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REVIEW ARTICLE

Infectious disease in the Pleistocene: Old friends or old foes?

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Abstract

The impact of endemic and epidemic disease on humans has traditionally been seen as a comparatively recent historical phenomenon associated with the Neolithisation of human groups, an increase in population size led by sedentarism, and increasing contact with domesticated animals as well as species occupying opportunistic symbiotic and ectosymbiotic relationships with humans. The orthodox approach is that Neolithisation created the conditions for increasing population size able to support a reservoir of infectious disease sufficient to act as selective pressure. This orthodoxy is the result of an overly simplistic reliance on skeletal data assuming that no skeletal lesions equated to a healthy individual, underpinned by the assumption that hunter-gatherer groups were inherently healthy while agricultural groups acted as infectious disease reservoirs. The work of van Blerkom, Am. J. Phys. Anthropol., vol. suppl 37 (2003), Wolfe et al., Nature, vol. 447 (2007) and Houldcroft and Underdown, Am. J. Phys. Anthropol., vol. 160, (2016) has changed this landscape by arguing that humans and pathogens have long been fellow travelers. The package of infectious diseases experienced by our ancient ancestors may not be as dissimilar to modern infectious diseases as was once believed. The importance of DNA, from ancient and modern sources, to the study of the antiquity of infectious disease, and its role as a selective pressure cannot be overstated. Here we consider evidence of ancient epidemic and endemic infectious diseases with inferences from modern and ancient human and hominin DNA, and from circulating and extinct pathogen genomes. We argue that the pandemics of the past are a vital tool to unlock the weapons needed to fight pandemics of the future.

KEYWORDS
ancient DNA, human evolution, infectious diseases, metagenomics, pandemics

1 | INTRODUCTION

Our understanding of infectious diseases in prehistory has to date been reliant on palaeopathology and hypothetical models. While careful micro- and macro- analysis of skeletal remains can reveal much about disease in past populations, it suffers from the effects of differential preservation of material in the archeological record, issues with accurate diagnosis of disease, the osteological paradox and, perhaps most crucially, the fact that the vast majority of infectious diseases typically have no skeletal involvement (Cohen et al., 1994; Wood et al., 1992). However, technological advances and increasing use of palaeogenetic and palaeoproteomic approaches to studying ancient human remains give us new ways to study human infectious diseases before the dawn of agriculture. By combining direct molecular and

proteomic evidence of infectious diseases with palaeopathology, phylogenetic analysis of extant pathogens, and analysis of patterns of selection within human genomes, we can test assumptions which have been taken as orthodoxy since at least the 1950s. Placing the first epidemiological transition in the Neolithic, recasting Omran's original epidemiological transition as the second instance of this phenomenon (e.g. Barrett et al., 1998; Zuckerman et al., 2014), continues to effectively negate the role of the disease in deep time. We can rethink what the landscape of Pleistocene infectious diseases may have looked like in different populations and identify important new research avenues.

When considering humans in the past the temptation is to forget about the impact of temporal and spatial patterns and to talk in terms of large-scale events that were seemingly the same across millennia and continents. The Neanderthal extinction debate is all too often spoken of in terms of 'climate' or 'conflict' and other such blanket terms which smother the actual challenges faced by individuals in real time. Pettitt (1999) argues for a series of Neanderthal extinctions as the result of a wide range of different factors, and places evolutionary ecology front and centre. The same complex interactions are also true of disease as a selective pressure, both positive and deleterious, in the distant and recent past. We are quick to forget that prior to Florey and Chain developing a method for synthesizing penicillin in the 1940s a simple cut could have been fatal. Prior to the introduction of antibiotics, bacteraemic skin infections had a mortality rate of 15% while *Streptococcus pneumoniae* carried a mortality rate of 30% (rising to 70%–90% if bacteraemic (Shlaes & Bradford, 2018)). The role of Neolithisation and the impact it had on human groups and population size was profound (Belfer-Cohen & Goring-Morris, 2020; Bocquet-Appel, 2011; Kuijt, 2000). The Neolithic therefore casts a very long shadow and the result, albeit unintentionally, has been the relegation of disease as a selective pressure, or indeed any sort of factor, when reconstructing human life prior to this. The Pleistocene, the crucible of the genus *Homo*, appears to be an aseptic environment in this model. Yet disease would have been an ever-present threat (Gurven & Kaplan, 2007): from animal-borne infections contracted during hunting, to dysentery-causing bacteria in water, it is unthinkable that there was ever a point when disease was not a constant factor in human experience. It is equally improbable that our ancestors did not develop mechanisms and strategies to ameliorate their effects. The profound impact of diseases on ancient forager populations is reflected by the fact that ~70% of deaths recorded among contemporary hunter-gatherer populations are the result of infectious, zoonotic and parasitic diseases (Gurven & Kaplan, 2007).

Genetics has transformed how we study human evolution. In 1987 Cann, Stoneking and Wilson analyzed mtDNA from 147 'geographically diverse' people and demonstrated a shared African origin for humans (Cann et al., 1987). While not without its issues, the paper kickstarted a revolution in the study of human evolution, adding a complex and rapidly developing genetic strand of data alongside the 'bones and stones' that had hitherto been the major foci of study. The following 30 years has witnessed an explosion of data: the first Neanderthal mitochondrial DNA sequence (Krings et al., 1997; Krings et al., 1999); the Neanderthal genome draft (Green et al., 2010) and high-coverage genome (Prüfer et al., 2014); the discovery of the Denisovans (Krause et al., 2010); and the identification of multiple introgression events between Late Pleistocene *Homo* species (Meyer et al., 2014; Posth et al., 2017; Schaefer et al., 2021). So rapid is the pace of archaeo/palaeogenetic development that a mere 32 years after Cann and colleagues analyzed 147 mt DNA samples, researchers are now able to sequence the ancient nuclear genomes of dozens of individuals from multiple archeological sites from a single valley. This was demonstrated by Mittnik and colleagues reporting the genomic sequences of 118 individuals from the Lech River valley in Germany, spanning the Late Neolithic to the Middle Bronze Age, across multiple burial contexts (Mittnik et al., 2019).

However, the increasing availability of ancient hominin genomes has also ignited new controversies. Some palaeoanthropologists had long suspected that archaic introgression (interbreeding between Neanderthals, humans and other hominins) had taken place in human prehistory, and ancient genomes from Neanderthals and Denisovans were able to confirm this hypothesis. Ancient DNA analysis revealed the presence of new hominin groups (Krause et al., 2010; Meyer et al., 2014), widespread interbreeding (such as a human with recent Neanderthal ancestor (Fu et al., 2015) and 'Denny' (Slon et al., 2018)) and hints of deep population structure (Jacobs et al., 2019; Posth et al., 2017), and population migration and replacement (Mafessoni et al., 2020; Meyer et al., 2016, 2014). Early human migrations out of Africa may or may not have left traces in the gene pool of humans alive today (Mondal et al., 2016; Skoglund et al., 2018; Teixeira et al., 2021). Given the time scales and the range of environments over which these changes and interactions took place, and the evidence of natural selection acting on introgressed DNA within the human genome, it is more important than ever to consider the role that infectious diseases would have played in the lives of our ancestors and related hominins during the Pleistocene.

