Mini Review: Dental Pulp as a Source for Paleomicrobiology

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Abstract

Paleomicrobiology is a recent science. Paleomicrobiology describes the history of infectious diseases through research of ancient microbes. The dentistry with the tooth in particular has contributed to the progress of this science. The dental pulp is very well protected in the centre of the tooth. Molecular techniques such as “suicide PCR” and Multiple Spacer Typing have identified and characterized micro-organisms in the ancient samples. The detection of bacterial DNA from ancient dental pulp provided the scientific evidences for diagnosis of the infectious diseases of the past. The cooperation of paleomicrobiology and paleodontology also contributed the new knowledge to human pathology.

Keywords: Paleomicrobiology; Ancient Teeth; Dental Pulp; Microbial Detection

Introduction

In 1993, after the first molecular detection of Mycobacterium tuberculosis in ancient skeletons (1), paleomicrobiology appeared as a new discipline for the identification and characterization of microorganisms in the ancient samples. Frozen tissues, mummies, skeletons or bones were the ancient remains often used in paleomicrobiological studies. However, the major limitations of the ancient DNA (aDNA) study consist in the degradation and fragmentation of aDNA, contamination of modern DNA or external sources. Moreover, aDNA extraction from mummified tissue and bones is complicated because rehydration and decalcification are necessary steps. Therefore, another source of ancient specimens that possibly limited the disadvantages above, were proposed: dental pulp (2), Figure 1. Vascularization of this organ is important, proportionally comparable with the human brain (3). Pathogenic bacteria circulating in blood may occur and colonize the dental pulp. A guinea-pig

model has confirmed the possibility to detect bacterial DNA in dental pulp after experimental infection by Coxiella burnetii. This experimental model has also confirmed that pathogenic bacteria in blood had penetrated and colonized the dental pulp and that could then be used for diagnosis of bacteremia (4,5). Moreover, Bartonella henselae DNA and Bartonella grahamii DNA had been isolated from the dental pulp of the cats that had been bacteremic for Bartonella sp. then euthenased and buried one year previously, according to French army veterinary records (6). These results indicate that dental pulp was an excellent specimen for diagnosis of ancient bacteremia.

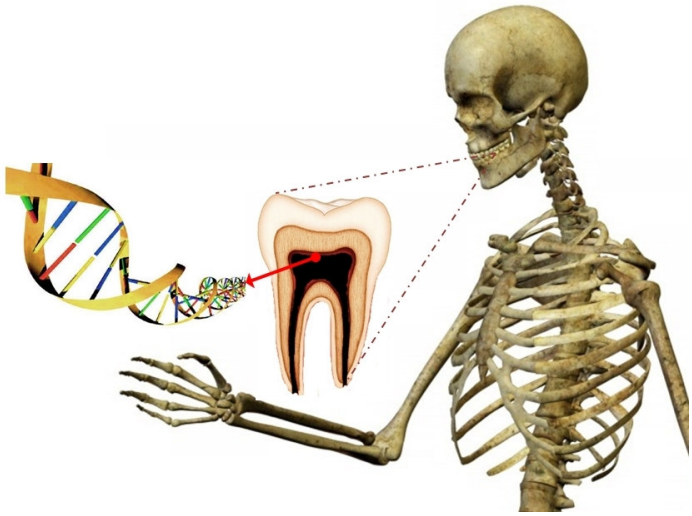

Figure 1 Ancient DNA recovered from dental pulp.

Paleomicrobiology of the dental pulp: methods

In 1998, a team of researchers in France had first introduced dental pulp collected from ancient teeth as a suitable tool for the diagnosis of ancient infectious diseases (7). The advantages of using dental pulp for paleomicrobiological study is its better preservation in a closed cavity. In the closed cavity, the soft tissues are preserved from environmental contamination and attacks of degrading agents. Moreover, the DNA extraction from soft tissues of the dental pulp is simple (2). An experimental animal model infected with Coxiella burnetii had also confirmed the possibility of finding bacterial DNA in dental pulp 20 days after bacteremia (4). A second guinea-pig model infected with Coxiella burnetii showed persistence of the bacteria in the dental pulp (5). The pulp can then be used as a tool for retrospective diagnosis when there was a bacteremia. Afterwards, this team proposed the “suicide PCR” for molecular identification of bacterial DNA from ancient teeth with strict criteria to avoid any risk of contamination and Multiple Spacer Typing for the genotyping of ancient bacteria (8). The important criteria of “suicide PCR” included: 1- The primers are used only once, 2-There were no positive controls, 3-The negative controls should be added, 4-Negative results are tested by other primers, 5-Positive results are confirmed by sequencing (9). The suicide PCR principle was used by another Greek team in 2006. This team using dental pulp has shown that the plague of Athens was in

