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Museums and disease: Using tissue archive and museum samples to study pathogens

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SUMMARY

Molecular studies of archival and fossil samples have traditionally focused on the nucleic acids derived from the host species. However, there has recently been an increase in ancient DNA research on the identification and characterization of infectious agents within the hosts. The study of pathogens from the past provides great opportunities for discovering the causes of historical infection events, characterizing host-microorganism co-evolution and directly investigating the evolution of specific pathogens. Several research teams have been able to isolate and characterize a variety of different bacterial, parasite and viral microorganisms. However, this emerging field is not without obstacles. The diagenetic processes that make ancient DNA research generally difficult are also impediments to ancient pathogen research and perhaps more so given that their DNA may represent an even rarer proportion of the remaining nucleic acids in a fossil sample than host DNA. However, studies performed under controlled conditions and following stringent ancient DNA protocols can and have yielded reliable and often surprising results. This article reviews the advantages, problems, and failures of ancient microbiological research.

1. Introduction

The study of ancient DNA has influenced many molecular biology based fields from molecular evolution to population genetics and genomics. The discipline has not had a smooth development from novelty to established methodology. Many of the studies from the early 1990s were later found to be erroneous due to the lack of rigorous controls and protocols for preventing contamination by foreign DNA. There was also a fundamental absence of basic theory concerning the characteristics of ancient DNA (i.e. fragmentation, deamination, etc.). This was universally the case for reports of DNA from samples over 1 million years old (Willerslev and Cooper, 2005). Thus, spectacular claims of dinosaur DNA sequences retrieved from amber inclusions are largely thought to have been laboratory artifacts (Paabo et al., 2004). As the process of DNA degradation over thousands of years has begun to unravel and the risks of contamination in the environment and laboratory taken seriously, proposals for general authentication were published (Cooper and Poinar, 2000). Some of the proposed criteria have been modified, and their necessity questioned over time, but adherence to most of these protocols are widely accepted and have been a common feature of ancient DNA reports since the 1990s.

Ancient microbiology and the characterization of ancient pathogens have emerged within the expanding field of ancient DNA. Since 1993, several researchers have reported the identification and characterization of different paleopathogens isolated from various types of samples and environments ranging from museum bone samples to permafrost tissues. The search for paleopathogens in ancient remains began with Spigelman and Lemma (1993) who reported the isolation of the *Mycobacterium tuberculosis* from skeletal remains of the 14th–16th century. The identification of microorganisms from ancient DNA developed along with the technologies that gave rise to the general discipline of ancient DNA and was duly designated paleomicrobiology (Drancourt and Raoult, 2005). The development of this field is continuing as technological advances in high throughput sequencing occur. However, there are serious impediments to this research. Pathogens will frequently be less well represented in a given sample than the mitochondrial and nuclear DNA of the host considering that during the course of infection, not every cell will be infected and thus, there will generally be less pathogen than host DNA present. In addition to the amount of pathogen present in the organism at the time of death, sample age, burial environment, DNA degradation and oxidation are factors that will determine whether or not infectious agents from the past remain detectable by PCR or a similar technique. Furthermore, ancient DNA based pathogen research is faced with the additional problem of sample contamination by related but poorly characterized microbes that are present in the environment. Museums also pose many problems as specimens may come

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	1993	1995	2000	2005	2011 [A]
Ancient Pathogen Publication Timeline	1	2	17	23	16

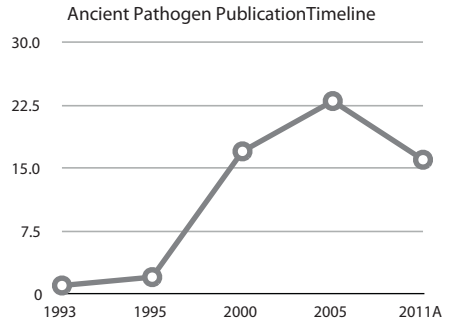

Fig. 1. Graph depicting the increase in ancient pathogen studies in the last 17 years.

into contact with each other and cross pollute the specimens with both host and microbe DNA. Thus, few target molecules for analysis and additional contamination risks pose unique hurdles for paleomicrobiology.

