium (frontal bone, occipital bone, and parietal bones medial
14
15 to the temporal lines) consistent with porotic hyperostosis, although the diploe is not
16
17 exposed so this cannot be confirmed. Medium-grade cribra orbitalia was also
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19 observed (after Stuart-Macadam 1985:392).
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26 M29

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28 MB07H2M29 (herein called M29) is a non-adult approximately 7 years of age at
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30 death (Oxenham et al. 2011). Buried supine, no clear grave goods were identified,
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32 with the presence of pottery sherds around the region of the sacrum (Fig. 1). The
33
34 cranium was not present and only the right side of the mandible (including teeth),
35
36 remained. The individual's postcranial axial skeleton is very well preserved, but the
37
38 hands and feet were virtually absent (with the exception of a single preserved
39
40 metacarpal). There were some hardened soil concretions on the surfaces of the long
41
42 bones and vertebrae, but this was minimal. Animal gnaw and puncture marks were
43
44 observed on some of the lower limb bones.
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51 M29 presents with diffuse multi-layered new bone across the diaphyses of all limb
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53 bones bilaterally, and on the single preserved metacarpal (**Fig. 3**). Nodes of
54
55 subperiosteal new bone are present at the midshafts of the right radius and ulna,
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57 proximal left ulna, entire shafts of tibiae and distal fibulae. Multiple lytic lesions with
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3 sclerotic margins, observed radiographically, are present on the proximal left ulna,
4
5 midshafts of the right radius and ulna, and inferior medial left clavicle. The lytic
6
7 lesions of the midshaft of the right ulna and radius have some postmortem damage to
8
9 the floor and margins. However, the preserved margins of the lesions indicate they did
10
11 not penetrate the cortex. The lytic lesions on the distal fibulae do penetrate the cortex
12
13 to the medullary canal. Radiographs also show well remodeled proliferative new bone
14
15 on the anterior crest of the tibiae indicating so-called saber shin. A unilateral
16
17 enlargement of the sternal end of the left clavicle without any macroscopic presence
18
19 of thick subperiosteal new bone deposit is evident and a thin cortex is appreciated
20
21 from the radiograph. Symmetrical mixed active and remodeled new bone and
22
23 abnormal cortical porosity is present in the supraspinous fossae of the scapulae.
24
25 Although the cranium is absent, remodeled abnormal cortical porosity of the right
26
27 coronoid process of the mandible is observed. Radiographically, all the long bones
28
29 present with radiodense metaphyseal plates in association with zones of radiolucency
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31 in the metaphyseal region.
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40 Deformity of some long bones is also present in M29. Symmetrical swellings of the
41
42 distal metaphyses and slight cupping of the femora occur (**Fig. 4c**). There is slight
43
44 bilateral medial “true” bowing of both tibiae confirmed by radiographs with slight
45
46 posterior protrusion of proximal metaphyseal plates suggesting depression and
47
48 deformity (**Fig. 4g**). Further, there is lateral bowing of the proximal third of the
49
50 humeral shafts, and depression and deformity of the metaphyses of the humeral heads
51
52 (**Fig. 4d**).
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58 Discussion

59 Differential diagnosis
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3 The differential diagnosis of the types and skeletal pattern of the lesions in these Man
4 Bac individuals included bone cancers, osteomyelitis, tuberculosis, brucellosis,
5
6 mycosis, leprosy, and treponematosi. These diseases are included in the differential
7
8 diagnosis due to the combination of proliferative and destructive lesions in the disease
9
10 pathophysiology. The bone response to metastatic cancers may produce a
11
12 combination of extensive subperiosteal and lytic response. However, these conditions
13
14 are less likely to cause such widespread involvement of the skeleton as that observed
15
16 here, and are not known to cause multiple and widespread superficial cavitations
17
18 (Ortner 2003:363-382, 503-544). Cancers such as multiple myeloma or Langerhan's
19
20 cell histiocytosis (LCH), which can result in systemic multifocal destruction of the
21
22 skeleton, most often appear as punched out lytic lesions without sclerosis, and can be
23
24 ruled out (Ortner 2003:362, 377). M20 and M29 do not appear similar to a possible
25
26 case of multiple myeloma or LCH identified by Domett and Buckley (2012) in Pre-
27
28 Angkorian Cambodia, and marginal sclerosis of the lytic lesions are rare and
29
30 subperiosteal new bone deposits are not characteristic of these diseases (Ortner
31
32 2003:363, 377). Because bone cancer in archaeological contexts is extremely rare
33
34 (Ortner 2003:363-382, 503-544), the presence of two individuals in the collection
35
36 with similar bone lesions (M20 and M29) further demonstrates that cancer can be
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38 ruled out as a possible cause.
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49 While subperiosteal and endosteal enlargement are non-specific inflammatory
50
51 responses to various diseases and trauma (Weston 2012), the widespread and non-
52
53 uniform nature of the enlargements in the Man Bac cases are more consistent with
54
55 that of a systemic infection than metabolic or traumatic etiologies. The subperiosteal
56
57 new bone lesions of metabolic disease tend to be uniform and symmetrical unlike the
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3 majority of the lesions of M20 and M29 (Brickley and Ives 2010; Pitt 1995; Resnick
4
5 1995b; Snoddy et al. 2018). Furthermore, subperiosteal new bone from trauma is most
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7 often localized, also unlike that observed in M20 and M29 (Rana et al. 2009).

