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Michelle Martin

The University of Western Ontario

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PALEOPATHOLOGICAL METHODOLOGY: MACROSCOPIC ANALYSIS FAILS TO MAKE THE GRADE

Michelle Martin

INTRODUCTION

Why do some people survive into adulthood and produce offspring while others die before reaching sexual maturity or are not able to produce offspring?

Many mechanisms influence adult survival and reproductivity, the most important being the environment and genetic predisposition. However, these mechanisms have no predictable reaction and tend to act together purely by chance. Through natural selection processes researchers have determined that genetic factors play a major role in both the way that humans respond to disease through immune mechanisms and in how humans adapt throughout history. Simultaneously, cultural mechanisms interact to either minimize the effects of disease or to continue those effects. Therefore, understanding the human cultural and biological responses to disease through time and space provides important data on the historical theoretical frameworks of human evolution and adaptation.

The study of paleopathology as a discipline looks to reconstruct past human behaviours by understanding disease and its impact on human populations. The initial discourse of paleopathology was descriptive, focusing on the identification of disease in antiquity and the presence of disease in one

human population and/or geographical region as compared to others (i.e. the origins of syphilis). These research questions only continue to pose further questions about disease transmission that have not been answered today. As the paleopathological literature becomes more abundant, researchers are moving toward questions about the evolutionary mechanisms involved in the relationship between humans and these disease agents. The focus is to reconstruct the impact of disease on past human populations and how this knowledge can explain modern health and disease issues.

Studying paleopathology is paramount in the attempt to reconstruct the impact of disease on past human populations; however, population samples of human remains are not representative of the living. Researchers should be careful when interpreting data and making references to living populations from past populations, as a significant difference might be evident. When using archaeological material to make inferences about disease within past populations the data obtained will contain sources of error and bias. For example, methodology used in paleopathology often only involves macroscopic analysis of lesions. Each observer identifies the pathology independently, thus interobserver and intraobserver error are common in biological anthropology.

Several theoretical issues arise in paleopathology, specifically the evolutionary mechanisms involved between humans and their response to disease, the scientific nature of paleopathology, the diagnostic limitations of paleopathological research models and the inferences that are currently being used to determine disease processes in the past (Ortner 1991).

Evidently many factors contribute to disease found in human populations, including the evolution of pathogens. It is

possible that pathogens found a viable host purely by accident and a human host was the last evolutionary host on a grand scale after the advent of domestication of animals (Aufderheide and Rodriguez-Martin 1998). The only evidence of past disease is its manifestation in skeletal material found within the archaeological record; however, skeletal lesions do not always tell the true story of how that individual lived and died. The absence of evidence in skeletal material does not necessarily indicate the absence of disease, but suggests one of three possibilities: the person died before the disease began to cause abnormalities to the skeleton; the person contracted a disease that does not leave skeletal lesions; or, the person's immune response may have controlled the disease before skeletal abnormalities could ensue (Wood *et al.* 1992). However, determining if any of these issues were factors of disease in the past is almost impossible.

Paleopathological methodology continues to evolve through the use of paleohistopathology and biomolecular analysis, which may identify disease in past populations that is not observable in bone. This brings us to the scientific nature of paleopathology. According to Ortner (2005:110) "...paleopathology is a reconstructive rather than an experimental scientific discipline", in other words, lesions on archaeological skeletal material allow for inferences to be made regarding the impact of disease on past populations. Because paleopathologists obtain their evidence from macroscopic lesions located on the skeleton they can only speculate as to the nature of the disease based on clinical knowledge of the pathogenesis of that particular disease. Lesions may actually represent another disease as some diseases have similar pathogenesis (i.e. treponematoses) leading to a misdiagnosis (Aufderheide and Rodriguez-Martin 1998). Therefore, clinical research of

infectious diseases needs to continue to focus on providing new information about etiology and pathogenesis of diseases that do cause manifestations in the skeleton. New findings will enable paleopathologists to produce more accurate and consistent diagnoses of disease in the past and to provide valid reconstructions of past human populations. According to Ortner (1991), the major cause of death in human populations throughout most of the Holocene was a result of infection, even though the skeletal evidence of infectious disease may be relatively uncommon.

