Part IV - Special Studies and Perspectives

28. Methods in Paleoepidemiology and New Perspectives in Paleoparasitology

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Methods in Paleoepidemiology and New Perspectives in Paleoparasitology

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Paleoepidemiology is similar to other sciences that study long periods of time, such as evolutionary biology, in that it relies on historical reconstruction and the elaboration of plausible retrospective scenarios. Unlike experimental physics and chemistry, paleoepidemiology can never rely exclusively on controlled experiments, although it frequently draws on recent advances in other branches of science such as molecular biology.

As highlighted by Mayr (2004), the interaction between historical reconstruction, deductive reasoning, and exhaustive exploration of empirical findings makes biology a “unique science”, an expression that serves as the title for his last book. Biology lies at the intersection between the natural sciences and history, especially in some of its branches such as evolutionary biology and diachronic ecology. The latter discipline focuses on the succession of ecosystems, analyzed by authors like Wilson (1999) in relation to the destruction and restoration of ecosystems, as with the volcanic eruption that shattered Krakatau Island in 1883.

What these branches of biology have in common with the sister science of paleontology and that of astronomy is the fact that they deal with phenomena produced by natural forces over thousands or even millions of years – in the case of astronomy, with time spans dating to the very origin of the universe (Sagan, 2006).

Modern epidemiology only recently began to analyze phenomena that spanned longer time periods. This was true for the epidemiology of infectious diseases, dealing with extended natural histories in chronic infections such as AIDS and hepatitis C, or the epidemiology of non-communicable diseases in modern studies on life histories and the incidence of diabetes mellitus and some cardiovascular diseases (Morabia, 2004). Still, although long time spans in modern epidemiology refer to contemporary genetic epidemiology (Khouri, Little & Burke, 2004), they reflect the time transpired in the life of only one or a few age cohorts.

Very recently, spectacular new techniques in molecular biology have spawned studies tracing genetic markers over many generations, combining data analysis from living tissues and the reconstruction of genetic material from deceased ancestors (Khouri, Little & Burke, 2004). Such research, for example on the genetic origin of Huntington's

chorea, mainly constitutes an exception to a markedly observational and prospective field of studies in terms of the nature and chronology of the phenomena under study.

The typical study designs of modern epidemiology – prospective cohorts and randomized clinical trials - aim to minimize the inferential biases of sampling procedures and risk estimation through designs that allow monitoring individuals over time, collecting and systematizing the findings in the course of observation periods. Modern epidemiology also includes studies in which information from the past is gathered and systematized. However, as mentioned above, such designs are usually limited to the time span of a single-generation cohort, and only more rarely to a few generations.

Epidemiology deals with short historical periods when compared to paleoepidemiology. Still, there is widespread agreement that retrospective studies are weaker than prospective ones and are more subject to various biases, such as those resulting from differential recall of past events and the correct attribution of the true exposure to parasites with high pathogenicity, or to harmful substances or sources of pollution (for a definition of the various biases in epidemiology, see Last, 1995).

Paleoepidemiology thus faces numerous challenges and great complexity, since it approaches phenomena that are observable after the fact, heavily determined by unique historical circumstances, and not amenable to exhaustive reconstruction. Furthermore, such phenomena suffer the wear imposed on compounds and beings by the same passing of historical time that shaped them, e.g., in the formation of fossils.

One of today's leading biologists, the physiologist and ecologist Jared Diamond, who circulates comfortably in both experimental biology and the analysis of phenomena with a long historical course, calls attention to the need for caution when using inferential statistical procedures in dealing with scarce observations, subject to the influence of different intervening variables (usually known only in part). Such methodological difficulties appear both in the sphere of their determination itself (causal determination chains) and in the transformation of their state over time, from the original occurrence to the recording of the event by the contemporary observer. Therefore, in Diamond's book dealing precisely with the collapse of ancient civilizations, the author recommends judicious use of empirical, descriptive, and/or statistical analysis and procedures from historical and anthropological studies, (re)constructing plausible scenarios by analyzing empirical evidence and formulating hypotheses (Diamond, 2005).

