



Chungara, Revista de Antropología Chilena

ISSN: 0716-1182

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Universidad de Tarapacá

Chile

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Chungara, Revista de Antropología Chilena, vol. 37, núm. 1, junio, 2005, pp. 85-90

Universidad de Tarapacá

Arica, Chile

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ASPECTS OF INGESTION TRANSMISSION OF CHAGAS DISEASE IDENTIFIED IN MUMMIES AND THEIR COPROLITES

TRANSMISIÓN DE LA ENFERMEDAD DE CHAGAS IDENTIFICADA EN COPROLITOS DE MOMIAS

Arthur C. Aufderheide*, Wilmar Salo*, Michael Madden*, John Streitz*, Katharina Dittmar de la Cruz**, Jane Buikstra***, Bernardo Arriaza****, and Lorentz E. Wittmers, Jr.*

Molecular study of *Trypanosoma cruzi* (*T. cruzi*) ancient DNA (aDNA) in the soft (nonskeletal) tissues of 283 naturally (spontaneously) mummified bodies from coastal sites located in southern Peru and northern Chile demonstrated a Chagas disease prevalence rate of about 41% over the past 9,000 years. This rate is similar to that of several endemic areas within this region prior to initiation of public health control programs. This report focuses on the presence of *T. cruzi* aDNA in the coprolites of some of these mummies. Review of the possible mechanisms that may explain the presence of this parasite in the coprolites indicates numerous antemortem and postmortem circumstances that conceivably could have been responsible. In given conditions, all of these may need to be considered. These considerations indicate that the presence of *T. cruzi* aDNA in mummy coprolites cannot categorically be considered as evidence of ingestion of the parasite.

Key words: coprolites, aDNA, american trypanosomiasis.

El estudio molecular del tejido blando de 283 de cuerpos momificados en forma natural proveniente de la costa del sur de Perú y norte de Chile revela la presencia de Trypanosoma cruzi a partir de 9.000 años atrás con una prevalencia de un 41%. Este valor es parecido a áreas de estas regiones donde la enfermedad era endémica, antes que se iniciaran los programas de control de la salud. Este ensayo discute la presencia de ADN de T. cruzi en los coprolitos de momias y revisa los posibles mecanismos de estos parásitos en los coprolitos. La presencia de T. cruzi en los coprolitos de momia no necesariamente indica que hubo una ingesta de este parásito.

Palabras claves: coprolitos, ADN, tripanosomiasis americana.

Rothhammer et al. (1984) reported on nine mummified cases with Chagas disease (American trypanosomiasis) symptomatology from Quebrada de Tarapaca in northern Chile. Their gross pathological finding included evidence of megacolon and cardiomegaly. The mummy's intestines were abnormally packed with feces, suggesting absence of peristalsis. These inhumations dated to 470 B.C. to 350 A.D. It is interesting that our biomechanical studies of Chagas disease in the coastal area of southern Peru and northern Chile is confirming the existence of this disease (Aufderheide et al. 2004). Using polymerase chain reaction (PCR) and molecular

probe methodology we tested for the presence of a segment of *Trypanosoma cruzi* ancient DNA (aDNA) in soft (nonskeletal) tissues dissected from 283 spontaneously mummified human remains dating to the period between 7,000 B.C. and A.D. 1,800 from that region. Results indicated that 41% of those populations had been infected with *T. cruzi*.

Not included in that report were the results of similar tests on coprolites from 27 of these mummies. This communication deals with the results of analysis of those coprolites and discusses their significance. In addition, head lice from heavily-infested hair of mummies of the Chiribaya cultural group (Tables 1 and 2) were also analyzed.

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Table 1. Results of ancient DNA from *Trypanosoma cruzi* sought in coprolites by amplification and hybridization with a molecular probe.
Resultados de análisis de ADN antiguo de Tripanosoma cruzi en coprolitos humanos, por amplificación e hibridación con pruebas moleculares.

