Dental calculus—a reservoir for detection of past SARS-CoV-2 infection

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Dear Sir/Madame,

The SARS-CoV-2 outbreak left all the scientific and medical community with tremendous doubts and difficulties in managing both symptomatic and asymptomatic patients, especially in case of seronegativity [1, 2]. In fact, in patients with mild symptoms, detectable IgG may not develop or progressively decrease [3]. The present letter aims to highlight an interesting finding, never mentioned before, that may help clinicians and epidemiologists in detecting previous SARS-CoV-2 infection. Learning from archaeological studies [4], we hypothesized that dental calculus, a mineralized oral plaque biofilm that contains biomolecules such as nucleic acids, could have preserved SARS-CoV-2 RNA after infection, regardless of the development and severity of symptoms.

Here, we present the results from a pilot study including five individuals with previous diagnosis of SARS-CoV-2 infection obtained by nasopharyngeal swab testing and subsequently healed within two months prior the study (POS), two individuals contact with a positive who developed mild symptoms and were negative at nasopharyngeal swab testing (SUSP), and five healthy individuals who denied contacts with positive subjects and had negative nasopharyngeal swabs (CTRL). All subjects underwent dental calculus sampling performed with sterile curettes. Samples were analyzed performing real-time reverse transcription polymerase chain reaction assays after nucleic acid extraction and amplification. The RdRp Institut Pasteur IP2 assay was

used, as previous studies demonstrated its high sensitivity and specificity [5]. The results showed that SARS-CoV-2 RNA was detected in all POS subjects, in the two SUSP subjects, and one among CTRL subjects. Ct values are reported in Table 1. Furthermore, one of the POS subjects resulted negative at a serologic assay while the other four subjects developed specific IgG antibodies. Samples were further analyzed using the CDC N1 assay, confirming the presence of SARS-CoV-2 RNA in one POS subject. The presence of SARS-CoV-2 RNA was confirmed by genomic sequencing of the amplicons obtained with the RdRp Institut Pasteur IP2 assay. Sanger sequencing was run at Eurofins Genomics facilities.

Dental calculus has been previously analyzed, mostly for microbiome definition in archaeological human samples and serving as *milieu* for the preservation of genomic information. Similarly, dental calculus may be helpful to detect SARS-CoV-2 traces in previously infected and possibly also in asymptomatic or mild symptomatic patients that may not develop detectable antibodies, therefore lacking a method to confirm the occurred infection. Further studies are needed to define method's reliability, cost/effectiveness, and suitability for large-scale epidemiological studies or post-mortem analysis. Although our study has some limitations due to the small number of samples, we believe that its interest resides in the fact that it is the first describing the presence of SARS-CoV-2 RNA in dental calculus.

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Table 1 Characteristics of the subjects included in the pilot study

Subject number	Group	Symptoms	Nasopharyngeal swab	Serological testing	Ct values (N1)	Ct values (IP2)
1	SUSP	Mild	Negative	Negative	ND	35,78
2	SUSP	Mild	Negative	Negative	ND	35,71
3	POS	Present	Positive	Positive	ND	37,28
4	POS	Present	Positive	Positive	ND	41,07
5	POS	Present	Positive	Positive	ND	33,37
6	POS	Present	Positive	Negative	36,19	34,73
7	POS	Present	Positive	Positive	ND	32,69
8	CTRL	Absent	Negative	Negative	ND	37,25
9	CTRL	Absent	Negative	Negative	ND	ND
10	CTRL	Absent	Negative	Negative	ND	ND
11	CTRL	Absent	Negative	Negative	ND	ND
12	CTRL	Absent	Negative	Negative	ND	ND

POS subjects with previous diagnosis of SARS-CoV-2 infection and healed at the time of sampling, *SUSP* individual contact with a subject with diagnosis of SARS-CoV-2 infection who developed mild symptoms, *CTRL* subjects who denied both contacts with positive subjects and symptoms, *N1* primers and probes for PCR amplification of the N gene 28287–28358* (US Centers for Disease Control and Prevention CDC, USA), *IP2* primers and probes for PCR amplification of the RdRp gene 12690–12797*, (Institute Pasteur, Paris), *ND* not detectable

Nucleotide numbering based on Wuhan-Hu-1 GenBank MN908997

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