Research Article

Hydatid disease (*Echinococcosis granulosis*) diagnosis from skeletal osteolytic lesions in an early seventh-millennium BP forager community from pre-agricultural northern Vietnam

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Abstract

Objectives Con Co Ngua is a complex, sedentary forager site from northern Vietnam dating to the early seventh millennium BP. Prior research identified a calcified *Echinococcus granulosis* cyst, which causes hydatid disease. Osteolytic lesions consistent with hydatid disease were also present in this individual and others. Hydatid disease is observed in high frequencies in pastoralists, and its presence in a hunter-gatherer community raises questions regarding human-animal interaction prior to farming. The objective of this paper is to identify and describe the epidemiology of hydatid disease in the human skeletal assemblage at Con Co Ngua.

Materials and Methods 155 individuals were macroscopically assessed for lesions. Of these, eight individuals were radiographed. Hydatid disease was diagnosed using a new threshold criteria protocol derived from clinical literature which prioritizes lesions specific to the parasite.

Results Twenty-two individuals (14.2%) presented with osteolytic lesions consistent with hydatid disease, affecting the distal humerus, proximal femur and forearm, and pelvis. Seven individuals radiographed (4.5%) had multilocular cystic lesions strongly diagnostic for hydatid disease. All probable cases had lesions of the distal humerus. The remaining lesions were macroscopically identical to those radiographed and were considered possible cases.

Discussion While hydatid disease has previously been found in pre-agricultural communities, the high prevalence at Con Co Ngua is non-incidental. We propose that the presence of wild canids and management of wild buffalo and deer increased the risk of disease transmission. These findings further reveal subsistence complexity among hunter-gatherers living millennia prior to the adoption of farming in Southeast Asia.

Introduction

Infectious diseases have played an important role in the discussion of health transitions with the introduction of agriculture in various regions of the world (Armelagos & Cohen, 1984; Armelagos, Goodman, & Jacobs, 1991; Cohen & Crane-Kramer, 2007). However, in recent decades the forager-farmer dichotomy has been questioned by ethnographic and archaeological research (Barker & Richards, 2013; Cramp et al., 2019; Gregg, 1988; Hudson, 2017; Junker & Smith, 2017). Specifically, the trade relationship between farmers and fringe foragers and large sedentary foraging groups demonstrates the complexities within shifts in subsistence, residential mobility and population density, all factors known to influence infectious disease epidemiology (Armelagos & Cohen, 1984; Junker & Smith, 2017; Larsen, 1995). For this reason, the identification of infectious diseases traditionally associated with agriculture or pastoralism, within archaeological assemblages of foraging groups, may provide a more nuanced understanding of the complexities that lie within what is more a continuum of lifeways than a dichotomy. The identification of skeletal evidence of these pathogens helps to shed light on the adaptability and resilience of human groups, and their relationship with their surrounding environments (e.g., Vlok et al. (2021) for Pre-Neolithic adaptation to malaria).

Oxenham et al. (2018) reported the presence of a calcified hydatid cyst within the soil matrix in the pelvis of an old adult male from the 2013 skeletal assemblage of Con Co Ngua, a Pre-Neolithic forager cemetery site from northern Vietnam (Figure 1). Hydatid cysts are formed in human tissue as a response to *Echinococcus granulosis*, a zoonotic tapeworm, the life-cycle of which is most commonly associated with domesticated canines and ruminants. This individual (CCN13M59a) from Con Co Ngua also had a circular osteolytic cyst-like lesion of the right and left distal humeri consistent with clinical descriptions of hydatid disease of bone (Song, Ding, & Wen, 2007). Multiple individuals were observed to have such lesions, frequently in the distal humeri, acetabula and distal femora of the 2013 assemblage (Buckley, Oxenham, Domett, Willis, & Hiep, 2018). Buckley et al. (2018) posited the possibility of hydatid disease as causing these lesions. The aim of this paper is to establish a diagnostic protocol for macroscopic and radiographic identification of hydatid disease in order to assess population level patterns and prevalence of hydatid disease at Con Co Ngua. The significance of this zoonotic disease in a pre-agricultural forager community is discussed. The zoonotic nature of hydatid disease also opens up discussion of human-animal interactions through a 'one health' perspective. A one health approach considers human and animal health as

dependent on the other if they share the same social and ecological space (Zinsstag, Schelling, Waltner-Toews, & Tanner, 2011). Where zoonotic diseases are concerned, animal health can directly influence the health of interacting human groups via transmission of pathogens or parasites.

The biocultural context of Pre-Neolithic Vietnam

Following a southerly route, anatomically modern humans (AMH) settled the southern and middle regions of East Asia and much of Island and Mainland Southeast Asia (MSEA) from at least 60,000 BP (Matsumura & Oxenham, 2014) if not earlier (Clarkson et al., 2017; Norman et al., 2018). Later, following a northern route, AMH appear in western Siberia at c. 46,000 BP and northern China c. 40,000 BP (Oxenham & Buckley, 2016). A significant increase in temperature and rainfall known as the Holocene Thermal Maximum (HTM) began c. 11,000 BP, and witnessed the independent development of farming in what is now northern (millet producing) and central (rice producing) China by c. 9,000 BP (Oxenham et al., 2018; Zhao, 2011). It is likely that these early farmers were genetically and phenotypically East Asian, presumably descendants of the original northern-route AMH migrants (Oxenham and Buckley 2016). Coincidentally, and also associated with the HTM, the large ecoregion Nam Viet covering southern China (Guangxi and western Guangdong provinces) and northern Vietnam saw the emergence of numerous high density, and for the most part sedentary, complex hunter-gatherer communities that were genetically and phenotypically Australo-Papuan, or descendants of the original southern-route AMH migrants (Matsumura et al., 2019; Matsumura, Hung, Zhen, & Shinoda, 2017; Oxenham et al., 2018). Subsequently, between 5000-4000 BP farming appears to have spread into southern China first, followed by northern Vietnam and much of MSEA relatively rapidly, both displacing and integrating with local forager communities (Bellwood & Oxenham, 2008; Higham, Guangmao, & Qiang, 2011). One outcome, particularly relevant here, is the apparent emergence or introduction of certain infectious diseases in MSEA with the development and/or intensification of farming practices (Oxenham, Thuy, & Cuong, 2005; Tayles & Buckley, 2004; Vlok et al., 2020), although it appears that one particular disease, malaria, was a significant burden prior to the adoption of farming (Vlok et al., 2021).

While numerous complex forager sites occur within the Nam Viet ecozone, few have attracted detailed analysis with only Con Co Ngua (n=272 individuals, Thanh Hoa Province, Vietnam), Dingsishan (n=331 individuals, Guangxi province, China), Huiyaotian (n=60

individuals, Guangxi province, China), and Liyupo (n=40 individuals, Guangxi province, China) being exceptions (Fu, 2002; Matsumura et al., 2017; Oxenham et al., 2018). Con Co Ngua and related sites in northern Vietnam along with other sites in Guanxi province, China, are representative of geographically extensive populations of a complex hunter-gatherer culture that had large open-air (as opposed to cave) cemeteries. These hunter-gatherer communities practiced complex funerary rites often associated with ritual body mutilation and (re)positioning, arguably performed by ritual specialists and ostensibly speaking to a highly sophisticated cosmology (Matsumura et al., 2018; Oxenham et al., 2018). Additionally, these populations are characterized by the production and use of pottery, polished stone tools, bone and shell implements, and the exploitation of a highly diverse range of aquatic (including riverine, estuarine, lacustrine and oceanic) and terrestrial plant and animal resources (Zhang & Hung, 2012). Furthermore, Con Co Ngua is particularly important in the context of evidence for the exploitation and management of large bodied wild bovids and cervids (Jones, Piper, Groves, et al., 2019), and the potential for transmission of hydatid disease (Oxenham et al. 2018).

Hydatid disease

The larvae of *Echinococcus* tapeworm species, including *E. granulosis* and *E. multilocularis*, can cause parasitic infection in human bone (Deplazes et al., 2017; Rausch & D'Alessandro, 1999). The *Echinococcus* tapeworm life cycle relies on both a definitive (end of life cycle) host (canid) and an intermediate host (ruminant) (Beggs, 1985). *E. granulosis* is the most common form of hydatid disease and is characterized by the development of parasitic cysts in the body (Beggs, 1985). A close relationship with domesticated dogs and ruminants is directly associated with human *E. granulosis* infection. Therefore, pastoralists are particularly at risk for this disease (Beggs, 1985; Deplazes et al., 2017). An alveolar form of hydatid disease (not related to alveolar bone) occurs with *E. multilocularis* (Brunetti, Kern, & Vuitton, 2010). This form is far more rare, more lethal, and does not cause the cyst-like changes in soft tissue that occur in *E. granulosis* (Brunetti et al., 2010; Rausch & D'Alessandro, 1999). However, alveolar and cystic hydatid disease (also called echinococcosis) are indistinguishable in bone (Merkle et al., 1997).

