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

12. Bacteria and Paleoparasitology

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Bacteria and Paleoparasitology

Joseli Maria da Rocha Nogueira • Ernesto Hofer

According to studies on ancient material, DNA from parasites can remain preserved together with the host for thousands of years. Not only frozen and mummified soft tissues, but also bones, tooth pulp, and coprolites can be used to study such microorganisms. In the environment, natural materials like resin and amber (Lambert et al., 1998) can also preserve useful material for studying bacteria.

Research techniques for ancient bacteria include microscopy, cell culture, immunology, and molecular biology. These techniques can also assist molecular typing in the epidemiological reconstruction of past epidemics and help improve current epidemiological models for emerging infections, thus contributing to the development of current preventive measures.

German naturalist Christian Gottfried Ehrenberg (1795-1876) introduced the word *Bacteria* (*Bacterium*) as a scientific term in 1838. The word originates from the Greek *Bakterion* (βακτηριον), which is the diminutive of *baktron*, or rod. The choice of the term is associated with the first observations of bacteria under the microscope, since the scientists thought such organisms looked like small rods. The nomenclature is used to this day (Harper, 2001).

Bacteriological paleoparasitology thus aims primarily to identify the bacterial agents that probably infected our ancestors. This allows a better understanding of the various habits and customs involved in ancient health-disease processes, thus identifying the origins of different bacterial infections and the main routes by which these agents spread among peoples and environments (Palhano-Silva & Nogueira, 2005).

We can diagnose diseases in extinct or ancient populations using various sources, such as ancient sculptures, drawings, and texts, abnormalities in bone records, tissues, and biological markers detected in coprolites or other organic remains (Oriel, 1973; Soulie, 1982; Duarte, Ferreira & Araújo, 2002; Manchester & Roberts, 1989).

Coprolites (Greek *copros*, feces; *litos*, rock), a vast source for these studies, are ancient feces naturally preserved by desiccation or mineralization. They often maintain physical or even molecular remains of organisms that were present in the intestines of the humans and/or animals that produced them (Araújo & Ferreira, 2000). Coprolites provide

data on health, diet, and agricultural practices (Rollo et al., 2002) and allow recovering extensive information on the paleoenvironment, even reconstructing part of the food chain among organisms (Andrews & Fernandez-Jalvo, 1998).

Until recently, bacteriological paleoparasitology was limited to the direct investigation of bone and tissue anomalies and their association with known diseases such as bartonellosis (Allison et al., 1974), syphilis (Rothschild & Rothschild, 1995), leprosy (Boldsen, 2001; Andersen, 1969), and tuberculosis (Morse, 1961; Allison, Mendoza, & Pezzia, 1973; Hartney, 1981). Most previous paleoparasitological studies with the detection of the etiological agent involved the microscopic identification of helminths. They used classic techniques of parasitological examination such as rehydration in trisodium phosphate aqueous solution (Callen & Cameron, 1960) followed by spontaneous sedimentation (Lutz, 1919) or some other concentration technique for microscopic examination of parasites eggs or cysts, or histological techniques with tissue preparation by paraffin inclusion or freezing (Ruffer, 1921; Reyman & Dowd, 1980; Ferreira, Araújo & Confalonieri, 1988).

Although these techniques are highly effective for the parasitological detection of helminth eggs and larvae, they overlook important groups of organisms such as protozoa, bacteria, and viruses, not detectable by these methods. The expansion of molecular biology and serological reactions opened new possibilities for studying previously undetectable organisms. Thus, interdisciplinary scientific collaboration provides the most feasible option for studies aimed at the detection and characterization of these agents (Araújo et al., 1998; Pääbo, 1991; Nogueira, 2008).

With the discovery of these new techniques, multidisciplinary studies around the world began to focus on the search for these organisms in an attempt to standardize methods and correlate findings with ancient epidemiological information.

CURRENT TECHNIQUES AND RECENT HISTORY

The use of serological techniques to identify bacteria in ancient feces allowed the successful indirect detection of *Salmonella* antigens (Sawicki, Allison & Dalton, 1976). With advances in the field, new possibilities have emerged.

ELISA (enzyme-linked immunosorbent assay) (Voller, Bidwell & Bartlett, 1976) with monoclonal antibodies has been used with promising results in studies of antigens in ancient material. In Brazil, Gonçalves (2002) used this technique and concluded that its sensitivity exceeded that of microscopic examination to detect *Giardia duodenalis*. The researcher, with collaborators (Gonçalves et al., 2004), used a commercial kit with *Entamoeba histolytica* anti-adhesin antibodies conjugated with peroxidase and showed the presence of protozoa in ancestral samples from 5,300 before present (BP), thereby raising the possibility of also detecting bacteria with this method in the same type of material.