Ancient pathogen DNA and advances in palaeopathology (ancient proteomics, lipidomics, CT scanning, host genome sequencing and our understanding of genetic disease risk) are ripe to underpin a similar transformation in our understanding of disease in the Pleistocene. They are also likely to prompt a significant re-evaluation of the prevailing 20th century models (Barrett et al., 1998; A. Cockburn, 1963; T. A. Cockburn, 1971; Omran, 1971) which point to the origins of human agriculture as the watershed moment for human infectious disease. New models are needed which incorporate changing environments for migrating hominins, hominin-hominin disease transfer, sedentism, and interactions with non-pastoral animals which occur in the Upper Paleolithic. The transfer of diseases between humans and (peri) domesticated animals, which is key to the first epidemiologic transition model (FET) as originally formulated, remains a crucial theme in ancient infectious disease research (Armelagos & Harper, 2005), but new models will need more nuance. Evidence is accumulating that epidemic and endemic infectious diseases cannot simply be divided into 'old friends and new foes' on either side of the transition to agriculture: humans have also been subject to endemic and epidemic infectious diseases (old foes) with significant selection associated pressures for far longer than the last 15ky.

The tool kit of palaeopathology is advancing but can only tell us about infections and inflammation which lead to skeletal pathology (e.g. lesions, healed or unhealed in bones or teeth). Some of these pathologies can be directly attributed to specific pathogens and diseases, but not all. However, problems remain when trying to use palaeopathological data as a reflection of the relationship between human populations and disease in the past. The osteological paradox, a phrase first coined by Wood and colleagues (Wood et al., 1992), highlights the difficulty of conflating absence of infection in a given skeleton (or indeed population) with a complete absence of infectious disease. Simply put, if an infection kills rapidly it leaves no skeletal markers and the individual will appear skeletally 'healthy'. Conversely an individual with multiple lesions caused by a long-term infection might be regarded as unhealthy or sick, but in fact had the stronger immune systems and was effectively much 'healthier' than the skeletons presenting no evidence of disease. As Siek (Siek, 2013) has argued, this can result in erroneous claims that infectious diseases were either very rare or completely absent in the deep past.

We are often dependent on case reports from Pleistocene burials, human and hominin, as a result (for example, evidence of bacterial infection of prostatic stones (Usai et al., 2017) and osteolytic pathology caused by *Echinococcus* (Vlok et al., 2021); for a more detailed summary, see (Houldcroft & Underdown, 2016)). Parasites may either cause disease directly (for example, helminthiasis (Crompton, 1999)), or they may act as vectors for an infectious disease (such as transmission of the typhus-causing bacteria *Rickettsia prowazekii*, which can be spread by human body lice, *Pediculus humanus* (Badiaga & Brouqui, 2012)). Palaeoparasite research may therefore be useful in detecting the presence of parasites which can act as disease vectors, but it may also detect parasites which are directly associated with human disease. The diversity of human and zoonotic parasites identified in the archeological record is substantial, and the palaeopathology and evolution of some of these parasites has been reviewed elsewhere (Katharina Dittmar et al., 2012; Ledger & Mitchell, 2022; Mitchell, 2013). As with palaeopathology, less is known about the palaeoparasite landscape of the Pleistocene than the Holocene (Mitchell, 2013), although there is evidence from France of the helminth *Ascaris lumbricoides* (a common human roundworm) at a site dated to 24–30 kya (Bouchet et al., 1996). These fields give a tantalizing but incomplete picture of Pleistocene infections.

Moving on from what we can learn from palaeopathology, we can also use our knowledge of the spread of infectious diseases today and make predictions about whether they would have been significant human pathogens in the past. These models may incorporate factors such as climate, population size, subsistence strategy, animal reservoirs, diet and the presence or absence of disease vectors (Brynildsrud et al., 2018; Dean et al., 2018; Fournié et al., 2017; Sattenspiel, 1990; L. A. White & Mordechai, 2020).

Genomics illustrates extant human and pathogen diversity, which can be analyzed for signals of past and current selection or demographic events. Metagenomics gives us the DNA of other organisms living in or on ancient humans and hominins, for example providing direct evidence of changes to the oral microbiome in mineralized biofilms such as dental calculus, which may include pathogens, commensal bacteria, and opportunistic infections. While refinements to the epidemiological transition model have been made in recent years (eg (Armelagos, 2009; Armelagos & Harper, 2005)), the increasing availability of ancient human and pathogen DNA leaves the assumptions underlying the first epidemiological transition ripe for reappraisal (Harper & Armelagos, 2013; Zuckerman et al., 2014).

Researchers also need to consider whether there are special ethical concerns for working with ancient pathogen material or diagnosing diseases on the basis of palaeopathology. For example, there may be a risk of stigmatization of particular communities or ancestries if particular diseases or pathogens are identified. For example, infectious diseases caused by mycobacteria, treponemes or poxviruses may lead to stigmatization of living individuals, and may also have been associated with stigma or ostracism in the past (Dofitas et al., 2022; Klaus & Ortner, 2014; Ogoina et al., 2019; C. A. Roberts, 2020). The ethics of studying ancient infectious diseases overlap with concerns about the appropriate treatment and study of human remains, and ethical considerations from modern microbiology (Johnson & Parker, 2020).

This review will predominantly, but not exclusively, focus on evidence for infectious diseases in the Pleistocene that has been gained from high-throughput and metagenomic sequencing efforts of pathogen and host. While approaches such as polymerase chain reaction (PCR), quantitative PCR (qPCR) and short-fragment sequencing have been used to great effect in many studies of ancient infectious diseases, they typically do not have the same agreed criteria for ancient DNA authentication, which have been reviewed in detail elsewhere (Key et al., 2017; Llamas et al., 2017; Salter et al., 2014; Warinner et al., 2017). PCR and short-fragment sequencing also convey different information to high-throughput sequencing and metagenomics.

These new sources of evidence need to be integrated (or better integrated) with palaeopathology and modeling to understand endemic and epidemic disease in the Pleistocene. Here we will discuss four of these new sources (Figure 1): ancient pathogen genomes sequenced from sub-fossil human remains; the genomes of pathogens circulating today and the genomes of living people; ancient hominin genomes and introgressed hominin DNA; and ancient biomolecules derived from dental calculus and sedimentary DNA (sedaDNA), which can be synergistic with the other categories.

