Struck by pestilence

Investigating the ancient pathogen landscape within and across archaeological sites using metagenomics and phylogenetics

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Abstract

How can we detect ancient pathogens in human remains? This thesis addresses the question by investigating the ancient pathogen landscape within and across European archaeological sites from the Middle Ages to the early modern period, a time when crowding, urban growth and mobility likely amplified transmission. Rather than focusing on a single pathogen, I approach each site as a bioarchaeological context to understand the life, infection and death of past communities, using phylogenetics only where warranted to place signals in time and space.

The introductory essay synthesises why detection succeeds or fails. Outcomes depend on pathogen ecology, load and genome size, as well as contamination, database composition and bioinformatics errors. Teeth are often considered to record bloodstream infections because they are well vascularised during life. Alternative routes, including ingress through caries or periodontal breaches and taphonomic transport, are likely contributors to detection as well, and these mechanisms are not mutually exclusive. I outline practical filters for presence and ancient status used in my analyses, many implemented in our metagenomics workflow, aMeta. I also show how secondary checks such as evenness-of-coverage proxies, competitive mapping to close relatives and sequence-similarity queries against NCBI's nucleotide database often support genuine presence more reliably than raw read counts or depth of coverage. Indeed, viruses have small genomes, variable loads and fast mutation rates, while eukaryotic parasites have very large genomes, which together limit coverage in metagenomic data.

In Chapter I, we present aMeta, co-developed with SciLifeLab, which narrows candidates with a *k*-mer classifier using taxa-specific DNA sequences of length *k* and then authenticates them with lowest common ancestor alignment metrics, reducing false positives and false negatives while supporting large reference databases. In Chapter II, I perform a context-focused metagenomic screening of the medieval necropolis of Las Gobas and describe multiple zoonoses, some novel and some rarely reported in ancient DNA, including variola virus, demonstrating the value of testing all that is present rather than only traditionally prioritised targets. In Chapter III, I examine an Islamic cemetery in Ibiza, where I identify and perform capture enrichment on a case of *Mycobacterium leprae* infection (the agent of leprosy) in an individual buried under conditions similar to the other burials. I also show that careful screening below conservative thresholds reveals common infections such as hepatitis B virus and human parvovirus B19, the latter often overlooked. In Chapter IV, in collaboration with researchers at the Karolinska Institute, we recover human parvovirus B19 of genotype 2 from the shipwrecks *Kronan* and *Mary Rose*, bridging a European temporal gap in its historical genomic record. In Chapter V, I detect malaria in several members of the *Mary Rose* crew, illustrating low-coverage eukaryotic pathogen detection and shedding light on species diversity within ague (the contemporary term for malarial fevers) in Tudor England.

Taken together, the chapters highlight the advantage of pathogen-agnostic investigation, favoured here by site-led screening, though the same logic scales to larger datasets. Site-focused disease detection, without privileging well-known or high-coverage taxa, provides archaeological clues that offer new insights into site histories, for example, zoonoses suggesting close contact with animals and low-coverage malaria in the *Mary Rose* crew. Investigating within sites, then across them, shows that many infections in medieval and early modern Europe were present but under-detected, and refines how we understand the pestilence past communities were plagued with.

Keywords: *Ancient pathogens, Ancient metagenomics, Zoonoses, Ancient DNA, B19V, Mycobacterium leprae, Variola virus, Erysipelothrix rhusiopathiae, Leptospirosis, Relapsing fever, HBV, Malaria, Phylogenetics, Archaeogenetics.*

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