In *E. granulosis* dogs serve as the definitive host whereas a number of ruminants including sheep, goat, cattle, and buffalo serve as the primary intermediate hosts (Beggs, 1985). In sylvatic (wild) strains of *E. granulosis*, wild animals such as deer are the intermediate hosts

whereas wolves, and other wild canids, can also serve as the definitive host (Rausch & D'Alessandro, 1999). For *E. multilocularis* foxes are more commonly the definitive hosts, with rodents serving as the intended intermediate host (Czermak et al., 2001).

Canids are infected through eating viscera of infected livestock, either through feeding domestic dogs offal of infected livestock, or scavenging of livestock carcasses (Eslami & Hosseini, 1998). The adult (a tapeworm) lives in the intestines of the definitive host. Hundreds of eggs are discharged by each hydatid worm into the intestines where the eggs are subsequently excreted in feces. Hydatid disease in canids is usually asymptomatic (Sheridan, 2009). Fecal matter is ingested by the ruminating intermediate hosts (Beggs, 1985). Humans are accidental intermediate hosts (Rausch & D'Alessandro, 1999; Vatankhah et al., 2015) with consumption of contaminated water or vegetables being the most common form of transmission (Beggs, 1985).

In intermediate hosts, parasitic embryos form and pass through the intestinal wall and spread throughout the body via the venous and lymphatic systems (Beggs, 1985). In *E. granulosis* larvae begin to grow in a vesicle of germinal tissue encased in laminated fluid filled cysts (Rausch & D'Alessandro, 1999; Vatankhah et al., 2015). The cyst has three layers: an outer layer (the pericyst) which develops from the intermediate host's inflammatory response and is comprised mostly of modified host cells, the middle laminated layer produced by the parasite which enables passage of nutrients but prevents invasion of bacteria, and the inner germinal layer which produces the laminations of the middle layer (Beggs, 1985). Concentric enlargement of the vesicle occurs until an approximate maximum size of 6 to 7cm (Rausch & D'Alessandro, 1999). In rare circumstances, these cysts can calcify and remain in the archaeological record (Beggs, 1985). Cysts can be simple, multifocal or multichambered and can proliferate in any organ, but are most commonly found in the liver (Rausch & D'Alessandro, 1999; Vatankhah et al., 2015). Up to 75% of cases involve the liver, followed in frequency by the lung (approximately 15%) (Beggs, 1985; Song et al., 2007; Vatankhah et al., 2015). Bone involvement ($\leq$4%) occurs through infiltration of larvae from adjacent infected tissue or directly from the bloodstream (Beggs, 1985; Song et al., 2007). A pericyst (the outer host-formed layer) does not form in bone with *E. granulosis* infection, and, due to a preference for vascularized areas such as trabecular bone and the medullary canal,

proliferation can be extensive and rapid, similar to the non-cystic malignant-like growth of *E. multilocularis* in soft tissue (Beggs, 1985).

Hydatid disease has been identified through the presence of calcified cysts in human skeletal assemblages and macroscopic analysis of faunal bone in a number of archaeological sites (Antikas & Wynn-Antikas, 2016; Calleja et al., 2017; Fornaciari et al., 2020; Oxenham et al., 2018; Rothschild & Surmik, 2020; Waters-Rist et al., 2014; Williams, 1985). However, no standardized diagnostic protocols in paleopathology exist for macroscopic bone lesions, nor have these bone lesions been documented in association with calcified cysts in prior paleopathological literature. In fact, possible human skeletal evidence of hydatid has not been reported, except in Oxenham et al. (2018). Waters-Rist et al. (2014) and Komar and Buikstra (2003) have previously established methods for differentiating calcified hydatid cysts from other calcified human tissues. A protocol for diagnosis in radiographs of archaeological human remains based on the well-established clinical diagnostic methods was applied here. A protocol for macroscopic diagnosis based on the radiographic literature and comparison to the radiographed archaeological cases was then subsequently produced.

Materials and Methods

Con Co Ngua site

The 2013 assemblage from Con Co Ngua was assessed for skeletal evidence of osteolytic lesions (n=155 suitably preserved individuals). First excavated in 1979/80 (n=100) (Bui, 1980; Oxenham, 2016), the site was re-excavated in 2013 with the recovery of a further 172 individuals (Oxenham et al., 2018). Radiometric dating indicates occupation of Con Co Ngua from the early seventh millennium BP. While approximately 30km from the ocean today, the site was likely coastal or situated upon an estuary during the HTM (Oxenham et al., 2018). Normative funerary rituals included laying out the body, postmortem mutilation (including head removal and breakage of major long bones), wrapping and placement in single (usually) earthen pits in a side-flexed or squatting position (see Oxenham et al., 2018 for images of typical burial forms). Currently, the climate in the region is subtropical, with two distinct seasons, a hot and cold season, where high levels of humidity are sustained year round (Oxenham, 2006; Oxenham, Matsumura, & Kim Dung, 2011). However, it is believed surface temperatures were up to 4°C warmer and rainfall as much as 100% greater than today (Renssen, Seppä, Crosta, Goosse, & Roche, 2012; Tao, Huijun, & Dabang, 2010).

The inhabitants of Con Co Ngua exploited a wide variety of resources in the surrounding landscape. Wild *Canarium* sp. (a genus which includes Chinese white and black olives) was a stable carbohydrate in the diet, while faunal remains include reptiles, fish, sharks, rays and birds from a diverse range of ecological habitats, including woodland, wetland, estuarine and offshore environments (Jones, Piper, Groves, et al., 2019; Oxenham et al., 2018). Of particular note is the observation that water buffalo (*Bubalis* spp.*),* and deer (Cervidae) to an extent, dominated the faunal assemblage with butchering marks on these animals indicating on-site processing (Jones, 2017; Jones, Piper, Groves, et al., 2019; Oxenham et al., 2018). Further, the frequency and type of healed skeletal trauma at Con Co Ngua suggests that wild bovids may have been managed or herded by the community (Scott et al., 2019). No evidence for domesticated ungulates or canids has been identified at Con Co Ngua, and faunal bones indicate the presence of wild ungulates and canids only (Jones, 2017; Jones, Piper, Groves, et al., 2019; Jones, Piper, Wood, Nguyen, & Oxenham, 2019; Oxenham et al., 2018).

Preservation

Individuals were predominantly partially (50 to 66%) to near complete (66 to 75%). As previously noted by Oxenham (2000), the postcranial axial skeleton and the pelvis were relatively poorly preserved at Con Co Ngua. Long bone shafts were better preserved with mixed excellent to poor preservation of the long bone epiphyses. Bones suffered significant post-mortem breakage, but most bones could be reconstructed with relative ease. Vertebrae and ribs were often fragmented. Surfaces exhibited erosion grades 2 to 3 (McKinley (2004).

Sex and Age Estimation

Non-metric standards for cranial and pelvis morphology (Buikstra & Ubelaker, 1994; Phenice, 1969; Walrath, Turner, & Bruzek, 2004), and sample-specific postcranial functions developed by Oxenham (2016) were applied for adult sex estimation. Subadult age-at-death was estimated using non-metric dental eruption and calcification standards (Moorrees, Fanning, & Hunt, 1963). Timing of epiphyseal fusion served as a primary form of age estimation in adolescents and young adults following Schaefer, Black, and Scheuer (2009). Standard non-metric adult age estimation criteria for pubic symphyseal face morphology (Brooks & Suchey, 1990) and sample-specific molar wear functions developed by Oxenham (2016) were used.

Pathological Recording and Differential Diagnosis

Each individual was assessed for osteoblastic and osteoclastic lesions of the cranium and postcranium. Fractures were recorded where they appeared to be secondary to other pathological processes. Radiographs were taken for eight individuals, seven of which presented with representative macroscopic osteolytic lesions, the other a distal humerus fracture. Due to logistical limitations not every lesion could be radiographed. Diagnostic threshold criteria were developed based on the recommendations of Snoddy et al. (2018) and Vlok et al. (2020) and applied to hydatid disease. Lesions are 'strongly diagnostic' if they are considered pathognomonic for the disease (unique for a specific condition) in the medical literature. They are 'diagnostic' if there is evidence-based clinical or anatomical association with these lesions to diagnosis of the disease, but on their own are not considered pathognomonic. Lesions are only 'suggestive' if they are typically associated with the disease but can result from a number of other diseases and therefore do not contribute to a distinct diagnosis in their own right. At minimum, a probable case requires one strongly diagnostic lesion and/or two diagnostic lesions. A possible case requires a minimum of one diagnostic lesion and/or two suggestive lesions for diagnosis. We acknowledge that a strongly diagnostic lesion does not mean the lesion cannot form in another disease, rather that the lesion very rarely occurs in other diseases (as opposed to recommendations by Appleby, Thomas, and Buikstra (2015) based on the Istanbul protocol for medical corroboration of a tortured individual's story, not medical diagnosis). The rarer conditions still need to be considered within the differential diagnosis as potential causes. An example of a well referenced 'pathognomonic' lesion in paleopathology is Pott's disease of the spine in tuberculosis (osteolysis of the anterior vertebral body resulting in anterior collapse of the spine) (Roberts, 2015; Vlok, Myagmar, Byambadorj, & Buckley, in press). However, in rare cases this condition can also occur in mycosis, pyogenic osteomyelitis, and brucellosis (Kakarla, Kalani, Sharma, Sonntag, & Theodore, 2011; Lehovsky, 1999; Pourbagher et al., 2006). Definite diagnosis is only possible through pathogen DNA analysis. For this reason, we avoid the terms *pathognomonic* and *definite diagnosis*. Diagnosis is restricted here to *possible* and *probable* cases only.