2 | ANCIENT PATHOGEN DNA FROM THE PLEISTOCENE

Ancient infectious diseases can increasingly be directly detected in human remains using approaches such as DNA sequencing and analysis of ancient biomolecules, i.e. proteins and lipids specific to certain pathogen species. These approaches may be used as means of confirming the presence of a pathogen present in aDNA data e.g. *Mycobacterium leprae* (Fotakis et al., 2020); or they may be used to indicate the presence of a pathogen when aDNA is not available, is too degraded or does not contain detectable frequencies of the pathogen of interest (Donoghue, 2017; Minnikin et al., 2015). Ancient

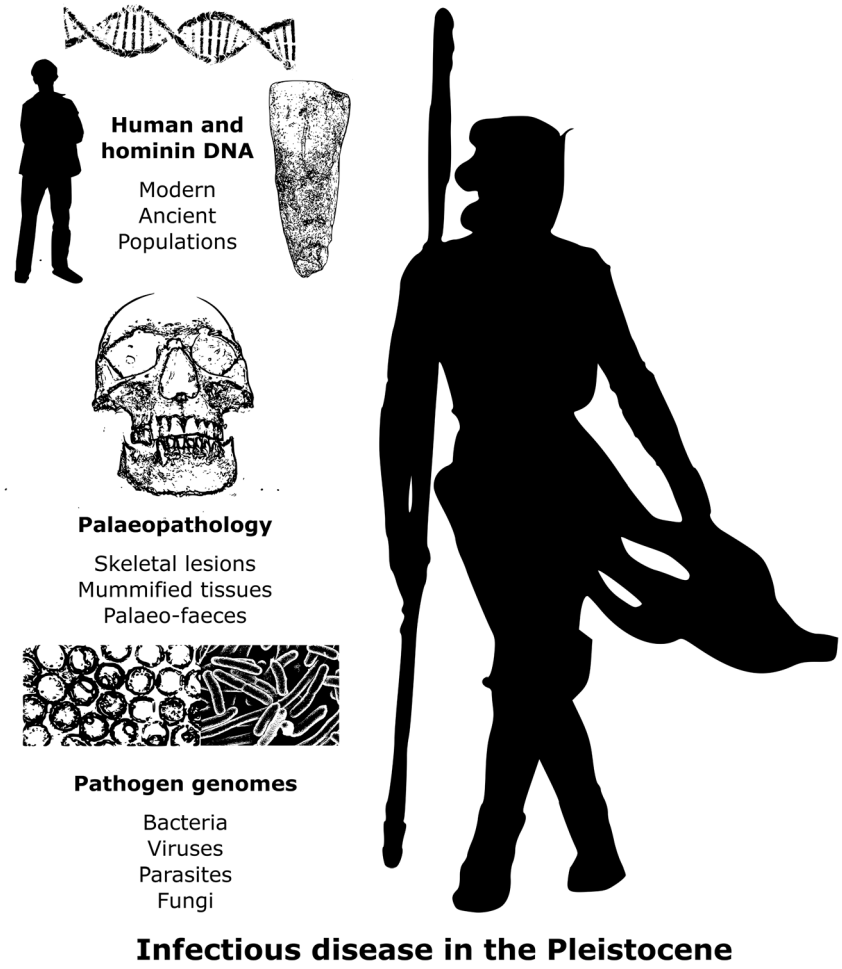

FIGURE 1 Sources of evidence for studying infectious diseases of humans and other Pleistocene hominins. From top to bottom: DNA analysis of humans and hominins, modern and ancient, including the analysis of genomes at a population scale; palaeopathology, such as osteolytic skeletal lesions resulting from infection, and the study of mummified tissues or palaeo-feces; and pathogen genomes, including bacteria, viruses, parasites and fungi encompassing the diversity of currently circulating lineages and ancient genomes.

pathogen research will increasingly utilize biomolecules in addition to DNA as techniques develop and authentication standards are agreed upon (Hendy, Welker, et al., 2018), but ancient pathogen DNA will remain a powerful tool in its own right. The identification of Pleistocene viral genetic material in sources as diverse as human teeth (Nielsen et al., 2021), rat palaeofaeces (Larsen et al., 2018) and glacier ice (Zhong et al., 2021) highlights the utility of deep-sequencing of ancient material.

Thanks to increased use of metagenomic sequencing of human remains, we now have a much more nuanced view of the infectious disease landscape of the Mesolithic and Neolithic. Some of the earliest pathogens directly identified by aDNA analysis are viruses, such as hepatitis B virus (HBV) and parvovirus B19 (B19V). B19V was present in European and Asian populations from at least 7kya (Mühlemann, Margaryan, et al., 2018). Directly dating these human-specific viruses using aDNA has suggested they began to emerge as human pathogens just before or around the time of the Neolithic revolution (Krause-Kyora et al., 2018; Mühlemann, Jones, et al., 2018; Mühlemann, Margaryan, et al., 2018), although additional ancient genomes may push this event further back in time. A growing collection of ancient *Salmonella enterica* Paratyphi C genomes, dating from 6.5 kya, show the evolution of this bacterium towards increasing human specialization (Key et al., 2020). The earliest *S. enterica* genome currently available is from Russia, from Murzikhinsky II. Individuals from this Eneolithic site were hunter-foragers who were transitioning towards pastoralism. This *S. enterica* genome is from a lineage able to infect and cause disease in many mammals, including horses; later *S. enterica* genomes from ancient agro-pastoralists are also associated with lineages which can infect pigs. For this pathogen, it appears that the Neolithic was an era of increased specialization as a pathogen of humans and perhaps also domesticated and companion animals, with increasingly rapid dissemination of related strains (Key et al., 2020). We have increasingly granular data on the molecular evolution of specific pathogens such as *Yersinia pestis* (plague) which have long evolutionary histories. The best and most abundant evidence for mortality caused by *Y. pestis* comes from the late Antique - early Medieval period onwards (Keller et al., 2019); it is harder to gauge the burden of morbidity and mortality associated with plague in prehistory with currently available evidence (Neumann et al., 2022; Spyrou et al., 2018; Susat et al., 2021). These studies help us to refine our understanding of models of the first epidemiological transition.

Studying ancient pathogen DNA can challenge our models of infectious disease ecology in unexpected ways. A case in point is the study of tuberculosis in the Americas. Modern MTBC (*Mycobacterium tuberculosis* complex) diversity in the Americas is clearly closely related to the MBTC strains which originate in Europe (Comas et al., 2013).

Therefore, circulating MTBC diversity is likely to be the result of the introduction of European TB strains during and after the colonial period of contact between the continents. There is, however, archeological evidence of MTBC in the Americas thousands of years before the arrival of European colonists and enslaved African peoples, particularly in the form of morphological and skeletal evidence in human remains and mummies from Chile and Peru (Arriaza et al., 1995; Blom et al., 2005; Mora et al., 2021). Following ancient DNA analysis, three sets of human remains from Peru, dated to 1028 CE to 1280 CE, showed evidence of infection not with human TB (*M. tuberculosis*) but with a mycobacterium most closely related to *M. pinnipedii* (Bos et al., 2014). This MTBC species is known to infect marine mammals including seals and sea lions (Cousins et al., 2003). This unexpected discovery of mammalian TB in South America raises questions: what was the broader landscape of TB (and indeed other infections (Guzmán-Solís et al., 2021; Ville N. Pimenoff & Houldcroft, 2021)) in the Americas before the arrival of European colonizers and enslaved Africans? This is particularly relevant to north American regions (Vågene et al., 2022), where fewer studies to recover human and pathogen aDNA have taken place thus far.

