International Journal of Paleopathology 53 (2026) 116–123

Contents lists available at ScienceDirect

International Journal of Paleopathology

journal homepage: www.elsevier.com/locate/ijpp

Unlocking the past: Dental calculus as key to understanding ancient health and disease through a One Health framework

Dulce Neves [a,b,*], Emanuela Cristiani [b], António Faustino Carvalho [c], Ana Maria Silva [a,d,e]

[a] University of Coimbra, Research Centre for Anthropology and Health, Department of Life Sciences, Calçada Martim de Freitas, Coimbra 3000-456, Portugal
[b] DANTE - Diet and ANcient TEchnology Laboratory, Department of Odontostomatological and Maxillofacial Sciences, Sapienza University of Rome, Italy
[c] CEAACP - Centro de Estudos de Arqueologia, Artes e Ciências do Património, ICArEHB - Interdisciplinary Center for Archaeology and the Evolution of Human Behaviour, F.C.H.S., University of Algarve, Campus de Gambelas, Faro 8000-117, Portugal
[d] UNIARQ - University of Lisbon, Portugal
[e] University of Coimbra, Centre for Functional Ecology, Department of Life Sciences, Calçada Martim de Freitas, Coimbra 3000-456, Portugal

A R T I C L E I N F O

Keywords:
Mineralized dental plaque
Diet
Health
Environmental contaminants
Medical practices
Residue analysis

A B S T R A C T

Objective: Ancient dental calculus (DC) serves as a critical biomolecular repository, preserving dietary and extra-dietary residues related to past health and disease. This review examines DC as a potential 'One Paleopathology' resource, highlighting the interconnectedness of past human-animal-environmental health. Through a One Health lens, it critically assesses current research and opportunities for the next 50 years of study.
Results: By synthesizing the available literature and the current state of the art, we critically evaluate how DC research bridges paleopathology, bioarchaeology, and modern health sciences, providing a unique lens for assessing health and disease within ecological and evolutionary contexts. We highlight methodological advances and limitations, while identifying critical gaps and opportunities for interdisciplinary integration, standardization, and forward-looking research to expand the field over the next 50 years.
Conclusions: DC research effectively integrates paleopathology, bioarchaeology, and modern health sciences enabling the reconstruction of health and disease within ecological and evolutionary contexts and highlights the interdependence of humans, animals, and the environment.
Significance: From a One Health perspective, dental calculus analysis provides a long-term perspective on interactions among humans, animals, microbes, and environments, informing past and contemporary health research.
Limitations: Interpretation is constrained by preservation biases, variability in analytical methods, and the destructive nature of sampling dental calculus.
Further Research: Future investigations should prioritize sustainable sampling, methodological standardization, and interdisciplinary collaboration. Emerging technologies and integrative approaches will place DC research at the forefront of 'One Paleopathology'.

1. Introduction

The One Health approach is an integrative framework that recognizes health as the outcome of interconnected relationships among humans, animals, and the environment (WHO, 2025a). Rather than viewing health solely in terms of the presence or absence of disease, One Health emphasizes overall well-being, resilience, and adaptation within complex ecological and social systems. Human health cannot be fully understood in isolation, as ecological settings, subsistence strategies, and interactions with other species shape susceptibility and resilience to disease. By integrating biological, environmental, and cultural evidence, the One Health framework treats health as a dynamic process shaped by nutrition, microbial communities, environmental exposures, and social practices, providing a deeper understanding of how these factors have shaped human health across time and space (Atlas, 2012; Bertram et al., 2024; Lerner and Berg, 2015; Pitt and Gunn, 2024; Wang et al., 2024).

In paleopathology, applying a One Health framework enables the examination of how past populations interacted with their ecosystems,

* Correspondence to: Research Centre for Anthropology and Health, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Coimbra 3000-456, Portugal.
E-mail address: dulceisabel.almeidaneves@uniroma1.it (D. Neves).

https://doi.org/10.1016/j.ijpp.2026.05.001
Received 30 December 2025; Received in revised form 3 May 2026; Accepted 7 May 2026
Available online 16 May 2026

not only in terms of disease emergence and zoonotic transmission but also through behaviors that shaped overall health outcomes (e.g., diet, medical treatments), either positively or negatively (Rayfield et al., 2023; Schug and Buikstra, 2025). This 'One Paleopathology', a concept first introduced by Buikstra et al. (2022), integrates evidence from multiple fields, including modern medicine and public health, bioarchaeology, zooarchaeology, and paleoecology, to reconstruct historical disease dynamics and their impact on human and other animal populations (Buikstra et al., 2025; Mitchell, 2024; Schug and Buikstra, 2025; Stamer et al., 2026).

While biocultural and ecological approaches have long emphasized interactions between human biology, culture, and environment, they have often remained analytically bounded, focusing on human populations, specific scales of analysis, or particular categories of evidence (Buikstra and Beck, 2017; Geller, 2021). In contrast, One Paleopathology advances these traditions by explicitly positioning health as multi-scalar, cross-species, and dynamically interconnected processes that combine human, animal, and environmental data within a unified interpretive framework. This shift is not only integrative in scope but also conceptual in orientation, since it emphasizes the co-evolution of hosts, pathogens, and environments, and seeks to link molecular, individual, populational, and ecological evidence in a way that allows more systematic comparison across space and time (Buikstra et al., 2025; Schug and Buikstra, 2025).

As such, One Paleopathology enables approaches that were not consistently achievable within earlier frameworks, including the integration of biomolecular and ecological data across scales, the explicit incorporation of non-human hosts and zoonotic pathways into reconstructions of past health, while contributing to the development of more predictive, comparative models of disease dynamics over deep time (Buikstra and Bolhofner, 2019; Mitchell, 2024).

The growing momentum and relevance of the integrative One Paleopathology framework offer a transformative opportunity for the field by promoting cross-disciplinary and multi-scalar health approaches, as evidenced by the increasing number of contributions in recent years (Gomes and Santos, 2026; Jones, 2024; Rayfield et al., 2023; Urban et al., 2021), driving the continued expansion in scope and methodological rigor within the field of paleopathology.

Despite its potential, integrating One Health with paleopathology presents challenges. Paleopathology offers critical historical perspectives on disease evolution, zoonotic transmission, and environmental influences on health. Yet, its reliance on fragmentary and often degraded archaeological evidence restricts direct comparisons with modern epidemiological data (Schug and Buikstra, 2025). Methodological constraints, such as the destructive nature of ancient DNA (aDNA) and other biomolecular analyses, raise ethical and sustainability concerns. Thus, bridging the two fields requires interdisciplinary collaboration, methodological refinement, and frameworks capable of translating ancient health data into meaningful insights for modern public health and disease prevention (Mitchell, 2024; Schug and Buikstra, 2025; Webster et al., 2016).

The analysis of ancient dental calculus (DC) is a particularly powerful tool in this context, since DC preserves co-occurring evidence of human, microbial, animal, and environmental interactions within a single archive. As a mineralized form of dental plaque, it accumulates over time and serves as a reservoir for various biomolecules, including starch, pollen grains, DNA, proteins, and metabolites. Moreover, it may contain preserved residues from both host and microbial sources, offering valuable information about past health and disease (Fagernäs and Warinner, 2023; Warinner et al., 2014). Through a One Health lens, DC research illuminates how ancient human health was shaped by interactions with animals, food sources, pathogens, and ecological changes, providing critical insights into zoonotic transmission, shifts in pathogenicity, medicinal practices, and broader ecological influences (D'Agostino et al., 2024; Fagernäs and Warinner, 2023; Radini et al., 2017).

While early DC research has been strongly focused on dietary reconstruction, studies addressing disease-related residues remain less extensive in comparison, although a growing number of contributions have begun to explore these aspects (e.g., Fiorin et al., 2024, 2019; Gismondi et al., 2018; Hardy et al., 2012). Similarly, while elements of integrative thinking are present within the broader literature, explicit engagement with One Health and One Paleopathology frameworks is still developing. Given the capacity of DC to preserve biomolecular evidence of human, animal, microbial, and environmental interactions, it represents a promising archive for more systematic interdisciplinary synthesis. However, these data streams are still often discussed in a relatively fragmented manner across different subfields. Strengthening conceptual integration across these domains would further enhance the interpretive potential of DC research within broader discussions of past health and disease.

