

A 9,000-year record of Chagas' disease

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Tissue specimens from 283 principally spontaneously (naturally) desiccated human mummies from coastal and low valley sites in northern Chile and southern Peru were tested with a DNA probe directed at a kinetoplast DNA segment of *Trypanosoma cruzi*. The time interval spanned by the eleven major cultural groups represented in the sample ranged from \approx 9,000 years B.P. (7050 B.C.) to approximately the time of the Spanish conquest, \approx 450 B.P. (\approx 1500 A.D.). Forty-one percent of the tissue extracts, amplified by the PCR reacted positively (i.e., hybridized) with the probe. Prevalence patterns demonstrated no statistically significant differences among the individual cultural groups, nor among subgroups compared on the basis of age, sex, or weight of specimen tested. These results suggest that the sylvatic (animal-infected) cycle of Chagas' disease was probably well established at the time that the earliest humans (members of the Chinchorro culture) first peopled this segment of the Andean coast and inadvertently joined the many other mammal species acting as hosts for this parasite.

For more than a century, examination of skeletal tissue from ancient human remains has demonstrated information useful for the understanding of some diseases in antiquity (1). Unfortunately, only a minority of human diseases leaves a detectable impact on bone. Hence, during the past few decades, efforts have been made to evaluate whether such other diseases could be detected by examination of the soft (i.e., nonskeletal) tissues in mummified human remains (2–4). Most of these studies have taken the form of individual case reports. These are valuable and will remain so for a long time. However, the study reported herein represents an effort to determine whether examination of such mummified human soft tissues can reconstruct the behavior of a disease among entire ancient populations. We selected American trypanosomiasis, more popularly known as Chagas' disease, as an appropriate candidate because of its high prevalence in the area of our study.

Initially, we attempted to identify the presence of *Trypanosoma cruzi* by methods of molecular biology that targeted a segment of the parasite's DNA in excess of 300 oligonucleotides length. Although we succeeded in that effort (5), the target segment proved to be too long for the sensitivity needed for a study involving a large number of specimens. A shorter segment involving a probe had the necessary sensitivity (6), but required considerable manipulation of the amplified product. Our final effort, described in detail in this report, used a short segment of kinetoplast DNA with lesser handling of our amplicon. This proved to have the sensitivity we needed with minimal manipulation to bring about the hybridization reaction. This was then applied to extracts of tissue specimens from 283 mummified human remains from a South Andean coastal zone. The results enabled us to construct the paleoepidemiology of Chagas' disease in that area over a 9-millennial interval, from the appearance of the first humans in that region until the near present.

A Brief Description of Chagas' Disease Today

Chagas' disease (American trypanosomiasis) is limited to Central and South America, where >10 million Latin Americans are

infected with the disease's cause, *T. cruzi*. A large fraction of these will die a premature death, usually of cardiac complications (7). The disease's reservoir lies in >100 different mammal species of wild animals. It is transmitted by several dozen insect species belonging to the family Reduviidae, subfamily Triatominae (reduviid or triatomine bugs; vincucha in Spanish). These insects hide in wild animals' nests or lairs, extracting their blood meals while the animal is sleeping (sylvatic cycle). Human activities sometimes may expose them to these insects. In addition, some of these insect species adapt to human dwellings where they hide in crevices, emerging at night for their blood meal, usually biting the subject in the facial area (domestic cycle). Within the insect's intestine, the trypanosome in the blood meal of the insect vector undergoes several successive developmental stages, terminating as a flagellated form living in the vector's rectum. Ingestion of the blood meal causes the vector to defecate. Upon awakening, the victim commonly rubs the itching bite area, pushing the trypanosome-laden feces into the bite wound or onto the conjunctiva. By these methods, the trypanosome gains access to the victim's blood stream, initiating the acute stage of the disease (8).