CONTRIBUTIONS BY PALEOPARASITOLOGY AND THEIR PALEOEPIDEMOLOGICAL IMPLICATIONS

The possibility of interpreting scarce data from paleoparasitology is illustrated by the intestinal parasite eggs found in some archaeological sites in the Americas. As discussed in Chapters 20 and 21, the findings produced new evidence on prehistoric migrations and the peopling of the continent. Various authors have interpreted these findings for different purposes. Examples include Sorenson & Johannessen (2009), who studied trade between the peoples of the New and Old Worlds before the Discovery, and Menzies (2008), with his study on Chinese seafaring voyages to America before the Europeans. Both drew on paleoparasitological data to support their theories on contacts between Asian and American populations before the Europeans “discovered” the New World.

Importantly, most paleoparasitological tests yield only scarce helminth egg finds. This paucity could result from taphonomic processes involving desiccation or even nematophagous fungi. The parasite burden may have been truly low in some cases, e.g., due to prehistoric peoples' use of plants with anthelmintic properties (Chaves & Reinhard, 2006). Another possibility would have been variations in oviposition due to evolutionary strategies adopted by

intestinal parasites in the parasite-host relationship. At any rate, the number of eggs found in coprolites or other ancient remains is generally very low. Several factors thus limit calculation of the parasite burden or formulation of consistent hypotheses on parasitic disease(s) in the individual. In the near future, such techniques as real-time polymerase chain reaction (PCR), allowing more precise quantification of the parasite's genetic material even in the absence of eggs, may provide an approximation of the true parasite burden and its impact on the host's health.

These inferences can be made more precise by combining data from historical documents with those obtained from paleoparasitology or paleopathology. For example, the description of epidemics and evidence of the parasite or its genetic material in organic remains reinforce studies on health aspects of a given human group.

Studies may also suffer from imprecision in the coprolite's origin. When researchers find diverse coprolites in a given site, they face doubts as to whether the feces were eliminated by various individuals during a given period, or even concerning the species of the animal that produced them, except when the host's diet is known in detail or the egg finds are from species-specific parasites (Chame, 2003), as discussed later in this chapter. Such imprecisions can be substantially minimized (although not eliminated) when the samples are processed painstakingly and according to the best excavation practices. Rigorous precautions must be taken when removing the material from its original site during excavation. Such measures increase the likelihood of determining whether the genetic material belonged to one or several hosts and help ensure its organic origin. This avoids wasting time and resources in collecting and analyzing what later proves to be useless material.

For some parasites, however, although the number of eggs found in coprolites is small in samples from pre-Columbian America, the same parasites are amply recorded elsewhere in the world. This is true for the intestinal parasites *Ascaris* and *Trichuris*, which allowed formulating a comprehensive paleoepidemiological perspective, potentially helping to reconstruct the evolutionary history of *Ascaris* and *Trichuris*.

Ascaris lumbricoides finds are especially rare in pre-Columbian South America, although recent molecular studies indicate that this parasite has been under-diagnosed (Leles et al., 2008). Even more intriguing is the large number of finds of *Trichuris trichiura* eggs when compared to *A. lumbricoides*, since females of these two nematode species lay an average of twenty thousand and two hundred thousand eggs per day, respectively. Assuming that the taphonomic effect could be eliminated, given that different processes prevailed in the various environments, and assuming that this difference still persisted, it would be possible to hypothesize an evolutionary change in these parasites in their strategies for reproduction and maintenance of infection in the human host.

