In search of earliest records of endemic Plague: Past research and new endeavors

Mark Simon [1], Quanchao Zhang [2], Martin Sikora [3], Hugh Willmott [4], Sharon DeWitte [5], Qian Wang [1]

[1]Department of Biomedical Sciences, Texas A&M University School of Dentistry, Dallas, TX, U.S.A., [2] School of Archaeology, Jilin University, Changchun, China; [3] Globe Institute, University of Copenhagen, Copenhagen, Denmark; [4] Department of Archaeology, University of Sheffield, Sheffield, U.K.; [5] Department of Anthropology, University of South Carolina, Columbia, SC, U.S.A.

[*] Correspondence to: Qian Wang, Ph.D., Department of Biomedical Sciences, Texas A&M University School of Dentistry, 3302 Gaston Ave, Dallas, TX 75246. Tel: 214-370-7002; Fax: 214-874-4835

ABSTRACT

Yersinia pestis, the bacterium responsible for at least three pandemics in the past, is still a threat to modern populations. The bacterium has potential to evolve rapidly and persists in natural animal reservoirs around the globe. Epidemic diseases such as plague can dramatically alter and shape human demography, biology, and socio-cultural practices. Through the synthesis of biomolecular analyses with bioarchaeological data, researchers have begun to uncover the effects of past epidemics on modern populations and are also searching for the origins of the *Y. pestis* bacterium. Understanding the origins, behaviors, and consequences of diseases with epidemic potential in the past can contribute to ongoing discourse in public health, social policy, economy, and biology, as well as inspire positive changes in living populations. We review here recent literature on *Y. pestis* ecology and evidence of the bacteria's evolution in prehistory before discussing ongoing research at the Hamin Neolithic settlement site that is suspected to have collapsed from an epidemic disease.

Keywords: Plague, epidemic diseases, prehistory, paleoepidemiology

Introduction

Plague, a disease of zoonotic origins caused by the bacterium *Yersinia pestis*, is a re-emerging infectious disease that has caused the death of millions of people for millennia. A persisting threat to modern populations around the world, plague has been the source of at least three pandemics: the First Plague Pandemic (6^{th}-8^{th} century CE, also known as the Plague of Justinian), the Second Plague Pandemic (14^{th}-19^{th} century) that included the Black Death (c. 1338-1353), and the Third Plague Pandemic (which started in 1772 before becoming global in 1894). Due to the global nature of the Third Pandemic, natural plague reservoirs are now present around the world and therefore remain an endemic threat to modern populations, as demonstrated as recently as the outbreak in Madagascar in 2017 (Nguyen, Parra-Rojas, and Hernandez-Vargas, 2018). The ongoing COVID-19 Pandemic, which has caused nearly 652,000,000 reported cases and over 6,650,000 reported deaths globally at the time of writing (15 December 2022 – https://coronavirus.jhu.edu), has strained modern societies differently, depending on preexisting health, demographic, socioeconomic, and political conditions. It therefore stands to reason that an effort to understand how natural and anthropogenic factors influencing the spread of diseases, like plague, retrospectively can inform the present for the future. By recognizing the lifeways before, during, and after an event such as an epidemic we can better understand the vulnerability and resilience within and between systems. As such, in this article we briefly review the different facets of recent plague research with the intention of describing what and how we know about plague in order to explore current research foci and future directions. We first describe the ecology of *Y. pestis* before discussing its clinical syndromes. After these points, we then discuss past and current trends in bioarchaeological research that lead to a better understanding of the plague's origin in human populations.

Plague Ecology

Y. pestis is primarily found in wild population reservoirs of mammals, most commonly in burrow-dwelling rodents (WHO, 2010; Mahmoudi et al., 2021). Spillover into human populations from wild reservoirs commonly occurs as a result of host-vector-host synchrony, i.e., pathogen levels within a wild mammalian host population are maintained at a stable level until the host population size drops to levels that drive the

insect vector to seek out new hosts (Hudson and Cattadori, 1999; Schmid et al., 2015; Stenseth et al., 2022). The most common transmission route today is by infected flea spillover from rodent epizootics. However, it has been demonstrated that human fleas or body lice are also able to transmit the disease with differing efficiency in human populations (Dean et al., 2018; Barbieri et al., 2020; Barbieri, Drancourt, and Raoult, 2021). Other pathways of animal-human infection have been demonstrated in various regions and ecosystems (WHO, 2010; Carlson, Bevins, and Schmid, 2022; Jullien, de Silva, and Garner, 2021).

