Part II - Parasite Remains Preserved in Various Materials and Techniques in Microscopy and Molecular Diagnosis

13. Viruses and Paleoparasitology

Hermann G. Schatzmayr

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SCHATZMAYR, H.G. Viruses and Paleoparasitology. In: FERREIRA, L.F., REINHARD, K.J., and ARAÚJO, A., ed. *Foundations of Paleoparasitology* [online]. Rio de Janeiro: Editora FIOCRUZ, 2014, pp. 199-203. ISBN: 978-85-7541-598-6. Available from: doi: [10.7476/9788575415986.0015](https://doi.org/10.7476/9788575415986.0015). Also available in ePUB from: <http://books.scielo.org/id/zngnn/epub/ferreira-9788575415986.epub>.

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Viruses and Paleoparasitology

Hermann G. Schatzmayr

Recent years have witnessed important growth in the field of paleoparasitology, or the study of infectious diseases in ancient humans and animals. A recent review by Nogueira et al. (2006) gives a precise description of the evolution of knowledge in the field, initially using tools from forensic medicine to describe signs of diseases in mummies from various countries. Such studies have recently gained great impetus with the availability of advanced methods for detecting nucleic acids and proteins in organic material (Konomi, Lebowitz & Zhang, 2002). These methodologies have allowed confirming (sometimes definitively) the macroscopic and microscopic data obtained from the study of preserved human tissues.

This development is particularly important when investigating data on viral infections, since viruses are fragile structures consisting of nucleic, ribonucleic, or deoxyribonucleic acid and protein envelopes. Viruses do not display resistant forms such as spores, which occur in various types of bacteria and other microorganisms.

Signs of viral infections have been found in human mortal remains in various parts of the world. This chapter briefly reviews the knowledge from paleoparasitology on several viral diseases.

SMALLPOX

Evidence shows that smallpox existed in Africa since 3,700 BC and in China since 1,100 BC, although it has not been possible to define exactly where the disease first appeared. According to the most widely accepted theory on the origin of smallpox, an animal virus from the Poxvirus group probably adapted specifically to humans.

When smallpox spread across Europe and the Americas along trade routes and through colonization, it became a major public health problem. Smallpox caused death and irreversible skin lesions, especially on the face, in millions of people over the course of the centuries, affecting all segments of the population. In Europe, smallpox killed monarchs, like Louis XV of France in 1775 (he thus escaped the guillotine, unlike his successor Louis XVI, executed by the French Revolution that began four years later).

The Spanish Conquistadores of Mexico used smallpox infection as a powerful biological weapon. The disease did not exist previously in the Americas. When the Europeans arrived, the virus was introduced deliberately into the continent by the English armies, for military purposes. This led to the decimation of indigenous populations in both Mexico and the United States. In Brazil, the same method was used to eliminate indigenous peoples that resisted construction of the Northwest Railway in the State of São Paulo. Due to the eclectic spread of smallpox, mummies of persons of higher social status have been found with viral particles or skin lesions indicative of the infection. During the 20th Egyptian Dynasty, a smallpox epidemic swept across the region. Lesions found on the face of the mummy of Ramses V (1156 BC), indicative of the infection, were one of the first observations of a viral infection in the field of paleoparasitology. Ruffer & Ferguson (1911) first reported these findings in the early 20th century.

The reign of Ramses V coincided with the exodus of the Jewish tribes to Israel, led by Moses. In the Old Testament, Leviticus chapters 13, 14, and 15 refer to skin lesions like pustules (boils), with recommendations for avoiding contagion to others, isolating the sick and requiring priests to examine infected individuals during their clinical course. The data suggest that the epidemic reached those population groups during their journey to Israel.

In the last three decades, studies on diseases in mummies from various regions of Italy have sparked great interest, describing calcification in large blood vessels, fibrotic pulmonary lesions suggestive of tuberculosis infection, diverticulitis, parasitic skin infections, tertiary syphilis, neoplasms, hernias, and other events (Fornaciari, 2006).