Larvae of *E. granulosis* and *E. multilocularis* are small enough to pass through the porous structures of skeletal tissue, termed microvesicular dissemination (Zlitni et al., 2001). As the larvae grow, the bone surrounding the cyst is destroyed in a spherical manner. The associated bone response varies from a lack of clear boundaries to marginal or well defined sclerotic

boundaries (Torricelli, Martinelli, Biagini, Ruggieri, & De Cristofaro, 1990; Zlitni et al., 2001). A combination of host response, cyst enlargement, and microvesicular dissemination via numerous daughter cysts (asexual reproduction) cause this osteolysis (Kalinova, Proichev, Stefanova, Tokmakova, & Poriazova, 2005). Large cysts rarely form in trabecular and cortical bone, due to the microvesicular source of new cyst growth in skeletal tissue. However, due to the free unlimiting space of the medullary canal, in rare cases they can increase in size. Bone cysts more commonly form as small and multifocal, and resemble a "bunch of grapes" on radiographs, a feature that is strongly diagnostic for hydatid disease (Table 1; Kalinova et al., 2005; Ortner, 2003, pp. 337-338; Zlitni et al., 2001, p. 76). Over time cortical bone adjacent to the medullary cyst may resorb, and penetration of the cortex may occur, and the larvae continue to invade the surrounding soft tissue and organs (Song et al., 2007; Zlitni et al., 2001). Macroscopic evidence of cortical penetration is, however, not clinically diagnostic for hydatid disease. Bone necrosis and pathological fractures due to failure of the structural integrity can occur in advanced cases (Zlitni et al., 2001). In regions such as the pelvis, osteoblastic bone response can occur as a cyst expands or proliferative new bone can form due to secondary osteomyelitis, therefore, the presence of new bone does not exclude a diagnosis of hydatid disease (Agarwal, Shah, Kadhi, & Rooney, 1992; Ortner, 2003, pp. 337-338).

Skeletal involvement in hydatid disease ranges from 0.5 to 4% of cases (Song et al. 2007; Zlitni et al. 2001) with regions of high trabecular volume and medullary cavities the most common sites of parasitic growth (Ortner, 2003, pp. 337-338; Zlitni et al., 2001). Most commonly, the disease is restricted to a single bone (Jaffe, 1972, p. 1073; Ortner, 2003, pp. 337-338) and rarely affects the subchondral (articular) bone of the epiphyses (Jaffe, 1972, p. 1073; Ortner, 2003, pp. 337-338). The dense subchondral bone provides a barrier for cyst enlargement. In severe cases the osteolysis can encroach around and into the margins of the subchondral bone of the epiphyses but the metaphyseal and non-subchondral (non-articular) epiphyseal regions (hereon referred to as the metaphyseal region) are always affected first (Figure 2) (Zlitni et al., 2001). While all bones of the skeleton can be affected, the vertebrae and the pelvis are the most frequent sites, followed by the metaphyses of the humerus, tibia, fibula and femur (Jaffe, 1972, p. 1073; Ortner, 2003, pp. 337-338; Zlitni et al., 2001). As the cystic bone lesions are located within the metaphyses of long bones, the spine and the pelvis, tuberculosis, mycosis, and osteomyelitis should be included in any differential diagnosis (Jaffe, 1972; Song et al., 2007). Furthermore, aneurysmal cysts, metastatic cancers,

osteochondritis dissecans and benign tumors can present very similar macroscopic circular destructive lesions in the long bone ends (Agarwal et al., 1992; Oxenham et al., 2005; Song et al., 2007).

Soft tissue hydatid cysts can calcify following the death of the parasite, and sometimes these cysts may survive in the archaeological record (Dervenis, Delis, Avgerinos, Madariaga, & Milicevic, 2005; Oxenham et al., 2018; Waters-Rist et al., 2014). If a calcified cyst is found, it must first be differentiated from calcified tumors and various benign cysts or soft tissue nodes within the body (Komar & Buikstra, 2003). The presentation of multiple layers of the calcified cyst wall, and the presence of septa, demonstrating evidence of daughter cysts, are crucial for the differential diagnosis of the calcified hydatid cyst (Komar & Buikstra, 2003; Oxenham et al., 2018). Energy dispersive X-Ray spectroscopy to identify the chemical composition may also accompany morphological analysis of the cyst (Fornaciari et al., 2020).

Table 1: *Diagnostic Criteria for Identification of Hydatid Disease (SD= strongly diagnostic, D= diagnostic, and S= suggestive).*

Pathology	Diagnostic Strength	Differential Diagnosis	Reference
Calcified Hydatid cyst: clear evidence of a double layered calcified pericyst, hollow, ellipsoid in shape with septa demonstrating evidence of daughter cyst formation.	SD	Calcified ovary, ovarian cyst or fibroma, lymph node, omental nodule, renal cyst or abscess; lithopaedion; urinary calculus; leiomyoma, mesenteric carcinoid tumor or cyst; lipoleiomyoma; fecalith; appendicolith; psammoma body; calcified neoplasm, alveolar *Echinococcus*	(Komar & Buikstra, 2003; Waters-Rist et al., 2014)
Small and numerous circular osteolytic cysts (multifocal or coalesced) resembling a bunch of grapes in regions of trabecular bone or marrow cavity with 1) minimal or no reactive subperiosteal new bone response, 2) lack of clear boundaries or marginal sclerosis 3) and lack of morphological changes to the bone. These lesions are not widespread throughout the body and typically do not cross articular regions as the cyst does not penetrate into the bone from the external subchondral epiphysis. Vertebrae, innominate, humerus, tibia, fibula and femur most commonly involved. Cysts can become large in the medullary space.	SD	Hyperparathyroidism	(Beggs, 1985; Jaffe, 1972, p. 1073; Ortner, 2003, pp. 337-338; Torricelli et al., 1990; Zlitni et al., 2001).
Combination of multilocular cysts and reactive sclerosis involving a large expansion of the pelvis.	D	Tuberculosis, neoplasms	(Agarwal et al., 1992; Song et al., 2007)
Radiographic: Thin cortices adjacent to the region of cystic lytic response	S	Various nutritional deficiencies	(Song et al., 2007; Zlitni et al., 2001)
Radiographic: Ischemic necrosis of the marrow area	S	Osteomyelitis, sickle cell disease, thalassemia	(Zlitni et al., 2001)

Pathology	Diagnostic Strength	Differential Diagnosis	Reference
Collapse of vertebral bodies	S	Tuberculosis, osteomyelitis, mycosis	(Ortner, 2003, pp. 337-338)
Secondary osteomyelitis or subperiosteal new bone at the site of cystic lytic response	S	Various infectious disease	(Agarwal et al., 1992; Ortner, 2003, pp. 337-338)
Pathological fracture associated with bone cyst	S	Extrinsic trauma	(Zlitni et al., 2001).
Large circular destruction of the cortex due to cortex breeching and continued infection of surrounding soft tissue.	S	Tuberculosis, mycosis, osteomyelitis, neoplasms, osteochondritis dissecans or erosive arthropathies of the long bones	(Song et al., 2007)

Results

Diagnosis of hydatid disease

Twenty-two individuals (two adolescents and 20 adults) presented with predominantly unilateral (sometimes bilateral) circular osteolytic cyst-like lesions with or without sclerosis of the margins and base (Figure 3). These osteolytic lesions were predominantly observed on the distal humeri, acetabula, and distal femora. Given that up to 14.2% (22/155) of the assemblage was affected, aneurysmal cysts, tumors, and congenital defects can be ruled out as causes of the osteolytic lesions, due to their rarity and their lesion patterning. Benign cortical defects tend to be shallow with clear sclerosis in the base. If they penetrate the cortex they are associated with osteoblastic enlargement of the outer cortex and macroscopically observed deformity (Kumar, Madewell, Lindell, & Swischuk, 1990). These characteristics do not match the circular osteolytic lesions observed in Con Co Ngua. While some benign tumors such as chondroblastomas and enchondromas present in dry bone as osteolytic lesions with well-defined margins, key differentiating features of these tumors were not present in the assemblage. Chondroblastomas produce circular osteolytic defects with sclerotic margins on long bone ends that penetrate the bone from the external epiphyses (superficial to deep), which was not observed here (Grauer, 2019; Hudson & Hawkins, 1981; Marques, 2019). Similarly, enchondromas present as circular osteolytic lesions without sclerosis in the medullary canals and metaphyses of long bones, but these lesions are associated with mineralized chondroid matrix and/or endosteal scalloping, again not observed in the Con Co Ngua assemblage (Flemming & Murphey, 2000; Marques, 2019).

