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CONTENT

EDITORIAL

The university journal PROSPECTIVA is recognized as an official document endorsed by the Directorate of Scientific Research and Technology of the Universidad Mayor de San Simón of Cochabamba, Bolivia (DICyT – UMSS). It stands out by publishing a wide variety of scientific articles. These are prepared by outstanding national and international academics who address a diversity of topics from different perspectives. This multidisciplinary approach, based on collaboration between different academic disciplines, has made it possible to enrich the value of the journal, as well as expand the breadth and depth of its thematic contents.

During its history, the DICyT- UMSS scientific journal has achieved a significant circulation that developed from the presentation of high quality academic and scientific research. PROSPECTIVA's key goal is to provide the university community, public and private institutions, as well as civil society, with a resource foundation of important thematic issues for generating strategies that enable the development of public policies. Through high academic and scientific quality, PROSPECTIVA has managed accomplish its established objective. To date, its multidisciplinary approach has generated debates around various topics such as sustainable development, urban planning, cultural heritage preservation, health, history, fossil exploration, mitigation of climate effects, and environmental protection. Included within this intellectual purview are archaeological research and paleopathological explorations such as the study of diseases in zooarchaeological and human remains from bioarchaeological contexts,

Given that approximately 79% of global publications are in English, the PROSPECTIVA journal has introduced, in this new issue, the publication of articles in two languages; English and Spanish. This approach enhances the scope and visibility for both researchers and interested readers and fosters greater opportunities for reading and citation which are the primary interests of any esteemed academic publication. Therefore, starting with this issue, the journal's publications will be available to the reading public in both languages.

Herein, the topic "Environment and Health since Antiquity" will be treated as a special issue in English, with the aim of covering a wider variety of fields and countries. This initiative seeks to satisfy the inclusion of English-speaking professionals who have contributed to the promotion of our institution in conferences, symposia, seminars and other events held in our city and by our professionals. In addition, it highlights the excellence of activities carried out in different contexts. Ultimately, it promotes scientific collaboration and interaction with international colleagues from around the world, thereby enhancing academic and research networks. At a global level, this strengthens ties between international universities with our academic institution.

It is essential to highlight the dedication and work carried out to prepare this document. It was enriched by an outstanding editorial board, recognized both nationally and internationally, in its review process. Additionally, it is important to highlight the valuable participation of expert translators in Applied Linguistics and Language Teaching from the Faculty of Humanities and Educational Sciences of the Universidad Mayor de San Simón. These professionals have provided their invaluable collaboration in the editing process of important English contributions submitted to this issue.

Like all scientific journals regularly produced at UMSS, including PROSPECTIVA, this issue was prepared by a team of professionals specialized in academic editing. They significantly help local and international researchers establish academic networks and provide quality platforms to share their research.

This special issue of the journal PROSPECTIVA highlights a selection of works presented during a prestigious international event held in 2023. The event, known as the "IX PAMinS,", is linked to the celebration of the Paleopathology Association Meeting in South America that took place in Cochabamba, Bolivia. The event's theme, "Climate events, environmental changes, mobility and health in the past", brought together leading experts in the field of research and analysis dedicated to the historical reconstruction of ancestral population's lives.

In this issue, Karl Reinhard, an expert in the field, presents research that exemplifies the historical context of these events and emphasizes the importance of complex research networks. These networks have played a fundamental role in the dissemination and expansion of various research lines and their interconnectedness. Moreover, the significant internationalization of research in events of this scale has been greatly supported by renowned academic institutions in Europe and the United States. Additionally, this article analyzes the program showcased at the congress and introduces the special issue of the journal PROSPECTIVA.

Multidisciplinary university journals play a crucial role in facilitating the exchange of essential scientific and research knowledge, contributing significantly to global academic discourse and promoting scientific progress. However, the management and editorial supervision of these journals are constantly at risk of instability, interruption, or even discontinuation. Consequently, to preserve the vital functions of university publications and ensure their continued existence and scholarly improvement, host institutions must establish effective incentives to encourage active and dedicated participation from academics.

We would like to take this opportunity to sincerely thank all the researchers for their distinguished and significant contributions that have made this issue an essential document in the field of scientific research. We are delighted to invite all those who are interested in exploring this specialized topic, which offers a wealth of enriching and inspiring perspectives. We hope that this material will serve as a comprehensive guide to fully comprehend the subject matter discussed in Paleopathology, and to gain a detailed understanding of the potential implications that may arise in modern societies.

Nancy Orellana Halkyer, Ph.D.
Editor-in-chief

PRESENTATION

The Paleoparasitology and Paleoepidemiology Research Group linked to Escola Nacional de Saúde Pública Sergio Arouca - ENSP, through the Department of Endemic Diseases Samuel Pessoa, is one of the oldest research groups at Oswaldo Cruz Foundation – FIOCRUZ that was formalized at CNPq – National Research Council of Brazil. It was registered in 1988 by Adauto Araújo, a very well-known paleoparasitologist, and an enthusiastic scientist of our times.

This research group was one of the pioneers who developed a line of research named by Luis Fernando Ferreira in 1978 PALEOPARASITOLOGY. Stimulated by Aidan Cockburn, one of the founders of Paleopathology Association, during his visit to Brazil, the group was pioneer in investigating the long history of parasitological diseases up to the present. Since then, the group´s trajectory has continued to expand the scope of its work and contributions from the different professionals as researchers, students and collaborators from different modalities. Today, working on a wide spectrum of topics and incorporating advanced techniques such as image studies, computed tomography, and genomics research, it maintains continuous scientific production in national and international books and journals.

A few decades later, and once again, our group was strongly encouraged by the team of the Paleopathology Association to go forward. Jane Buikstra, Della Cook, Mary Lucas Powel, Ana Luisa Santos and others were the mothers-in-law supporting the creation of a new meeting for the Paleopathology Association to be hosted in South America. This meeting should be an open door to promote professionals and students from our Continent, like has been done for years in Europe. Exactly 20 years ago, we had the honor to organize at the ENSP-FIOCRUZ the first PAMinSA – Paleopathology Meeting in South America, a memorable meeting with great international participation. The success of PAMinSA was ensured by the strong ties that already united colleagues inside and outside Brazil, and a great attractive congress to paleopathologists of Northern Hemisphere.

With a strong movement of national and international cooperation as its trademark, the FIOCRUZ Research Group in Paleoparasitology and Paleoepidemiology took charge as the main organizer of this first PAMinSA, whilst encouraging a strong spirit of cooperation and exchanges. For two decades, PAMinSA has developed a culture of shared production of knowledge, training of students and professionals, healthy coexistence and the joy of being South America, trying to expand its collaborative network inside and outside our continent. Since then, this has been the permanent feature of our meetings, the ninth edition of which took place in Cochabamba in 2023. In this volume of Prospectiva some works presented there are published.

As we approach 40 years of accreditation of this Research Group with the CNPq, the highest funding and accreditation agency for Brazilian researchers, we are proud to be this group whose legacy is represented by four main tracks: CPFERA – Paleoparasitological and Recent Animal Feces Collection, with thousands of ancient as well as modern specimen, one of FIOCRUZ's scientific collections; an expressive scientific production; a sustained compromise of permanent contribution to the talents and professional vocations; and last, but not least, the successful maintenance of a South American network contributing to knowledge dissemination activities, empowered by the biannual PAMinSA.

It is from our historical coherent place, and close to our 40th anniversary as a Research Group, that we congratulate Dr. Nancy Halkyer and the other colleagues who, embracing these purposes, have been helping us to sustain our meetings in South America to the present days, looking forward to celebrate in 2025 the 20th anniversary of PAMinSA!

Sheila Mendonca de Souza

Andean paleoparasitology: from the Tres Quixotes to PAMinSA IX

Paleoparasitología Andina: de Los Tres Quijotes a PAMinSA IX

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Abstract

The decades from 1990 through today have seen exciting paleoparasitology research in the Andes. Beginning in 1997, a research consortium was formed between the Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz (FIOCRUZ) and the School of Natural Resources, University of Nebraska (UNL). We conducted a paleoparasitological survey of central Andes. From 1997-2001 our focus was on samples from Chile and Peru. "Investigations of Chagas Disease" was the second topic which ran from 2002 to 2007. "Paleoepidemiological Transitions in Paleoparasitology" was a final project from 2012 to 2019. The published highlights from this work were a collection of articles published in the Memórias do Oswaldo Cruz (2003) and a volume *Fundamentos da Paleoparasitologia*. The latter work was awarded the Prêmio Jabuti Book of the Year in Natural Science in 2012 and was translated to English in 2014. Researchers at FIOCRUZ and UNL, participated in conferences and advisorial trips to labs in neighboring countries. The research presented at the 2023 PAMinSA shows sophistication of the field. There, participants presented work on animal parasites, mummy studies and arthropod parasitism showing continuation and expansion of older work. The application of AI, species distribution modeling, next-generation sequencing and shotgun DNA analysis are new approaches that offer high information yield. A new synthesis of Chagas disease tracing is ancient trypanosomid origins during continental drift to its current health status was presented. Significantly, paleoethnobotany and ethnography were displayed essential areas for understanding human adaptation to parasitic disease. The PAMinSA IX shows labs with diverse approaches strengthened the field in Argentina, Bolivia, Brazil, Chile and Peru.

Keywords: Paleoparasitology, international connections, IX PAMinSA

Resumen

Desde 1990 hasta la actualidad se desarrollaron importantes aportes a la paleoparasitología. En 1997, se formó un consorcio de investigación entre la Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz (FIOCRUZ) y la Escuela de Recursos Naturales de la Universidad de Nebraska (UNL) con el objetivo de realizar estudios paleoparasitológicos en los Andes centrales. Se analizaron muestras de Chile y Perú, mientras se editaba un volumen sobre Paleoparasitología. "Investigaciones de la enfermedad de Chagas" fue el segundo tema financiado desarrollado entre 2002 y 2007. "Transiciones paleoepidemiológicas en paleoparasitología" fue un proyecto final que recibió apoyo de 2012 a 2019, que finalizó con la publicación de artículos en Memórias do Oswaldo Cruz (2003) y un volumen Fundamentos da Paleoparasitologia. Este trabajo recibió el premio Prêmio Jabuti Libro del Año en Ciencias Naturales en 2012, y fue traducido al inglés en 2014. Luiz Fernando Ferreira y Adauto Araújo, fueron fundamentales en este trabajo. FIOCRUZ y UNL recibieron investigadores, quienes participaron en conferencias y viajes de asesoramiento a laboratorios vecinos, promovimos la paleoparasitología. La investigación sobre parásitos de animales antiguos, la ampliación de los estudios de momias y la exploración del parasitismo de artrópodos. La aplicación de IA, el modelado de distribución de especies, la secuenciación de próxima generación y el análisis de ADN son enfoques nuevos que ofrecen un alto rendimiento de información. El congreso presentó un nuevo abordaje de la enfermedad de Chagas, que rastrea los orígenes de los tripanosomatideos durante la deriva continental hasta la situación actual. La paleoetnobotánica y la etnografía se mostraron como áreas esenciales para comprender la adaptación humana a las enfermedades parasitarias. El IX PAMinSA mostró diversos enfoques que han fortalecido está línea en Argentina, Bolivia, Brasil, Chile y Perú.

Palabras clave: Paleoparasitología, conexiones internacionales, IX PAMinSA

BEGINNING OF COLLABORATION

In the 1980s, a collaboration developed to trace the origins of parasitism in the Andes. Past reviews have focused on the important relationship between Brazilian institutions such as the Brazilan National School of Public Health, FIOCRUZ, and the Museu Nacional de Brasil with the Pathoecology Lab at the University of Nebraska – Lincoln (Araujo et al., 2011; Araujo et al., 2016). At these institutions, physical facilities dedicated to researching Latin American parasitism were constructed. The heart and soul of that collaboration was the participation of many more researchers and institutions in Brazil, Peru, Chile, Argentina, and the US. This personal review summarizes my memories of those interactions and highlights a new generation of researchers taking the field into directions only imagined by those of us who began the research into ancient parasites nearly 50 years ago.

Eve Cockburn and Art Aufderheide drew my attention to Luiz Fernando Ferreira, Adauto Araújo, and Ulisses Confalonieri at the Paleopathology Association conference in 1985., I had become aware of their work through their early papers (e.g., Ferreira et al., 1980; 1983; Confalonieri et al., 1981; 1985). I wrote to Ulisses and Luiz Fernando in 1985 and proposed a collaboration. We soon published our first paper together on methods (Reinhard et al., 1986) and planned to continue collaborating as I began my doctoral studies. The 1986 Paleopathology Association meeting was central to initiating research with Brazilian colleagues. I organized a session on Paleoparasitology that included contributions from Adauto Araujo and Ulisses Confalonieri from the Brazilian Oswaldo Cruz Foundation (FIOCRUZ). Ulisses Confalonieri was able to participate in person. In him, I found a colleague who was as intrigued as I by the ancient origins of parasitism. I was among many graduate students who gained guidance from this Brazilian paleoparasitology community.

In 1994, my partner, Deb Meier, the exhibits preparator for the University of Nebraska State Museum, and I met with Luiz Fernando, Adauto, and Sheila Mendonça de Souza in Rio de Janeiro. For a week we exchanged ideas about collaboration between our museums and laboratories. When we parted, we had formed a family of people with like interests and psyches. One commonality of interest was the parasitism of the prehistoric Andes. The Brazilian Lab had already collaborated with Lautaro Núñez on Chilean parasites (Araujo et al., 1983; Ferreira et al., 1984). In 1994, Deb and I were deeply into work in the Andes. As a group, we decided our goal would be to trace Andean human parasite origins and distribution. Deb and I had already been working from 1988 through 1996 with Jane Buikstra, Sonia Guillén, Art Aufderheide, Guido Lombardi, Bernardo Arriaza, and Calogero Santoro exploring the paleoepidemiology of human parasitism.

Some of our first University of Nebraska students were hosted by the Programa Contisuyo, Centro Mallqui, the Museo de Sitio Universidad de Tarapacá San Miguel de Azapa, and, eventually, the Instituto de Alta Investigación - Universidad de Tarapacá. Formal agreements allowed samples from some of these to be shared, and those collaborations formed the basis for the study of human parasite origins in the Andes. With funding from CNPq, in 1997-98, Adauto, Luiz Ferreira, and I analyzed those samples. In 2000, the University of Nebraska allowed me to be based at FIOCRUZ from January to August annually. In 2001, we initiated an exchange program began to bring Brazilian researchers to train in my laboratory at the University of Nebraska in cooperation with the Manter Laboratory of Parasitology at the University of Nebraska State Museum. In 1997, Deb and I spent some months with Sheila, Adauto, and Luiz Fernando, working in the Museu Nacional and I working in the Paleoparasitology Lab. In parallel, Sonia Guillén inspired Dennis J. Richardson and Dan Holliday to work on parasites of dogs and humans from Chiribaya Baja, Peru (Holiday et al., 2003; Richardson et al., 2012).

THE TRES QUIXOTES

These are the words of Luiz Fernando Ferreira from the Presentation of the *Foundations of Paleopathology* (Ferreira, Reinhard, Araújo, 2014). That was his perception of the three of us that emerged as we collaborated in the paleopathology lab or his office in the years between 1997 and 2010.

"The following tale, attributed to one Ludovicus Tertinus Guanabarinus, circulates in the laboratories and corridors of Manguinhos (the campus of the Oswaldo Cruz Foundation in Rio de Janeiro).

Before setting off for war, Don Quixote stopped to visit the local tavern. There he met a young man, whom he asked:

"Is your name Sancho?"

"No," replied the man. "My name is Quixote."

Don Quixote found this rather odd, but proceeded:

"I am going to war and need a squire. Will you follow me?"

"Yes," replied the other.

On their journey, they met another character who, oddly enough, was also named Quixote. He came from faraway lands. From Alaska, people said. And the three set out together to wage war for many long years."

FIGURE 1. Luiz Fernando Ferreira's perception of the Tres Quixotes.
Source: Eduardo Pucu de Araújo, (2014).

Throughout the years together, he reminded us of our identity as the Tres Cavalheiros de Paleoparasitologia and our Quixotesque goal of wrestling ancient parasites from the earth. We rode against the conventional wisdom of the time that prehistoric humans arrived in the New World unencumbered by parasites from their lands of origin. During the 1980s and 1990s, we found ourselves defending our scientific findings on battlefields such as *Transactions Royal Society of Tropical Medicine and Hygiene, Parasitology Today and Medical Anthropology*. In those decades, we learned that persistence as well as evidence is necessary to successfully challenge a prevailing world view.

Luiz Fernando was certainly our leader and guide (Figure 2). He was the older, established figure with an honorific history of landmark research.

He was charismatic, gregarious, flamboyant, and generous. He was highly respected and warmly, even deferentially, welcomed into research offices and scientific gatherings everywhere. My favorite moments with him were his introductions of me at professional conferences, "Gostaria de apresentar meu caro amigo . . ., pioneiro da paleoparasitologia . . . , figura importantíssima na ciência brasileira. . ., Karl Reinhard." The pauses in his introduction highlighted each honor he bestowed on me. While flattered, I was also aware that I was expected to live up to his kind words. His command of the audience and complimentary words always made me feel important and empowered me to present my work in broken Portuguese.

My cherished evenings with Luis Fernanddo began when we left FIOCRUZ after work and go to the city center. There we would haunt the neighborhoods of Cinelândia, Centro, Uruguaiana, Carioca, and surroundings where he taught me the history of those places. We stopped in the poorest pé sujos (very rustic corner restaurants) for sandwiches and coffee. He reveled in mingling with the less advantaged strata of the city and emphasized that all Carioca demographics had something to offer historically and practically. Because I was learning Portuguese, we only spoke in Portuguese. If I did not understand a detail, he patiently rephrased it over and over until I understood. I never knew he spoke English until Deb arrived two months into my grant period. I nearly fell off my lab stool to hear him say, "Welcome to Rio, Debbie, my house is your house" when she walked into our lab.

For me, his most endearing trait was his gravitation toward people of nearly invisible social status. In our last year working together, FIOCRUZ workers would gather in the Triagem neighborhood to take a daily charter bus to work. Most people went to upscale breakfast bars. However, one morning I was passing the poorest, smallest establishment that offered only coffee, bread, and sometimes eggs and there was Luiz Fernando chatting with the proprietor over coffee and a newspaper. I joined him daily after that. When I asked him why he preferred the poor restaurant and the pé sujos in the city center. He replied, "Eu sou um homem do povo, Karl". I realized then that his life of practicing medicine came from his empathy for people of all sorts and their struggles.

Luiz Fernando must have realized from our first meeting that Adauto and I were kindred spirits. Scientifically, we were perfectly matched. Aduato traveled the world to research paleoparasitology and I felt lucky to join him when I could (Figure 3). He was a true scholar and bibliophile. He had the latest literature as well as very old foundational works at the front of his brain and the tip of his tongue. In contrast, my strength was data collection in an interdisciplinary arena. His strengths were parasitology first and archaeology second, mine were archaeology first and parasitology second. This provided us with intermeshing interpretive constructs as we pondered the parasitological past, pursuing the archaeology of parasite transmission. We traveled across the Americas and drove thousands of km in the Southwest US to visit the "hotspots" of ancient parasitism.

Our friendship was based on much more than science. Our families meshed and we spent vacation time together. During extended exchanges, we shared each other's homes. Together, we feasted and discussed all topics from novellas to the environment. Adauto had loves for his wife and family, art, music, and the beauty of nature. I have similar loves. A unique character of Adauto was his universal acceptance by strangers we encountered in our life together. He genuinely cared about people. Because of that, all sorts of people warmed to him immediately at the first meeting. Whether in eateries, ranches, reservations, or cowboy hangouts, he was welcome. Even border guards were soften in his presence. In short, everyone we met in our travels recognized the special, quiet charisma that shone from Adauto. He exemplified what people should strive to be: intellectual, empathetic, nurturing, humorous, and gracious.

Adauto had the power of gentle persuasion and could do what neither I, nor Luiz Fernando could do: convince other parasitologists and scientists to join our paleoparasitology efforts. Adauto's encouragement could lead even the most recalcitrant, self-serving personalities into collaborations. He helped people understand they could benefit more from cooperation than isolation. In this way, he extended friendship and support across borders to create a global network of paleoparasitologists. There is no doubt that the unique humanity of Adauto Araújo built our field as we know it today.

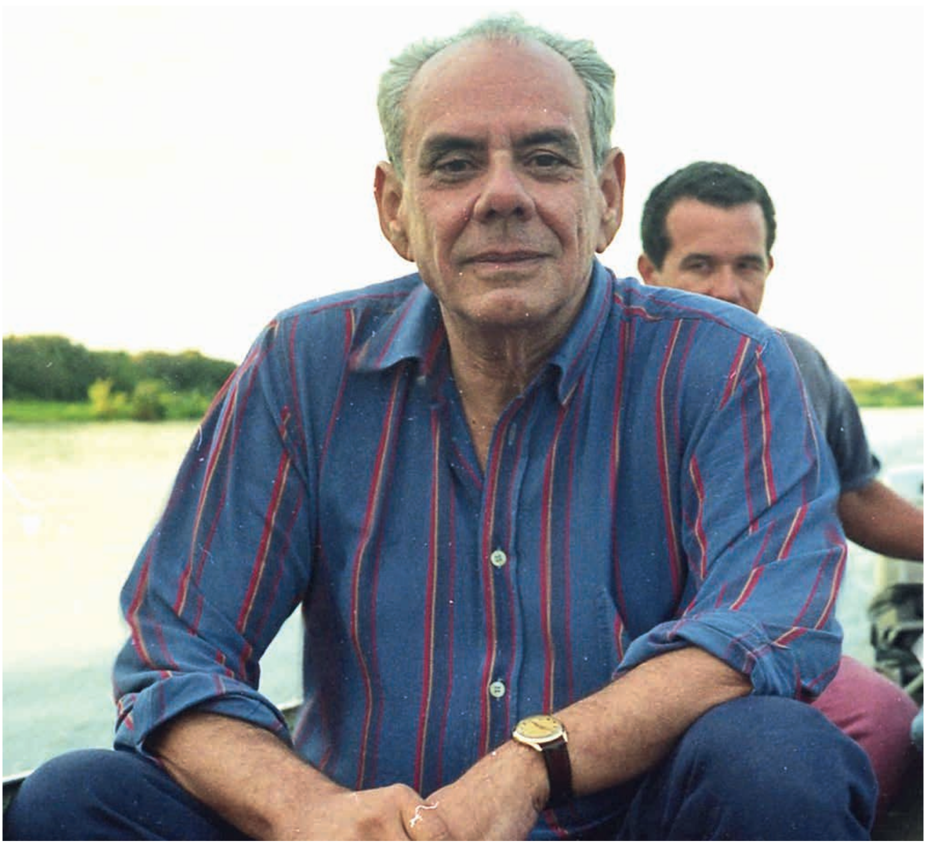

FIGURE 2. Luiz Fernando Ferreira on a vaccination campaign in the Pantanal with the Universidade Federal de Mato Grosso do Sul.

Source: Author´s file.

FIGURE 3. Adauto Araújo, Bernardo Arriaza and the author. Picture taken at the conclusion of a discussion that resulted in the publication of our ideas of ENSO affects on tapeworm infection (Arriaza et al, 2010). Bernardo Arriaza is a key colleague in studies of lice as well.

Source: Author´s file.

THE FOURTH QUIXOTE

I always felt that Sheila Mendonça de Souza was the "Fourth Quixote'. Her energy and gift for international collaboration were an engine for our group. She has backgrounds in archaeology, public health, and paleopathology that bridges paleoparasitology with the growing fields of archaeology and bioarchaeology in Brazil. Having positions with the National Museum and FIOCRUZ, she was key gear the research machinery between the institutions. She innovated the paleoepidemiology perspective that made parasitology relevant to pathology. Most importantly, she kept us all in communication. Each of us had his own intellectual trajectory which sometimes diverged. Sheila monitored us and found opportunities to engage us in discussions to ensure a blending of our ideas. Specific to me, I tend to eliminate from consideration my own research when it is inconclusive. Sheila skillfully persuaded me to return to topics that I had abandoned. In this way, I returned to important issues such as the relation of parasitism to porotic hyperostosis that I continue to explore with our joint advisee, Morgana Camacho.

In planning and conducting conferences, I was in awe of her organizational skills. In the past and today, she is a fireball of energy as she facilitates connections at conferences. I was always amazed at her persuasive power. I distinctly remember a conference for which she convinced me to give three presentations. "Ela está a explorá-lo" a friend observed. However, she never made me feel exploited and she always helped perfect my Portuguese. Indeed, she was determined that I share all of my areas of expertise. So, if I was exploited it was a joyful experience for me that benefited our sciences. Luiz Fernando empowered me, Adauto collaborated with me, and Sheila inspired me to communicate my mind as broadly as possible.

In the museum, Sheila hosted Deb to teach a workshop on mummy conservation, complete a conservation assessment on the Egyptian collection, and work on environment-context mummy displays. She collaborated with Deb on the conservation of mummies on display from Egypt and Brazil. Sheila recognized the need for conservation, parallel to Sonia Guillén and Guido Lombardi in Perú and Vicki Cassman in Chile.

With Adauto Araújo, we made our first WCMS appearances in a parasitology symposium at the 3rd Mummy Congress in Arica (1998). That meeting included a conservation workshop presented by Deb and Vicki. We maintained our participation in subsequent WCMS meetings and had our chance to host the 2013 congress in Rio de Janeiro. Claudia Rodrigues-Carvalho organized the logistics of the conference at the National Museum with the assistance of all members of the FIOCRUZ/UNL team.

By 2003, the "Four Quixotes" were committed to developing PAMinSA. These efforts culminated in the 2005 inaugural meeting of the association in Rio de Janeiro co-sponsored by FIOCRUZ and the National Museum. Claudia was critical in making a success of this meeting for the museum. This is appropriate because I believe she was the first person to focus her doctorate on paleopathology in Brazil. PAMinSA meetings were held every 2 years in different countries up to and including 2019 in São Paulo. For me, there is a direct line between the 1995 Annual PPA Meeting, establishing a research exchange between FIOCRUZ and UNL in 1997, to the initiation of PAMinSA in 2005 and the 8th WCMS in 2013.

FIGURE 4. Sheila with Adauto, Amélia Pucu de Araújo, Deb Meier and the author at the V World Congress on Mummy Studies in Turino, Italy, 2004. This was the anniversary of our first collaboration plans in 1994.

Source: Author´s file.

SCHOLAR EXCHANGE LEADING TO THE 2003 PALEOPARASITOLOGY MEMÓRIAS SPECIAL ISSUE

Between 1997 and 2002, with funding from CNPq, CAPES and the Fulbright Commission, an exchange of senior scholars between UNL and ENSP/FIOCRUZ began. One of our goals was defining parasite distribution and prevalence in the Andes. I worked for 14 months in 1997-1998 with Adauto and Luiz Fernando in the ENSP Paleoparasitology Lab analyzing samples (Table 1) and Adauto worked with me in the Pathoecology Lab at the School of Natural Resources and with Scott Gardner in the Manter Laboratory of Parasitology, University of Nebraska State Museum. In Nebraska, we edited papers for a special issue on Paleoparasitology for the Memórias do Instituto Oswaldo Cruz. With Fulbright Commission funding, I returned to FIOCRUZ to teach and to continue editing the special issue.

TABLE 1. Provenience of 140 samples analyzed in 1997-1998 and compiled by Reinhard and Araujo (Reinhard et al., 2016).

*= ENSP-FIOCRUZ collections. Many of these studies were negative. The positive findings were included in an overview of ancient parasites in global perspective.

Site, Date before present	Number Studied	Findings
Ring Site, Peru, 7,675±60–10,575±105	6	negative
Paloma, Peru 6,000	20	negative
Mono, Peru, 2300	28	*Enterobius vermicularis*
* Caserones, Chile, 2,400–1,200	10	*Enterobius vermicularis*
* San Pedro de Atacama, Chile, various contexts (Araujo et al., 2011)	48	Reanalysis of previously published work (Ferreira et al. 1984). No new findings.
* Antofogasta, Chile, various times (Araujo et al., 2011)	28	Reanalysis of previously published work. No new findings.

Source: Gonçalves et al., (2003)

MEMÓRIAS DO OSWALDO CRUZ, VOL 98, SPECIAL ISSUE

The 2003 Memórias special issue stands as a cornerstone in Andean paleoparasitology. In his overview of the volume, Aufderheide (2003) emphasized the new paleoepidemiology approach to the collection. Souza et al. (2003) formally established the paleoepidemiogy theme with a literature review and critique based on skeletal analysis. Their methodology and goals were readily applicable to archaeological parasitology, and in this way they defined paleoepidemiology as an interdisciplinary approach to develop epidemiological methods. Specifically, they suggest that paleo researchers apply methods in current use to past populations. Based in this approach, paleoepidemiology connects disease and health evidence of archaeological lifestyles and environments. Beginning with age and sex demographics, risks to subpopulations can be defined.

Reinhard and Buikstra (2003), applied this approach to lice on Chiribaya mummies from Peru's southern Osmore drainage. They found demonstrable differences in louse prevalence and infection intensities and between males and females, and, between adults and children. They tested their data for the negative binomial distribution of parasite verdispersion.

Overdispersion (also called aggregation) refers to the universal pattern of 80-90% of parasites distributed on 10-20% of hosts. Overdispersion was demonstrated, validating the reliability of the louse distribution.

Elizabeth Martinson's dissertation introduced the concept of pathoecology (Martinson et al., 2003). This is the reconstruction and study of biotic, abiotic, and cultural determinants of infection. For the Osmore Valley of southern Peru, they described the geography, sites, and each site's role in the broader economy. They reported *Trypanosoma cruzi, Leishmania sp.*, *Adenocephalus pacificus* (formerly *Diphyllobothrium pacificum*), and T*richuris trichiura*. They explained the pathoecology of each species and assessed the disease risk posed by each species. Finally, they summarized data from guinea pig, llama, and dog mummies to establish a complete picture of the diversity of parasite-host ecological interaction in the valley. This approach has been amplified and widely applied by Araujo and Reinhard (Araujo and Reinhard, 2021; Arriaza et al., 2010; Reinhard and Araujo, 2015; 2016).

Each pathoecological analysis reveals host-parasite relations specific to a time and place. For Chiribaya lice and endoparasites in the Osmore Valley, both environment and behavior determine the ecology of parasite transmission. Santoro et al., (2003) presented an example of extreme mediation of transmission by empire. At the time of the Memórias special issue, there were substantial theoretical perspectives that had significant time depth (Black, 1975; Boyden, 1970; Cockburn, 1971). With excavations in the Lluta Valley of Chile, these theories could be evaluated with evidence-based perspectives on the influence of the Inca Empire expansion on the area. These were based on coprolites recovered from both Inka (Late Period) and pre-Inka (Late Intermediate Period) settlements. Four helminth species were recovered, *T. trichiura, A. pacificus*, a Hymenolepidid tapeworm and the pinworm, *Enterobius vermicularis*. Of these species, only pinworms showed significant variation. From the Late Period, 21% of the samples had pinworm eggs. In contrast, none of the Late Intermediate Period samples were positive. Pinworms reflect behavioral changes associated with Late Period Inka expansion. The Inka resettled the indigenous population from smaller, scattered habitations to large towns. Because pinworms are crowd parasites, it proliferates in large, condensed populations. The LIP settlements were small, family units that were dispersed in the valley. LP settlements were large villages housing several hundred or more people.

Alena Iñiguez led two research teams in Brazil in the search for ancient DNA (hereafter aDNA) in coprolites (Iñiguez et al., 2003a; b). They demonstrated that aDNA was recoverable from coprolites (2003a) and, in the second paper, that *E. vermicularis* aDNA could provide evidence of infection independent of microscopic identification of eggs. These papers marked the beginning of the 'molecular revolution' in Andean paleoparasitology.

Dittmar et al., (2003) reported aDNA from mummified fleas (Pulex). Their contribution addressed the diagenetic processes that affect insect chitin structure, protein preservation, and the problem of bacteria proliferation, and potential for arid land preservation. They addressed the considerations of aDNA extraction in detail as they presented their positive results of recovering aDNA. They considered their results as preliminary steps towards two overall goals. The first was to clarify the taxonomic relationship between *P. simulans* and *P. irritans*. The second was to evaluate the *Pulex*-complex as vectors of prehistoric infectious agents, such as plague. This led them to the exciting conclusion that flea aDNA study had the potential of addressing both goals.

Sergio Chaves contributed a paper derived from his dissertation research on medicinal plant pollen found in coprolites from Pedra Furada, Piauí, Brazil, 8450 ± 80 BP - 7230 ± 80 BP (Chaves and Reinhard, 2003). That paper summarized the diversity of botanical remains that are recoverable from coprolites and urges researchers to collect dietary data as they conduct parasite data. They then focus on the value of medicinal plant data by emphasizing that parasitism was a universal human condition. People developed herbal medicines to treat parasites as part of their pharmacopoeias derived from their local environments. They showed that it is possible to recover evidence of medicinal plants to treat symptoms of parasitic disease. They concluded that a multidisciplinary approach must be innovated involving paleoethnobotanists, paleoparasitologists, paleopathologists, and pharmacologists.

BOTANICAL ARCHAEOLOGY AS A COMPLEMENTARY FIELD WITH THE FULBRIGHT PROGRAM

Based on Chaves's *Memórias* contribution, I was funded as a Fulbright Senior Specialist in Botanical Archaeology to serve in Perú and Brazil. In both countries, the grants served to develop methods for botanical analysis. The Brazilian Fulbright workshop organized by Sabine Eggers (USP) was attended by Célia Boyadjian and Verônica Wesolowski who derived their graduate research from that experience (Boyadjian et al., 2007, 2016; Wesolowski et al., 2007, 2010). Chaves and I trained our paleoparasitology students in botanical analysis increasingly from 2005 onwards. The integration of parasitology and dietary studies became hallmarks of some of our students (Camacho and Reinhard, 2019; Pucu et al., 2020; Teixeira-Santos et al., 2015). Isabel Teixeira-Santos championed dietary analysis for our lab and contributed to studies in Brazil, Chile, Sicily and the USA (Piombino-Mascali et al., 2013; Reinhard et al., 2013; 2019; Sianto et al., 2012; Verostick et al., 2019; Vinton et al., 2009). Through her, our lab had a broad geographic application of botanical archaeology on three continents.

INVESTIGATIONS OF CHAGAS DISEASE

As we were submitting the final edits of the *Memórias* special issue, we received our first grant to work on the origins of ancient Chagas Disease. These grants supported us and our colleagues, particularly Katharina Dittmar and Ana Maria Jansen, from 2002-2007. My interest was stimulated by my findings of megacolon in a prehistoric mummy found on the border of Texas and Coahuila and then in 2 of 28 Chiribaya mummies in the Osmore Valley. Thus, by 1990 I was aware of megacolon in North and South America. If Luiz Fernando and Adauto agreed with me that if those cases were related to *T. cruzi* infection, we needed a new theoretical framework to explain Chagas in both South and North America.

As Tres Quixotes, we sometimes speculated about the history of science if Charles Darwin had not suffered from Chagas Disease. During his most productive years, his daily work was stymied by lost hours in sickness. Would his work have been completed earlier? Would he have taken an active role in popularizing his theory? Although Chagas disease and *T. cruzi* were not described during his life, Chagas disease probably caused his chronic disease. He took notes at the time he was infected by a kissing bug while riding across the Argentine pampas. "We slept in the village, which is a small place, surrounded by gardens, and forms the most southern part, that is cultivated, of the province of Mendoza; it is five leagues south of the capital. At night I experienced an attack (for it deserves no less a name) of the Benchuca (a species of Reduvius), the great black bug of the Pampas. It is most disgusting to feel soft, wingless insects, about an inch long, crawling over one's body. Before sucking, they are quite thin, but afterward become round and bloated with blood, and in this state are easily crushed. They are also found in the northern parts of Chile and in Peru" (Darwin, 1845: 330). As evolutionary biologists, we Tres Quixotes were interested in at least determining whether Darwin's nemesis had a prehistoric genesis.

We began our published research at the turn of the 20th century with a paper describing

T. cruzi aDNA based on endoscopically recovered tissue from Chilean mummies conserved at the Museo Arqueologico de San Pedro de Atacama (Ferreira et al, 2000). This was one of three seminal papers on the topic based on an analysis of Chilean mummies, two by a separate team (Guhl et al., 1999; 2000). As we investigated the paloparasitology of *T. cruzi*, research into its molecular biology was advancing by other researchers as reviewed by Jansen et al., (2020). This research led to the discovery of seven discrete *T. cruzi* typing units (DTUs), TcI to TcVI plus TcBat (Jansen et al., 2020). It appeared that all DTUs had a common ancestor that evolved into two groups, one constituted by TcII and the other comprising the variants TcI, TcIII, and TcIV (Barbera at al., 2024). TcV and TcVI then evolved via hybridization between TcII and TcIII. TcBat diverged from all other DTUs although retaining an affinity to TcI. Thus, we had a molecular taxonomy to incorporate in a new theory of the origin and spread of Chagas.

The first regional focus for Chagas paleoparasitology research was the Andes (for review see Barbera et al., 2024). Gross pathology was described from the autopsy evidence from 35 mummified remains from a site at Tarapaca, Chile, dating from 470 B.C. to A.D. 600 (Rothhammer et al., 1985). Nine exhibited clinical megacolon symptoms. Two of these showed cardiomegaly and a third an enlarged esophagus. This initial investigation spurred intensive research for that eventually led to the large sample landmark study that revealed *T. cruzi* DNA in pre-Columbian mummies (Aufderheide et al., 2004). Those researchers analyzed 283 naturally desiccated mummies from northern Chile and southern Peru. They represented many phases of cultural development dating from 9,000 years ago to the times of the Spanish conquest. *Trypanosoma cruzi* aDNA was found in 115 individuals. Across all time periods, 40.6% of the samples were positive for infection. This demonstrated the long association of humans and Chagas disease through established sylvatic cycles before the first human settlement (Aufderheide et al., 2004), in hosts that became reservoirs upon the arrival of humans. Continued research indicated that the domiciliation of the transmission began with permanent occupations (Guhl et al., 2014). Permanent habitations and animal domestication favored the establishment of wild triatomines within the dwellings. Following a long period of cohabitation and transmissibility within homes, the cycle became domesticated.

Orellana-Halkyer (2008) expanded *T. cruzi* DNA to Bolivia. She analyzed 29 mummified individuals for aDNA. Eleven of these individuals were positive. Of these, ten came from modern endemic areas for Chagas disease. One, however, was from Tiwanaku. This individual was probably infected due to commercial exchange. He was probably infected somewhere beyond Tiwanaku in an endemic area and then died in the highlands of Tiwanaku. Thus, studies traced *T. cruzi* to a variety of environments and cultures in the Andes.

Once DTUs were characterized, paleoparasitology revealed the parasite's diversity and population structure across time and geography. Chinchorros became infected with DTUs TcI and TcIV, associated with sylvatic cycles (Guhl et al., 2014). Chinchorro lifestyle was characterized by fishing/ hunting/gathering and small ephemeral settlements. These infections were probably fortuitous, as susceptible humans intruded into the original disease cycle involving wild mammals and triatomines.

DTUs were traced through molecular analysis of mummies from the Azapa Valley, Chile (review by Barbera et al., 2024). By 1,000 BCE, agriculture and pastoralism were important for the subsistence foundation. Villages were composed of stable walls of adobe around a reed core. These were ideal triatomine niches. Domestic animals in close proximity served as reservoir *T. cruzi* hosts. From this time, two individuals tested positive for infection with *T. cruzi* II.

Today, TcII has a strictly domiciliated cycle and is strongly associated with the domestic triatomine vectors. From the Middle Horizon (ca. 550–1000 CE) and on to the Late Intermediate period (ca. 1000–1450 CE), TcII was joined by TcV, and TcVI. These strains are only observed in domiciled infections. Thus, finding them 1,600 years ago (Cabuza, Chile) demonstrates a long history of the intradomiciliary cycle. Srprisingly, TcBat was identified from the heart tissue of two individuals dating Cabuza (A.D. ~400) and Camarones (A.D. ~900–1350). In modern times, it was isolated only once in humans (Barbera et al., 2024), perhaps suggesting the potential extinction of an ancient parasite lineage.

As for the Tres Quixotes, we were intrigued by the possibility that Chagas disease was a problem beyond the prehistoric Andes. If discovered outside of the Andes, that would change the prevailing theory of the origins of Chagas.

It was the prevailing 20th century theory that Chagas disease in humans emerged 8,000 to 6,000 years ago when Andean peoples domesticated guinea pigs. By raising the animals indoors, people created a household environment that attracted hematophagous insect vectors of *T. cruzi*. Then, the development of grain storage attracted granivorous mammals that contributed to establishing Chagas disease in early agriculturalists. Wood and packed earth were used to make house walls which were ideal environments for triatomine 'kissing bug' vectors of *T. cruzi*. Thus, the ideal conditions domiciliation of Chagas disease were created according to this theory. Triatomines took blood meals from humans and domestic animals. Importantly, the long-term adaptation of triatomines human dwellings, over centuries, resulted in irreversible genomic alterations and the emergence of variants of *T. cruzi* that tended towards human specificity. We came to believe that the prevailing hypothesis of Chagas origins was wrong, and began to search for evidence in Brazil and North America,

Far from the Andes, a case of megacolon was found by a rancher in the 1930s in the Lower Pecos Canyonlands at the Coahuila-Texas border. I had presented this case as probable evidence of Chagas disease. In 2002, Adauto and I drove the 3,200km roundtrip from the University of Nebraska to Langtry, Texas. It was in the Lower Pecos where I became aware of Adauto's unique acceptance by seemingly everyone. For the last 680km of the drive to Langtry, there is only one city, San Antonio, and one large town, Abilene. We enjoyed stopping at the smallest communities for food and fuel. Across the plateaus and into the canyons, we saw only the rugged individualists that populate west Texas. They were fascinated by the convivial Brazilian who proactively engaged with people in all places we stopped. Even the border patrolmen were taken with Adauto as he explained that he was in the USA to study paleoparasitology and mummies. One patrolman removed his sunglasses when he heard Adauto say Chagas disease and began listening attentively. Chagas disease is an endemic in the Texan desert. They continue to talk about the importance of mummies. I realized then that Adauto charmed people trained to be uncharmable. Mummies, Chagas disease, and paleoparasitology were the common topics of conversations with strangers across the arid lands of west Texas.