Cemetery	Tomb No.	Culture	Time Period	Soft Tissues	Coprolites
Mo1-6	27	Chinchorro	2,000 B.C.	+	Neg
Mo1-6	29	Chinchorro	2,000 B.C.	+	Neg
Cam-15D	9	Chinchorro	2,000 B.C.	+	Neg
AZ-75	85	Alto Ramírez	A.D. 400	+	Neg
AZ-75	133	Alto Ramírez	A.D. 400	+	Neg
CHA-1	1	Chiribaya	A.D. 1000	+	Neg
SR-1	1	Maitas	A.D. 1200	+	Neg
Mo1-6	16	Chinchorro	2,000 B.C.	+	+
Mo1-6	28	Chinchorro	2,000 B.C.	+	+
Mo1-6	32	Chinchorro	2,000 B.C.	+	+
Mo1-6	55	Chinchorro	2,000 B.C.	+	+
Mo1-6	U3	Chinchorro	2,000 B.C.	+	+
AZ-75	12	Alto Ramírez	A.D. 400	+	+
CHA-3	325	Chiribaya	A.D. 1,000	+	+
CHA-7	707	Chiribaya	A.D. 1,000	+	+
			Total	15+	8+

For cemeteries: Mo1-6 = Morro 1-6; Cam-15D = Camarones-15D; AZ-75 = Azapa-75; CHA-1,3,7, = Chiribaya Alta-1,3,7; SR-1 = Sin Referencia-1. All these sites are in northern Chile, except the CHA cemeteries; the latter are in southern Peru at the mouth of the Osmore Valley near the seaport Ilo and represent the South Peruvian Chiribaya population. The term Maitas is applied to a related Chiribaya population in the Azapa Valley of northern Chile.

Table 2. Results of ancient DNA from *T. cruzi* sought in coprolites by amplification and hybridization with a molecular probe.
Resultados de análisis de ADN antiguo de T. cruzi en coprolitos humanos, por amplificación e hibridación con prueba molecular.

Cemetery	Tomb No.	Culture	Time Period	Soft Tissues	Coprolites
Mo1	12	Chinchorro	5,000 B.C.	Neg	Neg
Mo1-6	18	Chinchorro	2,000 B.C.	Neg	Neg
Mo1-6	39	Chinchorro	2,000 B.C.	Neg	Neg
Mo1-6	46	Chinchorro	2,000 B.C.	Neg	Neg
Mo1-6	56	Chinchorro	2,000 B.C.	Neg	Neg
Mo1-6	U7	Chinchorro	2,000 B.C.	Neg	Neg
Cam-15D	23	Chinchorro	2,000 B.C.	Neg	Neg
AZ-75	57	Alto Ramírez	A.D. 400	Neg	Neg
ALG-1	342	Tiwanaku	A.D. 800	Neg	Neg
CHA-1	38	Chiribaya	A.D. 1,000	Neg	Neg
CHA-3	304	Chiribaya	A.D. 1,000	Neg	Neg
Cam-9	13	Inca	A.D. 1,400	Neg	Neg
			Total	12 Neg	12 Neg

In addition to some of the same cemeteries as indicated in the legend for Table 1, the cemetery abbreviated as Mo-1 = Morro-1, near the mouth of the Azapa Valley in northern Chile; Cam-9 = Camarones-9, near the mouth of the Camarones Valley dated to the Inca Period (though probably composed principally of local mariners descended from the Late Regional Development Period); and ALG-1 = Algodonal, a site from the lower Osmore Valley near Ilo, southern Peru, some of whose tombs had Tiwanaku-related features (as did the one we tested), while others had features of southern Peru's Chiribaya population.

Material and Methods

Details of the mummies and the biochemical procedures employed have been reported previously (Aufderheide et al. 2004). Briefly, the mummy soft tissue specimens were acquired by dissection principally with permission of the Dept. of Anthropology of the University of Tarapaca in Arica, Chile. The lice were studied by permission of Jane Buikstra, Ph.D., director of the archaeological excavation of the Chiribaya burial sites in the Osmore Valley of southern Peru near the seaport of Ilo in 1990 as part of Project Cuntisuyu. Ancient DNA was extracted, PCR amplified and characterized by hybridization. Specimens of lice were collected as reported in Dittmar de la Cruz (2003). The collected lice were retained in a refrigerated solution of 100 % ethanol until analyzed. A single louse was removed from the ethanol and allowed to dry 20 minutes. The louse was transferred to the PCR tube using sterile forceps. The louse was then crushed with a flame-sealed Pasteur pipette and the PCR solution was added. Subsequent processing was the same as for tissue specimens except that the amplicon was characterized by electrophoresis. Positive results were confirmed by sequencing the isolated band. Each of the 57 lice was studied in this way. Coprolite samples were pulverized under liquid nitrogen in a mechanical mill (Spex CertiPrep Freezer/Mill (Spex International, Metuchen, New Jersey, USA). 400 mg of the powdered sample was then selected for analysis and processed in the same manner as were the soft tissue samples (Aufderheide et al. 2004).