The biggest (and to our minds, most interesting) questions in infectious disease ecology and evolution focus on the Pleistocene, before widespread sedentism and domestication of pastoral animals. This is the time period in which our models of how infectious diseases have impacted human evolution could be revolutionized. The first direct evidence of human viral pathogens from the Pleistocene comes from the Yana Rhinoceros Horn site (RHS) in Siberia (Nielsen et al., 2021). To date, Yana RHS is the oldest known human settlement in the Arctic Circle, dated to the Upper Paleolithic (Pitulko et al., 2004). Among the remains recovered from this site were isolated deciduous teeth from two children who lived around 32,000 years ago. These teeth were deeply sequenced to recover not just high-depth host genomes (Sikora et al., 2019) from these individuals, but also the pathogens present within their teeth, most likely in the blood (Nielsen et al., 2021). This study recovered a range of pathogenic and commensal viruses, including two different genotypes of adenovirus species C, which is associated primarily with upper respiratory tract infections. There was also evidence that the children were infected with multiple herpesviruses, from all three subfamilies of human herpesviruses: HHV1 (herpes simplex virus 1, cause of cold sores), HHV4 (Epstein–Barr virus, cause of infectious mononucleosis), HHV5 (cytomegalovirus, typically leading to silent primary infection), and HHV6B and HHV7 (associated with rash and fever in children upon primary infection).

Evidence from ancient human populations in Siberia (Sikora et al., 2019, 2017) from the Upper Paleolithic reveals relatively large effective populations sizes of up to 500 people, and contemporary individuals from the same site did not show signs of inbreeding or even close relationships. These are factors likely to promote the persistence and spread of more diverse infectious disease packages, although insufficient for 'crowd diseases' such as measles (Black et al., 1974). Sikora and colleagues suggest that this genetic evidence "reinforces the view that wide-ranging mate exchange networks were present among Upper Palaeolithic foragers across the pre-LGM landscape" (Sikora et al., 2019). This would also promote the spread of infectious diseases, especially persistent/latent infections, which is reflected in the pathogens recovered from the Yana RHS remains (Nielsen et al., 2021).

Analysis of hundreds of skeletons from Europe and the Americas, dating back to 11kya, identified hepatitis B virus as a pathogen widespread during the early Neolithic in Europe and the Americas, and with the most recent common ancestor (MRCA) of European and American HBV strains dating to the late Pleistocene (Kocher et al., 2021). HBV has also been identified in Neolithic (~7kya), Bronze Age and Iron Age human remains from Europe and Asia (Krause-Kyora et al., 2018; Mühlemann, Jones, et al., 2018), with evidence of viral lineage replacement in Europe between the Mesolithic and the Neolithic (Kocher et al., 2021). Ancient HBV genomes also point to contact (leading to HBV transmission) between the ancestors of European and Native American populations until as recently as 13-15kya (Kocher et al., 2021). We suggest that these studies raise the prospect of HBV as a widespread disease of the late Pleistocene, with perhaps further and more significant impacts in the Neolithic.

Data from modern human remains demonstrates that there is scope for recovery of a number of viruses not just from teeth, but also from bones such as the femur. In recently deceased individuals, it was possible to detect herpesviruses (herpes simplex virus-1, varicella-zoster virus, Epstein–Barr virus; cytomegalovirus; HHV6B and HHV7), polyomaviruses (JC polyomavirus and Merkel cell polyomavirus), papillomaviruses (HPV), parvovirus B19V, hepatitis B virus and torque teno viruses (TTV), using a mixture of quantitative PCR and deep sequencing (Toppinen et al., 2020). A 1.6x B19 V genome was recovered from a petrous bone dating to 880–1000 CE (Mühlemann, Margaryan, et al., 2018) and Merkel cell polyomavirus DNA has been recovered from the cement that attaches human head lice nits to the hair follicle (Pedersen et al., 2022), demonstrating that aDNA studies may be able to recover other ancient virus DNA from human remains even when no teeth are available. It seems only a matter of time, sample size and depth of sequencing until ancient human TTV, or HPV sequences are published. Dental calculus may be another promising source of ancient virus DNA or proteins: it has already provided a Pleistocene bacterial genome from Neanderthal teeth, dated to 48 kya (Hendy, Warinner, et al., 2018; Weyrich et al., 2015; Weyrich et al., 2017).

Whether the detection of a given pathogen in ancient human remains reflects the presence of disease symptoms remains a subject of debate. For a given pathogen, primary and chronic infection may be associated with very different pathogen load dynamics, and be highly variable in their associated morbidity and mortality; and so the significance of the detection of pathogen DNA in ancient human remains must be appraised pathogen by pathogen. It is important to note that detecting DNA viruses in blood in living individuals is a relatively rare event, suggesting that viraemia for common DNA viruses is well controlled in immune competent hosts (Moustafa et al., 2017; Williams et al., 2022). Higher virus loads in blood are associated with poorer clinical outcomes for a number of viral pathogens, such as HBV

(Trépo et al., 2014) and adenovirus (Zhang et al., 2021), although the relationship between virus load and severity of symptoms is less clear cut for other pathogens, such as EBV (Odame et al., 2014).

RNA viruses will be much harder to study using ancient genomics, partly because of the more rapid degradation of aRNA, and also because current aDNA library preparation methods are not designed for RNA molecules. Early studies recovered ancient RNA virus fragments from, for example, cases of influenza from the 1918 pandemic (Basler et al., 2001; Reid et al., 1999; Taubenberger et al., 1997), and plant viruses from herbaria specimens (Fraile et al., 1997; Malmstrom et al., 2007). A partial genome of an RNA virus has been recovered from caribou feces, preserved in a sub-arctic permanent ice patch (Ng et al., 2014). Specimens of this type, essentially cryopreserved at a low constant temperature for hundreds of years, will be the exception rather than the rule, but they demonstrate that aRNA recovery is possible (Guy, 2014; Smith & Gilbert, 2018); and studies of plant remains have identified aRNA preserved at more ambient temperatures ranges for 1000 years (Peyambari et al., 2019).

3 | CAN WE USE CONTEMPORARY PATHOGEN GENOMES TO UNDERSTAND ANCIENT DISEASES?