This review critically synthesizes current research on health-related residues in ancient DC and frames findings within the One Health perspective. We evaluate methodological strengths and limitations, highlight key insights into diet, disease, and medicinal practices, and consider how DC research bridges paleopathology, bioarchaeology, and modern health sciences. Unlike previous reviews (e.g., Fagernäs and Warinner, 2023; Forshaw, 2022; Putrino et al., 2024; Radini et al., 2017; Radini and Nikita, 2023), this paper adopts a One Health framework to critically evaluate how dental calculus reveals human-animal-environment interactions, disease dynamics, and medicinal practices, at the same time as highlighting methodological gaps and future directions for the next 50 years. Beyond assessing its current potential, we discuss future opportunities and challenges, including the sustainability of destructive analyses and the integration of DC studies into predictive, interdisciplinary models of human, animal, and environmental health.

2. Ancient dental calculus: a brief summary

DC forms through the mineralization of dental plaque, driven by interactions between saliva and oral bacteria. Once calcified, it contains a wide variety of minerals (e.g., apatite, whitlockite) and organic components (e.g., proteins, peptides, carbohydrates). The etiology of DC, or the factors contributing to its formation, is multifaceted and involves various biological and behavioral influences. Diet and reduced oral hygiene habits are critical factors as they promote the accumulation of dental plaque, which mineralizes into DC. Additionally, there are individual factors that also play a role in DC formation, such as salivary composition (e.g., salivary flow rate, pH, and mineral content), oral microbiome, genetics, age, oral hygiene practices, or health conditions (Aghanashini et al., 2016; Fagernäs and Warinner, 2023; Fons-Badal et al., 2020; Radini et al., 2017).

Due to its durability and its ability to trap micro-debris, DC serves as a key biomolecular source, refining archaeological and bioanthropological data. Besides the macroscopic examination of DC (focusing on the quantity and location of calculus deposits on teeth), other methods have developed to address and identify the residues trapped in its matrix, such as microscopy (analyzing entrapped debris) and, more recently, metagenomic and biomolecular (with focus on the oral microbiome and biomolecular identification) (Forshaw, 2022; Radini et al., 2017; Radini and Nikita, 2023).

Warinner et al. (2014: 343) recognized it as 'one of the richest biomolecular sources in the archaeological record'. Initially used to reconstruct diets (e.g., Capasso et al., 2024; Cristiani et al., 2021, 2016), DC analysis now extends to studying behaviors, such as the use of teeth as 'third-hand' (e.g., Sperduti et al., 2018), self-medication or medical practices and oral hygiene (e.g., Fiorin et al., 2024, 2026; Hardy et al., 2012; Radini et al., 2016), or to help trace the evolutionary history of oral bacteria or detect pathogens that affected past populations (e.g., Olsen, 2016; Warinner et al., 2015, 2014). Moreover, its entrapment of micro-debris offers insights into paleoecology, particularly when other forms of environmental evidence, such as macrobotanical remains, are absent. Beyond individual biographical details, DC provides a broader perspective on historical living conditions and environmental interactions (e.g., D'Agostino et al., 2024, 2022, 2021; MacKenzie et al., 2021).

3. Diet and health

Diet is the most extensively studied topic in DC research, yet it is seldom explicitly linked to health outcomes in past populations, with some notable exceptions (Buckley et al., 2014; Hardy et al., 2012). Within a One Health perspective, diet is a central factor shaping human well-being, disease susceptibility, and ecological interactions. The World Health Organization highlights that a healthy diet underpins overall health and disease prevention while also requiring ecological sustainability (Forde and Decker, 2022; WHO, 2025b).

Ancient dietary reconstruction using bioarchaeological and paleopathological approaches (e.g., stable isotopes, dental paleopathology, dental microwear) provides essential baseline evidence for dietary reconstruction and its relationship to health (Buikstra and Beck, 2017; Larsen, 2015). However, these methods primarily capture broad dietary patterns rather than fine-grained dietary components. DC adds a complementary dimension by preserving microremains such as starch granules, pollen, and phytoliths (microscopic silica-rich plant structures), which reveal the range of consumed foods and access to environmental resources (Fagernäs and Warinner, 2023; Radini and Nikita, 2023).

Integrating DC studies with paleopathological and bioarchaeological approaches allows dietary practices to be interpreted not only as subsistence behavior but also as part of broader adaptive strategies linking environment, food procurement, and health outcomes. Beyond identifying food types, diet-related residues can inform discussions on food security, zoonotic disease transmission, and environmental sustainability in both (pre)historical and modern contexts (Bourbou, 2024; Lewis et al., 2023; Littleton et al., 2022; Rayfield et al., 2023). For example, the works of Cristiani et al., (2021); (2016), González-Rabanal (2022), and González-Rabanal et al. (2022) have documented the introduction of new resources or shifts between wild and domesticated resources, reflecting changes in ecological engagement of prehistoric populations that may have influenced adaptive strategies to local ecologies, nutritional quality, exposure to pathogens, and long-term physiological stress.

Another example comes from the works of Hardy et al. (2012), Henry et al. (2011), and Weyrich et al. (2017) on Neanderthal calculus. The evidence demonstrated the consumption of plant-based carbohydrates, including cooked starchy foods, indicating that these populations relied on a broader and more balanced diet than previously assumed. The inclusion of carbohydrate-rich plant foods has important implications for energy metabolism, nutritional diversity, and overall physiological resilience, challenging earlier interpretations of predominantly protein-based diets and highlighting the role of dietary breadth in supporting health.

By explicitly applying a One Health lens, DC research moves beyond simple dietary reconstruction. It reframes diet as a central interface through which ecological conditions, subsistence strategies, and biological outcomes are co-produced in human populations, rather than as an isolated behavioral variable.

4. Disease and pathogen history

Pathogens leave behind molecular and genetic traces in DC, enabling paleopathologists to track the deep-time evolution of infectious diseases and their interactions with human hosts. Ancient DNA (aDNA) retrieved from DC has provided direct evidence for pathogens that shaped global history, including *Mycobacterium leprae*, one of the causative agents of leprosy (Fotakis et al., 2020; Schuenemann et al., 2018). Such discoveries have revolutionized the reconstruction of historical epidemics by connecting skeletal, molecular, and contextual evidence, thereby offering a more comprehensive, dynamic picture of disease emergence, persistence, and adaptation across human populations.

The recovery of pathogen aDNA from DC demonstrates that this biomatrix preserves more than dietary or environmental debris. Its formation in close association with oral microbiota entraps both commensal and pathogenic species, creating a durable archive of complex microbial assemblages that reflect oral and systemic infection environments. Analyses of these biomolecular datasets have revealed the genomic diversity of ancient pathogens, the evolution of virulence factors, and long-term routes of zoonotic transmission (Ottoni et al., 2021; Weyrich et al., 2017, 2015). Comparative analyses between ancient and modern genomes further reveal how pathogens have adapted to shifting human ecologies, subsistence systems, and population structures (Putrino et al., 2024; Warinner et al., 2015, 2014).

In some cases, DC also preserves unexpected viral signatures, expanding the scope of detectable disease processes in paleopathology. The identification of a human exogenous betaretrovirus (MMTV-like) contributed to the discussion about its role in breast cancer and suggested possible cross-species viral transmission (Lessi et al., 2020). Thus, it captured previously unrecognized dimensions of ancient infection dynamics.

Exceptional cases further illustrate the DC's intersection of biology and behavior. Weyrich et al. (2017) identified *Enterocytozoon bieneusi*, a known gastrointestinal pathogen, in DC from a Neanderthal individual from El Sidrón (Spain). The same individual exhibited signs of an oral abscess and biochemical evidence of self-medication with plant-derived compounds, suggesting a complex behavioral response to infection. This exceptional case illustrates the DC's unique ability to bridge pathogen biology, human behavior, and ecological context, documenting not only the presence of pathogens but also human strategies to mitigate them. Understanding these ancient disease patterns enhances our knowledge of pathogen ecology, human adaptability, and the long-term consequences of human-animal-environment interactions.

4.1. Oral health and the oral microbiome

Metagenomic analysis of dental calculus provides direct access to ancient oral microbial communities, offering insight into long-term host-microbe relationships. These communities are composed of bacteria, fungi, and viruses that coexisted within the oral cavity and contributed to both local and systemic health processes. Shifts in microbial diversity and composition can either strengthen host defenses or, conversely, increase vulnerability to infections and chronic diseases (Koskella et al., 2017; Lamont et al., 2018; Warinner et al., 2015, 2014; Weyrich et al., 2015).