STANDARD METHODOLOGY

Paleopathologists are ultimately looking at skeletal material which provides evidence of abnormalities that could be disease related. According to Ortner (2005:45) "...abnormal bone formation is always the result of an antemortem pathological process." However, these processes are not always evidence of infectious disease (i.e. trauma, vitamin deficiency or cultural or biological factors). Once a paleopathologist determines an abnormality is from a disease process this becomes the basis for their reconstruction of disease in a past human populations. Paleopathologists look for disease manifestations that affect groups of bones, specific bones, and lesions located at specific areas on bone because disease processes often have a set way of progressing (Aufderheide and Rodriguez-Martin 1998; Ortner and Putschar 1981). Paleopathologists traditionally use both macroscopic and radiological methods to determine disease prevalence of past human populations. These methods are useful for a preliminary diagnosis but, fail to provide accurate data. Inferences made from these data to modern human populations become not applicable.

How are we able to determine the type of leprosy found in the

bioarchaeological record just by macroscopic and radiological analysis? This is the reason why scholars sometimes choose not to differentiate between lepromatous leprosy and tuberculoid leprosy in their diagnosis of M. leprosy (Aufderheide and Rodriguez-Martin 1998). However, using biomolecular analysis and paleohistopathology, the researcher is able to differentiate between tuberculoid leprosy and lepromatous leprosy through presence, frequency and absence of bacilli in the lesions studied (Aufderheide and Rodriguez-Martin 1998; Schultz 2001). According to Aufderheide and Rodriguez-Martin (1998), tuberculoid leprosy contains rare frequencies of bacilli in the lesions and lepromatous leprosy lesions are 'teeming' with bacilli. The use of fluorescence microscopy or phase-contrast microscopy and scanning-electron microscopy is a new paleohistopathological analysis that is being utilized by palopathologist in Germany and the United States. According to Schultz (2001:120), this new method allows paleopathologist to study "...not only the specimens but also the vestiges and products of the organisms' growth." Studies have been conducted that illuminate the growth traces of actinomycetes, a very small bacterium that grows like fungus (Basset *et al.* 1980; Keith and Armelagos 1982, 1988, 1991; Schultz 2001).

**METHODOLOGY-
 PALEOHISTOPATHOLOGY**

Paleohistopathology provides a more reliable diagnosis of diseases and the relevant prevalence of those diseases because it can substantiate or correct a macroscopic diagnosis. In addition, paleohistopathology provides more accurate age determinations and helps with diagnostic analysis of possible environmental and cultural factors that may affect the skeletal material (i.e. heat and fire occurring around time of death) (Schultz

2001). In the case of small bone fragments, paleohistopathology allows for an accurate diagnosis between faunal and human remains (Schultz 2001).

According to Schultz (2001) scanning-electron microscopy (SEM) is a helpful tool in paleopathology; however, used by itself is an unreliable method because it only analyzes the surface of the bone. Transmission of light microscopy allows the observer to look through the bone tissue. Osteomyelitis, tuberculosis and treponemal diseases can macroscopically be mistaken for mechanisms of diagenesis since both cause alterations to bone that are products of non inflammatory periosteal reactions. For example, postmortem fungi growth is a diagenetic mechanism that often happens to archaeological remains usually causing relatively large destructive holes (Schultz 2001). These holes are often mistaken as osteoporosis or vestiges of a metastizing tumor. Thickened bone on the external surface can be easily mistaken as a periosteal reaction, either periostitis or osteolytic (Aufderheide and Rodriguez-Martin 1998). In addition, when bone is examined macroscopically, snake-like irregular grooves on the external skull vault can indicate osteomyelitis or treponemal diseases (Aufderheide and Rodriguez-Martin 1998). However, when microscopic analysis is conducted the diagnosis can be substantiated by excluding postmortem changes due to soil and water mechanisms (Schultz 2001). Microscopic analysis allows the researcher the ability to determine the paleopathology observed in archaeological remains and also provides a reliable diagnosis specifically as it pertains to the role that infectious diseases played within past populations. For example, sometimes parasites that affect the living may play a role in affecting skeletal remains. Flora and fauna found within the soil may be penetrating the skeletal remains causing