Results

T. cruzi aDNA was identified in 8 of 15 coprolites found in the colon of mummies whose soft tissues also harbored this parasite, but in none of 12 coprolites from mummies whose soft tissues yielded no trypanosomal DNA. In addition, this parasite's DNA was also present in two of 57 head lice (*Pediculus humanus*). Tables 1, 2 and 3 detail results of the coprolite and lice analyses.

Discussion

Coprolites were available from 15 mummies whose soft tissues had demonstrated the presence

Table 3. Identification of aDNA from *T. cruzi* sought in head lice by amplification and sequencing.

Identificación de ADN antiguo de T. cruzi en piojos de la cabeza, por amplificación y secuenciación

Chiribaya Mummy	Nº Lice Tested	Nº Positive	Nº Negative
CHB #1	20	0	20
CHB #2	23	0	23
CHB/1503	14	2	12

CHB = Chiribaya cemetery very near the seacoast at Ilo, southern Peru, at the mouth of the Osmore Valley, dated to the Regional Development Period.

of *T. cruzi*; eight of these coprolites revealed a positive reaction (Table 1). None of the coprolites were positive in the group of 12 mummies whose soft tissues tested negative for *T. cruzi* (Table 2). This difference is statistically significant; chi square analysis reveals that $p < .005$. The following comments are directed at the possible interpretation of these findings: how could *T. cruzi* aDNA have come to be incorporated into these eight coprolites?

Ingestion

Transmission of Chagas disease to humans normally is carried out by one of the blood-sucking insect vectors of the Reduviid family, usually *Triatoma infestans*. Defecation of trypanosome-laden vector's feces on the human's skin follows the vector's bite and blood meal. The trypanosomes gain access to the human tissues when the itching wound is rubbed, dragging the trypanosomes from the skin surface into the wound or onto the conjunctiva. However, oral ingestion is a well documented mechanism. It has occurred in humans as a laboratory accident while pipetting a solution of *T. cruzi* (Brener 1984). Experimental transmission has been observed by feeding an infected vector insect (*Rhodnius prolixus*) to hungry opossums (Yaeger 1971) and also by feeding both infected mice carcasses and infected vectors to wood rats (Bastien 1998:194; Marsden, 1962). Acha and Szyfnes (1991) suggest that the high frequency of infected cats in households with such vectors is the result of domestic cats feeding on infected mice.

Ingestion of infected, uncooked meat

In our tested mummies, oral transmission may have occurred by ingestion of infected, uncooked meat. One source of such meat may have been guinea pigs (*Cavia porcellus*). Many authors have noted that the common Andean practice of raising guinea pigs for consumption in cages within human dwellings invites infestation by infected Chagas insect vectors (Herrer 1964). From 10 to 61% of such guinea pigs have been found to be infected by *T. cruzi*. Guinea pigs thrive at altitudes up to 4000 meters and serve as hosts, reservoir and disseminators of this infection (Herrer 1964). At least some of our studied mummies were part of a cultural group (Chiribaya, A.D. 900-1,100) whose excavated dwellings included such guinea pig cages (Dittmar de la Cruz 2003). We know that at least some of our studied mummies' diets included consumption of uncooked meat because their coprolites contained ova of the fish tapeworm *Diphyllobothrium pacificum* (Araujo et al. 1983). Infected vectors prowling a house at night are also known to defecate on surfaces they traverse. These surfaces could include exposed food items, such as meat exposed to the open air to dry following the butchering of a large mammal. We may conclude that consumption of infected, uncooked meat is a candidate for explanation of finding *T. cruzi* aDNA in our mummy coprolites.