Until ancient viral RNA from humans is recovered, as discussed above, and until we have a wider availability of Pleistocene pathogen aDNA, we can use estimates of the substitution rate in extant pathogens to date when current diversity arose using approaches such as the molecular clock or maximum likelihood phylogenetic methods (Gojobori et al., 1990; Jenkins et al., 2002). Determining the age of human pathogens on the basis of circulating diversity alone often leads to underestimation of the age of the most recent common ancestor for many microbes (Duchêne et al., 2016), partly because rates of molecular evolution may vary over time (Bromham, 2009), and also because of the time-dependency of evolutionary rates: the apparent rate of molecular evolution slows as longer timescales are studied (Biek et al., 2015). There are also specific challenges and computational solutions required to study the molecular evolution of viruses (Ghafari et al., 2021). Including ancient DNA-derived genomes typically pushes estimates of the age of current circulating pathogen diversity further back in time (Membrebe et al., 2019). Ancient genomes also provide directly dated evidence for a particular pathogen being present at a given location and time period (eg bacterial and viral pathogens in early medieval Europe (Guellil, Keller, et al., 2022; Guellil, van Dorp, et al., 2022)). Genomic evidence may also reveal evidence of pathogen lineages which have gone extinct (Mühlemann, Margaryan, et al., 2018; Mühlemann et al., 2020). Unfortunately, poor preservation of aRNA makes it harder to apply these insights to RNA virus evolution.

There are other technical challenges involved in analyzing the evolution of RNA viruses. RNA virus genomes evolve more rapidly than DNA viruses, and thus all substitutable sites within the genome may have become saturated (mutated multiple times) within as little as hundreds of years (Aiewsakun & Katzourakis, 2016). This makes molecular clock approaches to dating the divergence of RNA viruses more difficult. However, new models of the evolution of RNA viruses which take account of and correct for the time-dependent rate phenomenon suggest that some human RNA viruses are truly ancient: hepatitis C virus may have begun to diverge into different genotypes more than 400,000 years ago, consistent with an out of Africa pattern of HCV migration (Ghafari et al., 2021). Additionally, the sarbecoviruses (the subgenus to which SARS-CoV-1, causative agent of SARS, and SARS-CoV-2, causative agent of COVID-19, belong) may have diverged from one another over 20,000 years ago (Ghafari et al., 2021). As we will discuss, this is consistent with evidence of coronavirus-related selection on the human genome on certain human populations before the origins of agriculture (Souilmi et al., 2021).

4 | DEEP TIME AND DISEASE IN AFRICA

Current evidence suggests that the evolutionary history of hominins in Africa was strongly influenced by high levels of pathogen diversity and distribution across their habitats (V.N. Pimenoff et al., 2018; Van Blerkom, 2003; Wolfe et al., 2007). The deep-time frame of the shared human-disease coevolutionary relationship in Africa strongly suggests that disease will have acted as a strong selective pressure, and the role of disease acting as a constraint on host-populations has been widely recorded in Sub-Saharan hunter-gatherer populations (Ferwerda et al., 2007; Kwiatkowski, 2005; Linz et al., 2007; Tallavaara et al., 2018; Tanabe, Mita, Jombart, et al., 2010). This selective pressure not only constrained populations but also influenced patterns of dispersal out of, and within Africa, but without ancient DNA or skeletal lesions, remains a largely invisible force (Linz et al., 2007; Tanabe, Mita, & Balloux, 2010; Wolfe et al., 2007).

The evolution of malaria parasite species is a key area of research when considering ancient infectious disease pressure in Africa, and is a disease for which host, mosquito vector and malaria parasite genomics can provide synergy with findings from palaeopathology. Although we await a paper which analyses all three genomes together, studies which have started to integrate palaeopathology and ancient DNA analyses have much to offer (eg. Pfeiffer et al., 2019). There is currently only indirect evidence for the emergence of human malaria parasites during the Pleistocene, but it points to areas of important future study for aDNA researchers. By analyzing the genomes of extant human and gorilla malaria, scientists have been able to estimate when these two parasites began to speciate (and thus specialize in their specific primate hosts). The divergence of human malaria (*Plasmodium falciparum*) and western lowland gorilla malaria (*Plasmodium praefalciparum*) parasites began around 40-60 kya, with a population bottleneck for human *P. falciparum* around 5 kya (Otto et al., 2018). It is unclear what drove this reduction in *P. falciparum* population size, which may have been related to its mosquito vector, human population sizes or behavior, and/or environmental change, among many possibilities. *Plasmodium vivax* has also experienced population bottlenecks in humans but not in great apes, and its

evolutionary history is less clear than that of *P. falciparum* (Loy et al., 2018). Studying the genomic histories of the mosquito vectors that carry human malaria parasites (such as *Anopheles gambiae* and *A. funestus*) gives us another window into the risk exposure profile of our ancestors. For example, were mosquito vectors present in a particular region; did their populations expand at a similar time to human populations in that region, or following a change to subsistence patterns (Morgan et al., 2011; B. J. White et al., 2011)? Sequencing modern, historical and ancient disease vectors such as *Anopheles* mosquitos more broadly complements our understanding of the evolution and geographic distribution of human vector-borne diseases by showing us regions with the correct vectors for diseases, even if we are unable to identify whether a specific pathogen was present (Daszak et al., 2000; Humphrey et al., 2010; Reed et al., 2004).

Sedimentary ancient DNA (sedaDNA) is another source of ancient biomolecules that offers the possibility of opening a window into human disease exposure in deep time. SedaDNA can be created, especially in, but not limited to, archeological contexts, via a wide range of sources such as flakes of hair and skin, bodily fluids, feces, eggshells, feathers, leaves, pollen, seeds, and prokaryotes and viruses present within organic matter (V.N. Pimenoff et al., 2018). By analyzing sedaDNA we can recover fragments of viral, parasitic and bacterial DNA (Côté et al., 2016; Madeja et al., 2009; Ng et al., 2014; Tian et al., 2016) to create a more detailed understanding of human exposure to disease during the Pleistocene. Similarly, human or hominin palaeo-feces are a source of host, dietary, microbiome and pathogen DNA (Gilbert et al., 2008; Poinar et al., 2001; Rifkin et al., 2020). They are also important material for the study of ancient parasites, including microscopic and immunological detection methods (K. Dittmar & Steyn, 2004; Gonçalves et al., 2002; Mitchell, 2013). Looking beyond teeth and skeletal material as sources of genetic material will increase our understanding of human pathogens and commensal species, as well as potentially providing a source of aDNA which is more acceptable to some groups who have beliefs or concerns around destructive sampling of human remains. Working collaboratively, equitably and ethically with local researchers and populations should be at the heart of all such research (Fleskes et al., 2022; Gibbon, 2020; Somel et al., 2021).