markers of paleopathology (Schultz 2001). Therefore, the microscopic identification of these flora and fauna specimens helps in the differential diagnosis process of skeletal remains.

According to Schultz (2001) periosteal reactions are found on the surface of ancient long bones and can be analyzed microscopically. The range of diseases that could be responsible for these newly built bone formations include osteomyelitis, tuberculosis, leprosy and treponemal. Each disease has an individual characteristic microscopic marker that is evident to the trained observer.

Osteomyelitis at the macroscopic level indicates periosteal reactions that entail inflammatory processes that affect both the compact bone and medullary cavity (Aufderheide and Rodriguez-Martin 1998). In osteomyelitis this process starts from the medullary cavity and continues to the periosteum. According to Schultz (2001:126), "...when bone is affected by inflammatory process, its character will be altered macroscopically and radiologically becoming sclerotic but it will also start remodeling and thus typical morphological features can be observed at the micro level."

According to Schultz (2001), polsters are characteristic features that are found at the microscopic level in chronic treponemal diseases. Polster-like structures that are rudimentarily developed and relatively flat can also be observed in leprosy, whereas polsters are never found in osteomyelitis of the long bones (Schultz 2001). In addition, leprosy and treponemal diseases display resorptive holes and corroded structures (i.e. incomplete trabeculae), as well as extensive remodeling processes but these microscopic diagnostic features are not found to this extent in osteomyelitis (Schultz 2001).

Schultz (2001) compares a two to three year old child, from burial 246 of the

Ikiztepe cemetery that suffered from anemia with a six to twelve month old infant, from burial 78 of the same cemetery that suffered from osteomyelitis of the skull vault. Both display similar porotic changes macroscopically; however, when compared microscopically the surface of the infant from burial 78 is porotic due to inflammatory process (i.e. osteomyelitis).

Similarly Jackes and colleagues (2001) skeletal material from Muge, Portugal display alteration to bone as a result of bacterial action and is readily visible in the scanning-electron microscope (SEM). If skeletal lesions are located on the surface of remains then paleohistopathology should be applied as a preliminary method to identify the presence of disease and to distinguish which infectious disease is evident. After this procedure the researcher should apply biomolecular methods (i.e. ancient DNA analysis) that furthers the diagnosis and confirms the preliminary results of disease. It seems as though when researchers rely solely on one method for their examinations their results are usually ambiguous and even insufficient for a proper pathological diagnosis. According to Roberts and Manchester (1995), paleohistopathology provides a secondary diagnosis to macroscopic and radiologic observations that is relatively easy to control for the effects of diagenesis.

METHODOLOGY- BIOMOLECULAR ANALYSIS

Differential diagnosis is further complicated because differences exist within observations of macroscopic analysis as compared to that of biomolecular analysis using ancient DNA. In recent years, scholars profess that lesions of the ribs are indicators of tuberculosis (Roberts *et al.* 1994; Kelley and Micozzi 1984; Pfeiffer 1991). However, the study conducted by Mays *et al.* (2002), indicates otherwise. It is a common consensus among the clinical

literature that rib lesions are a result of hematogenous dissemination of tuberculosis through remote soft-tissue foci (Poppel *et al.* 1953; Wolstein *et al.* 1974; Wiebe and Elwood 1991; Asnis and Niegowska 1997).