Ingestion of infected head lice

We also considered the possibility of transmission by head lice (*Pediculus humanus*). The usual Chagas disease vector in domiciliary settings such as *Triatoma infestans*, tolerates completion of the *T. cruzi* life cycle within its intestines after it ingests an infected blood meal. The parasite flourishes in the vector insect's rectum ("posterior station"). When it ingests a blood meal, the insect expels its rectal contents (teeming with *T. cruzi*) on the victim's skin surface. The *T. cruzi* are carried into the wound or nearby conjunctiva when the victim rubs the itching wound area. *T. cruzi* cannot survive the head louse's intestinal environment (Khachorian and Arakelian 1978). However, if the head louse were ingested by a human shortly after its bite, the *T. cruzi* released in the ingesting human's intestine could infect that individual. Precedence for such a sequence of events has been

reported by Argañaraz et al. (2001) where infected lice, ingested during observed "grooming" (removal of lice by a primate from another primate and then promptly eats the lice), apparently spread the infection throughout a baboon primate research colony. Some of our mummy populations, especially the Chiribaya (listed in Tables 1 and 2) had a heavy affliction of head lice. Testing of 57 individual lice collected from the mummy hair of that cultural group demonstrated *T. cruzi* aDNA in two of these that had a positive reaction (Table 3).

While the lice infestation was obvious in some of our tested mummy populations, we have no direct evidence that such individuals indulged in grooming. We do know that some Native North Americans did indulge in grooming practices. Of these, the most well-documented is by Samuel Hearne (1958:128-129) during his famous trek from the Hudson Bay post to the Coppermine River and back. Thus, while the evidence is weak, we cannot exclude ingestion of infected head lice as an explanation for finding *T. cruzi* aDNA in mummy coprolites.

Indirect ingestion

A third possible source of *T. cruzi* ingestion (indirectly) is the conceivable lodging of peripheral blood flagellated *T. cruzi* parasites in pulmonary capillaries. Their size is such that this might occur occasionally. Some of these could escape from the capillary of the lung's interalveolar septum, migrate into the intra-alveolar space, enter the terminal bronchiole and be swept upward on the cilia-propelled mucous blanket lining the bronchi, being ultimately discharged into the pharynx and then swallowed, entering the intestinal tract. While this is conceivable, it has no supportive or experimental evidence.

Parasitemic Invasion

Experimental evidence in acutely infected chagasic mice has demonstrated histological lesions of acute, necrotizing arteriolitis in the smooth muscle of the intestinal wall (Okumura et al. 1960). Extension to the gut mucosa of such necrotic areas could provide *T. cruzi* access to the intestinal content. Our test results (Table 1) indicate that *T. cruzi* was found only in coprolites of mummies whose soft tissues were also positive. Certainly

parasitemic invasion of the intestinal wall remains a candidate for explaining our results.

T. cruzi DNA incorporation into the host genome

Investigations by Teixeira et al. (1994) and Nitz et al. (2004) have indicated that *T. cruzi* kinetoplast and also nuclear DNA can be integrated into the host genome. The cells of the human gut mucosa turn over rapidly, with complete replacement every 4 to 6 days (Kumar et al. 2005:829). If such DNA integration occurred in our tested mummies, *T. cruzi* DNA may have been carried into the tested coprolites by exfoliated cells of the intestinal mucosa. Until the host genome of our mummies is investigated for such possible integration, this mechanism remains a possible explanation of our results.

Postmortem migration of T. cruzi parasites

Most of our tested mummies became desiccated via spontaneous mummification. Under these conditions the intestinal mucosa degenerates rapidly immediately after death prior to arrest of decay by desiccation in many of the other body tissues. This

reduces the gut wall to a thin, translucent membrane. The mucosa with its blood vessels that may contain parasitemic *T. cruzi* parasites liquifies after death and can be absorbed into the coprolite in the gut lumen. This appears to be the most probable of the possibilities discussed above, a conclusion supported by the statistically significant observation that *T. cruzi* aDNA was present in coprolites only in those mummies in whom it was also present in the mummies' soft tissues.

Conclusions

T. cruzi aDNA was found in coprolites of spontaneously mummified mummies' intestines. A review of the possible routes of *T. cruzi* transport, reaching intestinal coprolites includes ingestion, parasitemic dissemination, host genome incorporation of *T. cruzi* aDNA and postmortem migration. While any of these are conceivable, the postmortem route appears to be the most probable. It is clear that the presence of *T. cruzi* aDNA in the coprolites of mummies cannot always be considered evidence of *T. cruzi* ingestion during life.

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