It is useful to consider whether any of the Pleistocene pathogens so far identified could be considered pandemics or to have pandemic potential. Herpesviruses are extremely ancient pathogens, dating to the origins of the animal kingdom (Baker et al., 2005; McGeoch & Gatherer, 2005; Novoa et al., 2016; Savin et al., 2010); they have low mortality rates in immune competent hosts, and would have been endemic in the earliest human populations, which is reinforced by the detection of multiple HHVs in a child at Yana RHS (Nielsen et al., 2021). This makes them unlikely candidates for an ancient pandemic pathogen. Despite earlier estimates (Comas et al., 2013) of an ancient dispersal of the *Mycobacterium tuberculosis* complex (MTBC) out of Africa, *Mycobacterium tuberculosis* now seems - on the basis of aDNA evidence and a revision of the molecular clock - to be too young to qualify as a Pleistocene pathogen, although this does make its apparently relatively recent worldwide distribution and burden of disease more striking (Bos et al., 2014). Human adenoviruses have a number of characteristics which give them pandemic potential but the risk is proposed to be greatest when a host-switch occurs or a new recombinant genotype arises (Kremer, 2021). The presence of circulating adenovirus C genotypes around the world today suggests that an adenovirus pandemic has occurred during human history, likely predating the ancient genomes recovered at Yana RHS, but we have no idea of its severity (Kremer, 2021; Nielsen et al., 2021).

Given the global distribution of HBV indicated by aDNA studies, this virus is a contender for a Pleistocene pandemic pathogen. We suggest, in contrast to the authors (Kocher et al., 2021), that chronic infection caused by HBV *would* have been a cause of both morbidity and mortality for late Pleistocene populations (Kocher et al., 2021). The frequency of HBV found in archeological samples, which represent only a fraction of the original population, must have been at epidemic levels in some settings. HBV DNA is detected in blood during primary infection, or during reactivation in chronic HBV carriers (Yim & Lok, 2006); archeological remains in which HBV DNA has been detected are therefore only a subset of the total infected population. While 90% of adults infected today clear their HBV infections over time, it is unclear if this was true in pre-modern settings and to what extent other infections and environmental exposures such as aflatoxins might exacerbate the clinical course of primary and chronic HBV infection (Chang, 2000; Fouché et al., 2020; Kew, 2003; Rehermann & Nascimbeni, 2005). In contemporary populations, the presence of HBV DNA in blood is associated with poor outcomes, such as progression to liver fibrosis and cirrhosis, with the greatest risk in adults over the age of 40 (Iloeje et al., 2006; Mendy et al., 2010). Perinatal transmission of HBV in particular is associated with poor health outcomes (Trépo et al., 2014) and reduced ability to clear a HBV infection (McMahon, 2009). Two possibilities therefore present themselves: either perinatal HBV infection was common or even endemic (leading to widespread chronic carriage and increased risk of poor outcomes 10-30 years after infection (Fattovich, 2003; Indolfi et al., 2019)); or primary infection in adult life was either endemic or epidemic, with age at first infection influencing the risk of disease progression. HBV is not a human heirloom pathogen (Harper et al., 2013), based on current evidence (Kocher et al., 2021). Therefore, it may have had an epidemic or pandemic phase during its emergence as a human pathogen, rather than achieving endemicity immediately. Introductions of HBV into remote communities have been known, in contemporary societies, to reach hyperendemicity within the course of one-three generations (for example, in parts of Nigeria (Forbi et al., 2010)), so an epidemic or pandemic phase may have been brief but nevertheless significant.

Malaria is another important candidate but the evidence for its antiquity comes from studies of the genomes of extant *Plasmodium* species and the genomes of living people. We do not yet have ancient genomes to refine molecular dating or provide direct evidence of malaria in the Pleistocene, nor do we know for certain the geographic range of different malarial parasites from palaeopathology alone. It is also unknown whether malaria was always endemic in some regions (an heirloom pathogen), or whether an epidemic period preceded the

endemicity seen in parts of sub-Saharan Africa today (Harper et al., 2013; Ryan et al., 2020).

We are at an exciting moment in our understanding of Pleistocene infectious diseases, thanks to the addition of ancient biomolecules to existing data from palaeopathology. Further ancient pathogen genomes from the Pleistocene and early Holocene will enrich our understanding of infectious disease in the deep past, and help us to understand the evolution of pandemics on a range of timescales. This includes the evolution of the pathogen over time, and the change in the impact of the pathogen on populations because of host factors (Valtueña et al., 2022).

5 | INDIRECT EVIDENCE OF ANCIENT EPIDEMICS AND PANDEMICS FROM THE HUMAN GENOME

Where ancient pathogen biomolecules or skeletal evidence have not survived, we can also look to human and ancient hominin genomes for evidence of intense selection pressure due to infectious diseases in prehistory, including the portions of our genomes which derive from other hominin ancestors through introgression, allowing us to further unpick the complex history of human disease dynamics in the past.

Living people with predominantly non-African recent ancestry derive approximately 1%–2% of their genome from a Neanderthal ancestor; Denisovan ancestry is found at a frequency of <1% in the genomes of people from south and south-east Asia, reaching 5% in people of Papuan ancestry in New Guinea (Choin et al., 2021; Sankararaman et al., 2016; Vespasiani et al., 2020). This introgressed DNA has consequences for human health. We and others (Barreiro & Quintana-Murci, 2020; Houldcroft & Underdown, 2016; Kerner et al., 2021) have previously reviewed the role of adaptively introgressed Neanderthal and Denisovan loci, discussing both specific loci with functions in immunity to pathogens, and broader trends such as an over-representation of Neanderthal-like sites involved in immune functions in the genomes of living Asian populations (Khrameeva et al., 2014). Studies comparing electronic medical records and human genotype data have uncovered further associations between Neanderthal variants and their impacts on immunity and auto-immunity in different human populations (Dannemann, 2021; Simonti et al., 2016). Subsequent studies have shown that in Pacific populations, Denisovan ancestry is enriched in genetic pathways associated with protection from infection and other immune functions, with consequences for gene expression in relevant immune cell subtypes (Vespasiani et al., 2020; Zammit et al., 2019). We cannot, however, tell whether these introgressed Neanderthal and Denisovan variants are providing protection from the same pathogens, or types of pathogens, in living populations as in our ancestors (anatomically modern, Neanderthal or Denisovan).

We are able to study proteins which interact with pathogens more broadly, increasing our ability to study the host genomic footprint of unknown infections which have been selective agents in human evolution. This set of proteins can then be cross-checked against regions of the human genome which have been inherited from other hominins, i.e. Neanderthals and Denisovans. This approach has been applied to viral pathogens to identify virus-interacting proteins (VIPs) within the human genome. VIPs which give protection from RNA virus infection and disease are enriched for Neanderthal ancestry (Enard & Petrov, 2018). This suggests that Pleistocene hominins were under immunological and evolutionary pressure from RNA virus infections, and that some of these variants have evolutionary advantages for living humans, following introgression from Neanderthals. What is much harder to discern is which specific RNA viruses drove the evolution of human and Neanderthal immunity genes.