Aneurysmal cysts are cystic cavities filled with blood that cause circular osteolysis in the metaphyseal and/or diaphyseal regions of long bones (Capanna et al., 1985). Aneurysmal cysts can develop in the medullary space or external cortex of long bones and encroach into the metaphyseal region when they are large. The lesions form through a process of slow erosion, allowing enough time for the margins around the lesion to remodel and develop characteristic sclerotic smooth walls (Rizzo, Dellaero, Harrelson, & Scully, 1999; Vergel De Dios, Bond, Shives, McLeod, & Unni, 1992). Severe osteolysis that penetrates the cortex due to an aneurysmal cyst often results in pathological fracture, or significant enlargement of the bones with clear macroscopically observed deformity (Capanna et al., 1985; Rizzo et al., 1999). Additionally, more than half of the cases are associated with subperiosteal new bone deposits, and adjacent bones are frequently affected (Vergel De Dios et al., 1992), which is not the case with hydatid disease. While macroscopic observation alone cannot rule out aneurysmal cysts in some of the Con Co Ngua cases, the characteristic thick sclerotic smooth walled margins of the osteolytic lesion and associated subperiosteal new bone associated with an aneurysmal cyst does not meet the criteria of the lesions observed. Osteolytic lesions from Con Co Ngua that exhibited sclerotic margins do not resemble the degree of sclerosis in aneurysmal cysts. In addition, most individuals affected with aneurysmal cysts are under 20 years of age, and more frequently occur in childhood (Vergel De Dios et al., 1992), not consistent with the adult demographic of affected Con Co Ngua individuals. Joint diseases, such as the erosive arthropathies, can also be ruled out as it is clear the lesions in the Con Co Ngua assemblage only encroach into the subchondral bone when the lesions are large and erosive joint diseases initially affect the superficial surfaces of the subchondral bone within the synovial joint capsule.

In the context of Southeast Asia, melioidosis, a tropical respiratory disease, needs to be considered in the differential diagnosis. Melioidosis is a respiratory disease caused by the bacteria *Burkholderia pseudomallei* that is endemic to Southeast Asia (Chierakul et al., 2005). The disease spreads to the lungs through inhalation or contact of open wounds with contaminated soil or water and is regularly fatal (Chierakul et al., 2005). Septic arthritis and osteolysis of the metaphyses and epiphyses are characteristic of this disease (Chong & Fan, 1996). The knee, ankle, and elbow are the most commonly affected regions. While osteolysis of the metaphyses is consistent with the lesions observed in the Con Co Ngua individuals, significant subchondral bone involvement was distinctly absent, which does not fit the clinical findings of skeletal involvement in melioidosis (Chong & Fan, 1996).

Given the context of forager exploitation of the surrounding environment, another potential consideration is mycotic infection. *Penicillium marneffei* is a mycotic organism known to lead to osteolysis of the bony prominences of the long bones (Chan & Woo, 1990). The fungus itself rarely infects humans, therefore a prevalence of >10% in a skeletal assemblage would be extremely unusual. However, the fungus is endemic to Southeast Asia so should be considered (Chan & Woo, 1990). The lesions formed in response to *P. marneffei* do not exhibit sclerosis on the base, as was observed in a number of the lesions at Con Co Ngua (Figure 3), and *P. marneffei* infection results in destructive lesions in multiple bones, whereas the osteolytic lesions observed here were predominantly restricted to a single bone (Qiu et al., 2015). Additionally, the osteolysis of this disease presents as a "moth-eaten' appearance rather than being cystic in form and joint involvement has been observed with this mycosis (Qiu et al., 2015). The cyst-like osteolytic lesions observed in the dry bone at Con Co Ngua are then *suggestive* for hydatid disease, but macroscopic observation of cortex penetration alone is not sufficient for diagnosis (Table 1).

Radiographs of seven (out of eight) individuals with macroscopic cyst-like lesions identified small and numerous multifocal cystic lesions resembling a "bunch of grapes" within the marrow and trabecular regions of the bone. This radiographic sign is strongly diagnostic for hydatid disease. Therefore, 4.5% (7/155) of the Con Co Ngua assemblage demonstrated lesions consistent with probable hydatid disease. As shown in Figure 3b, the bases of the osteolytic lesions are multilocular (this lesion is radiographically observable in Figure 4a). Although with most individuals, cortical penetration was not extensive enough to observe this macroscopically. Of these individuals, five also presented with ischemic necrosis of the marrow area surrounding the multifocal osteolytic lesions (Figure 3b, 4c). This condition was observed in individuals diagnosed with thalassemia in the Con Co Ngua assemblage (Vlok et al., 2021). However, in the individuals with osteolytic lesions associated with necrosis no other lesions consistent with thalassemia were present, and the necrosis was clearly associated with the path of osteolytic formation in the bone. One individual (CCN13M21a, adult female) displayed a pathological fracture at the lesion site (left distal humerus) which is known to occur in hydatid disease (Zlitni et al., 2001).

Radiographically, the osteolytic lesion of one individual (CCN13M113a, middle aged adult male) appears to originate within the subchondral bone of the distal right femur which is not

consistent with hydatid disease where infection occurs from within the marrow space or internal trabeculae. Rather, this lesion may be more characteristic of osteochondritis dissecans. As such, this individual was not diagnosed with hydatid disease. While the remaining 14 individuals were not radiographed, it is likely that they also had hydatid disease given the morphological similarities to the strongly diagnostic lesions observed through radiographs. Figure 4 presents the skeletal expression of individuals diagnosed with hydatid disease by radiographs, as well as the macroscopic expression of all 21 individuals (13.5% of assemblage) with possible or probable hydatid disease.

While radiographs were not available to determine a probable diagnosis, the remaining osteolytic lesions were observed in regions of the skeleton that hydatid disease prefers (with exception of the vertebrae). Particularly, a higher prevalence of affected pelvic bones and distal humeri are consistent with the skeletal expression of hydatid disease (Figure 5; Table 2). In hydatid disease the vertebrae are most commonly involved followed by the pelvis (Jaffe, 1972, p. 1073; Ortner, 2003, pp. 337-338; Zlitni et al., 2001). However, this was not the case at Con Co Ngua, with the pelvis being the most commonly affected skeletal element. The postcranial axial skeleton was heavily fragmented in this assemblage, likely influencing the lack of observation of lesions in this region of the body. Only one individual presented with a possible hydatid cyst of the superior aspect of the first sacral body. While the pelvis was also poorly preserved, more robust elements such as the acetabular regions survived, and lesions were observed on these elements (Figure 6).

Table 2: *Individuals with macroscopic osteolytic lesions consistent with hydatid disease*

Individual ID CCN13	Age	Sex	Bone affected	Portion of bone affected	Presence of Sclerosis	Macroscopically observed?	Radiographed
M12a	20-29	M	Right humerus	Posterior distal metaphysis	Yes	Yes	Yes
M59a*	50+	M	Left and right humerus	Lateral distal metaphysis, encroaches into capitulum	Very slight	Yes	Yes
M19a	40-49	M	Left humerus	Distal metaphysis	No	Yes	Yes
M21a	20-29	F	Left humerus	Distal metaphysis	No	No- pathological fracture with reactive bone and a possible cloaca (actually a cortex breach) was recorded macroscopically.	Yes
M28a	30-39	F	Left cuboid		Yes	Yes	No
M31a	20-29	M	Right os coxa	Acetabular fossa	No	Yes	No

M32a	30-39	I	Right humerus	Lateral distal metaphysis	Yes	Yes	Yes
M33a	20-29	I	Right os coxa	Acetabular fossa	No	Yes	No
M45a	50+	I	Right os coxa	Acetabulum	Very slight	Yes	No
M65a	30-39	F	Right os coxa	Pubis	No	Yes	No
M72a	15-19	I	Left humerus	Posterior distal metaphysis	Yes	Yes	Yes
M79a	50+	M	Left humerus	Posterior distal metaphysis	Very slight	Yes	No
M83a	50+	F	Right os coxa	Acetabular fossa	Yes	Yes	No
M100a	20-29	F	Right femur	Intercondylar space encroaching into subchondral bone	Very slight	Yes	No
M115a	50+	F	Left humerus	Distal metaphysis	No	Yes	Yes
M118a	20-29	F	Right humerus	Distal metaphysis	No	Yes	No
M142a	20-29	M	S1	Superior body	Yes	Yes	No
M92a	50+	F	Left ulna	Proximal ulna encroaching into trochlear notch	Yes	Yes	No
M1a	Adult	F	Right ulna and humerus	Proximal ulnar and distal humeral metaphyses	Yes	Yes	No
M35a	30-39	M	Left radius	Left proximal radius encroaching into subchondral bone	No	Yes	No
M67a	15-19	I	Right humerus	Distal metaphysis	Yes	Yes	No

CCN13M113a was excluded from the list as the radiographs indicate the lesion was not consistent with hydatid disease. Sex: M= Male, F= Female, I= indeterminate due to ambiguous features and poor preservation of sex estimation features of the cranium and the pelvis. *= a calcified hydatid cyst was found in the pelvic cavity of this individual.