Kelly and Micozzi (1984) described rib lesions as existing on the visceral surface of the ribs and consisting mainly of periostitis. They found an association between rib lesions and known cases of skeletons diagnosed with tuberculosis at death (i.e. Hamman-Todd collection). It is believed that the lesion visible on the visceral surface arose from sub adjacent infection of the lungs or pleura. Roberts *et al.* (1994) also conducted a study of similar proportions using the Terry collection. In this analysis of 255 individuals that supposedly died of pulmonary tuberculosis, 157 (62%) exhibited visceral surface rib lesions; however, in an analysis conducted by Kelley and Micozzi (1984), the sample of 445 individuals that supposedly died from tuberculosis, only 39 (9%) exhibited rib lesions.

To further exacerbate the problem, more recent evidence seems to substantiate the notion that evidence of rib lesions on skeletal remains is associated with diagnosed tuberculosis. Santos and Roberts (2001) conducted a study observing rib lesions on juveniles from a 19th century skeletal collection from Portugal that were diagnosed with pulmonary tuberculosis at death. They found that of the 11 known tuberculosis deaths, 10 individuals displayed visceral surface rib lesions.

However, according to Mays *et al.* (2002), the use of ancient DNA of *Mycobacterium tuberculosis* demonstrates that macroscopic diagnosis of tuberculosis and biomolecular diagnosis are at odds with each other. Using a medieval skeleton collection from Wharram Percy, a deserted medieval village, 687 skeletons were excavated of which 360 are adult and 322

are juvenile; Mays *et al.* (2002) applied ancient DNA analysis to determine if macroscopic diagnosis was accurate. If tuberculosis was associated with macroscopic rib lesions, then those skeletons with rib lesions would have a higher frequency of PCR-positives for tuberculosis. Mays and colleagues (2002) examined all specimens from the Wharram, Percy assemblage and only seven individuals exhibited pathological changes to the visceral surface of the ribs. The results showed that only one of the pathological rib samples, from burial G658 proved PCR-positive for tuberculosis. In addition, two of the control samples tested PCR-positive for tuberculosis, however exhibited no macroscopic evidence of rib lesions. Therefore, only one out of seven skeletons that showed macroscopic rib lesions tested positive for tuberculosis.

According to Mays *et al.* (2002), although it cannot be completely excluded, contamination and poor preservation are highly unlikely causes of the negative results because a previous study (Mays *et al.*, 2001) indicates that the *M. tuberculosis* complex DNA survives very well in diseased bone under the burial conditions associated with Wharram Percy. However, they do suggest that these individuals may not have been infected by inhalation but rather by *M. bovis* which would not indicate PCR-positives for *M. tuberculosis*. *M. bovis* is transmitted by animal to human contact. Mays and colleagues (2002) dismiss *M. bovis* as a possibility because at Wharram Percy there is no evidence for anything other than *M. tuberculosis*; however, lack of evidence does not indicate absence of disease. Instead, it indicates the possibility that people infected with *M. bovis* may develop different pathological conditions at different time intervals that may or may not be displayed on skeletal remains, which is dependent on time of death and degree or severity of

symptoms experienced by the disease (Wood *et al.* 1992).

One interesting finding is that one of the seven skeletons that exhibited rib lesions (G658) also displayed osteological lesions throughout the post cranial and cranial skeleton, as well as evidence of resorption of the inferior costal margins of the ribs. This skeleton displayed periosteal reaction of many long bones including the left femur, the tibia and the fibula as well as to the main vertebral bodies that display multiple circumferential resorptions on the anterior and lateral surfaces. Skeleton G658 also displayed irregular resorption lesions on the distal metaphysis of the left femur and proximal metaphysis of the left tibia underlying the epiphyseal plate.