Much speculation has focused on whether natural and synthetic medicines may be more effective against *Ascaris* as compared to *Trichuris* (Keiser & Utzinger, 2008). For microparasites, it is known that long periods of parasite-host association do not necessarily result in beneficial associations or greater stability in this relationship, especially vis-à-vis the parasite's reproduction rate (Ewald, 1994). However, little is known in relation to macroparasites (as *Ascaris* and *Trichuris* are classified). This dynamic proves to be complex and results from the interrelationship between the two species' adaptive capacity, the parasite's pathogenicity, the interaction between the parasite and the host's immune system, and the parasite's life cycle inside and outside the host, among other factors (Ewald, 1994; Frank, 2002).

One possible explanation for the fact that *A. lumbricoides* increased its egg production over time is that the increase resulted from the use of medicinal plants by prehistoric peoples, thereby producing selective pressure. Since *A. lumbricoides* is more easily eliminated from the intestinal tract than *T. trichiura*, it tended to develop an evolutionary strategy of increasing egg production to ensure its transmission when humans began to ingest anthelmintic plants (whether as food or for intentionally medicinal purposes).

The question remains: is paleoparasitology pointing to a strategic change in the evolution of *Ascaris*, which increased its reproducibility, partly due to pharmacological pressure on its evolutionary trajectory? Such questions may soon be answered by studies of genetic material recovered directly from the archaeological record, as discussed in various chapters of this book. Whether or not changes occurred in gene sequences over time, and whether these are related to the parasite's behaviors, it will be possible to trace evolutionary processes in both the parasite and its host.

However, other aspects of paleoparasitology can be analyzed with greater precision. For example, the finding of human intestinal parasites that originated by the phylogenetic route and were thus inherited from African ancestors constitutes irrefutable proof that the hosts brought them from other lands during their prehistoric migrations.

As discussed in Chapter 8, geohelminths require specific climatic conditions to develop in the soil and be transmitted from host to host. Based on analysis of these restrictive conditions, one can infer (with a good degree of certainty) the paths taken by their hosts, that is, whether such paths favored maintenance of the infection over the course of the host-environment-host life cycle. What matters more in this case is an accurate diagnosis rather than the number of eggs. The fact that one correctly diagnoses eggs of *Enterobius vermicularis*, for example, means that this parasite, which definitely originated in African ancestors, followed the prehistoric migration routes of its human hosts, with whom it reached the territories it eventually occupied.

Equally interesting is that even rare egg finds of a given parasite in organic remains from humans or other animals means that this parasite was transmitted in this group of individuals. This can lead to a series of inferences. Cockburn (1967) was one of the first authors to use parasite biology to reconstruct ancient epidemiological scenarios. He studied the possibility of the measles virus having infected prehistoric populations, especially nomadic hunter-gatherers, discussing the minimum number of individuals capable of maintaining the virus in circulation without interrupting the transmission chain. Measles, among other viral infections, can only be maintained in population groups with more than two hundred thousand members, or the sufficient number of individuals to contain the minimum amount of susceptible and infected individuals capable of maintaining transmission to new hosts. Contemporary medical geography features classical studies on this subject, such as the work by Cliff and his research group on the emergence and extinction of epidemics on islands (Cliff, Hagget & Smallman-Raynor, 2000).

Likewise, the wide dispersal of most felines in forests would explain why these animals display no major epidemics, but only infections caused by some parasites showing greater infectivity and resistance during their cycles outside the host, i.e., greater capacity to survive for prolonged periods in the environment (Anderson & May, 1992).

Analogously, one can reconstruct the epidemiological scenario of the hunter-gatherers. Nomadic or seminomadic groups of hunter-gatherers consisted of small numbers of persons, some 25 to 30 individuals circulating in extensive, borderless territories, although the spaces might be occupied by different bands (Campbel, Loy & Cruz-Urbe, 2005). First, the reduced number of persons would limit many parasitic infections. Assuming that in a band of 30 individuals, all were susceptible to (and exposed to) infection with the measles virus, they would soon all become infected and would quickly be cured and immune to new infection, or have died of the disease. The virus would no longer circulate (Cockburn, 1967).