The famous rancher, Jack Skiles, had preserved the mummy that was discovered by his father. Jack played a significant part in the eventual diagnosis of *T. cruz*i from the mummy. Jack was a botanist and developed a botanical garden of native plants in Lagtry. He also wrote a history of Langtry. In the 1800s wild west, Langtry was the "only law west of the Pecos". Jack preserved the archaeology on his land and hosted researchers and excavations. In prehistory, the canyons saw a proliferation of hunter-gatherers over 11,700 years. They left a rich archaeology in the arid caves of the area. They are most famous for rock art, but their textiles, stonework, and portable paintings were also intriguing and became more elaborate, especially between 7,000 and

2,000 years ago. The mummy was radiocarbon dated 780 years ago, relatively recent in the chronology of the area.

Katarina Dittmar joined us, and Jack led us through his Eagle Nest Canyon to visit rock shelters and the habitats of triatomines. We captured a triatomine to be tested for *T. cruz*i. Also, Jack allowed us to sample the mummy for a molecular test of *T. cruzi*. In 2003, we published the positive results for the mummy, corroborating the megacolon diagnosis of Chagas Disease (Dittmar et al., 2003). Thus, from this perspective, the Andean origin hypothesis was disproved.

Years later, with funding from CNPq and CAPES, Isabel Teixeira-Santons and Elisa Pucu de Araujo were able to carry on analyses of sediments from Eagle Cave on Jack Skiles ranch. Isabel excavated samples and completed a botanical and ecological study (unpublished). Using these samples, Elisa completed a molecular analysis for *T. cruzi*. Because of taphonomic conditions, no diagnostic aDNA was recovered (Pucu de Araujo, 2019).

Adauto had long suspected that Chagas disease present in ancient Brazilian hunter-gatherers. He wrote (Araujo et al., 2009:11), "In 1984, during excavations in the Boqueirão da Pedra Furada, archeological site in the Serra da Capivara National Park in southeast state of Piauí (PI), Northeast Brazil, coordinated by Niéde Guidon (Guidon and Delibrias, 1986; Guidon, 1989), we witnessed the archeologists constantly being attacked by triatomines while they studied the rock paintings on the sandstone walls of the rock shelter in the prehistoric camp. The paintings were all over the wall and, at that time, without the current technologies, it was necessary to copy them for subsequent study. Both in the early morning and in the hotter hours of the day, with temperatures reaching 42-45ºC, as well as at night, when the temperature drops to 8-10ºC, *Triatoma brasiliensis* nymphs and adults emerged, attempting to feed on the blood of archeologists, students, and workers. Some of the captured specimens were infected with *T. cruzi*. We postulated then that the ancient artists and other inhabitants of the rock shelters were also attacked by the vector and infected with the parasite".

Molecular studies found evidence of *T. cruzi* in remains from two sites in the Peruaçu Valley, Minas Gerais State (Araujo et al., 2009). First, the partially mummified body of a 35-40 individual was recovered and dated to 560 years ago. The preservation was good. This body was in fetal position and covered with baskets and leaves and the head was wrapped in leaves. There was partial preservation of extremities and abdomen. arms, legs, abdominal skin and musculature of some body parts are well preserved. Megacolon was evidenced by a large coprolite mass obstructing the descending and sigmoid colons. A PCR test was positive for *T. cruzi* of the TCII genotype and TCI genotypes, suggesting a mixed infection (TCI/TCII). A second study (Araújo et al., 2005), used mitochondrial 18S rDNA (126bp and 159 bp) and 12S rDNA (148 bp) to confirm the TCII infection. The analysis showed that *T. cruzi* disseminated throughout the body with a high parasite load, typical of acute disease. Megacolon is typical of the chronic phase (Fernandes et al. 2008). If I understand the papers correctly, it is likely that this individual experienced sequential reinfections, chronic and acute, TCI and TCII.

Extremely old infection with *T. cruzi* comes from TCI genotype evidence in a bone fragment dating to 7,000-4,500 BP from a different site in the same region (Lima et al., 2008). Therefore, *T. cruzi* infected hunter-gatherers and subsequent agriculturalists in the region. The data from Minas Gerais and Texas disproved the Andean origin of Chagas disease. As of today, there are no data to support Adauto's hypothesis that Chagas was present in the caves of the sertão Serra da Capivara National Park. There is transmission in pau-a-pique houses in the area today. Future paleoparasitologists will likely find the needed evidence.

To understand the transmission of *T. cruzi* among prehistoric hunter-gatherers. Adauto and I adopted my father's (hereafter KR) concept of pathoecology (Reinhard, 1972; 1974) to explain the interaction of human behavior and natural cycles of *T. cruzi*. KR was an epidemiologist who worked on the emergence and control of parasitic and bacterial disease in the Arctic. He proposed that understanding disease transmission in times that predated quantitative records required ecological and behavioral data for accurate estimations of the environmental transmission conditions. Adauto and I used that perspective to describe Chagas disease emergence in the Lower Pecos Canyonlands of Texas and the Cerrado of Minas Gerais of Brazil. Our final work, long delayed in publication, is *Mummies, Parasites, and Pathoecology* in the *Ancient Americas which appeared* in the *Handbook of Mummy Studies* (2021).

REVISED HYPOTHESIS 2007

The paleoparasitological data revealed changed the classically accepted theory drastically. A century after Carlos Chagas' discovery, a new history of Chagas disease began to emerge, starting thousands of years before the discovery of the disease. The description of lesions typical of Chagas disease and PCR-positive for *T. cruzi* in pre-Columbian 9,000-year-old mummies from Andean countries proves the antiquity of Chagas infection and disease in humans (Rothhammer et al., 1985; Fornaciari et al., 1992; Guhl et al., 1997; 1999; 2000; Ferreira et al., 2000; Martinson et al., 2003); Aufderheide et al., 2004). The scenario that has unfolded supports the hypothesis that *T. cruzi* existed in wildlife hosts before its presence in the Americas and the emergence of Chagas disease probably occurred rapidly when humans arrived on the scene (Barbera et al., 2024; Reinhard et al., 2024).

QUANTIFICATION AND PALEOEPIDEMIOLOGY

In 2012 onward, CNPq founded a new research project. Simultaneously, I was awarded a CAPES Ciências sem Fronteiras grant that facilitated the direct exchange of scholars between the institutions for three years. Both grants were for "Paleoepidemiological Transitions in Parasitology". The prospectus read, "During the development of the project, master's and doctoral students from the University of Nebraska and the Sergio Arouca National School of Public Health will have the opportunity to exchange ideas since the two laboratories involved have been cooperating since 1984 and their researchers have been producing scientific work together ever since. Expected results: The proposed studies will complement aspects of prehistoric life in the Americas, especially regarding health conditions." These funds facilitated intense research that focused primarily on the Americas including Peru, Chile, Brazil, Mexico and the southwestern US. In Nebraska, participants interacted in laboratory research, Archaeoparasitology Class, and Parasitology Seminar sponsored by the School of Natural Resources and the Manter Laboratory of Parasitology.

This project generated discussion of approaches and methods among participating faculty and students. A focus on egg-per-gram (epg) quantification was developed to perfect the paleoepidemiological approach based on a characteristic of parasites – overdispersion. Overdispersion is the statistical description of the universal pattern that the majority of parasites occur in a minority of hosts. This concept is fundamental in parasitology. As a rule of thumb, 10-20% of host population is infected with 70-80% of the parasite population. We believed that real epidemiological data were retrievable from archaeological sites and epg quantification should be fundamental to providing comparative foundations for comparison across archaeological contexts and will be fundamental to modern studies.

The epg methods were developed in labs in Argentina, Brazil and the US (summarized by Reinhard, 2017). Camacho et al., (2018) evaluated which methods were most reliable and accurate. They also explained the importance of demonstrating overdispersion in data sets to validate important measures such as infection prevalence and intensity (Camacho et al., 2018). They then demonstrated that when quantification is properly done, a comparison of infection patterns between sites and times is feasible. Teixeira-Santos, Morgana Camacho,and Elisa Pucu reviewed samples from the Andes between 2015 and 2019 (Reinhard et al., 2023a; b).

PARASITE PALEOEPIDEMIOLOGY OF THE ANDES

LICE

Pediculus humanus capitis nits and eggs attached to the hair shafts of deceased individuals are an ideal source for paleoepemiological research (Araujo et al., 2000; Arriaza et al., 2012; 2013a; 2013b; 2022; Reinhard and Buikstra, 2003; Reinhard et al., 2020). The "Paleoepidemiological Transitions" project presented cultural factors associated with louse prevalence and infestation intensity (Reinhard et al., 2023a). Building on the work of Reinhard and Buikstra study (2003), The 2003 study was deficient due to the small sample size of one site, Algodonal. For the Paleoepidemiological Transitions study, the sample size was increased. With general support from the Fulbright Foundation and logistical support from Bruce Owen (Anthropological Studies Center, Sonoma State University) and Bernado Arriaza (Instituto de Alta Investigación, Universidad de Tarapacá), Nicole Searcey was able to collect louse infestation data at the Centro Mallqui, El Algorrabal. The increased sample size and application of refined archaeological statistics that address small sample sizes and parasitology analysis packages specific to defining overdispersion, prevalence, and intensity confirmed that the distribution of nits and eggs on Chiribaya hosts was overdispersed.

Reinhard and Buikstra (2003), made several important observations. Men had a higher infestation prevalence than women due to men's more elaborate hairstyles and the use of hats. Louse infestation intensity for some people was more pronounced at the scalp than along the hair shafts a few centimeters from the scalp. That suggested that louse infestation was controlled by hygienic measures such as louse combing. Children were least infested, a fact that contrasts with modern societies in which children are more infested than adults.

The infestation intensity and prevalence of parasites were influenced by social and demographic factors at three archaeological sites located in the southern Peruvian Osmore River Valley, Ilo, near the Pacific coast (Reinhard et al., 2023a). The three sites are only approximately contemporaneous, between cal 900 to 1400 CE. Yaral is located much further inland in the middle valley at a higher elevation well outside the coastal fog belt. Algodonal and Chiribaya Alta are situated in the coastal portion of the Osmore River valley. Yaral and Chiribaya Alta are associated with the Chiribaya culture. Algodonal belongs to the Ilo-Tumilaca/Cabuza (ITC) culture. The sites are distinct in the style of their ceramics, wooden spoons, clothing, and house construction. Cultural affiliation might be expected to affect epidemiology. For example, ITC and Chiribaya diets differed, and Chiribaya families lived in dense communities of large, walled residential compounds with complicated, restricted floor plans. ITC people lived in small, freestanding houses of one or a few rooms (Owen, 2005).

Because the three sites had different characteristics, we could evaluate patterns in lice infestations for three variables: high vs. low socioeconomic status, inland vs. coastal location and environment, and cultural practices of the dominant, established Chiribaya vs. the immigrant, refugee Ilo-Tumilaca/Cabuza people. We also analyzed infestation patterns between sexes and across age groups. Louse infestations in coastal populations were about twice as common as they were inland at Yaral. No significant differences by sex or age group occurred within the Algodonal and Yaral sites. At Chiribaya Alta, louse infestation was significantly more prevalent among adults than among subadults. At Algodonal, subadults had significantly higher infestation prevalence than Yaral and Chiribaya Alta. In general, subadults, including infants, had low infestation intensities. However, ITC infants had significantly higher prevalence and intensity. Therefore, status affected infestation. Location in the lower valley was associated with higher prevalence. It is noteworthy that for the largest sample, at Chiribaya Alta, adult males had a higher prevalence (62%) than adult females (42%) than subadults (25%).

PACIFIC TAPEWORM, *Adenocephalus pacificus* (FORMERLY *Diphyllobothrium pacificum*)

The helminth analyses in the 2003 Memórias were, at the time, thought to be based on negligible sample sizes for the Lluta Valley, Chile and Osmore Valley, Peru. Sample sizes ranged as low as 15 to as high as 29. Jovani and Tella (2006) analyzed sample size and reliability of prevalence data for parasite studies. They conclude that uncertainty in results decreases as the sample size increases from 10 to 20. However, after acquiring a sample size of 15, a trade-off is reached between excessive data loss and maintenance of acceptable levels of uncertainty. New studies for the "Paleoepidemiological Transitions" project from these valleys, therefore focused on expanding sample sizes.

Pacific tapeworm infection prevalence is affected by cultural factors and environmental conditions. Increased prevalence in the Late Period of the Lluta Valley, Chile is associated with the Inka Empire expansion (Santoro et al., 2003). Village-level subsistence specialization resulted in pronounced prevalence variation in the Osmore coastal valley, Peru (Martinson et al., 2003). Arriaza et al., (2010) summarized the ecology, biology, and infection patterns in modern and prehistoric times of *A. pacificus* to evaluate the impact of ENSO climate fluctuations on infection.

Pacific tapeworm infections in Peru and Chile began about 6000 BP or earlier, with prevalence varying regionally, and cyclic variation probably associated with the ENSO phenomenon (Arriaza et al., 2010). *Adenocephalus pacificus* occurred at the inland site of Tiliviche village, Chile 6060-3900 BP (Ferreira et al., 1984). The chronological age of these findings corresponds with the El Niño events reported for Quebrada Los Burros (Fontugne et al., 1999). Variation in prevalence between Chinchorro cemeteries (Reinhard and Urban, 2003) may also be due to an ENSO phenomenon (Arriaza et al., 2006). Morro 1-6 dates range from about 4350-3550 BP and Morro 1 from about 4950-3750 BP and are just a few meters apart. The different dates suggest different populations may have been living under different ENSO conditions.

For the Paleoepidemiological Transitions analysis, we reviewed all samples available from published data (Holiday et al., 2003; Kuchta et al., 2015; Ramirez et al., 2021; Richardson et al., 2012). We also collected new data from prepared samples archived in our labs (Reinhard et al., 2023b). Isabel Teixeira-Santos took charge of directing the reanalysis of all extracted samples in the Pathoecology Lab, UNL by the 2016 Archaeoparasitology Class, School of Natural Resources UNL as part of her postdoc work. This effort was geared to collect epg data for all samples. Using these data, Morgana Camacho applied current statistical approaches to overdispersion and defining prevalence and infection intensity on a site-by-site level. This effort led to a diversified coprolite sample from Chile and Peru, showing variation in infection (Reinhard et al., 2003b).

We were able to statistically define a significant difference in prevalence between Chinchorro hunter-gatherer and Chiribaya mixed subsistence contexts. We confirmed that the most pronounced differences occur between populations within these groups. All epg data for each site exhibited overdispersion.

Unlike related species of *Diphyllobothrium*, plerocercoids of *Adenocephalus pacificus* do not occur in fish muscle. Rather, they encyst in the viscera, peritoneum, stomach wall, and gonads. Because muscles are pleroceroid-free, infection potential can be reduced significantly by removing viscera during the preparation of fish for eating. The most striking discovery, therefore, was the pronounced prevalence variations between 3 Osmore Chiribaya villages within 7 km of each other. The sites are roughly contemporaneous, dating to 900–1400 CE. The data from the sites reflects the pattern of subsistence specialization described for the area (Zaro, 2007). However, the infection prevalence is inconsistent with the presumed site function. San Geronimo (20% prevalence) is a coastal village that focused activities on marine resources including fish and shellfish. Shellfish do not host the Pacific tapeworm. The mixed marine subsistence resulted in relatively low infection. Chiribaya Baja (58% prevalence) focused on agriculture, and we expected a low prevalence for these farmers.

The high prevalence results are inconsistent with our prediction. Fish smoking and drying facilities that were found at this site. Obviously, this technology was not 100% effective at killing plerocercoid cysts in fish body cavities. Therefore, the high prevalence reflects the selection of intermediate host fish species for food, inadequate cleaning of the fish, and selection of preparation techniques that do not kill cysts. Chiribaya Baja inhabitants were thus at risk for high infections. Chiribaya Alta (4% prevalence) inhabitants had a diverse diet. Chiribaya Alta people likely selected fish species that were not tapeworm hosts, such as anchovies. Indeed, analysis of intestinal contents from 15 Chiribaya Alta mummies revealed that 5 contained anchovy bone and scale. In addition, they may have had more effective ways of cleaning and preparing fish that was consumed at the site.

The high infection at Chiribaya Baja thus may reflect an absence of folk knowledge for cleaning and preparing fish. In contrast, the near absence of infection at Chiribaya Alta resulted from a difference in fish preference and preparation. A portion of the elites interred at Alta may have come from outside of the region and, therefore, had a diet that excluded infected fish. This is supported by the strontium analysis of bones and teeth from burials at the 3 villages (Knudson et al., 2007).

The publications regarding *A. pacificus* show a remarkable spectrum of factors that affect infection prevalence and intensity. Empire expansion resulted in subsistence adaptation and the emergence of infection in Late Period Inca people of the Lluta Valley (Santoro et al., 2003). Variation was also affected by the movement of inland people to the coast (Reinhard et al., 2023b). Conversely, the movement of coastal people inland resulted in the deposition of *A. pacificus* eggs as many as 40 km inland (Ferreira et al., 1984). Selection of fish species for food, cleaning efficiency, and preservation methods were site-specific variables that defined remarkable variation between the Chiribaya villages of the coastal Osmore Valley, Peru. Clearly, trade between villages had the potential of creating infection nodes (Reinhard et al., 2023b). All these variations were defined and mediated by broad region ENSO phenomena (Arriaza et al., 2010). Every decade of Pacific tapeworm study has revealed new discoveries of how human behaviors interacted with *A. pacificus* biology in an ecological setting that created changes in prevalence and intensity.

PINWORM, *Enterobius vermicularis*

We learn the most about a region's paleoepidemiology from its most ubiquitous parasite. For the Americas, this is *Enterobius* *vermicularis*. It has been found in all parts of the Americas from low to high prevalence (Camacho and Reinhard, 2020). Infection is linked to a variety of behaviors and environmental conditions (Reinhard and Camacho, 2024).

The pinworm data from the "Paleoepidemiological Transitions" work was published in the form of a global review of all confirmed archaeological finds (Reinhard et al., 2016). Independently, a thorough survey of coastal and inland sites for helminth parasites was conducted (Ramirez et al., 2021). Taken together, these studies provide a good idea of pinworm proliferation and control.

Tiliviche, Iquique, Chile is the oldest site in our study area that was positive for pinworm. Samples date to Chinchorro times from 4,110-1,950 BCE. Significantly, a microscopic analysis of 26 samples resulted in no discoveries of pinworm, although Pacific tapeworm was found. Yet molecular analysis of two coprolites resulted in one positive diagnosis (Iñiguez et al., 2008).

This illustrates the challenge of diagnosing pinworm by microscopic analysis of coprolites. Microscopic analysis of coprolite always underrepresents the actual prevalence, because only 5-10% of pinworm-infected people pass eggs in feces (Camacho and Reinhard, 2020). Therefore, it was surprising that 11 of 16 (69%) samples from the Atacama Desert site of Tulán-54 were positive for eggs (Table 2). Because pinworm prevalence is related to population concentration, this suggests that Tulán-54 was home to a large number of people. Research in North American pueblo villages that housed dense populations shows that pinworm prevalence in coprolite samples can range up to 73%. However, the average prevalence is 40%. Compared to the prevalence for Andean sites (Table 2), Tulán-54 is an outlier.

Besides a remarkable pinworm prevalence, Tulán-54 was a unique ceremonial center. Núñez et al., (2017) excavated a temple with a surrounding perimeter wall made of vertically arranged blocks containing niches covered by lintels. Inside the perimeter were six walled structures. In the enclosed spaces and perimeter wall, they found 27 infant graves with offerings accompanied by blocks with zoomorphic (especially camelids), anthropomorphic, and linear petroglyphs. Evidence of feasts, ceremonies, and offerings showed that the site had important cultural and economic significance for the region's population. Chilean Early Formative societies; transitioned at both local and regional scales from hunter-gatherers to early pastoralist communities. Coprolites collected date towards the end of the site use, representing pastoral societies of increasing population density. The pinworm data attest to the use of Tulán-54 by a concentrated community in the latter phase of its existence. The resident population that used Tulán-54 was part of a complex of sites. Another important site, Tulán-122, was a large residential village with 153 structures, also conducive to pinworm transmission.

The Formative site of Caserones-1 (cal 20–1020 CE) has a lower positivity. It is associated with the cemetery of Tarapacá 40 (Tr-40) and the village site of Pircas-1. Coprolites were studied via PCR (Iñiguez et al., 2008) and microscopy (Araujo et al., 1985). One of the 11 coprolites was positive for parasite eggs through microscopy (Table 2). One of the two samples tested by PCR was positive. More recently, Ramirez et al., (2021) sampled coprolites from Tarapacá 40 burials. Two of 11 samples dating to cal 410–550 CE and 390–540 CE of the Formative Period, from individuals living at either Pircas-1 or Caserones-1, were positive.

The Late Intermediate Period site of Pica-8 is located near the current oases of Pica some 160km south of Tarapacá 40 on the plain of Pampa del Tamarugal (Ramirez et al., 2021). The oasis inhabitants developed intensive agriculture. The site had a wide trade network from the Pacific coast to San Pedro de Atacama, the Bolivian altiplano, and the eastern side of the Andes. One of three coprolites from burials from this site was positive for pinworms (Table 2)

ADNA POLYMORPHISM

It is significant that PCR analysis of coprolites from Tiliviche, Tulán-54 and Caserones revealed pinworm genetic polymorphisms (Iñiguez et al., 2008). Six archaeological sequences from all Chilean sites were found to be identical to *E. vermicularis* contemporary S11 RNA sequences. Two pinworm sequences from Tulán-54 had three nucleotide substitutions. Sequences from coprolites collected in Caserones exhibited one substitution. These polymorphisms could be evidence of a second parasite lineage.

EPG AND PALEOEPIDEMIOLOGICAL TRANSITIONS

The foundation for the 'Paleoepidemiological Transitions' portion of the pinworm work was established by archaeological work in Chile by Calogero Santoro and Bernardo Arriaza and in Peru by Jane Buikstra, Sonia Guillén, and Bruce Owen. These researchers sponsored parasitological work, the bases of which were published in the Memórias special issue. However, epg data collected for the 'Paleoepidemiological Transitions' effort, provided new perspectives for the prevalence and infection intensity studies.

Perhaps the most dynamic find builds on the published Late Intermediate and Late Period contrasts of the Lluta Valley, Chile (Santoro et al., 2003). The Late Intermediate Period Lluta Valley and the Osmore Coastal Valley had low or no pinworm prevalence (Table 2). Only one site, Chiribaya Alta, had positive samples for pinworm at a very low prevalence. The one positive sample contained just 20 epg. For the Lluta Valley, additional data collected by Souza et al, (2018) and Teixeira-Santos (unpublished) increased the sample size and revealed a 5% prevalence for the Late Intermediate Period and 24% for the Late Period (Table 2). This supports the original conclusion by Santoro et al. (2003) that housing changes associated with Late Period Inka expansion resulted in crowding and an increased prevalence. New epg data highlight an increased infection intensity which is an average number of parasites in infected individuals (Figure 5).

The intensity of 1440 epg exceeds the values of the highest Ancestral Pueblo values (Camacho and Reinhard, 2020), where values range from 139 to 701, averaging 435. They also exceeded the site at La Cueva de los Muertos Chiquitos, Durango, Mexico which had an infection intensity value of 122 epg. The Late Period inhabitants of the Lluta Valley thus experienced the highest pinworm intensity, and perhaps pathology, of any documented site.

WHY PINWORMS ARE IMPORTANT

During the 20th century pinworm infection was considered apathogenic. Recent clinical and population studies, however, have associated pinworm infection in high-prevalence areas of the world today with iron deficiency anemia, and aberrant ectopic infections causing a variety of organ pathology, some of them fatal (Table 3). It is likely that high prevalence sites such as Tulán-54 and Late Period Inca of the Lluta Valley experienced these conditions.

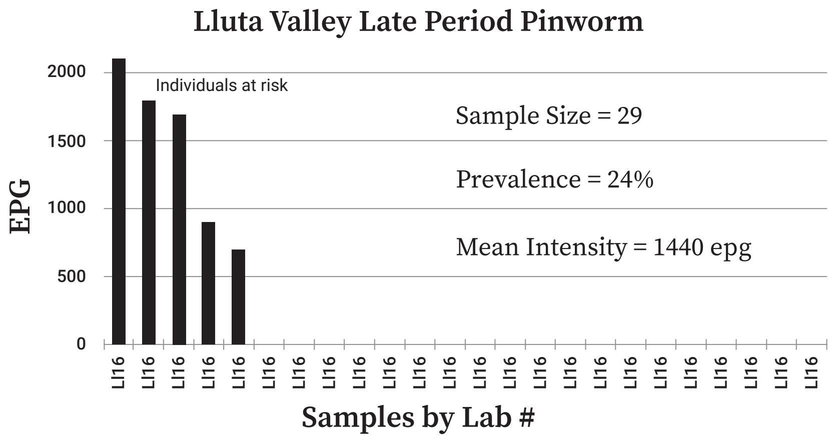

FIGURE 5. Pinworm distribution for Late Period coprolites from the Lluta Valley. The pinworms are overdispersed with 80% of the eggs in 10% of the hosts.

Source: Author´s elaboration.

TABLE 2. South American Pinworm Table: Caserones, Tarapacá-40 and Pica-8, Tarapacá-40 is a cemetery located in the lower course of the Tarapacá ravine, which is associated with the Formative sites of Pircas-1 (cal 370 BCE–530 CE) and Caserones-1 (cal 20–1020 CE), both defined as villages.

Site	Date: Period	PCR: number positive/ sample size	Microscopy: number positive/ sample size	References
Tiliviche, Iquique, Chile	4110-1950 BCE (~6100-4000 BP) Preceramic V	1/2	0/26	PCR Iñiguez et al., 2008; Micro Ferreira et al., 1984
Tulán-54, San Pedro de Atacama, Chile DNA polymorphism	3500–2400 BP: Early Formative 3360–2370 cal BP	4/20	11/16	PCR Iñiguez et al., 2008; Micro Ferreira et al., 1989
Caserones, Tarapacá Valley, Chile: DNA polymorphism	Cal 370 BCE–530 CE 2320-1420BP: Formative	1/2	1/10	PCR Iñiguez et al., 2008; Micro: Araujo et al., 1985
Tarapacá-40, Tarapacá Valley, Chile	Cal 390–540 CE for sample analyzed: Formative	n.a.	1/11	Ramirez et al., 2021
Pica-8, Tarapacá Valley, Chile	Cal 993–1414 CE for sample analyzed: Late Intermediate	n.a.	1/3	Ramirez et al., 2021
Chiribaya Alta, Peru	1,000–650 BP: Late Intermediate Period	n.a.	1/28	Martinson et al., 2003
San Geronimo, Peru	1,000–650 BP: Late Intermediate Period	n.a.	0/20	Martinson et al., 2003
Chribaya Baja, Peru	1,000–650 BP: Late Intermediate Period	n.a.	0/48	Martinson et al., 2003 and new analyses
Mitas Chiribaya, Lluta Valley, Chile	1,000–650 BP: Late Intermediate Period	n.a.	1/19	Santoro et al., 2003; Souza et al., 2017
Inca, Lluta Valley, Chile	539–483BP: Late Period	n.a.	7/29	Santoro et al., 2003; Souza et al., 2017

Source: Ad´an et al., (2013). Multiple Sources, Martinson et al., (2003 new analyses).

TABLE 3. Pinworm Pathology: 21st Century Studies relating *E. vermicularis* infection to pathology.

Iron deficiency anemia and/or vitamin B12 deficiency related to pinworm infection	
Study	**Outcome**
Dohan and Al-Warid, 2022	IDA significant correlation w/infection among 122 children with 74% prevalence
Al-Daoody and Al-Bazzaz, 2020	IDA significant correlation w/infection among 505 children with 27% prevalence
Olivares et al., 2002	B12 deficiency found in 40 infected children
Sadraei et al., 2007	Significant serum B12 depletion in 60 infected children
Mohamed et al., 2022	Significant serum depletion in 90 infected children
Ectopic Pinworm Pathology	
Rizvi et al., 2015	Fatal jejunum peritonitis case following intense inflammatory reaction to pinworm penetration
Rizvi et al., 2015	Thickening of the small intestine wall mimicking Koch's abdomen (abdominal tuberculosis) associated w/ pinworm perforation and peritonitis.
Rizvi et al., 2015	Degenerated chitinouse structures (eggs) embedded in intense inflammatory omental mass
Rizvi et al., 2015	Provisional clinical diagnosis of jejunum tuberculosis revealed to be necrotic omentum florid giant cell reaction to pinworm
Rizvi et al., 2015	Gravid worm infection caused ileio-ileal intussusception
Al-Shouli et al., 2003	Fatal infection due to gangrene from bowel vascular thrombosis and vasculitis leading to shock and death.
Serpytis and Seinin, 2012	Fatal case of bowel perforation with generalized bacterial peritonitis
Kamgarpour, 2003	Spinal cord, C3andC4 region, inflammatory mass tumor w/worms
Cateau, et al., 2010	Kidney inflammation w/ necrotic areas and granuloma formations from gravid pinworms represented by eggs
Craggs et al., 2009	Abscess and generalized peritonitis caused by pinworm in late pregnancy.
Raju et al., 2015	Initial diagnosis of cervical carcinoma revealed to be inflammatory response to gravid pinworm

Source: Author´s elaboration.

CONCLUSIONS

THE FUTURE OF PALEOPARASITOLOGY REPRESENTED AT PAMINSA IX

Paleoparasitology has a long history in South America. It is noteworthy that the foundational studies focused on the Andes (Araújo et al., 1983; 1985; Callen and Cameron, 1955; 1960; Ferreira et al., 1984). For the Tres Quixotes, these studies and experiences created a deep interest in the region. Most of our funded research, and much of our international collaboration, focused on the Andean region. Two tragedies occurred with the passing of Luiz Fernando (2018) and Adauto (2015). Also, there was significant damage to the main physical structure of the Museo Nacional de Brasil. Since those years there has been Covid-19 and the election of science-indifferent governments. The 2019 PAMinSA hosted by the Universidade São Paulo was the focus of farewell symposia to our wonderful, passed colleagues. These symposia presented the results of the "Paleoepidemiological Transitions" results and evidence of food and medicines collected during parasitological analyses. For me, the momentum of the Tres Quixotes's collaboration continued to the point when Elisa Pucu de Araújo was awarded her doctorate by Daniela Leles in 2019. Both Elisa and Daniela were Tres Quixotes advisees.

The 2023 PAMinSA conference showed that Paleoparasitology is very alive and healthy in South America. Adauto and Luiz Fernando had a special interest in the paleoparasitology applied to paleontology. María Beltrame has followed this interest for at least 15 years and her "Paleoparasitología" research group has elucidated parasites of animals from Pleistocene-Holocene Transition to historical times. Camelids, goats, sheep, rodents and carnivores area all in the area of their pervue. Their PAMinSA presentation presented new techniques, both flotation and genetic, for diagnosis. With these techniques, they found a greater number of positive samples and species identification of Eimeria. They also had enhanced success in identification of the zoological origin of the coprolites. They are designing a specific protocol for this approach.

Chagas Disease was a main theme. Orellana-Halkyer and Tellería's *La Enfermedad de Chagas: de la Prehistoria a la Actualidad* (2021), was distributed to conference attendees and accompanied by a complementary, expansive exhibit on the topic. This work is a landmark in tracing the diseases origin through Geologic Time to today's endemic challenge. In the symposium papers, Manneschi et al. applied Species Distribution Modelling to Chagas in the Tarapacá region. Guided by their successful model, paleoparasitologists will have fruitful discussion of the dispersion of endemic regional vectors of the disease. Pires et al. supported the Tres Quixotes's idea that Chagas had a distribution across Central and South America that put prehistoric susceptible humans at risk wherever they settled in endemic cycle areas. This replaced a previous theory that Chagas originated in permanent villages in the Andes. Pires et al. challenged the old notion by documenting *T. cruzi* in a prehistoric Brazilian population that predated sedentary villages dating to 4808 ± 546 cal BP. They also developed a methodology to diagnose *T. cruzi* in regions of poor preservation. Thus, PAMinSA IX showed the progress in theory and technology that will make paleoparasitology even more relevant to understanding the nuances of Chagas Disease in the past and present.

Lice were another theme of presentations. Aravena et al. presented behavioral and demographic features of infestation from Formative Period context. They analyzed 51 individuals from coast and valley sites were quantitatively. They found that 16.7% of the sample were heavily infested. Of 16 people who wore turbans, 93.8% were infested. Roughly comparable numbers of men (80%)

and women (85%) were infested. This study shows that behavior influenced infestation and also that, (in context of published studies), infestation prevalence varies between sites. Therefore, the paleoepidemiology of lice continues to be a fascinating topic. Using PCR- Sanger and Next Generation Sequencing (NGS) techniques, Skinner et al. were able to recover different louse aDNA sequences from five mummies. They found several clade A haplogroups as well as new haplotypes. Three mummies were infested with multiple louse haplogroups. The diverse findings may be able to trace prehistoric mobility in the region. To me, the novel variants are fascinating and suggest continued evolution of lice in the Americas after colonization by PaleoAmericans. These presentations show that parallel quantitative research into louse paleoepidemiology with molecular analysis has an exciting future in elucidating the details of infestation.

Refined and expanded diagnosis is being explored by combined microscopic and molecular analysis. Two presentations illustrated this with successful studies. Ramirez focused on well preserved camelid and human coprolites from mummies or stratified archaeological deposits. Microscopy revealed helminth and protozoa infections, and shotgun DNA analysis revealed the whole genetic community structure in these ancient samples. Parasites found in human samples did not pose a health risk. Camelid coprolites contained pathogenic species associated with health risks. Refined, multimethod diagnosis has been a theme of Iñiguez's research for two decades. In her presentation, she proposed the refinement cross-disciplinary study to a high degree. She and her colleagues synthesized Artificial intelligence/Machine Learning, Species Distribution Modeling and paleogenetic studies through next-generation sequencing (NGS). This presentation made a case that this refined approach will improve understanding of diverse paleoepidemiological and evolutionary scenarios in New World paleoparasitology.

Valverde et al., (2020) work to continue investigations of ancient parasites in coprolites and mummy digestive tracts, analyzing mummies preserved at the National Museum of Archaeology, La Paz, Bolivia. They diagnosed *Balantioide*s *coli* in one of the studied mummies. In combination with the previous study, *E. vermicularis,* capillarids and *B. coli* have been found. These findings illustrate the value of coprolite and mummy research in the discovery of novel parasites. In my opinion, these studies represent a new trend continued by Ramirez et al., (2021). The times when large excavations of mummies such as occurred in the 1990s and 1990s may be passed. Such excavations provided large sample sizes for the work of the Tres Quixotes. In replacement of large excavations, research in the last decade focuses on deriving prevalence data from analysis of mummies and coprolites in extant museum collections. As time goes on, this approach will be invaluable in tracing the ancient distribution of parasites. I think future research will be benefitted if the new EPG approach is applied to museum studies. Its importance to paleoepidemiology is illustrated in the Lluta Valley pinworm data and *Trichuris trichiura* data presented below. If EPG data is collected as well as presence/absence data, then a large comparative base can be assembled for assessment of pathology.

We can estimate the pathology caused by *T. trichiura* (whipworm) from publish clinical data linking epg values to pathology. In Table 4, data for 8 studies are presented. The range of values is extreme, from <100 to 51,630 epg. It is likely that only the mummies from Belgium and Sicily experienced pathology from infection. If epg data collection become standard, we can ask several questions. The first is individual based, i.e. did the sample represent a symptomatic individual. On a cemetery level, we can look at the prevalence and infection intensity of the population. On a broad regional level, we can ask if symptomatic infections varied between places and times. All of these questions, it seems to me, are valuable to ask.

TABLE 4. Egg per gram data were collected from mummies from various contexts. Based on published clinical data of pathology related to fecal EPG counts, one can estimate whether or not a mummy EPG counts represents pathology.

Context	*T. trichiura* EPG	Symptoms?
Nivelles, Belgium- mummy	51,630	yes
Piraino, Sicily- mummy	34,529	yes
Inca, Arica, Chile- coprolite	5,400	unlikely
Vilnius, Lithuania- mummy	4,779	unlikely
Chiribaya, Arica, Chile- coprolite	1,800	no
Zape, Mexico- coprolite	1,127	no
Chiribaya, Ilo, Peru- mummy	435	no
Zweeloo, Netherlands- mummy	<100	no

Source: Author´s elaboration.

The presentation by Orellana-Halkyer et al., expands considerably on the Tres Quixotes botanical archaeology approach. They relate paleoparasitological data, intestinal parasitism in indigenous peoples to ethnobotanical knowledge. They found that diverse helminth and protozoa infections are experienced in indigenous communities across the Andes, Amazonia and Chaco regions. Paleoparasitology suggests that these parasites were pre-Columbian problems. They concluded that with available regional medicine plants and knowledge in each eco-region, and that gained through exchange between regions, effective treatments were developed for intestinal infections. They conclude that ethnobotany is an important tool to identify medicinal plant knowledge passed down from the first populations in these regions.

My experience at PAMinSA showed me that the Tres Quixotes's approaches to the prehistory of infection have continued in labs in South America. Some of the older researchers may have been directly influenced by Adauto and Luiz Fernando in life. However, all have benefited from their careers-long published work in establishing Paleoparasitology as an international, collaborative effort.

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FIGURE 5. Location of the Paleopathology Meetings in South America.

Source: IX PAMinSA Bolivia brochure, (2023).

URL: https://paminsabolivia.umss.edu.bo/ix-paminsa-brochure/

Funeral preparation: historical mummified corpses from Minas Gerais, Brazil
Preparación funeral: cuerpos históricos momificados de Minas Gerais, Brasil

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Abstract

Hundreds of human bones are stored under the altar of the church of Santo Antônio de Itacambira (1754-1916), Minas Gerais, Brazil, including partially mummified specimens. A few of them were donated to Fiocruz, to the investigation of ancient diseases. Two of them, an infant and an adult, were subject of study of mumification. Parts of skin, muscles and other organs, even genitalia, were identified, despite having been exposed at the basement of the church and manipulated for decades. A cotton plug at the infant anus testifies to hospital preparation of her corpse. In the adult, preservation of arteries suggests the injection of products that could slow down cadaveric processes. The annotation in the church's burial books record most of the deaths during the 19th century. The absence of soil inside the mummy, the skin folding, and the flattening of their dorsal region point to burials in coffins, a practice that started around the middle of the same century. At that time, formalin was already in use in hospitals such as Santas Casas de Misericórdia, for funeral preparations. Without local medical resources, people from Itacambira, would be forced to seek health in Montes Claros, a neighboring city about 100 km. away from Itacambira, where one of the oldest Santas Casas was opened in Brazil. Some of the affluent residents of Itacambira may have been served there, when hinterland was plagued by deadly endemic diseases. Prepared, after death, for transfer and burial, these two artificially preserved bodies are probably part of a few unidentified testimonies of mobility in search for health in the Brazilian hinterland of the nineteenths.

Keywords: Mummification, 19th century, hygiene practices, funerals

Resumen

Cientos de huesos humanos se encuentran almacenados bajo el altar de la Iglesia de San Antonio de Itacambira (1754-1916), Minas Gerais, Brasil, incluyendo especímenes parcialmente momificados. Un lactante y un adulto fueron donados a la FIOCRUZ con el fin de contribuir a la investigación de enfermedades antiguas. Dos fueron estudiados con el fin de conocer sobre la momificación de sus cuerpos. Partes de la piel, músculos y órganos, incluso genitales, son identificables, a pesar de haber sido expuestas en el sótano de la iglesia y manipuladas durante décadas. Un tapón de algodón en el ano del infante atestigua la preparación hospitalaria de su cadáver. En el adulto, la preservación de las arterias sugiere la inyección de productos que podrían ralentizar los procesos cadavéricos. Las anotaciones en el libro de los entierros de la iglesia registran la mayoría de las muertes en el siglo XIX. La ausencia de tierra en la momia, las pliegues de la piel y aplanamiento del cadáver indican un entierro en ataúd, que comenzó a mediados del siglo XIX. En aquella época ya se utilizaba formol en hospitales para los preparativos funerarios, como la Santa Casa de Misericordia. Montes Claros, a 100 km. de Itacambira, fue una de las Casas Santa más antiguas de Brasil, donde algunos de los residentes de Itacambira pudieron haber sido atendidos, durante tiempos en que existian enfermedades endémicas mortales. Preparados, después de la muerte, para el traslado y el entierro, estos dos cuerpos conservados artificialmente se convirtieron en probables testimonios de movilidad en busca de salud del siglo XIX en el interior de Brazil.

Palabras clave: Momificación, siglo XIX, prácticas de higiene, funerales

INTRODUCTION

Thanks to their adaptability, humans have been able to surpass natural borders and occupy the planet. The human species is characterized by great mobility, moving continuously or not, directed or wandering, in migratory or transhuman movements, we just move. Mobility is associated to conflicts, resource depletion, geopolitical expansion, hunger or simple exploratory curiosity. Mobility is also closely related to health; either individuals or human groups may need to move seeking health. Sometimes we also move for funerals.

In general, health-related mobility is thought of as if human dislocations caused disease dispersion or was an effort to run off epidemic diseases. Modelling the consequences of mobility allows the prediction of development, planning of political and economic decisions, and distributing health assistance among others. Throughout history, supplying of medical services has been unequal, as it is still today. In general, smaller places have less access to sophisticated health services and professionals, thus mobility may be necessary to those ones whose life was in risk.

On the other hand, religious and magic concepts dominated for centuries the way people dealt with illnesses. Brazilian hinterland in the past centuries was plagued by infectious and nutritional diseases, some of which were still unexplained in the 20th century. The medical treatise describing the Brazilian epidemiologic conditions during the 19th century (Sigaud, 2009) gives an idea of the dramatic conditions lived by rural populations. In that vulnerable scenario, a catholic institution named Santa Casa de Misericordia was devoted to mitigating poverty and suffering. The first times, more than a true hospital, the place received the most ill and miserable beings. This was a place where diseases were disseminated and death was a companion, a place to where most of these people would not like to go.

When science met healthcare, some institutions adopted the best hygiene and medical practice, and privileged people increased the financial support to Santas Casas de Misericordia. Instead of being only a refugee for the underprivileged, those institutions adopted more and more scientific advances to provide medical attention, becoming a reference in health. Supported by increasing donations, those hospitals started to offer also private differential accommodation, attracting people from social segments who avoided hospitals, even when severely ill (Santos and Silva, 2014). An improving control of the therapeutic processes, as well as the reduction of contamination in the hospitals, contributed to the credit people devoted to science and the Santas Casas.

Considering the historical context, the analysis of mummified corpses found at the Santo Antônio de Itacambira church represents an opportunity to test the hypothesis that some of the deceased buried at Itacambira church could have been hospitalized, and if dead their corpses submitted to intentional treatment for funerals. In this sense, this paper aims to contribute to the discussions of historical and social aspects of Itacambira before the beginning of the 20th century.

