

The Paleoepidemiology of American Trypanosomiasis (Chagas Disease)

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Abstract

Ancient DNA methodology was applied to extract and amplify a segment of kinetoplast DNA of *Trypanosoma cruzi* in soft tissue specimens from about 300 spontaneously mummified human bodies from the Atacama Desert in northern Chile and southern Peru. A DNA probe was then employed to hybridize with the amplicon. Results indicate that about 41% of the population in that geographic area were infected with the trypanosome over the past 9000 years. The epidemiological implications of these findings are discussed. It is also emphasized that this and several other paleoepidemiological studies in progress have established that population-study cohorts of mummies now can generate statistically valid paleoepidemiological investigations capable of testing hypotheses. These reflect the maturation of the academic discipline of the scientific study of mummies.

Introduction

The scientific study of mummified human remains is an emerging academic discipline. Like most new areas of research, this discipline is passing through a descriptive stage. For mummy studies, this takes the form of individual case reports. These are valuable, because they document the presence of a specific disease at a known date in a precise geographical area. Eventually the accumulation of a large number of such reports may suggest a disease pattern. However, case reports cannot be employed to test hypotheses. This requires large cohorts, generally population comparisons. Few examples of such studies employing mummy soft tissues in cohorts large enough to

generate statistically valid epidemiological parameters have been reported (Aufderheide, 2003:2). Detailed below is a summary of a paleoepidemiological study of a South American parasitic disease characterized by infection with *Trypanosoma cruzi* (American trypanosomiasis or Chagas disease).

The disease

This microscopic parasite's reservoir is composed of at least a hundred different species of feral animals principally in South America's forest areas. It is transmitted by the bite of a blood-sucking winged insect of the Reduviid family. In this manner the disease is passed from insect to host animal to insect, maintaining the disease in the forest («sylvatic» cycle). Some vector species learn to live in crevices of poorly-built human houses, preying at night on its human and domesticated animal occupants («domestic cycle»).

Following the bite from an infected insect, in humans the parasite is distributed throughout the body via the blood. In some the parasite enters heart muscle or brain, causing heart failure or meningoencephalitis. This is called the acute stage lasting about one month during which time about 10% die. The survivors pass into the chronic stage that commonly lasts several decades. During this stage patients suffer intermittent parasitemia with attendant malaria-like fever and muscle aches. Heart involvement with eventual fatal heart failure often terminates this disease's course. No curative antibiotics are available.

Materials and methods

This study has been published in detail (Aufderheide et al., 2004) and therefore only the principal features will be itemized here. Specimens from the 283 mummies of nine different cultural groups radiocarbon-dated from 7000 BC to about AD 1800 that were examined in this study came from human bodies excavated from coastal or near-coastal sites of the Atacama Desert in northern Chile between about 18°-23° South Latitude. This has an extremely arid climate with essentially no rainfall. Bodies buried in this soil commonly become completely desiccated before the soft

tissues decay, generating spontaneous mummification. The studied specimens were acquired by anatomic dissection. The chemical procedure is detailed in the original report (Aufderheide et al., 2004). Single-strand synthetic molecular probes targeting four conserved regions of *T. cruzi* DNA were prepared and chemically attached to a series of polystyrene wells. The target aDNA was then extracted from the 283 mummy tissues, and the targeted kinetoplast regions were amplified by nested PCR. Treatment of the PCR reaction with T7 gene 6 exonuclease yielded the single-strand products complementary to the probes. Subsequent hybridization was visualized with Ambion's BrightStar system.

Results

Of the 283 specimens tested, 41% had a positive result, indicating the presence of *Trypanosoma cruzi* in the specimen. No statistically significant differences in positive results could be identified when the nine cultural groups were compared with each other in chi-square (X^2) and one-way NOVA methods. Similarly, no differences were found when time periods over this 9000-year period were compared, nor were the results different between male and female groups. Only infants less than two years of age had a statistically significant different (27%) percent of positive results. Similar results have been reported in modern populations about 25-35 years ago in endemic regions of Bolivia and even rural northern Chile.

Discussion

The most plausible interpretation of our findings is that long before the first humans appeared in the studied area of South America, this disease was well-established in the forest areas, the insect vector serving to maintain the infection among the many different mammal species

(sylvatic cycle). When humans arrived they became just another mammal host for the infection, and when humans built houses some insect vector species adapted to the swellings, establishing a domestic cycle within them.

Significance of the study

In addition to the generated information unique to American Trypanosomiasis (Chagas disease), this study calls attention to at least two other features:

1. Given the availability of specimens, the technique used in this study can be expected to be effective also for the study of most other bacterial or viral infections in antiquity.
2. This study, as well as several others currently in progress (Fletcher et al., 2003; David, 1998) report cohorts of mummies large enough to lend themselves to statistically valid paleoepidemiological investigations. The concordant ability of such studies to test hypotheses represents a «rite of passage» for mummy studies, reflecting its progression into the status level of a legitimate scientific discipline.

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