Paleoepidemiology

Paleoepidemiological analysis was carried out for the radiographically diagnosed cases only and thus provide minimum estimates for prevalence at Con Co Ngua. The disease affected both sexes, all adult age groups, and adolescents 15-19 years of age (Table 3). No individual under 15 years of age was affected. The Odds ratio for probable hydatid disease by sex demonstrated no statistically significant difference (n=88, OR=1.7, CI: 0.45-6.3, p=0.4). Similarly, there are no clear trends across adult age-at-death with less than 15% of individuals affected in all age cohorts. There is a slight trend towards older age, but this was not statistically significant when the prevalence between individuals 15-29 and 30+ years was tested (n=101, OR=0.5, CI: 0.09-3.04, p=0.5).

Table 3: Prevalence of probable hydatid disease across age and sex

	Affected	Observed	(%)
15 to 19 years	1	17	5.9
20 to 29 years	2	30	6.7
30 to 39 years	1	22	4.5
40 to 49 years	1	16	6.3
50+ years	2	16	12.5
Total nonadults	1	71	1.4
Total adults	6	84	7.1
Males	3	54	5.6
Females	2	34	5.9
Total assemblage	7	155	4.5

Discussion

The application of this new diagnostic method for hydatid disease identified the condition in at least seven individuals. Consideration of skeletal expression of the macroscopically observed osteolytic lesions further extended the overall disease prevalence. It is clear, however, that diagnostic confidence is reliant on access to radiography of the osteolytic lesions. Furthermore, systematic radiography of the spine, pelvis and long bone ends commonly affected (such as the distal humerus), may identify a higher prevalence of the disease, including individuals where the hydatid cysts had not penetrated the cortex. The prevalence estimated from the skeletal lesions is considerably higher than the reliance on the identification of hydatid disease strictly from calcified cysts, which is the current method for identification in the literature (up to 13.5% compared to 0.01% of the Con Co Ngua assemblage). It is apparent from the outcomes of this research, particularly when considering that only up to 4% of cases with hydatid disease will manifest lesions skeletally (Song et al. 2007; Zlitni et al. 2001), that hydatid disease was a significant disease burden for the Con Co Ngua community. The higher prevalence of bone hydatid disease in Con Co Ngua than the clinical average may be due to three factors. Firstly, the medical literature documents the number of symptomatic cases that report to a hospital or clinic and not asymptomatic cases of bone disease which could be significantly higher. Additionally, if asymptomatic bone hydatid cysts rupture and lead to anaphylactic shock, the cause of death may not be correctly attributed to hydatid disease. Secondly, the sedentary nature of the Con Co Ngua inhabitants

may increase the risk of infection via water sources contaminated with parasites. While estimates for hydatid disease in humans are lower due to modern food hygiene practices, higher prevalence rates (over 45%) have been reported in sedentary cattle in Australia who share the same water source (Gemmell, 1990). Lastly, the osteological paradox is likely a factor influencing the prevalence of skeletal hydatid disease in the Con Co Ngua assemblage as bone disease is frequently fatal increasing the proportion of cases in the assemblage.

In individuals over the age of 15 years, there was no significant relationship between age-at-death and the presence of hydatid disease at Con Co Ngua. While this disease is recognized to have a high mortality rate once it has affected the skeleton, it often lies dormant in organs for decades prior to skeletal infection. Therefore, skeletal hydatid is considered a disease of adults only (Zlitni et al., 2001). The relationship of skeletal hydatid disease to age-at-death is determined by the age of onset (Song et al., 2007). Adolescents and adults of all age cohorts were infected in this population, as were both males and females, suggesting that at the very least all adults were at risk of exposure to hydatid disease. Young children may also have been infected without progression of the disease to a skeletal stage which occurs much later in life.

Hydatid disease is usually associated with pastoralism requiring a contiguous relationship between human groups, domestic dogs and ungulates for humans to become an accidental intermediate host (Beggs, 1985). As mentioned, sylvatic (wild) strains do occur, but today they rarely infect humans. Strains that affect deer as intermediate hosts and wolves (and other wild canids) as definitive hosts are also currently only found in the northern hemisphere (Rausch & D'Alessandro, 1999). Buffalo and deer dominate the faunal assemblage at Con Co Ngua and evidence of butchery marks on these remains indicate processing of the full carcass at the site which could suggest hunting of animals close to places of residence (Oxenham et al., 2018). Buffalo are also known to be intermediate hosts for *E. granulosis* non-sylvatic strains (Beggs, 1985). The frequency and patterning of healed trauma at Con Co Ngua, similar to that seen among agropastoralists, suggests a close relationship with buffalo through the management of wild herds (Scott et al., 2019). As proposed by Oxenham et al. (2018), these two species (deer and buffalo) are posited as plausible intermediate hosts for hydatid disease at Con Co Ngua, and a number of carnivorous animals such as civets, jackals, wolves, leopards, and foxes may have served as the definitive host (Jones, 2017; Oxenham et al., 2018). In both sylvatic and non-sylvatic strains of *E. granulosis*, contamination of the water

source is a primary route of accidental infection in humans (Beggs, 1985). It is plausible that water sources shared with wild buffalo and/or deer, and carnivores, led to the infection of the Con Co Ngua inhabitants with hydatid disease, likely that of a sylvatic strain. However, the high prevalence of skeletal hydatid disease suggests it was endemic in the community, and therefore was unlikely to be a result of incidental encounters with wild herds alone. Prolonged use of shared water sources resulting in hydatid disease in humans at Con Co Ngua, mimic cycles observed in non-sylvatic strains in pastoralist communities, which further supports the hypothesis for management of wild herds including buffalo and possibly deer by this sedentary foraging group. From a 'one health' perspective it is evident that the ecological interactions between humans and these ungulates and canines was detrimental to the health of all species involved.

Zoonotic disease and the foraging-farming dichotomy

Recent research has begun to question the simple narratives of foraging versus farming in East and Southeast Asia, with the adoption of farming practices having been highly variable at a local level (Guedes et al., 2020; Hsiao-chun, 2019; Junker & Smith, 2017; Oxenham, 2015; Oxenham et al., 2015; Pan, Zheng, & Chen, 2017). For example, early inhabitants of Khok Phanom Di, an early Neolithic site in southern Thailand (dated to 4000-3500BP), appear to have predominantly foraged wild resources, with their diet supplemented by rice likely traded from neighboring agriculturalists (Higham et al., 2011). These individuals are morphologically similar to the groups who introduced farming to the region rather than the indigenous foragers such as the inhabitants of Con Co Ngua (Matsumura & Oxenham, 2014).

Both foraging and farming practices are dynamic and the intensity of these practices fluctuate over time, with human groups highly sensitive to their environmental surroundings (Pan et al., 2017) and as such, the forager spectrum is exceptionally diverse. Reliance on cultivation, horticulture, or trade in domesticated food products to supplement hunting, gathering, and fishing endeavors are commonplace amongst modern and historical foraging groups such as the Vedda of Sri Lanka who supplement their foraging with rice (Kelly, 2013; Roberts et al., 2018). This is also the case for documented prehistoric sedentary foraging groups. For example, the Jomon Pre-Neolithic hunter-gatherers of Japan are known to have relied on the cultivation and storage of chestnuts and acorns particularly as floral resources became scarce during autumn and winter (Habu, 2004; Matsui & Kanehara, 2006). Similarly, large pottery

vessels presumably used for storing food and liquids were abundant at Con Co Ngua and other Dingsishan-Da But sites (Fu, 2002; Nguyen, 2005; Oxenham et al., 2018).