Beyond dietary reconstruction, the oral microbiome offers a unique lens for exploring the deep-time coevolution of humans and their microbial symbionts. Studies of DC show that microbial communities have not been static but evolved alongside cultural, environmental, and subsistence transitions. By comparing ancient microbiomes with those of modern humans, researchers have traced the emergence of disease-associated taxa, antibiotic resistance genes, and metabolic pathways that underpin both oral and systemic pathologies (Fellows Yates et al., 2021; Ottoni et al., 2021; Warinner et al., 2015; Weyrich et al., 2017, 2015).

A particularly illustrative example is the transformation of the oral microbiome across the shift from foraging to agriculture. The work of Ottoni et al. (2021) demonstrated that the adoption of agriculture and subsequent industrialization profoundly reduced microbial diversity while promoting the dominance of cariogenic and periodontal bacteria, such as *Streptococcus mutans* and *Porphyromonas gingivalis*. These changes correlate with increased rates of dental caries, periodontal disease, and systemic conditions associated with inflammation. The decline in microbial diversity reflects a narrowing of dietary breadth and the introduction of refined carbohydrates, highlighting the ecological sensitivity of the human microbiome to diet and cultural innovation. This transition represents a pivotal moment in human biocultural evolution, linking subsistence shifts, microbial ecology, and health outcomes.

The oral microbiome, therefore, serves as a model system for understanding how microbial ecosystems mediate the relationship between human biology, diet, and environmental change over time. Its study offers valuable parallels between ancient and modern health challenges, from infectious diseases to antibiotic resistance (Olsen, 2016; Putrino et al., 2024; Warinner et al., 2015). Looking forward, combining ancient microbiome data with paleopathological evidence will enable predictive modeling of disease origin and emergence, microbial resistance, and host–microbe coevolution, linking deep-time insights to contemporary One Health challenges and advancing our understanding of the evolutionary resilience and fragility of human health systems.

5. Respiratory health, hygiene habits, and public health issues

Dental calculus preserves inhaled and ingested particulate matter, including soot, pollen, dust, and mineral particles derived from both natural and anthropogenic environments. These inclusions provide evidence of long-term exposure to airborne contaminants that are often not detectable in skeletal remains (MacKenzie et al., 2021; Radini et al., 2017; Radini and Nikita, 2023).

Several occupational activities can produce dust with significant levels of particulate matter, including stone-working, pottery-making, food processing, and woodworking (Chow et al., 2006; Inthavong, 2020; Se et al., 2010). Inhalation of fine particles, particularly those smaller than 10 micrometers, is associated with increased respiratory risk because they can reach deep into the lungs (Se et al., 2010; Hardy et al., 2016). The presence of such particles in DC provides a direct record of environmental exposure linked to daily activities, habits, and labor practices, enabling the reconstruction of past respiratory risk, pollutant burden, and disease susceptibility (Buckley et al., 2021; Hardy et al., 2016; Neves et al., 2025, 2024; Radini et al., 2017). For instance, the presence of conifer wood tissue in DC from a Neanderthal at El Sidrón, Spain, suggests the use of toothpicks, a behavior supported by the presence of interproximal grooves (Radini et al., 2016). Additionally, calculus can trap fungal spores, parasites, and other microorganisms, offering further insight into infections, dietary exposures, and living conditions (Afonso-Vargas et al., 2015; Fiorin et al., 2023; Juhola et al., 2019; Neves et al., 2024).

By bridging environmental, behavioral, and medical evidence, calculus provides a tangible link between past human life and the One Health framework, emphasizing the interconnectedness of human health, behaviors, and surrounding ecosystems. By examining DC from ancient populations, we gain valuable perspectives that resonate with contemporary public health concerns, providing a long-term view of how humans have interacted with their environments over millennia. Such insights can illuminate the historical origins of respiratory diseases linked to air pollution, the evolution of oral hygiene behaviors, and the cumulative impact of environmental exposures on health (e.g., the burden of respiratory irritants). Understanding these patterns informs contemporary public health strategies by contextualizing modern challenges in air quality, infection control, and occupational health.

6. Medicinal practices and drug use

Dental calculus preserves molecular traces of plant-based and pharmacologically active compounds used in past medicinal practices. These include bioactive molecules derived from herbs, resins, and naturally occurring drugs, providing direct evidence of therapeutic behaviors (Cristiani et al., 2025; Fiorin et al., 2026, 2024, 2022, 2019; Hardy et al., 2012). These discoveries allow for the reconstruction of ancient medicinal knowledge, highlighting how humans have long harnessed natural substances for healing, pain relief, and disease management.

Archaeological, historical, and anthropological evidence from diverse sources, such as material culture, osteological studies, archaeobotanical analyses, and textual references, has long suggested that ancient peoples used plant-based remedies. Recent detection of medicinal plants and drug-related residues entrapped in DC provides a direct molecular corroboration, further substantiating long-held hypotheses about early medicinal practices and the complex use of natural resources by ancient people (Allende and Samplonius, 2022; Fiorin et al., 2024, 2019; Gismondi et al., 2020, 2018; Hardy et al., 2012; Weyrich et al., 2017). These findings mark a significant advance in paleopathology and molecular archaeology, as they enable researchers to identify the specific plants, bioactive compounds, and even the pharmacological purposes involved in ancient therapies.

The identification of the medicinal use of psychotropic substances in DC dates back to 1999 with the presentation of a pioneer case-study at the II Congrés del Neolític a la Península lbèrica (Valencia, Spain). Through a multi-pronged approach to DC—which combined light microscopy, scanning electron microscopy, radioimmunoassay, and gas chromatography/mass spectrometry—Juan-Tresseras and Villalba (1999) identified the use of opium poppy (*Papaver somniferum* L.) by a male individual buried in the Neolithic site of Can Tintorer (Gava, Barcelona). As the individual (male, ~30 y) presented signs of an antemortem double trepanation, the authors interpreted the possible application of the opium poppy in surgical practices due to its narcotic, analgesic, and sedative properties.

More recent research continues to strengthen the link between DC and ancient pharmacology. Fiorin et al. (2024) identified biomolecular residues consistent with ginger (*Zingiber officinale*) in medieval individuals afflicted with leprosy, providing molecular support for its documented use in historical medical manuscripts as a treatment for this condition. Similarly, other studies have detected residues of antimicrobial and anti-inflammatory plants, such as chamomile (*Matricaria chamomilla*) and yarrow (*Achillea millefolium*), highlighting the persistent reliance on phytotherapeutic compounds in premodern societies (Gismondi et al., 2020; Fiorin et al., 2019).

The analysis of drug entrapment in DC remains a developing field (Guerra-Doce, 2015), but it has already yielded significant insights into both historical and forensic contexts. Sørensen et al. (2021) demonstrated that DC can preserve traces of various pharmacological and psychoactive substances, including opioids, cannabinoids, cocaine, and nicotine. Their identification is particularly relevant for forensic cases, yet it also offers new opportunities to study substance use over time. In archaeological contexts, such findings can reveal individual behaviors or social practices, such as tobacco consumption in early colonial North America (Eerkens et al., 2018), while in modern forensic cases, they may assist in reconstructing drug exposure histories (Sørensen et al., 2021).

Beyond psychoactive or medicinal compounds, DC can also accumulate heavy metals and other inorganic residues, which can reflect both toxic exposures and therapeutic applications. Charlier et al. (2010) and Zhang et al. (2019) detected cadmium in DC from modern populations, linking its presence to smoking and betel-quid chewing—behaviors associated with elevated cancer risk. In other cases, heavy metal detection reveals past medical practices: mercury, a known antimicrobial but toxic element, was found in the DC of medieval individuals affected by leprosy, aligning with historical documentation of mercury-based treatments (Fiorin et al., 2026, 2022).

These findings demonstrate that therapeutic practices were embedded within broader ecological and cultural systems, where healing, toxicity, and environmental exposure were closely interconnected. More than documenting therapeutics, disease management, and unintended consequences of medical and environmental interactions, DC research can add to long-term assessments of the use and efficacy of traditional medicines while also highlighting potential pathways for modern pharmacological research grounded in ethnobotanical heritage.

7. Sustainability and limitations of dental calculus research

Sustainability in archaeological and anthropological research often involves the use of destructive methods on bioarchaeological remains, including dental calculus. Although DC is sometimes treated as a secondary deposit (ectopic growth) rather than primary skeletal tissue (MacKenzie et al., 2021; Mackie et al., 2017b; Radini and Nikita, 2023), it nevertheless derives from human remains and therefore should fall within broader ethical frameworks governing the destructive analysis of archaeological human-derived materials, including principles of respect, proportionality, and minimization of sampling.