Widely distributed via the blood stream, the trypanosome sheds its flagellum and penetrates tissue cells. Proliferating by binary fission within the cells (especially myocardium and meninges), the amastigotes eventually cause the cell to burst, liberating more organisms. The death rate for persons in the acute stage is $\approx 10\%$ due to myocarditis or meningoencephalitis. Variations in the disease's severity may be the effect of genetic polymorphism (9) or expression of transsialidase by the parasite (10). Survivors of the acute stage progress either to the indeterminate (asymptomatic) or to the chronic stage. The latter is characterized by a commonly decades-long interval during which asymptomatic periods alternate with febrile episodes of parasitemia and/or a variety of other (including cardiac) symptoms. These often reflect a myocardium progressively damaged by extensive chronic inflammation and fibrosis. Its terminal phases usually present as dilated cardiomyopathy. In a smaller fraction, the gut's ganglion cells are destroyed, leading to segmental paralysis of the esophagus or colon that may terminate as achalasia or intestinal obstruction (11). The nature of the myocardial changes in the chronic stage has been considered by some to be an autoimmune phenomenon based on antigenic mimicry in the form of an antibody targeting *T. cruzi* polypeptides (12). More recently, however, persistence of the parasite in the tissues has been demonstrated (13). Nevertheless, consensus regarding the mechanism of the myocardial lesion in humans with chronic trypanosomiasis has not yet been reached (14). Although nifurtimox or benznidazole produce a variable response in the acute stage, no effective therapy to control the chronic stage is available.

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Table 1. Culture sequence of studied populations, related time periods, and distribution of samples

Culture	Time range	No. tested	Percent positive
Early Chinchorro	7050–3000 BC	18	39
Late Chinchorro	3000–1500 BC	53	43
Early Alto Ramirez	1000 BC–0	16	25
Late Alto Ramirez	0–400 AD	20	35
Cabuza	400–1050 AD	27	41
Maitas	1000–1250 AD	25	40
Chiribaya	1050–1250 AD	70	47
M8 (upper Chiribaya)	1050–1250 AD	16	19
San Miguel	1250–1350 AD	9	33
Inca	1450–1550 AD	26	50
Colonial	1550–1850 AD	3	67
All cultures	7050 BC–1850 AD	283	40.6

Conventional radiocarbon ages were determined by radiocarbon dating. No. tested, total number of mummies tested from each group; Percent positive, the percent of tested samples in each group that hybridized with our probe.

Materials and Methods

The Mummies and Their Tissues. All of the sampled mummies were from archaeological sites between $\approx 18^{\circ}\text{S}$ to 23°S (Ilo, Peru to Antofagasta, Chile) along South America's coastal area of the Atacama desert in southern Peru and northern Chile. Unique climatic and hydrologic features have combined to produce one of the world's most arid deserts here (the Atacama). Burial of a human body in this region's soil results in such rapid tissue dehydration that soft tissue decay often is arrested long before many soft tissues disintegrate. Virtual absence of rainfall there, even during el Niño events, provides survival of desiccated soft tissues until they are excavated as much as millennia later. Some bodies were recovered from sandy beach sites, but most are from low valley sites within 25 km of the ocean.

The succession of cultures in this coastal zone is presented in Table 1. The Chinchorros are the earliest people identified as settling this region. Their artifacts appear from ≈ 7500 B.C., and the earliest mummy (Acha Man) recovered from the Acha valley has been radiocarbon dated at $8,970 \pm 225$ years B.P. (7020 B.C.) (15). Theirs was a maritime subsistence strategy, living at river mouths at the band or extended family level. Their housing was a round, originally conical structure, probably covered with reed mats and a mat floor. A radical form of anthropogenic ("artificial") mummification of their dead involving extensive excarnation and reconstruction was practiced during the first half of their 5,000-year reign on this coast (16). This form of mummification was gradually simplified and finally abandoned in ≈ 2000 B.C. (17).

The subsequent succession of cultures that succeeded the Chinchorros was characterized by an increase of agriculture and pastoralism, practices that, at this latitude, originated in the Andean highlands. These appear on the coast ≈ 1000 B.C. and increase progressively at the expense of harvesting of marine resources. Of these, the first was the group called Alto Ramirez. These people lived in houses of stone platforms with cane or reed walls, and roofs of unknown material. The influence of the highland's Tihuanaco empire was felt in this coastal area ≈ 400 A.D. (called Cabuza culture on the coast) that survived until that empire's collapse ≈ 1000 A.D. The political chaos after that collapse found the Maitas of northern Chile and the far southern Peru's Chiribaya (together with the latter's M8 site at Moquegua) evolving into agriculturally based societies (18, 19). The following San Miguel and Inca groups improved the technology of their agriculture.

The tissue specimens of the mummies were acquired by dissection, placed into plastic containers, and stored in desiccated chambers. Aseptic techniques were used to sample these archived specimens. The tissue type selected from each mummy was based on available abundance and gross tissue integrity. Tissues sampled included heart, lung, liver, kidney, ileum, colon, muscle, and brain.

