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«Molecular identification of infectious diseases in ancient human remains»

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The molecular identification of infectious diseases in skeletal and mummified human remains allows a unique insight into the presence, frequency and evolution of pathogens in ancient populations. The analysis of ancient DNA, including extraction, amplification and characterization of specific DNA fragments, made it possible to detect a wide range of infectious diseases in mummies and skeletons from different time periods and geographic origins. This holds particularly true for the identification of the *M. tuberculosis* complex, which seems to be more robust than other microbes due to its waxy, hydrophobic and lipid-rich cell wall. Our analysis of ancient tuberculosis cases provided useful information about the occurrence, but also frequency of this disease in former populations.

The application of modern molecular technologies, such as next-generation sequencing, represents a new approach for the study of infectious diseases in ancient populations. Recently, we carried out a whole-genome analysis of the 5300 years-old Tyrolean Iceman, commonly known as Ötzi. The data analysis revealed evidence for the presence of pathogens related to periodontal disease and sequences corresponding to the genome of *Borrelia burgdorferi* indicating the earliest human case of Lyme disease. This underlines the potential to detect pathogens by using metagenomic analysis on ancient human remains.
Previous investigations carried out in some parts of the 16-17th century AD series of Bácsalmás-Óalmás (southern Hungary) have already provided interesting paleopathological cases of tuberculosis (e.g. Molnár and Pálfi, 1994). These studies were essentially based on macromorphological analysis, biomolecular methods were used only in a few cases (e.g. Haas et al., 2000). From a macromorphological point of view, former investigations have only considered ‘classical’ tuberculosis (TB) alterations (advanced-stage lesions in common skeletal locations). However, due to the recent development of diagnostic criteria in the field of the paleopathology of infectious diseases, new approaches have been introduced in the identification of skeletal TB lesions (Pálfi et al., 1999; Maczel, 2003). Molecular methods for the detection of mycobacterial aDNA have also been developed considerably in recent years (e.g. Donoghue, 2008, 2011). The good state of preservation of the material, the important chronological period of the series and the relative high prevalence of TB in preliminary studies encouraged us to carry out a revision of TB-related lesions in the complete Bácsalmás-Óalmás series. A five year international research program – including both macroscopic and biomolecular studies of the series - was recently started.

The present study summarizes the results of a pilot project conducted to optimize the further systematic paleopathological and paleomicrobial studies. Skeletal material of 205 individuals was chosen for the macromorphological test-investigation, which was focused both on classical/advanced stage skeletal TB alterations (tuberculous spondylitis, tuberculous arthritis) and atypical/early-stage TB lesions (rib lesions, superficial vertebral changes, endocranial alterations, early-stage spondylodiscitis). In addition, the association of possible stress factors (long bone periostitis, cribra orbitalia, cribra cranii) were also considered. Paleomicrobiological analysis was used to study the presence of Mycobacterium tuberculosis ancient DNA (aDNA) in morphologically positive and negative cases. A comparative paleomicrobial analysis was carried out on different samples, to test the presence of MTB DNA in different skeletal regions.

Key words: paleopathology, aDNA, skeletal tuberculosis, Mycobacterium tuberculosis, Hungary

A large series of well documented, naturally mummified individuals came to light at the Dominican Church in Vác, Hungary, in 1994-1995. The coffins contained the remains of 265 individuals. The anthropological material is stored in the Department of Anthropology, Hungarian Natural History Museum, Budapest.

The studies of partially and completely mummified bodies and skeletal remains provided excellent material for the understanding of the origin and evolution of TB, and give an opportunity for the study of the population living in the 18th century, before the appearance of medical therapy and the resistance to antibiotics.

Morphological and radiological studies revealed some cases of vertebral tuberculosis. Skeletal and naturally mummified tissues were examined for the presence of Mycobacterium tuberculosis complex DNA. The research conducted by H.D. Donoghue and M. Spigelman discovered that overall, 67.7% individuals proved positive. The rate was 46.5% in children, 89.7% in middle-age individuals, and 69.6% in people older than 65 years. Investigation of samples of multiple sites revealed 78.5% positive individuals. Most of them had disease in the pulmonary region, 35.6% had disseminated, and 16.4% had extrapulmonary only.