One of the key advantages of studying DC is its long-term preservation. Due to its high mineralization, it can store and preserve diverse micro-debris in a process similar to 'fossilization', making it a reliable source of ancient organic and inorganic data even when other materials, like macro botanical remains, have deteriorated (MacKenzie et al., 2021; Radini et al., 2017). Indicative of its preservation potential is the fact that calculus has been found on teeth from Miocene hominids, as the *Sivapithecus* specimen dated between 12 and 8 million years ago (Hershkovitz et al., 1997), and hominins (Blumenschine et al., 2003; Henry et al., 2014, 2011; Radini et al., 2016; Weyrich et al., 2017).

Additionally, DC analysis is methodologically efficient. The high concentration of entrapped microdebris allows for meaningful analyses using very small sample sizes (often less than 50 mg), and calculus is frequently present on multiple teeth or in substantial quantities within a single individual. This enables targeted, minimally destructive sampling strategies that preserve unsampled material for future research. In this sense, DC sampling can—and should—be designed with long-term conservation of archaeological material in mind.

Despite these advantages, DC research does not eliminate broader sustainability challenges in archaeological science. The destructive nature of analysis requires careful consideration of sampling intensity, long-term preservation, and cumulative impacts across collections. As analytical techniques continue to develop, preserving material for future research remains essential (Mackie et al., 2017b). Although DC is often treated as a secondary deposit rather than primary skeletal tissue, we argue that it remains part of human-derived archaeological material and should therefore be subject to the same ethical principles governing destructive analysis, including minimization of sampling and long-term preservation considerations.

A further limitation concerns methodological standardization. Despite rapid growth in the field, DC research still lacks consistency across key analytical stages, including sample selection, decontamination, and decalcification protocols (as discussed in Gancz et al., 2023). This variability affects comparability and reproducibility, as differences in workflows can influence recovered biomolecular signatures and therefore downstream interpretations.

Methodological variability is compounded by contamination and selective preservation processes operating across the full *chaîne opératoire*. Contamination may be introduced during burial (soil chemistry, groundwater, microbial activity), excavation (handling and recovery), curation (storage and conservation), and laboratory analysis (reagents and procedures), particularly where protocols are not fully standardized. In parallel, DC is subject to inclusion bias, as its mineral matrix preferentially preserves chemically stable biomolecules while more labile components are underrepresented or lost. Together, these processes mean that DC represents a filtered and partially altered archive, shaped by both post-depositional processes and analytical constraints.

These factors introduce interpretative uncertainty. Observed variation in diet, microbiota, or pathogen presence may reflect not only biological and environmental differences, but also methodological choices and/or preservation bias. This limits cross-site comparability and constrains the development of large-scale synthetic datasets (Fagernäs and Warinner, 2023; Mann et al., 2023).

Because it preserves a selective and temporally compressed record, detected signals reflect preservation and detectability rather than their relative importance in past behavior or health. Co-occurring residues cannot be assumed to be functionally or causally related, as they may represent independent exposures. Moreover, the pathways by which debris becomes incorporated into dental calculus are not always unambiguous, making it difficult to distinguish between dietary, medicinal, environmental, or incidental ingestion, for example. This temporal and compositional compression increases the risk of conflating short-term events with broader behavioral or ecological patterns, requiring careful interpretation supported by independent lines of evidence.

From a sustainability perspective, methodological heterogeneity and interpretative uncertainty can also lead to redundant or non-comparable destructive sampling, reducing the long-term value of finite archaeological material. Addressing these challenges requires both methodological standardization and flexible best-practice frameworks that accommodate disciplinary diversity. Emerging models from aDNA and proteomics offer useful precedents, particularly in reporting standards, quality control metrics, and transparent methodological documentation (Bender et al., 2022; Chocholova et al., 2023; Fagernäs et al., 2020; Farrer et al., 2021; Mackie et al., 2017a).

In the context of DC research, minimum reporting standards for sampling, decontamination, and analytical workflows would significantly improve comparability without limiting methodological innovation. Cross-laboratory validation, shared reference materials, and open data practices would further enhance reproducibility and enable meta-analytical integration. Collectively, these measures would strengthen methodological consistency and improve the long-term sustainability of destructive sampling by maximizing interpretative value.

Taken together, dental calculus represents a high-value but finite archaeological resource whose scientific potential depends on careful ethical sampling, methodological standardization, and interpretative caution. Therefore, its effective use requires balancing analytical gain with long-term preservation, ensuring that its contribution to reconstructing past health and human-environment interactions remains robust, comparable, and sustainable over time.

8. Final considerations: the future of paleopathology

As outlined in the introduction, One Paleopathology has emerged as an increasingly relevant framework for interpreting health in deep time, although its application across different lines of archaeological biomolecular evidence remains uneven. Within this context, DC research has contributed substantially to reconstructing aspects of diet, disease, microbiomes, and environmental exposure, but these contributions are not always synthesized within a unified conceptual framework. This review builds on existing work by bringing these strands together through an explicit One Health and One Paleopathology perspective, offering a structured synthesis across multiple domains of DC research. Rather than proposing a fundamentally new direction, this article consolidates and reframes existing evidence in a way that highlights shared patterns across biological, environmental, and cultural dimensions of health. In doing so, it provides a foundation for future work that may further integrate DC data within broader comparative and interdisciplinary approaches to past, present, and future health.

Taken together, evidence from DC demonstrates that human health in the past was shaped by tightly interconnected biological, environmental, and cultural factors. Diet, pathogen exposure, oral microbial dynamics, respiratory irritants, hygiene practices, and medicinal or pharmacological interventions were not isolated phenomena but part of an integrated ecological system in which humans, animals, and the environment continuously interacted. As a result, researchers can explore the complex web of health determinants that influenced resilience, susceptibility, and adaptation across past populations.

Within a One Health perspective, DC offers a way of tracing the co-evolution of humans, microbes, and environmental exposures over time. By preserving biomolecular evidence of these interactions, it enables long-term perspectives on disease dynamics, ecological change, and human behavior that are difficult to access through other archaeological materials. These insights not only refine reconstructions of past lifeways but also contribute to broader discussions of disease emergence, antimicrobial resistance, and the ecological determinants of health.

At the same time, the growing body of DC research has not always been explicitly integrated within broader conceptual frameworks. This review has addressed this gap by synthesizing existing evidence through a One Health and One Paleopathology perspective, demonstrating how diverse lines of data can be brought together within a shared analytical framework. In this sense, the contribution of this study lies not in introducing entirely new data but in clarifying how existing evidence can be more effectively integrated to advance comparative and interdisciplinary approaches to health across deep time.

As we continue to uncover the secrets hidden within ancient DC, we gain not only a deeper appreciation of our evolutionary past but also knowledge that can directly inform contemporary medicine, global and public health initiatives, and sustainability strategies. Studying the past equips us to navigate the future more effectively, anticipating emerging diseases, tracking the evolution of antimicrobial resistance, and understanding the shifting relationships among humans, their microbiomes, and the broader environment. The insights gained from ancient DC highlight the power of interdisciplinary research and demonstrate how lessons from history can actively shape a healthier future.

Technological advances—ranging from high-resolution microscopy and metagenomic sequencing to proteomics and metabolomics—will continue to refine our understanding of microbial evolution, pathogen-host interactions, and dietary and medicinal adaptations over millennia. These tools enable predictive, cross-scale analyses that connect deep-time biological, ecological, and cultural processes to contemporary health and environmental challenges. DC preserves aDNA, proteins, metabolites, and environmental residues, providing a nuanced window into health, disease, and behavioral practices that skeletal analysis alone cannot capture.

Looking forward, continued advances in analytical techniques—including metagenomics, proteomics, and metabolomics—combined with improved methodological standardization and collaborative research frameworks, will further enhance the interpretive power of DC. These developments will support more robust, cross-scale analyses linking biological, ecological, and cultural processes, and will facilitate the integration of archaeological data into broader discussions of global and public health.

Future research should embrace sustainable, multi-site, and multi-omic studies, standardize methodologies, and foster interdisciplinary collaboration to unlock the full potential of dental calculus. In doing so, DC will not simply illuminate the past; it will actively guide the next 50 years of paleopathology, transforming our understanding of human-animal-environment interactions, the origins of disease, and the evolutionary trajectories that continue to shape health today, and connecting ancient insights to modern health, ecology, and public health strategies.