It has been proposed that the hunter-gatherer diet is dependent on the environmental contexts within which they live or have lived (Kelly, 2013). The abundance and variety of food resources available to Dingsishan-Da But culture communities possibly underlie the reasons for the emergence of large sedentary forager groups in the region. However, Kelly (2013) argues that sedentary foragers can arise when the population density increases to the point that the costs of mobility become too high. Indeed, the rate of natural population increase and fertility rates for Con Co Ngua, at least, suggest a steady growth in population similar to the later Bronze and Iron Ages in Southeast Asia (McFadden & Oxenham, 2018).

In northern Vietnam, seasonality (a dry and wet season) and climatic disruptions such as tropical storms may have resulted in annual fluctuations in food resource returns, and periods of wild resource scarcity (Oxenham, 2006). The management of wild herds may then have provided the necessary level of stability in food resources for the maintenance of these large sedentary forager communities. Certainly, the possibility of wild herd management at Con Co Ngua suggests a strategic response to climatic and social change that parallels the adoption of agriculture further north in the Yangtze and Yellow River valleys, without the necessity of the domestication of plants and animals (Oxenham et al., 2018). The skeletal evidence of hydatid disease presented suggests that zoonotic disease patterns emerged in these sedentary forager groups that mimic that of human relationships with domesticated species.

Conclusion

This paper presents strong skeletal evidence for the presence of the tapeworm *Echinococcus granulosis* responsible for hydatid disease in at least seven individuals from the sedentary forager community Con Co Ngua in northern Vietnam. A total of 13.5% of the assemblage was diagnosed with a possible or probable case of hydatid disease. One old adult male was found with a calcified hydatid cyst confirming the existence of the disease in this forager community. This individual also exhibited the osteolytic lesions strongly diagnostic for the disease. We argue that alongside distinct evidence for accidental trauma, and high frequencies of bovids and cervids in the faunal assemblage, that the Con Co Ngua foragers were likely managing wild animals, maintaining a contiguous zoonotic relationship with managed herds and wild canids through sharing contaminated water sources. This

relationship led to the unusually high burden of skeletal hydatid disease in the human inhabitants. The nature of the relationship between these foragers and wild herds further adds to the complexity that characterizes the forager-farming continuum in mainland Southeast Asia.

Data Availability Statement

Data presented in article

References

Agarwal, S., Shah, A., Kadhi, S., & Rooney, R. J. (1992). Hydatid Bone Disease of the Pelvis. A Report of Two Cases and Review of the Literature. *Clinical orthopaedics and related research*(280), 251-255.

Antikas, T., & Wynn-Antikas, L. (2016). Hydatidosis of a Pregnant Woman of the 3rd Century BC, Greece. *International Journal of Osteoarchaeology, 26*(5), 920-924.

Appleby, J., Thomas, R., & Buikstra, J. (2015). Increasing Confidence in Paleopathological Diagnosis–Application of the Istanbul Terminological Framework. *International Journal of Paleopathology, 8*, 19-21.

Armelagos, G. J., & Cohen, M. N. (1984). *Paleopathology at the Origins of Agriculture*: Academic Press Orlando (FL).

Armelagos, G. J., Goodman, A. H., & Jacobs, K. H. (1991). The Origins of Agriculture: Population Growth during a Period of Declining Health. *Population and Environment, 13*(1), 9-22.

Barker, G., & Richards, M. B. (2013). Foraging–Farming Transitions in Island Southeast Asia. *Journal of Archaeological Method and Theory, 20*(2), 256-280.

Beggs, I. (1985). The Radiology of Hydatid Disease. *American Journal of Roentgenology, 145*(3), 639-648.

Bellwood, P., & Oxenham, M. F. (2008). The Expansions of Farming Societies and the Role of the Neolithic Demographic Transition. In J. Bocquet-Appel & O. Bar-Yosef (Eds.), *The Neolithic Demographic Transition and its Consequences* (pp. 13-34): Springer Science.

Brooks, S., & Suchey, J. M. (1990). Skeletal Age Determination Based on the Os Pubis: A Comparison of the Acsádi-Nemeskéri and Suchey-Brooks Methods. *Human Evolution, 5*(3), 227-238. doi:DOI: 10.1007/BF02437238

Brunetti, E., Kern, P., & Vuitton, D. A. (2010). Expert Consensus for the Diagnosis and Treatment of Cystic and Alveolar Echinococcosis in Humans. *Acta tropica, 114*(1), 1-16.

Buckley, H., Oxenham, M. F., Domett, K., Willis, A., & Hiep, T. H. (2018). *Osteolytic Metaphyseal Lesions and a Calcified Cyst in a Pre-Farming Skeletal Assemblage from Northern Vietnam*. Paper presented at the 22nd European Meeting of the Paleopathology Association, Zagreb, Croatia.

Bui, V. (1980). *Bao Cao Khai Quat, Di Tich Van Hoa Da But, Dia Diem Khao Co Hoc: Con Co Ngua (Thanh-Hoa). [Cultural Vestiges at Da But Archaeological Site at Con Co Ngua (Thanh Hoa)]. Unpublished Excavation Report.* Institute of Archaeology Hanoi, Vietnam. (in Vietnamese).

Buikstra, J. E., & Ubelaker, D. H. (1994). *Standards for Data Collection from Human Skeletal Remains: Proceedings of a Seminar at the Field Museum of Natural History, Arkansas Archaeological Survey Research Series No. 44.* Fayetteville, USA: Arkansas Archaeological Survey.

Calleja, Á. M. M., Sarkic, N., López, J. H., Antunes, W. D., Pereira, M. F., de Matos, A. P. A., & Santos, A. L. (2017). A Possible Echinococcus granulosus Calcified Cyst Found in a Medieval Adult Female from the Churchyard of Santo Domingo de Silos (Prádena del Rincón, Madrid, Spain). *International Journal of Paleopathology, 16*, 5-13.

Capanna, R., Bettelli, G., Biagini, R., Ruggieri, P., Bertoni, F., & Campanacci, M. (1985). Aneurysmal cysts of long bones. *Italian journal of orthopaedics and traumatology, 11*(4), 409-417.

Chan, Y., & Woo, K. (1990). Penicillium marneffei Osteomyelitis. *The Journal of Bone and Joint Surgery. British Volume, 72*(3), 500-503.

Chierakul, W., Winothai, W., Wattanawaitunechai, C., Wuthiekanun, V., Rugtaengan, T., Rattanalertnavee, J., . . . White, N. J. (2005). Melioidosis in 6 Tsunami Survivors in Southern Thailand. *Clinical Infectious Diseases, 41*(7), 982-990.

Chong, V., & Fan, Y. (1996). The Radiology of Melioidosis. *Australasian Radiology, 40*(3), 244-249.

Clarkson, C., Jacobs, Z., Marwick, B., Fullagar, R., Wallis, L., Smith, M., . . . Carah, X. (2017). Human Occupation of Northern Australia by 65,000 Years Ago. *Nature, 547*(7663), 306-310.

Cohen, M. N., & Crane-Kramer, G. M. M. (2007). *Ancient Health: Skeletal Indicators of Agricultural and Economic Intensification*. Gainesville, USA: University Press of Florida.

Cramp, L. J., Ethier, J., Urem-Kotsou, D., Bonsall, C., Borić, D., Boroneanţ, A., . . . Whelton, H. L. (2019). Regional Diversity in Subsistence among Early Farmers in Southeast Europe revealed by Archaeological Organic Residues. *Proceedings of the Royal Society B, 286*(1894), 20182347.

Czermak, B. V., Unsinn, K. M., Gotwald, T., Waldenberger, P., Freund, M. C., Bale, R. J., . . . Jaschke, W. R. (2001). Echinococcus multilocularis Revisited. *American Journal of Roentgenology, 176*(5), 1207-1212.

Deplazes, P., Rinaldi, L., Rojas, C. A., Torgerson, P., Harandi, M., Romig, T., . . . Cringoli, G. (2017). Global Distribution of Alveolar and Cystic Echinococcosis. In *Advances in Parasitology* (Vol. 95, pp. 315-493): Elsevier.

Dervenis, C., Delis, S., Avgerinos, C., Madariaga, J., & Milicevic, M. (2005). Changing Concepts in the Management of Liver Hydatid Disease. *Journal of Gastrointestinal Surgery, 9*(6), 869-877.

Eslami, A., & Hosseini, S. H. (1998). Echinococcus granulosus Infection of Farm Dogs of Iran. *Parasitology Research, 84*(3), 205-207.

Flemming, D. J., & Murphey, M. D. (2000). *Enchondroma and Chondrosarcoma.* Paper presented at the Seminars in Musculoskeletal Radiology.

Fornaciari, A., Gaeta, R., Cavallini, L., Aringhieri, G., Ishak, R., Bruschi, F., & Giuffra, V. (2020). A 13th-Century Cystic Echinococcosis from the Cemetery of the Monastery of Badia Pozzeveri (Lucca, Italy). *International Journal of Paleopathology, 31*, 79-88.