ITACAMBIRA

Itacambira is a city in the northwestern hinterland of Minas Gerais State, Brazil. Its history began in the 17th century, when it was supposed to be a post to provide food for the travelling adventurers and explorers searching for precious stones and minerals. During the 18th and 19th centuries Itacambira followed subsequent economic cycles of minerals extraction, cattle raising and agriculture (cotton). Nestled in a dry region, foothills of the Jequitinhonha´s river valley, the city is placed at an altitude of around 1,000 m. Its rocky soil is rich in mica, which gives to the hills in the vicinities a peculiar shine, justifying the nick name of "Shining Mountains". Having always been a small hinterland town, Itacambira had periods of more and less autonomy in the political, economic and religious dimensions. Its links to larger poles such as Montes Claros and Grão Mongol are still important (Pires, 1999; Cotrim, 2014).

The church of Santo Antônio de Itacambira (Figure 1a) begins to be built in 1707 (Cotrim, 2014). According to the church´s books (Figure 1b) available on the https://www.familysearch.org website, the first burials are dated to 1754. At the time, people were buried inside the church. After decades, a small cemetery was constituted at the churchyard, receiving more burials until 1916. From the twentieth century onwards, the burials moved to the secular cemetery of the municipality, created in the urban plan, a few blocks from the church headquarters. Described as Baroque style, it displays a peculiar internal decoration of oriental motifs. It is listed by the Institute of Historical and Artistic Heritage of Minas Gerais - (IEPHA MG, 2014), for being a single example of that period in Minas Gerais. In spite of having been restored, it has never been the object of an archaeological research project. Except for what is known in the oral tradition and what is reproduced by local historians, little is known about the burial ground, or how it was used over time

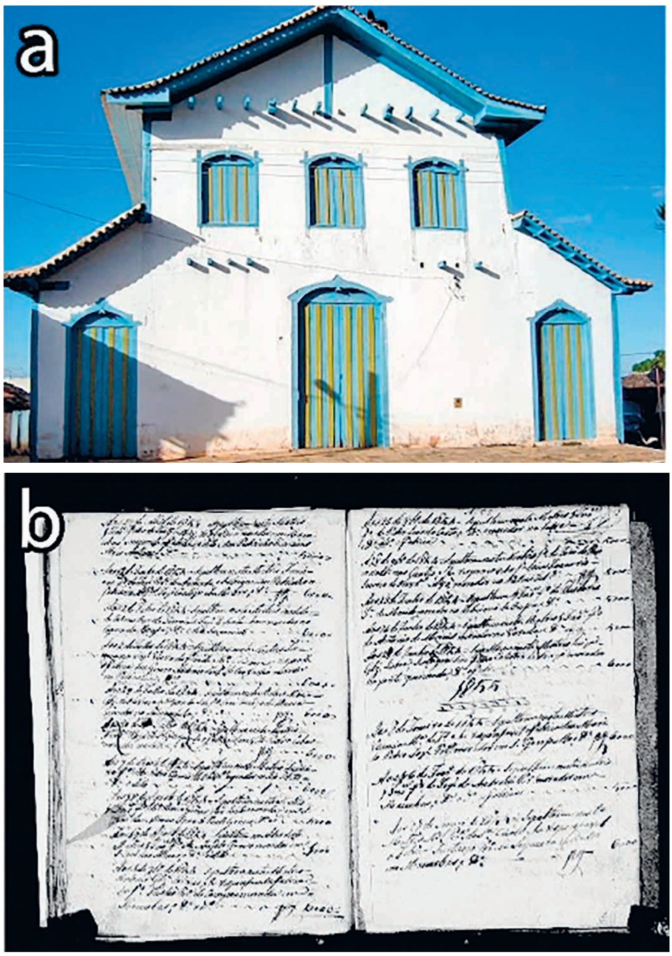

FIGURE 1. (a) Frontal view of the church of Santo Antônio de Itacambira, in Itacambira, Minas Gerais State, Brazil, that is one of the churches that are protected by the State Institute of Historic and Artistic Heritage of Minas Gerais (IEPHA-MG); (b) Book of burial records of the same church.

Source: (a) IEPHA - Instituto Estadual do Patrimônio Histórico e Artístico. (b) Author´s file.

HUMAN REMAINS

From the mid-20th century onwards, the church became famous for the finding of human bones and mummified bodies. According to the oral tradition, all those remains came from exhumation of some church burials, which had to be moved to the crypt under the altar (Figures 2a; 2b; 2c). Their discovery, years later, was accidental when the old wooden floor collapsed. No document was ever found to explain the history of the remains, and the explanations rest only in the narrative and memories of some old residents of Itacambira (Pires, 1999; Cotrim, 2014). Today the bones and mummies have become an additional tourist attraction for their economy, focused on the local rugged and natural beauties and adventure tourism.

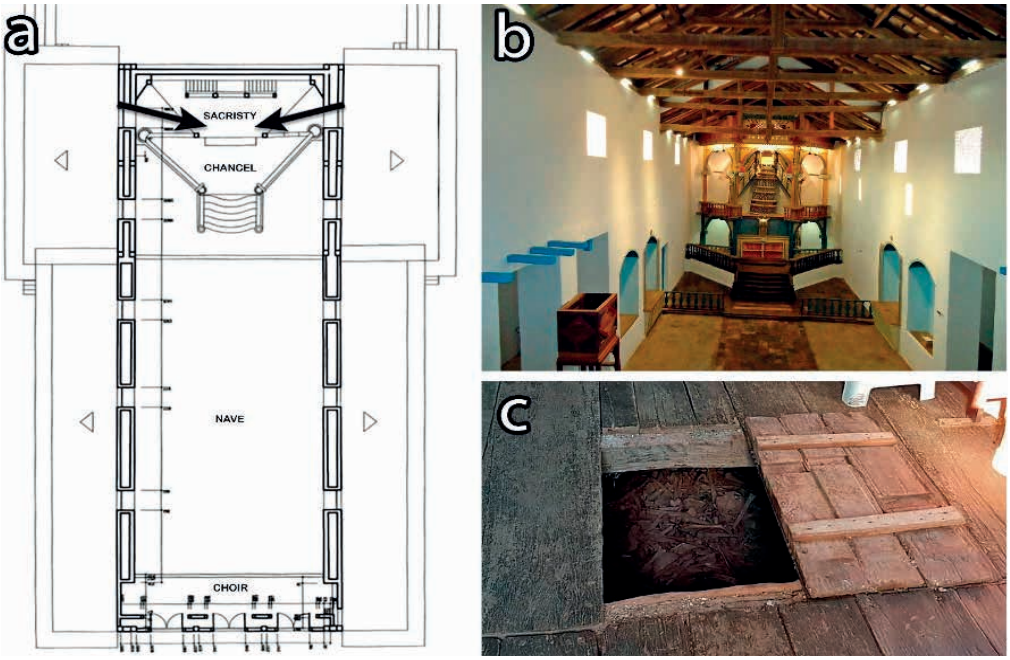

FIGURE 2. (a) Floor plan of the church of Santo Antônio de Itacambira, arrows indicating where the human remnants were found; (b) View inside the church, showing the chapel; (c) Trapdoor behind the altar, giving access to the crypt where the human remnants were found.

Source: IEPHA - Instituto Estadual do Patrimônio Histórico e Artístico.

During the 1980s, an engineer and local historian named Simeão Ribeiro Pires, living in Montes Claros, registered the local history of Itacambira in books. He also developed a private museum, that was well known by archaeologists working in Minas Gerais at the time. Contacted by Fiocruz paleoparasitologists, Dr. Ulisses Confalonnieri and Dr. Luis Fernando Ferreira, he provided information about the Itacambira mummies and donated part of what he had in his private museum (parts of 3 mummified bodies and some loose bones) to the Eduardo Marques Paleoparasitology Laboratory of the National School of Public Health. Subsequently, these remains of what is estimated to be parts of a minimum of 6 and a maximum of 10 individuals, were incorporated into the CPFERA scientific collection of Fiocruz. These remains are studied for different purposes, such as the presence and antiquity of local diseases caused by parasites (Araujo et. al., 1988; Bastos et. al., 2015), taphonomy (Braga et al., 2015), cranial morphology and bioarcheology (Liryo et al., 2020) and archaeobotanical (Souza et al., 2024), among others.

Local scientists have discussed a possible unusual preservation of those human corpses. The preservation of soft parts, clothing and footwear, as well as other objects, was generally supposed to be caused by the arid conditions of the region. Incorruptibility of bodies became part of the Itacambira legends, reinforced by the oral tradition of the need to wetting the graves to help decomposition of the bodies (Pires, 1999).

For a long time, probably decades after exhumation, the Itacambira human remains were exposed in the church basement, commingled and spread on the soil, without packing or the protection of coffins. They were progressively dismembered, mixed, stripped of their accompaniments, subject to different risks of damage and loss. Taphonomic analysis at Fiocruz CPFERA found evidence of recent damage, like cracking, insects' colonization, fungus, dismembering, among others. Pictures of the basement show different kinds of debris associated with the human remains. Most of them are clean bones, either skeletonized, or displaying no more than a few dry stripes of soft tissues. Even the mummified segments of corpses are partially decomposed, probably a consequence of long contact with soil and humidity for decades. In that condition, it is surprising that some of them have well-preserved soft parts and organs, confirming their differential preservation.

METHODOLOGY

Two partially mummified specimens from Itacambira in the CPFERA collection were analyzed: the corpse of a female infant(#A0077), and part of the corpse of an adult man (#A0075). Their preservation caught the attention of their collector Simeão Ribeiro Pires, as well as the attention of Fiocruz paleoparasitologists, who considered their potential to keep traces of former parasites. Their preservation motivated the present investigation.

Even in modern exhumations it is not unusual to find fragments of ligaments and tendons still attached to the bones. That is what is in most Itacambira bones, especially close to the joints, where fibrous resistant anatomical structures usually remain. Considering the arid Itacambira climate, the effect of quick dehydration could have preserved a few anatomical parts (Tibett and Carter, 2008), like other findings in archaeological sites (Souza et. al., 2002). In Itacambira, on the other hand, a few well-preserved individuals have more than tendons and parts of the skin, they are true mummified testimonies of a special preservation.

Treating corpses to slow down decomposition is an old cultural practice, described for many human groups (Aufderheide, 2004). In the context of Catholicism (Souza et. al., 2006) some intentional mumification practices were applied. They were fashionable during the 18th and 19th century, when priests of different brotherhoods, as well as health professionals, developed their special techniques (Leonzo and Almeida, 2018), contributing to those changes in funeral practices. In the context of a scientifically based practice, formalin and aseptic solutions were used in the 19th century for the preparation of corpses for their funerals. In the absence of historical evidence of overt clerical activity in the small Itacambira, sanitary practices perhaps provide the best explanation for the finding of mummified remains in the church cemetery.

Morphological comparative analysis of their anatomic aspects was used to identify them and to describe the characteristics of each body, their pathological condition and state of preservation (Standring, 2010).

The first specimen, #A0077, consists of two segments of the corpse of the same individual. The largest segment is the almost complete mummified trunk and limbs of an infant completely covered by mummified skin, containing part of the skeleton in the original anatomical position. Damage related to previous manipulation and recent sampling for scientific paleoparasitological investigation explain the absent parts of the corpse. The smallest segment is represented by part of the facial skin of the same individual, retaining delicate bones, including the maxilla, mandible and teeth. Although these segments have been disconnected, the donor Simeão Ribeiro Pires, attested their previous connection, which was confirmed by the present analysis.

The second specimen (#A0075) corresponds to the body segment of an adult male, represented by a complete pelvis with the thighs strongly attached. It is covered in resistant and mummified skin of a leathery appearance. Under the skin it is possible to distinguish bundles of dehydrated muscles, attached by tendons to their insertions. In addition, vascular structures, cartilage and vessel-like structures can be identified, as well as poorly identifiable amorphous materials, suggesting that the mummification process penetrated deeply within the body of the deceased. Fragments of cotton textile (Souza et. al., 2024 – in print) from the garment or shroud, are still adherent to the skin.

As in the previous specimen, the macroscopic analysis was performed by detailed examination of the entire skin surface, cavities, organs and exposed bone surfaces.

The analyses were based on the comparative method. Possible taphonomic processes and lesions, as well as the presence of foreign materials, infestations (fungi) and various signs of bioactivity were also investigated with the help of a ProScope digital magnifier (50x). The acquisition and first processing of the tomographic images was performed at the Imaging Service of the Evandro Chagas National Institute of Infectious Diseases, at the Manguinhos Campus of Oswaldo Cruz Foundation (Ministry of Health), Rio de Janeiro. The radiologic documentation was processed to confirm the integrity and position of the anatomical structures, also providing osteometric analysis. The CT scanning protocol used for both the infant and adult was 50 KV (kilovoltage) and 20 MAS (milliamperage), these values being the minimum in the CANON medical equipment.

The subsequent processing, image reconstructions and analyses were helped with three different software's. The first software was the AURORA@ used at the Institute Evandro Chagas for the first recordings. The other two, both free, were INVESALIUS@, recommended by the Brazilian Ministry of Science, Technology and Innovation, and the 3D software SLICER@. The last one provided better reconstructions from the original DICOM images. Sections of 1.0 mm and spacing of 0.5 mm were used for the 3D reconstructions.

RESULTS

EVIDENCE OF INTENTIONAL PRESERVATION

#A0077 – Body and head of a mummified infant

The specimen (with the segment of the face positioned at the upper end of the body) measures approximately 56 cm. Only part of the face is preserved, with no cervical segment (Figure 3a). The skin of the face has part of the frontal, zygomatic, nasal, maxilla and mandible still adherent. The maxilla and mandible keep part of the dental germs. Fragments of cloth and remnants of clothing are still attached to the face. Their manufacture, fabrics and decoration are like those preserved in the trunk and limbs, confirming their previous connection.

The corpse is covered by an almost complete elastic skin beige-colored, with no signs of pleating, blistering or insect colonization; in this sense, it differs from other specimens from Itacambira described in Braga et al. (2015). Skin folds at the elbows and knees are broken, probably because of manipulation of the rehydrated limbs. Straight cuts have removed parts of the abdominal skin, opening the abdominal cavity for paleoparasitological sampling in the 1980s. Most of the inner contents were removed, including some chest bones such as ribs and vertebrae.

There is no sediment from the local soil either inside the body, or in the skin folds and damaged areas where the skin is decomposed. No trapped sediments were found in the fabrics and clothing of that individual. The dorsal flattening of the body is suggestive of contact with a hard and even surface. Both pieces of evidence suggest that the body was buried in a coffin, not having been in direct contact with the local soil. Residues of dust and other materials are deposited on the specimen, possibly associated to decades of exposition under the wooden floor of the church.

The limbs´ are fixed within the mummified skin pointing to the original position of the body for funeral (Figure 3b). The arms were aligned with the torso, the forearms flexed at 90° were resting on the chest, and the hands were extended at right angles to the forearms, with both palms in contact and the fingers flexed, as if holding an object. The lower limbs are fully extended, and both feet are preserved almost complete. Careful observation of the mummified skin confirms that it has collapsed towards the back of the body, following the soft tissues as fat and delicate muscles became unraveled. At the dorsal region the flat compressed skin confirms the supine position. Skin preserves the impressions of the clothing and fabrics, some of which are still partially attached to the body, as described by Souza et al., (2024).

FIGURE 3. (a) Dorsal view of the partially mummified body of the female infant (#A0077). Parts of the cotton dressing are still attached to the skin. (b)The mummified face is detached from the body and the bones of the neurocranium have been lost, but the mandible, maxilla, zygomatic bones, part of the frontal bone and teeth are adherent to the skin. Notice the preserved contours of the mouth, nostrils and eye socket.

Source: Author´s file.

Concerning the skeleton, some vertebrae, clavicles, scapulae, and ribs are in their anatomical situation, humeri, radii, ulnae, and some bones of the hands are preserved. Part of the lumbar vertebrae and sacrum are still connected to the pelvic bones, continuous to the lower limbs. Femora, tibiae, fibulae, and major foot bones are aligned and anatomically connected. The knees were partially damaged, what explains detachment of the epiphyses on the right leg (distal epiphysis of the femur and proximal epiphysis of the tibia). The middle and distal phalanges of the second and third toes of the right foot are partially absent, the aspect suggesting that they may have been bitten, possibly by scavengers.

The bones, visualized both directly and on CT scans, have a normal morphology for age. Some linear measurements of the long bones could be estimated from the 3D images, based on the reconstructions obtained with the 3D SLICER@.

The results were considered for the estimation of biological age, although teeth provided a more precise estimation. The value for each bone can be seen in Figure 4a.

Odontogenesis was initially estimated by direct examination. As dental germs are partially hidden inside the mandible and maxilla, the tomographic images were decisive for the final age estimation (Figures 4b e 4c). Figure 4b shows the right and left portions of the mandible, not fused yet at the time of e death. The dental germs visualized in the upper arch are the deciduous central incisors, the deciduous lateral incisors, the deciduous canines, the primary first molars, the deciduous second molars, and the permanent first molars.

In the lower arch the dental germs are the deciduous lateral incisors, the deciduous canines and the primary first molars, the deciduous second molars and the permanent first molars. In Figure 4c, the three-dimensional reconstruction (3D SLICER@) allows us to visualize the enamel surface of the dental crowns in formation, as well as the incipient tooth roots.

The absence of the lower central incisors´ teeth germs (Figure 4b), is explained by their possible dislocation during exhumation, or during the subsequent handling. The rotation of the dental germs of the first permanent molars may have the same explanation.

The set of visualized teeth (Figure 4c) allowed us to estimate dental age based on Ubelaker (1989) and Scheuer and Black (2000) at 9 months, +/- 3 months. However, the observation of the still incipient formation of the teeth cusps, confirmed by the length of the large, long bones, suggests that the age is close to the lower limit of the estimated confidence interval, that´s to say child would be a little bit older than 6 months.

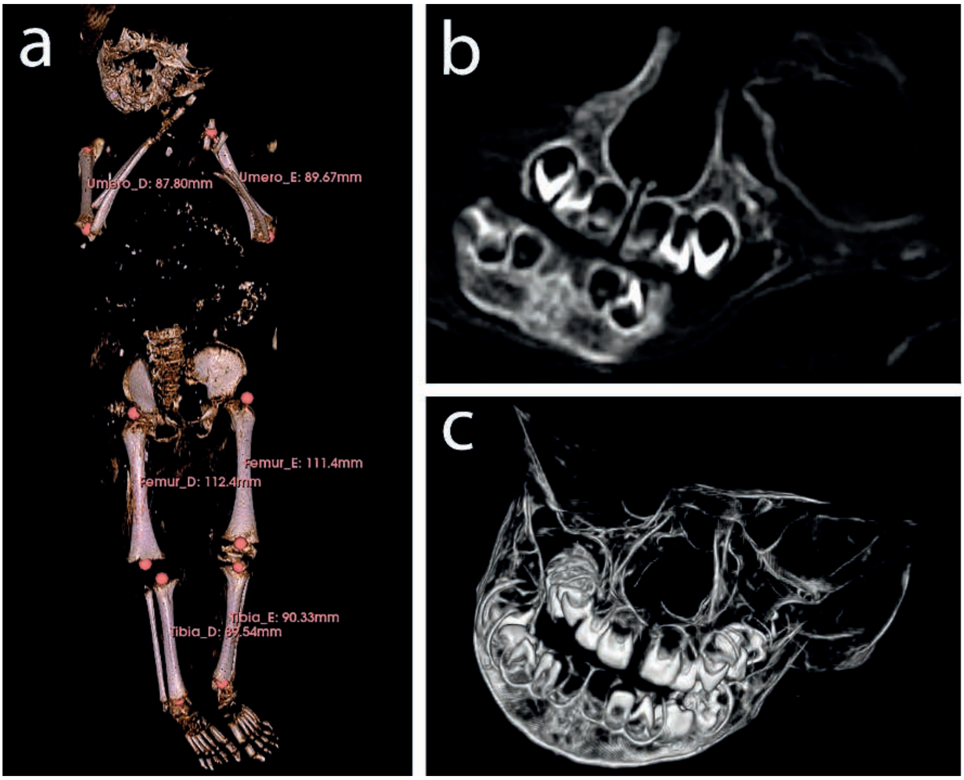

FIGURE 4. (a) Three-dimensional (3D) reconstruction of the infant's skeletal structure and specially the well-preserved long bones, and their length measurements. Notice the missing bones, such as some phalanges of the right toes, most of the ribs and vertebrae. The flexed arms with hands placed on the chest is typical of a catholic burial; (b) CT slice image of the preserved face of the same infant show mandible and maxilla bones, besides the stage of development of the dentition. (c) Three-dimensional (3D) reconstruction of the infant's face highlights dental structure confirming an age estimation between 6 and 9 months.

Source: Author´s file.

The direct examination of the body also brings to light a wad of cotton fibers, a plug or tampon, yellowish in color, retained between the gluteal folds of the infant´s mummified corpse. A closer examination of this material confirmed that it had been intentionally inserted into the body and was retained there thanks to the preservation process. The CT images helped to define the contours of this low-density material. It´s microscopic examination confirmed it was made up of cotton fibers in natura (Souza et al., 2024). Its position between the gluteal folds, introduced in the anus region and penetrating the rectal ampulla, and its contours spindle shape (approximate 24 mm in length, 7 mm in width, and 15.5 mm in height) was consistent with the anatomical situation (Figures 5a, 5b, 5c, 5d). Plugging the orifices of the body as preparation for funerals is a well-known practice in hospitals. Closer examination of the mouth and the vagina, where other possible tampons could be found, was negative for cotton fiber residues.

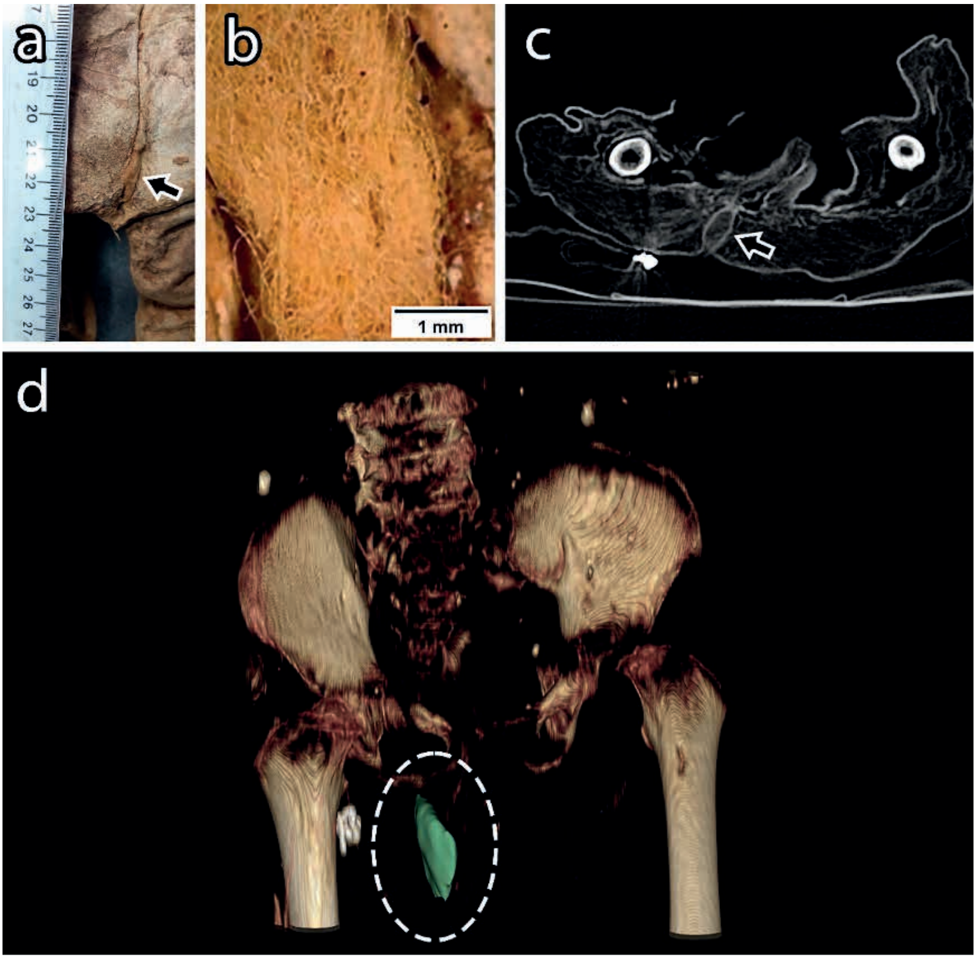

FIGURA 5. (a) Infant's lumbar view, arrows indicating the cotton tampon between the gluteal folds; (b) Detail of the tampon cotton fibers (ProScope digital microscope 50x); (c) CT slice at hip level of infant's mummy with the arrow highlighting the volume inside the rectum, an indication of an intentional funeral preparation; (d) Three-dimensional (3D) reconstruction of the infant's hip region, the cotton tampon is highlighted by the dashed circle.

Source: Author´s file.

#A0075: Adult male pelvis and thighs

The adult male pelvis and thighs preserve the genitalia, confirming the sex as male. Otherwise, the penis has been partially mutilated by a sharp cut that removed part of it, leaving less than 2 cm of its base. The aspect of the mutilation suggests that it occurred after the exhumation of the corpse. It may be associated with the habit of some residents who often disposed of the mummified bodies in disrespectful jokes, as related by local historians of Itacambira (Pires, 1999).

The skeleton has well-articulated bones, with the thighs extended, fixed in the position in which the body was laid out for burial. The internal organs are firmly attached by the mummified skin which, except for a partial destruction at the lumbosacral region, is preserved. There are also areas of loss and/ or tearing of soft tissues at the lower and posterosuperior portions of the left thigh. These losses, which occurred after mummification, have possible causes associated with exposure of the corpse to wet surfaces, as inside the basement. Recent pictures of the deposit show the bones and mummified parts spread at the bottom of the basement, on the soil surface, without any protection. Another damage is a large tear in the medial part of the left thigh that is suggestive of the action of scavenger fauna, a possibility since the basement was not totally isolated from the outside environment. Tooth marks of rodents were found in other specimens of the same CPFERA collection.

The skin of the thighs is tightly contracted around the remaining set of muscles, tendons, and other dehydrated structures It is also possible to notice that the the skin folds and pending towards the posterior part of the body, confirming the desiccation took place with the body in the supine position, and so was possibly the burial position (Figure 6a). There are no signs of blistering on the skin, nor evidence of insect colonization of the body, internally or externally, contrary to what was found by Braga et al. (2015) in other Itacambiraremains.

The upper part of the specimen is opened at the level of the iliac crests, and the thoracolumbar segment is absent, exposing the interior of the pelvic cavity. Remnants of soft organs are partially preserved inside, but no abdominal viscera can be recognized. As in other cases, here the untimely manipulation and vandalism damaged the specimen. The 5th lumbar vertebra is fixed in position by mummified ligaments of the base of the sacrum, and so are the iliac bones. At the opposite end, corresponding to the knees, the patellae are also attached to the femora, their position confirming the knees were extended for burial. The other bones of the lower limb are absent.

The first examination of the bones was direct, but detailed analysis was only possible with the help of CT scan 3D images, providing elements for sex and age estimation, including angular and linear measurements. In addition, longitudinal measurements of the femora helped to estimate height for this individual.

The maximum femoral length (Figure 6b) on both sides was 460.5 mm (right) and 460.4 mm (left). Based on Trotter (1970), the stature was estimated between 1.67 m and 1.71 m, considering a possible error of about 6 to 8 cm. These values would be in the interval variation of the average male height of Brazilians and considers that the individual could be of different origins, European, Asian or African. The shape and proportions of the bony pelvis, confirmed by the tomographic images, are consistent with those of a male (Figure 6c). The hip bones of predominant vertical dimensions are observed, with sciatic notch angles at 47º. On the other hand, the subpubic arch, with straight sides and a 60º angle, and the narrow pelvic opening are typical of male skeletons (Ubelaker, 1989).

The skeleton showed no signs of degenerative changes at the joints. At the metaphysis, parts of the epiphyseal fusion plates were still radiologically visible. This suggests that the individual was a young adult; however, examination of the 3D images corresponding to left and right symphysial surfaces, showed the formation of the annulus with persistence of the hiatus in their ventral margin, besides bone growths in their lower part. These details (Figure 6d), confirming phase IV of the Suchey-Brooks age changes (Brooks and Suchey, 1990), which leads to change the age estimate to middle adult, around 35 years (+/- 12 years).

Remnants of cotton fabric can still be seen adhering to the skin, testifying to the last garment or shroud, as described in Souza et al., (2024).

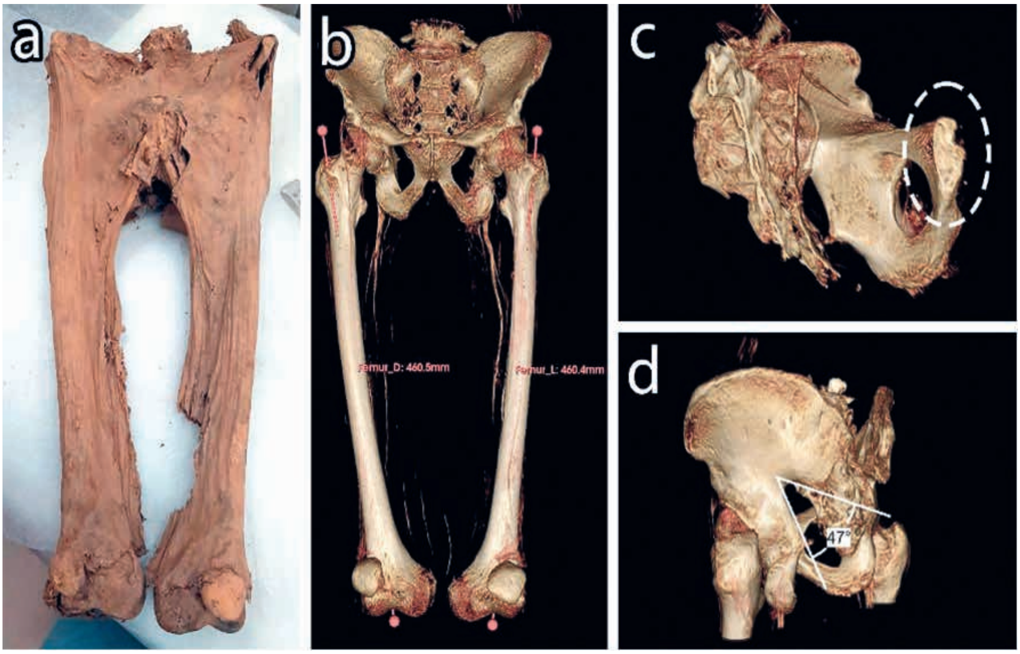

FIGURE 6. (a) Hip and thighs of the adult male (#A0075) with mummified intact skin and other preserved anatomical structures like muscles, vases, nerves and bones. Part of the left thigh is thorn off and the penis has been cut off. (b) Three-dimensional (3D) reconstruction highlights the bone structure of the hip and thighs of an adult male. Note the preservation of the pelvic bones, femurs and patellae, as well as the presence of the fifth lumbar vertebra. (c) Three-dimensional (3D) reconstruction of the left symphyseal face, highlighted by the dashed cycle, with characteristics of stage IV (35 years +/- 12 years) of the Suchey-Brooks age changes; (d) Three-dimensional (3D) reconstruction of the hip showing the gluteal surface view of the left hip bone, with the angle of the greater sciatic notch less than 90o, characteristic of males.

Source: Author´s file.

In addition to the observation of bone morphology, the three-dimensional colorized reconstructions, contrasting the muscle, bone and other components, exposed symmetrical cord like structures of radiological intermediate density both sides of the pelvis. The images of those structures (Figure 7a) confirmed they followed a downward path along the hip and thighs, on each side of the mummified corpse, resting on the anterior border of the hip bone, between the iliopubic eminence and the anterior inferior iliac spine. After crossing in front of the femoral head, both cordlike structures follow the medial portion of the thighs and disappear between their upper and middle thirds between muscles (Figure 7b). In the radiological image, it presents a higher density at the edges and a lower density in the center, suggestive of a structure endowed with a lumen, what is evidence in favor of a blood vessel.

Direct observation in situ and measurement of the cord-like structure gives 5 mm in maximum diameter (proximal end), and this value is maintained along the structure suggesting that it is the iliac and femoral segments of the artery. Other anatomical structures are visible in the pelvis and thigh, being recognized as thinner-walled vessels of larger diameters compatible with veins. Large filamentous structures compatible with nerves are also identified, as well as muscles and their tendons (Standring, 2010).

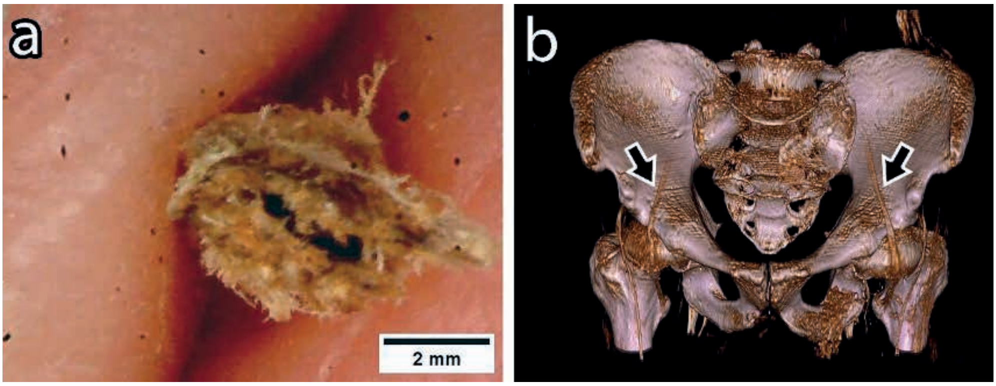

FIGURE 7 . (a) Microscopic view of the cross-section of the left femoral artery taken with a ProScope digital microscope (50x); (b) Three-dimensional (3D) reconstruction of the hip with arrows pointing to the femoral arteries.

Source: Author´s file.

A fortuitous finding during the analysis of the tomographic images was the presence of an approximately spherical structure, close to the linea aspera of the left femur, without continuity with that bone. Its size (7.6 mm x 3.5 mm), shape and location led to the hypothesis of a calcified cyst of cysticercosis, but its identification awaits specialized analysis.

DISCUSSION

NATURAL OR ARTIFICIAL?

Under the wooden floor of the altar of the church of Santo Antônio de Itacambira, in Minas Gerais, dozens of skeletonized remains, including a few mummified ones, were accidentally discovered in the middle of the 20th century. Possibly exhumed from the old church cemetery, they were moved inside a crypt, possibly attending to the Christian practice of saving the exhumed remains close to the chapel of the same church where they had been buried (Nascimento, 2013)

Two of the mummified humans remains in the series at CPFERA collection, together with a few other specimens at the basement of the church of Santo Antonio de Itacambira, have well preserved soft organs. Compared to the remains of another dozen individuals stored in the same basement, they have differential preservation. More than having a few dehydrated parts of tendons, cartilage or ligaments, they are true mummified corpses. The local explanations for the famous "mummies" of Itacambira suggest that the arid local climate and soil conditions are the only explanation for their preservation. The breakdown of a cadaver depends on autolysis, a chemical process affected by temperature and moisture and starts minutes after death; putrefaction, associated with the proliferation of microbes in the anaerobic environment of the dead body, causes changes in color and bloating/rupture of the cadaver; and decay, when the proliferation of aerobic microorganisms accelerate decomposition. The balance between decomposition and desiccation defines the probability of mumification (Aufderheide, 2004), but the desiccation as a natural factor to determine mummification is accepted only for the most extreme environments, as desertic sands of Egypt, Peru and Siberia, for instance (Tibet and Carter, 2008). In Itacambira, the average temperature of 22 o C associated to a silty and sandy soil would help draining the decomposition liquids, but does not explain a natural mummification process, although the association of both could explain a slower decay (CPRM, 2004).

New scientific evidence related to differential preservation and human intervention for the preservation of those corpses is now to be considered. The following discussion presents some hypothesis as to what has possibly halted the decomposition of these bodies before their burials, contributing to a better preservation.

The specimens analyzed here are the corpses of a an adult male and a female infant which have partially preserved soft tissues, including the frailest anatomical parts. The infant´s body #A0077 has a flexible skin and even delicate segments like the 2008 genitals can be recognized. Details of the face, eyelids, eyeballs and even the lips are reserved. A cotton plug inside the rectum points to a hospital preparation for funeral. Considering the adult male #A0075, special attention is drawn to the good preservation of muscles and other non-mineralized structures under the skin, being possible to identify cartilage, tendons, vessels, nerves and the genitalia. The vesels preserved are arteries, strongly suggesting that the iliac-femoral arteries were used for perfusion of a formaldehyde solution.

The use of formaldehyde for preservation of dead bodies began in the middle of the 19th century (Leonzo and Almeida, 2018) through injection in the vascular system following blood drainage. By reducing the processes of cadaveric decomposition and the unpleasant sensorial effects, that preparation gave time for prolonged transfers and wakes. Depending on the concentration and amount of formalin injected, impregnation acted on the organic components, dehydrating and eliminating part of the bioactive microbiota, thus interrupting the first chemical reactions of cadaveric decomposition. The gas and liquefaction phases were completely aborted, as confirmed in these two mummified specimens, without of signs of blisters on the skin. Unhappily, the chemical detection of traces of formaldehyde solutions, after such a long time, is very difficult, due to the volatility and transformation of the residues into other organic compounds. The pictures of the crypt under the altar confirm that the proportion of truly mummified corpses is very small, given the large number of skeletonized skulls and other bones. Both cases of body preservation, contrasting with the great number of skeletonized remains, preserving no more than scarce fibrous fragments of soft parts, strongly point to the intentional preparation of the two corpses. Their burials, at a cold dry region like Itacambira certainly contributed to their prolonged conservation.

The records of burials in the church books (https://www.familysearch.org) confirm that the cemetery of the church of Santo Antônio de Itacambira was used for almost two centuries. Those books have already been the source for demographic (Fonseca and Reis, 2012) and other projects of local history investigation. Thus, the exhumed remains deposited in the basement under the altar represent a small fraction of the individuals that have been buried there. Throughout the history of this locality, successive generations lived there, as confirmed by the census, and the evidence that hundreds of burials took place in that church is registered in the three death registers, containing their names, ages and other information, an incredible source for future research.

According to the same church death records, the former cemetery was used during all of the 19th century, a period when Itacambira had its most affluent economic cycles and population growth. It was only during the second decade of the 20th century that the church cemetery stopped to be used, and a new secular cemetery was opened at Itacambira. Counting the dead, it is possible to confirm that most of the funerals in that church were performed during the 19th century, probably the century when those ones described here were buried. A clue for their possible chronology is the flattening of their backs, and the absence of sediments inside and outside the mummified remains, suggesting that the bodies had been protected inside a coffin, and not directly deposited on the soil of the grave. The practice of using wooden coffins for burials started in the middle of the 19th century, partially motivated by sanitary considerations, as mentioned above, as a consequence of the plagues and epidemics of those difficult times.

The evidence points to the 19th century as the most possible period for the funerals of those two individuals. The 19th century was also the period when practices were changing, and hygiene principles were strongly introduced for funerals. Concerning the special treatment of the dead for funerals, the most distinctive finding was the presence of the anal plug in the infant. This finding confirms the procedure used in hospitals to prepare the bodies of the deceased for transport and burial. By closing the orifices of the body, like nostrils, mouth, vagina and anus, it was possible to reduce the discomfort of emanations and loss of fluids from the corpse, and this was also supposed to reduce the risk of contagion. Otherwise, that practice was unusual when people died at home, and should be considered here a conclusive element to the hypothesis of hospitalization, reinforcing the hypothesis of a preservation practice.

However, the hypothesis of intentional preservation makes it necessary to explain how some people could be submitted to an efficient technique of preservation in a small place like Itacambira. A small town like that seems not to have had resources to keep a hospital. The demographic census of 1872, late 19th century, (https://www.biblioteca.ibge.gov.br) does not mention resident professionals such as dentists, physicians, pharmacists, nurses, or even barber surgeons, who could eventually perform such formulation process. Until the beginning of the 20th century, itinerant doctors and barber-surgeons would occasionally visit and care for the sick in their homes. Healers, midwives, prayers and practitioners of all kinds would work trying to save bodies and souls. On the other side, the catholic church was modest, often under the administrative dependency of other churches like the one at Montes Claros (Pires, 1999), so it should not be expected that local religious groups could have practiced the preservation of the dead for funerals. Indeed, during the last centuries a varied practice for preserving bodies after death encompassed dozens of different methods. According to Aufderheide (2004), the resumption of such practices in the 18th and 19th centuries, in Western countries, manifests itself as fashions, in cycles associated with economic conditions, sanitary guidelines and other motivations. Different products were employed by those professionals in charge, and those practices were in force in Brazil at the end of the 18th century and in the years 1900. There are records of procedures to reduce cadaveric processes using formaldehyde solutions in the Santas Casas de Misericordia (Leonzo and Almeida, 2018). Temporarily stabilizing the decomposition of the corpses, those practices ensured more time and comfort for transfers and wakes. They were also believed to offer greater security against the transmission of diseases due to “cadaveric emanations”. The exaggerated use of this practice has even led to complaints that, in some places, bodies "did not decompose", to the point its practice had to be controlled. Thus, during the 19th century, the preparation of cadavers was in practice, though not available to everybody.

Hinterland areas like Itacambira would be subject to a condition that accumulated long-standing health problems (Sigaud, 2009; Silveira, 2011; Souza, 2020). According to different authors the population suffered from diseases ranging from the so-called "worms", to tuberculosis, goiter, yaws, among others. Silveira (2011), emphasizes two relevant health crises during the second half of the 19th century, caused by the epidemics of smallpox and cholera. The burial records of the church of Santo Antônio de Itacambira (https://www.familysearch.org) attest also to the high mortality of infants and children, as well as the precocious death of adults in that population.

The period of epidemics, as period of social crisis (Silveira, 2011), also precipitates changes, and possible the search for new resources as well as changes in the mortuary practices, should be associated to new times and practices. Some of those practices following the epidemic crisis are certainly the use of wooden coffins for burial, special care for the transportation of dead bodies, and a leading role of Santas Casas de Misericordia attending the ill and dead.

Montes Claros is historically and geographically a larger urban center, not very far from Itacambira, but with more historical, social and economic prominence. In that town there was one of the oldest Santas Casas de Misericordia in Brazil (Santos and Silva, 2014), dated to 1839. The distance between Montes Claros and Itacambira is estimated at about 100 km, which in due course would be overcome by simple transport, on mountain roads, which would require no less than one or two days of travel.