Chemical Methods. Primers/probes. All primers and probes were purchased from IDT (Coralville, IA). The primers were: primer A, TATATTACACCAACCCCAATC; primer B, ACTTTTGGGGCGGAAATTCAT; primer C, CGAACCCACCTC-CCCGTAAA; and primer D, Biotin*G*C*G*G*AAATTC-ATGCATCTCCC.

Note in primer D that the last four 5'-nucleotidyl and 5'-biotinyl groups are linked through phosphorothioyl groups, as indicated by the asterisks. The probes represent four conserved segments (segments 1–4) of the kinetoplast minicircles of *T. cruzi*. All contained a 5' amino group, allowing them to be covalently attached to the wells of titer plates (see below). The probes were probe P1, ATCCGCCAAATCCATAAAATAATGTACGG; probe P2, TTCCGCCAAATCCATAAAATAATGTACGG; probe P3, ATCCGCAATTTAGCCAAAATAATGTACGG; and probe P4, ATCGGTAAAATGCCAAAATAATGTACGG.

An additional 5' amino and 3'-biotinylated oligonucleotide, T1 (GTTGTG), was made to test for reactivity of the plates for linking the probes and to test the final detection step. Finally, a 5'-biotinylated oligonucleotide H1, complementary to P1/P2, was made to test hybridization and to determine sensitivity.

Sample preparation. Samples of mummy tissue specimens were pulverized under liquid nitrogen by using a Spex CertiPrep Freezer/Mill (Spex Industries, Metuchen, NJ). Whenever possible, 400 mg of pulverized tissue was used for DNA extraction.

DNA extraction. DNA was extracted from the pulverized tissue samples by using the GeneClean for Ancient DNA kit (Bio 101, Vista, CA). The DNA was then used as template in the PCR amplification.

PCR. Nested PCR was performed by using primer sets A and B in the initial round followed by primer sets C and D in the second round. The PCR conditions used were as described (6), with mimic DNA template in the first round. Two milliliters of extract were used as template in the initial round, and 2 μl of that reaction were used as template for the nested round. A final round of PCR was done employing primer sets C and D and 2 μl of the nested round reaction. Negative controls of Egyptian mummy tissue and water were run with each set of PCRs.

Hybridization/detection. The final PCR products were treated with T7 gene 6 exonuclease to convert them into the single-stranded, probe-complement form. The 5'-phosphorothioate linkages in primer D protected the probe-complement strand from degradation (20). Probes P1, P2, P3, and P4 were reacted for 16 h at 37°C under saturating conditions with the wells of Reacti-Bind Maleic Anhydride Activated Polystyrene plates (Pierce), according to the manufacturer's prescribed protocol. The amounts of each probe incubated in each well were 10 μl of 100 μM stock of each P1, P2, P3, and P4 in a final volume of 100 μl of PBS. For each set, one well was reacted with T1 as a test to be sure the reactivity of the plates had not deteriorated.

Plates were then rinsed with blocking buffer and water and then prehybridized in $5\times$ SSC/ $5\times$ Denhardt solution/ 0.1% SDS/ 0.1 $\mu\text{g}/\mu\text{l}$ salmon sperm DNA for 1 h at 50°C . Prehybridization solution was removed, plates were washed three times in water, and 75 μl of hybridization solution with 25 μl of exonuclease-treated DNA was added. Final hybridization solution concentrations were the same as prehybridization solution, except that the Denhardt solution was omitted. Hybridization was carried out at 50°C for 16 h with slow rotation.

Detection procedure. Detection was done by using Ambion's BrightStar BioDetect Nonisotopic Detection kit (Ambion, Austin, TX). Hybridization plates were washed, blocked, and detected as per Ambion's protocol. Light emission was detected by using an Alpha Innotech MultiImage Light Cabinet. The plates were exposed for 30 min, and the images were recorded. It was conservatively calculated that this system, PCR, hybridization, and detection had a minimal sensitivity for between 5 and 50 cells of *T. cruzi*.

Evidence of hybridization of an amplified PCR product with our probe is herein referred to as a "positive" test result.