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10 However, some of the subperiosteal lesions in both M20 and M29 are symmetrical
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12 and a metabolic etiology contributing to the overall expression of subperiosteal
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14 lesions in both individuals is not ruled out and is discussed further below.
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19 Pyogenic osteomyelitis is a non-specific infection of the bone producing a
20
21 characteristic suite of lesions of bone death from loss of blood supply (sequestrum),
22
23 combining a lytic lesion (circular pus-draining cloaca) and a proliferative healing
24
25 response (involucrum) (Ikpeme et al. 2010). The major causative agents are
26
27 *Staphylococcus* or *Streptococcus* bacterial species although other pathogens can infect
28
29 bone (Acosta et al. 2004; Ikpeme et al. 2010). Initial transmission generally occurs
30
31 through soft tissue infection of open wounds and, less frequently, secondary to
32
33 respiratory, food-borne or water-borne diseases (Giaccari and Idriss 1952; Honda and
34
35 McDonald 2009; Jaffe 1972:1015-1046; Miller et al. 1963; Vohra et al. 1997). The
36
37 pathogenic agent enters the bone through the bloodstream and infiltrates through
38
39 nutrient arteries (Jaffe 1972:1015-1020). Proliferation within bone generally begins in
40
41 the metaphyseal region, and eventually spreads to the cortex through the Haversian
42
43 canals (Ikpeme et al. 2010; Jaffe 1972:1020). Osteomyelitis can result in nodes of
44
45 new bone only, like treponemal disease (Hackett 1976:424). However, it is
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47 particularly diagnostic in its pyogenic form. Substantial deposits of subperiosteal new
48
49 bone occur particularly in the long bones resulting in a shell of new bone termed the
50
51 involucrum (Ikpeme et al. 2010; Kharbanda and Dhir 1991). Disruption to blood
52
53 supply by the involucrum results in bone death (sequestrum) (Ikpeme et al. 2010;
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3 Kharbanda and Dhir 1991). Finally, cloacae develop within the involucrum in order to
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5 drain pus that has accumulated within the medullary canal (Ikpeme et al. 2010;
6
7 Kharbanda and Dhir 1991). Dactylitis, as observed in M29, is known to occur in
8
9 osteomyelitis, but is frequently associated with direct infection to the surrounding soft
10
11 tissue due to trauma or localized infection (Jaffe 1972:1018). Osteomyelitis is rare in
12
13 adults but can result from reoccurrence of infection from adolescence (Ortner
14
15 2003:187; Resnick and Niwayama 1995). However, the superficial focal cavitations
16
17 on the external cortex as observed in the left fibula of M20 are unknown to occur in
18
19 osteomyelitis and are incompatible with the pathophysiology of cloacae in pyogenic
20
21 bone infection. While necrotic abscesses have been observed in osteomyelitis, they
22
23 occur within the medullary canal and not the external cortex (Jaffe 1972:1020).
24
25 Although two lesions in the distal fibulae of M29 penetrate to the medullary canal and
26
27 are reminiscent of cloacae, such lesions have also been observed in cases of
28
29 treponemal disease where gummatous lesions are associated with advanced endosteal
30
31 enlargement (Hackett 1975). It is clear the entire bone of the fibulae shafts has been
32
33 involved in this child with the inner cortex appearing porous. There is a lack of
34
35 association of involucrum or sequestrae with the lytic lesions in M29, as would be
36
37 expected with osteomyelitis, and more extensive proliferative new bone response also
38
39 would be expected (Hackett 1976:92). Furthermore, it would be unusual for cloacae
40
41 to occur symmetrically such as observed in M29 as formation of cloacae are
42
43 determined by the nature of the involucrum development and, therefore, do not appear
44
45 in predictable patterns (Jaffe 1972:1018; Roberts 2019:298). Systemic non-localized
46
47 skeletal expression of both the lytic and proliferative lesions in M20 and M29 without
48
49 focus of the infection in the metaphyseal regions of bone make osteomyelitis as the
50
51 underlying cause extremely unlikely. However, given the non-specificity of
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3 osteomyelitis, particularly the non-pyogenic form, a secondary bacterial, viral or
4
5 fungal infection cannot be ruled out.
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10 The *Mycobacterium tuberculosis* complex (MTBC) is a group of infectious slow-
11
12 growing gram positive bacteria that can cause granulomatous necrotizing abscesses
13
14 throughout the entire body, including bone (Flynn and Chan 2001; Haque 1990;
15
16 Knechel 2009). The MTBC consists of *Mycobacterium* strains that infect a number of
17
18 mammalian primary hosts but can all cause infection of humans. These include: *M.*
19
20 *tuberculosis* (humans), *M. bovis* (domestic livestock), *M. canetti* (humans), *M. microti*
21
22 (voles, hyrax, llama, rarely humans), *M. caprae* (goats), *M. pinnipedii* (seals and sea
23
24 lions, rarely humans), and *M. africanum* (humans) (Roberts 2012). The most common
25
26 form is *M. tuberculosis*, which is primarily a respiratory disease affecting the lungs
27
28 (De la Rua-Domenech 2006; World Health Organization 2013). *M. tuberculosis* is
29
30 most often spread through air droplets from coughing, sneezing and talking (Knechel
31
32 2009; World Health Organization 2013), whereas zoonotic forms such as *M. bovis*
33
34 tend to spread to humans through ingestion of meat or dairy (De la Rua-Domenech
35
36 2006).
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45 In its chronic form, tuberculosis can cause systemic granulomatous skeletal
46
47 destruction with minimal new bone response, predominantly in regions of trabecular
48
49 bone and articular surfaces, such as the vertebral bodies (Jaffe 1972:956; Key 1940;
50
51 LaFond 1958; Ortner 2003:228). Therefore, bone destruction in tuberculosis most
52
53 commonly affects the spine above all other skeletal elements, followed in frequency
54
55 by involvement of the hip and knee joints (Jaffe 1972:956; LaFond 1958; Ortner
56
57 2003:228). In the most advanced form, destruction of the vertebral bodies results in
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1
2
3 collapse referred to as gibbus formation (or structural kyphosis) seen in Pott's disease,
4
5 which has been considered pathognomonic for tuberculosis (Davidson and Horowitz
6
7 1970; Jaffe 1972:958-961; Turgut 2001). While circumferential deep focal destruction
8
9 can occur in the long bones in tuberculosis, it is more characteristic of childhood
10
11 tuberculosis and, even then, is focused in long bone ends rather than shafts (Lewis
12
13 2017:161; Ortner 2003:245; Teo and Peh 2004). Sclerotic margins of the superficial
14
15 focal lesions on the external shafts of the long bones of M20 and M29 accompanied
16
17 by cortical enlargement is not reminiscent of the destruction of tuberculosis which,
18
19 while chronic, elicits minimal remodeling of the lesion margins (Jaffe 1972:976).
20
21 Proliferative new bone response is known to occur, particularly in the early stages of
22
23 disease, as a systemic inflammatory response to the pathogen or due to psoas abscess
24
25 formation adjacent to the lumbar spine, but is infrequently found in direct association
26
27 with the destructive lesions in adults (Davidson and Horowitz 1970; Ortner 2003:232;
28
29 Ridley et al. 1998). In the case of tuberculosis, dactylitis is rare but can occur in
30
31 juveniles such as that observed in the single metacarpal of M29 (Teo and Peh 2004).
32
33 However, in M29 the degree of expression is not severe and lacks the cystic
34
35 destructive form more common to childhood tuberculosis (Lewis 2017:162; Teo and
36
37 Peh 2004). As there is no evidence of spinal, trabecular or joint involvement of which
38
39 pathogens in the MTBC complex are selective for, tuberculosis is ruled out as a
40
41 possible cause for the lesions in M20 and M29.
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51 Brucellosis is a zoonotic disease commonly associated with animal husbandry.
52
53 Human disease is mainly caused by four species of bacteria *Brucella abortus*, *B. suis*,
54
55 *B. canis* and *B. melitensis* (Al-Shahed et al. 1994; Franco et al. 2007). The bacteria
56
57 are transmitted mostly through consumption of animal products such as dairy, but can
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1
2
3 be transmitted through skin lesions in circumstances of close contact with animals
4
5 (d'Anastasio et al. 2011; Franco et al. 2007; Mehmet et al. 2002). The *Brucella*
6
7 bacteria bind to cells of the mononuclear phagocyte system, a group of cells that
8
9 include bone marrow progenitors, blood monocytes, and macrophages found in
10
11 hematopoietic regions of the body including in bone (Campbell et al. 1994; Hume
12
13 2006). Once chronic, it can affect the skeleton in focal or diffuse forms (Al-Shahed et
14
15 2006). Once chronic, it can affect the skeleton in focal or diffuse forms (Al-Shahed et
16
17 al. 1994). Less has been described about the skeletal pathology of brucellosis than
18
19 tuberculosis, and questions remain about how to differentiate definitively between the
20
21 two, and whether this is even possible in many cases (Al-Shahed et al. 1994). As is
22
23 the case in tuberculosis, focal destruction of the spine is a primary characteristic of
24
25 this disease (Al-Shahed et al. 1994). Brucellosis can cause widespread destruction of
26
27 the skeleton, and skeletal involvement is common (Al-Shahed et al. 1994; Mehmet et
28
29 al. 2002). Destructive lesions are often but not always accompanied by sclerotic
30
31 reaction due to their slow development, which is not characteristic of tuberculosis
32
33 (Roberts and Buikstra 2019:231). Remodeling can result in the presence of parrot's
34
35 beak osteophytes on the superior margin of the anterior vertebral bodies (Al-Shahed
36
37 et al. 1994). While collapse of the spine can occur, it is unusual for the destruction to
38
39 advance to the severity seen in tuberculosis (Roberts and Buikstra 2019). Similar to
40
41 tuberculosis, osteolytic lesions develop in regions of hematopoiesis including the hip,
42
43 knee and ankle joints (Al-Shahed et al. 1994; Mehmet et al. 2002). Unlike in
44
45 tuberculosis, following the spine, the sacroiliac joint and knee are most commonly
46
47 affected (Al-Shahed et al. 1994). For similar reasons as stated in the consideration of
48
49 tuberculosis, it is unlikely that brucellosis is responsible for the skeletal appearance of
50
51 disease observed in M20 and M29. Although brucellosis is known to result in focal
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53 lesions with sclerotic response, superficial lesions of the external cortex, particularly
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3 in relation to cortical enlargements of long bone shafts have not been observed in the
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5 pathophysiology of this disease.
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10 Various fungal infections are known to result in skeletal involvement. Diseases
11 including coccidioidomycosis, blastomycosis, histoplasmosis, aspergillosis, and
12 cryptococcosis rarely infect the musculoskeletal system, but are more common in
13 immunocompromised individuals (Corr 2011). Transmission often occurs through
14 open wounds or through the respiratory tract whereby hematogenous spread occurs
15 (Corr 2011). Infection is more common in the spine, but can occur in any region of
16 the skeleton, particularly in regions of hematopoiesis (Corr 2011; Toone Jr and Kelly
17 1956). For this reason, there is a predilection for axial skeletal involvement
18 (Taljanovic and Adam 2011). When long bones are involved, the metaphyses are
19 more commonly affected, with joints affected if destruction is extensive, at times
20 resulting in septic arthritis (Taljanovic and Adam 2011). The pathological response is
21 primarily in the form of focal punched out or deep granulomatous osteolytic lesions
22 with or without sclerotic margins (Corr 2011; Jones and Goodwin Jr 1981; Taljanovic
23 and Adam 2011; Toone and Kelly 1956). Proliferative new bone response to the
24 extent observed in M20 and M29 have not been reported for mycotic infections.
25 However, given the systemic inflammatory response observed in fungal infections
26 such as coccidioidomycosis, the potential for subperiosteal involvement in mycotic
27 infections cannot be ruled out (Corr 2011; Taljanovic and Adam 2011). Finally,
28 clinical reports of skeletal mycotic infections describe lesions that tend to be
29 unilateral and assymmetrical throughout the skeleton (Jones and Goodwin 1981;
30 Taljanovic and Adam 2011; Toone and Kelly 1956). Given the predilection for the
31 axial skeleton such as in the case of tuberculosis and brucellosis, destruction is
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3 associated with hematopoiesis in the skeleton, whereby deep osteolysis is more
4
5 consistent with the pathophysiology of this disease group rather than superficial lytic
6
7 lesions in the appendicular skeleton as observed in M20 and M29. The symmetrical
8
9 lytic lesion pattern in the Man Bac individuals is also not consistent with clinical
10
11 reports of mycosis. Mycosis is therefore ruled out as a possible cause.
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17 Leprosy is a bacterial infection caused by *Mycobacterium leprae* or *M. lepromatosis*
18
19 that affects the skin, nasal mucosa and peripheral nerves (Eichelmann et al. 2013;
20
21 Saonere 2011). The symptoms of the disease vary along a spectrum and are highly
22
23 dependent on individual immune response (Eichelmann et al. 2013). Tuberculoid
24
25 leprosy is a milder form that is often non-contagious, whereas lepromatous leprosy is
26
27 more systemic, infectious, and results in more severe symptoms (Eichelmann et al.
28
29 2013; Saonere 2011). Major skeletal changes in leprosy involve naso-facial
30
31 destruction with inflammatory pitting, and resorption of the terminal phalanges of the
32
33 hands and feet secondary to loss of sensation from peripheral nerve or circulatory
34
35 degeneration caused by the pathogen (Møller-Christensen 1961, 1978; Ortner
36
37 2003:264-265). Slow progression of resorption of the naso-palatal margins, including
38
39 the anterior nasal spine, and alveolar process of the maxillary incisor area, with
40
41 associated proliferative subperiosteal new bone and cortical pitting are considered
42
43 strongly diagnostic features of this disease (Møller-Christensen 1961, 1978; Møller-
44
45 Christensen et al. 1952). While new bone can occur in leprosy, particularly in the
46
47 lower legs, and skin granulomas can also occur, leprosy is not known to cause focal
48
49 granulomatous-like lytic lesions of the long bones such as that observed in M20 and
50
51 M29 (Faget and Mayoral 1944). Osteomyelitis secondary to peripheral nerve
52
53 destruction in advanced stages of the disease is well documented; but, as described
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2
3 above, the superficial lytic lesions of M20 and M29 are not characteristic of
4
5 osteomyelitis (Faget and Mayoral 1944; Maas et al. 2002). The suite of cranial lesions
6
7 observed in M20 is not characteristic of leprosy, as neither destructive lesions of the
8
9 cranium nor proliferative or cortical pitting lesions of the nasal region were observed.
10
11 Furthermore, no destructive changes to the preserved tubular hand and feet bones of
12
13 M20 and M29 were observed. The absence of the cranium and the presence of only
14
15 one metacarpal means leprosy cannot be ruled out as a co-morbidity in the case of the
16
17 child. However, it is improbable that leprosy is responsible for lesions observed in
18
19 both M20 and M29.
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26 Treponematosi