When costal lesions alone are present, differential diagnosis is problematic as adequate paleopathological criteria do not at present exist to allow us to distinguish on the basis of the bony pathology present, the various conditions, pneumonia, bronchitis, emphysema, pleurisy (Roberts and Lewis 1994) and actinomycosis (Molto 1990) which may be associated with visceral surface rib lesions indicating that not always do the observations of such lesions represent a tubercular infection. These results should highlight the need to obtain or examine as much of the skeleton as possible and that osteological study should be combined with biomolecular observations in order to provide the best diagnosis in each case.

According to Santos and Roberts (2001) it is not uncommon to observe periosteal reaction on the long bones of juveniles known to exhibit early tuberculosis lesions. Baker (1999) also suggests that the appearance of visceral rib lesions in conjunction with vertebral body changes is representative of early stage skeletal tuberculosis.

Mays and colleagues (2002) observation of both vertebral and cranial

changes may be manifestations of tuberculosis, although it could also be a coincidental finding. The two control specimens EE002 and EE005 that tested PCR-positive for tuberculosis displayed no lesions so it is possible that they were exposed to tuberculosis that only affected the soft tissues, but left no macroscopic evidence on the skeleton. According to Mays and colleagues (2002) the most obvious reason that six out of seven cases are PCR-negative for *M. tuberculosis* is because differential diagnosis exists for the pathology observed.

PRESERVATION AND DIAGENESIS

When discussing the possibilities of biomolecular analysis as it pertains to understanding paleopathology in skeletal evidence, one must consider the preservation capabilities of skeletal remains within the archaeological record. The survival of organic matter in bone will surely determine a researcher's ability to provide accurate and valid results. Much has been written and reviewed in the literature as to the mechanisms of preservation and structure of bone that allows survival for biomolecular analysis (Child 1995; Collins *et al.* 1995, 2002; Shipman 1981). Since bone is of organic matter it is susceptible to biomolecular deterioration through bone diagenetic mechanisms (Collins *et al.* 2002).

According to Collins *et al.* (2002) collagen begins degrading during an individual's life and continues its decline upon deposition. Therefore, researchers must not only be aware of this degradation, but must incorporate these discrepancies into the data analysis. It has already been discussed that tuberculosis and leprosy DNA has been found within bone samples from the archaeological record, therefore it is possible that the bacterial DNA of these diseases may also be altered or eliminated completely from the specimen under examination through biodegradation.

According to Collins *et al.* (1995) where bone is exposed to decomposition, through diagenetic mechanisms, collagen slowly undergoes chemical hydrolysis until the chains are so small that over a relatively short archaeological time span they leach out.

The findings from this study demonstrate that using biomolecular analysis can help answer researchers' questions pertaining to bone and the effects of bacteria on that bone. More importantly it emphasizes that methods of analysis need to be more readily combined. According to Child (1995:173) "...histological examination of the source material and experimentally treated bone may confirm preliminary results from both biomolecular and macroscopic analysis." In addition, researchers should not rely solely on biomolecular or ancient DNA analysis. As shown by Burger and colleagues (1999), the preservation of DNA material varies and is most often influenced by diagenetic mechanisms that include temperature, soil pH, humidity and especially the presence of microorganisms. DNA preservation can be largely affected both qualitatively and quantitatively. Burger *et al.* (1999) concluded that ancient DNA could be detected sporadically within the designated samples (i.e. Lichtenstein Cave, Shimal site and Mustang site), but those results are rarely reproducible. The Mustang site, which entailed a large degree of microorganism action, only produced 20% of ancient DNA quantity and failed to produce any percentage of quality of reproducibility. According to Burger *et al.* (1999) microorganisms and their metabolites can completely destroy aDNA. It is only logical that if aDNA can be destroyed than it is possible that aDNA of infectious diseases could also be destroyed by diagenetic mechanisms including microorganisms.

Furthermore, no general conclusions could be made about the amplifiability length of aDNA. According to Burger and colleagues (1999) these findings show that the short tandem repeat (STR) locus longer than 200bp are capable of providing proper amplification of aDNA; however, only those samples that came from good burial conditions (i.e. Lichtenstein cave groups 1-4) can be quantified.