Fornaciari & Marchetti (1986) identified a smallpox infection based on macroscopic characteristics, indirect immunofluorescence, and scanning electron microscopy. They detected particles characteristic of the virus in one of these 16th century mummies, a two-year-old child from Naples. Other researchers (Marennikova et al., 1990) confirmed this finding.

Attempts failed to induce infections in the laboratory using original material, thus demonstrating that the virus lost its capacity to infect cells. This finding was important, since some smallpox victims have died and been buried in cold regions of the planet like northern Russia and Alaska, raising the hypothesis that infectious virus could be recovered from these sources, with potential implications for illegal activities such as bioterrorism.

Kieron et al. (2006) later found lesions attributed to smallpox in mummified tissues deposited in the British Museum.

Due to the characteristic lesions of smallpox, nearly always on the patient's face, and the relative resistance of the virus, hopefully more data will be obtained in the future on smallpox infection in preserved human tissues.

INFLUENZA

The influenza virus has caused successive epidemics and epizootics over the centuries, with birds and domestic animals as its main reservoirs. The human disease appears and spreads rapidly, due to mutations and molecular rearrangements in the virus, in its two surface proteins, H (hemagglutinin) and N (neuraminidase), generating virulent strains for humans. These modifications have emerged in Asia and spread elsewhere, as occurred recently with the H5N1 strain in China and even more recently with H1N1. Better knowledge of these mutations is highly important for predicting future epidemics and reducing their impact.

Influenza has characteristic signs and symptoms, a seasonal pattern, and often high case-fatality, thus allowing recognition of clinical descriptions of influenza since remote antiquity. The name suggests a relationship with various types of "influences" that were blamed for the epidemics, such as the position of the stars and similar inferences (Schatzmayr & Corrêa, 2005).

Lesions caused by the influenza virus are not recognized specifically in mummified tissues. However, as mentioned above, research methods for viral nucleic acids have made a major contribution to unlocking the genetic composition of viral samples in human tissues.

The most important contribution of paleoparasitology in influenza involved studies to retrieve the viral genome in patients that died during the great pandemic of 1918-1919.

From September 1918 to April 1919, at least 20 million people died across the globe, the equivalent of nearly 4% of the world population at the time. In Rio de Janeiro alone, influenza killed 15 thousand people in only a few months, including President Rodrigues Alves. People initially believed that the virus showed such high case fatality because it found a population debilitated by World War I, without adequate hospitals or medicines. However, mortality was equally high in the United States and in other countries like Brazil, which had not suffered directly from the war in Europe.

The longstanding dream of unveiling the structure of the strain responsible for the 1918 pandemic was achieved by retrieving fragments of the viral genome from paraffin blocks containing lung samples from fatal cases in New York and fragments from the remains of an Inuit woman buried in Alaska.

The Inuit woman was buried in the permafrost on Seward Peninsula on the western coast of Alaska, close to the Bering Strait, together with 72 other members of the same village, all of whom had succumbed to influenza (Lederberg, 2001).

Fragments of viruses present in the lung were sequenced in the laboratory. That is, their internal structure was determined and several of their genes were studied by their insertion into modern influenza viruses.

As occurred with fragments of smallpox virus found in the Italian mummy, the influenza virus was not capable of infecting animals, using the original fragments. More recently, a research group demonstrated that the addition of various genes from the 1918 sample to current influenza samples leads to a significant increase in the new sample's virulence (Tumpey et al., 2004).

In fact, the simple knowledge that the virus from 1918 presented the basic H1N1 composition, a structure still circulating today in nature, was a major stride, calling attention to the potential risk of this presentation of the viral genome.

HEPATITIS B

Hepatitis virus infections are a serious public health problem in Asia that has been reduced in places that have implemented widespread vaccination, such as Japan. However hepatitis is still an important problem in the other Asian countries.