A milestone in the history of bacterial detection in archaeological material was the discovery of deoxyribonucleic acid (DNA) from *Mycobacterium tuberculosis* in a skeleton found in an archaeological site (Spigelman & Lemma, 1993). This unprecedented work encouraged other groups to conduct molecular studies (Salo et al., 1994; Baron, Hummel & Herrmann, 1996; Donoghue et al., 2004). Various studies had already been performed to detect bacterial diseases in mummies, but the majority were limited to searching for evidence from bone morphology (Larsen, 2002), potentially raising confusion with other diseases causing similar lesions. Adopting the same line, Rafi et al (1994) and later other researchers demonstrated the presence of *Mycobacterium leprae* in bones.

With advances in these techniques, researchers used materials such as feces (Ubaldi et al., 1998), mummified tissues (Lowenstein, 2004; Castillo-Rojas, Cerbon & Lopez- Vidal, 2008), and dental pulp (Raoult et al., 2000) to detect various bacteria.

In Brazil, Iñiguez (1998) demonstrated the possibility of retrieving bacterial DNA from artificially desiccated feces using PCR. After inoculating *Vibrio cholerae* and *Bacillus sphaericus*, she successfully viewed identifiable genetic material from these microorganisms.

Prat & Mendonça de Souza (2003) published a commentated review on findings of tuberculosis in populations from the prehistoric Americas.

Various studies investigating bacteria in these materials have been conducted in Brazil, based mainly on molecular biology (Chart 1).

Chart 1 – Summary of various findings in bacteriological paleoparasitology and the possibility of methodological authenticity

Source	Site in body	Mode of preservation	Date	Method	Strength/ quality of evidence
<i>Mycobacterium tuberculosis</i>					
Bison (Rothschild et al., 2001)	Metacarpus	Buried	17,000 BP	Molecular biology	A/I
Human (Salo et al., 1994)	Lung, lymph node	Mummified	1,000 BP	Molecular biology	B/III
Human (Crubézy et al., 1998)	Bone	Mummified	5,400 BP	Molecular biology	A/III
Human (Taylor et al., 1999)	Metacarpus, lumber vertebra	Buried	Medieval	Molecular biology and spoligotyping	B/III
Human (Mays, Fysh & Taylor, 2002)	Rib	Buried	Medieval	Molecular biology	B/III
Human (Arriaza et al., 1994)	Vertebra	Buried	1,000 BP	Molecular biology	B/III
Human (Haas et al., 2000a)	Mandible	Buried	1400–1800 AD	Molecular biology	B/III
Human (Haas et al., 2000b)	Vertebra, femur, ankle, rib, pleura	Buried	7 th –8 th , 17 th century	Molecular biology	B/III
Human (Donoghue et al., 1998)	Lung, pleura	Buried	600 AD	Chromatography, molecular biology	B/III
Human (Gernaey et al., 2001)	Bone	Buried	1,000 BP	Chromatography, molecular biology	B/III
Human (Mays & Taylor, 2003)	Vertebra, rib	Buried	400–230 BC	Molecular biology, spoligotyping	B/II
Human (Zink et al., 2003)	Bone, soft tissues	Mummified	2050–500 BC	Molecular biology	A/I
Human (Spigelman & Lemma, 1993)	Bone	Buried	Not informed	Molecular biology	C/III
Human (Fletcher et al., 2003)	Lung, pleura, abdomen, tooth, ribs, hair	Mummified	18 th –19 th century	Molecular biology	A/I
Human (Taylor et al., 1996)	Lung, lumbar vertebra	Buried	14 th – 16 th century	Molecular biology	C/III

Chart 1 – Summary of various findings in bacteriological paleoparasitology and the possibility of methodological authenticity (continued)

