Comment

Pathogenic microbial ancient DNA - a problem or an opportunity?

The recent review on ancient DNA in this journal by Willerslev and Cooper (2005) is timely. It follows more than a decade of intensive research in this area, and others have also seen the need to review progress and reflect on the future (Pääbo *et al*. 2004; Cipollaro *et al*. 2005; Nicholls 2005). Since the first papers in this field, the majority of studies have dealt with population genetics, diet, domestication, and evolution of mammals and birds. The study of ancient microbial DNA developed shortly after, and has led, for example, to studies of bacteria in amber (Bada *et al*. 1999; Greenblatt *et al*. 2004) and microbes retrieved from the depths of the permafrost (Christner *et al*. 2003).

A distinctive branch of ancient DNA research is the study of pathogenic microbial DNA within the bodies of infected host species, primarily humans. There is increasing interest in this field, but in order to critically assess this body of research, readers should be aware of the differences caused by the characteristics of the microbial pathogens, and their distribution within the host.

Microorganisms differ in the susceptibility of their DNA to the initial decay processes and the physico-chemical changes over time. Mycobacteria, including the causative agents of tuberculosis and leprosy, have resistant hydrophobic cell walls that have been shown to physically persist for at least 250 years (Donoghue *et al*.2004). In addition, they are members of a family with DNA rich in guanine and cytosine which confers greater stability (Pääbo *et al*.2004). Therefore, it is not surprising that *M. tuberculosis* and *M. leprae* are the subjects of the majority of ancient microbial pathogen papers (Donoghue *et al*.2004; Drancourt & Raoult 2005). In contrast, *Treponema pallidum*, the causative agent of syphilis, is an exquisitely

environmentally sensitive spirochaete, and its DNA very labile. Gram-negative bacteria are a little more robust and the DNA may persist in especially protected sites such as dental pulp.

Pathogenic microorganisms and their DNA will always be a minor component within a host, so it is necessary to know the sites where the particular pathogen will be localised. Initially, attention was directed to bony lesions in suspected tuberculosis cases, yet even in the pre-antibiotic era these were rare, occurring in only 3-6% of cases. The majority of patients died from disseminated disease before lesions had time to form. Lack of understanding this point led to classical palaeopathologists querying reports that *M.tuberculosis* DNA could be detected in bones without lesions (reviewed by Donoghue *et al*.2004; Zink *et al*. 2005). We have detected *M.tuberculosis* DNA in over 50% of ribs from an 18th century Hungarian population yet <5% showed any pathological changes (updated data from Fletcher *et al*. 2003a). Here, the visceral surface closest to the lung was examined, not the inner tissue, and usually the upper ribs where a primary focus of infection is most likely.

Other diseases are highly localised. *M. leprae* DNA is primarily found in the *cavum nasale* and maxilla; diarrhoeal diseases are restricted to the intestinal tract, although they may spread to the liver, e.g. *Schistosoma mansoni* (Rutherford 1999). In meningitis, pathogens are restricted to the CSF and meninges, so only vertebrae and skull samples may be positive. Septicaemic diseases, such as bubonic plague and disseminated tuberculosis, will result in pathogen DNA being distributed around the body via the blood. In these cases bone marrow and dental pulp should be examined (Wiechmann & Grupe 2004; Drancourt & Raoult 2005). These sites should also be examined in suspected cases of blood-borne parasites, for example, of malaria (Sallares & Gomzi 2001).

Syphilis was an early focus of interest because of the clear palaeopathological markers of the disease. However, this is a disease where knowledge of its natural history is crucial.

Although there is a septicaemic stage of the disease with high numbers of organisms, this is rarely the cause of death. It is tertiary syphilis that kills, yet any medical textbook will point out that the characteristic lesions of tertiary syphilis contain few, if any spirochaetes. This, together with the fragility of the pathogen, explains the negative findings (Bouwman & Brown 2005).

Examination of human tissues for microbial pathogens requires modification of procedures used for extraction of host DNA. Pathogenic species are not normally present in healthy individuals, and several, such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* have no known environmental reservoir. Therefore, their detection indicates infection and the site may demonstrate whether this was localized or disseminated. Hemi-nested and nested PCR are commonly used, due to the initial low number of target molecules. More rigorous DNA extraction procedures may be necessary for microbes with persistent cell walls. Contamination from investigators is less of a problem, compared with the need to exclude modern human DNA from ancient DNA studies. Even so, strict precautions against cross-contamination are taken and it is common practice for independent verification to be carried out, often in more than one other laboratory (Spigelman *et al*. 2002; Fletcher et al 2003a,b; Donoghue *et al* 2005). The use of species-specific loci for PCR is widespread, and for those used in clinical diagnostic microbiology, there are extensive data on their stability and specificity. This has led to the possibility of molecular epidemiological studies (see Donoghue *et al*. 2004, Drancourt & Raoult 2005) that throw light on the evolution and spread of microbial pathogens in the past.

In conclusion, rather than ancient microbial DNA being a problem (Willerslev & Cooper 2005), we agree with Pääbo *et al*. (2004) that this field, in particular that of pathogenic microbial ancient DNA, is potentially very exciting, and is already reaping results.

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