Santas Casas were religious institution designed for Mercy care, not as a true hospital. In fact, people in general would prefered to be treated at home, not going to hospitals even when very ill. This is especially true for people who had possessions, as Silveira (2013) recalls, in such a way that "(...) those who had possessions were treated at home, under the care of family members and under the guidance of a private doctor". From the mid-nineteenth century onwards this view of society tended to change, to the extent that hospitals also began to undergo modifications, under the new airs of science and hygiene, and "(...) Alongside the infirmaries for the free care of the poor, private pavilions began to appear, for the sick who could afford them. It would not be an exaggeration to say that this practice would last until the passage between the 19th and 20th centuries, especially when we examine the Brazilian case", as reminded by Silveira (2013). In the case of Itacambira, besides the fear of hospitalization, most people would not have the appropriate conditions to be taken to a distant hospital like the Montes Claros Santa Casa de Misericórdia.

The examination of the accompaniments preserved in the infant's body, garments and fabrics of different types, provide more clues for the interpretation of this case: her mummy was dressed in a sophisticated and well-finished garment, where cotton fabrics predominated. Additionally, a piece of fine fabric, possibly silk, was associated to her (Souza et al., 2024). These findings were considered indicative of a higher social origin for the infant. Access to the Santa Casa de Montes Claros would depend on a long trip, and a small infant, only a few months of age, would be followed by adults to care for her. To take such a young person to a far-distant hospital possibly means her social status, in times of high infant mortality, babies were expensive, and saving a child could be the difference between having or not having heirs.

FINAL COMMENTS

Changes in medical, hospital, and funeral practices reflect the change in the relationship between the society of that time and the institutionalization of health care, represented by the Misericórdia and its services. The findings in the two mummified bodies from the church of Santo Antonio de Itacambira, Minas Gerais State, Brazil, give us a glimpse of a historical moment when mobility and science started to change everyday´s life, witnessing a moment of important new social choices affecting even the small town of Itacambira. Bioarchaeological results here are consistent with the evidence of a changing society and its relationship with death.

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Bioarchaeology of infectious diseases in the Cathedral of Panama Viejo (1519-1671 A.D.) [1 2]
Bioarqueología de las enfermedades infecciosas en la Catedral de Panamá Viejo (1519-1671 A.D.)

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Abstract

This study examines the frequency and prevalence of lesions associated with non-specific infectious diseases in an osteological excavated at the first Spanish foundation on the Pacific coast of the Americas. According to historical sources, the settlement of this city in a humid and hot landscape could be a factor in the spread of several infectious illnesses, suggesting potential links with environmental adaptation processes. We conducted systematic and standardized macroscopic analyses on 175 individuals excavated from the Cathedral of Panama Viejo (1519-1671 AD), representing a range of sexes, ages, and biogeographic origins. Periosteal reactions were diagnosed through differential diagnosis by recording the type of reaction, location, distribution, severity, and lesion healing. Additionally, we performed Chi-squared and Fisher statistical tests to verify significant differences between subgroups within the sample. The prevalence of lesions associated with non-specific infectious diseases is 26.29%. However, a higher incidence is observed among men *(X^2: 9.08; df: 1; p: 0.00257)*. The high prevalence of these lesions in individuals aged 35-55 years in the osteological series (*54.55%*) suggests that they are typical of cumulative age-related processes. There are no significant differences concerning biogeographic origin. The results suggest that the living conditions of Panamanian populations were impacted by several factors that contributed to the prevalence of non-specific infectious diseases, such as the particularities of the landscapes, difficulties in the processes of environmental adaptation, exposure to pathogens.

Keywords: Infections, environmental adaptation, historical bioarchaeology

Resumen

Este trabajo busca aportar datos sobre la frecuencia y prevalencia de las lesiones asociadas a enfermedades infecciosas en una serie osteológica del periodo colonial excavada en la primera fundación española en el Pacífico americano. Las fuentes históricas señalan que la ciudad se asentó en un paisaje húmedo y caluroso, lo cual podría ser la causa de varias enfermedades infecciosas, sugiriendo posibles relaciones con los procesos de adaptación ambiental. Se realizaron análisis macroscópicos sistemáticos y estandarizados en 175 individuos excavados en la Catedral de Panamá Viejo en Panamá (1519-1671) de diferentes sexos, edades y orígenes biogeográficos. Para analizar las reacciones periósticas se realizó diagnosis diferencial registrando el tipo de reacción, localización, distribución, severidad y cicatrización de las lesiones. Adicionalmente se hicieron pruebas estadísticas para verificar diferencias significativas en subgrupos de la muestra. Se observa una prevalencia de 26.29% para lesiones asociadas con infecciones inespecíficas. Sin embargo, los hombres fueron más afectados (*X^2: 9.08; df: 1; p: 0.00257*). La alta prevalencia de estas lesiones en individuos entre 35-55 años *(54.55%)*, sugiere procesos acumulativos propios de la edad. No hay diferencias significativas en relación con el origen biogeográfico. Los resultados sugieren que las condiciones de vida de la población panameña se vieron afectadas por varios factores que incidieron en la prevalencia de las enfermedades infecciosas inespecíficas, que incluyen las particularidades del paisaje, las dificultades a los procesos de adaptación ambiental y la exposición a agentes patógenos.

Palabras clave: Infecciones, adaptación ambiental, bioarqueología histórica

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INTRODUCTION

The contact between Europe and the Americas has prompted extensive discussions regarding the unique characteristics of the process and the factors contributing to the success of the conquest. These considerations encompass elements such as the deployment of more advanced weaponry and transportation technologies, changes in labor and production systems, and perhaps the most significant impact on the American demographic—the introduction of disease (Betrán Moya, 2006).

In this regard, Mrozowski (2006) highlights how multiple studies have addressed the topic of biological impact, particularly in interpreting the role of disease during the exploration and subsequent implementation of the European colonial model from the 15th century onwards. During this period, the introduction of new pathogens had a profound effect on the living conditions, health, and disease patterns of local populations, leading to a demographic crisis.

Panama was not exempt from these circumstances. As the first city founded on the Pacific coast of the Americas in 1519, it quickly became one of the most significant ports in the Spanish Empire in the West Indies. This city served as a convergence point for Indigenous peoples, Europeans, and Africans, who had to adapt to new living conditions, environmental challenges, and several diseases. The original settlement remained until 1671, when it was besieged by Henry Morgan, leading to its relocation to Ancón in 1673 (Mena García, 1997). However, the initial site successfully established key colonial institutions, including the Cathedral, which functioned not only as the main religious temple but also as the seat of ecclesiastical authority.

During the colonial period, churches, convents, chapels, and hospitals became primary burial sites for deceased individuals (Aram et al., 2020; Martín-Rincón, 2002; Martín-Rincón and Díaz Pérez, 2000; Rivera-Sandoval, 2006, 2014). In specific contexts, these locations continued to be used as burial grounds until the early 20th century (Rivera-Sandoval, 2006). This study presents findings from archaeological excavations conducted at the Panama Viejo Cathedral between 2017 and 2018 as part of an interdisciplinary project involving historians, archaeologists, bioanthropologists, and geneticists aiming to reconstruct the living conditions of the population buried in this church (Aram et al., 2020). The explorations identified two distinct phases of cathedral construction. The earlier phase (1519-1541) was situated south of the main plaza and was characterized by a wooden structure, later relocated to the eastern side of the plaza (1542-1671), where it was rebuilt using stone materials (Aram et al., 2020; Hernández et al., 2021). In both buildings, burials of city inhabitants were identified, reflecting the population diversity at the port from its inception, including individuals of various origins.

This study offers reflections on the investigation of infectious diseases within bioarchaeology and presents the results from paleopathological analyses conducted on the osteological series recovered during archaeological excavations at the two sites occupied by the Panama Viejo Cathedral (Figure 1). The focus was on lesions indicative of non-specific infections. Through this analysis, the research identified the potential challenges faced by the inhabitants of the first Pacific port in adapting to the emerging environmental, sanitary, and sociocultural conditions established from 1519 onward, which influenced the population as it began to form. These interpretations are expected to be corroborated in the future through comparison with other contemporaneous collections, the integration of additional information sources, and the application of advanced technologies in the analysis of infections within bioarchaeology.

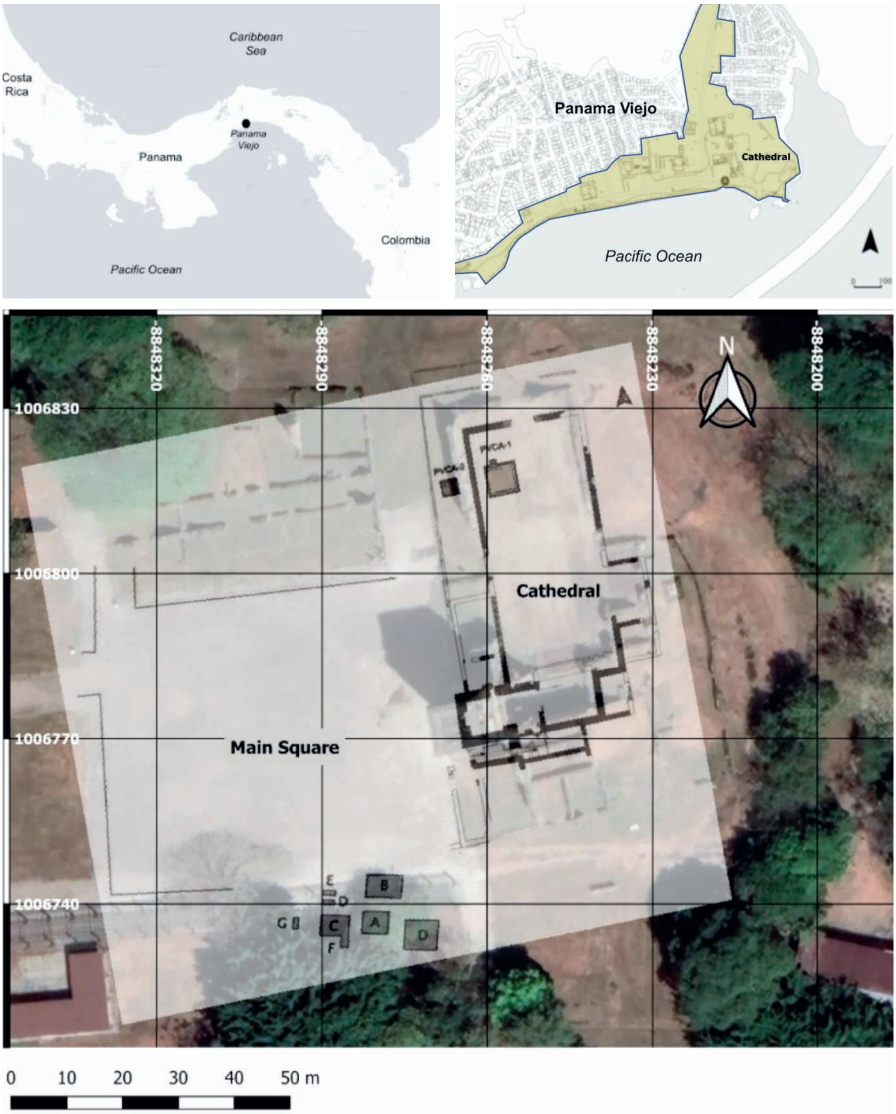

FIGURE 1. Location of the excavated areas of the Panama Viejo Cathedral.
Source: Author's elaboration based on information obtained from Hernández, (2022); Hernández et al., (2020).

THE STUDY OF DISEASES IN BIOARCHAEOLOGY

Human skeletal remains are regarded as the most significant source of information for interpreting the living conditions of past populations, their adaptive processes, and biocultural practices (Larsen, 2015). These remains also offer insights into individual life processes and the cumulative impact of life experiences, which play a role in the cultural construction of the body (Sofaer, 2006: 23).

From a biocultural perspective, the emergence of diseases within a population is contingent upon a complex interplay of factors that connect individuals with their ecological environment and the cultural context in which populations evolve. These interactions are dynamic and subject to change, resulting in evolutionary and adaptive processes (Goodman, 2013; 1993; Goodman et al., 1988; Rodríguez, 1999; 2006).

Adopting this interpretative model, the disease is understood as the outcome of the interaction between biological, environmental, and cultural factors that act as stressors, potentially disrupting homeostasis and manifesting in bone and dental tissues as stress markers (Luna, 2006). The health-disease status is a critical factor in assessing the living conditions of a population, as it reflects the degree of adaptability, the quality of diet, and the effectiveness of demographic regulatory mechanisms.

To understand the processes associated with diseases in ancient populations, it is crucial to consider the context in which the osteological remains were discovered, as this enables a more reliable interpretation. Additionally, such observations allow for the explanation of processes with significant temporal depth, as well as how these groups generated responses to the various factors that caused the lesions. However, some challenges limit the reconstruction of a paleopathological and paleoepidemiological profile of past populations, including the absence of soft tissue that could provide insights into the symptomatology of diseases, the limitations in diagnostic accuracy when relying on a few bone fragments, and the bone's non-specific or uniform response to various stressors that can produce similar lesions (Luna, 2006: 259).

In this regard, the work of Wood and colleagues (1992) highlights the complexities involved in analyzing osteological samples, emphasizing the importance of considering the characteristics of these samples and their implications for constructing paleodemographic and paleopathological profiles. Both types of profiles require population-based reference frameworks, which are conditioned by the ability to reconstruct the sample distribution according to criteria such as sex, age, biogeographic origin, or social status. However, with the introduction of the osteological paradox (Wood et al., 1992), certain considerations emerged that need to be considered in such studies, given the nature of the samples and the uncritical application of some assumptions that had been previously used in bioanthropology. Thus, the osteological paradox challenged a descriptive model by introducing the possibility of creating interpretative frameworks and testable hypotheses, but always within a population-based perspective (Wright et al., 2003: 43-44).

In paleoepidemiology, the osteological paradox also affects interpretative models through two key concepts: selective mortality and hidden heterogeneity in frailty (DeWitte and Stojanowski, 2015; Wood et al., 1992). In essence, these considerations began to challenge interpretations of osteological series previously deemed healthy due to the absence of skeletal responses indicative of disease. Wood and his colleagues demonstrated that populations exhibiting extensive responses to disease—manifested as stress indicators—had sufficient time for their skeletal structures to react. In contrast, groups lacking defense mechanisms against pathogen exposure did not show such skeletal evidence.

When considering the population-based nature of this reconstruction model, it becomes almost essential to quantify the sample according to sex and age group distribution. This issue has been a point of contention in academic circles, where the lack of precision in the data is frequently criticized. However, employing more flexible models that incorporate statistical techniques allows for the generation of working hypotheses and interpretations that enhance the data provided by archaeological contexts. It is important to clarify that interpretations made in bioarchaeology are conditioned by the sample analyzed, making it impossible to generate expectations regarding the living population to which the sample belonged (Luna, 2006: 274).

The approaches and projections reconstructed from the data can be helpful when analyzing an osteological series, helping to avoid confusion that may arise from using more rigid models that do not adequately capture the health, life, and mortality conditions of past populations. For instance, the application of statistical tests may have limited sensitivity to low values, which are represented by slight variations (Mendonça de Souza et al., 2003:23).

The consideration of other variables may address this issue, beyond the statistical representativeness of the sample. A thorough evaluation of the archaeological context would provide insight into the biocultural landscape in which the individuals under study interacted, bringing us closer to constructing a profile that more accurately reflects the conditions faced by a human group during a specific period. In summary, it is essential not only to consider the information provided by quantitative data but also to conduct a parallel and cross-referenced review with qualitative data.

METHODOLOGY

THE RECORDING OF INFECTIOUS LESIONS IN PALEOPATHOLOGY

In recent years, research in bioarchaeology and paleopathology has refined the documentation of lesions caused by infectious diseases, incorporating interpretative models from a biocultural perspective. Efforts have also been made to elucidate the etiology, transmission routes, and response mechanisms of pathogenic agents (Larsen, 2015: 66). However, osteological evidence of such infections is not always present, necessitating the development of a robust methodology for accurate lesion recording. This includes employing reliable diagnostic tools, utilizing appropriate terminology, ensuring systematic recording, applying stringent criteria for diagnostic certainty, and incorporating differential diagnosis and threshold criteria in the diagnostic process (Buikstra et al., 2017; Vlok, 2023).

The incomplete preservation of skeletal remains, often encountered in bioarchaeological contexts, limits paleopathological observations, particularly in cases of infection, which are classified as specific or non-specific. The latter are more frequent due to the bone's uniform response, typically affecting the outer layer known as the periosteum. In the literature, such lesions are referred to as periosteal new-bone formation, periostitis, and periostosis (Weston, 2012). In more severe cases, lesions like osteitis and osteomyelitis may develop, affecting even the medullary cavity of the bone structures (Aufderheide and Rodríguez-Conrado, 1998; Fornaciari and Giuffra, 2009; Larsen, 2015; Waldron, 2009). The challenge in identifying the stressor of these non-specific lesions lies in their potential origins, which could include various infectious pathogens, as well as vascular, nutritional, neoplastic, or traumatic factors, among others.

On the other hand, specific infections typically elicit a systemic skeletal response, where the assessment of multiple osseous indicators together can be considered pathognomonic for a particular disease. This category includes diseases such as tuberculosis, scurvy, treponematoses, and leprosy, all of which were prevalent during the colonial period.

Given the characteristics of osseous indicators, it is essential to employ differential diagnosis, which involves the accurate description, recording, quantification, and interpretation of a lesion (Buikstra et al., 2017; Vlok, 2023). In this context, the recording of infectious lesions in the sample selected from the Panamá Viejo Cathedral was conducted on skeletal structures with more than 50% representation. However, some individuals with lower representation were included if a periosteal reaction consistent with infection was evident. The documentation was based on the type of periosteal reaction observed (surface modification or bone destruction), the location on the bone, the distribution in the affected area (focal or diffuse), the percentage of involvement, the severity of the lesion, and the type of healing observed (Buckley and Tayles, 2003; Weston, 2008; 2012). Lesions were classified as consistent with infection if they exhibited a diffuse distribution, involved more than one skeletal structure, and were characterized by specific patterns of bone formation. Additionally, we performed Chi-square and Fisher's exact tests to compare prevalence rates by sex, age, and biogeographic origin, and to assess significant differences between subgroups within the sample.

MATERIALS AND OSTEOLOGICAL ANALYSIS

The sample analyzed in this study comprises 175 individuals excavated in 2017 and 2018 from the two locations that housed the Cathedral of Panamá Viejo (Table 1). Of these, 159 were found in the nave and atrium of the Cathedral (1542-1671), while the remaining 16 were in the southern part of the main square (1519-1541), where the city's main temple was initially established (Aram et al., 2020; Hernández et al., 2021; Hernández 2022).

TABLE 1. Distribution of the sample analyzed by sex and age cohorts.

Age	Male		Female		Indeterminate		Unobservable		TOTAL	
	N	%	N	%	N	%	N	%	N	%
Infant I (0-6 years)	0	0,00	0	0,00	5	2,86	0	0,00	5	2,86
Infant II (7-12 years)	0	0,00	0	0,00	4	2,29	0	0,00	4	2,29
Juvenile (13-17 years)	2	1,14	3	1,71	2	1,14	0	0,00	7	4,00
Young Adult (18-34 years)	31	17,71	57	32,57	0	0,00	3	1,71	91	52,00
Middle Adult (35-55 years)	8	4,57	14	8,00	0	0,00	0	0,00	22	12,57
Adult (+18 years)	9	5,14	22	12,57	5	2,86	10	5,71	46	26,29
Total	50	28,57	96	54,86	16	9,14	13	7,43	175	100

Source: Author's elaboration.

The osteological analysis included information on the sex and age of the individuals, as well as biogeographical origin or phenotypic affiliation in cases where this observation was feasible. These three criteria are addressed to determine whether significant differences in the prevalence of infectious lesions can be identified within the sample based on gender, age, or ethnic affiliation. Sex determination was based on dimorphic characteristics of the pelvis and skull (Acsadi and Nemeskeri, 1970; Buikstra and Ubelaker, 1994; Phenice, 1969). Age estimation involved examining changes in the pubic symphysis and auricular surface of the ilium (Brooks and Suchey, 1990; Buckberry and Chamberlain, 2002; Lovejoy et al., 1985) and, secondarily, the closure of cranial sutures (Acsadi and Nemeskeri, 1970; Meindl and Lovejoy, 1985) when more diagnostic structures were unavailable. For biogeographical origin estimation, the study considered theoretical, methodological, and ethical challenges associated with this type of analysis (Dunn et al., 2020). However, it is essential to address this variable given the sociohistorical particularities of Panama during the 16th and 17th centuries, a period marked by the formation of a city shaped by the first wave of globalization. This era saw the interaction of Indigenous groups with European, African, and Asian populations (Aram et al., 2020). In this context, complex relationships were established at various levels of daily life, where access to resources, occupational roles, and socioeconomic status may have influenced risk factors for developing infectious diseases. In this way, it is important to clarify that the aim was not to classify individuals into racial categories, which are not recognized within the discipline, but rather to identify geographical patterns that could provide insights into the biological origins and diversity of human populations. Accordingly, methods based on prediction algorithms using craniometric observations (Navega et al., 2015), morphological features of the facial skeleton (Hefner, 2009), and non-metric dental traits (Scott and Irish, 2017; Scott et al., 2018) were employed.

Although few, juvenile (4%) and infant (5.14%) individuals recovered during the archaeological interventions were included in the sample. For these individuals, dental development stages were recorded for age estimation (AlQahtani et al., 2010; Demirjian et al., 1973; Mincer et al., 1993; Moorees et al., 1963; Ubelaker, 1989), followed by the assessment of bone maturation and epiphyseal union (Schaefer et al., 2008; Scheuer and Black, 2000). In the case of infant individuals (0-12 years), no observations on sex were made due to the incomplete maturation of diagnostic skeletal elements.

RESULTS

Table 2, presents the results from the paleopathological analysis of non-specific infectious lesions recorded for the Cathedral sample, showing an overall prevalence of 26.29% (46/175). Examination of prevalence by sex reveals a significant disparity, with a higher proportion affecting men (46%; 23/50) compared to women (21.88%; 21/96). This contrasts with findings reported for the human skeletal collection from the colonial period of Panamá Viejo (Rojas-Sepúlveda et al., 2011), recovered from the Hospital of San Juan de Dios, the Church of the Society of Jesus, the Convent of La Concepción, and the Cathedral, where infectious diseases affected 20% of men (3/20) and 18.75% of women (4/16), figures relatively closer to those observed for females buried in the Cathedral. However, the frequencies reported by Rojas-Sepúlveda et al. (2011) are generally low, which may have influenced the results. Nevertheless, it is important to consider these data, as they pertain to chronologically contemporaneous contexts with populations similar to those identified in the Cathedral.

TABLE 2. Prevalence of infectious lesions according to sex, age, and biogeographic origin.

Sex	n/N	%
Female	21/96	21,88
Male	23/50	46,00
Indeterminate	0/16	0,00
Unobservable	2/13	15,38
Total	**46/175**	**26,29**
Age	**n/N**	**%**
Infant I (0-6 years)	0/5	0,00
Infant II (7-12 years)	0/4	0,00
Juvenile (13-17 years)	2/7	28,57
Young Adult (18-34 years)	26/91	28,57
Middle Adult (35-55 years)	12/22	54,55
Adult (+18 years)	6/46	13,04
Total	**46/175**	**26,29**
Biogeographic Origin	**n/N**	**%**
Predominantly European	11/33	33,33
Predominantly African	11/40	27,50
Predominantly Indigenous	2/16	12,50
Indeterminate	22/86	25,58
Total	**46/175**	**26,29**

Source: Author's elaboration.

Regarding age, infections exhibit a higher prevalence in middle-aged adults (35-55 years) at 54.55% (12/22), compared to young adults (18-34 years) and juveniles (13-17 years), each accounting for 28.57%. This may be attributed to the cumulative nature of such diseases, but it is also essential to consider the implications of the osteological paradox, where certain individuals do not survive long enough to develop observable skeletal responses to disease (Wood et al., 1992), with younger populations being more affected. In this sample, for instance, children do not exhibit observable lesions associated with infections. Concerning biogeographic origin, the most affected groups were those of European descent (33.33%; 11/33), followed by individuals of African origin (27.50%; 11/40), and to a lesser extent, those of Indigenous filiation (12.5%; 2/16).

Considering the age groups and the degree of lesion healing (active, healed, and mixed), along with individuals without lesions, Table 3 reveals that middle adults exhibit the highest proportion of individuals with fully healed lesions (22.73%), followed by young adults (8.79%), with no cases observed in the juvenile group. Similarly, for mixed lesions—those displaying both reparative and active processes—middle adults show the highest proportion (31.82%), while juveniles and young adults each account for 14.29%. However, the distribution changes for active lesions, with the highest percentages observed among juveniles (14.29%), followed by young adults (5.49%), and no cases recorded in middle adults. This supports the idea of cumulative processes associated with age. It is important to interpret the data for the juvenile population cautiously, as only two individuals in this group represent periosteal reactions.

TABLE 3. Frequency of individuals with periosteal reactions based on the degree of healing or absence of lesions

Age	Absent (%)	Active (%)	Mixed (%)	Healed (%)	Total
Infant (0-12 years)	9 (100)	0 (0)	0 (0)	0 (0)	9
Juvenile (13-17 years)	5 (71.42)	1 (14.29)	1 (14.29)	0 (0)	7
Young Adult (18-34 years)	65 (71.43)	5 (5.49)	13 (14.29)	8 (8.79)	91
Middle Adult (35-55 years)	10 (45.45)	0 (0)	7 (31.82)	5 (22.73)	22
Adult (+18 years)	40 (86.96)	3 (6.52)	0 (0)	3 (6.52)	46
Total	**129 (73.71)**	**10 (5.14)**	**21 (12)**	**16 (9.14)**	**175**

Source: Author's elaboration.

When comparing the data by sex and age, statistically significant differences are observed between men and women (X2: 9.08; df: 1; p: 0.00257), suggesting that males were exposed to conditions that favored the development of infectious lesions. Similarly, comparing young adults and middle-aged adults reveals significant differences (X2: 5.35; df: 1; p: 0.0206), supporting again the notion of cumulative processes with age. This pattern is also observed when contrasting data for young adult females with young adult males (X2: 5.41; df: 1; p: 0.0199), but no such differences are seen when comparing middle-aged females and males (Table 4). Furthermore, no significant differences are found in the comparisons by biogeographic origin and sex, suggesting that phenotypic filiation did not play an important role in the development of infections.

TABLE 4. Chi-square tests, Fisher and P-values, for the lesions observed.

Differences between Sex and Age	X^2	*p*
Female-Male	9,0882	0,002573
Young Adults-Middle Adults	5,3552	0,02066
Young Adult Female-Young Adult Male	5,4122	0, 019996
Medium Adult Female-Medium Adult Male	-	0, 6749*
Differences between Sex and Biogeographic origin		
African-Europeans	0,2922	0,58879
Indigenous-European	-	0,1741*
African-Indigenous	-	0,3078*
African Female-European Female	-	1,00*
African Female-Indigenous Female	-	0,6563*
Indigenous Female-European Female	-	0,64*
African Male - European Male	-	0,6517*
African Male-Indigenous Male	-	0,2657*
Indigenous Male-European Male	-	0,6126*
* Values are Fisher's F-test.		

Source: Author's elaboration.

DISCUSSION

Periosteal new bone formation results from various factors that stimulate a response in the superficial layer of bone, including vascular reactions, traumatic events, neoplasms, or systemic infections. In bioarchaeological studies, such formations are typically associated with non-specific infections; however, clinical literature has demonstrated that they may also arise from other processes (Weston, 2008; 2012). This highlights the necessity and the challenges of conducting accurate differential diagnoses of lesions, as outlined in the methodology section.

These challenges are further compounded by taphonomic damage to the material, the representativeness of skeletal structures, and the bone's uniform response to diverse stressors. Despite these difficulties, the study of the sample recovered from the Cathedral of Panama Viejo involved a systematic recording and differential diagnosis to identify bone manifestations consistent with infections while excluding periosteal reactions associated with other etiologies (Figure 2).

FIGURE 2. Periosteal reaction consistent with non-specific infections, showing different stages of healing in several individuals.

Source: Author's elaboration.

The sample analyzed from the Cathedral of Panama Viejo exhibits a moderate prevalence of periosteal reactions associated with infections (26.29%), which exceeds by approximately ten percentage points the prevalence previously reported by Rojas-Sepúlveda et al., (2011) for Panamá Viejo (15.62%). This difference may be attributed to the smaller sample size in the earlier study. However, a clear trend is observed, with male individuals exhibiting more cases than females, albeit with a smaller gender difference than that observed in the Cathedral sample. At the time, this pattern was interpreted as a consequence of the adaptation processes to the environmental conditions of Panamá experienced by foreign groups (Europeans and Africans) and the impact on local populations following contact with populations from the other side of the Atlantic.

Table 5, presents data reported for other osteological series from historical contexts in Hispanic America comparable to those from Panama. Among these, it is noteworthy that in the city of Campeche, Mexico, during the 16th and 17th centuries, an absolute prevalence of 30% of individuals exhibited such lesions. However, subgroup analyses revealed that juveniles, foreign populations, and males were the most affected (Rodríguez Pérez, 2010). This situation contrasts with Panama, where no significant differences were observed regarding the biogeographic origin, and the highest prevalence of lesions was found among middle-aged adults. Researchers in Campeche interpreted these findings as linked to nutritional deprivation, which, alongside chronic exposure to stressors, influenced the development of such lesions in specific population sectors, particularly concerning their ethnic origin.

In the case of the Royal Hospital of San Jose de los Naturales, Mexico, spanning the 16th to 18th centuries, a total prevalence of 28.12% was recorded, with males, juveniles, and young adults being the most affected. However, researchers emphasize that this may be related to the characteristics of the sample, where these demographic groups were the most represented (Meza-Peñaloza, 2023). Although individuals of diverse biogeographical origins were identified, the study did not report specific data regarding periosteal lesions. Nevertheless, the findings are interpreted in the context of the profound changes brought about by establishing the colonial structure, which affected various sociocultural domains. It is also noted that the prevalence may have been underestimated due to the potential for acute conditions experienced by the population.

Even with a similar prevalence, studies conducted on the Convent of San Francisco church in Quito, Ecuador, reported that 26.78% of the sample exhibits periosteal new bone formation (Ubelaker, 1994). However, the chronological range is broader than previous cases, including individuals buried between the 16th and 19th centuries. This broader temporal span complicates the analysis of the impact of these lesions on a more precise timescale. Additionally, how the data are presented does not allow for the observation of prevalence by subgroups such as sex, age group, or biogeographical origin.

TABLE 5. Periosteal lesions reported in other studies of historical bioarchaeology in Hispanic America.

Archaeological Site	Chronology	Prevalence of periosteal lesions	Reference
Cathedral of Panama Viejo, Panama	1519-1671	46/175 (26,29%)	Present study
Panama Viejo, Panama	1519-1671	10/64 (15,62%)	Rojas-Sepúlveda et al., 2011
Puruchuco-Huaquerones, Peru	1532-1540	14/162 (8,64%)	Williams and Murphy, 2003
Mórrope, Perú	1534-1760	28/67 (41,8%)	Klaus and Tam, 2009
Royal Hospital of San José de los Naturales, Mexico	1531-1761	124/441 (28,12%)	Meza-Peñaloza, 2023
San Martín de Timucua, Florida, USA	1608-1656	32/80 (40%)	Stojanowski, 2013
Cathedral of Campeche, Mexico	16th-17th centuries	54/180 (30%)	Rodríguez Pérez, 2010
Convent of San Jerónimo, Mexico	16th-19th centuries	159/184 (86,41%)	Bautista Martínez and Jaén Esquivel, 2023
Church of the Convent of San Francisco, Quito, Ecuador	16th-19th centuries	15/56 (26.78%)	Ubelaker, 1994
Church of La Caridad, Mendoza, Argentina	16th-19th centuries	8/34 (23,52%)	Mansegosa, 2016
Central Cemetery of Bogotá, Colombia	1850-1950	260/1455 (17,9%)	Rojas-Sepúlveda et al., 2020
La Candelaria Mission (appendicular periosteal reaction), Tierra de Fuego, Argentina	1893-1948	13/15 (86%)	Laborde et al., 2024
La Candelaria Mission (costal periosteal reaction), Tierra de Fuego, Argentina	1893-1948	10/18 (55%)	Laborde et al., 2024

Source: Author's elaboration.

Regarding healing processes, although no statistically significant differences were found between age groups (*X2: 5.9522; df: 2; p: 0.050991*), the data suggest that middle adults are more likely to exhibit healing processes, whether mixed or fully completed, compared to young adults or juveniles. This pattern is associated with these individuals' resistance and survival mechanisms, without ruling out the possibility that they may have had access to medical treatments that could have more effectively addressed these types of diseases compared to younger individuals. In other bioarchaeological studies on non-specific infections, such as those conducted by DeWitte (2014), it is noted that no individuals over 30 years of age with active lesions lacking evidence of healing were reported. A similar observation is made for individuals over 35 years old in the Panama Cathedral sample. These findings suggest that individuals who survived to this age developed mechanisms of resistance to the various stressors associated with infectious diseases, in contrast to young adults or juveniles, where low prevalence or absence of healing is observed.

In this context, it is essential to consider the framework of the osteological paradox (Wood et al., 1992; DeWitte and Stojanowski, 2015) and the concept of hidden heterogeneity, which posits that younger individuals are more susceptible to death from infectious diseases. These individuals may not develop any observable bone response in acute infections, as was the case with the subadult individuals in the Panama Viejo Cathedral sample, none of whom exhibited periosteal reactions consistent with infections. Consequently, those who survived the earliest stages likely possessed greater resistance and, therefore, would be expected to exhibit higher prevalence rates of healed or healing lesions.

On the other hand, when considering environmental factors as a model to explain the prevalence of periosteal lesions, the results of the study suggest a relationship between infectious diseases and adaptive processes to the warm, humid environment characteristic of Panama, an aspect also documented in historical sources, along with difficulties in obtaining potable water in the city. In this context, a recent study on the diet of the population of Panama Viejo (Martin et al., 2021), which incorporates historical, isotopic, and paleobotanical analyses, identifies a broad range of plant and animal products that were part of the diet during the colonial period. This finding rules out the hypothesis that changes in diet caused trauma, the introduction of new products, or shifts in resource access mechanisms resulting from socioeconomic models implemented from the 16th century onwards. However, the same study reports paleopathological data indicating a relatively high prevalence of porotic lesions in the cranial bones and linear enamel hypoplasia, which, while nonspecific indicators, could, when cross-referenced with other evidence, reflect metabolic stress responses due to poor water quality and susceptibility to parasitic diseases. This situation, in turn, would have affected the individuals' nutritional status, weakened their immune systems, and made them more vulnerable to infectious diseases.

The presence of these types of diseases, can be associated with the challenging environmental conditions in which the first cities in the Americas were established during the 16th and 17th centuries. In this regard, some historians have pointed out that at that time, there were various perspectives on the selection of locations for these settlements, emphasizing the attributes they should have, including sufficient sources of water and food (López-Ríos Fernández, 1993: 77). The environmental conditions were also considered following the humoral theory of Galenic and Hippocratic medicine, where it was recommended to choose healthy locations with pure air and temperate climates (Alzate Echeverri, 2007: 48). However, it was necessary to adapt to the geographical, climatic, and historical particularities of the New World. From the humoral perspective, a set of ideas began to take shape regarding the effects of the environment on human life, including aspects related to disease contagion and the geographical predisposition to certain illnesses.

These ideas persisted throughout the entire colonial period, and they even found some parallels with the indigenous perception of the relationship between humans and the environment, which eventually merged with Western medical discourse. In this context, disease was defined as an organic imbalance caused by the influence of the environment on the individual, a concept linked to the ideological constructs of the humoral and temperamental models. These frameworks conditioned not only the bodies of individuals but also those of cities (Campos Rodríguez, 1997).

This explains how government authorities began to issue a series of regulations and recommendations for selecting the most favorable sites for the foundation of settlements. However, such recommendations did not always characterize the locations where many urban settlements in Panama were established. For instance, during the 16th century, there was mention of high mortality due to endemic diseases, exacerbated by the heat and humidity of the region. Additionally, the lack of resources during the early years of the city further contributed to this, as they only had access to products from the sea, which only partially met their nutritional needs (Mena García, 1992:37-38). However, this situation appears to have stabilized according to the isotopic and paleobotanical studies mentioned above (Martin et al., 2021).

Similarly, authors such as Rubio (1947) and Susto (1955) argue that the visibility of these problems became evident very early on. There are even references to comments suggesting the potential relocation of the city of Panama by Antonio de Gama in 1531, and by Juan de Tejeda and Juan Bautista Antonelli in 1591. These precedents reflect the dissatisfaction with the city's location, indicating that the site chosen for Panama Viejo was selected hastily without adequately considering the sanitary and sustainability conditions required for the settlement. This situation led to the rapid decline of many early foundations on the mainland, such as Belén, Santa María la Antigua del Darién, San Sebastián de Urabá, Acla, and Nombre de Dios (Mena García, 1996).

However, the Spanish Crown had other motivations for maintaining the settlement of Panama, which were unrelated to urban planning or the environmental and sanitary conditions under which the city developed (Mena García, 1996). Even the city's founder, Pedrarias Dávila, and his companions described these lands as unhealthy and waterlogged. The persistence of the settlement was driven by political and economic interests, with the isthmus serving as a crucial link in interoceanic and intercontinental trade. Complaints about the swampy areas and the lagoon located to the north of the city were common (Figure 3), though these features also functioned as natural barriers that helped limit and protect the settlement (Chaunu, 1983: 152). Nevertheless, in 1538, there was consideration of draining this 'lagoon,' which formed during Panama's long rainy season, due to the diseases it caused and the limitations it imposed on the city's expansion in that direction (Rivera-Sandoval, 2021).

Additionally, potable water sources were quite limited, considering that the Gallinero and Algarrobo rivers did not provide sufficient water to meet the city's needs. Shafroth (1953: 8) points out that the supply of drinking water was always a challenge, as it had to be brought from streams located far from the city. While it is true that many homes in Panama had wells, many of them produced brackish water, which became yet another factor contributing to the spread of diseases.

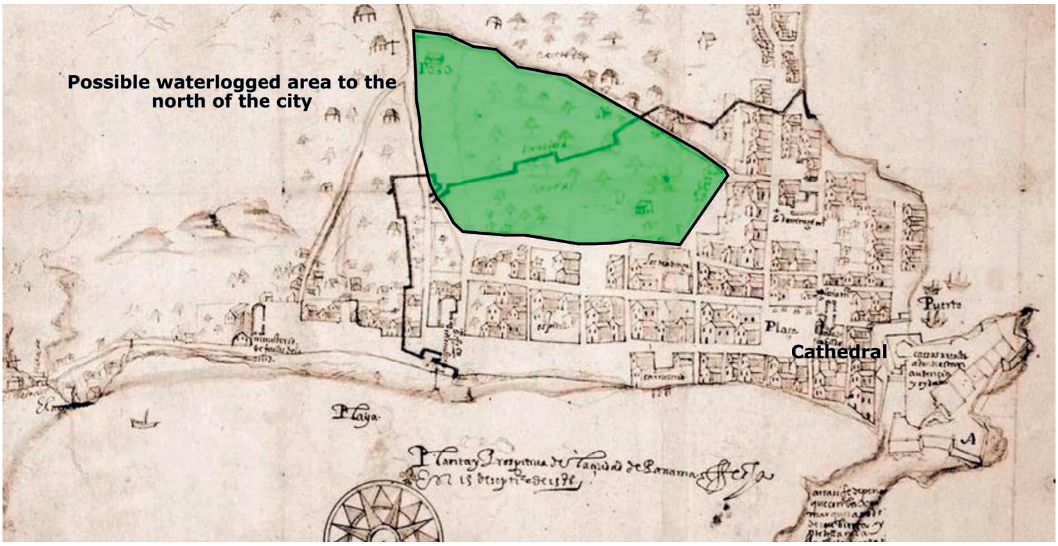

FIGURE 3. Map of the city of Panama by Bautista de Antonelli (1586), indicating the possible area that experienced flooding problems during the rainy season.

Source: Author's elaboration. Modified from an image courtesy of the Archivo del Museo Naval, Madrid, Spain.

By the late 16th century, concerns about the city's location and its impact on the health of its inhabitants continued to be raised, once again relying on arguments that referenced environmental conditions. An example of this can be seen in the report prepared in 1591 by Don Juan de Tejeda and the military engineer Bautista Antonelli:

> *"It would also be beneficial to the service of Your Majesty and the health of those involved in the Peru trade, as well as the residents of Panama, to relocate the city of Panama from its current location, as the site is unhealthy and at times experiences conditions as unhealthy as those in Nombre de Dios. The cause is that the city is situated in a lowland where all the rainwater gathers, and about fifteen hundred paces away, there are stagnant water swamps. With the intense heat, these waters rot and release harmful vapors into the city, causing fevers.*" (Carles 1969: 9, translate by the author).[2]

The adaptation to the environmental conditions in which the city of Panama was founded posed significant challenges, greatly affecting the living conditions of its inhabitants. This would explain the increase in infectious diseases, with the leading causes being the lack of potable water, poor sanitary and hygienic conditions in the city, population growth, and proximity to swampy areas that became centers of infection

CONCLUSIONS

After conducting a systematic review of infection-compatible lesions in the Panama Viejo sample, the data suggest a significant impact on the living conditions of the Panamanian population during the 16th and 17th centuries. The data also suggest that men and middle-aged adults (35-55 years) were more affected by these lesions compared to women and other age groups. However, attention must be drawn to the potential impact of these diseases on individuals with weakened immune systems, who may have died without exhibiting osseous responses, as

2 Original text: "También convendría al servicio de Vuestra majestad y a la salud de los que tratan en este comercio del Perú y de los vecinos de Panamá que se mudase la dicha ciudad de Panamá de donde está de presente por ser el lugar malsano y a veces suele tener tan poca salud como Nombre de Dios; y causa es estar situada la dicha ciudad en un bajo a donde vienen a morir todas las aguas que llueven y como a mil quinientos pasos tienen unas ciénagas de agua que con los grandes soles vienen a podrirse las aguas y dan malos vapores a la ciudad y esto causa calenturas." (Carles 1969:9).

proposed by the osteological paradox (Wood et al., 1992). Although higher prevalences in the male population have also been reported in other bioarchaeological contexts of Hispanic America, the underlying causes remain unclear. It is likely that, since women were the ones who remained permanently in the city, they may have adapted more effectively to the specific environmental conditions of Panama, resulting in greater resistance to infectious diseases. In contrast, men, who were primarily a transient population and frequently traveled to different territories, were exposed to various sources of infection.