General procedures. The various stages of this work, sample preparation, extraction of DNA, PCR, exonuclease treatment, and hybridization were carried out in physically separate rooms or work areas (hoods, DNA-disruptive work stations; e.g., Clone Zone, USA Scientific, Ocala, FL). All pipetting was done with aerosol trapping filtered pipette tips. Controls, as described above, were run with each set of samples.

Results

Comparisons by Culture. One hundred and fifteen (40.6%) of the entire group of 283 mummies demonstrated a positive reaction (i.e., hybridized) with the probe for *T. cruzi*. No statistically significant differences ($P > 0.05$) in the frequency or proportion of positive test results were noted between any of the cultural groups as determined by χ^2 test and one-way ANOVA. Pooling the values for Early and Late Chinchorro into a single group and similarly for Early and Late Alto Ramirez groups did not alter the results.

Comparisons by Age Groups. Thirteen of 47 (27.7%) tested infants below 2 years of age had a positive test, whereas 28 of 56 (50.0%) of those aged 3–15 years were positive. In adults (>15 years), 69 of 165 tested (41.8%) were positive. These values indicate that the frequency of positive results among infants is significantly lower ($\chi^2 = 4.22$, $df = 1$, $P = 0.04$) than that for all ages above 2 years (including adults). Lower rates among younger age groups also have been found in modern populations (21).

Comparisons by Sex. Thirty-five of 88 (39.8%) tested females had positive tests, whereas 52 of 123 (42.3%) tested males were positive. These values for the entire database indicate that the prevalence of Chagas' disease in females was not different from that in males. These findings are similar to those in modern Brazil (21), although this feature has geographic variations (22). Female/male positive reaction ratios within cultural groups did not differ.

Comparisons by Time Periods. When the values (expressed as percent positive) are arranged according to their radiocarbon dates (years B.P.), a slight trend toward higher values in the more recent years is suggested. However, the derived regression equation was $y = 45.940 - 0.0023x$, and its low R value ($R = 0.32$) reflects the wide scatter of the plotted values, especially in the more recent periods.

Comparison of Tissue Sites. Only the 37 positive muscle specimens of the 70 that were tested (52.9%) were significantly different from the values of the remainder of the tissue locations tested ($\chi^2 = 5.46$, $df = 1$, $P = 0.019$).

Comparison of Weight of Tested Specimens. The specimens from which DNA was extracted were separated into two weight groups: 0–399 mg ($n = 22$) and 400+ mg ($n = 261$). The frequency of positive reactions in these two groups did not differ.

Discussion

Items of Epidemiological Considerations. Prevalence. The study revealed a prevalence rate for Chagas' disease in the entire population of 40.6% with no significant differences among the individual cultural groups. This finding suggests the presence of a very common infectious disease with a large, accessible reservoir of hosts and an essentially unchanging, effective transmission mechanism resulting in a static disease pattern over a period of 9 millennia. How realistic could the picture painted by these values be?

Modern human infectious diseases are the product of interactions between the biology of the infectious agent(s), the environment, and human behavior/biology. If these potential variables do not change, the expression of the disease within a population can be expected to remain constant. Significant alteration in one or more of these variables, however, can cause the disease to present itself in a very different fashion. During the recent past, physicians have struggled with a host of human infectious diseases that either had no precedence or represented rapid increase of previously rare conditions. These have been termed "emerging infectious diseases," and include such examples as coronavirus (which causes severe acute respiratory syndrome, SARS), hantavirus, AIDS, and even a resurgence of tuberculosis in some American locations. Many have been associated with a major change in one or more of the above variables. Arguably, these emerging infectious diseases could be dealt with greater assurance if we had knowledge of how they behaved during antiquity under often dramatically different circumstances.

These stand in contrast to our reconstruction of the behavior of Chagas' disease during the past 9,000 years. The sylvatic form of this disease appears to have been well established when humans first entered our study region 9 millennia ago. Its transmission depends on the ability of the insect vector to infest the wild animals' nests or lairs, providing opportunities for the insect's blood meal and transmission of the infectious agent (*T. cruzi*). Many of the animals adapted to the trypanosome's presence, manifesting little or no effect from the trypanosomes circulating in their blood, suggesting that this disease among feral animals probably has a far greater antiquity than the period covered by our study.