Fu, X. (2002). The Dingsishan Site and the Prehistory of Guangxi, South China. *Bulletin of the Indo-Pacific Prehistory Association, 22*, 63-72.

Gemmell, M. A. (1990). Australasian contributions to an understanding of the epidemiology and control of hydatid disease caused by Echinococcus granulosus—past, present and future. *International journal for parasitology, 20*(4), 431-456.

Grauer, A. L. (2019). Circulatory, Reticuloendothelial, and Hematopoietic Disorders. In J. Buikstra (Ed.), *Ortner's Identification of Pathological Conditions in Human Skeletal Remains* (pp. 491-529): Elsevier.

Gregg, S. A. (1988). *Foragers and Farmers: Population Interaction and Agricultural Expansion in Prehistoric Europe*. Chicago, USA: University of Chicago Press.

Guedes, J. d. A., Hanson, S., Lertcharnrit, T., Weiss, A. D., Pigott, V. C., Higham, C. F., . . . Weber, S. A. (2020). Three Thousand Years of Farming Strategies in Central Thailand. *Antiquity, 94*(376), 966-982.

Habu, J. (2004). *Ancient Jomon of Japan* (Vol. 4). Cambridge, UK: Cambridge University Press.

Higham, C., Guangmao, X., & Qiang, L. (2011). The Prehistory of a Friction Zone: First Farmers and Hunters-Gatherers in Southeast Asia. *Antiquity, 85*(328), 529-543.

Hsiao-chun, H. (2019). Prosperity and Complexity Without Farming: The South China Coast, c. 5000–3000 BC. *Antiquity, 93*(368), 325-341.

Hudson, M. J. (2017). The historical ecology of colonialism and violence in Hokkaido, Sakhalin, and the Kuril Islands, AD 1200–1900. In J. Habu, P. V. Lape, & J. W. Olsen (Eds.), *Handbook of East and Southeast Asian Archaeology* (pp. 695-706). New York, USA: Springer.

Hudson, T., & Hawkins, I. (1981). Radiological Evaluation of Chondroblastoma. *Radiology, 139*(1), 1-10.

Jaffe, H. L. (1972). *Metabolic, Degenerative, and Inflammatory Diseases of Bones and Joints*. London: Lea and Febiger.

Jones, R. K. (2017). *Transitions to Animal Domestication in Southeast Asia: Zooarchaeological Analysis of Cồn Cổ Ngựa and Mán Bạc, Vietnam.* (PhD). Australian National University, Canberra, Australia.

Jones, R. K., Piper, P. J., Groves, C. P., Anh, T. N., Thi, M. H. N., Thị, H. N., . . . Oxenham, M. F. (2019). Shifting Subsistence Patterns from the Terminal Pleistocene to Late Holocene: A Regional Southeast Asian Analysis. *Quaternary International, 529*, 47-56.

Jones, R. K., Piper, P. J., Wood, R., Nguyen, A. T., & Oxenham, M. F. (2019). The Neolithic Transition in Vietnam: Assessing Evidence for Early Pig Management and Domesticated Dog. *Journal of Archaeological Science: Reports, 28*, 102042.

Junker, L. L., & Smith, L. M. (2017). Farmer and Forager Interactions in Southeast Asia. In J. Habu, P. V. Lape, & J. W. Olsen (Eds.), *Handbook of East and Southeast Asian Archaeology* (pp. 619-632). New York: Springer.

Kakarla, U. K., Kalani, M. Y. S., Sharma, G. K., Sonntag, V. K., & Theodore, N. (2011). Surgical Management of Coccidioidomycosis of the Spine. *Journal of Neurosurgery: Spine, 15*(4), 441-446.

Kalinova, K., Proichev, V., Stefanova, P., Tokmakova, K., & Poriazova, E. (2005). Hydatid Bone Disease: A Case Report and Review of the Literature. *Journal of Orthopaedic Surgery, 13*(3), 323-325.

Kelly, R. L. (2013). Cambridge: Cambridge University Press.

Komar, D., & Buikstra, J. E. (2003). Differential Diagnosis of a Prehistoric Biological Object from the Koster (Illinois) Site. *International Journal of Osteoarchaeology, 13*(3), 157-164.

Kumar, R., Madewell, J. E., Lindell, M. M., & Swischuk, L. E. (1990). Fibrous Lesions of Bones. *Radiographics, 10*(2), 237-256.

Larsen, C. S. (1995). Biological Changes in Human Populations with Agriculture. *Annual Review of Anthropology, 24*(1), 185-213.

Lehovsky, J. (1999). Pyogenic Vertebral Osteomyelitis/Disc Infection. *Best Practice & Research Clinical Rheumatology, 13*(1), 59-75.

Marques, C. (2019). Tumors of Bone. In J. Buikstra (Ed.), *Ortner's Identification of Pathological Conditions in Human Skeletal Remains* (pp. 639-717): Elsevier.

Matsui, A., & Kanehara, M. (2006). The Question of Prehistoric Plant Husbandry during the Jomon Period in Japan. *World Archaeology, 38*(2), 259-273.

Matsumura, H., Hung, H.-c., Higham, C., Zhang, C., Yamagata, M., Nguyen, L. C., . . . Oktaviana, A. A. (2019). Craniometrics Reveal "Two Layers" of Prehistoric Human Dispersal in Eastern Eurasia. *Scientific Reports, 9*(1), 1451.

Matsumura, H., Hung, H.-c., Zhen, L., & Shinoda, K. i. (2017). *Bio-anthropological Studies of Early Holocene Hunter-Gatherer Sites at Huiyaotian and Liyupo in Guangxi, China*. Tokyo, Japan: National Museum of Nature and Science Tokyo.

Matsumura, H., & Oxenham, M. (2014). Demographic Transitions and Migration in Prehistoric East/Southeast Asia Through the Lens of Nonmetric Dental Traits. *American Journal of Physical Anthropology, 155*(1), 45-65. doi:DOI: 10.1002/ajpa.22537

Matsumura, H., Willis, A., Hung, H.-c., Nguyen, K. T. K., Li, Z., Hiep, T. H., & Oxenham, M. F. (2018). *Dismemberment and Post-Mortem Body Manipulation: Early-Mid Holocene Forager Mortuary Practices in Southern China and Southeast Asia*. Paper presented at the 21st Congress of the Indo-Pacific Prehistory Association, Hue, Vietnam.

McFadden, C., & Oxenham, M. F. (2018). Rate of Natural Population Increase as a Paleodemographic Measure of Growth. *Journal of Archaeological Science: Reports, 19*, 352-356.

McKinley, J. (2004). Compiling a Skeletal Inventory: Disarticulated and Co-Mingled Remains. In M. Brickley & J. McKinley (Eds.), *Guidelines to the Standards for Recording Human Remains* (Vol. IfA Paper no.7). Reading: BABAO/ Institute of Field Archaeologists.

Merkle, E., Kramme, E., Vogel, J., Krämer, S., Schulte, M., Usadel, S., . . . Brambs, H.-J. (1997). Bone and Soft Tissue Manifestations of Alveolar Echinococcosis. *Skeletal Radiology, 26*(5), 289-292.

Moorrees, C. F., Fanning, E. A., & Hunt, E. E. (1963). Formation and Resorption of Three Deciduous Teeth in Children. *American Journal of Physical Anthropology, 21*(2), 205-213.

Nguyen, V. (2005). The Da But Culture: Evidence for Cultural Development in Vietnam during the Middle Holocene. *Bulletin of the Indo-Pacific Prehistory Association, 25*, 89-94.

Norman, K., Inglis, J., Clarkson, C., Faith, J. T., Shulmeister, J., & Harris, D. (2018). An Early Colonisation Pathway into Northwest Australia 70-60,000 Years Ago. *Quaternary Science Reviews, 180*, 229-239.

Ortner, D. J. (2003). *Identification of Pathological Conditions in Human Skeletal Remains* (2nd ed.). San Diego, USA: Academic Press.

Oxenham, M. F. (2000). *Health and Behaviour: During the Mid-Holocene and Metal Period of Northern Viet Nam.* Northern Territory University,

Oxenham, M. F. (2006). Biological Responses to Change in Prehistoric Viet Nam. *Asian Perspectives, 45*(2), 212-239.

Oxenham, M. F. (2015). Mainland Southeast Asia: Towards a New Theoretical Approach. *Antiquity, 89*(347), 1221-1223.

Oxenham, M. F. (2016). *Bioarchaeology of Ancient Northern Vietnam* (Vol. 2781): Archaeopress.