Natural plague reservoirs, which are areas where host, vector, and pathogen can and do survive indefinitely, are located in relatively arid areas or those that have a continental climate (Dubyanskiy and Yeszhanov, 2017; Mahmoudi et al., 2021; Stenseth et al., 2022). Examples of these climates are the Eurasian Steppe and the plains savannahs of the western United States. Additionally, due to the development and spread of human populations, symbiotic rodent populations in cities have also been described as foci despite possibly being in wet or tropical climates (Dubyanskiy and Yeszhanov, 2017). If humans are eliminated from the reservoir paradigm momentarily, these "natural plague reservoirs" would limit the range of *Y. pestis* globally, as demonstrated by Stenseth et al. (2022). However, once introduced by humans to an unnatural geography, Stenseth et al. (2022) suggest that temporary reservoirs may have been established and allowed the bacteria to remain in geographic areas that allowed for multiple reintroductions to human populations in the past, thus allowing pandemics to persist in locations such as Europe. Previously, Schmid et al. (2015) had demonstrated that fluctuations in climate allowed for plague bacterium to be introduced into Europe repeatedly every 14-16 years during the Second Pandemic and concluded that there was no support for a "permanent" reservoir during medieval Europe. These two scenarios should not be in opposition with one another; both cyclical introduction and temporary reservoir establishment should likely be considered in the case of plague in Europe and elsewhere where a natural reservoir is not established. These two scenarios allow us to further question the adaptability of the *Y. pestis* bacterium and its ability to persist within differing environments both genotypically and phenotypically in future research.,

Clinical Manifestations

Plague occurs in various clinical forms in human populations, and the three most common types are: bubonic, septicemic, and pneumonic. Without intervention, bubonic plague has a mortality rate of 50-90% while primary septicemic and pneumonic forms have a near 100% mortality rate (Prentice and Rahalison, 2007; Stenseth et al., 2008). The bubonic variant is a primary infection that concentrates within the lymphatic system creating its characteristic buboes due to cutaneous or mucous membrane exposure via arthropod vectors. However, with modern medical interventions, bubonic plague has a mortality rate of 10-20% and is the principal clinical form of the disease accounting for 80-95% of global cases reported (Prentice and Rahalison, 2007; WHO, 2021). A small subset of bubonic plague victims (10-20%) have been known to develop a secondary septicemic plague that increases the chance of mortality to 22% (Prentice and Rahalison, 2007). In a 2015 study, Kugeler et al. described that 10% of plague infections in the United States from 1900-2012 were primary septicemic infections that 9% of which were acquired from flea bite. Having a nearly 100% mortality rate if left untreated, primary pneumonic plague is the rarest form of the disease but is the most contagious variation. Pneumonic plague may also be developed secondary to bubonic or septicemic plague if the infection spills over into a patient's pulmonary tissues.

Currently, the nations with the highest disease incidence are the Democratic Republic of the Congo, Madagascar, and Peru. However, 95% of all cases since 2000 have occurred in Africa, with the most notable outbreak being 2017's outbreak of pneumonic plague in Madagascar (WHO, 2021). Despite its high mortality rate, even in modernity, the global cases of infection numbered 38,310 with 2,845 deaths from 1989 to 2003, and since 2005 only cases of the pneumonic variation and infection occurring outside of endemic zones require notification to the World Health Organization (Eisen et al., 2010; Barbieri et al., 2020; WHO, 2021). Detection, treatment, and containment are the priorities for clinical research on plague. Due to its historical relevance and epidemiological potential, genetic analyses of plague bacterium have progressed in recent years with multi-disciplinary teams.