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28 Treponemal disease is a group of infections caused by a spirochete bacterium that
29
30 primarily affects the skin but can also affect bones and other organs (Hook and Marra
31
32 1992). Four diseases including yaws (*Treponema pallidum pertenu*), pinta (*T.*
33
34 *carateum*), venereal syphilis (*T. pallidum pallidum*) and endemic syphilis (*T. pallidum*
35
36 *endemicum*) have similar bacterium morphology and share similar pathogenesis
37
38 (Hook and Marra 1992; Marks et al. 2014; Willcox 1974). Pinta is the only
39
40 treponemal disease that does not affect bone (Willcox 1974). Each treponeme
41
42 occupies a different ecological niche (Marks et al. 2014). For example, endemic
43
44 syphilis is restricted to warm arid regions of the world whereas yaws thrives in
45
46 subtropical and tropical regions (Willcox 1974). Yaws, pinta, and endemic syphilis
47
48 are non-venereal treponemal diseases which are transmitted primarily by skin contact,
49
50 whereas venereal syphilis has evolved to be spread primarily through sexual contact,
51
52 (Willcox 1974). A primary skin lesion forms at the site of inoculation within a few
53
54 weeks of initial infection and subsequently resolves itself (Peeling and Hook 2006). If
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3 left untreated, the disease almost always progresses to a secondary phase. In the
4
5 secondary phase, the skin rash reappears, becoming more diffuse and is associated
6
7 with swelling of lymph nodes (lymphadenopathy) (Peeling and Hook 2006). The
8
9 secondary phase is then followed by an asymptomatic latent phase, where the
10
11 infection is contained within granulomas by the immune system (Hackett 1953b).
12
13 Latency can last from 3 to 10 years beyond the initial infection (Hackett 1953b).
14
15 Following the period of latency, except in the case of pinta, a tertiary stage begins,
16
17 where necrosis of the granulomas occurs, resulting in *gummatous* lesions of skin,
18
19 internal organs, and bones (Hackett 1953b; Powell and Cook 2005:15-51). Formation
20
21 of the tertiary stage has been historically documented to occur in one in three
22
23 untreated cases (Hackett 1953b). The tertiary stage is distinctive for its gross
24
25 destruction of the face, skull, limbs, and, in the case of venereal syphilis, arterial
26
27 vessels and organs (Hackett 1951; Peeling and Hook 2006). During the tertiary stage,
28
29 secondary lesions stop forming (Hackett 1953b). While early treponemal lesions can
30
31 heal within a few months, the late (tertiary stage) lesions can last for more than a year
32
33 and leave permanent changes in bone (Hackett 1978).
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42 These granulomatous, gummatous lesions are distinctive and specific to all
43
44 treponemal diseases, and are universally accepted as strongly diagnostic in
45
46 paleopathology (Harper et al. 2011; Ortner 2003:286). The so-called *caries sicca*
47
48 lesions on the skull are the most well recognized lesions of the disease and are
49
50 clinically and paleopathologically documented in all skeletal forms of treponemal
51
52 disease (Hackett 1951:159-165; 1974; Sandison 1980). Gumma outside of the
53
54 cranium can develop as focal lesions termed “superficial cavitations” within
55
56 proliferative new bone on outer surfaces of long bones (Hackett 1976:362-396, 429-
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3 433). All tertiary lesions of treponemal disease are also associated with a sclerotic
4
5 smoothing of the margins and base of the lesion (Hackett 1976:362-396, 429-433).
6
7 Gross destruction of the palate and the nasal aperture can also occur (Hackett 1946).
8
9 In the case of venereal syphilis, trans-placental transmission, congenital syphilis,
10
11 causes distinctive bone and dental deformations in infants and young children up until
12
13 puberty (Jaffe 1972:908-910; Lewis 2017:176-177). Congenital forms are rarer in the
14
15 endemic treponemes but have been reported in endemic syphilis from the Ramadi
16
17 District of Iraq, and in yaws in central Java, Indonesia (Akrawi 1949; Csonka 1953;
18
19 Engelhardt 1959). The three treponemes that affect bone cannot be distinguished by
20
21 their skeletal expression (Harper et al. 2011).
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29 As the above diseases are unlikely causes of the lesions, the presence of superficial
30
31 cavitations within nodes of new bone in M20 and M29, strongly suggest that the
32
33 etiology of the lesions expressed is treponematosi s (**Fig. 5**). The presence of saber
34
35 shin and dactylitis supports this diagnosis. We also note here that the lytic lesions and
36
37 distinct nodes of new bone in M20 and M29 correspond with dissemination through
38
39 lymph nodes argued by Buckley and Dias (2002) to be the primary form of non-
40
41 cranial dissemination of the *Treponema pallidum* spirochete into bone. Unilateral
42
43 enlargement of the sternal end of the clavicle, as observed in M29, has been noted in
44
45 cases of late onset congenital syphilis (Dax and Stewart 1939; Frangos et al. 2011;
46
47 Harper et al. 2011; Lewis 2017:179; Yang 1940). This so-called *Higoumenakis sign*
48
49 presents as layers of proliferative new bone on the sternal end of the clavicle
50
51 unilaterally (Frangos et al. 2011). The lack of macroscopic or radiographic trace of
52
53 proliferative subperiosteal new bone or cortical enlargement on the sternal end of
54
55 M29's left clavicle makes the identification of this pathology as a clear Higoumenakis
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3 sign difficult. However, a rationale for this is explained below in consideration of co-
4 morbidity with nutritional stress.
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10 To allow for standardization of diagnosis of treponemal diseases, we here present
11 threshold criteria for the designation of possible and probable cases (Table 1).
12 Standard criteria have been presented previously by Harper et al. (2011) using a 5-
13 score system for inclusion or exclusion of cases. This system attributes a score
14 between 1 and 5, dependent on the specificity of the lesion to treponemal disease.
15 Non-specific lesions such as subperiosteal new bone deposit are classified as a score
16 of 1 and lesions specific to treponemal disease, such as gummatous lesions and late
17 stage caries sicca of multiple skeletal elements, are provided a score of 5. However,
18 the authors do not give a minimum point score required for a sufficient diagnosis of
19 treponemal disease in dry bone. The standardization presented here builds upon work
20 by Harper et al. (2011) by introducing a "threshold approach" to diagnosis as
21 recommended by Brickley and Ives (2010) for their criteria for diagnosis of scurvy,
22 rickets and osteomalacia and Snoddy et al. (2018) for their criteria for scurvy
23 diagnosis. As described above, we have employed the minimum criteria of one
24 strongly diagnostic or two diagnostic lesions for a probable diagnosis of treponemal
25 disease, which both of these cases demonstrate.
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49 A recent review of global evidence for treponemal disease by Baker et al. (2020)
50 includes a diagnostic protocol similar to the one presented here. The Baker et al.
51 (2020) protocol divides lesions into three diagnostic strengths, consistent with
52 treponemal infection but not diagnostic (C), strongly suggestive (ST), and
53 pathognomonic (P). The criteria presented by Baker et al. (2020) illustrates the
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2
3 consensus among paleopathologists to work toward standards of diagnosis of
4
5 treponemal disease. The two cases presented here would score 5 out 5 using the
6
7 Harper et al. (2011) criteria for diagnosis, and would be scored as having
8
9 pathognomonic lesions using the Baker et al. (2020) criteria, all consistent with
10
11 probable diagnoses using the threshold criteria presented here.
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17 Nutritional disease

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19 Not all lesions of M20 and M29 are consistent with infectious disease only (Table
20
21 S5). The discrete deposits of new bone and cortical porosity on the posterior and
22
23 anterior aspects of the zygomatic bones, and the external greater wings of the
24
25 sphenoid bones and the temporal bone squama in M20 are more consistent with
26
27 repeated microtrauma and hematoma formation characteristic of scurvy and not of
28
29 treponemal infection where diffuse subperiosteal new bone across the face and
30
31 cranium would be expected (Samarkos et al. 2011; Snoddy et al. 2018). This
32
33 individual also has evidence of anemia in the form of cribra orbitalia and porotic
34
35 hyperostosis of the ectocranial vault which may be a consequence of parasitic,
36
37 infectious, and/or nutritional stress in childhood. A synergistic relationship between
38
39 infectious and nutritional diseases is well documented (Chan 2000; Larsen 1995;
40
41 Mata 1975; Nussenblatt and Semba 2002; Pelletier et al. 1995; Roberts 2000; Roberts
42
43 and Brickley 2018; Ruiz et al. 1994) and co-morbidity between these would not be
44
45 unexpected at Man Bac given the biosocial context of subsistence transition occurring
46
47 at the site (Larsen 1995; Oxenham et al. 2011).
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56 Similar to M20, there are lesions present in M29 that are more likely to result from
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58 metabolic or developmental defects than infection (Fig. 4). As outlined above, various
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3 deformities are present in the long bones of M29. While it is possible that the swelling
4 of the distal femora is a result of the infection, the combination of bending
5
6 of the distal femora is a result of the infection, the combination of bending
7
8 deformities, cupping, and swelling of distal ends of multiple long bones suggest
9
10 alternative etiologies for these pathologies and are particularly indicative of a
11
12 demineralization disorder, such as rickets (Brickley and Ives 2010:103-107).
13
14 Interactions between infectious and nutritional diseases during childhood growth is an
15
16 area of ongoing study in paleopathology, and it is possible that a combination of these
17
18 factors caused the long bone bowing identified in M29. The described porotic and
19
20 new bone lesions of the mandible and scapulae are all diagnostic macroscopic signs of
21
22 scurvy (Snoddy et al. 2018). The radiographs also revealed ground-glass osteopenia,
23
24 and Trummerfeld zones (radiolucent bands within the metaphyses) in association with
25
26 white lines of Frankel (radiodense lines on the metaphyseal plates of the long bones)
27
28 (Fig. 4), which are further diagnostic signs of Vitamin C deficiency (Brickley and
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30 Ives 2010; Snoddy et al. 2018; Snoddy et al. 2017).
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38 The likelihood of co-morbidity of treponemal disease with nutritional deficiency in
39
40 M29 including scurvy as well as deficiency of hormones, minerals and/ or vitamins
41
42 related to the mineralization process (Vitamin D, calcium, phosphorus, and/or
43
44 parathyroid hormones) may be causing the concurrent thinning of cortices impacting
45
46 the visibility of a clear Higoumenakis sign of congenital syphilis. As gummatous
47
48 lesions usually form within localized nodes of new bone, the presence of a small lytic
49
50 process on the medial end of M29's left clavicle may suggest a well remodeled new
51
52 bone deposit in a child with a high bone turnover rate also undergoing nutritional
53
54 stress. The presence of a congenital expression of treponemal disease at Man Bac is
55
56 then possible. In summary, M29 probably suffered from tertiary treponemal disease in
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3 association with multiple nutrient deficiencies affecting both osteoid formation and
4 mineralization at various points in the child's lifetime. In light of more definitive
5 evidence of nutritional stress in M29, an argument could be made for a co-morbidity
6 of treponemal disease and Vitamin C deficiency in M20 as well.
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14 Possible treponemal disease?

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16 Lesions in five other individuals are also consistent with treponemal disease.
17
18 However, the skeletal expression of their lesions does not provide enough diagnostic
19 strength to allow a probable diagnosis. MB05M5 is an infant approximately 18
20 months of age with endosteal enlargement of the long bones associated with
21 superficial lytic lesions. While the radiographs demonstrate a superficial focal
22 sclerotic response in the distal metaphysis of the left humerus and superior acromial
23 end of the clavicle, these lesions are small (lesion on humerus is 3.9x1.7mm, and on
24 clavicle is 3x3mm), impeding confidence that these lesions are superficial focal
25 cavitations as described by Hackett (1976:429-433). MB05M29 is a middle-aged
26 adult male with dactylitis and diffuse new bone in the forearms and legs associated
27 with endosteal enlargements. Two focal superficial lesions with sclerotic response are
28 present on the medial distal right fibula. However, these lesions are not within a
29 distinct node of new bone and therefore do not strictly fit the definition of superficial
30 cavitation by Hackett. A further three individuals present with endosteal
31 nodes/enlargements, and/or dactylitis which are also consistent with treponemal
32 disease (see Table S6). All lesions follow a postcranial pattern of lymphatic
33 dissemination consistent with treponemal disease (Buckley and Dias 2002).
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58 Pathophysiology of treponemal disease in M20 and M29

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3 The presence of gummatous lesions in M20 and M29 is strongly diagnostic for
4 treponemal disease in this early Neolithic community and, therefore, is sufficient
5 evidence for Pre-Columbian treponemal disease outside of the Americas, and
6 represent the oldest cases in the Asia-Pacific region. The presence of a possible
7 Higoumenakis sign in M29 attributed to the late onset congenital form of treponemal
8 disease does suggest the possibility of transplacental transmission in this case.
9
10 Transplacental transmission of yaws has been known to occur, albeit rarely
11 (Engelhardt 1959). Children infrequently develop gummatous lesions, but they also
12 have been documented to occur in yaws (Hackett 1951). Given the age of M20 it is
13 possible that initial infection occurred in late childhood to adolescence, which is
14 epidemiologically consistent with all treponemal diseases.
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31 Which treponemal disease was present in Neolithic Man Bac?