The question of pre-neolithic tuberculosis is still open in paleopathological perspective. One of the major interest is to explore what type of infection could have existed prior to the domestication and at its early stages. Description of paleopathological lesions observed on skeletons coming from sites representing pre and early domestication phases in the geographical cradle of agriculture can help in answering this question.

Paleopathological study of two neolithic individuals from Syria: 1) adult skeleton dating from the second half of the 11th millennium BP calibrated (Early PPNB) of the predomestication neolithic site of Dja’d El-Mugara (Northern Syria), 2) immature skeleton coming from the site Tell Aswad (Southern Syria) dating from 9800-8600 BP calibrated (Middle PPNB).

Laser scanning and microCT scan have been applied. Detection of mycolic acids is in progress; aDNA analyses are planned. Dja’d adult skeleton exhibits lesions that are typical of an infectious spondylodiscitis: the inferior part of the 9th thoracic vertebrae is completely destroyed, the upper plate of the 10th thoracic vertebrae shows lytic rounded cavitations expanding to the vertebral body. Tell Aswad immature individual exhibits plurilamellar periosteal reactions of all the long bones, associated with spina ventosa of the right ulna. This two paleopathological cases strongly suggest the presence of tuberculous infection before domestication as well as in its early stages. Mycolic acids are positive for Dja’d sites. Molecular analyses (aDNA) are planned in order to better explore the past history of human tuberculosis.
In populations, the presence of linkage disequilibrium (LD) implies a non-random association of genes from different locus. As a consequence, some alleles combinations are more frequently observed than expected with a randomized gene association. When two or more populations with different allele frequencies mixed together, we observe a linkage disequilibrium in the recently admixed population. The slow disequilibrium decrease is potentially interesting in mapping genes that are involved in susceptibility to some diseases. Analyzing this LD in parallel with demographic factors (such as population size and demic expansion) would be of interest to better understand the role of these factors in human population responses to infectious diseases.
Understanding how pathogen can become adapted to humans is fundamental in public health to understand (and mitigate) the explosiveness of epidemics. It is now accepted that the first epidemiological "transition" took place 10,000 years ago with the development of agriculture. Today, we observe a new epidemiological "transition" associated to antibiotics resistance, globalization of human transport and biodiversity loss. After developing arguments for the importance of agriculture development in the transfer of pathogen from domestic animals to humans 100 years ago, I show what is the current knowledge on this adaptation process. Through a mathematical modeling, I show how biodiversity can increase the probability of human adaptation. Finally, I discuss on the importance to consider the animal species that cannot transmit the pathogen in public health strategies.
Human tuberculosis, Animal TB, which one existed first? Has this question a sense? Perhaps, if we want to know if there is a "responsible", and where he is to be found, in Man or Animals. TB has been diagnosed independently in Man (H. erectus) as far as 500,000 years and in wild American Ungulates some 18,000 years ago, that is 5,000 years before any human presence on the American continent. Besides, ADN studies show that *Mycobacterium tuberculosis* is much older than previously thought (ca 35-40,000 years BP), and results from multiple horizontal gene transfers (that is hybridizations) between protoTB bacilli. “Modern” human TB appears to be at the origin of (domestic) animal TB, extending the disease to wild species and back to man, after, say, 6,000 years BC.

May archeozoological data help to confirm this hypothesis? The rarity of adequate tissues (essentially bone) conservation and contextual (i.e. animal + human) findings make it very fortuitous any advance in our knowledge.
Animal domestication was certainly a long process, occurred in different areas of the world and concerned a few animal species. The word "domestication" may even represents many situations as, for instance, if the house fly is called *Musca domestica*, or if a large population of the house mouse is called *Mus musculus domesticus*, it is difficult to pretend that they have been domesticated on purpose. However, today, we are all, more or less, living in the same house, under a single roof. So these sanitary consequences will here include impacts of both truly domesticated species as well as commensal or parasites animals. A few examples will be presented.