Interestingly, early human settlers built houses of wattle and thatch that provided ideal opportunities for nesting reduviid bugs. Evidence of residence in coastal, bat-inhabited caves is also extant (23). Subsequent cultural groups improved the quality of houses, but persisted in the use of construction material friendly to the Chagas vector right up to the present in many rural areas. The common inclusion of domestic animals (dogs, guinea pigs) within the dwelling added further opportunity to facilitate the disease's cycle within the domestic environment. In short, humans unwittingly offered the Chagas vector a physical environment for access to a blood meal that was equivalent to the nests and lairs of feral animals. Although many aspects of their life changed dramatically, the features of dwelling structure and habitation conducive to reduviid bug infestation were not altered significantly. The result was maintenance of an equilibrium between the biology of the trypanosome and its vector, the environment, and human behavior/biology. The consequence was an unchanging prevalence of the disease over the entire interval as reflected by the results of our study.

Possible error sources. One possible source of error could arise from the fact that we tested only one tissue sample from each mummified body. It is conceivable that further testing of other tissues might sometimes have yielded a positive result. If so, then this would have increased the prevalence value; thus, our values can be viewed as minimal rates for the studied groups.

Fig. 1 indicates that all age groups are represented in appro-

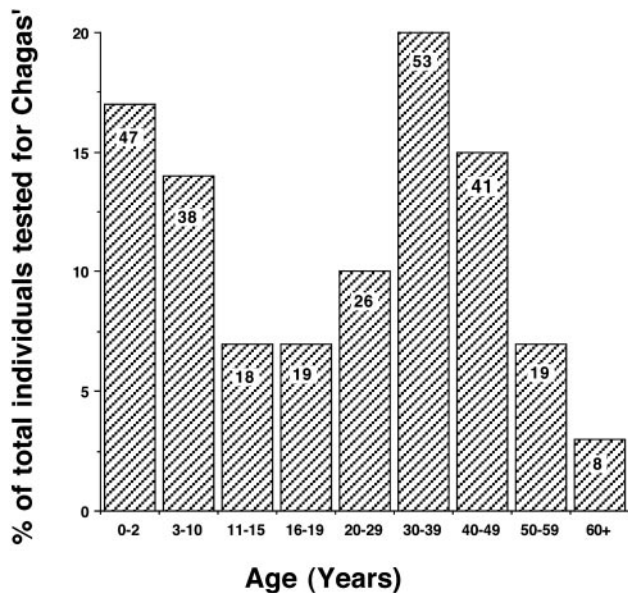


Fig. 1. Distribution of "age-at-death" for the entire database of all groups. The high infant mortality and adult deaths' frequency, peaking in the fourth decade, is characteristic of ancient populations. The number on each bar indicates the number of individuals tested.

priate proportions. Ancient populations commonly had $\approx 40\%$ subadult deaths (36% in our study group), and adult deaths peaked in the fourth and fifth decades. Males and females are equally represented and have the same rates of positive tests.

Comparison with today's prevalence in the same region. How does the 40.6% prevalence of Chagas' disease in antiquity compare with rates for modern populations of this same region? Such comparisons must keep in mind that a country with sufficient infrastructure to produce reliable disease statistics probably has already altered significantly those factors integral to the prevalence of Chagas' disease, such as quality of housing, socioeconomic standards, and probably even control programs (insecticide paint, etc.). The country in this region whose rural circumstances have probably been altered least by such actions is Bolivia. In the late 1970s, 20% of the total Bolivian population (both urban and rural) of that country was estimated to be infected with *T. cruzi*, and rural areas provided prevalence rates from 40 to 80% (22). In rural northern Chile (the source of most of our ancient specimens), 20% of the population in the early 1980s had positive serological tests for *T. cruzi*, and endemic areas had values ranging from 15 to 60% (23), although vigorous control programs since then have lowered these values significantly. Far southern Peru identified prevalence rates of 12–20% (24). Certain rural areas of northern Venezuela, such as the state of Belén, had prevalence rates of $\approx 50\%$ (25).

The megavisceral syndromes. Based on the gross evidence of megacolon and megaesophagus found during dissection of 22 mummies from the Tarapaca Valley, Rothhammer *et al.* (26) suggested that megavisceral syndromes may have been present in 41% of the pre-Columbian populations in northern Chile. They cautioned, however, that their sample was small and also noted that geography may have been an important variable because only one case of megacolon had been identified in ≈ 500 mummies exhumed from the Azapa Valley (the origin of many of our mummies). A recent study suggests that the puzzling inconstancy of the megavisceral complication may be the consequence of variations in the parasite's genetic structure (13).