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33 We offer no attempt to diagnose which treponemal disease is responsible based solely
34 on skeletal expression. Discussion on the uniformity of skeletal lesions of different
35 treponemes has been dealt with elsewhere (Buckley and Dias 2002). However, the
36 epidemiological, social, and environmental contexts of the disease do allow some
37 discussion on the treponeme responsible. Climatically, Vietnam is within latitudinal
38 boundaries where yaws has been historically documented (Mitjà et al. 2013). Prior to
39 the eradication attempts of yaws by the World Health Organization in the 1950s, yaws
40 was endemic to Vietnam, and reports as late as the 1990s demonstrate a recent history
41 of the disease in the region (Meheus and Antal 1992). With consideration of all
42 possible and probable cases at Man Bac, it is clear the lesions are predominantly in
43 non-adults, with only two adults presenting lesions consistent with treponematosi
44 and only one of these with strongly diagnostic lesions (Table S6). The
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3 epidemiological distribution in terms of age does suggest a non-venereal form such as
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5 yaws, where initial infection most often occurs between the ages of 2 and 15 years
6
7 (Mitjà et al. 2013). Therefore, yaws is a possible candidate for the treponemal disease
8
9 present at Man Bac. However, the climate of this region is marginal for the successful
10
11 maintenance of yaws in a community. Yaws thrives in regions with average yearly
12
13 temperatures of 27°C, but can persist in areas of, at minimum, 21°C yearly average
14
15 temperature (Hill 1953). While northern Vietnam fits into this range, in regions where
16
17 temperatures are not consistent year round, yaws is even less likely to thrive (Hill
18
19 1953). In January, the minimum average temperature in Northern Vietnam is 12°C
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21 and 14.3°C specifically for Ninh Binh province where Man Bac is situated (Oxenham
22
23 et al. 2011). The temperature during the early Neolithic of Vietnam may have been
24
25 slightly elevated compared to the present day due to the termination of the mid-
26
27 Holocene Thermal Maximum at around 3000B.C., which was marked by higher
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29 temperatures and rainfall (Oxenham et al. 2018). While there are no historic reports of
30
31 endemic syphilis in the region, North Vietnam becomes cold and dry during the
32
33 winter months, which is more suitable for the endemic form. In summary, the
34
35 ecological and climatic context of Man Bac allowed for the presence of all of the
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37 treponemal conditions. However, given the age of the site and epidemiology in regard
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39 to the ages of affected individuals, it is more likely a non-venereal rather than
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41 venereal form.
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52 Social and biological conditions promoting treponemal disease at Man Bac

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54 The ecological and social contexts of Man Bac fostered an environment that was
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56 advantageous to the spread of non-venereal treponemal disease in the community.
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58 Agricultural transitions such as that which occurred at Man Bac have been universally
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3 documented to have resulted in epidemiological transitions as farming practices
4 encouraged sedentism, population growth and increased susceptibility to infection due
5 to nutritional stress (Armelagos and Cohen 1984; Cohen and Crane-Kramer 2007;
6 Larsen 2006). In northern Vietnam, this subsistence change may have led to different
7 epidemiological pressures in the communities with no distinct decline in health as
8 pre-Neolithic sedentary foragers also had high disease burdens (Oxenham et al. 2018).
9 Signs of multi-nutrient deficiency throughout the life of M20 and M29 is further
10 supported by considerably high levels of stress identified in other dental and skeletal
11 evidence from the site (McDonnell and Oxenham 2014; Oxenham and Domett 2011).
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26 Coastal habitations, such as prehistoric Man Bac, have been associated with higher
27 yaws incidence in historical contexts due to the abundance of water and vegetation
28 increasing population density (Hackett 1953a:135; Kazadi et al. 2014). Furthermore,
29 pre-industrialized agricultural communities have also been associated with increased
30 incidence of yaws (Guimarães 1953; Hackett 1953a:135). The coastal region is also
31 slightly warmer and humid than inland northern Vietnam and therefore more
32 conducive to the spread of yaws (Hill 1953).
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45 The increase in fertility and population growth at Man Bac was likely associated with
46 an increase in infants and children within the community, who are known to be the
47 primary transmitters of yaws (McFadden et al. 2018; Mitjà et al. 2013). Given the
48 age-based hierarchy, possible relocation of resources to older individuals in the
49 community may have promoted malnutrition in younger individuals further
50 encouraging disease transmission in children (Hill 1953; Oxenham et al. 2011). A
51 degree of social group cohesiveness may have further encouraged the transmission of
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3 a non-venereal form of treponematosi s spread through close skin contact. The
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5 presence of a profoundly disabled individual with quadriparesis following Klippel-
6
7 Feil syndrome, who required continuous and intensive care, highlights that the
8
9 community was one in which individuals were highly co-operative (Oxenham et al.
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11 2009; Tilley and Oxenham 2011).
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17 Origins of treponemal disease at Man Bac

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19 It is not possible at this point to determine confidently whether treponemal disease
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21 was introduced by the farmers from southern China. However, spread of treponemal
22
23 disease into MSEA with migrant farmers during the Neolithic is plausible when
24
25 considering the extent of the migration, and the sedentary agricultural contexts they
26
27 brought with them (Bellwood and Oxenham 2008). There is no archaeological
28
29 evidence for contact with other groups apart from southern Chinese farmers outside of
30
31 MSEA at this time. Admixture with indigenous foragers in northern Vietnam may
32
33 have enabled further transmission of treponemal disease throughout MSEA, although
34
35 currently no further evidence for prehistoric treponemal disease exists in Southeast
36
37 Asian skeletal collections. While we recognize that absence of evidence is not
38
39 evidence of absence and a deep antiquity of treponemal disease in MSEA is possible
40
41 prior to the agricultural transition, there is no identification of human to human
42
43 transmissible diseases (such as tuberculosis, treponematosi s, or leprosy) prior to this
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45 time in MSEA despite intensive bioarchaeological research in the region (Buckley
46
47 and Oxenham 2016). The evidence of infectious disease in MSEA considerably
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49 increases from the Bronze and Iron Ages (Oxenham et al. 2005; Tayles and Buckley
50
51 2004). If the treponemal disease at Man Bac is in fact yaws, the route of transmission
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53 throughout the continent would be restricted to tropical and subtropical zones.
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3 Although Man Bac is positioned at the present day upper geographical limit of the
4 survival of yaws, documented cases of yaws existed in southern China and Taiwan
5 prior to worldwide eradication attempts where the climate is similar to northern
6 Vietnam (Hill 1953). In light of pre-eradication, clinical evidence of yaws in this
7 region, a possible origin for the spread of treponemal disease into MSEA from
8 farmers originating in southern China from approximately 4000 years ago is
9 plausible. Given the presence of treponemal disease at Man Bac, regional interactions
10 throughout the Red River delta also presents possible routes of transmission to and
11 from other Phung Nguyen sites where archaeological material indicates contact with
12 agricultural groups of southern China (Khoach 1980; Nguyen 2008). The implications
13 of migration and human population interaction on the potential spread of treponemal
14 disease into northern Vietnam is an area for further research, particularly with regard
15 to the broader context of the timing of the introduction of treponemal disease into
16 MSEA. Further investigation of existing and newly excavated skeletal assemblages in
17 southern China and MSEA may provide further insight into the origins and antiquity
18 of this disease within the region.
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43 It can be hypothesized that, with the demographic and subsistence transition resulting
44 in nutritional instability, and possibly the introduction of new infectious diseases with
45 migration, the social and environmental contexts of Man Bac were suitable for the
46 spread of treponematosi. The high rate of natural population increase at Man Bac
47 was likely fueled by the supplementation of agricultural foods enabling larger groups
48 to thrive, and increasing the number of individuals in the community and further
49 encouraging the spread of treponematosi (McFadden et al. 2018). The identification
50 of two cases of probable tertiary treponemal disease in a northern Vietnamese
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3 Neolithic community further supports the contextual evidence for a population
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5 undergoing accelerated growth, significant social change, necessitating the navigation
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7 of interaction between two populations and one of a unified and close community
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9 wherein a treponeme could spread with relative ease.
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16
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28
29

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31

32 **Author Contributions**

33
34 M.V wrote majority of the manuscript, and performed data collection and
35 processing. H.R.B contributed to research design, assisted in data processing,
36 and contributed substantially to the manuscript. M.F.O and K.D contributed to
37 research design, provided preliminary data and contributed to the manuscript.
38 T.T.M facilitated access to collections, assisted with the data collection and
39 provided Vietnamese translation of the abstract. N.T.M, H.M, H.H.T and
40 N.T.H facilitated access to collections and provided essential background
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Figure Legends

Fig. 1. The burial position and associated artefacts of M20 (left) and M29

(right). Both individuals were buried extended and supine. The burials are in excellent skeletal condition with the exception of the cranium of MB07H2M29 which was not present during excavation. (Image: M. Oxenham)

Fig. 2. Expression of lesions related to infectious disease in M20. Preserved

bones are outlined in black, with absent bones outlined in light grey. The skeletal extent of osteoblastic lesions is indicated by dark grey fill. a) and b) Superficial focal cavitations with smooth sclerotic margins not within distinct nodes of new bone on the anterior and posterior lateral epicondyle of the right humerus. c) Remodeled cortical enlargements throughout the shafts of the tibiae with striated nodes of new bone on the distal fibulae. d) Radiographs of the tibiae and fibulae: a distinct node of new bone with medullary intrusion is present on the medial middle shaft of the right tibia. The nodes of new bone of the distal fibulae are radiolucent due to the extent of pathological change to the internal cortex of the bone. e) Superficial focal cavitation not within a distinct node of new bone on the proximal right fibula. f) Two focal superficial cavitations on the lateral aspect of the right distal fibula within a distinct node of striated new bone. g) Focal superficial cavitation on the medial aspect of the right distal fibula within a distinct node of striated new bone. h) Focal superficial cavitation on the medial left calcaneal body, not within a distinct node of new bone. (Image: M. Vlok)

Fig. 3. Expression of lesions related to infectious disease in M29. Preserved

bones are outlined in black, with absent bones outlined in light grey. An undetermined metacarpal is also present but not demonstrated in the figure as its skeletal position is unknown. The skeletal extent of osteoblastic lesions is indicated by dark grey fill. a) Subperiosteal new bone deposit on the distal shafts and metaphyses of the humeri. b) There are slight cortical enlargements visible on radiographs throughout the humeral shafts. c) Remodeled enlargement of the medial left clavicle. The metaphyseal plate is bulging. d) Radiographs do not demonstrate a distinct cortical enlargement of the left medial clavicle. The internal trabeculae appear radiolucent and a distinct oval lytic lesion with a radiodense sclerotic margin is present on the inferior margin of the medial metaphysis. Some increased calcification is present on the medial metaphyseal plate. e) Diffuse subperiosteal new bone deposit across the radii and ulnae. f) Two oval cavitations within nodes of new bone on the midshafts of the right radius and ulna. While significant damage has occurred to the lesion floor, the margins of the lesion are preserved and indicate superficial cavitation. g) Large node of new bone on the proximal left ulna. Radiographs demonstrate a small oval lytic lesion with sclerotic margins within the node. h) Prolific subperiosteal new bone across the tibiae and fibulae. Fibular cortical enlargements are thickest towards the distal shaft. Note the rounded margins of the anterior shaft of the tibiae indicating saber shin deformity. i) Radiograph of the right tibia demonstrating cortical enlargement of the anterior crest indicating saber shin. j) An unknown metacarpal with significant post-