CONCLUSIONS

Even though there have been some ground breaking discoveries in paleopathological methodology, the discipline needs to continue to diagnose disease in the past by actually recording the evidence of those diseases. The new methods (i.e. paleohistopathology and biomolecular analysis) being used by paleopathologists are providing more accurate and valid conclusions than macroscopic analysis alone. However, literature available on the preservation of ancient DNA, including how long it survives in the archaeological record and in what conditions promote its survival, is lacking.

It is important to mention that other biomolecules are potentially preserved better and may provide further diagnosis within paleopathology. Collagen, haemoglobin, albumin, osteocalcin and Human leukocyte antigens (HLA) may provide evidence to the discipline of paleopathology if they are able to successfully survive diagenesis, microorganisms and other burial phenomena (Roberts and Manchester, 1995). For instance, HLA antigens are now known to be associated with specific diseases including the B27 antigen and its association to ankylosing spondylitis.

The Cambridge Encyclopedia of Human Paleopathology is an excellent reference guide to diseases of the past; however it fails to provide adequate photographs and radiographs and does not mention paleohistopathology or

biomolecular methods. Ortner and Putschar (1981) provide a comprehensive recording of photographs and radiographs, but fail to provide a complementary breakdown of disease pathology, pathogenesis and differential diagnosis. Ortner and Putschar (1981) do not mention the methodology of paleohistopathology nor biomolecular analysis.

Currently, there is no standardization of recording pathological lesions. Once researchers approach paleopathological examination with a standard methodology of what and how to record abnormalities, it will be possible to provide accurate recreations of human behaviour in the past. Some attempts have been made to provide recommendations to create a skeletal database (Rose *et al.* 1991) and provide a standard manual of diseases encountered in the skeletal record with photographs of typical pathological lesions. However, they are not nearly comprehensive in nature and consistent reference material is not utilized by all paleopathologists. No actions have been taken to move towards the goal of a standardized skeletal database network. Many other disciplines have similar types of databases (i.e. York System – Faunal Remains and FORDISC – Forensic Identification) which do not always accomplish perfect recording procedures but it is a starting point for the development of a more comprehensive recording system in the future. Providing the means for paleopathologists to move forward toward a common goal of interpreting and understanding the mechanisms involved in pathology of past populations and the consequences for future populations is essential. According to Roberts and Manchester (1995) the movement toward a standardized recording method is of utmost importance because as skeletal material becomes more limited due to acts of repatriation around the world,

paleopathologists will lose the opportunity to examine those skeletal remains in the future.

Paleopathologists need to move past a descriptive and methodological approach to diseases of past populations and consider biocultural mechanisms that help to explain human adaptations to disease within certain environmental contexts. Some people and populations tend to be more susceptible to some diseases and others are more predisposed to the manifestations of those diseases. A comprehensive study of the diseases found within certain environments, including rural as compared to urban centers and the aspects of poverty and its involvement in disease as well as the differences of diseases associated with each gender. “Infective diseases are not solely microbiological entities but are a composite reflection of individual immunity, social environmental and biological interaction.” (Roberts and Manchester, 1995:159)

Multiple factors come into play when interpreting disease including gender, immune response, diet, living conditions, stress, climate, and poverty. These mechanisms need to be fully considered by paleopathologists. Just as any discipline has its limitations so does paleopathology, and it is important to be aware of those shortcomings. Newer methodologies have increased the viability of the discipline in providing other forms of evidence in the reconstructions of the history of disease and its effects on past human behaviours. According to Roberts and Manchester (1995:202, my emphasis) the discipline of paleopathology “...shows how health has changed through time and what we might expect from the future if we have a change in diet, environment, climate or living conditions. It is by its nature a holistic discipline which has direct relevance to future populations.”

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