Source	Site in body	Mode of preservation	Date	Method	Strength/ quality of evidence
<i>Mycobacterium leprae</i>					
Human (Montiel et al. 2003)	Foot bones	Buried	12 th century	Molecular biology	B/II
Human (Spigelman & Donohue, 2001)	Metacarpi	Buried	300–600 AD	Molecular biology	B/II
Human (Donoghue, Holton & Spigelman, 2001)	Nasal bone tissue	Buried	1,100 BP	Molecular biology	B/II
Human (Donoghue, Holton & Spigelman, 2001)	Skulls	Buried	1400–1800 AD	Molecular biology	B/II
Human (Haas et al. 2000a)	Hard palate and skulls	Buried	1400–1800 AD, 10 th century	Molecular biology	B/II
<i>Enteric bacteria</i>					
Mastodon (Rhodes et al., 1998)	Intestine	Frozen	12,000 BP	Culture	C/II
Human (Zink et al., 2000)	Metatarsus	Mummified	1400 BC	Molecular biology	B/II
Human (Fricker, Spigelman & Fricker, 1997)	Stomach content	Bog	300 BC	Molecular biology	C/III
<i>Treponema pallidum</i>					
Human (Kolman et al. 1999)	Bone	Buried	240 BP	Immunodetection; Molecular biology	B/II
<i>Borrelia burgdorferi</i>					
Ticks (Matuschka et al. 1996)		Dry	1884 AD	Molecular biology	A/III
Rodents (Marshall et al., 1994)		Dry	19 th century	Molecular biology	B/II
<i>Other spirochetes</i>					
Termite (Wier et al., 2002)	Intestinal tissue	Amber	Miocene	Microscopy	A/II
<i>Bartonella quintana</i>					
Human (Drancourt et al., 2005)	Dental pulp	Buried	4,000 BP	Molecular biology	A/II
<i>Bartonella henselae</i>					
Cat (La et al., 2004)	Dental pulp	Buried	13 th –18 th centuries	Molecular biology	A/II

Chart 1 – Summary of various findings in bacteriological paleoparasitology and the possibility of methodological authenticity (continued)

<i>Yersinia pestis</i>					
Human (Drancourt et al., 2004)	Dental pulp	Buried	5 th -14 th centuries	Molecular biology	A/II
Human (Drancourt et al., 1998)	Dental pulp	Buried	1590-1722 AD	Molecular biology	B/III
Human (Raoult et al., 2000)	Dental pulp	Buried	1348 AD	Molecular biology	A/II

BC – Before Christ; AD – Anno Domini; BP – Before Present

A – High evidence of authenticity; B – Moderate evidence of authenticity; C – Low evidence of authenticity

I – Independent teams and concordant protocols; II – Single team and concordant protocols; III – Single team without protocol concordance.

Source: Drancourt & Raoult (2005).

Importantly, the results do not always truly express the detection of ancient bacteria. Drancourt & Raoult (2005) highlight the importance of extremely painstaking work, since any contamination with contemporary material can shed doubt on the research.

Rigorous control of the results' authenticity and quality is extremely important. Level A strength of evidence is defined as the detection of one or more original identified sequences, two identified and unrelated sequences, or at least one detected original sequence and another unrelated molecule. Level B is defined as moderate authenticity, with the identification of a specific sequence or biological molecule. Level C expresses low evidence of authenticity, in studies with the detection of a sequence or biological molecule with low specificity. As for the quality of evidence, the same authors proposed index I for studies in which two independent teams worked with concordant protocols, II for a single team using concordant protocols, and III for a team not using protocol concordance.

Importantly, when searching for bacteria in archaeological material that has already been handled, such as coprolites, the sample should be pretreated with UV irradiation to avoid contemporary contamination (Santos, 1996; Nogueira et al., 2006a).

According to a new and simple line of research for potential isolation of bacteria in ancient material, various studies have suggested isolating viable bacteria from organic material preserved in ice, salt crystals, or amber, considering the tolerance of spores to heat and low humidity over time (Seaward, Cross & Unsworth, 1976; Vreeland, Rosenzweig & Powers, 2000; Nicholson, 2003; Christner et al., 2003; Gatson et al., 2006; Cano & Borucki, 1995).

In Brazil, Nogueira (2008) used external cleaning, irradiation, perforation, direct isolation, and culture to successfully retrieve viable bacteria from bacterial spores inside ancient South American coprolites in the Paleoparasitology Collection of the Sergio Arouca National School of Public Health, Oswaldo Cruz Foundation (ENSP/FIOCRUZ). After isolating the bacterial colonies in solid medium, she conducted biochemical studies to identify each of these microorganisms (Nogueira, 2007). She then performed molecular tests including PCR, cloning, and sequencing to indicate the various species in this material. The same researcher suggested the presence of different species from those currently circulating, due to the impossibility of typing them with the currently available methods (Nogueira, 2008).