Meanwhile, other parasites would have experienced no difficulty in surviving in these small groups, even with low parasitism. An example is *Enterobius vermicularis*: this parasite developed various modes of host-to-host transmission, quite effective for adaptation. *E. vermicularis* can transmit from one host to another, and its eggs remain infective in the soil for several months. Thus, even if bands of hunter-gatherers roamed across extensive territories, when they returned to camp at previously occupied sites, the eggs in the soil could infect new individuals or re-infect previously infected ones.

Reinhard (1992) studied pinworm infection in seminomadic hunter-gatherer/agriculturalist peoples of the Arizona Desert in North America. He explains the parasite's transmission inside the adobe dwellings built in the rock shelters by the Ancestral Puebloans. Similar studies (Hugot et al., 1999) have discussed different transmission routes and parasite burdens, suggesting that the aerial route, considered a novelty in transmission to humans, was favored by the change in behavior from nomadic hunter-gatherers to a more sedentary lifestyle, initially associated with cave-dwelling, with more limited ventilation.

Tuberculosis is also interesting for studying diseases in pre-Columbian America. Considered nonexistent among the native peoples of the Americas, TB was purportedly introduced by the Europeans when they discovered and colonized the New World. In the case of Brazil, it was assumed that Jesuit priests played a key role in transmitting *Mycobacterium tuberculosis* to indigenous peoples. When they came to catechize the natives, the Jesuits also intended to enjoy what they considered a healthy environment, favorable for curing their maladies, as in the case of Father Anchieta. The Jesuit priests were thus considered the main culprits in the introduction of *M. tuberculosis* to Native American peoples (Ruffino Netto, 1999).

Paleoparasitology has disproven the introduction of *M. tuberculosis* infection exclusively during the colonial period. Typical cases of skeletal tuberculosis and recovery of genetic material from the bacillus have been described in various archaeological sites in the Americas, dated far before the Europeans' arrival. Tuberculosis already existed in pre-Columbian America (Souza, Carvalho & Lessa, 2003). At the time, there were huge differences between the various peoples inhabiting the continent (Heckenberger et al., 2008). The population contingent varied greatly from region to region.

The peoples that inhabited the Andean Region increased in number when compared to the groups in the South American lowlands. But there were also substantial differences among the latter population groups. For example, the Brazilian coast was inhabited by populations with considerable numbers of individuals, more than two hundred thousand, for example (Gaspar et al., 2008), capable of maintaining various parasites in circulation, including viruses, bacteria, protozoa, helminths, and others.

In fact, the Europeans' arrival produced enormous changes in the organization of the life of these peoples and their interaction with the natural and social environment. The Jesuit Reductions were one of these transformations. This form of protection of indigenous peoples led to the clustering of villages in cramped territories, facilitating circulation of parasites. Even if the priests cannot be blamed for introducing infections like tuberculosis into the New World, they contributed (although involuntarily) to facilitating its circulation.

Chapters 5 and 20 of this book present an interesting example. Chapter 5 discusses the peopling of Patagonia and its colonization process. Chapter 20 analyzes the transition resulting from changes in the diet of indigenous groups and the parasites found in the coprolites left by them. Extensive evidence in food remains indicates reliance on wild resources, with the presence of parasites of animals (whether or not establishing true infection in the human host) and the emergence, resurgence, and accumulation of other parasite eggs such as *A. lumbricoides* and *T. trichiura* after the Europeans' arrival.

Meanwhile, the first Spanish and English that arrived and set up colonies in southern Patagonia also attempted to adapt to the environment, but were not initially successful. The paleoparasitological record includes data that elucidate these events.