In our study, 53 adult mummies had specific reference to the state of the colon included in the dissection report. In one of

these (Chiribaya autopsy no. 96), the colon was massively dilated and filled with coprolites. A positive hybridization result was obtained on a lung specimen from this body. Marked dilatation of colon, stomach, or ileum was found in eight others, but absence of coprolites in these suggested that the dilatation was the product of postmortem bacterial growth with gas formation. Only one of these samples hybridized with our probe.

In a more recent study, four of six mummies from San Pedro de Atacama in northern Chile at an altitude of 2,436 m above sea level were found to have Chagas' disease as determined by PCR amplification of a *T. cruzi* kinetoplast DNA segment, but no megavisceral lesions were present (27).

Given the variables mentioned above, these prevalence rates for relatively recent Andean communities as well as several small samples of ancient mummies reported by others from our region of interest are compatible with the 40.6% rates in our studied ancient groups.

Transmission. The six mechanisms of transmission of *T. cruzi* described in modern circumstances include the following: domestic cycle, sylvatic cycle, peridomestic cycle, congenital transmission, blood transfusion, and ingestion. Given our archaeological, paleoenvironmental, and laboratory-generated database, how do the results of our study fit into these patterns?

The domestic cycle. Today, *Triatoma infestans* is the almost universal vector responsible for domestic cycle transmission in our study region, and is only very rarely involved in the sylvatic cycle, although numerous other *Triatoma* species are. Hence, *Triatoma infestans* is viewed as a species that has become highly adapted to the environment of the human dwelling. Hosts in addition to humans in this cycle include animals that commonly share human dwellings, including dogs, cats, mice, guinea pigs, and others (28).

Because we recovered no ancient vectors from our examined mummies or animals, we do not know the vector involved in the transmission of Chagas' disease to our studied populations. However, we do know that our ancient mummies' dwellings were of a construction with which *Triatoma infestans* would have been very comfortable. Because the region was and is devoid of rainfall, simple dwellings were created principally to provide shelter from wind and sun, buffer the cool air of winter nights, store personal belongings, and allow privacy. These needs were easily supplied by the conical dwellings of the early Chinchorros (29), the cane-walled houses of the subsequent Alto Ramirez people, the Tihuanaco-related Cabuza group (30, 31), and even the more structured dwellings of the Chiribaya and later groups (32).

So, was *Triatoma infestans* and its related domestic cycle the principal transmission mechanism in deep antiquity just as it is today? Lacking samples of ancient vectors, we cannot answer that question with certainty at this time. The following items, however, are worthy of consideration. Presumably, a substantial period would be required for a sylvatic cycle vector to become exposed and adapt to a human dwelling environment. Yet the first archaeologically defined human presence in the studied area, the Chinchorros, had a Chagas' disease prevalence rate of nearly 40%, statistically the same as their successor groups. The oldest body from that Chinchorro group has been radiocarbon dated as $\approx 9,000$ years old (15), and that body was infected with *T. cruzi*. The origin of the Chinchorro group remains unknown. However, unless they brought with them a completely house-adapted set of domestic-cycle vectors when they first arrived to settle this coastal segment, it would not appear that sufficient time would have been available to evolve such a cycle after their arrival and still produce a 40% prevalence in that earliest group of settlers. Because the Chinchorros occupied that coastal strip for the subsequent 5,000 years, such a domestic cycle could well have become established by the time their cultural successors replaced them or during the coastal residence period of any of

the groups after the Chinchorros. This suggests that no such domestic cycle operated in this area at the time of the initial appearance of the Chinchorro settlers, but at some as yet undetermined point, the domestic cycle evolved to the dominant role it plays today.

The sylvatic cycle. The sylvatic cycle includes only wild animals as the reservoir with only an occasional human intruder. Although many reduviid insects effectively transmit *T. cruzi* in the sylvatic cycle, the principal vectors are *Rhodnius prolixus* (northern South America) and *Panstrongylus megistus* (the forest of coastal Brazil) (24). In contrast to *Triatoma infestans*, these vectors can function in both the domestic and sylvatic cycles (33). They have become prominent participants in the domestic cycles principally in regions where humans have substantially altered the local ecotopes. Because of these features, they are commonly viewed as having had a relationship to humans for a lesser interval than *Triatoma infestans*. The reservoir hosts in the sylvatic cycle number >100 species (see check list in ref. 34) that include armadillo, opossum, sloth, rabbit, mouse, rat, squirrel, porcupine, bat, fox, skunk, coati, and many others. The vectors for these infest nests or dens normally occupied by these host animals. Thus, nests in hollow trees, birds' nests, fallen logs, fronds of palm trees, beneath loose bark, and similar locations commonly harbor these vectors.