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3 mortem damage. However, it is clear the entire shaft of the metacarpal
4
5 has undergone prolific pathological change indicative of dactylitis. The
6
7 internal margins of the cortex are porous. k) and l) Focal cavitations of
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9 the right and left distal fibulae. Margins of the lesions are smooth and
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11 within nodes of new bone. The surrounding bone has undergone
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13 complete prolific pathological change with the internal cortex appearing
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15 porous. Osteoblastic lesions considered in the diagnosis of nutritional
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17 disease is presented in Fig. 4. (Image: M. Vlok)

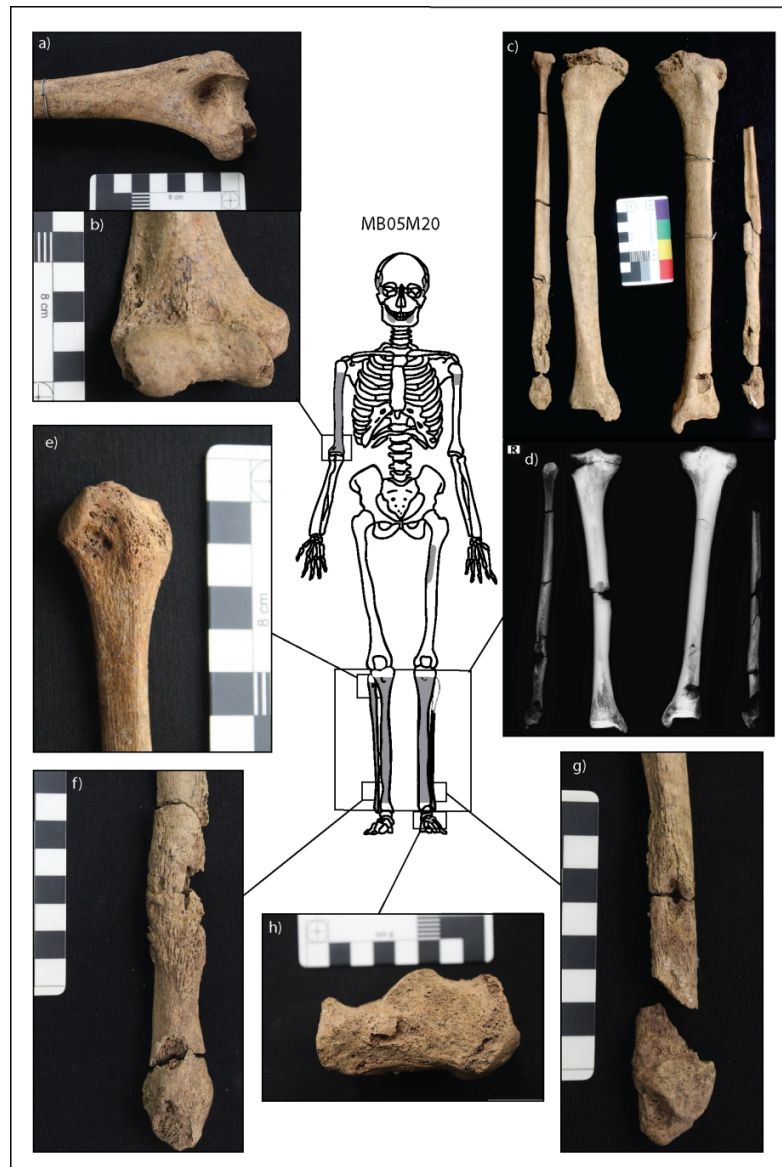
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21 **Fig. 4. Expressions of lesions related to nutritional disease in M29.**

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23 Preserved bones are outlined in black, with absent bones outlined in
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25 light grey. An undetermined metacarpal is also present but not
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27 demonstrated in the figure as its skeletal position is unknown. The
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29 skeletal extent of osteoblastic lesions possibly related to nutritional
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31 disease is indicated by dark grey fill. a) Mixed active and remodeled
32
33 discrete deposit of subperiosteal new bone and abnormal cortical
34
35 porosity medial to the coronoid process of the mandible. b) Symmetrical
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37 mixed active and remodeled discrete deposit of subperiosteal new bone
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39 in the supraspinous fossa of the scapulae. c) Bilateral swelling and slight
40
41 cupping of the distal femur d) Depression of the humeral heads which
42
43 are reminiscent of coxa vara deformities of the femoral heads (see
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45 Brickley and Ives 2010: 102). e) Trummerfeld (scurvy) line and white
46
47 line of Frankle on the distal radius and ulna. These radiographic changes
48
49 were identified in all metaphyses of long bones. f) Slight medial bending
50
51 deformities of the tibial shafts. g) Posterior bending of the proximal
52
53 metaphyseal plate of the tibia. (Image: M. Vlok)

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3 **Fig. 5.** Strongly Diagnostic and Diagnostic Macroscopic Lesions for
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5 Treponemal Disease in M20 and M29. a) Superficial focal cavitation of
6
7 the medial left calcaneus (M20, diagnostic). b) Superficial focal
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9 cavitation of the anterior lateral epicondyle of the right humerus (M20,
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11 diagnostic). c) Superficial focal cavitation of the posterior lateral
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13 epicondyle of the right humerus (M20, diagnostic). d) Superficial focal
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15 cavitations within a distinct node of new bone of the right distal fibular
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17 shaft M20, strongly diagnostic). e) Superficial focal cavitations within a
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19 distinct node of new bone of the right distal fibular shaft (M20, strongly
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21 diagnostic). f) Focal cavitation of the right distal fibular shaft with
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23 sclerotic margins within a distinct node of new bone (M29, strongly
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25 diagnostic). g) Superficial focal cavitation of the proximal right fibula
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27 (M20, diagnostic). h) Focal cavitation of the right distal fibular shaft
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29 with sclerotic margins within a distinct node of new bone, same lesion as
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31 5f. (M29, strongly diagnostic). i) Focal cavitations of the right mid ulnar
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33 and radial shafts with sclerotic margins within a distinct node of new
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35 bone (M29, strongly diagnostic). (Image: M. Vlok)
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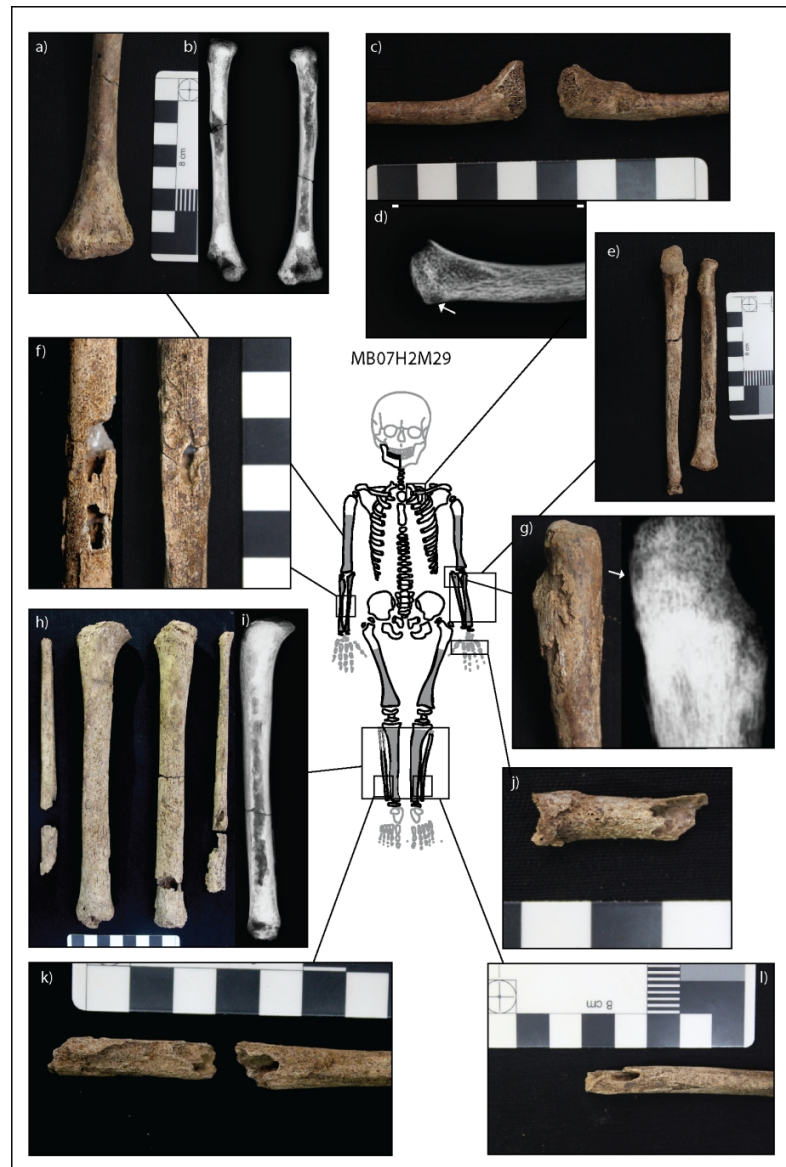
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45 Fig. 2. Expression of lesions related to infectious disease in M20. Preserved bones are outlined in black, with
 46 absent bones outlined in light grey. The skeletal extent of osteoblastic lesions is indicated by dark grey fill.
 47 a) and b) Superficial focal cavitations with smooth sclerotic margins not within distinct nodes of new bone on
 48 the anterior and posterior lateral epicondyle of the right humerus. c) Remodeled cortical enlargements
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 55 aspect of the right distal fibula within a distinct node of striated new bone. h) Focal superficial cavitation on
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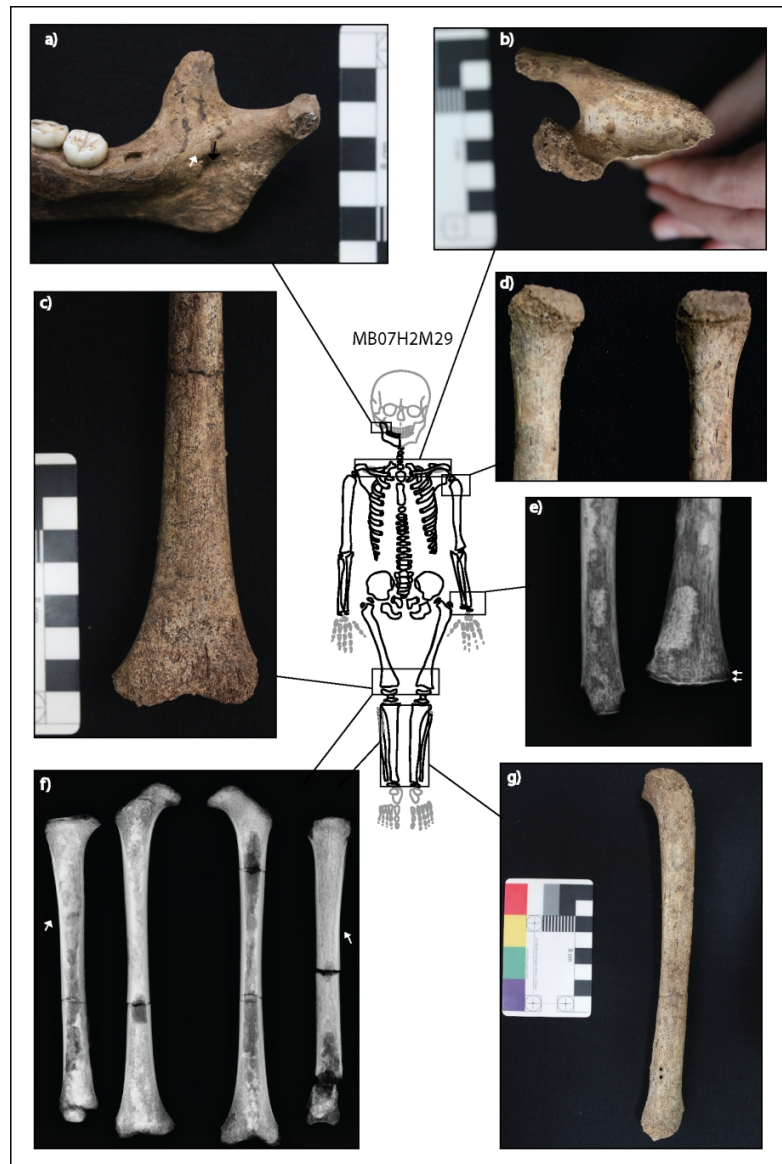