On the other hand, the relatively high frequency observed in middle-aged adults (54.55%) suggests that they may be responding to cumulative processes associated with aging. However, when comparing the data concerning biogeographic origin of these individuals, no significant differences are found, indicating that, regardless of population affiliation, there was a generalized exposure to pathogenic agents.

The analysis also highlights the prevalence of non-specific infections compared to those with a more evident pathognomonic profile, indicating the need for immunological, histological, and radiological tests to confirm the diagnoses made in this study.

Finally, the data obtained from the Cathedral of Panama not only illustrate the impact these diseases had on the population's living conditions during the 16th and 17th centuries but also suggest a strong relationship with the environmental conditions of the site chosen for the city's foundation. This is supported by historical documentation and recent archaeological and bioarchaeological studies (Aram et al., 2020; Hernández et al., 2021; Martin et al., 2021).

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Plant use from Jabuticabeira II southern brazilian shellmound (3137–2794 to 1860–1524 cal BP): A paleogenomic approach

Uso de plantas del montículo de conchas Jabuticabeira II del sur de Brasil (3137–2794 to 1860–1524 cal BP): Un enfoque paleogenómico

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Abstract

The study of ancient DNA (aDNA) in archaeological contexts has been a valuable source of information for the identification of plant microremains. This study proposes an integrative methodological approach that combines metadata with a customized flora database, an unprecedented initiative thus far. The applied methodology involved the use of Sanger sequencing and High-Throughput Sequencing (HTS) for ancient DNA (aDNA) analysis in sacral sediments and control samples from six burials at the Jabuticabeira II *sambaqui* (shellmound) archaeological site (JABII), located in southern Brazil, comparing the results with databases constructed based on information from the Flora do Brasil platform. In total, the two applied methods identified 28 families corresponding to 200 possible species, including species with utility for consumption, use as tools, and medicinal properties. This study presents an innovative methodology for the integration of floristic data in paleogenetic analysis, contributing to a more comprehensive understanding of the role of plants in the cultural practices of *sambaqui* people in southern Brazil.

Keywords: Ancient DNA, Flora,High-Throughput Sequencing, *Sambaquis*

Resumen

El estudio del ADN antiguo (ADNa) en contextos arqueológicos ha sido una valiosa fuente de información para la identificación de microrestos vegetales. Este estudio propone un enfoque metodológico integrador que combina metadatos con una base de datos de flora, una iniciativa sin precedentes hasta el momento. La metodología aplicada implicó el uso de secuenciación Sanger y secuenciación de alto rendimiento (HTS) para el análisis de ADNa en sedimentos sacros y muestras de control de seis entierros en el sitio arqueológico Jabuticabeira II *sambaqui* (JABII), ubicado en el sur de Brasil. Estos datos fueron comparados con bases de datos construidas a partir de información de la plataforma Flora do Brasil. En total, los dos métodos aplicados identificaron 28 familias correspondientes a 200 posibles especies, incluidas especies con utilidad para el consumo, para la construción de herramientas y de propiedades medicinales. Este estudio presenta una metodología innovadora para la integración de datos florísticos en el análisis paleogenético, contribuyendo a una comprensión más completa del papel de las plantas en las prácticas culturales del pueblo *sambaqui* en el sur de Brasil.

Palabras clave: ADN ancestral, Flora, Secuenciación de alto rendimiento, *Sambaquis*

INTRODUCTION

The identification of flora from plant microremains has been a challenge due to the complexity and lack of more comprehensive reference collections, which limits taxonomic identification at the species level (Boyadjian, 2012; Haslam, 2004; Scheel-Ybert, 2003). Genetic and genomic studies on archaeological material are carried out to retrieve information on plant remains often not identified in microarchaeobotanical analyses (Haslam, 2004; Lentz et al., 2021). In recent years, paleogenetics and paleogenomics research have played a key role in archaeological studies, focusing on the application of ancient DNA (aDNA) (Pääbo, 2014; Sawafuji et al., 2020; Weyrich et al., 2017). These studies have been applied to archaeological materials to obtain complementary information on the food composition and diversity of plant items used by past populations for food, toolmaking, rituals, and medicinal purposes.

We propose an integrative methodology that combines genetic metadata with a customized database of the local flora of the Jabuticabeira II *sambaqui* (shellmound) archaeological site with radiocarbon dating that indicates a construction period between 2880±75 years BP (3209-2779 cal years BP) and 1805±65 years BP (1864-1534 cal years BP) (Bianchini et al., 2011), located in the south of Brazil. This novel approach aims to establish a precise correlation between the plant genetic data obtained in sacral and control samples of JABII individuals, as well as the information contained in the classical literature and local flora database based on the Flora Brasil platform. The methodology involves the analysis of ancient aDNA from archaeological sediments using Sanger sequencing and High Throughput Sequencing (HTS) techniques, comparing the results with the constructed database.

This approach allows for a deeper understanding of the relationship between plant species and the archaeological context, contributing to research in paleogenetics and paleogenomics, as well as to the comprehension of the cultural practices of the *sambaqui* people.

METHODOLOGY

PRELIMINARY SURVEY OF FLORISTIC INFORMATION

A preliminary survey of the main plant families described in the JABII literature was conducted, using selection criteria for native species of the Atlantic Forest in Santa Catarina. As of May 2023, 389 species of native plants have been identified, belonging to 21 distinct families. These species encompass a variety of vegetation types, including restinga, mangrove, and dense, mixed ombrophile forest. Subsequently, all identified native species were searched in the GenBank/NCBI database to verify the availability of genetic sequences. Among the 389 species identified, 225 had information available on GenBank/NCBI.

A custom database was created from a literature review of *sambaqui* studies in the southern and southeastern regions of Brazil, utilizing data from four distinct databases: Web of Science, Scientific Electronic Library Online – SciELO, Pubmed, and Capes Journals. This stage was carried out to answer the following question: "Which plants composed the diet and landscape of the JABII *sambaqui* and other *sambaquis* of southern Brazil?".

Search criteria were established to identify families, genera, and native species of the Atlantic Forest biome in three ecosystems in the region of Jaguaruna, Santa Catarina state, where JABII was located. The data filtering process used the Flora do Brasil database and included eight selection categories: "Name", "Description", "Life forms", "Range", "Distribution", "Vegetation", in addition to the selection of taxa with scientifically accepted or non-scientifically accepted names. After the identification of the species in the Flora of Brazil, a search was carried out in the GenBank/NCBI to determine which genetic sequences were deposited during the period of elaboration of the research, using the Taxonomy Browser to browse the taxonomic information available in the database. In the context of this study, the Flora of Brazil database played a fundamental role in the identification of families, genera, and species of plants native to the region under study. The use of this platform strengthened the scientific basis of the study, allowing the comparison and correlation of the results obtained with taxonomic and botanical information available in the database.

PALEOGENETIC ANALYSIS

Recommendations and criteria for authenticity in DNA studies were followed to avoid contamination with modern DNA. Paleogenetic collection was carried out to avoid contamination of archaeological samples and post-excavation degradation. At each collection, personal protective equipment (PPE), sterile materials, transportation of cold material, light protection, and disposable materials, such as gloves, lab coats, masks, caps, and shoe cover, were exchanged. The paleogenetic analyses were carried out in the exclusive space for working with aDNA of the Laboratório de Parasitologia Integrativa e Paleoparasitologia of the Instituto Oswaldo Cruz, FIOCRUZ/Brazil.

Sediment samples from the pelvic regions of JABII individuals were subjected to rehydration with aqueous solution of 0.5% trisodium phosphate (Na_3PO_4) for 72 hours (Callen and Cameron, 1960) at 4ºC. The rehydrated samples were submitted to the spontaneous sedimentation technique (Figure 1). A total of 200µl of rehydrated sediment was reserved for paleogenetic/paleogenomic analysis. Ancient DNA was extracted (QIAamp DNA investigator – QIAGEN) from sacral sediments and control samples that were collected from 6 JABII burials. The Reconstructive Polymerization technique (Iñiguez et al., 2021), a pretreatment that reconstructs and amplifies the aDNA, was applied to all extracted samples. PCR (Go*Taq*® G2 Hot Start *Taq* Polymerase – PROMEGA) was conducted to amplify the *rbc*L target of the chloroplast with a final volume of 25µl, containing 1X of Buffer, 2.5mM of $MgCl_2$, 0.2mM of dNTP, 100ng of each primer, 2.5U of Platinum *Taq* (Promega) and 5µl of aDNA or 5-10ng concentration (Figure 1). For the amplification of the *rbc*L gene, the following cycles were applied: 95ºC to 3 minutes, 40 cycles of 30 seconds to 95ºC, 30 sec to 45ºC, 30 sec to 72ºC and an extension of 72ºC to 7 min.

PALEOGENOMIC ANALYSIS

The aDNA reactions submitted to Sanger sequencing were performed using the Big Dye™ Terminator v3.1 kit on the DNA sequencing platform/Fiocruz (Sequencer Applied Biosystems ABI 3730). Sequence analysis was performed using BioEdit v.5.0.9 and SeqMan v. 7.00 – DNASTAR Lasergene and compared using BLAST/NCBI with the Genbank database. A mixture of PCR amplicons was submitted to HTS in the MiSeq sequencer (Illumina) located at the Instituto Nacional de Cáncer– RJ/Brazil. Between 20-50ng of aDNA mass were used with Illumina's TruSeq® Nano DNA Library Prep kit, following a protocol adapted for aDNA study (Figure 1). There was no fragmentation of the DNA, as the aDNA is already naturally fragmented. Sequencing was done on the MiSeq System (Illumina) sequencer using the MiSeq Reagent Nano Kit v2 500-cycle kit. The quality control of the reads was performed with a script customized by the Fiocruz Bioinformatics Platform, using TRIMMOMATIC and FastQC. The paired reads were concatenated into a single sequence and searched for similarity with BLAST against a customized database containing all the amplified gene entries available in GenBank (Figure 1). This allowed us to identify the species with the highest percentage of identity and coverage concerning each concatenated sequence. Genetic data were compared with a personalized database created from records of the flora of Brazil, obtained from the Global Biodiversity Information Facility, initially focusing on species native to the southern region of Brazil. The reads were analyzed and compared with the local flora using the Taxonomyzer (Figure 1).

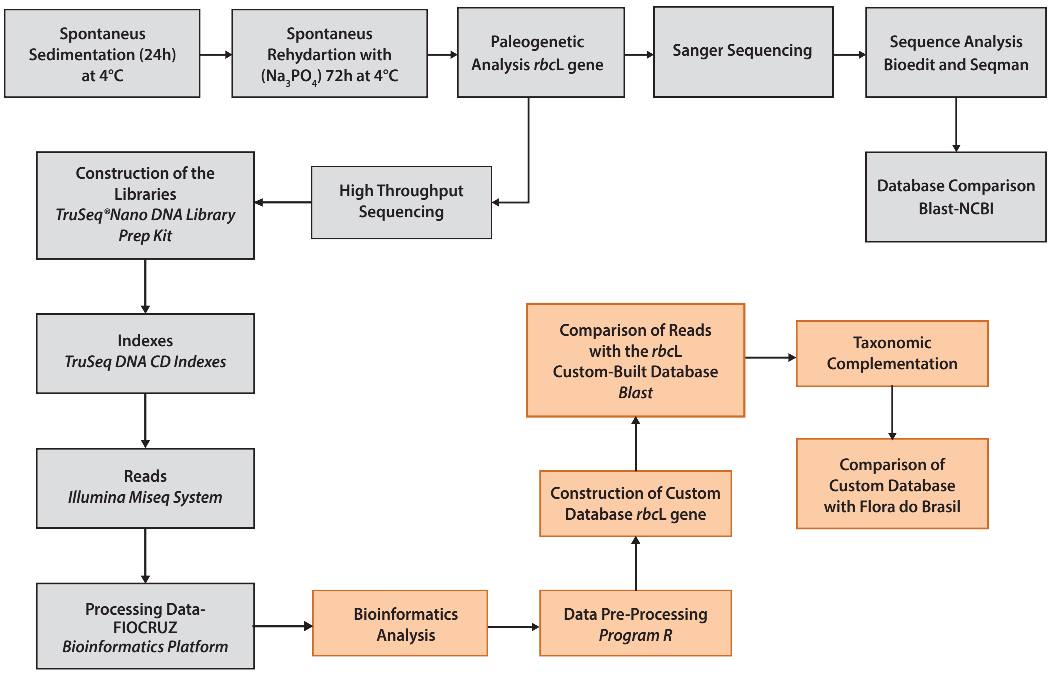

FIGURE 1. Flow diagram of paleogenetic and paleogenomic analyses. The arrows in gray indicate the steps of the paleogenetic analysis, while the arrows in orange represent the steps of the bioinformatics analysis.

Source: Author´s elaboration.

RESULTS AND DISCUSSION

The methodology used in this study, based on literature review and the construction of customized databases for the *rbc*L gene, proved to be fundamental. The applied integrative approach allowed not only the identification of potential genera and species of the native flora of the study region but also brought to light new perspectives on the plant composition that could have integrated the diet or landscape of the JABII archaeological site. By using Sanger sequencing, it was possible to confirm the presence of Lauraceae families, previously described in the literature of the JABII archaeological site, which includes species used in the production of cuttings for funeral events (Bianchini and Scheel-Ybert, 2007) In addition, families such as Chloranthaceae and Solanaceae were identified, which were not previously mentioned in the literature for shellmounds in the southern region. The aDNA metabarcoding approach enabled a broader identification, revealing a total of 28 families. Of these families, 19 had already been mentioned in the literature for microtraces in coastal sites in southern Brazil. In addition, we identified 9 families for the first time in a *sambaqui* context.

The combined application of the aDNA metabarcoding approach with established computational analyses resulted in the suggestion of a total of 200 species belonging to 28 identified families. Among these families, 19 had been previously described or suggested in the literature on microremains from archaeological sites in the southern coastal region of Brazil, including Euphorbiaceae, Fabaceae, Araceae, Cyperaceae, Apiaceae, Acanthaceae, Lamiaceae, Poaceae, Malvaceae, Proteaceae, Asteraceae, Melastomaceae, Lauraceae, Loganiaceae, Apocynaceae, Bromeliaceae, Cucurbitaceae, Marantaceae, and Myrtaceae. Of these, 15 families were specifically reported in previous studies of the Jabuticabeira II *sambaqui*, such as Euphorbiaceae, Fabaceae, Lamiaceae, Poaceae, Malvaceae, Proteaceae, Asteraceae, Melastomaceae, Lauraceae, Loganiaceae, Apocynaceae, Cucurbitaceae, Marantaceae, and Myrtaceae. Of the identified families, 60 genera were observed. Only six of these genera (*Myrcia sp.*, *Euphorbia sp.*, *Eugenia sp., Ocotea sp., Roupala sp.* and *Myceugenia sp.*) have been previously described in the microremains literature for *sambaquis* in the southern coastal region of Brazil.

CONCLUSIONS

The innovative methodological approach not only enabled the identification of plant resources but also provided new perspectives on the paleoenvironment and eating habits of ancient populations. Furthermore, the information generated significantly contributes to the development of reference collections, promoting advances in understanding this community's cultural practices. The continuous enrichment of these reference collections is essential to support comparative research and foster future discussions in the field. The results of this research provided valuable data that expanded the understanding of the cultural, environmental, and economic aspects associated with the way of life of the populations that occupied the Jabuticabeira II *sambaqui*.

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Ancestry of intestinal parasitosis control in indigenous communities: a paleoparasitological and ethnobotanical approach in Bolivia

Ancestralidad del control de parasitosis intestinales en comunidades indígenas: un enfoque paleoparasitológico y etnobotánico en Bolivia.

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Abstract

The frequence of intestinal parasites found in Indigenous communities settled in different ecoregions of South America is similar in each region included in this review. Indigenous communities over the Bolivian Andes, Amazonia and Chaco suffered of intestintal infections caused by Ancilostomideos, Ascarideos, Trichuridios, Enterbiosis and *Giardias*. Paleoparasitological data also suggests that these parasites were also reported in different ecoregions over South America during prehistory. On the other hand, indigenous communities developed control strategies during history, with the available medicine plants in each ecoregion, the active exchange of products and knowledge between different human groups since prehistoric times allowed the treatment of different illness, including intestinal infections.

Cultural practices and health control methods used by this indigenous populations are part of the ancient knowledge that persisted over time. Therefore, the results presented in this paper show the wide distribution of intestinal parasites in indigenous communities of South America and medicine plants use for intestinal illness known as antihelminthics in different ecoregions of Bolivian territory. The paper also included paleoparasitological data, evolutive approach, paleopharmacology, ethnobotanical data and the discussion of predominant factors and cultural traditions that predispose the presence and distribution of intestinal parasites.

Keywords: Intestinal parasites, Southamerican indigenous communities, ethnobotany, paleoparasitology, Bolivia.

Resumen

La frecuencia de parásitos intestinales encontrados en comunidades indígenas asentadas en diferentes ecorregiones de América del Sur es similar en cada región incluida en esta revisión. Las comunidades indígenas de los Andes, la Amazonia y el Chaco bolivianos sufrieron infecciones intestinales causadas por Ancilostomideos, Ascarideos, Trichuridios, Enterbiosis y Giardias. Los datos paleoparasitológicos sugieren que estos parásitos también fueron reportados en diferentes ecorregiones de América del Sur durante la prehistoria. Por otro lado, las comunidades indígenas desarrollaron estrategias de control durante la historia, con las plantas medicinales disponibles en cada ecorregión. El intercambio activo de productos y conocimientos entre diferentes grupos humanos desde tiempos prehistóricos permitió el tratamiento de diferentes enfermedades, incluidas las infecciones intestinales.

Las prácticas culturales y los métodos de control sanitario utilizados por estas poblaciones indígenas son parte de conocimientos ancestrales que persistió en el tiempo. Por lo tanto, la información presentada muestra la amplia distribución de parásitos intestinales en comunidades indígenas de América del Sur y el uso de plantas medicinales para enfermedades intestinales conocidas como antihelmínticas en diferentes ecorregiones del territorio boliviano. En el documento se presentan datos paleoparasitológicos, se integran aspectos evolutivos, información paleofarmacológica, datos etnobotánicos y se discutieron las tradiciones culturales que pudieron predisponer la presencia y distribución de parásitos intestinales.

Palabras clave: Parásitos intestinales, comunidades indígenas sudamericanas, etnobotánica, paleoparasitología, Bolivia.

INTRODUCTION

Parasitism depends on three subsystems that evolved together: the parasite, the host, and the environment. If one of these components is disturbed, disease takes place. This phenomenon of parasitism occurs in every organism on earth and is as old as earth. The history of humankind was shaped by evolutive process and the association between parasites and humans is still as strong as it was at the beginning of history. In fact, intestinal parasites have evolved with humans throughout history (Alum, 2010). This relationship had also been influenced since prehistoric times by climatic changes, that today are more frequent and still affect human socio-cultural spectrum (Orellana -Halkyer and Navarro, 2010; Alum, 2010. Human evolution and parasitic infections have shaped selection on parasite transmission, which lead coevolution of host-parasite systems. These interactions were also shaped by local environments and other host factors that influence transmission risk.

During modern human's history, many different parasites were acquired and despite the pressure of drastic environmental conditions, and other relationship forms as symbiosis, parasitism, and virulence have persisted over the time. The evolutive strategies used by living organisms as microorganisms to not perish, must have depended on their ability to jump from one species to another, or to develop structures that can protect them from drastic environmental changes to stay long periods awaiting the opportunity to infect new hosts (Greenblatt and Spigelman 2003; Cox, 2002; Cox, 2003).

Parasites were acquired by two lines, by phylogenetic line when parasites were inherited from pre-hominids, and shared with other phylogenetically close host species, and by ecological line, when parasites were acquired in each colonized environment, during prehistory (Hawash, 2016). Therefore, human migrations around the world, emergence of agriculture and animal domestication, changes in human behavior, social organization, population growth, together with latter formation of social conglomerate settlements are situations that favored changes in the pathoecology of emerging diseases (Marcogliese, 2004). So that, some diseases may be new, most of them have been in unexplored niches, awaiting an ecological signal or an accidental situation to come forth and multiply, as happened with some pandemics in history, such as the last one caused by COVID-19 (Destoumieux-Garzon, 2021).

The way that infectious diseases were acquired, whether phylogenetically or by ecological route, have a long history (Alum, 2010), so that human populations since prehistory learned to confront them with different strategies, including components of the flora available, found in each colonized region. This ancestral knowledge was transferred over time between and through different indigenous populations.

On this way, the paleopharmacology, allowed us to know what plants were used in the past to deal with gastroenteritis and/or other pathologic symptoms from archaeological records, ethnographic investigation, plant chemistry, physiology, textual information about ancient cultures, and pharmacopoeias (Holloway, 1983, Reinhard, 1998).

Ancestral medical control strategies used by ancient cultures were described in the chroniques, some detailed the pharmacopoeia used by the Aztecs. Three documents cited by Miranda and Reinhard (2003:207) contain information about plants used for medical purpose in the past: *Primeros Memoriales*, *Codex Matritense*, and the larger *Florentine Codex*. They also cited, Ortiz De Montellano (1975), who identify the active ingredients for many medicinal plants used by the Aztecs, such as *Chenopodium* species employed as a treatment for parasite infection. Likewise, Riley (1993) reviewed the archaeological evidence of this anthelminthic found at coprolite analysis. Five North American tribes were documented as using various species of *Chenopodium* as a vermifuge: the Cherokee, Rappahannock, Houma, Koasait, and Natchez. Later, Reinhard et al. (1985) reported the results of coprolite analysis of ancient populations, which suggested that some species of *Chenopodium* served in Archaic Period as prophylaxis for parasite infection. Subsequently, coprolite analysis of prehistoric Anasazi agriculturalists, evidence the use of *C. graveolens* and *C. botrys* as antihelminthics.

Riley (1993) suggests that in Salts and Mammoth Caves, Kentucky ancient hookworm and *Ascaris lumbricoides* infection resulted in the use of *Chenopodium* species to cure worm infection in the Southeast (In de Miranda and Reinhard, 2003:207). On the other hand, Miranda and Reinhard (2003), found in coprolites, by experimental studies, an important quantity of pollen grains of plants used to treat parasitic infection symptoms.

At the Peruvian coast, pollen and vegetable remains of *Pavonia paniculata* (malva-malva) were found in coprolites. This species was used to treat stomach disorders (Weir and Bonavia, 1985). They also reported pollen and vegetable remains of *Chenopodium*, they could not identify the species in a few cases, but they suggested it was probably ingested with medical porpoise.

Holloway (1983) was the first individual to look at pollen as evidence of medicinal plant use. Other researchers have also followed his approach. Ephedra (Mormon tea) pollen was found in Mojave Desert, Chihuahua Desert, and Colorado Plateau hunter-gatherer coprolites (Reinhard et al. 2003, Sobolik and Gerick 1993) ranging from tens of thousands to hundreds of thousands of grains per gram of coprolite. Although *Ephedra* and *Larrea* are remedies used today by American Indian cultures to remedy diarrhea (Moerman, 1986). Holloway (1983) as well as Moerman (1983), found pollen of *Larrea* in coprolites. Perhaps parasites were the cause of diarrhea treated with *Larrea* and *Ephedra* in the past.

De Miranda and Reinhard (2003) reported that the trunk of *Bauhinia cheilanta* ("miroro"), covered by bark can be included in a vermifuge infusion and it was found in brasilian coprolites. They also reported that some species of *Chenopodium* have vermifuge property and both seeds and foliage can be used for medicinal pourpose. The leaves and fruits of *Caesalpinea ferrea* ("pau-ferro") are anti-dysenteric. *Terminalia sp*. ("maçarico") heals dysentery. *Borreria sp*. ("cabeça-de-velho") can be used as an infusion to facilitate digestion (Miranda and Reinhard, 2003: 211).

So, different native communities value this ancestral knowledge and continue using medicinal plants mainly for the treatment of different diseases including digestive system disorders (Ballero et al., 1998; Bustos et al., 1996; Madhava Chetty et al., 1998; Heinrich et al., 1992), such as amoebic dysentery, presence of intestinal worms, diarrhea due to bacterial infection, among others. Also, the treatment of gastrointestinal disorders is increasingly important in developing countries - such as Bolivia - where these conditions occur largely in rural areas of the distinct ecoregions (Andes, Valleys, Amazon and Chaco). To this purpose, there are several works on medical ethnobotany explaining the use of medicinal plants for the treatment of these conditions (Gallo, 1996; Montaño, 1997; Arrázola et al. 2002; Bourdy et al. 2004; Vandebroek, 2008).

The available medical ethnobotanical data for the management of intestinal infections by Bolivian indigenous communities provides us with a starting point for establishing a relationship between intestinal parasites and Prehistoric and actual populations on this territory.

The aim of this document is to present an analysis and a review of the historical and ethnobotanical information, regarding the management of intestinal parasitism by Bolivian indigenous communities today and its relationship with the presence of parasitosis also in the South American territory to elucidating the spatiotemporal dimensions of intestinal parasitism from prehistory to the present in the Bolivian territory.

ANCESTRY OF INTESTINAL PARASITES IN CURRENT AMERICAN TERRITORY

The transition of the subsistence of hunter gathering to agriculture and animal domestication, and the acquired cultural habits, during sedentarism facilitated parasite transmission during prehistory (Barret, 1998). In prehistory, *Enterobious vermicularis* was also transmitted directly from one individual to another, with no need of a stage in soil. Therefore, they are not influenced by environmental changes. These parasites were found in either small or large groups of people, inhabiting form very cold regions or warm climates. They are called heirloom parasites and have accompanied humans during migrations and territory colonization (Araujo et al., 2013). Other parasites were also originated in African and evolve together with human ancestors of *Homo sapiens*. *Enterobious vermicularis* and *Trichiuris trichiura* (hookworms), are examples of parasites that have accompanied ancient human migrations from Africa to Europe, Asia and America. Therefore, these two parasites are considered as probes to trace human prehistoric migrations (Araujo et al., 2003).

Likewise, *Ascaris lumbricoides* is another intestinal parasite of humans that has also accompanied humans during evolution. *A. lumbricoides* infection was also commonly found in prehistoric groups, both in North and South Americas. Prehistoric human migrants should have brought the parasite to American continent, and most of the nematodes that infect the human intestinal tract were inherited from human ancestors and came to America with the first migrants. Not all intestinal helminths were introduced by Bering route, this cold region would make them perish, and the condition of transmission that these helminths found in the Americas were also completely different to their original territories (Reinhard, 2013; Araujo et al., 2010; Frías et al., 2012).

HELMINTHS

Enterobious vermicularis, Trichuris trichiura and *Ascaris lumbricoides*, found in archaeological sites both in North America and South America, dated as old as 9.000 years ago (Araujo et al. 2008, Arriaza et al. 2012). Paleoparasitological data showed that they were prevalent among prehistoric populations in the Americas. The number of eggs found in coprolites of Brazilian, North American prehistoric populations were always low, indicating low prevalence (Leles et al., 2012).

The first studies in South American human coprolites. Hookworm (ancylostomideus), whipworm *(Trichiuris trichiura)*, pinworm *(Enterobius vermicularis)*, and roundworm *(Ascaris lumbricoides)* were found infecting prehistoric populations both in North and in South America mainly in the actual territories of Argentina, Brazil, Peru, Chile and Bolivia) (Allison et al., 1974; Ferreira et al., 1980). Some plant remains and pollen of medicinal plants found in coprolites are still used until today for intestinal parasites (Figure 1).

FIGURE 1. Helminths and Protozoa in diferent actual ecoregions of SouthAmerica during Prehistory.
Source: Author´s elaboration.

PROTOZOAN

Protozoan parasites have been found in ancient remains throughout the world, but the first findings in America were possible by serological tests, identifying protozoan cyst of *Giardia duodenalis*, *Giardia intestinalis* and *Entamoeba histolytica*. Antigens of *Cryptosporidium sp.*, *Cyclospora sp.*, and the bacteria *Helicobacter pilori*, were identified in Andean mummies dated up to 3.000 years by fluorescent microscopy (Peruvian and Chilean Mummies) (Table 1) (Allison et al., 1999; Ortega and Bonavia, 2003).

As a matter of fact, Paleoparasitological research proved that common intestinal parasites infected pre-Columbian people long before European and African migrations (Gonçalves et al. 2003). During American prehistory, people were infected with common bacteria, protozoa, and helminths. Although hookworms, pinworms, shipworms, and roundworm infection were the same, and prevalence rates among pre-Columbian populations and transmission dynamics should behave differently, according to environmental conditions of each region influenced by the acquired habits of each culture.

TABLE 1. Helminths and Protozoan in different ecoregions of current South America and Bolivian territories, during prehistory.

Current Geographical area	Current Ecoregions	HELMINTHS						PROTOZOAN							
		lumbricoides	*T. ttrichiria*	*Hookworm*	*E. vermicularis*	*Taenia. spp*	*Fasciola*	*Chilomastic mesnili*	*Entamoeba spp.*	*Cryptosporidium parvum*	*Isospora beli*	*Cylospora cayetanensis*	*Sarcocystitis hominis*	*Balantioides coli*	*Capillaria sp.*
SOUTH AMERICA	ANDES	●	●	¬	●	◊	◊	▼	▼	▼	▼	▼	▼	¬	¬
	CHACO	¬	●	●	●	◊		¬	¬	¬	¬	¬	¬	¬	¬
BOLVIA	ANDES	¬	¬	¬	¬	◊	¬	¬	¬	¬	¬	¬	¬	▼	▼

* Phylum: ●=Helminths; ◊= Platyhelminthes; ▼=Protozoa; ¬ = No records found.

Source: Author´s elaboration.

INTESTINAL PARASITES IN CURRENT SOUTH AMERICAN AND BOLIVIAN INDIGENOUS COMMUNITIES

Around 800 indigenous groups (Albó et al., 2009), are disseminated in the South America territory, around of 800 indigenous groups (Albó et al., 2009), ancestral heirs of pre-Columbian communities preserving their cultural and social acquired traditions within their natural ecosystems, initiated in some time of their ancestral history, (Toledo, 1999; Kim, 2009; Zimmerer, 2002) which, probably could have a strong influence in the acquisition and dissemination of intestinal parasites between them. (Ebrahim, 1981; Sattenspiel, 2000) On this regard, the available ethnobotanical evidence reinforces this statement, due to the use of medicinal plants, many of them with antiparasitic properties, by indigenous communities, knowledge that has been inherited by oral transmission for several generations (Gallo, 1996; Montaño, 1997; Arrázola, 2002; Bourdy 2004; Vandebroek, 2008).

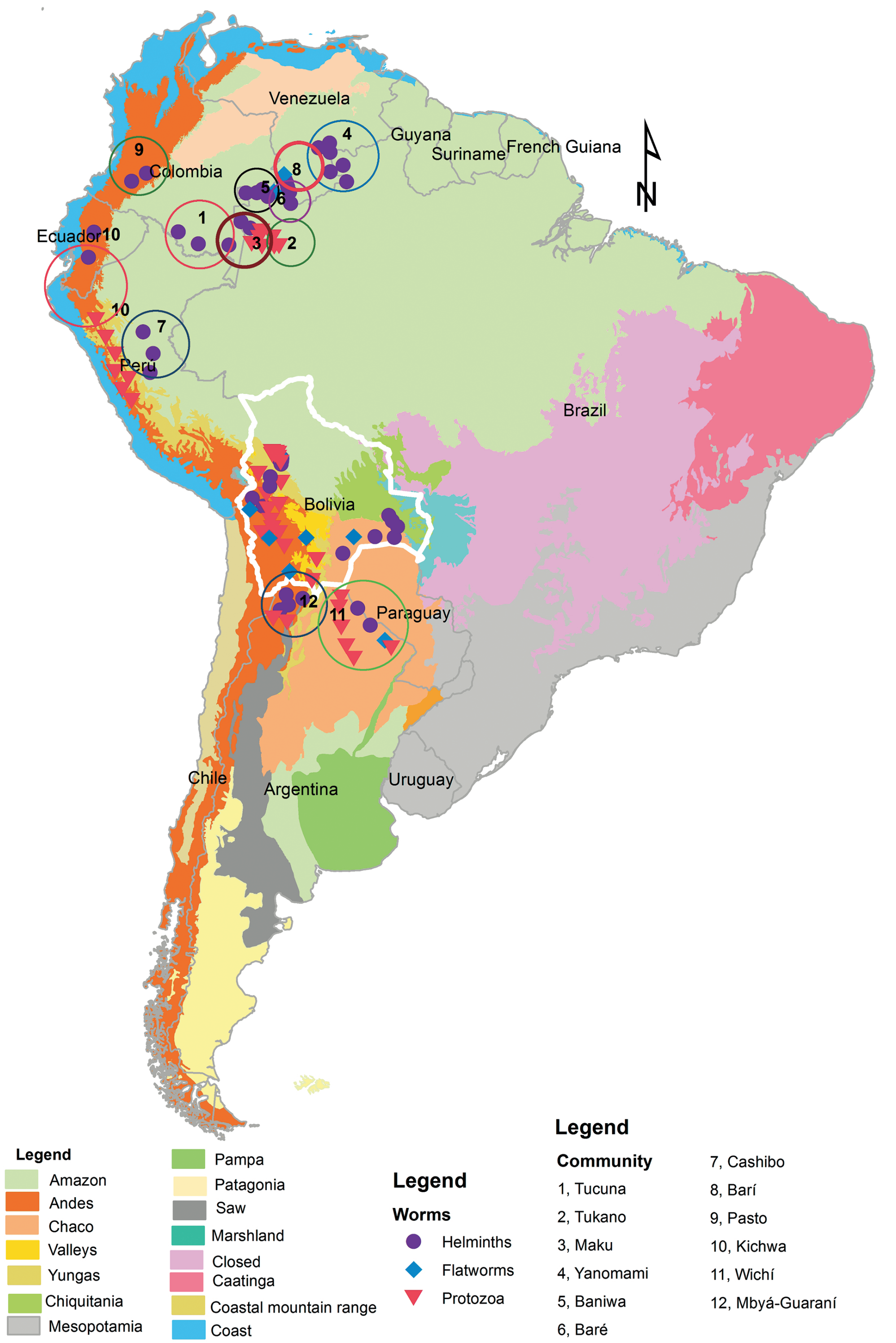

FIGURE 2. Frequency of intestinal parasites (Helminths and Protozoa) found in indigenous communities settled in different ecoregions of South America.

Source: Author´s elaboration.

HELMINTHS

The available information regarding the presence of intestinal helminth parasites between indigenous groups of South American territory (Table 2), reflects the presence mainly of *Ascaris lumbricoides, Trichuris trichiura,* and Ancylostimidae through different eco-regions. These helminths are the more frequent between indigenous *Tucuna*, settled in the Colombian amazon and *Tukano* and *Maku*, settled into Brazilian Amazon (Figure 2) (Schwaner, 1974; Chernela, 1989). The geographical proximity between these indigenous groups could represent an important way that favoring the dissemination and persistence of these intestinal helminthiasis between them, through the different type of social interactions. Interestingly, these helminthic parasitosis are also present in indigenous communities in the Andean region of Venezuela, Colombia, Ecuador, Peru and Bolivia and among those settled in "Chaco" eco-region corresponding to the territories of Argentina, Paraguay and Bolivia (Figures 2, 3), geographically distant of Amazonian indigenous communities. A feasible explanation about the presence of these helminthiasis could be attributable to the cultural and social practices as cited previously (Toledo, 1999; Kim, 2009; Zimmerer, 2002). These helminths parasites share as part of their life cycle a stage in the soil with adequate humidity, temperature and shade (Benesh, 2021), such conditions can be achieved in different eco-regions through practices that allow to generate this and the presence of compact human conglomerates.

Humans and animals share the same water sources contaminated with feces. The food is washed with contaminated water, which increases the risk of contracting parasitic, bacterial or viral diseases, which provides poor health conditions (Esteban, 1998; Villegas, 2005).

The Bolivian indigenous communities Aymara and Kichwa settled in the Andean ecoregion; the smaller indigenous population Tupi-Guaraní and Arahuaco concentrated in the Chaco ecoregion (Villegas, 2005; Andersen, 1999) and twenty-four other indigenous communities settled in the Amazonian ecoregion (Albó, 2009) presented also different species of intestinal helminth parasites, standing out, regardless of the indigenous community and ecoregion, the prescence of *Ascaris lumbricoides, Trichuris trichiura,* Ancylostimidae and also *Strongyloides. stercoralis* and *Hymenolepis nana,* (Table 2 and Figure 3) reinforcing the previous analysis carried out with the findings reported in indigenous communities settled in South American territory. Other parasite species present in some indigenous groups which are also reviewed in paleoparasitological data, (Table 1) are acquired through diet, when animals are consumed without cooking, and surely due to some hygiene habits. Also, the geophagy, an habit in some indigenous groups, related to a physiological need for acquisition of mineral micronutrients reported also in archeological findings, allowed the infection and presence of some parasites (Walker, 1997; Ghorbani, 2009). It is also important to emphasize that *Ascaris lumbricoides, Trichuris trichuria, Enterobius vermicularis*, and the protozoan *Giardia lamblia, Entoamebidae*, and *Cryptosporidia*, can resist dry environmental conditions, or extreme conditions in soil, so it can be used as migration markers over time (Alum, 2014; Davis, 2018).

PROTOZOAN

Regarding protozoan parasites, the reviewed publications provide data on the presence of *Giardia lamblia, Entamoeba coli, Endolimax nana, Entamoeba histolytica, Iodoameba butchilly,* in indigenous communities settled in the Amazon region of Brazil; the Andean region of Colombia and Ecuador and the Chaco region of Argentina (Chernela 1989; Taranto 2003; Rinne 2005; Zonta, 2010; Salcedo-Cifuentes, 2012), as well as other species of protozoan parasites (Figure 2 and Table 2).

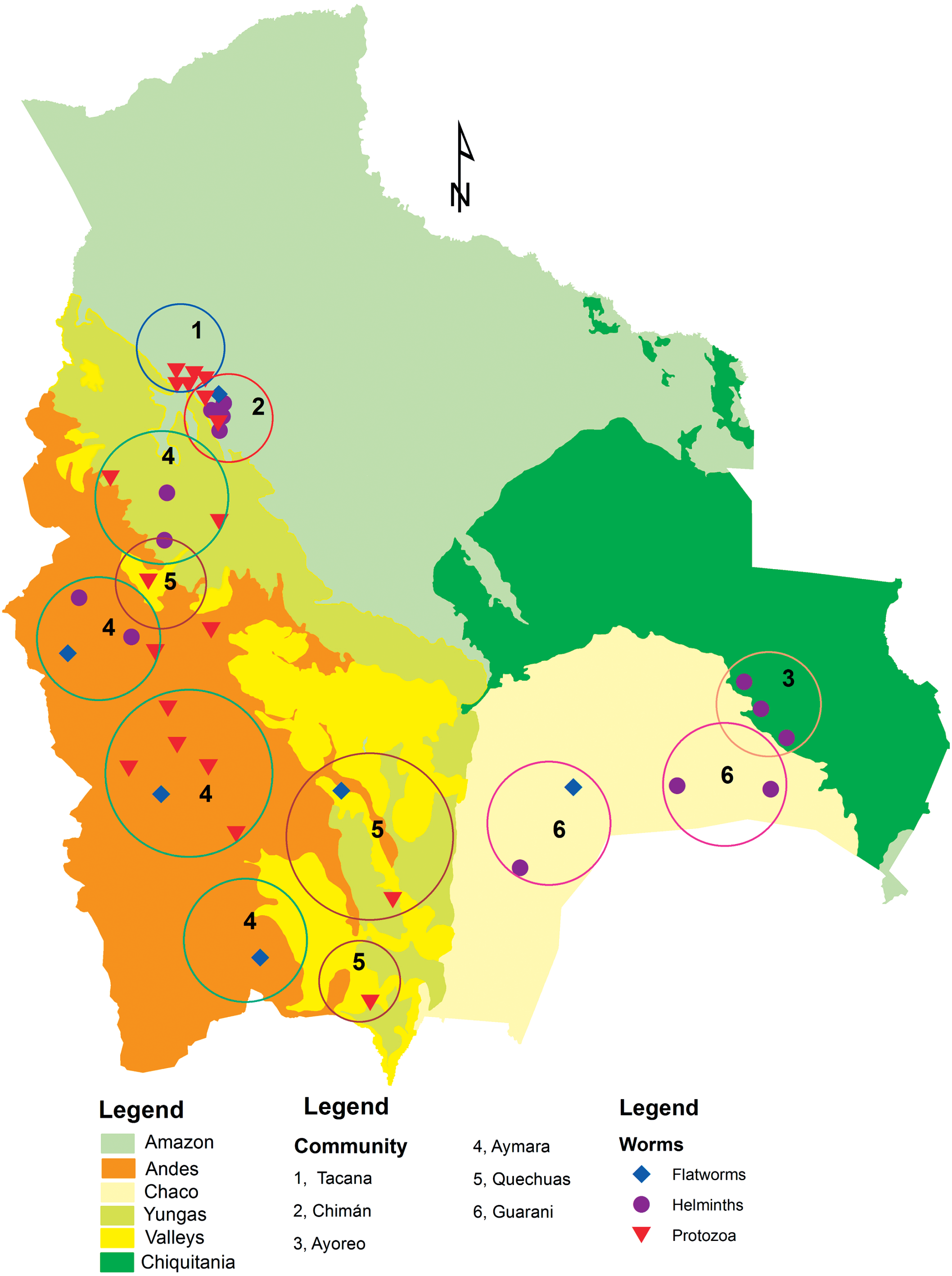

FIGURE 3. Presence of intestinal parasites (Helminths and Protozoa) in Bolivian indigenous populations settled in different ecoregions.

Source: Author´s elaboration.

TABLE 2. Helminths and Protozoan in different ecoregions in indigenous communities settled in South America and Bolivian territories.