Peridomestic cycle. The peridomestic cycle is simply extension of a sylvatic cycle participant, such as opossum, that is drawn into an area of human habitat that already has an established domestic cycle.

Blood transfusion. Blood transfusion has no application to our studied, ancient populations.

Congenital transmission. Congenital transmission occurs in 0.5–6.3% of infants born to Chagas'-infected mothers (11, 35). There is no reason to expect these rates to have been less in antiquity.

Ingestion. Oral ingestion of trypomastigotes is capable of transmitting Chagas' disease. This has been demonstrated in experimental animals where consumption of infected vectors rendered opossums infective (36) and has occurred as laboratory accidents (24). Cats often have a high rate of Chagas' infection in homes where a domestic cycle is established, probably because they eat infected mice. Early hunters may have become infected by butchering and eating the uncooked meat of acquired, infected prey, such as rodents. The Chiribaya indulged in the common Andean practice of raising guinea pigs in corrals within their homes. Domestic cycle vectors usually quickly establish residence within such corrals, and these animals become part of the domestic cycle. The Early Chinchorro practice of completely disassembling the body of a recently deceased member and subsequently reconstructing it into a mummy (16) must have exposed them to trypanosome-containing blood, some of which may have been ingested inadvertently.

Other atypical vectors can function under certain circumstances. Naturally infected ticks (*Rhipicephalus sanguineus*) have transmitted the disease to dogs. Although bedbugs (*Cimex lectularius*) cannot support the full parasite cycle after experimental feeding on infected animals, their feces were infective to mice (37).

Thus, in addition to the other methods noted above, direct

ingestion of trypanosomes as described as well as certain atypical vectors may have played a role also in antiquity.

Body Site Distribution of *T. cruzi*. Positive tests found in most soft tissue specimen locations imply that most of these individuals were parasitemic at time of death or that the trypanosomes were located within tissue cells. Clinical investigation has established that individuals in the chronic or indeterminate (asymptomatic) stage have a low frequency of parasitemia. If the trypanosomes we found in these late stages actually are intracellular, the parasitized cell type would need to be present in most of the body's tissues that we tested; e.g., the endothelial cell would be a good candidate (38). Alternatively, multiple cell types may be involved.

Our Reconstruction of the Paleoepidemiology of Chagas' Disease Among Northern Chile's Coastal Populations

The use of a DNA probe targeting a segment of *T. cruzi* kinetoplast DNA extracted from nearly 300 Andean human mummified remains has reconstructed the behavior of American trypanosomiasis (Chagas' disease) during the past 9,000 years in the southern Andean coastal area. The resulting epidemiological pattern is that of a static disease with a nearly constant (40.6%) prevalence rate during this entire time period. The most plausible explanation for this phenomenon suggests that, before human occupation of the studied region of South America, the sylvatic cycle of Chagas' disease involved a vast reservoir among wild mammals and that the disease was transmitted by a large number of insect vectors that existed before human occupation of the studied region of South America. Upon settlement of this coastal segment ≈9,500 years ago, humans intruded upon and became participants in this sylvatic cycle, perhaps augmented by various forms of trypanosome ingestion, including consumption of infected food. Gradually, the domestic cycle evolved, leading to its current status today.

The data are consistent also with several other conceivable but less probable scenarios. The Chinchorros may have established a sedentary lifestyle elsewhere that included a domestic cycle before they moved to the northern Chile coast, bringing the vector and the disease with them. However, present archaeological knowledge provides no evidence of links between the Chinchorros and any other postulated site. The proposed intrusion of the earliest Chinchorro people into a fully developed sylvatic cycle with gradual transition into a domestic cycle in subsequent generations or cultures appears to us to be the most plausible interpretation of our findings.

This study also demonstrates that comparative population investigations can now be carried out on ancient soft tissues for paleoepidemiological purposes on emerging or other infectious diseases, emphasizing the enormous value and potential of archived ancient human soft tissues.

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