fact typhoid fever (10). Rapid Diagnostic Test was an immunodetection method developed by another team for special diagnosis of plague from ancient remains (11). From 1998 to the present day, several independent teams using these methods, detected multiple pathogens from ancient dental pulp to confirm the epidemics of the past, Table 1.

Table 1 Microorganisms detected from ancient teeth

Microorganisms	Infectious diseases	References
Anelloviridae	Virus	25
Bartonella henselae	Cat-scratch disease	24
Bartonella quintana	Trench fever	17-19;21;22
Mycobacterium leprae	Leprosy	23
Mycobacterium tuberculosis	Tuberculosis	23
Rickettsia prowazekii	Typhus	21;22
Samonella enterica serovar Typhi	Typhoid fever	10
Yersinia pestis	Plague	7-9;11;13-18

Paleomicrobiology of the dental pulp: contributions

In history, the three plague pandemics were cited as humanity's disaster, in which an epidemic known as Black Death occurred in 1347 and then spread from the Caspian Sea to almost all European countries. Over the next few years, Black Death had killed an estimated 17 – 28 million Europeans, representing approximately one third of the population at the time (12). In 1998, Yersinia pestis DNA had first been identified in dental pulp of the ancient teeth collected from skeletons excavated from 16th and 18th century graves of persons suspected of having died of plague (7). Genotyping had then yielded Y. pestis Orientalis strain (8,13). These results were later reinforced by other independent teams to confirm the presence of plague in Medieval Europe (11,14-16). Interestingly, two recent studies detected co-infection of the body louse, Bartonella quintana, along with Yersinia pestis in ancient dental pulp, suggesting that the body louse may have been a vector for interhuman transmission of medieval plague pandemics (17-18).

Bartonella quintana is the causative agent of trench fever disease and transmitted by lice, fleas and ticks to humans. Molecular detection of B. quintana in a 4000-year-old human tooth (southeastern France) indicated that B. quintana bacteremia had occurred in prehistoric humans 19. Typhus epidemic had been demonstrated in the 20th century as "a best friend" of wars. In the past, this epidemic spread rapidly in Europe from Spain to France and Italy following the wars between Emperor Charles V and King François I (20). The city of Douai in northern France was besieged from 1710 to 1712 during the war of Spanish succession when France and Spain opposed the other nations. The causative agent of typhus, Rickettsia prowazekii strain Madrid E type B, and B. quintana DNA had been isolated from ancient dental pulp of the individuals buried in Douai. This molecular detection

allowed the researchers to confirm the presence of the typhus epidemic in Douai from 1710 – 1712 (21). Typhus was later a constant companion of Napoleon's army in all European wars. In 2006, evidence of louse-transmitted diseases in soldiers of Napoleon's Grand Army in Vilnius was reported when R. prowazekii and B. quintana DNA had been amplified by "suicide PCR" from dental pulp collected from the remains of the soldiers. These results showed that louse-borne infectious diseases had affected nearly one-third of Napoleon's soldiers buried in Vilnius and suggested a possible cause of the French retreat from Russia (22), Figure 2 and 3.

Mycobacterium tuberculosis and Mycobacterium leprae had also been detected in ancient teeth recovered from a 1st century tomb in Jerusalem, Israel. The findings had suggested the co-infection of these two mycobacteria in ancient human populations (23). Moreover DNA of Samonella enterica serovar Typhi had been successfully identified in dental pulp of ancient teeth collected from Kerameikos cemetery. This discovery had demonstrated that typhoid fever could be the cause of the plague of Athens in 430 – 426 BC (10). Interestingly, molecular detection of Bartonella henselae in dental pulp of 800-year-old cats suggested the occurrence of Cat-scratch disease within a medieval population in France because archaeological and historical data indicated that these cats had lived in close contact with people (24). A recent study had surprisingly reported the detection of an ancient virus (Anelloviridae DNA) from 200-year-old dental pulp recovered from ancient teeth of Napoleon's soldiers in Kaliningrad, Russia (25).