Geographical area	Ecoregions	Indigenous commun	HELMINTHS										PROTOZOAN											
			A. lumbricoides	*T. ttrichiria*	*S. stercoralis*	*Hookworm*	*H. nana*	*H. dimminuta*	*Taenia. spp*	*E. vermicularis*	*Toxocara*	*Fasciola*	*Chilomastic m.*	*Entamoeba coli colispp.*	*Entamoeba histol.*	*Entamoeba haer.*	*Iodameba b.*	*Cryptosporidium*	*Giardia lamblia*	*Blastocystis. h.*	*Endolimax nana*	*Balantidium coli*	*Retortomonas*	*Dientamoeba f.*
SOUTH AMERICA	AMAZON	1	●	●	¬	●	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬
		2	●	●	¬	●	¬	¬	¬	¬	¬	¬	▼	▼	▼	¬	▼	¬	▼	¬	▼	▼	▼	¬
		4	●	●	●	●	¬	¬	¬	●	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬
		5	●	●	¬	●	◊	¬	¬	●	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬
		6	●	●	¬	●	◊	¬	¬	●	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬
		7	●	●	●	●	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬
	ANDES	8	●	●	●	●	◊	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬
		9	●	●	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬
		10	●	●	¬	¬	¬	¬	¬	¬	¬	◊	▼	▼	▼	¬	▼	¬	▼	▼	▼	¬	¬	¬
	CHACO	11	●	●	●	●	◊	¬	¬	●	●	¬	¬	▼	¬	▼	¬	▼	▼	¬	¬	¬	¬	¬
		12	¬	¬	●	●	¬	¬	¬	●	¬	¬	¬	▼	¬	¬	▼	¬	¬	▼	▼	▼	¬	¬
BOLVIA	AMAZON	1	●	●	●	●	¬	◊	¬	¬	¬	¬	▼	▼	¬	¬	▼	¬	▼	▼	▼	¬	¬	¬
		2	●	●	●	●	◊	¬	¬	¬	¬	¬	¬	▼	¬	¬	▼	¬	▼	▼	▼	¬	¬	¬
	ANDES	3	●	●	●	●	¬	¬	◊	●	¬	◊	▼	▼	▼	¬	¬	▼	▼	▼	▼	▼	▼	▼
		4	●	¬	¬	¬	◊	¬	¬	¬	¬	◊	▼	▼	▼	▼	▼	¬	▼	▼	▼	¬	¬	¬
	CHACO	5	¬	¬	●	●	¬	¬	◊	¬	●	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬
		6	¬	●	●	●	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬	¬

SOUTH AMERICA: 1=*Tucuna*; 2=*Tukano*; 3=*Maku;* 4=Yanomami; 5=Baniwa; 6=Baré; 7=Cashibo; 8=Barí; 9=Pasto; 10=Kichwa; 11=Wichí; 12=Mbyá-Guaraní. **BOLIVIA:** 1=Chiman; 2=Tacana; 3=Aymaras; 4=Kichwa; 5=Ayoreo; 6=Guaraní.

Phylum: ●=Helminths; ◊= Platyhelminthes; ▼=Protozoa; ¬ = No records found.

Source: Author´s elaboration.

The registers published previously, summarized in Table 2, shown the presence of *Chilomastic mesnilli, Giardia lamblia, Blastocystis hominis, Endolimax nana, Iodameba bütschlii and Entomoaeba coli* in the indigenous communities settled in Amazon and Andes region, and *Entamoeba histolytic* only in Andes communities. However, the absence of records on protozoan parasites in Table 2 only indicates that no information on the subject was found in the literature review. So, the presence of parasites not reported on this document should be considered possible in all indigenous communities.

USE OF MEDICINAL PLANTS BY CURRENT BOLIVIAN INDIGENOUS COMMUNITIES

A non-systematic review of literature about medicinal plants, focused in gastrointestinal diseases caused by protozoan and /or bacteria, taken account the symptoms such as profuse diarrhea, cramps and intense abdominal pain, used by Bolivian indigenous communities, (Riester, 1976; Ortiz, 1986; Wentzel, 1989; Diez Astete, 1991; Toledo, 1995; Bourdy, 2000; Vandebroek, 2004; Bourdy, 2004; Díez, 2011; Navarro, 2011; ME and VCandT, 2013; Quiroga, 2017) made it possible to find fifty-two medicinal plants (Scarpa, 2002; Scarpa, 2009) with this properties between indigenous communities settled in Chaco ecoregion, *Izoceño-Guaraní* (13 type of plants), *Weenhayek, Tapiete* (11 type of plants), *Itika Guasu*. Between communities settled in Amazon ecoregion were reported in *Yuracaré-Trinitario* (18 type of plants), *Chimán* (10 type of plants), Tacana; also, was reported the use of different medicinal plants between Aymara and Kichwa in Andes ecoregion and Chiquitanos (Pantanal ecoregion) (Scarpa, 2002; Scarpa, 2009).

The usual practice for the consumption of medicinal plants, regardless of the settlement site of the indigenous communities, is decoctions and infusions, from which a liquid product results and would be consumed in different proportions, according to the criteria of the traditional healers (Scarpa, 2002).

The decoction is prepared with a large amount of water (half a liter, a liter or more), at room temperature and in different doses throughout the day (it would be the equivalent of "low doses" of the medicinal plant) (Scarpa, 2002). Taking a remedy in tea form means drinking the infusion as hot as possible in small quantities (a cup or less) and only once a day, often on an empty stomach (this would be the equivalent of "high doses") (Scarpa, 2002). Also, they know that the continuous use of a certain plant could lead to intoxication, especially with plants from the Solanaceae family. (Arrázola et al, 2002) According to local informants, the consumption of the plant remedy should be for a prudent period, following the instructions of a traditional healer (Arrázola 2002).

The comparative values of the Sorensen coefficient expressed in Table 3 show the relationship of similarity between the absolute and relative quantities of medicinal plants shared by all the indigenous peoples considered. As can be observed, there is a higher percentage of shared plants between the Weenhayek and Izoceños (50%), as well as between the Yuracré and Chimanes (46%); the sharing of medicinal plants in a minor percent is evidenced between Chimanes and Tacana, both settled in Amazon ecoregion and between Chiquitano (Pantanal ecoregion) and Tacana (Amazon ecoregion). Meanwhile, the Aymaras and Kichwas would have little or no percentage of comparability of medicinal plant species used.

TABLE 3. Percentage of medicinal plants shared between some Bolivian indigenous groups..

Indigenous communities	*Aymara*	*Chiman*	*Chiqquitano*	*Izoceño*	*Pantanal*	*Kichwa*	*Tacana*	*Weenhayek*	*Yuracare*
Aymara	100	0	0	8,7	0	20	0	0	0
Chiman		100	11,11	9,09	0	10,53	28,57	0	46,15
Chiquitano			100	0	13,33	0	28,57	0	23,08
Izoceño				100	10,53	8,7	0	50	6,67
Pantanal					100	0	0	15,38	0
Kichwa						100	0	0	7,41
Tacana							100	0	18,18
Weenhayek								100	0
Yuracare									100

Source: Author´s elaboration.

The Figure 4, shows a dendrogram where values related to the coincidences of medicinal plants used by the nine indigenous peoples were grouped together. In it, three groups can be observed: The first group, made up of Aymara and Kichwa, showing a 42% similarity; a second group made up of Izoceños, Weenhayek and Chiquitanos from the Pantanal and Chaco ecoregions with a 42% similarity between them, but at the same time, the Izoceños and Weenhayek show a 72% similarity between them. Finally, the third group would show a 36% similarity and in turn showing greater similarity between Chimán-Yuracaré (68%) both, settled in Amazon ecoregion and Chiquitano-Tacana (61%) settled in Pantanal and Amazon ecoregions respectively. The dendrogram, also suggests that the medicinal uses attributed to the treatment of parasitosis among both the Aymara and Kichwa are more similar to each other, both corresponding to the Andean zone (high plateau, valleys); and in turn, the medicinal uses of the Izoceños-Weenhayek and Chimán-Yuracaré are more similar to each other, populations that are located both in the Chaco plain and in the Amazon ecoregion respectively.

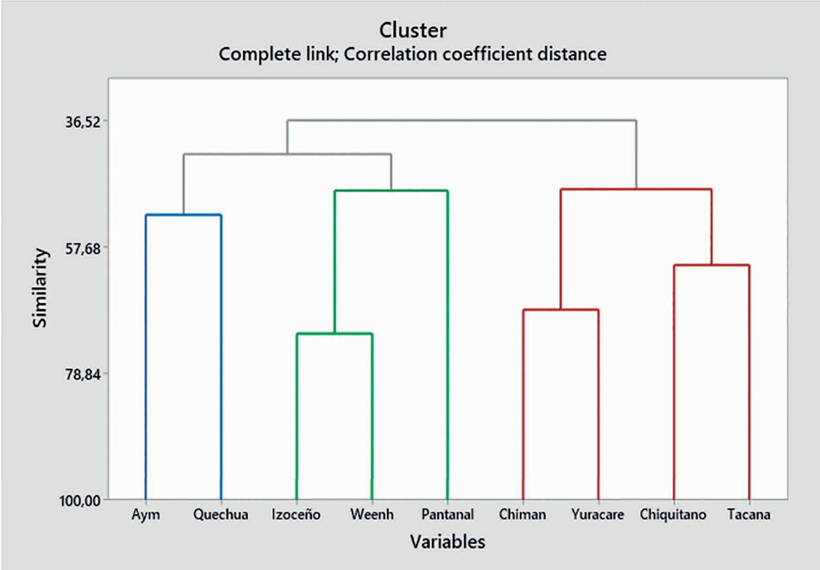

FIGURE 4. Comparative analysis of the coincidences about the use of medicinal plants in nine indigenous communities using the cluster method.

Source: Author´s elaboration.

INTESTINAL PARASITISM FROM PREHISTORY TO PRESENT IN SOUTH AMERICA AND BOLIVIA REGIONS AND ITS RELATIONSHIP WITH THE USE OF MEDICINAL PLANTS BY CURRENT BOLIVIAN INDIGENOUS COMMUNITIES AS AN ANCESTRAL KNOWLEDGE

The human intestinal parasitism is as ancient as humanity itself (Cox, 2002). So, to explain this ancient relationship between human and intestinal parasites, paleoparasitological research has made important contributions, allowing the understanding of parasites evolution in different ecological context since prehistory period. In fact, this kind of research allow to know intestinal parasites distribution, and the available evidence in bioarchaeological contexts, the ecology of infectious disease in different prehistoric periods, and adequate detection techniques were the keys to identify many parasites. Most of the species found are present until today, but certainly this long relationship was important to developed control strategies between indigenous populations. Indigenous communities are still using this ancestral knowledge to control parasite infections based on symptoms and many medicinal plants are known between different cultures.

In this context, Reinhard (1998: 445) cited, *"One of the significant aspects of genus Chenopodium studies is the description of the development of pharmaceuticals in prehistory. Species of Chenopodium were used by ancient hunter-gatherers and agriculturalists primarily as a food source. As knowledge of various species of Chenopodium progressed, there came the recognition that certain species had a pharmacological value"*.

Otherwise, many species of *Chenopodium* in American prehistory have been used until today in traditional medicine for a variety of ailments. They are used predominantly as laxative, diuretic, anthelmintic, use to treat dysentery and it can also help with abdominal pain (Kasali et al., 2021). Many of these symptoms are assumed as indicative of intestinal parasite infections by indigenous communities settled in the different ecoregions of Bolivian territory (Table 4).

TABLE 4. Medicinal plants used for gastrointestinal disorders by indigenous communities of Bolivia..

Ecoregion	Botanical families of medicinal plants	Indigenous Communities	Medicinal use
AMAZON	*Anacardiaceae; Arecaceae Chenopodiaceae; Cariaceae Rubiaceae; Polygonaceae Salicaceae; Ulmaceae Myrthaceae; Phytolaccaceae Fabaceae; Lauraceae Eritroxylaceae; Caricaceae Phytolaccaceae*	Yuracaré Moseten Chiman Tacana	Stomach inflam-mation, abdominal pain, diarrhea, dysentery.
ANDES	*Salicaceae Lauraceae*	Kichwa	Stomach inflam-mation, abdominal pain, diarrhea, dysentery
CHACO	*Poaceae; Rosaceae Polygonaceae; Rubiaceae Sopotaceae; Santalaceae Ulmaceae; Zingiberaceae Urticaceae; Plygonaceae Olocaceae; Myrthaceae Rhamnaceae; Lauraceae Bromelia-ceae; Bignoniaceae*	Tapiete Huareta Itika guasu Izoceño Weenhayek	Stomach inflam-mation, abdominal pain, diarrhea, dysentery

Source: Author´s elaboration.

This ethnobotanical information is an ancestral knowledge transmitted from generation to generation by indigenous population. This information has been developed since prehistory in response to health problems. Ancestral knowledge is important because it is the key to the conservation of biodiversity, since indigenous population have lived with nature for millennia and have a caring relationship with it, respecting what nature give to them in terms of ethnobotanical and traditional medicine (WWF, 2023).

The ethnobotanical information regarding the different bioclimatic floors in Bolivia (Annex 1) shows the use of different plants to treat intestinal conditions, which according to the indigenous groups are used when it comes to stomach pain and diarrhea, and other symptoms, that according to their knowledge are indicatives of intestinal parasites infection. The symptoms allow traditional healers to decide which plant and dose should be used. These symptoms or discomforts may be related to some pathogenic agent, poisoning or food allergy that, according to the knowledge of traditional healers, is recognized and treated through infusions and decoctions. From Chaco pharmacoea, it has been described that thanks to active ingredients existing in the leaves of *"Guayaba" (Psidium guajaba), "Tala" (Celtis sp.)* and *"Algarrobillo" (Libidibia paraguariensis),* it is possible to control profuse diarrhea (Gallo, 1996; Arrázola et al, 2002; Quiroga et al, 2009).

This inherited knowledge is also shared between indigenous groups, so that the same plants described above for Chaco indigenous populations are also used by populations of other ecoregions of Bolivia. Compartment percentages of medicinal plants among lowland indigenous populations (Chaco, Amazon and Pantanal) range between 18% and 50% (Table 3). These compartment percentages decrease when compared with populations from the Andean region (Aymaras, Kichwa), achieving up to 20% similarity, especially between the mentioned Andean populations. These variations are due to the differences between the ecological systems of highlands and lowlands, as well as the well-marked cultural differences between both areas. On the other hand, it is interesting to observe the existence of intestinal parasites such as *Enterobius vermicularis, Ascaris lumbricoides, Entoamoeba coli,* both in low and high lands; and that can be treated through herbal medicine. The first settlers arrived, bringing some parasites that they took to the places where they settled, which managed to adapt to the new environments.

The cluster obtained from the conglomerate (Figure 4) agrees with the information about the Sorensen Coeficient (Table 3) showing a relationship between the indigenous populations cluster and the territorial ecological characteristics of the land occupied by them. In fact, according to the use of their medicinal plants, they cluster according to the altitudinal and rainfall gradient; for example, Kichwa and Aymara (Bolivian Andes ecoregion); Izoceños, Weenhayek (Bolivian Chaco ecoregion) together with human groups from the Pantanal and finally a third group made up of Chimanes, Yuracaré, Tacana (Bolivian Amazon ecoregion) and Chiquitano (Bolivian Pantanal ecoregion).

From a significant number of plant species with healing properties, which have frequent medicinal use within the pharmacopoeias of the indigenous populations, registered here, can be inferred that over time the local inhabitants in the different regions of the country were verifying the effectiveness of these medicinal plants, also establishing a generational transmission of local knowledge.

Thus, we have the assessments of Alvarsson (1993) and Diez (2011), which indicate the existence of oral transmissions of knowledge between communities of the same indigenous people and transmissions of interethnic knowledge (from one indigenous community to another). This transmission would have occurred over time, gradually establishing the most optimal plant remedies and routes of administration to treat various diseases, including those that afflict the digestive system. However, Alvarsson (1993) and VAIPO (2000) also explain that the new generations in the Weenhayek indigenous population, are no longer interested in learning about their culture, especially about traditional medicine, so unfortunately this knowledge is being lost. The same phenomenon can occur in other indigenous populations, both in the lowlands and highlands, who are adopting the habits of urban inhabitants, preferring drugs made by the Western medicine over natural products obtained from herbal medicine.

CONCLUSIONS

Many important parasites found today not only existed in ancient times, but also were widespread before written records began. Our early ancestors must have been aware of the presence of the largest and most common worms that cause some diseases, which pushed them to find strategies to deal with different diseases. The phenomenon of parasitism evolved together with their host and depends on the relationship with the environment. These three components on earth modeled the modern human history.

Ancient populations surely know how to treat many pathologies, and some symptoms produced by different microorganisms, using plants found in their environment or by the exchange of knowledge and plants of other regions.

Paleoparasitological data, show that in many archaeological sites of South America, situated in different ecoregions, prehistoric populations were also infected by the same parasites found in the indigenous population mentioned in the paper.

The document shows the distribution of intestinal parasites in South America and in Bolivia, both today and in prehistory. This information is compared with ethnobotanical data from Bolivia and paleopharmacological information available from South America, which shows the ancestrality of this medicinal knowledge that was acquired during history in different colonized regions and transferred from generation to generation.

The importance to analyze ethnobotanical information, traditions and health control methods of the indigenous communities in different ecoregions, will give important information about ancestral knowledge that could be considered by public health programs. Analyzing cultural traditions would also be important to understand the persistence of some intestinal parasitic infections. So, it is important to promote the development of paleopasitological studies to identify the remains of plants and pollen in coprolites that may have been used by the populations of South America in the past to treat ancient intestinal parasites and other pathologies.

On the other hand, currently the displacement and presence of indigenous populations in urban regions, due to the impact of climate change, the degradation of soils, the unconsciousness that has caused imbalances in nature and has forced indigenous populations to migrate, causes the loss of cultural identity, and forces them to adapt to the new territories. Without the possibility of receiving multiple benefits from nature, much of their ancestral knowledge is being lost, including ethnobotanical knowledge.

The Law No. 27811 of the Plurinational State of Bolivia establishes a special regime for the protection of the collective knowledge of indigenous peoples linked to biological resources, where medicinal plants are also considered as part of the worldview.

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ANNEX 1. Medicinal plants used for gastrointestinal disorders by indigenous communities of Bolivia.
Abbreviations: L=Leaf; **Fl**=Flower; **Fr**=Fruit; **Cr**=crust; **Mu**=Mucilage; **Se**=Seed; **Br**=Branch; **Ro**=Root; **St**=Steam.

Family	Plant parts used for medical purpose	Way of consumption	Medicinal use	Bolivian Indigenous groups	Ecoregion
ALLIACEAE					
Allium sativum L.	St	Direct consumption	Intestinal worms	Aymara	Andes
ANACARDIACEAE					
Anacardium occidentale L	L	Infusion	Diarrhea, dysentery	Tacana	Amazon
	L	decoction	Diarrhea	Chiquitano	Pantanal
Astronium urundeuva	L	decoction	Diarrhea	Pantanal	Pantanal
	Cr	decoction	Dysentery	Chiquitano	
Mangifera indica L	L	decoction	Diarrhea	Yuracare	Amazon
	L	decoction	Dysentery	Chiquitano	Pantanal
Spondias mombin L.	Cr	decoction	Diarrhea	Pantanal	Pantanal
APOCYNACEAE					
Aspidosperma rigidum rusby	Cr	decoction	Diarrhea parasites	Yuracare	Amazon
ARECACEAE					
Attalea phalerata Mart. ex Spreng.	Ro	decoction	Dysentery	Yuracare	Amazon
	Ro	decoction	Dysentery	Chiman	Amazon
ASTERACEAE					
Artemisia absinthium L.	Br	decoction	Intestinal parasites	Aymara	Andes
Baccharis genistelloides	Whole plant	decoction	Anthelminthic	Kichwa	
Schkuhria pinnata Lam (Kuntze) Allium sativum L.		decoction	Anthelminthic	Kichwa	Andes
	Ro	decoction	Intestinal worms	Aymara	
Pereskia sacharosa Griseb.	L; Mu	decoction	Diarrhea, dysentery	Izoceño	Chaco
CAPPARIDACEAE					
Capparis tweediana	L	decoction	Stomach pain, diarrhea	Weenhayek	Chaco
CARICAEAE					
Carica papaya L.	Fl; Se	decoction	Intestinal worms, diarrhea	Izoceño	Chaco
	Ro	¬	Diarrhea	Chiman	Amazon
	Se	decoction	Intestinal worms,	Yuracare	

Jacaratia digitata Solms, Fl. Bras. (Martius)	Ro	decoction	Intestinal worms,	Yuracare	Amazon
CELASTRACEAE					
Maytenus scuteoides Griseb. Lourt and O Donell	L ,Cr,Fr	decoction, maceration	Diarrhea, dysentery	Izoceño	Chaco
CHENOPODIACEAE					
Chenopodium ambrosoides L.	L,whole plant	decoction	Intestinal worms, stomach ache	Izoceño	Chaco
	L,Ro,whole plant	Infusion	Diarrhea	Yuracare	Amazon
	Br,whole plant	decoction	Diarrhea antiparasitic	Quechua	Andes
COSTACEAE					
Costus beckii	Whole plant		Dysentery	Chiman	Amazon
CYPERACEAE					
Scleria sp	L	Infusion	Diarrhea, dysentery	Yuracare	Amazon
ERYTHROXYLACEAE					
Erythroxylum coca	L	Infusion	Diarrhea, dysentery	Chiman	Amazon
FABACEAE					
Acacia polyphylla DC.	Cr	decoction	Diarrhea, dysentery	Pantanal	Pantanal
Caesalpinia spinosa			Diarrhea	Kichwa	Andes
Dipteryx alata Vogel	Cr	Infusion	Diarrhea	Pantanal	Pantanal
Geoffroea decorticans (Gillies exHook &Arn.) Burkart	Fr,Fl	decoction	Diarrhea	Weenhayek	Chaco
	L,Fl,Cr	decoction	Diarrhea, stomachache	Izoceño	
Libidibia paraguariensis (D.Parodi) G.P.Lewis	Cr	decoction	Diarrhea	Weenhayek	
	L,Cr,,Fr	decoction	Diarrhea, dysentery, stomachache	Izoceño	
Machaerium hirtum (Vell.) Stellfeld	Cr	Infusion	Diarrhea, dysentery	Pantanal	Pantanal
Ormosa coccinea	Se		Dysentery	Tacana	Amazon
Prosopis flexuosa DC.	L	decoction	Diarrhea	Izoceño	Chaco
Pterodon emarginatus Vogel.	Fr	decoction	Diarrhea	Chiquitano	Pantanal
Senna morongii	¬	¬	Diarrhea	Kichwa	Andes
Vatairea macrocarpa (Benth) Ducke	L,Cr,Ro	decoction	Dysentery	Chiquitano	Pantanal

IRIDACEAE					
Sisyrinchium sp	L	Infusion	Diarrhea	Aymara	Andes
LAMIACEAE					
Mentha viridis (L.)L.	L	decoction, Infusion	Diarrhea, Intestinal worms	Aymara	Andes
Minthostachys sp	L	Infusion	Anthelminthic	Kichwa	Andes
Stachys pusilla (Wedd.)	L	Infusion	Diarrhea	Aymara	Andes
LAURACEAE					
Aniba canelilla	Cr	decoction	Diarrhea, dysentery	Kichwa	Andes
Persea americana	Cr,L	decoction	Dysentery	Yuracare	Amazon
	Cr,L	decoction	Diarrhea	Chiman	
	Cr,L	decoction	Diarrhea	Tacana	
MALVACEAE					
Abutilon herzogianum R. E. Fries	L	Maceration	Diarrhea, dysentery	Izoceño	Chaco
MORACEAE					
Ficus insipida	Re	Direct consumption	Diarrhea	Yuracare	Amazon
MYRTHACEAE					
Plinia cauliflora (DC.) Kausel	Cr,Fr	decoction	Diarrhea	Chiquitano	Pantanal
Psidium guajava L.	Cr	Maceration Cooking	Diarrhea	Yuracare	Amazon
	Cr	decoction	Diarrhea	Chiman	
	Cr	decoction	Diarrhea	Tacana	
	Cr, Fr	decoction	Diarrhea	Chiquitano	Pantanal
OLACACEAE					
Ximenia americana	Cr	decoction	Diarrhea	Izoceño	Chaco
	L, Cr	decoction	Diarrhea	Weenhayek	
PHYTOLACCACEAE					
Gallesia integrifolia	Cr	decoction	Diarrhea	Yuracare	Amazon
Petiveria alliaceae	Ro	Maceración	Diarrhea, parasites	Yuracare	

POACEAE					
Hordeum vulgare L.	Se	decoction	Diarrhea, dysentery	Aymara	Andes
POLYGONACEAE					
Ruprechtia triflora	L, Cr	decoction	Diarrhea, stomachache	Izoceño	Chaco
	Cr	decoction	Diarrhea	Weenhayek	
Triplaris americana	Cr	decoction	Diarrhea	Yuracare	Amazon
	Cr	decoction	Diarrhea	Chiman	
Polygonum sp		¬	Diarrhea	Kichwa	Andes
RHAMNACEAE					
Zizyphus mistol	L, Cr	Direct application infusion	Wounds Diarrhea	Izoceño	Chaco
ROSACEAE					
Rubus uriticifolius	L	decoction	Diarrhea, dysentery	Aymara	Andes
RUBIACEAE					
Cinchona pubescens	Cr	decoction	Diarrhea	Weenhayek	Chazo
Faramea multiflora	Cr	Infusion	Dysentery	Yuracare	Amazon
Uncaria guianensis	Cr	Infusion	Diarrhea	Yuracare	
	Cr	Infusion	Diarrhea	Chiman	
	Cr	Infusion	Dysentery	Yuracare	
Citrus medica L.	L	Infusion	Diarrhea	Chiquitano	Pantanal
Citrus sinensis (L.)	Fr	decoction	Dysentery	Chiquitano	
SALICACEAE					
Salix humboldtiana	Cr	decoction	Diarrhea, dysentery, intestinal parasites	Yuracare	Amazon
	Cr	decoction	Diarrhea	Chiman	
	Cr	decoction	Diarrhea, dysentery	Quechua	Andes
SAPOTACEAE					
Bumelia obtusifolia	L, Cr	decoction	Diarrhea	Izoceño	Chaco
SOLANACEAE					
Cestrum parqui	L	Maceration	Diarrhea	Izoceño	Chaco
	L		Diarrhea	Quechua	Andes
	L	Infusion	Dysentery, stomach infections	Aymara	

ULMACEAE					
Celtis spinosa	L	Infusion	Diarrhea	Izoceño	Chaco
	L	decoction	Diarrhea, stomachache	Weenhayek	
Celtis iguanaea	L	decoction	Diarrhea	Yuracare	Amazon
URTICACEAE					
Urtica urens L.	L, Ro	decoction	Diarrhea, parasites	Aymara	Andes
VERBENACEAE					
Vitex cymosa Bertero ex Spreng.	L	decoction	Diarrhea	Pantanal	Pantanal
ZINGIBERACEAE					
Zingiber officinale	Ro	decoction	Diarrhea	Tacana	Amazon

Source: Author´s elaboration.

Ethnobotany, an ancestral knowledge used during the pandemic COVID-19 in Cochabamba, Bolivia
Etnobotánica, un conocimiento ancestral utilizado durante la pandemia COVID-19 en Cochabamba, Bolivia

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Abstract

This study addresses the use of medicinal plants in Cochabamba, Bolivia, during the COVID-19 pandemic. Faced with the lack of specific pharmacological treatments, local communities turned to traditional medicine to alleviate respiratory symptoms of the virus such as cough and nasal congestion. Among the most commonly used plants were eucalyptus *(Eucalyptus sp.)* with 32%, "wira wira" *(Achyrocline saturejoides)* with 17%, and "matico" *(Piper aduncum)* with 12%.

The study also highlights that knowledge about the use of these plants was passed down intergenerationally, mainly from parents and grandparents. Most respondents were herbalists (57%) and naturopaths (34%), who collaborated with doctors (60%) and biochemists (27%) to integrate traditional knowledge with scientific approaches, ensuring the safety and efficacy of the treatments.

Although plants proved effective in some cases, the study emphasizes the need to use them under medical supervision, especially for severe diseases like COVID-19. The resurgence of herbal medicine, not only in Bolivia but also in other countries, reflects the revaluation of ancestral knowledge in the search for accessible solutions amid global health crises.

Keywords: Medicinal plants, COVID-19, Traditional medicine

Resumen

Este estudio aborda el uso de plantas medicinales en Cochabamba, Bolivia, durante la pandemia de COVID-19. Ante la falta de tratamientos farmacológicos específicos, las comunidades locales recurrieron a la medicina tradicional para aliviar los síntomas respiratorios del virus como la tos y la congestión nasal. Entre las plantas más utilizadas se encuentran el eucalipto (*Eucalyptus sp.*) con un 32%, la wira wira (*Achyrocline saturejoides*) con un 17% y el matico (*Piper aduncum*) con un 12%.

El estudio también destaca que el conocimiento sobre el uso de estas plantas se transmitió intergeneracionalmente, principalmente de padres y abuelos. La mayoría de los encuestados eran herbolarios (57%) y naturópatas (34%), que colaboraban con médicos (60%) y bioquímicos (27%) para integrar el conocimiento tradicional con enfoques científicos, garantizando la seguridad y eficacia de los tratamientos.

Aunque las plantas resultaron eficaces en algunos casos, el estudio subraya la necesidad de utilizarlas bajo supervisión médica, especialmente en enfermedades graves como la COVID-19. El resurgimiento de la fitoterapia, no sólo en Bolivia sino también en otros países, refleja la revalorización de conocimientos ancestrales en la búsqueda de soluciones accesibles en medio de las crisis sanitarias globales.

Palabras clave: Plantas medicinales, COVID-19, medicina tradicional

INTRODUCTION

The COVID-19 pandemic, which began in December 2019, has had an unprecedented impact on global public health, driving an intense search for treatments and preventive measures. Despite advancements in modern medicine, many communities have turned to traditional practices, such as the use of medicinal plants, to prevent and treat the symptoms of the disease. Herbal medicine, used for centuries as an alternative and complementary therapy, has resurged strongly during the pandemic due to its accessibility, low cost, and the empirical knowledge accumulated across various cultures about its therapeutic properties (Yupanqui and Lizana, 2020; Cárdenas et al., 2022).

In this context, medicinal plants have garnered new interest due to their potential to strengthen the immune system and alleviate some symptoms associated with COVID-19. Throughout history, diverse cultures have employed these plants for their healing properties. However, it is crucial to emphasize that the use of medicinal plants can not replace the preventive measures established by health authorities to combat COVID-19. While the potential of these plants has been researched for the prevention and mitigation of respiratory diseases that could alleviate symptoms such as fever, cough, and difficulty breathing, there is no conclusive scientific evidence regarding their specific effectiveness against this virus (de los Ángeles et al., 2020).

The use of these plants should be considered as complementary or alternative to the prevention and relief of respiratory diseases, leveraging ancestral knowledge and the rich biodiversity of native flora in different regions worldwide. This resurgence of herbal medicine reflects not only the search for accessible alternatives in the absence of specific pharmacological treatments for COVID-19 but also a revaluation of traditional knowledge in disease management (de los Ángeles et al., 2020).

In Bolivia, as in other countries, particularly in Cochabamba, various medicinal plants have been researched in the context of COVID-19. Some of the most studied plants include eucalyptus *(Eucalyptus sp.)*, garlic *(Allium sativum)*, chamomile *(Matricaria chamomilla)*, matico *(Piper aduncum)*, wira wira *(Achyroclina saturejoides)*, muña *(Minthostachys mollis)*, lemon *(Citrus limon),* onion *(Allium cepa)*, ginger *(Zingiber officinalis)*, borage *(Borago officinalis)*, coca leaf *(Erythroxylum coca),* maca *(Lepidium meyenii)*, cat's claw *(Uncaria tomentosa)*, nettle *(Urtica dioica)*, stonebreaker *(Phyllanthus niruri)*, dragon's blood *(Croton lechleri),* quinoa *(Chenopodium quinoa),* paico *(Dysphania ambrosioides)*, annatto *(Bixa orellana)*, valerian *(Valeriana officinalis),* and ayahuasca *(Banisteriopsis caapi*).

It is important to note that while these plants have been traditionally used and may offer potential benefits, it is essential to consult a healthcare professional before starting any treatment with them, especially in the case of serious illnesses such as COVID-19.

This study, conducted through surveys of herbal, tea vendors and traditional doctors authorized by the Departmental Health Secretariat (SEDES-Cochabamba), investigates which medicinal plants were most commonly used, which parts of the plants were employed, at what stage of the disease were most effective, and how it should be used in the treatment of COVID-19.

THEORETICAL SECTION:

Since the beginning of human history, there has been a close relationship between humans and natural resources. Currently, according to the World Health Organization (WHO), 80% of the world's population relies on traditional medicine to meet their primary healthcare needs. With the emergence of the new coronavirus (COVID-19) in late 2019 and throughout 2020, there was a significant increase in the global mortality rate. Health professionals, researchers, and those affected by the virus were disoriented in the face of an unknown threat. Despite having sophisticated equipment, large health centers worldwide were overwhelmed, and pharmacies ran out of essential medications, generating a sense of helplessness and desperation.

In response to this crisis, many people turned to the use of medicinal plants and explored various preparation methods to treat the disease. Scientifically, COVID-19 is an infectious disease caused by the SARS-CoV-2 virus, which leads to severe acute respiratory syndrome or coronavirus (WHO, 2020). This virus was first identified in December 2019 in Wuhan, Hubei, China, and declared a pandemic by the WHO on March 11, 2020 (Guo et al., 2019). By September 2020, more than 326 million cases had been reported in 188 countries and territories, with over 990,000 deaths.

The epidemiological situation of COVID-19 in Bolivia, compared to other South American countries, highlights that Bolivia ranks sixth in the number of cases with 1,198,404 confirmed cases. Bolivia ranks eighth with an incidence rate of 9,972 accumulated cases per 100,000 inhabitants. Bolivia also ranks seventh in total deaths, with 22,383 deaths. The mortality rate per 100,000 inhabitants was lower in Bolivia than in the rest of South American countries, with Bolivia's cumulative fatality rate ranked fifth at 19% (Mamani, 2023).

Around 225 million people have recovered from this disease through ongoing treatments (Guo et al., 2019). The virus spreads primarily between people in close proximity through the air via small droplets or particles, even spreading through speech, coughing, and sneezing. Common symptoms include fever, cough, fatigue, shortness of breath, and loss of senses such as smell and taste. The incubation period can range from one to fourteen days (Perez et al., 2020).

Local transmission of the disease has occurred in most countries across the six WHO regions (WHO, 2020). The main preventive measures recommended include maintaining a social distance of at least one meter, frequent handwashing, staying home when symptomatic, covering the mouth with a mask, and avoiding touching the face (Perez et al., 2020). To date, there are no proven vaccines or specific treatments for COVID-19, although several are under development. Management involves treating symptoms, supportive care, isolation, and experimental measures (ECDC, Europe, 2020).

People with COVID-19 generally experience viral-like illness, with symptoms ranging from mild respiratory infections to severe acute infections, similar to flu-like symptoms. In some cases, shortness of breath (dyspnea) develops, typically between 4 to 8 days after symptom onset, though it can appear after 10 days. This respiratory difficulty warrants medical attention (Llover, 2021). Patients who experience dyspnea generally suffer from lung and respiratory dysfunction, and even after recovering from COVID-19, they may present with sequelae such as physical impairment, difficulty in oxygen transfer, shortness of breath during exertion, and muscle atrophy (April et al., 2020).

The resurgence of the use of medicinal plants during the COVID-19 pandemic underscores the importance of integrating ancestral and traditional knowledge with modern scientific research. In many cases, communities have relied on medicinal plants due to limited access to conventional medications, especially in rural areas or countries with fragile healthcare systems (Leyns and Azcarruns, 2020).

METHODOLOGY

For data collection, a survey was conducted with traditional doctors from various specialties and herbal tea vendors authorized by the Departmental Health Secretariat (SEDES-Cochabamba). The participants answered a questionnaire in person, after providing informed consent. The questionnaire included the following questions:

- What is the respondent's specialty?
- What types of medicinal plants do you consider effective in treating the symptoms of COVID-19?
- How did you acquire knowledge about the use of medicinal plants?
- What other pathologies can be treated with these plants?
- What are the benefits of the medicinal plants used?
- What parts of the plant are used, what is the recommended dosage, the duration of use, and the method of preparation?

Once the surveys were completed and collected, the data was entered into a database using Excel. Statistical analysis was carried out using R Studio software.

Ethical considerations: The research was approved and certified by the Ethics Committee of the Albina R. de Patiño Hospital, Cochabamba-Bolivia. Before participating, the respondents attended an informational workshop where the purpose and benefits of the study were explained in detail, as well as instructions for completing the questionnaire. Confidentiality and anonymity of the participants' information were guaranteed.

This research will provide valuable data on the use of medicinal plants, contributing to the advancement of scientific knowledge and informed decision-making in public health.

RESULTS

Out of the 67 respondents, 50.7% were women and 49.3% were men, with different specialties:

TABLE 1. Specialty of respondents

Specialty	Frequency	Percentage %
Herbalist	38	57
Naturalist	23	34
Nutritionist	2	3
Jampiri	2	3
Midwife	2	3

Source: Author´s elaboration.

The distribution of specialties among traditional doctors is shown in Table 1, with the most common specialty being herbalists, accounting for 57%. This group specializes in the use of herbs for various aliments. The second most common specialty is naturopaths, with 34%, consisting of therapists who have knowledge and experience in the practice of natural medicine and its principles. The less common specialties are nutritionists, jampiris, and midwives, each representing 3% of the results.

Regarding the respondents' use of plants in response to COVID-19, we have:

TABLE 2. Plants for covid-19

Plants	Frequency	Percentage %
Eucalyptus sp.	22	32
Achyroclina saturejoides	12	17
Piper aduncum	8	12
Matricaria chamomilla	7	10
Zingiber officinale	4	6
Allium sativum	2	3
Other plants	11	16
Propolis and *Aloe vera*	3	4

Source: Author´s elaboration.

In Table 2, we can see that the most commonly used plant, with 32% of cases, is *Eucalyptus sp.* (eucalyptus), followed by *Achyroclina saturejoides* (wira wira) at 17%, *Piper aduncum* (matico) at 12%, *Matricaria chamomilla* (chamomile) at 10%, and *Zingiber officinale* (ginger) at 6%. These plants are also relatively common for the treatment of COVID-19. *Allium sativum* (garlic) and propolis combined with *Aloe vera* are used less frequently, at 3% and 4% respectively. The "other plants" category includes a variety of less commonly used plants such as muña, molle, onion, horsetail, pine, oregano, guava leaf, cat's claw, sugarcane, and some fruits.

TABLE 3. Other medicinal uses of plants for various pathologies

Pathologies	Frequency	Percentage %
Respiratory diseases	29	43
Hematological disorders	5	7.5
Urinary diseases	5	7.5
Digestive diseases	12	18
Various pathologies	16	24
Total	**67**	**100**

Source: Author´s elaboration.

In Table 3, an overview of the frequency and percentage of different medicinal uses is presented. The most common condition for which medicinal plants are used is respiratory diseases, with 29 recorded cases, representing 43%. This is followed by hematological conditions, with a frequency of 5 cases, which equates to 7.5%, similar to urinary diseases with 5 cases (7.5% of the total). Various other pathologies, where this category groups less common conditions for which medicinal plants are used, account for a total of 16 cases, corresponding to 2.4%.

On the other hand, regarding the effectiveness of medicinal plants against mild and severe COVID-19 symptoms, many respondents mentioned that *Eucalyptus sp.* (eucalyptus) was effective in the mild phase with 34% and in the severe phase with 20.8%. For *Achyroclina saturejoides* (wira wira), they mentioned that only 16% found it effective in the mild phase and 8% in the severe phase. The use of *Piper aduncum* (matico), *Matricaria chamomilla* (chamomile), *Zingiber officinale* (ginger), *Allium sativum* (garlic), and propolis was indifferent in the acute or mild phase, with similar percentages in these cases. Respondents also indicated that there is a small percentage of effectiveness (13%) when using 'other plants (Figure 1, See Table 4)

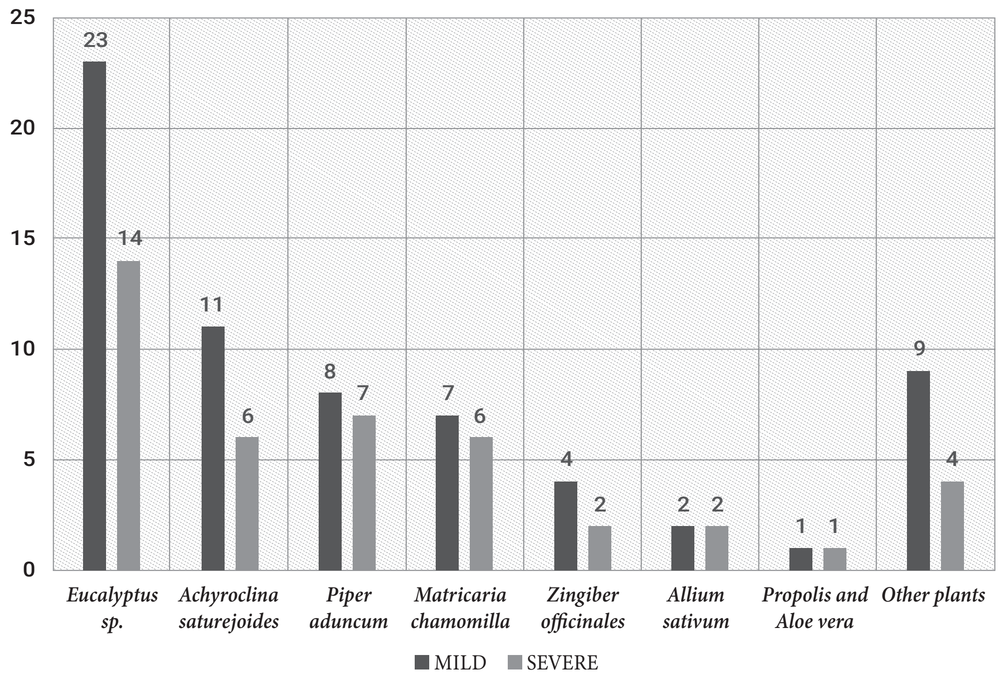

FIGURE 1. Effectiveness of medicinal plants in mild and severe symptoms of COVID- 19.
Source: Author´s elaboration.

TABLE 4. Contingency table of medicinal plant type and its effectiveness on COVID-19 symptoms.

Contingency table of medicinal plant type and its effectiveness									
Efficacy	**Medicinal plants**								**Total**
	Eucaliptus sp.	*Achyroclina saturejoides*	*Piper aduncum*	*Matricaria chamomilla*	*Zingiber officinale*	*Allium sativum*	*Propolis and aloe vera*	*Other plants*	
Mild	9	5	1	1	2	0	0	5	23
Mild and severe	14	6	7	6	2	2	1	4	42
Severe	0	0	0	0	0	0	1	1	2
Total	**23**	**11**	**8**	**7**	**4**	**2**	**2**	**10**	**67**

Source: Author´s elaboration.

A Chi-square test was conducted to determine if there is indeed a relationship between the type of plant used and its effectiveness on mild or severe symptoms. Taking Ho = There is no relationship between these variables and Ha = There is a relationship between these variables, with 95% confidence. The analysis was performed using R Studio software, yielding a P-value of ($0.0268 < 0.05$), concluding that there is a quite significant relationship between both variables.