CRediT authorship contribution statement

António Faustino Carvalho: Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Ana Maria Silva:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Dulce Neves:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Conceptualization. **Emanuela Cristiani:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgements

This work was supported by the FCT – Fundação para a Ciência e Tecnologia [2021.04560.BD, DOI: 10.54499/2021.04560.BD, awarded to DN; DOI: 10.54499/UIDB/00283/2020, awarded to CIAS – Research Centre for Anthropology and Health], PRIN 2022 project ROMAN CALCULUS - Ancient *Materia Medica*, Modern Therapeutic Substances, and Drugs of Abuse Through Dental Calculus, and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme [Grant agreement No 639286—HIDDEN FOODS awarded to EC].

The authors thank CIAS - Research Centre for Anthropology and Health (University of Coimbra, Portugal) and DANTE - Diet and Ancient Technology Laboratory (Sapienza' University of Rome, Italy). The authors also acknowledge the anonymous reviewers, whose valuable comments helped improve the manuscript.

References

Afonso-Vargas, J., Serna-Ramos, I.L., Arnay-de-la-Rosa, M., 2015. Fungal spores located in 18th century human dental calculi in the church "La Concepción" (Tenerife, Canary Islands). Journal Archaeological Science Reports 2, 106–113. https://doi.org/10.1016/j.jasrep.2015.01.003.

Aghanashini, S., Puvvalla, B., Mundinamane, D.B., Apoorva, S., Bhat, D., Lalwani, M., 2016. A comprehensive review on dental calculus. J. Health Sci. & Res. 7, 42–50. https://doi.org/10.5005/jp-journals-10042-1034.

Allende, M.K.-G., Samplonius, A., 2022. Dental anthropological report: Exploring plant-based treatments through the analysis of dental calculus and sediment of dental caries in a woman from the Late Preceramic period, Peru. Ann. Anat. - Anat. Anz. 240, 151849. https://doi.org/10.1016/j.aanat.2021.151849.

Atlas, R.M., 2012. One health: its origins and future. In: Mackenzie, J.S., Jeggo, M., Daszak, P., Richt, J.A. (Eds.), One Health: The Human-Animal-Environment Interfaces in Emerging Infectious Diseases, Current Topics in Microbiology and Immunology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1–13. https://doi.org/10.1007/82_2012_223.

Bender, O., Megreli, D., Gavish, T., Meyrom, N., Zamir, N., May, H., Sarig, R., Bar, D., 2022. The hidden secrets of the dental calculus: calibration of a mass spectrometry protocol for dental calculus protein analysis. Int. J. Mol. Sci. 23, 14387. https://doi.org/10.3390/ijms232214387.

Bertram, M.G., Costi, M.P., Thoré, E.S.J., Sabo-Attwood, T., Brooks, B.W., 2024. One health. Curr. Biol. 34, R517–R519. https://doi.org/10.1016/j.cub.2024.04.025.

Blumenschine, R.J., Peters, C.R., Masao, F.T., Clarke, R.J., Deino, A.L., Hay, R.L., Swisher, C.C., Stanistreet, I.G., Ashley, G.M., McHenry, L.J., Sikes, N.E., Van Der Merwe, N.J., Tactikos, J.C., Cushing, A.E., Deocampo, D.M., Njau, J.K., Ebert, J.I., 2003. Late pliocene *Homo* and Hominid land use from Western Olduvai Gorge, Tanzania. Science 299, 1217–1221. https://doi.org/10.1126/science.1075374.

Bourbou, C., 2024. The byzantine "ecosystem": evidence from the bioarchaeological record. in: A Companion to the Environmental History of Byzantium. Brill, pp. 83–108. https://doi.org/10.1163/9789004689350_005.

Buckley, S., Power, R.C., Andreadaki-Vlazaki, M., Akar, M., Becher, J., Belser, M., Cafisso, S., Eisenmann, S., Fletcher, J., Francken, M., Hallager, B., Harvati, K., Ingman, T., Kataki, E., Maran, J., Martin, M.A.S., McGeorge, P.J.P., Milevski, I., Papadimitriou, A., Protopapadaki, E., Salazar-García, D.C., Schmidt-Schultz, T., Schuenemann, V.J., Shafiq, R., Stuijts, I., Yegorov, D., Yener, K.A., Schultz, M., Spiteri, C., Stockhammer, P.W., 2021. Archaeometric evidence for the earliest exploitation of lignite from the bronze age Eastern Mediterranean. Sci. Rep. 11, 24185. https://doi.org/10.1038/s41598-021-03544-w.

Buckley, S., Usai, D., Jakob, T., Radini, A., Hardy, K., 2014. Dental calculus reveals unique insights into food items, cooking and plant processing in prehistoric Central Sudan. PLOS ONE 9, e100808. https://doi.org/10.1371/JOURNAL.PONE.0100808.

Buikstra, J.E., Beck, L.A., 2017. Bioarchaeology: The Contextual Analysis of Human Remains. Routledge.

Buikstra, J.E., Bolhofner, K.L., 2019. Bioarchaeologists speak out: an introduction. In: Bioarchaeologists Speak Out: Deep Time Perspectives on Contemporary Issues, Bioarchaeology and Social Theory. Springer Nature Switzerland AG, pp. 1–18.

Buikstra, J.E., O'Donnell, L., Uhl, E., 2025. One paleopathology: raising awareness and advancing knowledge. In: McKenzie, C.J., Tilley, L., Mack, J.E., Evis, L.H. (Eds.), Applied Bioarchaeology, Bioarchaeology and Social Theory. Springer Nature Switzerland, Cham, pp. 59–79. https://doi.org/10.1007/978-3-032-04692-5_4.

Buikstra, J.E., Uhl, E.W., Wissler, A., 2022. Big pictures in 21st-century paleopathology: interdisciplinarity and transdisciplinarity. In: Grauer, A.L. (Ed.), The Routledge Handbook of Paleopathology. Routledge, pp. 625–636.

Capasso, G., Neves, D., Sperduti, A., Cristiani, E., Manzo, A., 2024. Direct evidence of plant consumption in Neolithic Eastern Sudan from dental calculus analysis. Sci. Rep. 14, 4278. https://doi.org/10.1038/s41598-024-53300-z.

Charlier, P., Huynh-Charlier, I., Munoz, O., Billard, M., Brun, L., Grandmaison, G.L. de la, 2010. The microscopic (optical and SEM) examination of dental calculus deposits (DCD). Potential interest in forensic anthropology of a bio-archaeological method. Leg. Med. 12, 163–171. https://doi.org/10.1016/j.legalmed.2010.03.003.

Chocholova, E., Roudnicky, P., Potesil, D., Fialova, D., Krystofova, K., Drozdova, E., Zdrahal, Z., 2023. Extraction protocol for parallel analysis of proteins and DNA from ancient teeth and dental calculus. J. Proteome Res. 22, 3311–3319. https://doi.org/10.1021/acs.jproteome.3c00370.

Chow, J.C., Watson, J.G., Mauderly, J.L., Costa, D.L., Wyzga, R.E., Vedal, S., Hidy, G.M., Altshuler, S.L., Marrack, D., Heuss, J.M., Wolff, G.T., Arden Pope III, C., Dockery, D. W., 2006. Health effects of fine particulate air pollution: lines that connect. J. Air & Waste Manag. Assoc. 56, 1368–1380. https://doi.org/10.1080/10473289.2006.10464545.

Cristiani, E., Radini, A., Edinborough, M., Borić, D., 2016. Dental calculus reveals Mesolithic foragers in the Balkans consumed domesticated plant foods. Proc. Natl. Acad. Sci. USA 113, 10298–10303. https://doi.org/10.1073/PNAS.1603477113.

Cristiani, E., Radini, A., Zupancich, A., Gismondi, A., D'agostino, A., Ottoni, C., Carra, M., Vukojičić, S., Constantinescu, M., Antonović, D., Price, T.D., Borić, D., 2021. Wild cereal grain consumption among Early Holocene foragers of the Balkans predates the arrival of agriculture. eLife 10. https://doi.org/10.7554/ELIFE.72976.

Cristiani, E., Vaiano, F., Ottolenghi, L., Sperduti, A., Cecconi, V., Dimitrova, A., Fiorin, E., Gentile, I., Giordano, A., Ndokaj, A., Neves, D., 2025. Dental Calculus as a Forensic and Archaeological Archive: Methodological Advances from the PRIN Project Roman Calculus.