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Fig. 3. Expression of lesions related to infectious disease in M29. Preserved bones are outlined in black, with absent bones outlined in light grey. An undetermined metacarpal is also present but not demonstrated in the figure as its skeletal position is unknown. The skeletal extent of osteoblastic lesions is indicated by dark grey fill. a) Subperiosteal new bone deposit on the distal shafts and metaphyses of the humeri. b) There are slight cortical enlargements visible on radiographs throughout the humeral shafts. c) Remodeled enlargement of the medial left clavicle. The metaphyseal plate is bulging. d) Radiographs do not demonstrate a distinct cortical enlargement of the left medial clavicle. The internal trabeculae appear radiolucent and a distinct oval lytic lesion with a radiodense sclerotic margin is present on the inferior margin of the medial metaphysis. Some increased calcification is present on the medial metaphyseal plate. e) Diffuse subperiosteal new bone deposit across the radii and ulnae. f) Two oval cavitations within nodes of new bone on the midshafts of the right radius and ulna. While significant damage has occurred to the lesion floor, the margins of the lesion are preserved and indicate superficial cavitation. g) Large node of new bone on the proximal left ulna. Radiographs demonstrate a small oval lytic lesion with sclerotic margins within the node. h) Prolific subperiosteal new bone across the tibiae and fibulae. Fibular cortical enlargements are

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3 thickest towards the distal shaft. Note the rounded margins of the anterior shaft of the tibiae indicating
4 saber shin deformity. i) Radiograph of the right tibia demonstrating cortical enlargement of the anterior
5 crest indicating saber shin. j) An unknown metacarpal with significant post-mortem damage. However, it is
6 clear the entire shaft of the metacarpal has undergone prolific pathological change indicative of dactylitis.
7 The internal margins of the cortex are porous. k) and l) Focal cavitations of the right and left distal fibulae.
8 Margins of the lesions are smooth and within nodes of new bone. The surrounding bone has undergone
9 complete prolific pathological change with the internal cortex appearing porous. Osteoblastic lesions
10 considered in the diagnosis of nutritional disease is presented in Fig. 4. (Image: M. Vlok)

11 207x306mm (200 x 200 DPI)



45 Fig. 4. Expressions of lesions related to nutritional disease in M29. Preserved bones are outlined in black,
46 with absent bones outlined in light grey. An undetermined metacarpal is also present but not demonstrated
47 in the figure as its skeletal position is unknown. The skeletal extent of osteoblastic lesions possibly related to
48 nutritional disease is indicated by dark grey fill. a) Mixed active and remodeled discrete deposit of
49 subperiosteal new bone and abnormal cortical porosity medial to the coronoid process of the mandible. b)
50 Symmetrical mixed active and remodeled discrete deposit of subperiosteal new bone in the suprascapular
51 fossa of the scapulae. c) Bilateral swelling and slight cupping of the distal femur d) Depression of the
52 humeral heads which are reminiscent of coxa vara deformities of the femoral heads (see Brickley and Ives
53 2010: 102). e) Trummerfeld (scurvy) line and white line of Frankle on the distal radius and ulna. These
54 radiographic changes were identified in all metaphyses of long bones. f) Slight medial bending deformities of
55 the tibial shafts. g) Posterior bending of the proximal metaphyseal plate of the tibia. (Image: M. Vlok)

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Fig. 5. Strongly Diagnostic and Diagnostic Macroscopic Lesions for Treponemal Disease in M20 and M29. a) Superficial focal cavitation of the medial left calcaneus (M20, diagnostic). b) Superficial focal cavitation of the anterior lateral epicondyle of the right humerus (M20, diagnostic). c) Superficial focal cavitation of the posterior lateral epicondyle of the right humerus (M20, diagnostic). d) Superficial focal cavitations within a distinct node of new bone of the right distal fibular shaft M20, strongly diagnostic). e) Superficial focal cavitations within a distinct node of new bone of the right distal fibular shaft (M20, strongly diagnostic). f) Focal cavitation of the right distal fibular shaft with sclerotic margins within a distinct node of new bone (M29, strongly diagnostic). g) Superficial focal cavitation of the proximal right fibula (M20, diagnostic). h) Focal cavitation of the right distal fibular shaft with sclerotic margins within a distinct node of new bone, same lesion as 5f. (M29, strongly diagnostic). i) Focal cavitations of the right mid ulnar and radial shafts with sclerotic margins within a distinct node of new bone (M29, strongly diagnostic). (Image: M. Vlok)

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Table 1: *Diagnostic Criteria for Identification of Treponematoses (SD= strongly diagnostic, D= diagnostic, and S= suggestive).* One SD lesion or two D lesions are required for diagnosis consistent with a probable case. One D lesion or two S lesions are required for diagnosis consistent with a possible case. Some lesions are provided for congenital cases and have been observed only in infants and children. The final column indicates which lesions were present in M20 and M29.

Lesion	Diagnostic Strength	Differential Diagnosis	Clinical Reference	Paleopathological Reference	Lesion Present
Caries Sicca Stages 4-8: circumvallate cavitation, radial scars, serpiginous cavitation, nodular cavitation and caries sicca of the ectocranium	SD		(Hackett 1975; 1976:362-396)	(Harper et al. 2011)	-
Gummatous lesions on any skeletal element: focal superficial cavitations in direct relation with nodes or expansions (enlargements) of new bone	SD		(Hackett 1975; 1976:429-433)	(Harper et al. 2011)	M20 M29
Hutchinson's incisors (congenital)	SD	Normal morphology	(Hackett 1976:441; Pessoa and Galvão 2011; Stokes and Gardner 1923)	(Harper et al. 2011; Hillson et al. 1998; Lewis 2017:179)	-
Moon's Molars (congenital)	SD	Growth disruption	(Hackett 1976:441)	(Harper et al. 2011; Hillson et al. 1998; Lewis 2017:179)	-
Congenital syphilis (Caffey's) triad: osteochondritis, bilateral thick periosteal reaction of the long bones and osteomyelitis of metaphyseal ends (early congenital)	D	Non-specific osteomyelitis, infantile cortical hyperostosis, Paget's disease.	(Caffey 1939; Jaffe 1972:910)	(Lewis 2017:178)	-
Caries sicca sequence stages 1-3: clustered pits, confluent clustered pits, focal superficial cavitation of the ectocranium or focal superficial cavitation of the cortex of long bones not within a distinct node or expansion (enlargement).	D	Mycosis, tuberculosis, Langerhans's cell histiocytosis, multiple myeloma and other metastatic neoplasms	(Hackett 1975; 1976:362-396)		M20
Gross rhinomaxillary and palatal destruction (gangosa)	D	Leprosy, tuberculosis, mycosis, destructive neoplasms,	(Fiumara and Lessell 1970; Hackett 1951:164-168; 1975;	(Lewis 2017:175)	-

		trauma, leishmaniasis.	1976:399- 401)		
Wimberger's corner sign (congenital): bilateral widening band of decreased calcification of the metaphyses leading to destruction	D	Non-specific osteomyelitis, trauma, scurvy	(Brackett and Standley 2019; Rasool and Govender 1989)	(Harper et al. 2011; Lewis 2017:178)	-
Mulberry (Fournier's) molars	D	Mercury use, growth disruption		(Harper et al. 2011; Hillson et al. 1998; Lewis 2017:179)	-
Thick periosteal new bone deposition and exostoses on the maxillae (goundou)	S	Scurvy, osteomyelitis, leprosy, infantile cortical hyperostosis, Paget's disease, genetic anemias		(Buckley 2016:30; Harper et al. 2011; Lewis 2017:175)	-
Higoumenakis' Sign: unilateral enlargement of the sternal end of the clavicle (congenital)	S	Trauma, non-specific osteomyelitis, normal variant	(Dax and Stewart 1939; Yang 1940)	(Frangos et al. 2011; Harper et al. 2011; Lewis 2017:179)	M29
Saber shin (pseudobowing of the tibia without bowing of the medullary canal)	S	Leprosy, osteomyelitis, various benign and malignant bone tumors, Paget's disease, hyperflourosis	(Hackett 1951:28)	(Harper et al. 2011)	M20 M29
Boomerang leg: True tibial bowing with bowing of medullary canal (until adolescence)	S	Rickets, skeletal dysplasias, osteogenesis imperfecta, fracture		(Harper et al. 2011; Lewis 2017:175)	-
Dactylitis (subperiosteal new bone enlargement of the hand and feet bones)	S	Tuberculosis, non-specific osteomyelitis, genetic anemias	(Hackett 1951:30; Rasool and Govender 1989)	(Harper et al. 2011; Lewis 2017:178-179)	M20 M29
Charcot's joint (resorption of weight bearing joints)	S	Septic arthritis, diabetes, osteoarthritis, syringomyelia, tuberculosis	(Hackett 1976; Johns 1970; Sequeira 1994)	(Harper et al. 2011; Lewis 2017:179)	-
Opera glass fingers: diaphyseal tapering of the bone	S	Leprosy, rheumatoid arthritis, syringomyelia, diabetes, osteomyelitis, erosive osteoarthritis	(Hackett 1951:153-154; Jones 1972; Swezey et al. 1972)	(Ortner 2003:277)	-
Radiographic: Saw tooth appearance of metaphyseal ends or macroscopic	S	Rickets, scurvy, trauma, tuberculosis	(Jaffe 1972:910-912; Rasool and	(Harper et al. 2011; Lewis 2017:178)	-

symmetrical destruction of metaphyses attributed to osteochondritis (early congenital)			Govender 1989)		
Thick deposit of mixed active and remodelled new bone on the long bones, thickest at the midshaft (congenital) or long bone endosteal nodes or expansions (enlargements) with medullary canal intrusion	S	Paget's disease, non-specific osteomyelitis, metastatic bone tumors, tuberculosis	(Hackett 1951, 1975; 1976:411-433)	(Harper et al. 2011; Lewis 2017:178-180)	M20 M29
Saddle nose (congenital)	S	Leprosy, trauma, non-specific osteomyelitis	(Fiumara and Lessell 1970; Pavithran 1987)	(Harper et al. 2011; Lewis 2017:177)	-
High palatal arch (congenital)	S	Various congenital deformities including: Turner's syndrome and Klippel-Feil syndrome.	(Pavithran 1987)	(Lewis 2017:177)	-

Supplementary Material

Text S1: Radiocarbon dating of Man Bac Human Remains

Radiocarbon dates for charcoal throughout the stratigraphic layer above the sterile layer within which the burials were found date to between 4016- 3524 cal BP (95% CI) and relative dating of archaeological material provides supportive evidence for a secure window of time for the burials in question in this paper (Oxenham et al. 2011). Archaeological material indicates a contemporaneous relationship with the burials and the stratigraphic layer wherein the charcoal was obtained for radiocarbon analysis.