Figure 2 and 3 Vilnius, 1812, Pictures of Yann ARDAGNA (UMR 6578 CNRS Université de la Méditerranée-CNRS-EFS

Perspectives

X-rays were used to detect and locate the target teeth in the jaws (Figures 4 and 5). The ancient tooth was usually bisected longitudinally for the recovery of dental pulp (Figures 6, 7 and 8). This method led to the destruction of its morphological structure and possible external contamination . “Orthograde entrance technique” is an “original method” to collect dental pulp with morphological conservation of ancient teeth that will be used afterwards by anthropologists (26). However, this technique has some disadvantages due to the relatively fragile, permeable and weak nature of the tooth root. The first method is probably the simplest and most effective despite the disadvantage of morphological changes of the tooth, it is necessary to restore the tooth by bonding techniques.

The ancient proteins seem to be more resistant than aDNA. Lipids such as mycolic acids of M. tuberculosis also appear to be particularly robust in ancient remains (27). Mass spectrometry had successfully identified specific bacterial proteins of M. tuberculosis from archeological bone samples (28). Therefore, this method, detecting microbial non-nucleotidic biomolecules from ancient dental pulp, may hereafter become a promising approach to complement aDNA analysis in paleomicrobiology.

In conclusion, detection of pathogen traces from ancient teeth by using many paleomicrobiological methods has provided the scientific proofs to elucidate several infectious diseases in the past. This great cooperation of paleomicrobiology and paleodontology has also contributed new knowledge of human pathology as well as evidence of human history.

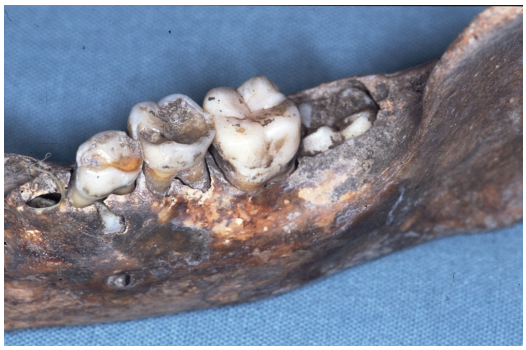

Figure 4 Mandible of a child about 9 years (Observance massgrave-Marseille 1724)

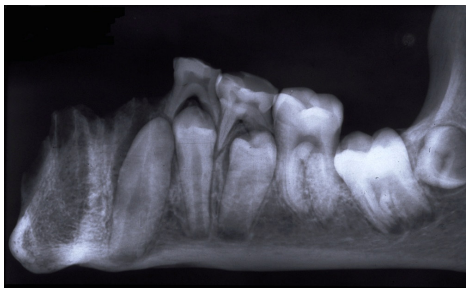

Figure 5 Radiography of the mandible and choice of the tooth (34 non-eruptive)

Figure 6 Realization of the fracture using a carborundum disc on handpiece

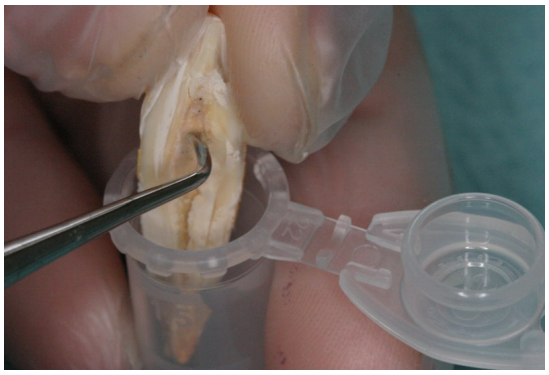

Figure 7 Recovery of the dental pulp

Figure 8 After the fracture and recovery of the pulp, tooth is ready to be glued

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