Another process of environmental modification in the social ecology of infectious diseases (Mayer & Pizer, 2007) was perhaps more important and contributed considerably to the spread of infection among Amerindian peoples and

colonizers. The reproduction of European urban models used houses whose architecture aimed to protect the residents from purported miasmas. The colonizers built their living quarters with the typical blinds or jalousies and the alcove (bedroom) located at the center of the residence, with no lighting, ventilation, or communication with the outdoors, and with virtually no facilities for running water or sewage disposal (Cavalcanti, 2003).

Brazilian colonial towns were built packed together, with people circulating in cramped spaces. The indigenous peoples also migrated to the towns, and there was a constant increase in the African slave trade, with the slaves living in abject servitude, under adverse health conditions (Sousa, 1998). The majority were occupied in urban labor, a context in which the “house slave” emerged. In the early 19th century, an average colonial family in Rio de Janeiro had an estimated seven to eight negro slaves living in the home (Ruffino Netto & Pereira, 1981), and with them, new parasites, habits, and customs.

Syphilis also has an old history in the Americas. Extensive literature discusses the origins of the different *Treponema* species. Whether syphilis originated in the Americas and spread from there to Europe with sailors in Columbus’ returning fleet (the Columbian hypothesis) or already existed in the Old World, with different clinical forms (the pre-Columbian hypothesis) is still subject to debate.

Epidemic syphilis irrefutably occurred in late 15th century Europe, initially with a highly virulent form. However, there are two conflicting hypotheses for its origin. The first contends that syphilis already existed in the Old World before Columbus’ voyage to the Americas, but that it was absent from New World indigenous groups. Infected sailors were responsible for spreading the infection when they reached the New World.

The second hypothesis is exactly the opposite: the indigenous peoples infected the sailors, who returned to Europe and spread syphilis, previously unknown in the Old World. The support for this second hypothesis comes from the purported lack of European human remains with syphilitic lesions prior to 1493, in contrast to abundant evidence after this date. However, skeletons with signs of *Treponema* infection, some clearly suggestive of syphilitic lesions, have recently been discovered in the Old World with dates prior to the 15th century.

According to the Columbian hypothesis, syphilis was introduced into the Old World by Columbus’ fleet upon their return from the Americas. In Europe, the infection found favorable cultural, social, and biological conditions for its spread, including urban crowding, major population shifts and contingents (mainly associated with wars), and changing sexual behaviors, favoring the establishment of a susceptible population in permanent interaction (Celentano et al., 2007). This hypothesis appears to be corroborated by the large number of findings of individuals with treponemal lesions in New World prehistoric material, contrary to the Old World, and by descriptions of lesions consistent with treponemal diseases in Native Americans by the first Europeans that reached the Americas (lesions that were considered nonexistent in Europe). Added to this was the sudden description of symptoms, contagion, and course of the infection that emerged in the European literature after the 15th century.

Considering these two conflicting hypotheses in the international literature, this remains an open question. It may soon be answered using other tools, most recently from molecular biology (Steinbock, 1976; Rothschild et al., 2000; Sefton, 2001; Meyer et al., 2002). Molecular assays have been applied to skeletal archaeological material with syphilitic lesions. However, the results so far require a critical stance (Kolman et al., 1999; Bouwman & Brown, 2005). The first studies with a phylogenetic approach in the attempt to elucidate the origin of syphilis point to the Columbian hypothesis (Harper et al., 2008).

One of the most interesting questions on the origin of treponemal diseases was raised by epidemiologist Robert Desowitz in his book *Who Gave Pinta to the Santa Maria?* (1998). He discusses the various clinical manifestations of

syphilis, highlighting aspects of its pathogenicity, virulence, and case-fatality in the European population from 1493 to 1510, in contrast to its purportedly benign course in the pre-Colombian Amerindian peoples.