D'Agostino, A., Di Marco, G., Marvelli, S., Marchesini, M., Rizzoli, E., Rolfo, M.F., Canini, A., Gismondi, A., 2022. Neolithic dental calculi provide evidence for environmental proxies and consumption of wild edible fruits and herbs in central Apennines. Commun. Biol. 5. https://doi.org/10.1038/s42003-022-04354-0.

D'Agostino, A., Di Marco, G., Rolfo, M.F., Alessandri, L., Marvelli, S., Braglia, R., Congestri, R., Berrilli, F., Fuciarelli, M.F., Ferracci, A., Canini, A., Gismondi, A., 2024. Microparticles from dental calculus disclose paleoenvironmental and palaeoecological records. Ecol. Evol. 14, e11053. https://doi.org/10.1002/ece3.11053.

D'Agostino, A., Di Marco, G., Rubini, M., Marvelli, S., Rizzoli, E., Canini, A., Gismondi, A., 2021. Environmental implications and evidence of natural products from dental calculi of a Neolithic–Chalcolithic community (central Italy). Sci. Rep. 11, 10665. https://doi.org/10.1038/s41598-021-89999-3.

Eerkens, J.W., Tushingham, S., Brownstein, K.J., Garibay, R., Perez, K., Murga, E., Kaijankoski, P., Rosenthal, J.S., Gang, D.R., 2018. Dental calculus as a source of ancient alkaloids: detection of nicotine by LC-MS in calculus samples from the Americas. Journal Archaeological Science Reports 18, 509–515. https://doi.org/10.1016/j.jasrep.2018.02.004.

Fagernäs, Z., García-Collado, M.I., Hendy, J., Hofman, C.A., Speller, C., Velsko, I., Warinner, C., 2020. A unified protocol for simultaneous extraction of DNA and proteins from archaeological dental calculus. J. Archaeol. Sci. 118, 105135. https://doi.org/10.1016/j.jas.2020.105135.

Fagernäs, Z., Warinner, C., 2023. Dental calculus. in: Handbook of Archaeological Sciences. John Wiley & Sons, Ltd, pp. 575–590. https://doi.org/10.1002/9781119592112.ch28.

Farrer, A., Wright, S., Skelly, E., Eisenhofer, R., Dobney, K., Weyrich, L., 2021. Effectiveness of decontamination protocols when analyzing ancient DNA preserved in dental calculus. Sci. Rep. 11, 7456. https://doi.org/10.1038/s41598-021-86100-w.

Fellows Yates, J.A., Velsko, I.M., Aron, F., Posth, C., Hofman, C.A., Austin, R.M., Parker, C.E., Mann, A.E., Nägele, K., Arthur, K.W., Arthur, J.W., Bauer, C.C., Crevecoeur, I., Cupillard, C., Curtis, M.C., Dalén, L., Díaz-Zorita Bonilla, M., Díez Fernández-Lomana, J.C., Drucker, D.G., Escribano Escrivá, E., Francken, M., Gibbon, V.E., González Morales, M.R., Grande Mateu, A., Harvati, K., Henry, A.G., Humphrey, L., Menéndez, M., Mihailović, D., Peresani, M., Rodríguez Moroder, S., Roksandic, M., Rougier, H., Sázelová, S., Stock, J.T., Straus, L.G., Svoboda, J., Teßmann, B., Walker, M.J., Power, R.C., Lewis, C.M., Sankaranarayanan, K., Guschanski, K., Wrangham, R.W., Dewhirst, F.E., Salazar-García, D.C., Krause, J., Herbig, A., Warinner, C., 2021. The evolution and changing ecology of the African hominid oral microbiome. Proc. Natl. Acad. Sci. 118, e2021655118. https://doi.org/10.1073/pnas.2021655118.

Fiorin, E., Moore, J., Montgomery, J., Lippi, M.M., Nowell, G., Forlin, P., 2023. Combining dental calculus with isotope analysis in the Alps: New evidence from the Roman and medieval cemeteries of Lamon, Italy. Quat. Int. 653654 89–102. https://doi.org/10.1016/J.QUAINT.2021.11.022.

Fiorin, E., Perrot, V., Roberts, C.A., Chapelain De Seréville-Niel, C., Gao, Y., Snoeck, C., Cristiani, E., 2026. Mercury treatment in late medieval European leprosaria? New data from human dental calculus. J. Archaeol. Sci. 185, 106444. https://doi.org/10.1016/j.jas.2025.106444.

Fiorin, E., Roberts, C., Cristiani, E., 2022. Exploring medical care through dental calculus analysis: St Leonard's medieval leprosy hospital, Peterborough, England.

Fiorin, E., Roberts, C.A., Baldoni, M., Connelly, E., Lee, C., Ottoni, C., Cristiani, E., 2024. First archaeological evidence for ginger consumption as a potential medicinal ingredient in a late medieval leprosarium at St Leonard, Peterborough, England. Sci. Rep. 14, 2452. https://doi.org/10.1038/s41598-024-52422-8.

Fiorin, E., Sáez, L., Malgosa, A., 2019. Ferns as healing plants in medieval Mallorca, Spain? Evidence from human dental calculus. Int. J. Osteoarchaeol. 29, 82–90. https://doi.org/10.1002/OA.2718.

Fons-Badal, C., Fons, A., Labaig-Rueda, C., Solá-Ruiz, M.F., Selva, E., Agustín-Panadero, R., 2020. Analysis of predisposing factors for rapid dental calculus formation. J. Clin. Med. 9, 858. https://doi.org/10.3390/jcm9030858.

Forde, C.G., Decker, E.A., 2022. The importance of food processing and eating behavior in promoting healthy and sustainable diets. Annu. Rev. Nutr. 42, 377–399. https://doi.org/10.1146/annurev-nutr-062220-030123.

Forshaw, R., 2022. Dental calculus - oral health, forensic studies and archaeology: a review. Br. Dent. J. 233, 961–967. https://doi.org/10.1038/S41415-022-5266-7.

Fotakis, A.K., Denham, S.D., Mackie, M., Orbegozo, M.I., Mylopotamitaki, D., Gopalakrishnan, S., Sicheritz-Pontén, T., Olsen, J.V., Cappellini, E., Zhang, G., Christophersen, A., Gilbert, M.T.P., Vågene, Å.J., 2020. Multi-omic detection of *Mycobacterium leprae* in archaeological human dental calculus. Philos. Trans. R. Soc. B 375, 20190584. https://doi.org/10.1098/rstb.2019.0584.

Gancz, A., Wright, S., Weyrich, L., 2023. Ancient human dental calculus metadata collection and sampling strategies: recommendations for best practices. Am. J. Biol. Anthropol. https://doi.org/10.1002/ajpa.24871.

Geller, P.L., 2021. Theorizing bioarchaeology, Bioarchaeology and Social Theory. Springer Nature Switzerland AG.

Gismondi, A., D'Agostino, A., Canuti, L., Di Marco, G., Martínez-Labarga, C., Angle, M., Rickards, O., Canini, A., 2018. Dental calculus reveals diet habits and medicinal plant use in the Early Medieval Italian population of Colonna. Journal Archaeological Science Reports 20, 556–564. https://doi.org/10.1016/j.jasrep.2018.05.023.

Gismondi, A., D'Agostino, A., Di Marco, G., Martínez-Labarga, C., Leonini, V., Rickards, O., Canini, A., 2020. Back to the roots: dental calculus analysis of the first documented case of coeliac disease. Archaeol. Anthropol. Sci. 12. https://doi.org/10.1007/s12520-019-00962-w.

Gomes, R.A.M.P., Santos, A.L., 2026. Cribra orbitalia and cribra cranii in perspective: rethinking etiology through life course and ONE paleopathology approaches. Int. J. Paleopathol. 52, 108–118. https://doi.org/10.1016/j.ijpp.2026.01.008.

González-Rabanal, B., 2022. Dinámicas de población, dieta y prácticas funerarias de los últimos cazadores-recolectores y primeras sociedades campesinas de la Región Cantábrica (PhD thesis). Escuela de Doctorado de la Universidad de Cantabria, Santader.

González-Rabanal, B., Marín-Arroyo, A.B., Cristiani, E., Zupancich, A., González-Morales, M.R., 2022. The arrival of millets to the Atlantic coast of northern Iberia. Sci. Rep. 12. https://doi.org/10.1038/s41598-022-23227-4.

Guerra-Doce, E., 2015. The origins of inebriation: archaeological evidence of the consumption of fermented beverages and drugs in Prehistoric Eurasia. J. Archaeol. Method Theory 22, 751–782. https://doi.org/10.1007/s10816-014-9205-z.