We can also examine the effect of Neanderthal and Denisovan ancestry on susceptibility to specific infections when large, genotyped patient cohorts exist, as is the case for SARS-CoV-2. Analysis of human genetic variants which modulate the risk of severe COVID-19 have identified Neanderthal variants which both protect against and predispose infected individuals to more severe SARS-CoV-2 symptoms. A Neanderthal isoform of 2′–5′ oligoadenylate synthetase 1 (*OAS1*) found in some Europeans is associated with protection from infection, hospitalization, the need for mechanical ventilation, and death (Zhou et al., 2021). A further region on chromosome 12 has been found to be of Neanderthal origin and is associated with reduced risk of severe SARS-CoV-2 outcomes (Zeberg & Pääbo, 2021). In contrast, variants on chromosome 3 which are associated with increased risk of severe SARS-CoV-2 symptoms are also of likely Neanderthal origin, and are found at high (50%) frequency in some Asian populations, and in 16% of Europeans (Zeberg & Pääbo, 2020). There has been considerable interest in why humanity's admixed ancestry may contribute to the range of symptom severity caused by this coronavirus, as estimates of the age of circulating Sarbecovirus diversity are still tens of thousands of years too young to suggest these were Neanderthal or Denisovan pathogens (Ghafari et al., 2021). The Neanderthal variants identified by these authors have known (Kristiansen et al., 2010; Sams et al., 2016) or likely (Zeberg & Pääbo, 2020) pleiotropic roles in response to other infections, or differing roles within the human immune system, making it difficult to directly link these variants to a specific ancient coronavirus epidemic.

There is evidence, however, of an ancient coronavirus epidemic preserved in the genomes of today's East Asian populations. Souilmi and colleagues studied VIPs which are involved in the response to coronavirus infection, and then further analyzed whether these coronavirus interacting proteins had evidence of being under selective pressure (Souilmi et al., 2021). Across 26 human populations, multiple East Asian populations had evidence of selective sweeps at coronavirus interacting protein loci, but a similar pattern was not seen in the genomes of other populations, including those from South Asia. The most likely explanation for this trend is that a coronavirus or a virus which interacts with a highly similar set of VIPs was a strong selective pressure on the ancestors of people living in East Asia, estimated to have taken place 25 kya (870 generations ago). This may even have been a series of different coronaviruses or repeated coronavirus epidemics. These methods are unable to reliably detect selective sweeps

taking place before 30 kya; it is therefore possible that earlier selective sweeps in response to viral outbreaks have also impacted the genomes of human, Neanderthals and Denisovans, but we do not currently have the tools to detect them (Souilmi et al., 2021).

Human genomic diversity within Africa can help us to understand the pathogen selection pressures experienced by modern humans both before and after populations began to migrate out of Africa. Malaria (particularly malaria caused by *P. falciparum*) is a significant selection pressure in Africa (Kwiatkowski, 2005), because most of the burden of mortality is experienced by children, and serious childhood infections have implications for the development of an effective adult immune system and may lead to lifelong health consequences, for example reduced growth (Halcrow et al., 2020). It may even be the most significant selection pressure humans experienced during our evolution (Kwiatkowski, 2005). Comparing the frequencies of human genetic mutations which are protective against malaria in different African groups points towards a Pleistocene origin for the most famous host genetic response to malaria: the sickle cell trait. Research by Laval and colleagues suggests that the HBB (beta-globin) βS sickle cell-causing mutation arose in the ancestor of African agriculturalist groups 22 kya and subsequently spread to rainforest hunter gatherers during the Holocene (Laval et al., 2019). This would be compatible with the estimates of the timing of the emergence of *P. falciparum* as a human-specific pathogen discussed above (Otto et al., 2018). As more African ancient genomes become available, dating the timing and spread of these mutations across the continent will become easier. It is crucial that this aDNA is not only ethically collected and fully integrates and acknowledges the expertise of local researchers and community stakeholders (and not just as names buried in the middle of dozens of authors on a paper), but that it is also properly plugged into models of human-disease coevolution.

The way we respond immunologically to infectious diseases may also have changed since the onset of the Neolithic (Domínguez-Andrés et al., 2021). Over time, we might hypothesize that both the specific infectious diseases we have been exposed to, and the *intensity* of that exposure, has changed. We have some evidence that during human evolution, the same types of pathogens have elicited different immune responses from modern humans as we have evolved towards traits such as immune tolerance or increased inflammation towards specific infections compared to our ancestors (Domínguez-Andrés et al., 2021). Domínguez-Andrés and colleagues characterized the cytokine response to infectious stimuli of hundreds of individuals of European ancestry, and established genotype–phenotype correlations between particular genetic loci and the cytokines produced in response to stimuli from different kinds of pathogen. This could then be used to predict whether the response of extinct individuals who shared these loci was skewed towards inflammation or tolerance to that type of pathogen. They found changes in the predicted immune responses of individuals who lived before and after the Neolithic. After the onset of the Neolithic, individuals were predicted to have more tolerant immune responses (decreased inflammatory cytokine production) towards intracellular pathogens of likely zoonotic origin (*Coxiella* and TB); but to have pro-inflammatory responses, associated with a robust immune response, towards extracellular infections such as the fungal pathogen *Candida albicans* and bacterium *Staphylococcus aureus*. The authors interpret this data as showing that "human immune responses need to adapt to a new landscape of infectious agents depending on the geographical location and types of microbe encountered" (Domínguez-Andrés et al., 2021). Further analysis of predicted genetic susceptibility to infectious diseases suggests that selection pressure from infectious diseases became more intense during the Bronze Age (Kerner et al., 2023). This suggests that dividing diseases into pre and post farming or pre and post industrialization is no longer sensible, because we are not comparing like with like immunologically: we have evolved, our pathogens have evolved, *and* specific infectious disease packages in distinct geographical regions have changed, on a variety of time scales (Kerner et al., 2023).

6 | WHAT CAN ANCIENT INFECTIOUS DISEASES TEACH US ABOUT EMERGING INFECTIOUS DISEASES?

The SARS-CoV-2 global pandemic that began in 2019, and at the time of writing continues, is a salutary reminder of the power that infectious disease can have on humanity. A small piece of genetic code surrounded by a protein coat effectively shut down the planet for 2 years. While the interconnectivity of life in the 21st century was unimaginable even 75 years ago, let alone 75,000, the impact of a modern epidemic like SARS-CoV-2 can be measured in relatively small degrees of difference, not types, from those during the Pleistocene. The impact of SARS-CoV-2 at the continental level is arguably less than the impact of, for example, a haemorrhagic fever on a Pleistocene human group numbering a few hundred. Infectious disease matters now, infectious disease mattered then.

2022 saw the increased global spread of mpox virus (MPXV) outside of endemic regions of west and central Africa (Zumla et al., 2022). MPXV is an orthopoxvirus related to variola virus (VARV), the causative agent of smallpox; VARV aDNA can give us valuable insights in to the long-term evolution of human poxviruses as we witness the apparent emergence of a poxviruses with the capacity to become globally endemic (Sklenovská & Van Ranst, 2018).