As discussed in Chapter 26 (on Chagas disease in prehistoric America), *Trypanosoma cruzi* must have infected humans as soon as they occupied habitats favorable to the vector and animal reservoirs. Outside the Andean Region, in the United States and Brazil, cases of megacolon have been described in mummified bodies with a considerable accumulation of feces. This severe condition was the probable cause of death in the two individuals described by Reinhard, Fink & Skiles (2003) and Fernandes et al. (2008). Chagasic infection existed in prehistoric groups in Brazil some 7,000 years ago (Lima et al., 2008). It affected both nomadic hunter-gatherers and agriculturalist hunter-gatherers as long as 1,200 years ago (Fernandes et al., 2008). For shelter, both types of groups used the caves in the limestone outcropping of the *cerrado* (savannah) in what is now Minas Gerais State. These caves are inhabited by wild triatomine species that transmit *T. cruzi* and by mammalian reservoirs. The caves and rock shelters served not only as the band's temporary dwellings or camps, but also for the agriculturists to store food and bury their dead.

The remains left by these groups included baskets of food, especially grain, which must have attracted small animals like rodents, some of which were efficient reservoirs for *T. cruzi*. The conditions thus existed in pre-Columbian groups for the transmission of *T. cruzi* and cases of acute or chronic Chagas disease. What manifestations or impact might Chagas disease have produced in these populations? Chronic cases are nicely illustrated by the North American finding described by Reinhard, Fink & Skiles (2003). The individual died at around 35 to 45 years of age and received an elaborate funeral, with various accoutrements, the most evident of which was a leather band wrapped around his abdomen, showing the special care taken with that individual.

The body had a huge amount of feces accumulated in the intestine, and his companions apparently attempted to relieve his suffering by wrapping him with the leather band to contain his abdominal distension. Tests indicate that he died of a ruptured intestine and systemic infection (Reinhard, Fink & Skiles, 2003). The case shows that this prehistoric group had the knowledge and experience to deal with a chronic health problem (specifically Chagasic megacolon) that must have been relatively common at the time.

Other situations illustrate the potential of paleoparasitology to elucidate longstanding questions. For example, the PhD dissertation by Diana Maul de Carvalho on the Athens plague (Carvalho, 1996) analyzes the different possible diagnoses for the disease while reconstructing life in ancient Greece. However, more recent findings suggest a different diagnosis for the epidemic, discussing the finding of genetic material from *Salmonella enterica* serovar typhi and its possible role in the Athens plague (Papagrigorakis et al., 2006).

Recent studies by Bianucci et al. (2007, 2008) point to new research paths using the rapid diagnostic dipstick test for *Yersinia pestis* F1 antigen to investigate the plague in ancient human remains. Samples studied thus far cover periods of the plague epidemic in 16th, 17th, and 18th-century France. Such advances definitely open new and interesting analytical prospects, capable of answering questions on the parasite's evolution and possible changes in its pathogenicity over time (Bianucci et al., 2007, 2008). Chapters 18 and 19 of the current book explore these issues quite thoroughly, dealing with the recovery of genetic material from ancient remains, but the same issues cut across virtually all of the chapters. As highlighted in the opening chapters, the phenomenon of parasitism belongs to the domain of ecology and is thus eminently evolutionary.

FINAL REMARKS

Despite all the potential shortcomings in the application of epidemiological methods to studies on infections and diseases of the past, as discussed by Souza, Carvalho & Lessa (2003), the new findings in paleoepidemiology unveil absolutely unprecedented possibilities for the reconstruction of ancient events. The currently available techniques allow recovering genetic material to back interpretations dated to such times. There is no doubt that the past can be understood through reliable diagnostic methods for parasitic infections, reconstructing specific scenarios for each human group inhabiting the different environments where *Homo sapiens* roamed and settled.

Since the trajectory of parasites and hosts is inseparable, the future will reveal a new evolutionary history of paths that date back to the very origins of life (Araújo et al., 2003), when the intermolecular relations were shaped that allowed the emergence of life on the planet.

As invariably happens with the various fields of contemporary science, modern epidemiology has much to learn from the history of humankind and Earth, and especially from paleoepidemiology.

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