Hardy, K., Buckley, S., Collins, M.J., Estalrrich, A., Brothwell, D., Copeland, L., García-Tabernero, A., García-Vargas, S., De La Rasilla, M., Lalueza-Fox, C., Huguet, R., Bastir, M., Santamaría, D., Madella, M., Wilson, J., Cortés, Á.F., Rosas, A., 2012. Neanderthal medics? Evidence for food, cooking, and medicinal plants entrapped in dental calculus. Naturwissenschaften 99, 617–626. https://doi.org/10.1007/S00114-012-0942-0.

Hardy, K., Radini, A., Buckley, S., Sarig, R., Copeland, L., Gopher, A., Barkai, R., 2016. Dental calculus reveals potential respiratory irritants and ingestion of essential plant-based nutrients at Lower Palaeolithic Qesem Cave Israel. Quat. Int. 398, 129–135. https://doi.org/10.1016/j.quaint.2015.04.033.

Henry, A.G., Brooks, A.S., Piperno, D.R., 2014. Plant foods and the dietary ecology of Neanderthals and early modern humans. J. Hum. Evol. 69, 44–54. https://doi.org/10.1016/j.jhevol.2013.12.014.

Henry, A.G., Brooks, A.S., Piperno, D.R., 2011. Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy I and II, Belgium). Proc. Natl. Acad. Sci. USA 108, 486–491. https://doi.org/10.1073/PNAS.1016868108.

Hershkovitz, I., Kelly, J., Latimer, B., Rothschild, B.M., Simpson, S., Polak, J., Rosenberg, M., 1997. Oral bacteria in MioceneSivapithecus. J. Hum. Evol. 33, 507–512. https://doi.org/10.1006/jhev.1997.0149.

Inthavong, K., 2020. From indoor exposure to inhaled particle deposition: a multiphase journey of inhaled particles. Exp. Comput. Multiph. Flow. 2, 59–78. https://doi.org/10.1007/s42757-019-0046-6.

Jones, C.A., 2024. Expanding the diagnostic scope of paleopathology: identification of Q fever (*Coxiella burnetii*) using a one paleopathology approach. Int. J. Paleopathol. 47, 43–53. https://doi.org/10.1016/j.ijpp.2024.10.001.

Juan-Tresseras, J., Villalba, M.J., 1999. Consumo de la adormidera (Papaver somniferum L.) en el Neolítico Peninsular: el enterramiento M28 del complejo minero de Can Tintorer. In: Il Congrés Del Neolític a La Península Lbèrica, SAGVNTVM-PLAV, pp. 397–404.

Juhola, T., Henry, A.G., Kirkinen, T., Laakkonen, J., Väliranta, M., 2019. Phytoliths, parasites, fibers, and feathers from dental calculus and sediment from Iron Age Luistari cemetery, Finland. Quat. Sci. Rev. 222, 105888. https://doi.org/10.1016/J.QUASCIREV.2019.105888.

Koskella, B., Hall, L.J., Metcalf, C.J.E., 2017. The microbiome beyond the horizon of ecological and evolutionary theory. Nat. Ecol. Evol. 1, 1606–1615. https://doi.org/10.1038/s41559-017-0340-2.

Lamont, R.J., Koo, H., Hajishengallis, G., 2018. The oral microbiota: dynamic communities and host interactions. Nat. Rev. Microbiol 16, 745–759. https://doi.org/10.1038/s41579-018-0089-x.

Larsen, C.S., 2015. Bioarchaeology: Interpreting Behavior from the Human Skeleton, 2nd ed. Cambridge University Press, Cambridge.

Lerner, H., Berg, C., 2015. The concept of health in one health and some practical implications for research and education: what is One Health? Infect. Ecol. & Epidemiol. 5, 25300. https://doi.org/10.3402/iee.v5.25300.

Lessi, F., Grandi, N., Mazzanti, C.M., Civita, P., Scatena, C., Aretini, P., Bandiera, P., Fornaciari, A., Giuffra, V., Fornaciari, G., Naccarato, A.G., Tramontano, E., Bevilacqua, G., 2020. A human MMTV-like betaretrovirus linked to breast cancer has been present in humans at least since the copper age. Aging 12, 15978–15994. https://doi.org/10.18632/aging.103780.

Lewis, C.M., Akinyi, M.Y., DeWitte, S.N., Stone, A.C., 2023. Ancient pathogens provide a window into health and well-being. Proc. Natl. Acad. Sci. U. S. A. 120, e2209476119. https://doi.org/10.1073/pnas.2209476119.

Littleton, J., Karstens, S., Busse, M., Malone, N., 2022. Human-animal interactions and infectious disease: a view for bioarchaeology. BI. https://doi.org/10.5744/bi.2021.0002.

MacKenzie, L., Speller, C.F., Holst, M., Keefe, K., Radini, A., 2021. Dental calculus in the industrial age: human dental calculus in the post-medieval period, a case study from industrial Manchester. Quat. Int. https://doi.org/10.1016/J.QUAINT.2021.09.020.

Mackie, M., Hendy, J., Lowe, A., Sperduti, A., Holst, M., Collins, M., Speller, C., 2017a. Preservation of the metaproteome:: variability of protein preservation in ancient dental calculus. STAR Science & Technology Archaeological Research 3, 74–86. https://doi.org/10.1080/20548923.2017.1361629.

Mackie, M., Radini, A., Speller, C., 2017b. The Sustainability of Dental Calculus for Archaeological Research, in: Shallow Pasts, Endless Horizons: Sustainability & Archaeology. Presented at the Proceedings of the 48th Annual Chacmool Conference, The Chacmool Archaeological Association of the University of Calgary.

Mann, A.E., Fellows Yates, J.A., Fagernäs, Z., Austin, R.M., Nelson, E.A., Hofman, C.A., 2023. Do I have something in my teeth? The trouble with genetic analyses of diet from archaeological dental calculus. Quat. Int. Food Environ. Adv. Anc. Hum. Dent. Calc. Res. 653–654, 33–46. https://doi.org/10.1016/j.quaint.2020.11.019.

Mitchell, P.D., 2024. Using paleopathology to provide a deep-time perspective that improves our understanding of one health challenges: exploring urbanization. Res. Dir. One Health 2, e5. https://doi.org/10.1017/one.2024.1.

Neves, D., Cristiani, E., Carvalho, A., Silva, A., 2025. Addressing accidental inhalation of air pollutants through ancient dental calculus: A case-study from Late Neolithic Cova das Lapas (Portugal).

Neves, D., Silva, A., Carvalho, A., Cristiani, E., 2024. Health and environmental contaminants of Prehistoric Iberian communities: insights from ancient dental calculus analysis.

Olsen, I., 2016. The oral microbiome in health and disease. In: Lynge Pedersen, A.M. (Ed.), Oral Infections and General Health: From Molecule to Chairside. Springer International Publishing, Cham, pp. 97–114. https://doi.org/10.1007/978-3-319-25091-5_10.

Ottoni, C., Borić, D., Cheronet, O., Sparacello, V., Dori, I., Coppa, A., Antonović, D., Vujević, D., Price, T.D., Pinhasi, R., Cristiani, E., 2021. Tracking the transition to agriculture in Southern Europe through ancient DNA analysis of dental calculus. Proc. Natl. Acad. Sci. 118, e2102116118. https://doi.org/10.1073/pnas.2102116118.

Pitt, S.J., Gunn, A., 2024. The one health concept. Br. J. Biomed. Sci. 81, 12366. https://doi.org/10.3389/bjbs.2024.12366.

Putrino, A., Marinelli, E., Galeotti, A., Ferrazzano, G.F., Ciribè, M., Zaami, S., 2024. A journey into the evolution of human host-oral microbiome relationship through ancient dental calculus: a scoping review. Microorganisms 12, 902. https://doi.org/10.3390/microorganisms12050902.

Radini, A., Buckley, S., Rosas, A., Estalrrich, A., Rasilla, M. de la, Hardy, K., 2016. Neanderthals, trees and dental calculus: new evidence from El Sidrón. Antiquity 90, 290–301. https://doi.org/10.15184/aqy.2016.21.

Radini, A., Nikita, E., 2023. Beyond dirty teeth: integrating dental calculus studies with osteoarchaeological parameters. Quat. Int. 653–654, 3–18. https://doi.org/10.1016/j.quaint.2022.03.003.