Oxenham, M. F., & Buckley, H. (2016). The Population History of Mainland and Island Southeast Asia. In M. F. Oxenham & H. Buckley (Eds.), *The Routledge Handbook of Bioarchaeology in Southeast Asia and the Pacific.* (pp. 9-23). London: Routledge.

Oxenham, M. F., Matsumura, H., & Kim Dung, N. (2011). *Man Bac: The Excavation of a Neolithic Site in Northern Vietnam The Biology, Terra Australis 33*. Canberra, Australia: ANU ePress.

Oxenham, M. F., Piper, P. J., Bellwood, P., Bui, C. H., Nguyen, K. T. K., Nguyen, Q. M., . . . Sarjeant, C. (2015). Emergence and Diversification of the Neolithic in Southern Vietnam: Insights from Coastal Rach Nui. *The Journal of Island and Coastal Archaeology, 10*(3), 309-338.

Oxenham, M. F., Thuy, N. K., & Cuong, N. L. (2005). Skeletal Evidence for the Emergence of Infectious Disease in Bronze and Iron age Northern Vietnam. *American Journal of Physical Anthropology, 126*(4), 359-376. doi:DOI: 10.1002/ajpa.20048

Oxenham, M. F., Trinh, H. H., Willis, A., Jones, R. K., Domett, K., Castillo, C., . . . Kells, A. (2018). Between Foraging and Farming: Strategic Responses to the Holocene Thermal Maximum in Southeast Asia. *Antiquity, 92*(364), 940-957. doi:DOI: 10.15184/aqy.2018.69

Pan, Y., Zheng, Y., & Chen, C. (2017). Human Ecology of the Early Neolithic Kuahuqiao Culture in East Asia. In J. Habu, P. V. Lape, & J. W. Olsen (Eds.), *Handbook of East and Southeast Asian Archaeology* (pp. 347-377): Springer.

Phenice, T. W. (1969). A Newly Developed Visual Method of Sexing the Os Pubis. *American Journal of Physical Anthropology, 30*(2), 297-301.

Pourbagher, A., Pourbagher, M. A., Savas, L., Turunc, T., Demiroglu, Y. Z., Erol, I., & Yalcintas, D. (2006). Epidemiologic, Clinical, and Imaging Findings in Brucellosis Patients with Osteoarticular Involvement. *American Journal of Roentgenology, 187*(4), 873-880.

Qiu, Y., Zhang, J., Liu, G., Zhong, X., Deng, J., He, Z., & Jing, B. (2015). Retrospective Analysis of 14 Cases of Disseminated Penicillium marneffei Infection with Osteolytic Lesions. *BMC Infectious Diseases, 15*(1), 1-7.

Rausch, R. L., & D'Alessandro, A. (1999). Histogenesis in the Metacestode of Echinococcus vogeli and Mechanism of Pathogenesis in Polycystic Hydatid Disease. *Faculty Publications from the Harold W. Manter Laboratory of Parasitology*, 400-418.

Renssen, H., Seppä, H., Crosta, X., Goosse, H., & Roche, D. M. (2012). Global Characterization of the Holocene Thermal Maximum. *Quaternary Science Reviews, 48*, 7-19.

Rizzo, M., Dellaero, D. T., Harrelson, J. M., & Scully, S. P. (1999). Juxtaphyseal aneurysmal bone cysts. *Clinical Orthopaedics and Related Research®, 364*, 205-212.

Roberts, C. A. (2015). Old World Tuberculosis: Evidence from Human Remains with a Review of Current Research and Future Prospects. *Tuberculosis, 95*, S117-S121.

Roberts, P., Gillingwater, T. H., Lahr, M. M., Lee-Thorp, J., MacCallum, M., Petraglia, M., . . . Wainnya-Laeto, U. (2018). Historical Tropical Forest Reliance Amongst the Wanniyalaeto (Vedda) of Sri Lanka: An Isotopic Perspective. *Human Ecology, 46*(3), 435-444.

Rothschild, B., & Surmik, D. (2020). Echinococcal Hydatid Cysts in a Pleistocene Camel. *Historical Biology*, 1-5.

Schaefer, M., Black, S. M., & Scheuer, L. (2009). *Juvenile Osteology: A Laboratory and Field Manual*: Elsevier, Academic Press.

Scott, R. M., Buckley, H. R., Domett, K., Tromp, M., Trinh, H. H., Willis, A., . . . Oxenham, M. F. (2019). Domestication and Large Animal Interactions: Skeletal Trauma in Northern Vietnam during the Hunter-gatherer Da But Period. *PLoS One, 14*(9).

Sheridan, L. (2009). Important helminth endoparasites of dogs and cats. *Veterinary Nursing Journal, 24*(9), 23-26.

Snoddy, A. M. E., Buckley, H. R., Elliott, G. E., Standen, V. G., Arriaza, B. T., & Halcrow, S. E. (2018). Macroscopic Features of Scurvy in Human Skeletal Remains: A Literature Synthesis and Diagnostic Guide. *American Journal of Physical Anthropology, 167*(4), 876-895. doi:DOI: 10.1002/ajpa.23699

Song, X., Ding, L., & Wen, H. (2007). Bone Hydatid Disease. *Postgraduate Medical Journal, 83*(982), 536-542.

Tao, W., Huijun, W., & Dabang, J. (2010). Mid-Holocene East Asian summer climate as simulated by the PMIP2 models. *Palaeogeography, Palaeoclimatology, Palaeoecology, 288*(1-4), 93-102.

Tayles, N., & Buckley, H. R. (2004). Leprosy and Tuberculosis in Iron Age Southeast Asia? *American Journal of Physical Anthropology, 125*(3), 239-256. doi:DOI: 10.1002/ajpa.10378

Torricelli, P., Martinelli, C., Biagini, R., Ruggieri, P., & De Cristofaro, R. (1990). Radiographic and computed tomographic findings in hydatid disease of bone. *Skeletal Radiology, 19*(6), 435-439.

Vatankhah, A., Halász, J., Piurkó, V., Barbai, T., Rásó, E., & Tímár, J. (2015). Characterization of the Inflammatory Cell Infiltrate and Expression of Costimulatory Molecules in Chronic Echinococcus granulosus Infection of the Human Liver. *BMC Infectious Diseases, 15*(1), 530.

Vergel De Dios, A. M., Bond, J. R., Shives, T. C., McLeod, R. A., & Unni, K. K. (1992). Aneurysmal bone cyst. A clinicopathologic study of 238 cases. *Cancer, 69*(12), 2921-2931.

Vlok, M., Buckley, H. R., Miszkiewicz, J. J., Walker, M., Domett, K., Matsumura, H., . . . Oxenham, M. F. (2021). Forager and Farmer Evolutionary Adaptations to Malaria evidenced by 7000 Years of Thalassemia in Southeast Asia. *Scientific Reports, 11*, 5677.

Vlok, M., Myagmar, E., Byambadorj, B., & Buckley, H. R. (in press). Pott's Disease in a Liao Dynasty (10th to 12th Century) adult from Mongolia. *Bioarchaeology International*.

Vlok, M., Oxenham, M. F., Domett, K., Tran, M. T., Mai Huong, N. T., Matsumura, H., . . . Buckley, H. R. (2020). Two Probable Cases of Infection with *Treponema pallidum* during the Neolithic Period in Northern Vietnam (ca. 2000-1500B.C.). *Bioarchaeology International, 4*(1), 15-39.

Walrath, D. E., Turner, P., & Bruzek, J. (2004). Reliability Test of the Visual Assessment of Cranial Traits for Sex Determination. *American Journal of Physical Anthropology, 125*(2), 132-137.

Waters-Rist, A. L., Faccia, K., Lieverse, A., Bazaliiskii, V. I., Katzenberg, M. A., & Losey, R. J. (2014). Multicomponent Analyses of a Hydatid Cyst from an Early Neolithic Hunter–Fisher–Gatherer from Lake Baikal, Siberia. *Journal of Archaeological Science, 50*, 51-62.

Williams, J. A. (1985). Evidence of Hydatid Disease in a Plains Woodland Burial. *Plains Anthropologist, 30*(107), 25-28.

Zhang, C., & Hung, H.-C. (2012). Later Hunter-Gatherers in Southern China, 18 000–3000 BC. *Antiquity, 86*(331), 11-29.

Zhao, Z. (2011). New Archaeobotanic Data for the Study of the Origins of Agriculture in China. *Current Anthropology, 52*(S4), S295-S306.

Zinsstag, J., Schelling, E., Waltner-Toews, D., & Tanner, M. (2011). From "one medicine" to "one health" and systemic approaches to health and well-being. *Preventive Veterinary Medicine, 101*(3-4), 148-156.

Zlitni, M., Ezzaouia, K., Lebib, H., Karray, M., Kooli, M., & Mestiri, M. (2001). Hydatid Cyst of Bone: Diagnosis and Treatment. *World Journal of Surgery, 25*(1), 75-82.