Genetic analysis of historical preserved African striped squirrel museum specimens dating back to 1899 has already pushed back the date of the earliest identified MPXV (Tiee et al., 2018). Similarly, sequencing of historical samples from museums and pathological specimens with characteristic lesions or associated contextual data has allowed multiple VARV genomes from smallpox cases to be successfully sequenced, covering a period of ~400 years (Duggan et al., 2016; Ferrari et al., 2020; Porter et al., 2017). The most recent common ancestor of these VARV genomes is estimated to date to the late 16th to early 17th centuries (Duggan et al., 2016; Porter et al., 2017). However, metagenomic sequencing of human remains dating to the Northern European Viking era revealed the presence of an unknown, extinct VARV clade (Mühlemann et al., 2020). The Viking

VARV and smallpox-associated VARV clades are estimated to have diverged around 1700 years ago. This Viking VARV clade experienced different gene inactivating mutations to the smallpox-associated clade, and it is unknown whether it caused the same human disease as later VARV genomes which caused smallpox. We do not know why or when this Viking clade went extinct, and whether there was competition between VARV clades. Studies such as this should reinforce the need for caution when trying to predict the evolution of MPXV as it spreads globally in new populations, particularly where virulence in concerned. We do not argue, based on current evidence, that either MPXV or VARV were human pathogens in the Pleistocene (Duggan et al., 2016; Forni et al., 2022; Mühlemann et al., 2020; Patrono et al., 2020), but they are important examples of bringing together historical and ancient pathogen genomes to better understand current disease outbreaks or infectious diseases with pandemic potential.

Taken together, these studies show that we need to consider the history and evolution of epidemics and pandemics holistically: pathogen, person, place. Where specific identification and even genomic analysis of a pathogen is possible, this provides complementary data to studies of endemic, epidemic or pandemic disease which may previously have only been identifiable through changes in mortuary practices or burials, non-specific skeletal lesions, or syndromic descriptions (Bianucci et al., 2015; Roberts, Davies, et al., 2022; Roberts, Scollard, & Fava, 2022; Schuenemann, Kumar Lankapalli, et al., 2018). By thinking about the person affected, this can include a genomic and bioarchaeological 'biosketch' of the person (Barquera et al., 2020; Guellil, van Dorp, et al., 2022; Guichón et al., 2015; Guzmán-Solís et al., 2021), which takes into account known genetic risk factors for infectious diseases, co-infections and 'health' writ large. 'Place' encapsulates not just geographic location, but also environment, climate conditions and biome (Crowl et al., 2008; Gottdenker et al., 2014). Models of this type have been important in understanding infant and child health, disability and mortality in prehistory (Halcrow et al., 2020), or the collapse of particular societies (Wright & White, 1996), and aDNA will add further dimensions to our understanding. It is almost impossible to fully understand the impact of infectious diseases in the Pleistocene without a clearer picture of the variation and interaction of these three factors. Ancient and modern DNA studies have also made evident that it is unreasonable and lazy to think that epidemics (or even pandemics) are an exclusive feature of the Neolithic onwards. Many independent lines of evidence from host and pathogen suggest they were experienced by our hunter-gatherer ancestors too (Houldcroft & Underdown, 2016; Souilmi et al., 2021). The selective pressures faced by humans in the past were diverse and largely immediate (Kwiatkowski, 2005; Shlaes & Bradford, 2018). The continued obsession within the field of trying to correlate millennial scale patterns to the lives of our ancestors is reductive and removes nuance from the equation. Rainfall patterns changing over a period of 10,000 years would have been of far less importance than, for example, tick borne diseases or a festering cut in the everyday lives of our Pleistocene ancestors. The demographic impact of this type of stochastic infection would have been profound but, because they lack a palaeopathological signature, are invisible and thus excluded from explanations of the past.

Another important aspect of disease ecology that needs to be considered when understanding ancient and emerging epidemics is the impact of age at primary infection. Multiple studies have shown that SARS-CoV-2 infection outcomes, morbidity and mortality are strongly negatively affected by age (O'Driscoll et al., 2021). This is not a unique aspect of SARS-CoV-2 immunobiology: for many diseases, bacterial and viral, there is a 'J' shaped relationship between the age at primary infection and the mortality rate. Infection is least serious in children between the ages of approximately 4–10 years of age (Glynn & Moss, 2020), with typically higher mortality in infants, and increasing mortality rates in older children, adolescents and adults. This is not immunosenescence, the waning of immunity with older age (Aw et al., 2007), but an apparently wider pattern of increasing disease severity with age following the onset of puberty. Delaying the age at primary infection of even ancient pathogens such as Epstein–Barr virus (Fourcade et al., 2017) and varicella-zoster virus (Malavige et al., 2008) may lead to mild childhood infections becoming severe diseases of adulthood (Glynn & Moss, 2020). Vaccination is a powerful tool to ameliorate the disease burden of infections, diminishing disease risk in all age groups and protecting in particular the very youngest and the very oldest. Developing effective vaccines against pathogens such as respiratory syncytial virus would be particularly appealing for this very reason (Shi et al., 2021, 2017), but it is important that strategies for vaccinating the elderly do not delay infection to an even more vulnerable age group (Malloy et al., 2013).

Multiple studies of the genetics underlying the human immune system suggest that human populations have experienced multiple episodes of selection pressure from infectious diseases (Benton et al., 2021; Deschamps et al., 2016; Fumagalli et al., 2011; Klunk et al., 2022), some intense enough to leave putative signals of selection tens of thousands of years later (Souilmi et al., 2021). These signals of intense selection should remind us of the vital importance of technologies like vaccination and improved hygiene, which we hope will spare us the mortality associated with survival of the fittest (Galvani & Slatkin, 2003; Immel et al., 2021; Karlsson et al., 2014; Laayouni et al., 2014).

7 | CONCLUSIONS

Ancient DNA and the study of human and other hominin genomes has profoundly challenged our understanding of infectious disease before the genesis of agriculture and pastoralism. While many pathogens may be older than we think (Ghafari et al., 2021), direct dating of ancient pathogen genomes has also revealed surprises, such as significant question marks over the ages of *M. tuberculosis* and *M. leprae* as human pathogens (Gagneux, 2018; Schuenemann, Avanzi, et al., 2018). Some questions can only be answered by integrating modern and ancient pathogen genomes into a shared analytical framework. Finally, studying ancient and modern human genomes has shown the importance of including immune system evolution in our models of ancient infectious diseases, considering whether the balance between inflammation and tolerance of certain pathogens has changed over time, as well as the package of diseases.

Infectious disease is, and has always been, a fellow traveler of humanity. Yet its near complete dismissal from any pre-Neolithic setting has massively reduced our ability to understand human evolution during the Pleistocene. Ultimately, without proper focus on the triumvirate of pathogen, person and place, and the proper application of ancient and modern disease data we cannot properly understand humans. To slightly paraphrase Hippocrates, "*It is more important to know people suffered from disease than to know what sort of disease people suffered from*."

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