In te following table, we can see the detailed and summarized results regarding the usefulness of medicinal plants.

TABLE 5. Plants for COVID-19: plant part, dosage, duration of use, and preparation method.

Family	Scientific name	Common name	Part of the plant used	Preparation method	Duration of use	Therapeutic applications
MYRTACEAE	*Eucalyptus sp.*	Eucalyptus	Leaf	Infusion, vaporization	1-3 times/day	Cough, nasal congestion, muscle pain
ASTERACEAE	*Achyroclina saturejoides*	Wira Wira	Branch	Infusion	1-3 times/day	Anti-inflammatory, antispasmodic, antitussive
PIPERACEAE	*Piper aduncum*	Matico	Leaf	Infusion, tincture	1-3 times/day	Respiratory and digestive infections, astringent, healing
ASTERACEAE	*Matricaria chamomilla*	Chamomile	Flower	Infusion	1-3 times/day	Anxiety, insomnia, inflammation
ZINGIBERACEAE	*Zingiber officinale*	Ginger	Root, rhizome	Infusion, tea	1-3 times/day	Anti-inflammatory, antiviral
AMARYLLIDACEAE	*Allium sativum*	Garlic	Bulb	Raw, infusion, oil	1-3 times/day	Antibacterial, antiviral

RUTACEAE	*Citrus limon*	Lemon	Leaf, fruit	Juice	1-3 times/day	Immune system booster
AMARYLLIDACEAE	*Allium cepa*	Onion	Bulb, leaf	Raw, infusion	1-3 times/day	Antibacterial, anti-inflammatory
BORAGINACEAE	*Borago officinalis*	Borage	Flower	Infusion	1-3 times/day	Colds, bronchitis, cough, nasal congestion
ERYTHROXYLACEAE	*Erythroxylum coca*	Coca leaf	Leaf	Infusion, tea	1-3 times/day	Analgesic, digestive
BRASSICACEAE	*Lepidium meyenii*	Maca	Root	Infusion, decoction, powder	1-3 times/ day	Aphrodisiac, fertility, revitalizing, antidepressant, sedative
RUBIACEAE	*Uncaria tomentosa*	Cat's Claw	Leaf, bark, roots	Infusion	1-3 times/ day	Arthritis, antiviral, anti- inflammatory, diuretic
URTICACEAE	*Urtica dioica*	Nettle	Leaves, stems	Infusion	1-3 times/ day	Diuretic, anti-anemic, anti-inflammatory
PHYLLANTHACEAE	*Phyllanthus niruri*	Chancapiedra	Whole plant, leaves, stems	Tea, infusion	1-3 times/ day	Antiviral
EUPHORBIACEAE	*Croton lechleri*	Dragon's Blood	Bark, leaves, latex	Decoction, infusion, poultice	1-3 times/ day	Antiviral, anti-inflammatory, disinfectant, wound healing, ulcers
AMARANTHACEAE	*Chenopodium quinoa*	Quinoa	Seed	Cooked seed	1-3 times/ day	Nutritive, diuretic, expectorant
AMARANTHACEAE	*Dysphania ambrosioides*	Paico	Leaf, berries, root	Infusion	1-3 times/ day	Anti-inflammatory, antidiarrheal, digestive, bronchitis
BIXACEAE	*Bixa orellana*	Achiote	Leaf, fruit, roots	Gel	1-3 times/ day	Urinary infections, kidney function regulation
CAPRIFOLIACEAE	*Valeriana officinalis*	Valerian	Roots	Infusion	1-3 times/ day	Anti-stress, migraine, menstrual pain, fever, convulsions, rheumatic pain
MALPIGHIACEAE	*Banisteriopsis caapi*	Ayahuasca	Leaves	Decoction, mate	1-3 times/ day	Antidepressant

Other medicinal plants						
CUCURBITACEAE	*Cucurbita sp.*	Pumpking	Seed, leaf, pulp and fruit	Infusion	1 to 3 times a day	Strengthens the immune system, lung protection.
CACTACEAE	*Opuntia ficus*	Tuna	Fruit and stalks	Poultice	1 to 3 times a day	Anti-inflammatory, analgesic, anti-fever, antioxidant
LAMIACEAE	*Minthostachys mollis*	Muña	Stem, leaf and flowers	Infusión	1 to 3 times a day	anti-inflammatory, carminative, antiseptic, analgesic in kidney and respiratory conditions.
ANACARDIACEAS	*Schinus molle*	Molle	Leaf, fruit, bark	Infusion	1 to 3 times a day	Antispasmodic and healing properties.

Source: Author´s elaboration.

The traditional doctors surveyed were also asked who taught them, and most responded that it was their parents and grandparents, as shown in the following table:

TABLE 6. Who taught you?

Who teaches?	Quantity	Percentage %
Mother/Father/Grandparents	39	58
Grandparents	17	25
Mother	3	4.5
Father	3	4.5
Father/Grandparents	1	2
Other	4	6
TOTAL	67	100

Source: Author´s elaboration.

According to Table 6, most respondents learned about natural medicine from their family members (mother, father, grandparents, or other relatives such as grandmother, uncles, and siblings). Most patients seeking treatment were adults and young people, representing 86.5% of the cases.

As complementary information, respondents were asked if they believed some plants lose their effectiveness in the presence of medications or foods; 94% of respondents believe this to be the case.

Regarding COVID-19 treatment, 60% of respondents coordinated with other professionals, with 60% working together with doctors and 27% with biochemists and nutritionists. They consider a biochemist to be the most suitable professional for advice, with 34.4% of the cases, and a biologist as the second option, with 23.9%.

As for the source of information for treating COVID-19, 65.7% of the respondents preferred the internet, while 14.9% favored online courses

DISCUSSION

Traditional medicine, passed down through generations, uses medicinal plants to treat various diseases, a knowledge that has been revitalized during the COVID-19 pandemic in rural regions of Bolivia, Mexico, and Cuba. In Mexico, traditional healers played an essential role not only in treating the physical symptoms of the disease but also in providing emotional support, highlighting the use of plants like eucalyptus and chamomile to treat respiratory illnesses and alleviate anxiety (Martínez and Sánche; 2023; Piña et al., 2022).

In Cochabamba, Bolivia, herbalists and naturopaths were key players, representing 57% and 34% of respondents, respectively. They used plants such as eucalyptus, wira wira, and matico in vapor treatments and infusions, following a pattern similar to that observed in Oaxaca, Mexico. The combination of these ancestral treatments with modern medicine helped strengthen the immune system and relieve respiratory symptoms (Martínez and Sánchez, 2023; Ministry of Health and Sports, 2023).

The Jampiri, a healer specialized in spiritual and energetic medicine, played an important role in Bolivia, akin to the Kallawayas of the Andes, who focused on both the emotional and physical aspects of the disease, emphasizing the importance of holistic well-being during the pandemic. Midwives also played a significant role in the care of pregnant women in rural communities, where access to hospitals was limited. This role was crucial to ensure comprehensive care during the health crisis (Martínez and Sánchez, 2023).

Collaboration between healthcare professionals and traditional medicine experts was fundamental. In Cochabamba, 60% of respondents collaborated with doctors and 27% with biochemists and nutritionists, ensuring the integration of traditional knowledge with scientific practices to ensure safe and effective treatments. Documents such as the Biosecurity Protocol and the Traditional Medicine Strengthening Model highlight collaboration with biochemists and doctors to ensure the safety of traditional therapies and their compliance with health regulations (Ministry of Health and Sports, 2020).

In Colombia, coordination between doctors, biochemists, and healers extended care to rural communities with limited access to formal medical services. Globally, the COVID-19 Response Mechanism highlights the importance of integrating traditional medicine into community health systems to ensure a resilient and effective response to future crises (Global Fund, 2021).

In this study, the most commonly used plants to treat COVID-19 were *Eucalyptus sp*. (32%), *Achyroclina saturejoides* (17%), *Piper aduncum* (12%), *Matricaria chamomilla* (10%), *Zingiber officinale* (6%), and *Allium sativum* (3%). In a study by Castillo (2020) in the Cajamarca region of Peru, the most commonly used plants were eucalyptus (66%), ginger (42%), garlic (38%), onion (30%), and plantain (12%).

García (2021), notes that eucalyptus vapor is an accessible non-pharmacological therapy against pulmonary fibrosis, a sequela of COVID-19. Villarreal et al., (2022) mention that eucalyptus leaves are used in infusions, tea, syrups, and vapor treatments, being important in popular knowledge.

Castañeta et al., (2022) highlight the reputation of wira wira in traditional Aymara medicine of the Bolivian highlands to treat respiratory conditions such as cough, cold, bronchitis, and pneumonia. Thanks to its expectorant properties, it is proposed as a phytopharmaceutical to prevent COVID-19 through infusions and vapor treatments. The respondents highly recommended wira wira to prevent and treat mild and acute phases of

COVID-19, although further studies are needed to validate its effectiveness.

Morales, (2020) indicates that ginger has anti-inflammatory, anti-allergic, and antioxidant properties useful for respiratory diseases, but its use must be medically supervised to avoid side effects such as bleeding. Ore (2021) reports that matico has healing, antimicrobial, and antifungal properties, but its antiviral action has not been confirmed, requiring further studies to evaluate its effectiveness in COVID-19. The respondents recommend the consumption of matico in infusion form for mild and acute phases of COVID-19. It is one of the most widely used plants in Bolivia to treat respiratory diseases, supported by ancestral knowledge.

Orantes et al., (2020) and Ríos et al., (2008) emphasize that chamomile (*Matricaria chamomilla*) has biological activities and has been used medicinally worldwide. In Bolivia, it is recommended for its antibacterial and anti-inflammatory properties to treat respiratory conditions, although no studies have yet supported its effectiveness against SARS-CoV-2. In our results, chamomile was frequently mentioned due to its numerous benefits in relieving COVID-19 symptoms in both the acute and severe phases. Its most common use is in infusion tea and vapor treatments, primarily to treat respiratory issues, reduce inflammation, and as a natural relaxant, reaffirming what has been indicated by the authors.

A study by Thuy et al., (2020) suggests that garlic oil may inhibit the replication of the SARS-CoV-2 virus. *Allium sativum* has traditionally been used as an antimicrobial and antidiarrheal agent (Espinoza et al., 2022). The surveys confirmed its use due to its antibacterial and antiviral properties in the treatment of COVID-19, consumed in infusion, macerated in oil, or raw, 1 to 3 times a day.

Schneegans et al., (2022) highlight that *Citrus limon* extract inhibits the development of fungi, bacteria, and viruses such as SARS-CoV-2 in vitro studies, suggesting its usefulness in preventing COVID-19. Our results confirm its antiviral potential and its role in strengthening the immune system, which could improve the body's resistance and recovery, positioning lemon as useful in preventing and reducing the severity of COVID-19.

Ayahuasca, a psychoactive and medicinal beverage, is prepared by decoction of P*sychotria viridis* and *Banisteriopsis caapi*. Its active compounds, obtained in infusion or maceration, generate relaxing effects that help alleviate COVID-19 symptoms (Castro et al., 2016). The Traditional Medicine Guide for COVID-19 in Bolivia recommends this plant to treat insomnia, headaches, anxiety, stress, fever, convulsions, and rheumatic pain. It should not be used during pregnancy, lactation, or in people with hypersensitivity, as it can cause headaches and palpitations (Ministry of Health and Sports, 2022).

Our results show that although ayahuasca (*Banisteriopsis caap*i) is not well known, it is used in decoction and tea form for its relaxing and antidepressant properties. It also has other medicinal benefits and is recommended for combating COVID-19, considering its contraindications.

Morocho (2022), notes that quinoa (*Chenopodium quinoa*) is beneficial for treating chronic diseases like diabetes, hypertension, and osteoporosis, which increase the risk of complications from COVID-19. Its consumption could reduce vulnerability to the virus, especially due to its properties in controlling triglycerides and cholesterol, crucial in a pandemic context.

The use of medicinal plants such as quinoa *(Chenopodium quinoa*) has been effective in managing chronic diseases that increase the risk of complications from COVID-19. According to Morocho (2022), quinoa helps control these conditions, reducing vulnerability to the virus. Our findings confirm that respondents consume it 1 to 3 times a day for its nutritional, diuretic, and expectorant properties, strengthening the immune system and improving respiratory symptoms.

The paico (*Dysphania ambrosioides)* has therapeutic antibacterial, antifungal, anti-inflammatory, and antiparasitic properties (León, 2009). It is used for colic, colds, pneumonia, and as an antitussive (Cáceres, 1994). Our survey participants, including traditional doctors and herbal tea vendors, confirm its use for treating bronchitis and digestive problems, consumed in infusions 1 to 3 times a day.

The achiote (*Bixa orellana*) is used in infusions and inhalations for its antiviral, anti-inflammatory, and expectorant properties, especially during the pandemic (Attalea, 2021). In our findings, its leaves, flowers, and roots are primarily used to treat urinary infections and as a renal function regulator, though its antiviral properties remain questioned. *Phyllanthus niruri* (stonebreaker), used in Ayurvedic medicine, may act as an immunomodulator, helping to reduce the cytokine storm caused by COVID-19 (Murugesan et al., 2021). Our study confirms its use in infusions as a natural remedy against COVID-19.

Huacachín and Huayta (2021) highlight that borage leaves strengthen the immune system, reduce blood pressure, and act as an anti-inflammatory and diuretic. In the context of COVID-19, its immune-boosting and anti-inflammatory properties are of particular interest, though further clinical research is needed. Our results show that respondents primarily use borage flowers in infusions 1 to 3 times a day to relieve colds, bronchitis, and nasal congestion, complementing the authors' indications of its benefits.

In the case of COVID-19, borage could strengthen the immune system and reduce inflammation, although more research is needed to determine its efficacy and safety before recommending its widespread use. According to Flores and Sánchez (2023), coca leaf has beneficial effects on the respiratory system, improving breathing and oxygenation. Traditional doctors note that it is used in infusions as an energizer and analgesic, but more scientific evidence is required to confirm its effectiveness against COVID-19.

King et al., (2020) highlight that dragon's blood (*Croton lechleri)* is used in traditional medicine to treat diarrhea, ulcers, wound healing, skin infections, and respiratory problems. It is one of the most common traditional medicines in Latin America, with its products available in Peru, North America, and Europe. In Bolivia, dragon's blood is known for its antiviral, anti-inflammatory, and healing properties, being used for respiratory problems such as COVID-19. Although studies have focused on its gastrointestinal and healing uses, anecdotal evidence supports its effectiveness in respiratory conditions.

Carvalho and Ribeiro (2019) and Wang and Zhu (2019) highlight that maca (*Lepidium meyenii*) improves sexual performance, has immunomodulatory properties, and offers protection against osteoporosis and UV radiation. Mendoza et al. (2014) and Wang and Zhu (2019) also mention its anti-inflammatory, antioxidant, hepatoprotective, and antiviral properties, suggesting its potential in treating COVID-19. The survey respondents confirm that maca, consumed in infusion or powdered form, has revitalizing and aphrodisiac effects.

Sabzian et al., (2023) emphasize the role of *Urtica dioica* (nettle) in COVID-19 due to its immunomodulatory and anti-inflammatory properties. Some studies indicate that certain components may inhibit viral entry. The respondents recommend infusions of nettle leaves and stems for their antiviral and diuretic effects, though moderate use is advised.

Hemeryth-Rengito et al., (2023) mention that nettle was used by 21 participants, mostly in infusions, and was effective for 66.7% without adverse effects for 90.5% of users. Although positively perceived, clinical evidence confirming its efficacy against SARS-CoV-2 is lacking, as previous studies suggest immunomodulatory effects.

Pumpkin oil has medicinal properties, such as antidiabetic and antioxidant benefits, and its seeds are rich in minerals (Lemus, 2019). According to our surveys, it is consumed in infusions to strengthen the immune system, though it is more valued for its nutritional properties than its medicinal uses for COVID-19.

Vicidomini and Roviello (2017) highlight the antiviral properties of the tuna cactus (*Opuntia ficus*), which could be useful against COVID-19. It also offers digestive and antioxidant benefits. While respondents recognize its anti-inflammatory and antipyretic properties, they are unaware of its potential antiviral benefits.

Silva et al., (2022) emphasize the analgesic and antiseptic properties of muña (*Minthostachys mollis*) to treat rheumatism and respiratory diseases. Our surveys align with the literature, noting its use for renal and respiratory conditions, though further studies are needed to explore its therapeutic potential.

Pessolato (2021) mentions that *Valeriana officinalis* is used for anxiety and insomnia and became popular during the pandemic due to its accessibility. While it offers an alternative to synthetic anxiolytics, its use must be supervised to avoid the risks of self-medication.

According to Delgado (2021), *Valeriana officinalis* is traditionally used in Peru to treat respiratory conditions such as bronchitis and cough, though mainly topically. Studies confirming its efficacy in treating severe respiratory diseases like COVID-19 are lacking.

CONCLUSIONS

The resurgence of traditional medicine during the COVID-19 pandemic highlights the need to integrate it with modern scientific research. In many instances, communities have relied on medicinal plants due to limited access to conventional pharmaceuticals, particularly in rural areas or countries with fragile healthcare systems. This reliance is not only driven by the accessibility of these plants but also by the deep-rooted empirical knowledge passed down through generations about their healing properties.

The most frequently used plants in Cochabamba, Bolivia, for the treatment of COVID-19 symptoms include *Eucalyptus sp.*, *Achyrocline saturejoides* (Wira wira), *Piper aduncum* (Matico), *Matricaria chamomilla* (Chamomile), and *Zingiber officinale* (Ginger). Each of these plants has been recognized for its potential to alleviate respiratory symptoms such as cough, nasal congestion, and inflammation. They have been used in various forms, such as infusions, inhalations, and topical applications, as remedies for mild and severe COVID-19 symptoms.

Our study demonstrated that the knowledge of these plants is transmitted intergenerationally, mainly from parents and grandparents, and that most herbalists and naturopaths collaborated with doctors and biochemists to ensure safe and effective treatments. This collaboration between traditional healers and modern healthcare professionals was essential in integrating both approaches, guaranteeing the safety of patients who sought alternative treatments during the pandemic.

However, while traditional medicine provided valuable support for symptom relief, it is critical to emphasize that medicinal plants cannot replace scientifically proven treatments or preventive measures. The use of these plants should always be done under the guidance of healthcare professionals, particularly in cases of serious illnesses like COVID-19. Their effectiveness, particularly for severe cases of the disease, requires further clinical research to substantiate their therapeutic potential.

The results also indicate that certain plants, such as *Eucalyptus sp.* and *Achyrocline saturejoides*, showed some effectiveness in treating both mild and severe symptoms of COVID-19. However, other plants, such as *Piper aduncum* and *Matricaria chamomilla*, were primarily used for less severe cases, indicating that their application might be more appropriate for symptomatic relief rather than treatment of severe conditions.

Moreover, the findings suggest that the most effective strategy for using these plants is through complementary or alternative approaches, ensuring that they do not interfere with conventional medical treatments. This aspect was particularly important, as some respondents believed that medicinal plants could lose their effectiveness when used alongside certain pharmaceutical drugs or foods, reinforcing the need for further investigation on potential interactions.

In conclusion, while traditional medicine offers promising avenues for symptom relief and immune system support, especially in underserved areas, it must be applied cautiously and always in conjunction with medical advice. As we continue to navigate the challenges posed by global health crises, the integration of ancestral knowledge and modern science may provide a more holistic approach to healthcare, benefiting communities in both rural and urban settings.

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Preliminary Paleogenomic Contributions in the Study of a Tropical pre-Columbian Archaeological Site from Brazil

Aportes paleogenómicos preliminares en el estudio de un sitio arqueológico precolombino tropical de Brasil

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Abstract

Due to prolonged exposure to weathering and erosion, ancient DNA (aDNA) present in archaeological samples becomes fragmented, erratic, and scarce, resulting in increased susceptibility to contamination with modern DNA and false-negative results. With the development of new technologies, methodologies from other scientific fields are employed in paleoparasitology and paleogenetics to minimize contamination and enhance the recovery of endogenous DNA from archaeological samples. In this study, we propose the application of next-generation sequencing/high-throughput sequencing (NGS/HTS) to archaeological samples severely degraded by intense taphonomic effects, such as those from coastal sites in tropical climates. For this purpose, 8 dental samples were selected from individuals buried in the *Malhada* archaeological site, Cabo Frio municipality, Rio de Janeiro state, Brazil. As a result, approximately 1.2 million high-quality reads were obtained according to the stipulated parameters. When compared to the context of Sanger sequencing applications, these results underscore the exploratory potential of high-throughput sequencing data analysis. The substantial volume of information allows for the recovery of endogenous aDNA, even from samples originating in contexts of low preservation, as evidenced by the archaeological site of *Malhada*. Looking ahead, the application of bioinformatics pipelines may reveal the taxonomic identification of these sequences at the species level and the antiquity of the reads, thereby minimizing the impacts of contamination.

Keywords: NGS/HTS, ancient DNA, bioinformatics

Resumen

Debido a la exposición prolongada a la intemperie y la erosión, el ADN antiguo (aDNA) de muestras arqueológicas se fragmenta, se vuelve errático y escaso, lo que hace que sea más suceptible a la contaminación con ADN moderno y a resultados falsos negativos. Con el desarrollo de nuevas tecnologías, se están empleando metodologías de otros campos científicos en la paleoparasitología y la paleogenética para minimizar la contaminación y optimimizar la recuperación de ADN endógeno de muestras arqueológicas. En este estudio, proponemos la aplicación de la secuenciación de nueva generación/ secuenciación de alto rendimiento (NGS/HTS) para muestras arqueológicas severamente degradadas por efectos tafonómicos intensos, como aquellas de sitios costeros ubicados en climas tropicales. Para este propósito, se seleccionaron 8 muestras dentales de individuos enterrados en el sitio arqueológico de *Malhada*, ubicado en el municipio de Cabo Frio, en el estado de Río de Janeiro, Brasil. Como resultado, se obtuvieron aproximadamente 1,2 millones de lecturas de alta calidad, según los parámetros estipulados. Estos resultados, al ser comparados con el contexto de las aplicaciones de la secuenciación Sanger, destacan el potencial exploratorio del análisis de datos de secuenciación de alto rendimiento. El volumen sustancial de información permite la recuperación de ADN antiguo endógeno, incluso a partir de muestras provenientes de contextos de baja preservación, como es el caso del sitio arqueológico de *Malhada*. En el futuro, la aplicación de pipelines bioinformáticos puede revelar la identificación taxonómica de estas secuencias a nivel de especie, así como la antigüedad de las lecturas, minimizando así los impactos de la contaminación.

Palabras clave: NGS/HTS, ADN antiguo; bioinformática

INTRODUCTION

The prospect of using ancient DNA (aDNA) obtained from archaeological samples as an alternative to gaining more comprehensive information about said individuals' biological and cultural patterns became a reality when Higuchi et. al., (1984) successfully extracted aDNA from an extinct animal, offering new research perspectives. The development and popularization of technologies such as Sanger sequencing (Sanger et al., 1977) allowed for the retrieval of previously unattainable biological information, providing means to observe genetic changes over time and enabling evolutionary analyses (Lan & Lindqvist, 2018). Additionally, with the advent of the Polymerase Chain Reaction (PCR) (Mullis & Faloona, 1987), paleogenetics has demonstrated the ability to recover aDNA from organic materials with various degrees of degradation.

For ancient DNA, the passage of time poses a significant threat to its preservation, with fragmentation often attributed to hydrolytic depurination and subsequent β-elimination, resulting in single-strand breaks (Lindahl, 1993). Additionally, cytosine deamination to uracil induces artificial transitions (Dabney et al., 2013), characteristic of aDNA. These factors are inherent to any biological remains found in archaeological sites; however, their intensity can vary. Covered archaeological sites, such as caves, generally exhibit milder effects, while open-air sites, such as coastal sites and villages, suffer more severe impacts due to greater exposure to weathering and other environmental phenomena (Brito, 2019; M. Camacho et al., 2016; Iñiguez, 2014; Souza, 2010). Despite the challenges in recovering this aDNA, the effort is justified by the invaluable insights it provides into the health and lifestyle of our ancestors. Working with such material necessitates a continual search for new tools and methodologies to enhance success rates and minimize risks, such as contamination with modern DNA (Hagelberg et al., 2015).

Advances in genome sequencing have led to the development of next-generation sequencing, also referred to as High Throuput Sequencing (NGS/HTS) technologies, that encompasses a collection of technologies that employ massively parallel sequencing approaches, producing millions of short-read sequences in a much shorter time with higher throughput compared to Sanger sequencing, which has vastly increased the amount of information that can be retrieved from archaeological and paleontological samples (Spyrou et al., 2019). This field, termed paleogenomics by Poinar et. al., (2006), offers greater sensitivity and precision to paleogenetic analyses due to the substantial number of reads generated by the sequencer. Additionally, it employs software capable of analyzing the degradation of the sample and potentially correcting substitutions, thereby ensuring the antiquity and authenticity of the sample results (Jónsson et al., 2013).

Due to these advancements, studies employing paleogenomic methodologies have gained significant power, introducing a substantial volume of information about the characteristics of past populations. These studies facilitate the establishment of familial relationships (Ning et al., 2021), aid in the reconstruction of paleoenvironmental conditions (Lentz et al., 2021; Moore et al., 2020), and recover paleoparasitological information (Chessa et al., 2020; Roche et al., 2020; Wiscovitch-Russo et al., 2020). Owing to the sensitivity of the technique, researchers have successfully recovered aDNA even from objects (Schablitsky et al., 2019) and sediments near burials (Sarhan et al., 2021). Thus, it is evident from the literature that NGS has established itself as a frontier methodology in archaeology, being exceptionally informative for understanding health conditions and interactions between humans and surrounding fauna.

Considering the high performance of paleogenomic techniques in archaeological samples compared to classical genetic sequencing methodologies applied to paleogenetics, the present study aimed to recover paleogenomic sequences from an archaeological site characterized by low taphonomic preservation. This endeavor sought to demonstrate the viability of these techniques in archaeological sites located in tropical regions, which are typically unfavorable for the preservation of genetic material.

METHODOLOGY

The archaeological site of *Malhada* (MA), located in the municipality of Cabo Frio (RJ), is a coastal site covering an area of 2400m^2 and reaching an elevation of 5.2m, with an antiquity ranging from 4020 to 710 years BP. Known since 1962, its excavation commenced in 1979 through various expeditions conducted by the Brazilian Institute of Archaeology (IAB) (Dias, 1992). Research at the *Malhada* archaeological site was driven by the significant quantity of animal bones identified as *Cavia sp*. (préa), suggesting the hypothesis of domestication of these rodents. Although this archaeological site features numerous human and animal burials, its location in a swamp (former paleolagoon) has contributed to the taphonomic degradation of the individuals recovered there. Such aDNA degradation was suggested in previous paleogenetic studies from our group, where PCR plus Sanger techniques were applied to identify *Trypanosoma cruzi* in the individuals from the archaeological site resulting in weak amplifications, nonspecific electrophoresis bands, and low-quality DNA sequences.

These samples were collected in collaboration with the archaeologists from the IAB responsible for the archaeological excavation. To prevent contamination during sample handling and cross-contamination between samples, paleogenetic collection protocols (Iñiguez, 2014) were strictly followed. These samples were then stored in the Paleoteca of the Paleogenetics at the *Laboratory from the Laboratório de Parasitologia Integrativa e Paleoparasitologia* (LPIP/IOC/Fiocruz) at -20ºC until the completion of paleogenetic and paleogenomic analyses. This laboratory maintains strict access control, allowing entry only to authorized personnel equipped with full personal protective equipment throughout their time in the facility. Furthermore, it is physically separated from other laboratories that process modern DNA and PCR products.

PALEOGENOMIC ANALYSIS

Dental samples from eight individuals were selected for paleogenomic analysis. These samples were pulverized using an analytical mill (Quimis Q298A) and subsequently subjected to aDNA extraction using the commercial DNA Investigator kit (Qiagen®) with specific modifications tailored for aDNA adaptation. Due to the low concentration observed following the aDNA extraction, the commercial kit GE Illustra GenomiPhi V2 was employed, for the reconstruction and amplification of the aDNA. PCR (Go*Taq*® G2 Hot Start *Taq* Polymerase – PROMEGA) was conducted to amplify the 18S rDNA and HVS-I mtDNA targets. For the 18S rDNA region, a final volume of 25µL, containing 1X of Buffer, 2.5mM of $MgCl_2$, 0.2mM of DNTP, 2.5U of Platinum *Taq* (Promega), 100ng of each primer (Kounosu et al., 2019), and 5µL (10~50ng/µL) concentration of template aDNA. For the amplification, the following cycles were applied: a cycle of 94ºC for 2 minutes, 50 cycles of 30 seconds at 94ºC, 30 seconds at 44ºC, and 30 seconds at 72ºC with an extension of 5 minutes at 72ºC. As for the amplification of the HVS-I mtDNA region, a final volume of 50µL, containing 1X of Buffer, 2.5mM of MgCl2, 0.2mM of DNTP, 2U of Platinum *Taq* (Promega), 100ng of each primer (Handt et al., 1996; Jaeger et al., 2012), and 5µL (10~50ng/µL) of template aDNA. As for the amplification, the following cycles were applied: a cycle of 94ºC for 3 minutes, 40 cycles of 30 seconds at 94ºC, 30 seconds at 55ºC, and 30 seconds at 72ºC with an extension of 5 minutes at 72ºC. A mixture of PCR amplicons was submitted to HTS in the MiSeq System facility (Illumina) located at the National Cancer Institute – RJ/Brazil. Between 20-50ng of aDNA mass per sample were used with Illumina's TruSeq® Nano DNA Library Prep kit, following a protocol adapted for aDNA study. There was no fragmentation of the DNA, as the aDNA is already naturally fragmented. Sequencing was done on the MiSeq System (Illumina) sequencer using the MiSeq Reagent Nano Kit v2 500-cycle kit.

BIOINFORMATIC ANALYSIS

The quality control of the reads was performed using a script customized by the Fiocruz Bioinformatics Platform, employing AdapterRemovalV2 and FastQC. For this stage, a minimum quality score of 25 in Phred score was required. Ends that did not meet these parameters were truncated until the criteria were satisfied and the counting of remaining reads was conducted using SeqKit. The obtained sequences were used as query for BLASTn (Camacho et al., 2009) software to align sequences against a custom-built reference database comprised of all 18S rDNA sequences available in the GenBank.

RESULTS AND DISCUSSION

All eight human archaeological samples submitted to HTS sequencing yielded the total number of reads necessary to infer the success of the sequencing, even in the absence of alignment against any database, presenting approximately 1.4 million reads in total distributed among the samples (Table 1). Once processed through the quality analysis pipeline, 1.2 million (~85,71%) reads were deemed of sufficient quality for subsequent analyses, and an even lower number was successfully aligned with the custom-built reference database (Table 1).

As a result, the majority (~99%) of the sequences were characterized as belonging to the kingdom Animalia. Among those, sequences related to the phylum Chordata were excluded from this analysis as they represent human DNA (endogenous aDNA and potential contamination with modern DNA), consistent with findings from our previous paleogenetic studies using Sanger sequencing. However, the application of high-throughput methodologies in this study demonstrated the capacity to overcome these limitations and identify previously unobtainable sequences (Table 2).

TABLE 1. Results obtained from HTS and bioinformatic processing from biological samples of *Malhada* archaeological site.

Sample ID	Total reads	Processed reads	18S rDNA hits
MA02	200.971	132.195	22.377
MA03	156.821	121.060	105.852
MA14	251.077	119.609	15.561
MA35	151.084	128.871	64.940
MA55	239.874	141.701	52.339
MA56	169.089	152.169	58.189
MA69	248.196	145.433	53.727
MA111	51.727	43.826	14.920

Source: Author´s elaboration.

TABLE 2. Proportion of non-human reads obtained from samples of the *Malhada* archaeological site.

Sample ID	Animal*	Bacteria	Plant	Fungi	Protist
MA02	0%	46,15%	23,08%	15,38%	15,38%
MA03	0%	93,10%	5,18%	0%	1,72%
MA14	0%	41,67%	50,00%	8,33%	0%
MA35	5,26%	42,10%	36,84%	15,79%	0%
MA55	11,11%	0%	66,67%	0%	22,22%
MA56	4,16%	41,67%	37,5%	16,67%	0%
MA69	2,32%	18,60%	9,30%	9,30%	60,46%
MA111	40,00%	10,00%	50,00%	0%	0%
**Human DNA sequences were omitted.*					

Source: Author´s elaboration.

These results, when compared to the context of Sanger sequencing applications, underscore the exploratory potential of high-throughput sequencing data analysis. The substantial volume of information allows for the recovery of endogenous aDNA, even from samples originating in contexts of low preservation, as evidenced by the archaeological site of *Malhada*. Future directions for this research include the construction of reference databases for alignment with the observed reads to further characterize into species level, which could contribute to understanding dietary habits, parasitic relations, and even the paleoenvironment in which those individuals once inhabited, as aligned with the study hypothesis. This will also enable us to determine the extent to which this volume is represented by endogenous aDNA or contamination and to authenticate their antiquity using tools such as MapDamage2.0 (Jónsson et al., 2013).

CONCLUSIONS

When compared to the context of Sanger sequencing applications, these results underscore the exploratory potential of high-throughput sequencing data analysis. The substantial volume of information allows for the recovery of endogenous aDNA, even from samples originating in contexts of unfavorable preservation conditions, as evidenced by the archaeological site of *Malhada*. These advances pave the way for detailed investigations into dietary habits, parasitic relationships, and paleoenvironmental reconstruction, along with integration with the usage of tools such as MapDamage2.0, which further reinforces the authenticity and antiquity of the results. In subsequent studies, comparative analyses of the aDNA reads identified at this archaeological site and those from other archaeological sites with varying taphonomic preservation conditions will be conducted. It is also important to emphasize that the presented results are preliminary and will undergo a revalidation process upon the accomplishment of each methodological stage outlined in these perspectives.

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Challenges of a paleogenomic approach to study pediculosis in chilean mummies
Desafíos de un enfoque paleogenómico para estudiar pediculosis en momias chilenas

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Abstract

Paleoparasitology and archaeology have revealed high rates of pediculosis in mummies from various cultures around the world. The louse *Pediculus humanus* is an ectoparasite that affects the genus *Homo*, and its evolutionary history reflects human mobility over time. Currently, *P. humanus* are genetically divided into six groups (haplogroups), labelled with the letters A to F. In this study, the application of high-throughput sequencing (HTS) methodology was evaluated for the genetic analysis of head lice from Chilean mummies. The metabarcoding approach using HTS was employed to analyze the mitochondrial ribosomal gene 12S rDNA of *Pediculus humanus* to identify the existence of multiple lice haplotypes in the same individual. The bioinformatics pipelines used to analyze the data generated by high-throughput sequencing are capable of filtering and tolerating a minimal number of errors in the sequences. However, as noted in the literature, the presence of a single SNP in a *Pediculus humanus* sequence leads to its consideration as a new haplotype. Therefore, the challenge of the analysis lies in the fact that, when dealing with haplotypes not deposited in the reference database, there is difficulty in distinguishing, in the results generated by HTS, those sequences that contain errors from those that have true SNPs and are new haplotypes.

Keywords: Head lice, Archaeology, Phylogenetics

Resumen

La paleoparasitologia y la arqueología han revelado altas tasas de pediculosis en momias de diversas culturas en todo el mundo. El piojo *Pediculus humanus* es un ectoparásito que afecta al género *Homo*, y su historia evolutiva refleja la movilidad humana a lo largo del tiempo. Actualmente, *P. humanus* está geneticamente dividido en seis grupos (haplogrupos), designados con las letras A a F. En este estudio, se evaluó la aplicacion de la metodología de secuenciación de alto rendimiento (HTS) para el análisis genético de piojos de la cabeza extraídos de momias chilenas. Se empleó el enfoque de metabarcoding, mediante HTS para analizar el gen ribosomal mitocondrial 12S rDNA de *Pediculus humanus* con el fin de identificar la existencia de multiples haplotipos de piojos en un mismo individuo. Los pipelines de bioinformatica utilizados para analizar los datos generados por la secuenciacion de alto rendimiento son capaces de filtrar y tolerar un número mínimo de errores en las secuencias. Sin embargo, como se ha señalado en la literatura, la presencia de un solo SNP en una secuencia de *Pediculus humanus* lleva a su consideración como un nuevo haplotipo. Por lo tanto, el desafio del análisis radica en que, al tratarse de haplotipos no depositados en la base de datos de referencia, generados por HTS, existe dificultad para distinguir aquellas secuencias que contienen errores con aquellas que tienen SNP verdaderos y son nuevos haplotipos.

Palabras clave: Piojos, Arqueología, Filogenética

INTRODUCTION

Pediculosis is a disease caused by the ectoparasite *Pediculus humanus* (the head and body louse). Archaeological and Paleoparasitological studies have revealed high rates of pediculosis in mummies from different cultures around the world. Arriaza et. at., (2012; 2014; 2022), in the context of the pre-Columbian Andean region, have investigated the presence of lice infestations in different human groups that occupied northern Chile. Reinhard and Buikstra, (2003) found variations in head lice infestation rates when analyzing mummies from different archaeological sites related to the Chiribaya Culture in southern Peru. According to the authors, differences in infestation rates may indicate that the site with the highest frequency of lice infestation was occupied by a larger number of individuals, which facilitated the transmission of the parasite.

Currently, two ecotypes of the human louse are known, *P. h. capitis* and *P. h. humanus* (Amanzougaghene et al., 2019; Boumbanda-Koyo et al., 2020). These two ecotypes are genetically subdivided into 6 haplogroups called A to F with varied geographic distributions (Amanzougaghene et al., 2020).

Classically, genotyping of *P. humanus* is performed using the Sanger sequencing method, and the *cyt*b, 12S rDNA, and cox1 markers. In the literature new lice haplotypes are described from the detection of 1 single nucleotide polymorphism (SNP) (Amanzougaghene et al., 2016; 2019; 2020; 2022; Boumbanda-Koyo et al., 2020; Kamani et al., 2023). Haplogroup A, the cosmopolitan and well-established, and B have already been reported in nits collected from the same Chilean mummy using the PCR-sanger sequencing method (Boutellis et al., 2013).

In this study, the application of High-Throughput Sequencing (HTS/NGS), a more sensitive technique than Sanger sequencing, aims to contribute to the discrimination of *P. humanus* haplotypes and identify the existence of multiple haplotypes of *P. humanus* infesting the same individuals, indicating human contact in places with high population density and consequent transmission of the ectoparasite. In recent years, the application of the HTS has been more frequent, including in the publication of studies on the detection of bacteria of which *P. humanus* may be a vector (Abd Majid et al., 2020; Bai et al., 2023).

METHODOLOGY

All samples (Figure 1) were transported to Brazil under permit No 339/2017 granted by the Chilean National Council of Monuments.

The samples were stored in the Paleoteca collection (-20°C) of the Laboratory of Paleogenetics (LP), part of the Laboratory of Integrative Parasitology and Paleoparasitology (LPIP/IOC/FIOCRUZ), until the paleogenetic and paleogenomic analysis was conducted.

The samples (Figure 1) analyzed were collected from mummies from six archaeological sites located in the Arica and Parinacota region and the Tarapacá region in the Atacama Desert, northern Chile. Quiani-7 (18º32'S 70º19'W) is the oldest archaeological site included in the study. It is part of the Archaic Period of northern Chile, dating to approximately 3590-3050 BP (Dauelsberg H., 1974; Muñoz et al., 2022). Tarapacá-40A (19º58'40''S 69º33'52''W) site belongs to the Initial Formative Period of northern Chile (2950-1450 BP) and is a sector of the cemetery located in the northern part of the Tarapacá ravine, Atacama Desert, Chile, which preserves over 100 intact tombs and is situated 65 km from the Pacific Ocean. It is interpreted that its burials belong to the ancient inhabitants of the neighboring archaeological site Caserones-1 (Uribe et al., 2015). Among the known archaeological sites in the Azapa Valley (18º35'S 69º30'W) are Azapa-71, Azapa-6, and Azapa-140 (Rothhammer and Santoro, 2001). According to Rothhammer and Santoro (2001), the occupants of Azapa-71 were the first of the three sites to settle and left material traces of occupation dating back to three chronological periods in northern Chile: Late Archaic, Formative, and Middle (3000 BP-700 BP). Near the mouth of the Camarones River in the Pacific Ocean (19° 11' 8.024" S, 70° 16' 25.198" W) is the archaeological site known as Camarones-9. It is a cemetery associated with the Inca Period (Late Period, 580–350 BP), which also has evidence of Hispanic contact, located approximately 100 km from Arica, Chile (Arriaza et al., 2022; Arriaza et al., 2022)

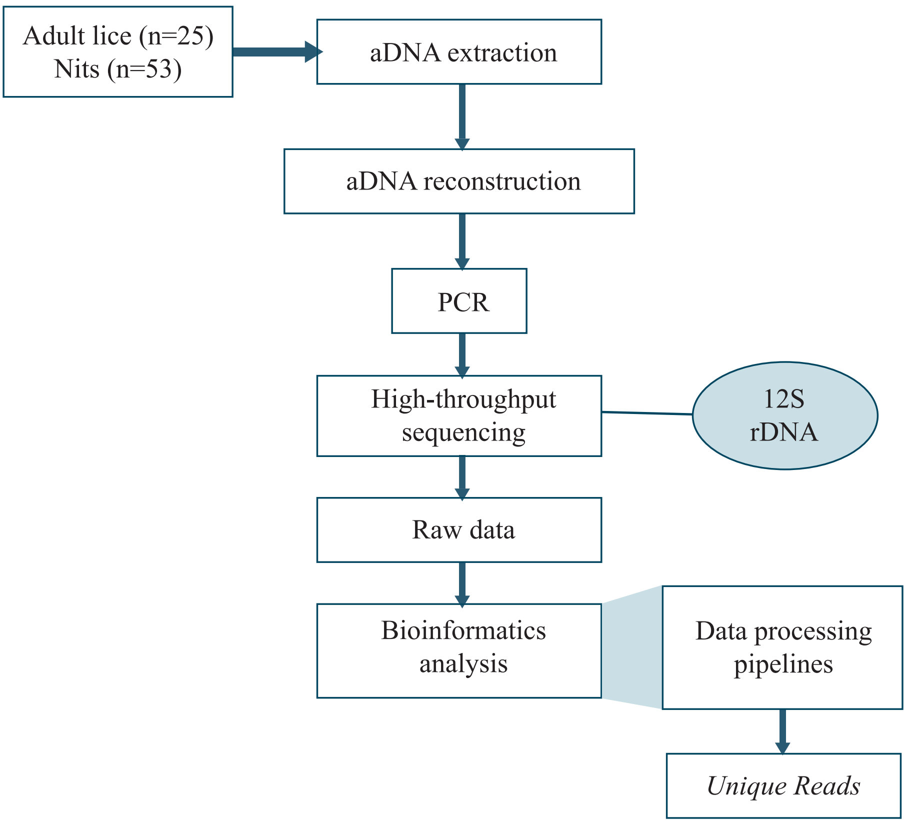

FIGURA 1. Methodological flow diagram of the application of the metabarcoding approach in High-throughput sequencing.