Radini, A., Nikita, E., Buckley, S., Copeland, L., Hardy, K., 2017. Beyond food: the multiple pathways for inclusion of materials into ancient dental calculus. Am. J. Phys. Anthropol. 162, 71–83. https://doi.org/10.1002/ajpa.23147.

Rayfield, K.M., Mychajliw, A.M., Singleton, R.R., Sholts, S.B., Hofman, C.A., 2023. Uncovering the Holocene roots of contemporary disease-scapes: bringing archaeology into one health. Proc. R. Soc. B. 290, 20230525. https://doi.org/10.1098/rspb.2023.0525.

Schuenemann, V.J., Avanzi, C., Krause-Kyora, B., Seitz, A., Herbig, A., Inskip, S., Bonazzi, M., Reiter, E., Urban, C., Pedersen, D.D., Taylor, G.M., Singh, P., Stewart, G. R., Velemínský, P., Likovsky, J., Marcsik, A., Molnár, E., Pálfi, G., Mariotti, V., Riga, A., Belcastro, M.G., Boldsen, J.L., Nebel, A., Mays, S., Donoghue, H.D., Zakrzewski, S., Benjak, A., Nieselt, K., Cole, S.T., Krause, J., 2018. Ancient genomes reveal a high diversity of Mycobacterium leprae in medieval Europe. PLOS Pathog. 14, e1006997. https://doi.org/10.1371/journal.ppat.1006997.

Schug, G.R., Buikstra, J.E., 2025. From ONE health to ONE paleopathology: deep-time perspectives on health in the face of climate and environmental change. Encyclopedia 5, 13. https://doi.org/10.3390/encyclopedia5010013.

Se, K., Mei, C., Inthavong, K., Tu, J., 2010. Inhalability of micron particles through the nose and mouth. Inhal. Toxicol. 22, 287–300.

Sørensen, L.K., Hasselstrøm, J.B., Larsen, L.S., Bindslev, D.A., 2021. Entrapment of drugs in dental calculus – detection validation based on test results from post-mortem investigations. Forensic Sci. Int. 319, 110647. https://doi.org/10.1016/j.forsciint.2020.110647.

Sperduti, A., Giuliani, M.R., Guida, G., Petrone, P.P., Rossi, P.F., Vaccaro, S., Frayer, D. W., Bondioli, L., 2018. Tooth grooves, occlusal striations, dental calculus, and evidence for fiber processing in an Italian eneolithic/bronze age cemetery. Am. J. Phys. Anthropol. 167, 234–243. https://doi.org/10.1002/AJPA.23619.

Stamer, J.R., Apata Mamani, M., Arriaza, B., Bendrey, R., Blevins, K., Campbell, T., Gottdenker, N., Gowland, R., Klaus, H., Lagia, A., Littleton, J., Maasch, K.A., Marques, C., Mauricio Llonto, A.C., Moore, J., Nelson, E.A., O'Donnell, L., Roberts, C., Sandweiss, D.H., Santos, A.L., Schuenemann, V.J., Shin, D.H., Snyder, T., Stone, A.C., Thomas, R., Tomasto-Cagigao, E., Van Schaik, K.D., Vega, M., Walser, J. W., I.I.I., Webster, E., Wilson, J.A., Wissler, A., Zuckerman, M., Robbins Schug, G., Uhl, E., Buikstra, J.E., 2026. Assessing the effectiveness of the one paleopathology workshop. Evol. Med Public Health 14, 1–10. https://doi.org/10.1093/emph/eoaf041.

Urban, C., Blom, A.A., Pfrengle, S., Walker-Meikle, K., Stone, A.C., Inskip, S.A., Schuenemann, V.J., 2021. One health approaches to trace mycobacterium leprae's zoonotic potential through time. Front. Microbiol 12. https://doi.org/10.3389/fmicb.2021.762263.

Wang, F., Xiang, L., Leung, K.S.-Y., Elsner, M., Zhang, Y., Guo, Y., Pan, B., Sun, H., An, T., Ying, G., Brooks, B.W., Hou, D., Helbling, D.E., Sun, J., Qiu, H., Vogel, T.M., Zhang, Wei, Gao, Yanzheng, Simpson, M.J., Luo, Yi, Chang, S.X., Su, G., Wong, B.M., Fu, T.-M., Zhu, D., Jobst, K.J., Ge, C., Coulon, F., Harindintwali, J.D., Zeng, X., Wang, H., Fu, Y., Wei, Z., Lohmann, R., Chen, C., Song, Y., Sanchez-Cid, C., Wang, Y., El-Naggar, A., Yao, Y., Huang, Y., Law, J.C.-F., Gu, Chenggang, Shen, H., Gao, Yanpeng, Qin, C., Li, Hao, Zhang, Tong, Corcoll, N., Liu, M., Alessi, D.S., Li, Hui, Brandt, K.K., Pico, Y., Gu, Cheng, Guo, J., Su, J., Corvini, P., Ye, M., Rocha-Santos, T., He, H., Yang, Y., Tong, M., Zhang, Weina, Suanon, F., Brahushi, F., Wang, Z., Hashsham, S.A., Virta, M., Yuan, Q., Jiang, G., Tremblay, L.A., Bu, Q., Wu, J., Peijnenburg, W., Topp, E., Cao, X., Jiang, X., Zheng, M., Zhang, Taolin, Luo, Yongming, Zhu, L., Li, X., Barceló, D., Chen, J., Xing, B., Amelung, W., Cai, Z., Naidu, R., Shen, Q., Pawliszyn, J., Zhu, Y., Schaeffer, A., Rillig, M.C., Wu, F., Yu, G., Tiedje, J.M., 2024. Emerging contaminants: a one health perspective. Innovation 5. https://doi.org/10.1016/j.xinn.2024.100612.

Warinner, C., Rodrigues, J.F.M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., Radini, A., Hancock, Y., Tito, R.Y., Fiddyment, S., Speller, C., Hendy, J., Charlton, S., Luder, H.U., Salazar-García, D.C., Eppler, E., Seiler, R., Hansen, L.H., Castruita, J.A. S., Barkow-Oesterreicher, S., Teoh, K.Y., Kelstrup, C.D., Olsen, J.V., Nanni, P., Kawai, T., Willerslev, E., Von Mering, C., Lewis, C.M., Collins, M.J., Gilbert, M.T.P., Rühli, F., Cappellini, E., 2014. Pathogens and host immunity in the ancient human oral cavity. Nat. Genet. 46, 336–344. https://doi.org/10.1038/NG.2906.

Warinner, C., Speller, C., Collins, M.J., 2015. A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. Philos. Trans. R. Soc. B 370, 20130376. https://doi.org/10.1098/rstb.2013.0376.

Webster, J.P., Gower, C.M., Knowles, S.C.L., Molyneux, D.H., Fenton, A., 2016. One health – an ecological and evolutionary framework for tackling neglected zoonotic diseases. Evolut. Appl. 9, 313–333. https://doi.org/10.1111/eva.12341.

Weyrich, L.S., Dobney, K., Cooper, A., 2015. Ancient DNA analysis of dental calculus. J. Hum. Evol. 79, 119–124. https://doi.org/10.1016/j.jhevol.2014.06.018.

Weyrich, L.S., Duchene, S., Soubrier, J., Arriola, L., Llamas, B., Breen, J., Morris, A.G., Alt, K.W., Caramelli, D., Dresely, V., Farrell, M., Farrer, A.G., Francken, M., Gully, N., Haak, W., Hardy, K., Harvati, K., Held, P., Holmes, E.C., Kaidonis, J., Lalueza-Fox, C., De La Rasilla, M., Rosas, A., Semal, P., Soltysiak, A., Townsend, G., Usai, D., Wahl, J., Huson, D.H., Dobney, K., Cooper, A., 2017. Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus. Nature 544, 357–361. https://doi.org/10.1038/nature21674.

WHO, 2025a. One health [WWW Document]. URL ⟨https://www.who.int/health-topics/one-health#tab=tab_1⟩ (accessed 2.28.25).

WHO, 2025b. Healthy diet [WWW Document]. URL ⟨https://www.who.int/health-topics/healthy-diet#tab=tab_1⟩ (accessed 3.19.25).

Zhang, B., Tan, X., He, X., Yang, H., Wang, Y., Zhang, K., 2019. Evaluation of cadmium levels in dental calculus of male oral SCC patients with betel-quid chewing in Hunan Province of China. Biol. Trace Elem. Res 191, 348–353. https://doi.org/10.1007/s12011-019-1639-4.