Considering the possibilities raised by these findings and recently introduced techniques, including molecular biology, the search for bacteria in archaeological material has huge potential to produce new data in the area of

epidemiological bacteriology, combining such detection with the search for evidence of infection and probable pathological manifestations in human or animal remains (Nogueira et al., 2006b).

In Brazil, the research group in paleoparasitology and paleoepidemiology at ENSP/FIOCRUZ now includes a variety of researchers and students interested in the knowledge generated by data obtained from ancient material. Bacterial studies in paleoparasitology are an integral part of this context, and as they develop they complement the findings from related lines of research.

Researchers are currently analyzing coprolites from the collection of the Paleoparasitology Laboratory at ENSP using monoclonal antibodies, molecular examination, and direct culture to detect bacteria and standardize the techniques for future studies. Further objectives include mapping the frequency of these agents in the material, correlating with the respective archaeological sites, co-infection with other agents, eating habits, and possible dispersal routes of the agents based on geographic data, besides dating the samples (Nogueira et al., 2005a).

Genomic analysis of these microorganisms should improve our understanding of the determination of infections and diseases in light of the complexity of parasite-host-environment relationships, as well as coevolution of the respective species.

INTERESTING PERSPECTIVES

The possibility of recovering bacterial spores from coprolites raises more new prospects. Even though a coprolite is a metabolically inactive structure, its interior maintains all of the microorganism's genetic information. The latent form, when cultivated using microbiological techniques, evolves to a vegetative form. The spores are made up of dipicolinic acid associated with calcium, and when compared to vegetative cells, they are extremely resistant to physical and chemical agents, and are refringent and highly tolerant to thermal alteration, oxidation, and desiccation, thus displaying a survival strategy (Setlow, 1999; Nicholson et al., 2000; Nicholson, 2003).

The bacterial organisms of clinical importance and capable of producing these structures include genera *Bacillus* and *Clostridium*. However, various other related bacterial genera such as *Paenibacillus*, *Amphibacillus*, *Desulfotomaculum*, *Sporosarcina*, *Sporolactobacillus*, *Sporohalobacter*, *Oscillospira*, and *Thermoactinomyces* also display this capacity (Bergey & Holt, 1994) and can be isolated, even after having been maintained in an inadequate environment for bacterial organisms (Nogueira, 2008)

ETHICAL ISSUES RELATED TO RESEARCH IN PALEOEPIDEMIOLOGY

Another increasingly important area in this new scientific frontier involves ethical reflections pertaining to the use of such ancestral material. An analysis of the current ethical concepts and research projects in biomedical fields shows that these issues have drawn growing attention, especially when they involve human beings and their mortal remains, meanwhile reflecting researchers' concern and interest in this issue (Coimbra & Santos, 1996; Schramm, 1995).

Holloway (1995) discusses the interaction between researchers, curators, and living descendants, the latter often demanding that the material used for research or museum exhibits be returned to the community. The author criticizes the measures that have been taken to protect disinterred remains. Once exposed, information that has remained buried for thousands of years can be lost rapidly. The main scientific commitment should be to the preservation of such material.

A proper ethical approach to the preservation of our ancestral cultural heritage should include all possible steps to preserve such materials not only for the coming generations, but also for future research and interpretation. We should give special emphasis to the documentation produced, since it lends meaning to the collections and allows the production of scientific knowledge. Such documentation should remain available and under safe conditions in the long term (Lima, 1997).

These essential issues must not be neglected, since they reflect the role of science in modern life, besides providing new ways to produce scientific knowledge (Nogueira et al., 2005b).

CONCLUSIONS

Based on the evidence and discussions above, the preservation of ancestral material and its use as a source for study can become allies in the reconstruction of ancestral history.

Part of the development of different causes in the health-disease process can be unveiled by the diverse data collected in these studies, ranging from historical records to new molecular techniques. However, such research can only be guaranteed if the researchers using the ancient material as a wellspring of knowledge take all the necessary steps to preserve and avoid destroying it, thus benefiting related fields in a conscientious and virtually never-ending process.