Human rib and tooth samples from both individuals (MB05M20 and MB07H2M29) were sent to the University of Waikato Radiocarbon Dating Laboratory. Insufficient collagen yields meant radiocarbon dating could not be attempted.

Radiocarbon dating of four human rib samples from other burials at Man Bac was undertaken at the Oxford Radiocarbon Accelerator Unit (ORAU) at the University of Oxford. Prior to AMS dating we screened bones for their %nitrogen content to assess collagen preservation. The majority were below our ideal threshold of ~0.76% Brock et al. (2010a). However values still indicated preserved collagen is likely to be present in some samples (Table S1).

Table S1: Samples analyzed for % nitrogen. Samples in bold were later successfully dated. Those in italics were attempted but failed to yield sufficient collagen for dating. Those not bold and italicized were not attempted.

Individual	Sample	wt %N
<i>MB05 M34</i>	<i>MB05 M34 13</i>	<i>0.434</i>
MB07 H2 M5	MB07 H2 M5 8	0.264
MB05 M31	MB05 M31 6	0.629
MB05 M20	MB05 M20 19	0.281
<i>MB07 H2 M30</i>	<i>MB07 H2 M30 5</i>	<i>0.402</i>
MB05 M28	MB05 M28 20	0.059
MB07 H2 M10	MB07 H2 M10 9	0.206
MB05 M15	MB05 M15 18	0.689
<i>MB05 M29</i>	<i>MB05 M29 1</i>	<i>0.730</i>
<i>MB07 H1 M9</i>	<i>MB07 H1 M9 21</i>	<i>0.448</i>
MB07 H2 M32	MB07 H2 M32 4	0.259
MB07 H2 M12	MB07 H2 M12 10	0.955
MB07 H2 M2	MB07 H2 M2 7	0.170
MB07 H2 M19	MB07 H2 M19 11	0.345
MB07 H1 M4	MB07 H1 M4 16	0.097
<i>MB07 H1 M11</i>	<i>MB07 H1 M11 14</i>	<i>0.595</i>
MB05 M11	MB05 M11 17	0.291
MB07 H2 M1	MB07 H2 M1 2	0.167
<i>MB07 H1 M10</i>	<i>MB07 H1 M10 15</i>	<i>0.443</i>
MB07 H2 M24	MB07 H2 M24 12	0.728

We extracted collagen from bones that passed or nearly passed the %nitrogen screening (those in bold, and italics in Table S1) using methods previously outlined in Brock et al. (2010b) using the following chemical pretreatment protocol (coded AF):

- Coarsely ground bone powder was loaded into a glass test tube (weights of bone are provided in Table S1);
- A sequence of 0.5 M HCl, 0.1M NaOH and 0.5M HCl was used to treat the bone, interspersed with rinsing with ultra-pure (MilliQ™) water between each reagent;
- Crude collagen was gelatinized in pH3 solution at 75°C for 20 hours;
- The gelatin solution was filtered using a polyethylene Eezi-filter™ whose pore size ranges between 45-90 μm, that is precleaned by thorough rinsing and ultrasonication and the insoluble residues discarded;
- The filtered gelatin was then pipetted into a precleaned ultra-filter (Sartorius Vivaspin™ 15 30kD MWCO) and centrifuged at 2500-3000 rpm until 0.5-1 mL of the >30 kD gelatin fraction remains (typically 20—40 min) (for the human bone this was not applied due to the low sample size of the recovered collagen);
- This gelatin was freeze-dried ready for combustion in a CHN analyzer.

The ultrafiltration step was originally described by Brown et al. (1988) and the ORAU has used the Sartorius and Vivaspin filters since 2000. The precleaning steps are undertaken after the protocols outlined in Brock et al. (2010a). Combusted gelatin samples were analyzed using a PDZ-Europa Robo-Prep biological sample converter (combustion elemental analyzer) coupled to a PDZ-Europa 20/20 mass spectrometer operating in continuous flow mode using an He carrier gas. This enables $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, nitrogen and carbon content and calculation of C:N atomic ratios. VPDB was the standard for $\delta^{13}\text{C}$ values. Graphite was produced by reacting the sample CO_2 over an iron catalyst in an excess H_2 atmosphere at 560°C. AMS radiocarbon measurement was carried out using the ORAU 2.5MV HVEE accelerator. Radiocarbon ages were calculated with reference to Stuiver and Polach (1977) in years BP.

Radiocarbon dates of bone are reported in Table S2 along with their context information. Bones were uniformly poorly preserved in terms of collagen, with all successfully dated samples >than 1% wt. collagen (the effective threshold in the ORAU). Six samples produced no collagen and therefore could not be dated (Table S3). All other analytical parameters measured, including the carbon to nitrogen atomic ratio, were acceptable.

We used OxCal 4.3 (Ramsey 2001) and the INTCAL13 calibration curve (Reimer et al. 2013) to calibrate the radiocarbon data.

Table S2 : ^{14}C age BP denotes the conventional radiocarbon age, expressed in years BP with BP being before 1950 AD. ‘AF’ is the ORAU pretreatment code for ultrafiltered collagen as outlined in Brock et al. (2010a). Stable isotope ratios are expressed in ‰ relative to VPDB and AIR with a mass spectrometric precision of $\pm 0.2\text{‰}$ for C and $\pm 0.3\text{‰}$ for N. Yield represents the weight of ultrafiltered collagen in milligrams. %Yld is the percent yield of extracted collagen as a function of the starting weight of the bone analyzed (“Used” also in mg). %C is the carbon present in the combusted gelatin. CN is the atomic ratio of carbon to nitrogen and is acceptable if it ranges between 2.9—3.5 Brock et al. (2010a).

OxA	P	Sample	^{14}C age BP	\pm value	PCode	Used	Yield	%Yld	%C	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	CN
X-2636-24	38655	MB05 M15 18	3539	29	AF	1050	4.53	0.4	37.6	-16.9	11.1	3.3
32378	38653	MB05 M31 6	3505	28	AF	1050	5.95	0.6	39.7	-17.6	11.3	3.4
32379	38658	MB07 H2 M12 15	3453	28	AF	950	5.67	0.6	39.6	-17.3	11.1	3.3
32380	38661	MB07 H2 M24 12	3406	35	AF	1010	5.67	0.6	39.9	-17.6	11.8	3.4

Table S3: Samples from Man Bac that failed to yield a radiocarbon determination. P number denotes the ORAU code.

P number	Sample	Lab comment
38652	MB05 M34 13	Failed due to no yield
38654	MB07 H2 M30 5	Failed due to very low yield
38656	MB05 M29 1	Failed due to no yield
38657	MB07 H1 M9 21	Failed due to no yield
38659	MB07 H1 M11 14	Failed due to very low yield
38660	MB07 H1 M10 15	Failed due to no yield

Calibration:

Calibration of the AMS determinations was undertaken using OxCal 4.3 (Ramsey 2001). We observe the possibility of a slight reservoir effect based on; 1) the presence of marine and freshwater fish and other mammals in the archeozoological assemblage and; 2) the stable isotopes of carbon and nitrogen that we measured as part of the radiocarbon dating procedure (see Table S2).

There is a dearth of stable isotope evidence from humans from this area in the prehistoric period which makes it difficult to obtain reliable estimates of reservoir uptake in humans of the period. Similarly, in terms of modern collected material of known age for calculation of the reservoir age, there is little reported by Reimer and Reimer (2001) for the Vietnam region. On Hon Tre Island, Bolton et al. (2016) report a measurement on a 1950 *Porites* sample, citing a reservoir age of 261 ± 22 BP, with a ΔR value of -9 ± 20 years.

Nam et al. (2011) have published fish carbon and nitrogen isotope results from modern catches to consider trophic level effects. This is important data in terms of

understanding the baseline for fish, shellfish and marine mammal isotopic values. Interestingly, the values are very similar to those we obtained from the humans from Man Bac raising the possibility that the people there might have been consuming quite significant quantities of fish, or that the fish have values that are not dissimilar to values for foods of terrestrial origin. The lack of a wider trophic level study of the food web and a baseline for marine and freshwater resources raises challenges to reliably correcting for the reservoir effect. We have therefore estimated a rather conservative 20% marine protein uptake for Man Bac with an uncertainty of 10% and used the ΔR value of -9 ± 20 years with a mixed marine curve approach in OxCal. We caution that the true calendar ages of the Man Bac samples may need to be revisited as more data becomes available that enables us to estimate more precisely the degree of marine food in the diet. For the time being this is our best estimate of the calendar age. The results of the analysis are shown in Figure S1.1 and also in Table S4.