According to available data for South Korea, some 12% of the population carries the hepatitis B virus, a figure far above the world average of around 5%. Recent paleoparasitological studies in South Korea have contributed important knowledge on the evolution of HBV infection in the region.

Until recently, no mummies were known to exist in South Korea. For religious reasons, preservation techniques are not applied to the bodies of deceased persons. However, in 2007, when a cemetery was being moved during an urban renewal project in Seoul, exceptionally well-preserved mummies were found, dated to c. 1400 AD.

In the decade prior to the deaths, the region had been ruled by a Buddhist state with the monks' absolute power. In 1392 the Confucionists took power. This period, known as the Joseon Dynasty, ushered in techniques for

treating mortal remains, according to the religious tenets that the corpse should disintegrate naturally, without the influence of worms or other external agents.

Corpses were thus kept on ice for several days and then placed in wooden coffins that seeped resins, and were buried in alkaline soil. These factors led to mummification of the corpses, not originally intended. The tissues remained pliable and in a better state of preservation than the Egyptian mummies, which were treated chemically to the point of total dehydration of the tissues.

Among the Korean mummies, researchers managed to recover the genome from hepatitis B virus from a child's liver. This finding raises the possibility of studying the evolution of hepatitis viruses, potentially elucidating the infection's pathogenesis.

HTLV-1 VIRUSES

A lymphoproliferative disease characterized as adult T-cell leukemia/ lymphoma was identified in the 1970s in Southwest Japan. In 1980 the first human retrovirus was isolated in the United States, called HTLV-I, the etiological agent of lymphomas similar to those observed in Japan.

In 1985, the same virus was shown to be related to a syndrome called tropical spastic paraparesis in Caribbean patients. Other geographic foci were discovered, and it was proven that the neurological disease could also be found in non-tropical regions.

The HTLV-1 viruses, belonging to the Retroviridae family, are the etiological agents of these severe human infections, in addition to uveitis and other manifestations like infectious dermatitis. These infections occur in well-defined regions of Japan (where the infection has been studied intensively), South America, and some regions of the Caribbean, besides Sub-Saharan Africa, Melanesia, and the Middle East.

Considering the distribution of the disease and the widely accepted hypothesis of Asian migration across the Bering Land Bridge to South America, mummies of inhabitants of Chile dated to 1,000 to 1,500 BP were studied, and fragments of HTLV-1 were identified in two of them (Hong-Shuan et al., 1999; Sonoda et al., 2000).

Molecular studies identified a significant similarity between the genome segments found in the mummies from Chile and the genome of HTLV-1 samples identified in Japan, as well as in the modern Andean population. These data confirm the arrival of this virus on the American continent before the Spanish conquerors, during Asian migration to the Americas.

OTHER OCCURRENCES AND CONCLUSIONS

Human papilloma virus, now definitively associated with carcinoma of the uterine cervix, was found in a 16th-century Italian mummy, demonstrating its ancient adaptation to humans (Fornaciari et al., 2003).

Another important finding involved orbital lesions in skeletons from Aborigine populations in Australia, indicating chronic eye infection. When these lesions are related to the distribution of trachoma in current populations from the same region and to other data such as the consistently advanced age of the affected individuals, one can conclude that this infection existed in Australia since remote times (Webb, 2005).

Another piece of information obtained from Italian mummies was the presence of the HLA-DRB allele in tissues from an individual dated to the 16th century. This allele expresses epitopes associated with Epstein-Barr virus glycoprotein 110, suspected to be responsible for the development of rheumatoid arthritis, also found in the same mummy (Fontecchio et al., 2006). The finding is also relevant to the origin of the disease, which previous paleopathological studies contended to have originated in America, in the Tennessee region, between 5000 and 500 BC.

In conclusion, the study of viruses within the field of paleoparasitology is just beginning. Hopefully, the use of advanced methodologies on a broader scale will help us learn more about the natural history of viral diseases, their distribution, and the evolution of viral genomes throughout the history of human diseases.

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