REFERENCES

- ALLISON, M. J.; MENDOZA, D. & PEZZIA, A. Documentation of a case of tuberculosis in pre-Columbian America. *American Review of Respiratory Diseases*, 107: 985-991, 1973.
- ALLISON, M. J. et al. A Case of Carrion's disease associated with human sacrifice from the Huari culture of Southern Peru. *American Journal of Physical Anthropology*, 41: 295-300, 1974.
- ANDERSEN, J. G. Studies on the medieval diagnosis of leprosy in Denmark: an osteoarchaeological, historical and clinical study. *Danish Medical Bulletin*, 16: 1-142, 1969.
- ANDREWS, P. & FERNANDEZ-JALVO, Y. Palaeobiology: 101 uses for fossilized faeces. *Nature*, 393: 629-630, 1998.
- ARAÚJO, A. & FERREIRA, L. F. Paleoparasitology and the antiquity of human host-parasite relationships. *Memórias do Instituto Oswaldo Cruz*, 95: 89-93, 2000.
- ARAÚJO, A. et al. Paleoparasitology: perspectives with new techniques. *Revista do Instituto de Medicina Tropical de São Paulo*, 40: 371-376, 1998.
- ARRIAZA, B. T. et al. Pre-Columbian tuberculosis in Northern Chile: molecular and skeletal evidence. *American Journal of Physical Anthropology*, 98: 37-45, 1995.
- BARON, H.; HUMMEL, S. & HERRMANN, B. *Mycobacterium tuberculosis* complex DNA in ancient human bones. *Journal of Archaeological Science*, 23: 667-671, 1996.
- BERGEY, D. H. & HOLT, J. G. *Bergey's Manual of Determinative Bacteriology*. 9th Ed. Baltimore: Lippincott Williams & Wilkins, 1994.
- BOLDSEN, J. L. Epidemiological approach to the paleopathological diagnosis of leprosy. *American Journal of Physical Anthropology*, 115: 380-387, 2001.
- CALLEN, E. O. & CAMERON, T. W. M. A prehistoric diet as revealed in coprolites. *New Scientist*, 8: 35-40, 1960.
- CANO, R. J. & BORUCKI, M. K. Revival and identification of bacterial spores in 25-to 40-million-year-old Dominican amber. *Science*, 268: 1.060-1.064, 1995.

- CASTILLO-ROJAS, G.; CERBON, M. A. & LOPEZ-VIDAL, Y. Presence of *Helicobacter pylori* in a Mexican pre-Columbian mummy. *BMC Microbiology*, 8: 119, 2008.
- CHRISTNER, B. C. et al. Bacterial recovery from ancient glacial ice. *Environmental Microbiology*, 5: 433-436, 2003.
- COIMBRA JR, C. E. & SANTOS, R.V. Ética e pesquisa biomédica em sociedades indígenas no Brasil. *Cadernos de Saúde Pública*, 12: 417-422, 1996.
- CRUBÉZY, E. et al. Identification of *Mycobacterium* DNA in an Egyptian Pott's disease of 5,400 years old. *Comptes Rendus de l'Académie des Sciences*, Série III, 321: 941-951, 1998.
- DONOGHUE, H. D.; HOLTON, J. & SPIGELMAN, M. PCR primers that can detect low levels of *Mycobacterium leprae* DNA. *Journal of Medical Microbiology*, 50: 177-182, 2001.
- DONOGHUE, H. D. et al. Tuberculosis: from prehistory to Robert Koch, as revealed by ancient DNA. *The Lancet*, 4: 584-592, 2004.
- DONOGHUE, H. D. et al. *Mycobacterium tuberculosis* complex DNA in calcified pleura from remains 1,400 years old. *Letters in Applied Microbiology*, 27: 265-269, 1998.
- DRANCOURT, M. et al. Detection of 400-year-old *Yersinia pestis* DNA in human dental pulp: an approach to the diagnosis of ancient septicemia. *Proceedings of the National Academy of Sciences*, 95: 12.637-12.640, 1998.
- DRANCOURT, M. & RAOULT, D. Palaeomicrobiology: current issues and perspectives. *Nature Reviews - Microbiology*, 3: 23-35, 2005.
- DRANCOURT, M. et al. Genotyping, Orientalis-like *Yersinia pestis*, and plague pandemic. *Emerging Infectious Diseases*, 10: 1.585-1.592, 2004.
- DRANCOURT, M. et al. *Bartonella quintana* in a 4,000-year old human tooth. *The Journal of Infectious Diseases*, 191: 607-611, 2005.
- DUARTE, N. A.; FERREIRA, L. F. & ARAÚJO, A. Paleoepidemiologia e paleoparasitologia. In: MEDRONHO, R. A. (Ed.). *Epidemiologia*. São Paulo: Atheneu, 2002.
- FERREIRA, L. F.; ARAÚJO, A. & CONFALONIERI, U. *Paleoparasitologia no Brasil*. Rio de Janeiro: PEC/Ensp, 1988.
- FLETCHER, H. A. et al. Widespread occurrence of *Mycobacterium tuberculosis* DNA from 18th-19th century Hungarians. *American Journal of Physical Anthropology*, 120: 144-152, 2003.
- FRICKER, E. J.; SPIGELMAN, M.; FRICKER, C. R. The detection of *Escherichia coli* DNA in the ancient remains of Lindow Man using the polymerase chain reaction. *Letters in Applied Microbiology*, 24: 351-354, 1997.
- GATSON, J. W. et al. *Bacillus tequilensis* sp. nov., isolated from a 2000-year-old Mexican shaft tomb, is closely related to *Bacillus subtilis*. *International Journal of Systematic and Evolutionary Microbiology*, 56: 1.475-1.484, 2006.
- GERNAEY, A. M. et al. Mycolic acids and ancient DNA confirm an osteological diagnosis of tuberculosis. *Tuberculosis* (Edinburgh), 81: 259-265, 2001.
- GONÇALVES, M. L. C. *Helminths, Protozoários e Algumas Ideias: novas perspectivas na paleoparasitologia*, 2002. PhD dissertation, Rio de Janeiro: Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz.
- GONÇALVES, M. L. C. et al. Amoebiasis distribution in the past: first steps using an immunoassay technique. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98: 88-91, 2004.
- HAAS, C. J. et al. Detection of leprosy in ancient human skeletal remains by molecular identification of *Mycobacterium leprae*. *American Journal of Clinical Pathology*, 114: 428-436, 2000a.
- HAAS, C. J. et al. Molecular evidence for different stages of tuberculosis in ancient bone samples from Hungary. *American Journal of Physical Anthropology*, 113: 293-304, 2000b.
- HARPER, D. *Online Etymology Dictionary*, 2001. Available at: <www.etymonline.com>. accessed 27/Oct/2005.
- HARTNEY, P.C. Tuberculosis lesions in a prehistoric population sample from southern Ontario. In: BUIKSTRA, J. E. *Prehistoric Tuberculosis in the Americas*, Northwestern University Archaeological Program, Evanston, 1981.