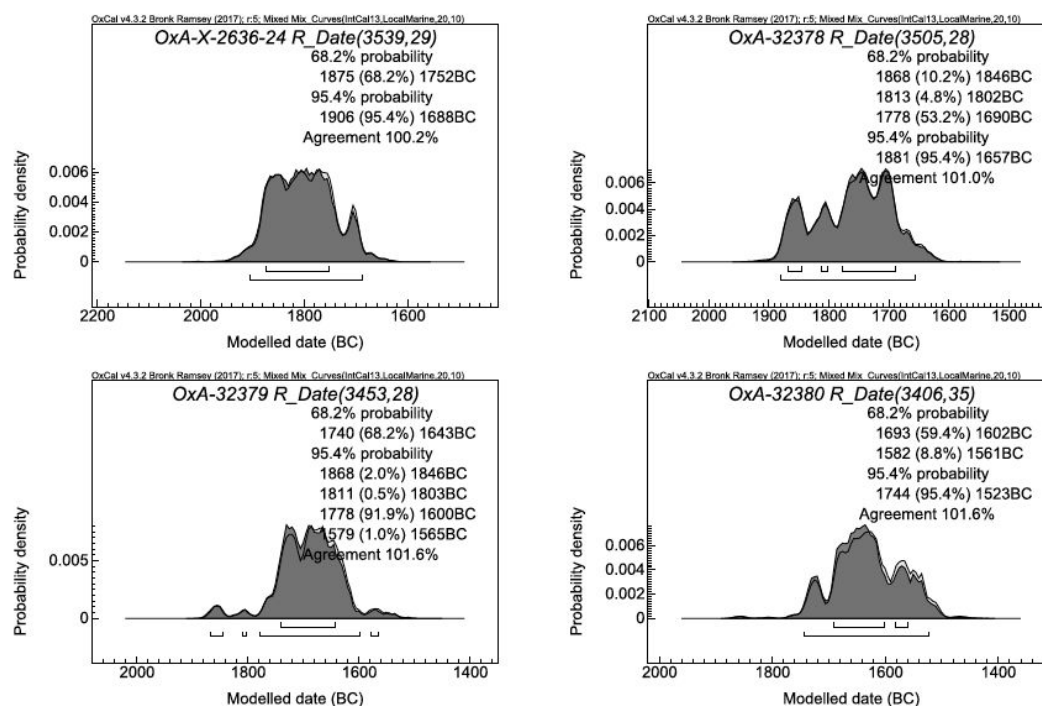


Figure S1: Calibrated results from the 4 AMS dates obtained from Man Bac. See text for details.

Table S4: calibrated age ranges for samples in Table S2, corrected using the data outlined in the text.

Name	years BC 68% range		years BC 95% range	
	from	to	from	to
R_Date OxA-X-2636-24	-1875	-1752	-1906	-1688
R_Date OxA-32378	-1868	-1690	-1881	-1657
R_Date OxA-32379	-1740	-1643	-1868	-1565
R_Date OxA-32380	-1693	-1561	-1744	-1523

Table S5. Lesions and Differential Diagnosis of M20 and M29 (SD= strongly diagnostic, D= diagnostic, S= suggestive, A= associated pathologies, SPNB= subperiosteal new bone deposit).

Elements	Lesions, associations
MB05M20- Young Adult Male	
Cranium	<ul style="list-style-type: none"> - Bilateral remodeling medium cribra orbitalia - Porotic hyperostosis of the frontal bone, occipital bone and parietal bones - Bilateral symmetrical discrete SPNB and abnormal cortical porosity on anterior and posterior zygomatics, external greater wings of sphenoid bones and temporal bones.
Mandible	<p>Scurvy= A, S, D, D^{1,2}, Rickets/Osteomalacia= A¹, Leprosy= A¹, Tuberculosis= A¹, Osteomyelitis= A¹, Treponemal Disease= A¹</p> <ul style="list-style-type: none"> - Remodeled symmetrical discrete SPNB and abnormal cortical porosity extending from the mental foramina onto the oblique lines
Humeri	<p>Scurvy= A¹, Rickets/Osteomalacia= A¹, Leprosy= A¹, Tuberculosis= A¹, Osteomyelitis= A¹, Treponemal Disease= A¹</p> <ul style="list-style-type: none"> - Two superficial focal cavitations with sclerotic reaction on the margins and base associated with remodeled SPNB on the anterior and posterior lateral epicondyle of the right humerus (17.6x 7.5mm and 6.7x 3.5mm) - Unilateral enlargement of the cortical shaft of the right humerus - Discrete deposit of SPNB on the anterior proximal left humerus <p>Treponemal Disease= D³, Scurvy= A²</p>
Femora	<ul style="list-style-type: none"> - Unilateral discrete SPNB on the medial proximal left femur <p>Scurvy= A²</p>
Tibiae	<ul style="list-style-type: none"> - Diffuse bilateral new SPNB with cortical enlargement of the tibial shafts - Saber shin - Discrete bilateral SPNB of the medial and lateral proximal tibiae <p>Scurvy= S², Treponemal Disease=S⁴</p>
Fibulae	<ul style="list-style-type: none"> - Symmetrical nodules of enlarged bone with multiple focal superficial focal cavitations with sclerotic margins and base (The lytic lesion diameters are: 9.9x7.1mm, 10.5x6.7mm, and 6.4x3.7mm for the right fibula and 12.7x7mm and 12.6x5.9mm for the left fibula) <p>Treponemal Disease= SD³</p>
Calcaneus	<ul style="list-style-type: none"> - Two focal superficial cavitations with sclerotic margins and base on the lateral and anterior-medial left calcaneus (18.48x9.45mm (lateral), and 7.71x 6.13mm (antero-medial)) <p>Treponemal Disease= D³</p>
Outcome	<i>Probable Scurvy, Probable Treponematosis</i>

Elements	Lesions, associations
MB07H2M29- Child Approx. 7 Years	
Mandible	<ul style="list-style-type: none"> - Remodeled abnormal cortical porosity on the right medial coronoid process, and discrete SPNB on the superior right incisive fossa
	Scurvy= D ²
Scapulae	<ul style="list-style-type: none"> - Mixed active and remodeled discrete SPNB and abnormal cortical porosity on the supraspinous fossae of the scapula
	Scurvy= D ²
Humerus	<ul style="list-style-type: none"> - Unilateral remodeled discrete SPNB lateral to the deltoid tuberosity on the left humerus - Bilateral diffuse active SPNB on distal shafts - Bilateral latera bending deformities of the humeral shafts, and depression deformities of the humeri
	Scurvy= A ^{1,2} , Rickets/Osteomalacia= A,D,D ^{1,5} , Leprosy= A ¹ , Tuberculosis= A ¹ , Osteomyelitis= A ¹ , Treponemal Disease= A ¹
Ulnae	<ul style="list-style-type: none"> - Bilateral mixed active and remodeled diffuse SPNB with cortical enlargement across shafts and proximal metaphysis - Superficial focal cavitation in nodes of enlargement on antero-lateral midshaft of right ulna (9.31x4.01mm) and lateral olecranon process of left ulna (<10mm).
	Treponemal Disease= SD ³
Radii	<ul style="list-style-type: none"> - Bilateral mixed active and remodeled diffuse SPNB with cortical enlargement across shafts and proximal metaphysis - Superficial focal cavitation in nodes of enlargement on antero-lateral midshaft of right radius (12.03x4.6.7mm)
	Treponemal Disease= SD ³
Hands	<ul style="list-style-type: none"> - Dactylitis
	Leprosy= S ⁶ , Tuberculosis= S ⁶ , Osteomyelitis= S ⁶ , Treponemal Disease= S ⁶
Femora	<ul style="list-style-type: none"> - Bilateral symmetrical SPNB with cortical enlargement - Slight cupping and swelling of the distal femora
	Rickets/Osteomalacia= D,D ⁵ , Leprosy= A ¹ , Tuberculosis= A ¹ , Osteomyelitis= A ¹ , Treponemal Disease= S ⁷
Tibiae	<ul style="list-style-type: none"> - Bilateral diffuse SPNB with cortical enlargement of the tibiae - Saber shin of the tibiae - Medial true-bowing of the tibiae - Posterior angulation (depression) of the proximal metaphyseal plates of the tibiae
	Rickets/Osteomalacia= D,D ⁵ , Leprosy= A ¹ , Tuberculosis= A ¹ , Osteomyelitis= A ¹ , Treponemal Disease= S ⁴
Fibulae	<ul style="list-style-type: none"> - Bilateral diffuse SPNB with nodes of enlargement on the distal fibulae - Symmetrical focal cavitations of the distal fibulae within in nodes of enlarged cortical bone (Left fibula lesion diameter: 9.07x3.94mm. Right fibula lesion diameter: 12.32x3.29mm)
	Treponemal Disease= SD ³
Clavicle	<ul style="list-style-type: none"> - Possible Higonmenakis sign of the left clavicle (unilateral enlargement of the sternal end)
	Treponemal Disease= S ⁸
All Limb Bones	<ul style="list-style-type: none"> - <i>Radiographic</i>: White line of Frankel - <i>Radiographic</i>: Trummerfeld Zone - <i>Radiographic</i>: Multiple Harris lines - <i>Radiographic</i>: Ground glass osteopenia

Elements	Lesions, associations
	Scurvy=S,D,D ⁹
Outcome	<i>Probable Scurvy, Probable Rickets, Probable Treponemalosis</i>

¹(Ormer 2003), ²(Snoddy et al. 2018), ³(Hackett 1975; 1976:362-396), ⁴(Hackett 1951:28), ⁵(Brickley and Ives 2010:103-107), ⁶(Lewis 2017:134, 162, 169, 179), ⁷(Hackett 1951, 1975; 1976:411-433), ⁸(Frangos et al. 2011; Harper et al. 2011; Lewis 2017:179), ⁹(Brickley and Ives 2010:65).

Table S6. Individuals from Man Bac with lesions consistent with treponematoses (SD= strongly diagnostic, D= diagnostic, S= suggestive, A= associated pathologies)

ID	Sex	Age	Gummatous lesions on any skeletal element: focal superficial cavitations in direct relation with nodes or expansions (enlargements) of new bone (SD) ¹	<i>Caries sicca</i> sequence stages 1-3; clustered pits, confluent superficial cavitation of the ectocranium <i>or</i> focal superficial cavitation of long bones not within a distinct node or expansion (enlargement) (D) ²	Saber shin (pseudobowling of the tibia without bowing of the medullary canal) (S) ³	Dactylitis (periosteal enlargement of the hand and feet bones) (S) ⁴	Long bone nodes of endosteal and periosteal enlargement (can be with medullary canal intrusion) (S) ⁵	Boomerang leg: True tibial bowing with bowing of medullary canal (juvenile) (S) ⁶	Systemic periosteal reaction of the long bones (A) ⁷	Diagnosis of Treponemal Disease	Notes
MB05M20	Male	15-29	x		x		x		x	Probable	
MB07H2M18	Possible Female	14-18					x		x	Possible	
MB05M5	Indeterminate	18 months (+/- 3 months)		x (acromial end right clavicle, medial distal right humerus)		x	x		x	Possible	Superficial lytic lesion of superior acromial end of right clavicle and distal right humerus with sclerotic response on margins and base. Fits Hackett (1976) early stage gummatous lesion development
MB05M10	Indeterminate	9 years (+/- 9 months)				x	x		x	Possible	
MB05M29	Male	30-39		x (right distal fibula)		x	x		x	Possible	Superficial lytic lesion of proximal fibula with sclerotic response on margins and base. Fits Hackett (1976) early stage gummatous lesion development
MB07H2M29	Indeterminate	7 years (+/- 9 months)	x		x	x	x		x	Probable	Possible Higurumakis sign
MB07H2M17	Indeterminate	12-18 years				x			x	Non-Diagnostic	Periosteal reactions without enlargements. Fits skeletal pattern of above individuals

¹(Hackett 1975; 1976:362-396), ²(Hackett 1975; 1976:362-396), ³(Hackett 1951:28), ⁴(Hackett 1951:30; Rasool and Govenader 1989), ⁵(Hackett 1976: 411-433), ⁶(Harper et al. 2011; Lewis 2017:175), ⁷(Ottner 2003).

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