The oldest domesticated animal species known is the wolf (*Canis lupus*) from which the dog aroused. Two issues can be discussed in connection with domestic dogs. First the importance today of rabies in man, nearly exclusively linked to rabid dog bites and the presence of *Pulex irritans* in human beings, the so called “human flea”.

Cattle have been sharing our lives for a longtime too. In this case, the wild ancestor, the wild ox (*Bos primigenius*) has been lost. Two main human diseases, one viral and one bacterial, have long been associated with them. Measles is seen today as the adaptation of rinderpest virus to human beings during the domestication process. The human disease is still highly prevalent, even in Europe, especially in France nowadays. Ironically, rinderpest has been officially declared as eradicated worldwide in 2012. The relationship between *Mycobacterium tuberculosis* and *M. bovis* is still hotly disputed. The first bacteria is the agent of human tuberculosis when the second one causes bovine tuberculosis.

In parallel with domestication, sedentarisation has been more and more important since early Neolithic times. The development of food stores was a real bonanza for local rodents. It is easy to understand how the house mouse “domesticated itself” in such conditions in the Middle-East. The black rat (*Rattus rattus*) followed a few millennia later. Its importance during human plague epidemics was major. The creation of water collections either as drinking water reservoirs or as wastes certainly had also many consequences on the presence of insects like mosquitoes. Knowing their importance in the transmission of different diseases, it is also possible that this step in human development was also at a sanitary cost. Mosquitoes are not the only ones.

The few examples will just to try to illustrate a few situations in which the known costs of domestication are mainly association with sanitary safety.
No less than in their epidemiologic identity, epidemics are sociologically and historically made of logics and temporalities heterogeneous in their essence: some are explosive (influenza), others repetitive (plague), sometimes even immobile (malaria). In addition, two historical models compete for apprehending and exploring epidemics, a biomedical model and a socio-constructivist one. Such complexities invite more mutual understanding from all the various disciplines that contribute to the study of epidemics.

Epidemics impact on societies in many ways. Ideas, beliefs, behaviors, social structure and institutions are hit by epidemic diseases, sometimes violently. One question is: do epidemics have a lasting effect on societies? Or is their imprint ephemeral? Considering the history of plague, one would be inclined to vote for the former; by contrast, flu would encourage the latter. Another question: must historians sanction contemporary notions and outlook for historical inquiry? Or should they stick to the present day taxonomies, therefore running the risk to impose our own notions on our predecessors?

Mass destruction, mass disorganization: here is the double template of an epidemic crisis currently having the upper hand among many epidemiologists and decision-makers. Thucydides bequeathed us this quintessential notion of an epidemic, adding moral and social havoc to cataclysmic suffering. Modern historians relished narratives of epidemics full of catastrophes and social disintegrations still very recently. Does current historiography validate such a template?

The issue of catastrophe has been clothed in a new language lately, namely the «fall of complexity» idea. Archeologists and historians of ancient societies in particular have drawn this concept from the catastrophe theory. Yet, what we see when considering the history of epidemics may be just the opposite: an increase in complexity. For instance, the Black Death that wiped out 30 to 60 per cent of the European population in the fourteenth century substituted for the medieval economic equilibrium: overpopulation, low wages, high rents, a new equilibrium: under-population, high wages, low rents. Far from a catastrophe, this would represent an «anastrophe», a fast move upwards, an increase in the complexity in the socio-economic domain. Similar move upwards can be cited in the artistic domain (e.g. Dance of Death).

We do not enjoy in the narrative of paroxysms of societal destruction that contented «gothic» epidemiology anymore. Current historiography inclines towards notion of gradual, partial and provisional changes. Former generations of historians liked to quake with fear imagining all the horrors of epidemic cataclysms. We, by contrast, have faith in the triumph of routines.