- HOLLOWAY, M. The preservation of the past. *Scientific American*, 272: 78-81, 1995.
- INÍGUEZ, A. M. *Análise do DNA Ancestral para o Estudo de Infecções Parasitárias em Populações Pré-históricas*, 1998. Master's thesis, Rio de Janeiro: Instituto Oswaldo Cruz, Fundação Oswaldo Cruz.
- KOLMAN, C. J. et al. Identification of *Treponema pallidum* subspecies *pallidum* in a 200-year-old skeletal specimen. *The Journal of Infectious Diseases*, 180: 2.060-2.063, 1999.
- LA, V. D. et al. Molecular detection of *Bartonella henselae* DNA in the dental pulp of 800-year-old French cats. *Clinical Infectious Diseases*, 39(9):1.391-1.394, 2004.
- LAMBERT, L. H. et al. *Staphylococcus succinus* sp. nov., isolated from Dominican amber. *International Journal of Systematic Bacteriology*, 48: 511-518, 1998.
- LARSEN, C. S. Bioarchaeology: the lives and lifestyles of past people. *Journal of Archaeological Research*, 10: 119-166, 2002.
- LIMA, T. A. A ética que temos e a ética que queremos (ou como falar de princípios neste conturbado fim de milênio). CONGRESSO DA SOCIEDADE BRASILEIRA DE ARQUEOLOGIA, 9, 1997. Rio de Janeiro.
- LOWENSTEIN, J.E. Paleodermatoses: lessons learned from mummies. *Journal of the American Academy of Dermatology*, 50: 919-936, 2004.
- LUTZ, A. O *Schistosomum mansoni* e a schistosomatose segundo observações, feitas In Brazil. *Memórias do Instituto Oswaldo Cruz*, 11: 121-155, 1919.
- MANCHESTER, K. & ROBERTS, C. The palaeopathology of leprosy in Britain: a review. *World Archaeology*, 21: 265-272, 1989.
- MARSHALL III, W. F. et al. Detection of *Borrelia burgdorferi* DNA in museum specimens of *Peromyscus leucopus*. *The Journal of Infectious Diseases*, 170: 1.027-1.032, 1994.
- MATUSCHKA, F. R. et al. Characteristics of Lyme disease spirochetes in archived European ticks. *The Journal of Infectious Diseases*, 174: 424-426, 1996.
- MAYS, S. & TAYLOR, M. A first prehistoric case of tuberculosis from Britain. *International Journal of Osteoarchaeology*, 13: 189-196, 2003.
- MAYS, S.; FYSH, E. & TAYLOR, G.M. Investigation of the link between visceral surface rib lesions and tuberculosis in a medieval skeletal series from England using ancient DNA. *American Journal of Physical Anthropology*, 119: 27-36, 2002.
- MONTIEL, R. et al. DNA sequences of *Mycobacterium leprae* recovered from ancient bones. *FEMS Microbiology Letters*, 226: 413-414, 2003.
- MORSE, D. Prehistoric tuberculosis in America. *The American Review of Respiratory Disease*, 83: 489-504, 1961.
- NICHOLSON, W. L. Using thermal inactivation kinetics to calculate the probability of extreme spore longevity: implications for paleomicrobiology and lithopanspermia. *Origins of Life and Evolution of the Biosphere*, 33: 621-631, 2003.
- NICHOLSON, W. L. et al. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiology and Molecular Biology Reviews*, 64: 548-572, 2000.
- NOGUEIRA, J. M. R. et al. Paleobacteriologia: novas possibilidades em estudos paleoparasitológicos. In: JORNADA CIENTÍFICA DE PÓS-GRADUAÇÃO DA FUNDAÇÃO OSWALDO CRUZ, 9, 2005, Rio de Janeiro. *Anais...* Rio de Janeiro, 2005a.
- NOGUEIRA, J. M. R. et al. The importance of ethical aspects in paleoparasitological research. In: PALEOPATHOLOGY ASSOCIATION MEETING IN SOUTH AMERICA – PAMINSA – HUMAN MIGRATIONS AND DISEASES, 1, 27-29 jul.2005, Rio de Janeiro: *Anais...* Rio de Janeiro: Ensp/Fiocruz, 2005b.
- NOGUEIRA, J. M. R. et al. Paleogenômica exploratória: sequenciamento aleatório de fragmentos de DNA de coprólitos oriundos do nordeste brasileiro. V Bienal de Pesquisa da Fiocruz, 2006a.