Source: Author´s elaboration.

PALEOGENETIC ANALYSIS

The analysis of the samples (Figure 1) was carried out at LP/LPIP/IOC, a restricted space for aDNA research, where no work had previously been done on this ectoparasite. All protocols were followed to avoid contamination by contemporary DNA and ensure the authenticity of aDNA results (Iñiguez, 2014; 2021). The specimens were counted and weighed. The aDNA from all samples was extracted using the commercial kit Dneasy® Plant Mini Kit (Qiagen) with some modifications established in the aDNA protocol. The modifications include a mechanical digestion step performed before the application of the commercial extraction kit, where 100µl of sterile MilliQ H2O was added to the samples, which were then submerged in liquid nitrogen and homogenized. Subsequently, the samples underwent chemical digestion, treated with Buffer AP1 (Tissue Lysis Buffer - Dneasy® Plant Mini Kit Qiagen), 0.5 mM EDTA (Invitrogen-©Thermo Fisher Scientific Corporation), and 20mg/ml Proteinase K (Invitrogen-©Thermo Fisher Scientific Corporation) at 65°C for 24 to 72 consecutive hours with agitation.

The Reconstructive Polymerization (Golenberg et al., 1996; Iñiguez et al., 2003), a pre-treatment for aDNA that rebuilds and amplifies it, was applied.

The detection of ectoparasite aDNA was conducted using the mitochondrial molecular target 12S rDNA (110 bp) as described in the literature (Amanzougaghene et al., 2016). For the detection of the molecular target, PCR reactions with a final volume of 25µl were carried out with 1X Buffer, 3.0mM MgCl2, 0.2mM dNTP, 10µM of each primer, 1U Go*Taq* G2 Hot Start (Promega), and 5µl aDNA (0.5ng-8ng). The cycling was 94°C for 10 minutes, and 45 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C with an extension of 7 minutes at 72°C.

High-throughput sequencing was conducted at the Illumina MiSeq platform facility of Instituto Nacional de Câncer – INCA, Rio de Janeiro. In the metabarcoding approach, PCR-amplified fragments are subjected to HTS that generates a substantial amount of data with specific targets within the genome.

The dataset of raw reads (aDNA sequences) obtained was processed using AdapterRemoval v2, for removing low-quality reads, short reads, and adapters, as well as removing duplicate sequences using SeqKit. Processed reads were compared with a custom-built reference database of *P. humanus* sequences of the mitochondrial gene 12S rDNA using Blastn.

RESULTS AND DISCUSSION

The application of High-throughput sequencing has contributed to genetic analysis by enabling the generation of massive amounts of DNA sequence data. This technology has been particularly effective in identifying known haplotypes that are already cataloged in databases such as the international GenBank. However, the detection of novel haplotypes, especially those distinguished by one or two nucleotide polymorphisms (SNPs) within *Pediculus humanus* sequences, poses significant methodological challenges within the HTS methodology.

One of the primary challenges arises from the potential for sequencing errors inherent in HTS data. These errors can arise from sources such as PCR sequencing artifacts. Consequently, distinguishing between genuine SNPs and sequencing artifacts becomes difficult, particularly when attempting to identify novel haplotypes.

In this study, through the analysis of data generated by HTS, it was possible to identify known haplotypes of *Pediculus humanus* and even the presence of more than one haplotype of the clade A in a single individual from an archaeological site with evidence of a high flow of people. Furthermore, HTS produced reads that contain single nucleotide polymorphisms (SNPs) suggestive of novel A haplotypes. However, it was not possible to determine whether these SNPs were genuine or the result of sequencing errors.

Despite its limitations in haplotype identification, HTS remains a valuable tool due to its capacity to generate large amounts of sequence data from one single sample, especially with archaeological remains that are fragile and limited materials. However, when the objective is to detect new haplotypes that may not be circulating in current molecular epidemiology, Sanger sequencing continues to be a more efficient method.

CONCLUSION

This study underscores the potential and limitations of high-throughput sequencing (HTS) in the genetic analysis of *Pediculus humanus*, particularly in contexts involving fragile and limited samples. HTS proved effective in identifying known haplotypes and detecting different haplotype lice within a single host. To face the HTS data analysis challenges concerning lice haplotype identification, innovative and improved methodological approaches are essential. This includes advancements in bioinformatic algorithms for error correction and a deeper understanding of the limitations and biases inherent in HTS data. These efforts are crucial for overcoming bioinformatic barriers and enhancing the accuracy and reliability of haplotype identification using high-throughput sequencing technologies.

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Historical overview of Chagas disease and its current situation in Bolivia
Reseña histórica de la enfermedad de Chagas y su situación actual en Bolivia

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Abstract

Chagas disease, also known as American trypanosomiasis, is a parasitic infection caused by the protozoan *Trypanosoma cruzi*. Discovered in 1909 by Brazilian researcher Dr. Carlos das Chagas. Over time, the disease has become widespread, particularly in Bolivia's rural areas, where living conditions favor its transmission. The first human case in Bolivia was diagnosed in 1916 by Dr. Rafael Torrico Montaño. In 1945, Bolivia created the National Service to Fight Chagas Disease (SNELCH) to combat the spread. In 1946, the first ophthalmoganglionic case was reported in a 14-year-old girl from Cochabamba, and by 1950, *Triatoma infestans* (vinchuca), the main vector, was found in the departments of Cochabamba, Santa Cruz, and Chuquisaca with alarmingly high infection rates. In 1986, Bolivia launched its First Regional Program for Comprehensive Control of Chagas Disease, which significantly expanded the country's efforts to reduce transmission. During the period 2000-2010, the "Chagas Initiative" further strengthened control measures across Latin America. Chagas disease remains present throughout Bolivia, with the highest incidence in the departments of Santa Cruz, Cochabamba, Tarija, and Chuquisaca. In 2010, the World Health Organization (WHO) certified the department of Tarija as free from vector transmission. Nevertheless, vector-borne transmission continues to be a major problem, especially in rural areas with precarious conditions. Bolivia has set an ambitious goal of eliminating vector-borne transmission by 2030 through strategies such as indoor fumigation, housing improvements, and health education campaigns.

Key words: Chagas, Historical Review, *Trypanosoma cruzi*

Resumen

La enfermedad de Chagas, también conocida como tripanosomiasis americana, es una infección parasitaria causada por el protozoo *Trypanosoma cruzi*. Fue descubierta en 1909 por el investigador brasileño Dr. Carlos das Chagas. La enfermedad se ha extendido en las zonas rurales de Bolivia, donde las condiciones de vida favorecen su transmisión. El primer caso humano en Bolivia fue diagnosticado en 1916 por el Dr. Rafael Torrico Montaño. En 1945, Bolivia creó el Servicio Nacional de Lucha contra la Enfermedad de Chagas (SNELCH). En 1946, se reportó el primer caso oftalmoganglionar en una niña de 14 años de Cochabamba. En 1950, *Triatoma infestans* (vinchuca), el principal vector, se encontraba en los departamentos de Cochabamba, Santa Cruz y Chuquisaca con tasas de infección alarmantemente altas. En 1986, Bolivia lanzó su Primer Programa Regional de Control Integral de la Enfermedad de Chagas, lo que amplió significativamente los esfuerzos del país para reducir la transmisión. Durante el período 2000-2010, la "Iniciativa Chagas" reforzó aún más las medidas de control en toda América Latina. La enfermedad de Chagas sigue presente en toda Bolivia, con la mayor incidencia en los departamentos de Santa Cruz, Cochabamba, Tarija y Chuquisaca. En 2010, la (OMS) certificó al departamento de Tarija como libre de transmisión vectorial. La transmisión vectorial sigue siendo un problema importante, especialmente en las zonas rurales con condiciones precarias. Bolivia se ha fijado el objetivo de eliminar la transmisión vectorial para 2030, mediante estrategias como la fumigación en interiores, la mejora de viviendas y campañas de educación sanitaria.

Palabras Clave: Chagas, reseña histórica, *Trypanosoma cruzi*

INTRODUCTION

Chagas disease, also known as American trypanosomiasis, is a neglected tropical disease with significant public health implications in Latin America. Caused by the protozoan *Trypanosoma cruzi*, the disease is primarily transmitted through the bite of triatomine insects, commonly referred to as "kissing bugs" (vinchucas). These insects are predominantly found in rural areas, where precarious housing conditions facilitate their reproduction and proximity to humans. The disease has two phases: an acute phase, which is often asymptomatic or presents mild symptoms, and a chronic phase, which can develop years after infection, leading to serious complications, particularly affecting the heart and digestive system. If left untreated, chronic Chagas disease can result in life-threatening conditions such as cardiomyopathy, heart failure, and digestive disorders, including megacolon and megaesophagus.

Historically, Chagas disease has been a significant challenge in Bolivia, where it continues to affect large segments of the population. The disease was first documented in the country in the early 20th century, and since then, it has become one of Bolivia's most pressing public the most affected, where traditional mud and thatch houses provide ideal breeding grounds for the triatomine vectors.

Over the years, Bolivia has made significant strides in combating Chagas disease. National and international efforts have been mobilized to control the spread of the disease, primarily through vector control programs. The government, in collaboration with international organizations like the Pan American Health Organization (PAHO) and the World Health Organization (WHO), has implemented large-scale fumigation campaigns aimed at reducing the population of *Triatoma infestans,* the main vector responsible for transmitting *T. cruzi*. Bolivia has one of the highest rates of Chagas disease in the world, with some regions reporting infection rates as high as 6-8% of the population in rural communities, particularly in the departments of Cochabamba, Santa Cruz, Tarija, and Chuquisaca. The efforts for the control of Chagas`disease have resulted in notable reductions in vector transmission in certain areas, and in 2010, Bolivia received certification from the WHO for having interrupted vector transmission in the department of Tarija (WHO, 2010).

Despite these achievements, the complete eradication of Chagas disease remains elusive. The geographic distribution of the triatomine insects, combined with Bolivia's vast and remote rural regions, poses significant logistical challenges for maintaining consistent control programs. Many communities are located in difficult-to-access areas, where healthcare infrastructure is limited, and regular fumigation and surveillance are challenging to sustain. Moreover, economic and social factors, such as poverty and migration, further complicate eradication efforts, as populations move from rural to urban areas, potentially spreading the disease.

In addition to vector transmission, other forms of transmission, including congenital transmission from mother to child and transmission through blood transfusions, also contribute to the persistence of Chagas disease in Bolivia. While screening of blood donations has greatly reduced transmission through transfusions, congenital transmission remains a significant concern. Bolivia has one of the highest rates of congenital Chagas in Latin America, underscoring the need for improved prenatal screening and early treatment programs.

The goal of this essay is to provide a comprehensive review of the historical evolution of Chagas disease in Bolivia, examining the progress made in controlling the disease as well as the ongoing challenges that hinder its eradication. The discussion will cover the historical context of the disease, including its discovery and spread, as well as the major public health strategies implemented to combat it. Furthermore, the essay will analyze the current epidemiological situation in Bolivia, focusing on the factors that contribute to the persistence of the disease, such as geographic isolation, poverty, and inadequate healthcare infrastructure. By exploring both the successes and limitations of existing programs, the essay aims to offer insights into the future prospects for Chagas disease control in Bolivia, highlighting the importance of sustained efforts in vector control, public health education, and improved access to healthcare services.

While Bolivia has made significant progress in reducing Chagas disease transmission in certain regions, the fight against the disease is far from over. The challenges posed by the geographic, economic, and social conditions of the country require a multifaceted and sustained approach to control and eventually eliminate the disease. As Bolivia continues to work towards the goal of eliminating vector-borne transmission by 2030, it is crucial to address the underlying factors that contribute to the persistence of Chagas disease in vulnerable populations, ensuring that future generations are protected from this debilitating condition.

ARGUMENT DEVELOPMENT

Bolivia has one of the highest prevalences of Chagas disease worldwide, with approximately 6% of the population infected by *Trypanosoma cruzi,* according to the World Health Organization (WHO). The disease mainly affects rural areas where poor living conditions provide ideal habitats for the triatomine insects that transmit the parasite, particularly in houses made of adobe and thatch.

Historically, Bolivia has made significant progress in diagnosing, treating, and controlling the disease through public health campaigns and vector control measures. However, several challenges persist that complicate total eradication. One key issue is the reinfestation of homes following fumigation efforts, particularly in areas where housing conditions have not been structurally improved. Many families cannot afford the necessary modifications, limiting the long-term success of fumigation programs.

Another major challenge is congenital transmission, where the parasite is passed from mother to child during pregnancy. Although there have been advances in diagnosing and treating infected mothers, access to prenatal care remains limited in rural and isolated regions, allowing the disease to persist across generations. Additionally, alternative transmission routes, such as through blood transfusions and oral ingestion of contaminated food, continue to be concerns in certain areas.

Despite progress, the eradication of Chagas disease in Bolivia is hindered by socioeconomic disparities, geographic isolation, and the persistence of alternative transmission routes. A more comprehensive and integrated approach is needed to address these broader challenges, including improvements in healthcare and housing infrastructure in the most affected areas.

VECTORS OF CHAGAS DISEASE IN BOLIVIA

Bolivia is recognized as the origin of *Triatoma infestans*, the primary vector of Chagas disease, commonly known as "vinchuca." This species is the most significant in transmitting *Trypanosoma cruzi*, the parasite responsible for the disease, primarily in rural areas of Bolivia. Although other vectors like *Triatoma sordida* and *Rhodnius prolixus* are also found in the country, *T. infestans* remains the most efficient vector. Its presence in both domestic and wild environments, especially in the Cochabamba region where it is associated with rodents, complicates eradication efforts (Noireau, 1999; Alfred et al., 1999).

The article also points out that vector transmission is the main route of infection in Bolivia, especially in rural areas. However, other transmission routes, such as congenital transmission, blood transfusions, and ingestion of contaminated food, also play a significant role and should be addressed in disease control efforts. While controlling *T. infestans* is key, a comprehensive approach that includes these other transmission pathways is necessary to effectively combat Chagas disease in Bolivia.

RESERVOIRS OF CHAGAS DISEASE IN BOLIVIA

In Bolivia, *Trypanosoma cruzi,* the causative agent of Chagas disease, infects a broad range of animal reservoirs, playing a critical role in sustaining the transmission cycle. These reservoirs include both domestic and wild mammals, which serve as hosts for triatomine vectors that transmit the parasite to humans.

Among domestic mammals, dogs and cats are key reservoirs in rural areas, with infection rates reaching up to 100% in dogs and 60% in cats. Guinea pigs raised inside homes in highland regions exhibit infection rates of up to 20%. Other domestic animals, such as rats (*Rattus norvegicus* and *Rattus rattus*), are significant due to their high population density and interaction with both humans and triatomines. Livestock like cattle, goats, pigs, and horses show lower infection rates due to less frequent contact with humans (Noireau, F., 1999).

Wild mammals also play an essential role in the maintenance of *T. cruzi*. Marsupials (*Didelphis marsupialis*) and armadillos (*Dasypus novemcinctus*) are notable for their high parasitemia, with infection rates reaching up to 50% in some areas. Other important wild reservoirs include rodents (*Galea musteloides*), monkeys (*Saimiri sciureus*), and bats, all of which help maintain the enzootic cycle of the parasite, particularly in forested and rural environments (Noireau, F., 1997, Teixeira et al., 2001).

These diverse animal reservoirs underscore the complexity of controlling *T. cruzi* transmission in Bolivia, as both domestic and wild species contribute significantly to the ongoing spread of Chagas disease.

Species reported in Bolivia: Infections have been identified in both domestic and wild animals, including marsupials,

A scientific essay on *Trypanosoma cruzi* reservoirs in Bolivia should focus on the diverse range of animal species that harbor the parasite and their role in the transmission cycle. armadillos, sloths, porcupines, monkeys, dogs, cats, and various species of rats in regions like the Yungas and Santa Cruz. These animals maintain the parasite in both sylvatic (wild) and peridomestic cycles, contributing to the persistence of Chagas disease in rural and forested areas (Noireau, 1999).

However, the sheer diversity of species complicates efforts to control these reservoirs. Targeting wild animals, such as armadillos and marsupials, which live in remote, forested regions, presents logistical and economic challenges. Domestic animals like dogs and cats, while commonly infected, may not be the most efficient focus for controlling the disease, as vector-borne transmission through triatomine insects is the primary route of human infection, rather than direct animal contact.

Furthermore, the sylvatic cycle, sustained by wild reservoirs, does not necessarily increase human infection risk in areas where vector control has been effective. Therefore, prioritizing human health interventions such as improving housing conditions, vector control, and public health education may be a more efficient and cost-effective approach. Focusing too heavily on controlling animal reservoirs may divert resources from these more impactful strategies for reducing human exposure to Chagas disease in Bolivia.

BACKGROUND OF THE DISEASE IN BOLIVIA

Chagas disease has likely affected Bolivia since pre-Columbian times, although its identification only became possible in the 20th century (Orellana-Halkyer, 2008). Early records by colonial chroniclers describe the living conditions of indigenous populations, but without understanding the involvement of *Trypanosoma cruzi*. Studies on ancient Andean mummies confirmed the long-standing presence of this parasite, demonstrating the disease's deep historical roots (Aufderheide et al., 2004).

The vector, *Triatoma infestans* (vinchuca), once fed on large mammals during the Cretaceous period but adapted to human hosts over time (Briones et al., 1999; Orellana-Halkyer and Telleria, 2021). The parasite was officially discovered by Dr. Carlos Chagas in Brazil in 1909, and in 1916, the first human case was diagnosed in Bolivia by Dr. Rafael Torrico. Significant progress occurred during the mid-20th century, particularly with the identification of clinical signs, such as the Romaña sign, that advanced early-stage detection of the disease (Torrico and Angel, 1950).

By the 1950s, researchers like Dr. Julio Rodríguez Rivas documented Chagas cardiomyopathy, marking a key moment in the country's understanding of the disease's severe chronic phase. With widespread vector control campaigns, including DDT fumigation, the prevalence of Chagas reduced, but the disease remained a major public health issue, with congenital transmission also emerging as a concern (Rivas, 1961).

Chagas disease continues to be a challenge in Bolivia, with the acute and chronic phases manifesting in a range of severe health complications, particularly heart failure and digestive system disorders. Although historical evidence places the parasite in pre-Columbian societies, the major impact of Chagas disease as a public health problem only gained recognition with modern scientific advancements and continues to be an area of focus for disease control efforts in Bolivia.

CONGENITAL CHAGAS

Congenital transmission of Chagas disease has become a major public health concern in Bolivia, especially in areas where vector control has been successful. Bolivia has one of the highest rates of congenital Chagas in Latin America, a reflection of the high seropositivity in pregnant women, sociocultural factors, and limited healthcare access. The disease, traditionally transmitted by *Triatoma* insects (vinchucas), can also pass from mother to child during pregnancy, which has emerged as a critical transmission route in endemic regions (Schmunis and Yadon, 2010; WHO, 2020).

Key studies have shown high rates of congenital Chagas in Bolivia, particularly among low-birth-weight newborns in areas with high vector infestation. Research indicates that congenital transmission is the third most important mechanism for contracting *Trypanosoma cruzi,* after vectorial and transfusional routes. The 2011 diagnostic and treatment guidelines for congenital Chagas emphasize early detection and treatment in newborns, with placental histopathology and serological testing playing pivotal roles (Ministerio de Salud y Deportes de Bolivia, 2018).

The National Chagas Program has implemented large-scale screening programs, revealing infection rates in pregnant women between 23.6% and 42.2%, depending on the region. In Santa Cruz de la Sierra, 23.6% of pregnant women were infected, while in Yacuiba, the rate reached 42.2%, with a congenital transmission rate of 6%. The program's follow-up strategy includes monitoring newborns for up to 12 months to confirm infection, though challenges remain in rural areas due to limited healthcare infrastructure (Torrico et al., 2004).

The high prevalence of congenital Chagas is attributed to several factors, including historical deficiencies in vector control programs, poor healthcare access in rural regions, and cultural barriers. Many women of childbearing age were infected during childhood due to insufficient vector control, and without addressing these root causes, congenital transmission will continue. Moreover, the difficulty of implementing widespread prenatal care and using sensitive diagnostic tools like PCR in remote areas poses a challenge for reducing congenital transmission rates (Carlier et al., 2012).

While congenital Chagas is an important issue, some argue that focusing exclusively on it may detract from more effective strategies such as vector control, improving housing conditions, and public health education. Addressing the root causes of the disease—particularly by investing in long-term vector control—could prevent future generations of women from becoming infected, thus reducing the risk of congenital transmission over time (PAHO, 2019).

Tackling congenital Chagas requires a comprehensive approach that addresses the high seropositivity rates in women, improves healthcare access, and continues vector control efforts. Without addressing these underlying issues, Bolivia will struggle to reduce both congenital and vector-borne transmission of Chagas disease effectively (Hotez et al., 2008; Pinto Dias, 2009).

PARASITOLOGICAL AND SEROLOGICAL DIAGNOSIS OF CHAGAS DISEASE

The diagnosis of Chagas disease relies on both parasitological and serological methods, with distinct approaches used depending on the disease phase. In the acute phase, *Trypanosoma cruzi* tripomastigotes can be detected in peripheral blood using optical microscopy and Giemsa-stained smears. Concentration methods, such as the Strout and microhematocrit techniques, allow for the detection of parasites by examining the buffy coat after centrifugation. The microhematocrit method is preferred for diagnosing congenital infections. Other indirect parasitological techniques include xenodiagnosis, hemoculture, and animal inoculation, which amplify the parasite but are time-consuming and require skilled personnel to identify the parasites morphologically (Schenone et al., 1981; Chiari et al., 1989).

Despite the value of these traditional methods, they present significant limitations in modern clinical practice. Their time-consuming nature delays critical treatment, especially in congenital infections. Furthermore, the requirement for trained professionals and complex intermediate steps, such as using triatomine insects or animals, adds logistical and ethical concerns. Molecular methods, like polymerase chain reaction (PCR), are becoming preferred due to their higher sensitivity, faster results, and ability to detect *T. cruzi* DNA even in low parasitemia cases, making them a more reliable diagnostic tool (WHO, 2002; Schijman, 2007).

In the chronic phase, where parasitemia is low and intermittent, serological methods are more effective. Enzyme-linked immunosorbent assays (ELISA) are widely used due to their high sensitivity and specificity, often employing total parasite lysates, purified antigenic fractions, or recombinant proteins. However, due to the antigenic diversity of *T. cruzi,* diagnostic algorithms recommend the concordance of two tests with different antigenic principles, and if results are inconclusive, a third test is required. This reliance on multiple tests delays results and can lead to follow-up loss, particularly in resource-limited settings (de Rissio et al.,2010).

While rapid diagnostic tests (RDTs) based on lateral flow immunochromatographic detection offer advantages like quick results and minimal infrastructure requirements, they have shown significant performance variability across different regions. In Mexico, for example, some

RDTs exhibit poor sensitivity, which is attributed to the genetic and antigenic diversity of *T. cruzi* strains. The genetic complexity of *T. cruzi*—with many strains expressing a limited number of antigenic determinants—further complicates the development of universally effective diagnostic tools (Ramírez et al., 2013).

While traditional parasitological and serological methods are valuable in diagnosing Chagas disease, they present significant challenges in terms of time, accuracy, and accessibility. PCR offers a faster, more sensitive alternative for acute cases, while improvements in serological tests and RDTs are needed to account for the antigenic diversity of *T. cruzi*. A region-specific approach to diagnosis, integrating both molecular and serological tools, is crucial for improving detection and treatment of Chagas disease, particularly in low-resource and endemic areas (Smith and Perez, 2023)

MOLECULAR BIOLOGY OF *Trypanosoma cruzi* AND ADVANCES IN DIAGNOSIS

Trypanosoma cruzi, the causative agent of Chagas disease, is a flagellated protozoan discovered by Carlos Chagas in 1909. Since then, research has progressed significantly, leading to a deeper understanding of the parasite's molecular biology, particularly its genetic structure and immune evasion mechanisms. These advances have played a key role in improving diagnostic methods, facilitating early detection and treatment, especially in endemic regions (Rassi and Marin, 2010).

ADVANCES IN THE MOLECULAR BIOLOGY OF *T. cruzi*

Molecular techniques have enabled the characterization of the *T. cruzi* genome, revealing six discrete typing units (DTUs) known as TcI to TcVI, each varying in virulence and geographical distribution. This genetic diversity has allowed for the development of diagnostic methods that differentiate strains and assess their impact on disease manifestation (Zingales et al., 2012).

GENOMIC CHARACTERIZATION AND GENETIC MARKERS

A landmark study by Mesri et al. (1990) cloned and characterized specific *T. cruzi* antigens, providing insight into the structure and function of parasite proteins. This discovery facilitated the development of serological tests that distinguish between different clinical phases of Chagas disease, aiding in the differential diagnosis of acute and chronic phases (Mesri et al., 1990).

IMMUNE EVASION MECHANISMS

T. cruzi is adept at evading the host's immune system by modifying its antigenic surface. This ability allows the parasite to persist for extended periods, complicating treatment. Identifying genes responsible for these mechanisms has opened new therapeutic avenues aimed at neutralizing the parasite's immune evasion (Mesri et al., 1990).

MOLECULAR DIAGNOSTIC TECHNIQUES

Molecular biology has revolutionized Chagas disease diagnosis. Techniques such as polymerase chain reaction (PCR) and real-time PCR (qPCR) offer highly sensitive and specific detection of *T. cruzi* DNA, even in low-parasitemia stages. These methods are crucial for tracking disease progression and treatment efficacy (Saavedra, 2013).

POLYMERASE CHAIN REACTION (PCR) AND QPCR

PCR and qPCR are the gold standard for detecting *T. cruzi* in blood and tissue samples. Beyond detecting parasite DNA, these methods also quantify it, providing valuable insights into disease progression and the effectiveness of treatment (Monteón et al., 1994).

INNOVATIVE TECHNIQUES: LAMP AND NANOTECHNOLOGY

Loop-mediated isothermal amplification (LAMP) has emerged as a promising alternative for molecular diagnosis in resource-limited areas due to its simplicity and high sensitivity. Nanotechnology is also making strides, with nanoparticles being used to enhance the capture and detection of *T. cruzi* antigens (Carrada and Bravo, 2004).

IMPACT OF *Trypanosoma rangeli* ON THE DIAGNOSIS OF CHAGAS

The coexistence of *T. rangeli* and *T. cruzi* in endemic regions complicates diagnosis due to serological cross-reactivity. However, advances in in situ hybridization and specific PCR techniques have improved the ability to distinguish between the two species, thus enhancing diagnostic accuracy (Zingales et al., 2012).

Advances in the molecular biology of *T. cruzi* have led to significant improvements in diagnosing and managing Chagas disease. Continued research into genetic markers, immune evasion, and innovative diagnostic methods is essential for improving outcomes in both acute and chronic cases of the disease (Coura and Borges, 2010).

CHALLENGES AND FUTURE PERSPECTIVES

Despite the advances made in the diagnosis and understanding of the molecular biology of *T. cruzi*, challenges persist in the implementation of these techniques in endemic regions with limited resources. The high genetic variability of the parasite and the overlap with other species complicate the implementation of a universally effective diagnosis. Furthermore, it is necessary to develop more accessible and economical diagnostic methods that can be applied effectively in clinical contexts with limited capacities (Schijman et al., 2024).

FUMIGATION AND CONTROL OF CHAGAS DISEASE

In endemic areas, controlling Chagas disease primarily relies on fumigating homes with insecticides to eliminate the vector, *Triatoma infestans*. However, reinfestation remains a significant challenge, necessitating complementary strategies such as community-based entomological surveillance. Involving local communities in vector monitoring and training local leaders helps sustain preventive actions and enhances awareness about the disease and its transmission. This integrated approach strengthens local capacities, ensuring a rapid response to new infestations (Gürtler et al., 2007).

Over the years, Bolivia has implemented various vector control strategies, including mass fumigation programs that significantly reduced transmission in some areas, especially in cooler regions like the highlands. However, in warmer, rural areas, where triatomines thrive, reinfestation is more common. Fumigation, while effective, presents several challenges such as high costs, the need for constant application, and emerging insecticide resistance in some triatomine populations. Thus, it is not a long-term solution on its own (Dias et al., 2002).

Bolivia's Chagas Disease Control Program (PCEC) has been instrumental in reducing vector-borne transmission, but residual hotspots and reinfestations highlight the need for sustained fumigation and surveillance efforts. Additionally, housing improvement programs, such as the "Housing Improvement Plan" (PMV), aim to prevent infestation by constructing safer homes using materials less prone to harboring vectors. Involvement from indigenous communities and the inclusion of educational programs have been critical for the success of these interventions, promoting yard and home cleaning and controlling livestock enclosures (Estívarez, 2005).

Despite these efforts, socio-economic and cultural factors complicate the sustainability of Chagas control measures. Limited access to healthcare, migration, and poverty hinder long-term solutions. Thus, continuous surveillance, housing improvements, and community education remain essential strategies for controlling the disease. The involvement of regional initiatives, such as those promoted by the Pan American Health Organization (PAHO), focuses on vector control and preventing congenital transmission through serological screening of blood donors and pregnant women (Jimeno et al., 2021).

While molecular advances in diagnosing *Trypanosoma cruzi,* such as PCR and LAMP, have improved disease detection, their application in resource-limited settings is constrained by infrastructure and cost. Therefore, a holistic, community-based approach remains crucial for sustainable disease control, addressing the social determinants of health that perpetuate the transmission of Chagas disease (Besuschio et al., 2020).

CURRENT CONTROL OF CHAGAS DISEASE IN BOLIVIA

Since 2000, Bolivia has implemented the National Chagas Program with support from the Inter-American Development Bank (IDB) and the Pan American Health Organization (PAHO/WHO). This program has been instrumental in reducing the transmission of *Trypanosoma cruzi,* the parasite responsible for Chagas disease, especially in the Department of La Paz, where home infestation rates by *Triatoma infestans* dropped from 52% in 1999 to 1.3% in 2010. Seroprevalence among children aged 1 to 5 years also significantly declined from 15.6% in 1990 to 0.8% in 2009, demonstrating the success of vector control efforts (PAHO, 2010).

Bolivia's Chagas control program includes mass fumigation campaigns, particularly in high-prevalence areas such as Cochabamba, where vector infestation was once as high as 90%. These efforts reduced infestation rates to between 1.1% and 3.2%. Other interventions, such as housing improvements and education campaigns, have complemented fumigation, especially in rural areas where poverty and poor living conditions increase transmission risks. However, these measures face challenges in areas like Chacopampa-Tupiza, where close contact with domestic animals and inadequate housing infrastructure continue to facilitate vector transmission (Espinoza et al., 2014).

While La Paz has achieved certification for the interruption of vector transmission, other regions still struggle with high infection rates, particularly due to rural-urban migration and congenital transmission. In Cochabamba, urban areas face ongoing transmission, with seroprevalence reaching 25% in some children aged 5 to 13 years. Poor housing and inconsistent control efforts in urban slums exacerbate the issue (Medrano et al., 2008).

Despite the progress made, challenges remain. The heavy reliance on fumigation, though effective in the short term, does not address the deeper socioeconomic factors that enable the persistence of *T. cruzi*. Inadequate housing, poverty, and limited infrastructure improvements allow triatomine bugs to reinfest treated homes. Long-term success in controlling Chagas disease requires a more comprehensive strategy that includes sustained community engagement, housing improvements, and public education alongside vector control measures.

ERADICATION PROGRAMS FOR THE DISEASE

The Bolivian government, in collaboration with international organizations such as the Pan American Health Organization (PAHO), has implemented several programs aimed at eradicating Chagas disease. One of the main objectives of these programs has been the elimination of triatomines from rural homes through fumigation campaigns and the improvement of housing conditions.

The National Chagas Program in Bolivia has made significant progress in reducing vector transmission in some regions of the country. However, the complete eradication of the disease remains a challenge due to the geographic dispersion of triatomines and logistical difficulties in maintaining control programs in remote rural areas.

ELIMINATION OF CONTAMINATED BLOOD TRANSFUSION

Another important strategy in the fight against Chagas disease has been the elimination of transmission through blood transfusion. In the past, many people became infected with *Trypanosoma cruzi* by receiving contaminated blood. However, the introduction of screening tests in blood banks has drastically reduced this risk in Bolivia.

Bolivian regulations require that all blood donations be screened for *Trypanosoma cruzi* before being used, which has contributed to the reduction of transmission through this means.

ESTABLISHED LAWS

In 1986, the First Regional Program for Comprehensive Control of Chagas Disease (CD) began, known as the Tupiza experience, in Bolivia. This program demonstrated the effectiveness of vector control to reduce disease transmission with community participation.

A decrease in the presence of the vector was achieved to 4.3%. The infestation rate was reduced to 58% until 1999. The Program to Fight Chagas Disease in Bolivia reached 93% coverage. As challenges and strategies, the constant use of insecticides in endemic areas generated resistance in vectors. In 1990, the National CD Control Program was created. In the years 2000-2010, the "Chagas Initiative" was implemented to control CD in Latin America.

In 2004, Bolivia reduced the presence of the vector from 75% in 1998 to 2.8% that year.

In 2006, Law 3374 was created, which declared CD as a national priority in all departments of the country. Bolivia is committed to eliminating vector-borne transmission of Chagas disease by 2030 (Chagas Coalition, 2019).

In 2010, Tarija received WHO certification as a departament free of vector-borne transmission of CD

In May 2019, following the decision of the 72nd World Health Assembly, World Chagas Disease Day was established, celebrated on April 14 (the date in 1909 when Carlos Chagas diagnosed the first human case of Chagas). in a two-year-old girl named Berenice). This day was celebrated for the first time in 2020, after the approval and support received by the world health assembly at the WHO in May 2019; in order to raise awareness about this neglected disease.

However, vector transmission is a major problem in rural areas with precarious living conditions. Bolivia is committed to eliminating vector transmission by 2030 and improving the strategies implemented, such as indoor fumigation and community education (Chagas Coalition, 2019).

As a review, it offers an overview of Chagas disease in Bolivia, from its history to the present, the progress it has made and the challenges that Bolivia faces in the control of Chagas disease.

In this review we conclude that Chagas disease in Bolivia continues to be in a constant fight, but with significant advances in its control.

BOLIVIAN GOVERNMENT LAWS ON CHAGAS DISEASE

Bolivia faces a high prevalence of Chagas disease, particularly in endemic areas. In response to this challenge, the Bolivian government has enacted important measures, such as Law No. 3374 in 2006, which declares the prevention and control of Chagas disease a national priority. This law facilitates resource mobilization and strategy implementation to reduce the incidence and mortality associated with the disease.

The government, in collaboration with international organizations like the Pan American Health Organization (PAHO) and the Inter-American Development Bank (IDB), has launched various health programs that have significantly reduced infestation rates. By 2005, infestation rates in endemic municipalities exceeded

3.9%, but after government interventions, they have been reduced to 1.3%. Furthermore, seroprevalence among children aged 1 to 5 years decreased from 15.6% in 1990 to 0.8% by 2009, indicating progress in controlling vector transmission (Ministerio de Salud y Deportes de Bolivia, 2010).

The Ministry of Health and Sports developed several manuals to guide healthcare professionals in diagnosing, treating, and managing Chagas disease. These include manuals for congenital Chagas, childhood Chagas, and recent chronic childhood cases, aligning with international recommendations to reduce disease complications.

Despite these advances, several challenges remain. Socioeconomic factors such as poverty, inadequate healthcare access, and infrastructure limitations continue to impede the full implementation of control measures, especially in rural and remote areas. Law 3374, while essential, cannot fully guarantee the success of public health programs without addressing these logistical barriers. Moreover, the focus on vector control has overshadowed the growing issue of congenital Chagas transmission, where 63% of cases go untreated due to insufficient healthcare coverage.

Additionally, Bolivia has made progress in preventing transmission through blood transfusions by mandating the screening of blood donors for *Trypanosoma cruzi*. However, the legal framework has been less effective in addressing the needs of prenatal screening and treatment for congenital Chagas, which is becoming more prominent as vector transmission decreases.

The inconsistent application of diagnostic and treatment manuals across regions highlights disparities in healthcare infrastructure and access to trained personnel, particularly in rural areas. While Bolivia's legal framework has raised national awareness about Chagas disease, community engagement remains a critical element in disease control, especially in rural areas where socioeconomic challenges limit participation in prevention programs (Ministerio de Salud y Deportes de Bolivia, 2010).

While Bolivia has made significant progress through its legal framework and public health programs, several obstacles remain. Addressing socioeconomic disparities, improving healthcare access, and engaging local communities are essential to the long-term success of Chagas disease control. A more integrated approach combining legal measures, community involvement, and sustained investment in infrastructure is necessary to fully combat the ongoing threat of Chagas disease in Bolivia.

CLINICAL MANIFESTATIONS AND DIAGNOSIS OF CHAGAS DISEASE IN BOLIVIA AND MEXICO

In Bolivia, the most common clinical manifestations of Chagas disease include chagasic cardiomyopathy and digestive complications such as megacolon and megaesophagus These complications depends of immune response of the affected populations, as well as limitations in the healthcare services available for diagnosing and treating these conditions.

In contrast, Mexico has achieved better control of the disease, resulting in lower rates of severe complications. This highlights disparities in the capacity to implement control programs and the resources available in both countries. While Mexico has maintained better control over vector transmission, Bolivia still faces high transmission rates, including congenital transmission.

The comparison between Bolivia and Mexico underscores how social, economic, and environmental factors influence the effectiveness of control measures. Although both countries have made valuable contributions in the fight against Chagas disease, each faces unique challenges that require tailored approaches to their epidemiological and geographical realities (Velasco and Rivas, 2008).

CONCLUSIONS

Chagas disease has been and continues to be a significant challenge for Bolivia, especially in rural areas where precarious conditions facilitate the spread of *Trypanosoma cruzi*. Over the years, the country has implemented various strategies, from the creation of the National Service to Fight Chagas Disease in 1945 to the World Health Organization's (WHO) certification of the elimination of vector-borne transmission in the department of Tarija in 2010. However, the persistent presence of the vector, particularly in rural and hard-to-reach areas, remains a crucial challenge.

Bolivia's commitment to eradicating vector-borne transmission of Chagas disease by 2030 requires sustained efforts in vector control, housing improvements, and public health education. Current strategies, including indoor fumigation and active community participation, are key to advancing toward this goal. Furthermore, it is essential to continue improving access to diagnosis and treatment, particularly in the most vulnerable areas, to break the transmission cycle of the disease.

Despite the challenges that remain, Bolivia has made significant progress in controlling the disease, demonstrating the effectiveness of public health policies implemented thus far. However, achieving the goal of total eradication will require continued collaboration at both international and national levels, as well as an intensification of preventive and treatment measures in the most affected areas.

DETAILED SUMMARY FOR A SCIENTIFIC ESSAY: RECOMMENDATIONS FOR CHAGAS DISEASE CONTROL

Chagas disease (*Trypanosoma cruzi*), a significant health threat in Latin America, affects 6-8 million people with approximately 75 million at risk. Its transmission occurs through various routes, including vector transmission via *Triatoma infestans*, congenital transmission, contaminated food, and blood or organ donations. Due to migration, cases have been reported in non-endemic countries such as the U.S., Europe, and Japan. Despite its broad impact, Chagas disease remains classified as a neglected tropical disease (NTD) by the World Health Organization (WHO), reflecting the need for increased attention and resources. A significant concern is that only 3% of *T. cruzi* carriers are diagnosed, and merely 1% receive treatment. This lack of diagnosis and treatment can lead to severe chronic cardiac and digestive complications in up to 30% of infected individuals.

In Bolivia, congenital transmission represents a major public health issue, emphasizing the need for universal screening for pregnant women and timely treatment to prevent further transmission to newborns. The World Health Assembly has urged the establishment of clear diagnostic algorithms to improve the detection and treatment of neglected tropical diseases like Chagas. Early detection is critical to expanding access to treatment and reducing the disease's burden.

Effective diagnosis varies depending on the disease stage and transmission route. Parasitological methods, while simple, have low sensitivity and require additional testing. Molecular methods, such as PCR, allow for automation and quantification, improving throughput and accuracy. Serological tests are useful but are challenged by the antigenic diversity of the parasite. The development of point-of-care testing is key to improving diagnostic accessibility, especially in resource-limited settings.

Chagas disease often goes unnoticed in its acute phase due to asymptomatic cases or mild symptoms resembling other febrile illnesses. In contrast, the chronic phase is characterized by low parasitemia, making diagnosis reliant on the immune response. The WHO's goal of eliminating Chagas as a public health problem by 2030 underscores the need for timely diagnosis and accessible treatment. However, current diagnostic algorithms, requiring multiple tests, face challenges in delivering timely results, particularly in low-resource environments. It is crucial to adopt innovative tools that streamline diagnostics.

The molecular understanding of *T. cruzi* has enabled advancements in diagnostics and pathogenesis knowledge. Continued research into these areas is essential for developing diagnostic methods tailored to the limitations of endemic regions. Integrating molecular techniques into both clinical and epidemiological practice will significantly improve the management and control of Chagas disease.

In addition to technological advancements, education and awareness campaigns are vital for reducing the stigma surrounding Chagas disease and encouraging broader participation in prevention and treatment programs. Preventative strategies should focus on reducing vector infestation and ensuring that newborns receive timely diagnosis and treatment to prevent long-term complications.

Through a combination of legal frameworks, health authority cooperation, innovative diagnostics, and public education, progress can be made toward eradicating Chagas disease and improving the quality of life for affected populations. (Vernal, 2015)

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ANNEX 1. Research and diagnosis activities for Chagas disease in endemic communitiees in the Department of Cochabamba, Bolivia.

SANITARY EDUCATION: Images of Chagas awareness campaigns in bolivian communities.
Source: Author´s elaboration

Houses in rural areas where vinchuca proliferates.
Source: Author´s elaboration.

Collection of triatomines in a house for the detection of *Trypanosoma cruzi*, field work.
Source: Author´s elaboration.

Collecting *Triatoma infestans* for *Trypanosoma cruzi* detection.
Source: Author´s elaboration.

Searching *Triatoma infestans* in domiciliarian structures.
Source: Author´s elaboration.

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