The promise of this new discipline is that it can provide evidence that may change our understanding of historical infection events in a way that cannot be done by examining modern pathogens. For example, many pathogens, viruses in particular, change so rapidly or can exchange genetic information so readily that determining their origin or relationships is very difficult or impossible using contemporary DNA analysis. Similarly, some pathogens may represent recent zoonotic or epizootic events which can directly be determined by accessing the appropriate tissue archival collections to look for the presence or absence at a particular time point. Like the species they inhabit, some microorganisms may also have become extinct with their hosts and thus the tremendous diversity of microbes observed today may itself be an underestimate of the diversity of the recent past. Paleomicrobiology can provide information on the introduction, development, host–microorganism interaction, and evolution of all detectable pathogens that either were or still are infecting humans and animals. The majority of the studies of microbes have been on bacteria and have concentrated on identification and classification. The list of identifiable paleopathogens has increased since 1993, but the majority of these reports are on the identification of *M. tuberculosis* from several different types of tissues (Fig. 1) (Salo et al., 1994; Mays et al., 2002; Rothschild et al., 2001). Investigations targeting *Yersinia pestis* as the cause of the "Black Death" (Raoult et al., 2000) have also been given the most attention and thus notoriety.

Virology has benefited from ancient DNA with the examination of various retroviruses (Calvignac et al., 2008; Worobey et al., 2008), which have provided tremendous insights into the origins and spread of viruses such as specific human immunodeficiency virus-1 (HIV-) subgroups and simian T cell leukemia virus-1 (STLV-1). A great success story in ancient virology is the determination of the complete genome of an RNA virus. The characterization of the notorious 1918 "Spanish" influenza virus (Reid et al., 2000; Taubenberger et al., 1997) has provided sequence information for a major pathogen that was effectively extinct but has been revived and characterized using ancient RNA. The complete genome has been sequenced and modern virological methods have been used to characterize this "revived" virus. It serves as an example of the largely untapped potential of museum collections and medical/veterinary tissue archives. The purpose of this review is to provide a thorough overview of the research on paleopathogens and to emphasize studies which do and do not adhere to rigorous ancient DNA authenticity standards. Much like organismal ancient DNA research, the microbiological research community has had to learn the advantages of rigorous contamination avoidance protocols by suffering some of the disadvantages and controversies that occur when the protocols are ignored.

2. Paper selection criteria

For the purposes of this review, we searched for all ancient DNA research on each bacterial, viral and parasite pathogens in order to obtain the most comprehensive coverage of the published literature on the subject. There has also been work on non pathogenic microorganisms such as bacteria from insects in amber inclusions that will not be covered here. Thus, articles were searched for in PubMed and Google Scholar using the following keywords in several different combinations: ancient DNA, archival samples, paleopathology, paleovirology, paleomicrobiology, bacteria, virus, parasites, trypanosome, retrovirus, mycobateria, *M. tuberculosis*, *Mycobacterium leprae*, *Mycobacterium bovis*, Endogenous retroviruses, *Y. pestis*, Spanish influenza, HIV, HTLV, STLV, *Salmonella enterica*, *Plasmodium falsiparum*, *Borrelia burgdorferi*, *Rickettsia prowazekii*, *Bartonella henselae*, *Bartonella quintana*, infectious diseases. For completeness, this review includes letters to the editor and comments, since we feel they provide additional information about questions of validity on specific reports. The only criterion that we used to exclude journals was language; we only included papers that were written in English. Any omission of articles is unintentional.

3. Authenticity criteria

Regardless of the goals of a given ancient DNA study, authenticating any sequences obtained is of crucial importance. Several papers have been published that recommend precautions and expected observations that, when followed, lead to results that have high confidence of authenticity. The most commonly used recommendations are physically isolated work area, negative control PCR amplifications, appropriate molecular behavior of amplified products, e.g. the larger the amplicon the weaker the product should be, reproducibility of results within a laboratory and in an independent laboratory, determining sequences from multiple clones and not direct sequencing, testing for general biochemical preservation of samples to be tested, quantitation of endogenous molecules in the same by quantitative PCR, and determining the presence of endogenous DNA in remains associated with the fossils of interest. Cooper and Poinar (2000) proposed these nine criteria as the gold standards of ancient DNA research. However, complete compliance with these recommendations does not guarantee authenticity of the results. For example, if a sample has been contaminated in the excavation/pre-analysis phase, one may retrieve reproducible results with all negative controls demonstrating lack of contamination. However, the result is still derived from a contaminant. Nonetheless, it is important to follow most of the suggested steps as they do aid in detecting common laboratory errors. For the purpose of this paper we decided to use seven out of nine criteria of Cooper and Poinar (2000, see Box 1) in order to compare the selected papers (Table 1). We decided to leave the biochemical preservation analysis and quantitative PCR out of the criteria used in our comparison table. Amino acid racemization analysis is a pre-extraction screening method used in order to demonstrate biochemical preservation in an ancient sample (Poinar et al., 1996). Amino racemization as a proxy for potential sample preservation state and retrievability of ancient DNA is largely not practiced, and has recently been called into question as an effective pre-screening