- NOGUEIRA, J. M. R. et al. Paleoparasitologia: revisão bibliográfica e novas perspectivas em estudos microbiológicos. *Revista de Patologia Tropical*, 35: 87-102, 2006b.
- NOGUEIRA, J. M. R. Paleoparasitologia bacteriana: recuperação de *Bacillus* sp. de esporos viáveis presentes em coprólitos sul-americanos. In: JORNADA DE PÓS-GRADUAÇÃO/REUNIÃO ANUAL DE INICIAÇÃO CIENTÍFICA DA FIOCRUZ, X/XV, 2007, Rio de Janeiro. *Novas Fronteiras da Educação e Saúde...* Rio de Janeiro, 2007.
- NOGUEIRA, J. M. R. *Paleoparasitologia: estudos associados à recuperação de organismos bacterianos de esporos viáveis presentes em coprólitos sul-americanos*, 2008. PhD dissertation, Rio de Janeiro: Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, 2008.
- ORIEL, J. D. Gonorrhea in the ancient world. *Paleopathology Newsletter*, 4: 7-9, 1973.
- PÄÄBO, S. Amplifying DNA from archaeological remains: a meeting report. *PCR Methods and Applications*, 1: 107-110, 1991.
- PALHANO-SILVA, C. S. & NOGUEIRA, J. M. R. Novas possibilidades em estudos paleoparasitológicos. *Revista da Sociedade Brasileira de Medicina Tropical*, 38, supl. 1: 253, 2005.
- PRAT, J. G. & MENDONÇA DE SOUZA, S. M. F. Prehistoric tuberculosis in America: adding comments to a literature review. *Memórias do Instituto Oswaldo Cruz*, 98, suppl. 1: 151-159, 2003.
- RAFI, A. et al. *Mycobacterium leprae* DNA from ancient bone detected by PCR. *The Lancet*, 343: 1.360-1.361, 1994.
- RAOULT, D. et al. Molecular identification by 'suicide PCR' of *Yersinia pestis* as the agent of medieval black death. *Proceedings of the National Academy of Sciences*, 97: 12.800-12.803, 2000.
- REYMAN, T. A. & DOWD, A.M. Processing of mummified tissue for histological examination. In: COCKBURN, A. & COCKBURN, E. (Eds.). *Mummies, Disease, and Ancient Cultures*. Cambridge: Cambridge University Press, 1980.
- RHODES, A. N. et al. Identification of bacterial isolates obtained from intestinal contents associated with 12,000-year-old mastodon remains. *Applied and Environmental Microbiology*, 64: 651-658, 1998.
- ROLLO, F. et al. Ötzi's last meals: DNA analysis of the intestinal content of the Neolithic glacier mummy from the Alps. *Proceedings of the National Academy of Sciences*, 99: 12.594-12.599, 2002.
- ROTHSCHILD, B. M. & ROTHSCHILD, C. Treponemal disease revisited: skeletal discriminators for yaws, bejel, and venereal syphilis. *Clinical Infectious Diseases*, 20: 1.402-1.408, 1995.
- ROTHSCHILD, B. M. et al. *Mycobacterium tuberculosis* complex DNA from an extinct bison dated 17,000 years before the present. *Clinical Infectious Diseases*, 33: 305-311, 2001.
- RUFFER, M. A. *Studies in Paleopathology of Egypt*. New York, Chicago: University of Chicago Press, 1921.
- SALO, W. L. et al. Identification of *Mycobacterium tuberculosis* DNA in a pre-Columbian Peruvian mummy. *Proceedings of the National Academy of Sciences*, 91: 2.091-2.094, 1994.
- SANTOS, A. K. C. R. *Diversidade de Linhagens de DNA Mitocondrial de Ameríndios da Amazônia: populações contemporâneas e ancestrais*, 1996. PhD dissertation. Ribeirão Preto: Universidade de São Paulo.
- SAWICKI, V. A.; ALLISON, M. J. & DALTON, H. P. Presence of *Salmonella* antigens in feces from a Peruvian mummy. *Bulletin of the New York Academy of Medicine*, 52: 805-813, 1976.
- SCHRAMM, F. R. A terceira margem da saúde: a ética 'natural'. *Ciência, História e Saúde – Manguinhos*, 1: 54-68, 1995.
- SEAWARD, M. R. D.; CROSS, T. & UNSWORTH, B. A. Viable bacterial spores recovered from an archaeological excavation. *Nature*, 261: 407-408, 1976.
- SETLOW, P. Bacterial spore resistance. In: STORZ, G. & HENGGE-ARONIS, R. (Eds.). *Bacterial Stress Responses*. Washington: American Society for Microbiology, 1999.
- SOULIE, R. Brucellosis: a case report dating from 650-700 AD. *Paleopathology Newsletter*, 38: 7-10, 1982.

- SPIGELMAN, M. & DONOGHUE, H. D. Brief communication: unusual pathological condition in the lower extremities of a skeleton from ancient Israel. *American Journal of Physical Anthropology*, 114: 92-93, 2001.
- SPIGELMAN, M. & LEMMA, E. The use of Polymerase Chain Reaction (PCR) to detect *Mycobacterium tuberculosis* in ancient skeletons. *International Journal of Osteoarchaeology*, 3: 137-143, 1993.
- TAYLOR, G. M. et al. Detection of *Mycobacterium tuberculosis* bacterial DNA in medieval human skeletal remains using polymerase chain reaction. *Journal of Archaeological Science*, 23: 789-798, 1996.
- TAYLOR, G. M. et al. Genotypic analysis of *Mycobacterium tuberculosis* from medieval human remains. *Microbiology*, 145: 899-904, 1999.
- UBALDI, M. et al. Sequence analysis of bacterial DNA in the colon of an Andean mummy. *American Journal of Physical Anthropology*, 107: 285-295, 1998.
- VOLLER, A.; BIDWELL, D. E. & BARTLETT, A. N. N. Enzyme immunoassays in diagnostic medicine: theory and practice. *Bulletin of the World Health Organization*, 53: 55-65, 1976.
- VREELAND, R. H.; ROSENZWEIG, W. D. & POWERS, D.W. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature*, 407: 897-900, 2000.
- WIER, A. et al. Spirochete and protist symbionts of a termite (*Mastotermes electrodomenicus*) in Miocene amber. *Proceedings of the National Academy of Sciences*, 99: 1.410-1.413, 2002.
- ZINK, A. et al. Molecular evidence of bacteremia by gastrointestinal pathogenic bacteria in an infant mummy from ancient Egypt. *Archives of Pathology and Laboratory Medicine*, 124: 1.614-1.618, 2000.
- ZINK, A. R. et al. Molecular study on human tuberculosis in three geographically distinct and time delineated populations from ancient Egypt. *Epidemiology and Infection*, 130: 239-249, 2003.