Bioarchaeological Studies of Plague

Despite its documented historical prevalence, plague as a disease caused by the *Y. pestis* bacteria was only identified in the late 19th century, and the term "plague" in historical documents was not necessarily specific to this disease. Given the ambiguity of the term "plague", excavation of mass burials associated with plague events in past populations spurred debates about whether the individuals interred in them were victims of "yersinial" plague or some other epidemic disease. However, partially due to this critical skepticism, large strides have been made in fields such as paleoepidemiology and paleogenomics. It is now well-established that *Y. pestis* was the etiological agent that caused the three pandemics, and *Y. pestis* has been identified in numerous historical burial sites by anthropologists and other scientists (Raoult et al., 2000; Achtman et al., 2004; Wiechmann and Grupe, 2005; Drancourt et al., 2007; Bos et al., 2011; Schuenemann et al., 2011; Willmott et al., 2020).

Since 2000 the *Y. pestis* genome has been reconstructed using multiple approaches and samples (Bos et al., 2011; Barbieri et al., 2020; Valtueña et al., 2022; Spyrou et al., 2022; Klunk et al., 2022). In tandem with the development of plague paleogenomics, new models and understandings about human populations living during plague pandemics were being developed (DeWitte, 2010; Harbeck et al., 2013; Wagner et al., 2014; Castex and Kacki, 2016; Keller et al., 2019; Godde, Pasillas, and Sanchez, 2020). Consequently, once foundations or nodes of research were developed, the next logical step was to develop connections within and between these different points. For example, Klunk et al. (2022) have demonstrated the selective pressure of and the genetic bottleneck created by the Black Death (the first wave of the Second Plague Pandemic) that has consequences for modern populations, specifically affecting susceptibility to autoimmune disorders. The findings from this research are but one example of how an understanding of plague has great potential to invoke change to societies and populations, which raises questions about the long-lasting effect of other disease-causing microbes in both the present and the future. Other examples of the power of paleogenomics abound and are being contextualized to better understand causes and consequences within and between populations, both past and present (Rasmussen et al., 2015; Damgaard et al. 2018; Mühlemann et al., 2020; Susat et al., 2021; Pedersen et al., 2021).

In a world that is still actively experiencing the COVID-19 pandemic and that has been able to recognize the role of social inequity during it, it should be time to recognize we are a part of nature and not separate from it. As such, research that allows us to discuss and develop proactive interventions instead of reactive responses is critical to benefit nature and people, develop equitable benefits for people, and empower the marginalized. With a strong biomolecular foundation and a growing pool of genetic data available to researchers, the opportunities to explore the evolutionary origins of and changes to pathogens such as *Y. pestis* are more abundant and critical than ever. A robust synergy is created by integrating biomolecular analyses with other disciplines, such as bioarchaeology. By contextualizing the risks of morbidity and mortality that are brought about biologically and socially, anthropologists can significantly contribute to dialogues that may improve health equality. Studies of plague in the past have created a powerful lens for paleoepidemiologists to develop and contribute to discourse in both the past and the present (DeWitte, 2010; DeWitte, 2018; Willmott et al., 2020; DeWitte and Wissler, 2022).

In bioarchaeology the idea of frailty has been adjusted from the clinical idea of comorbidity that predisposes an individual to poor health and to potential death into age-adjusted relative risk of death compared to the broader living population (DeWitte and Wood, 2008). As bioarchaeologists are utilizing skeletal populations regularly, they must contend with the fact that mortality e.g., death, is the ultimate expression of poor health and is therefore selective of frailty despite the hidden heterogeneity of individuals. Selective mortality patterns such as those demonstrated during the Black Death did not occur in a vacuum and had implications for individuals, communities, and populations (DeWitte and Wood, 2008; DeWitte, 2009; DeWitte, 2010; DeWitte et al., 2016; DeWitte and Lewis, 2020; Godde, Pasillas, and Sanchez, 2020; Willmott, 2020; DeWitte and Wissler, 2022; Klunk et al. 2022; Gopalakrishnan et al., 2022). At the same time, the choices and policies made in the past had consequences for more than just human bodies but also our social and biological environments. Prior to the Black Death in 14th century London, there is ample evidence that population health was worsening due to famines, war and increased social inequalities, and thereby inflated the mortality rate of the Black Death (DeWitte, 2015, 2018). However, in cases where a population is undocumented, as in many

archaeological contexts, the ability to detect and identify sites of past epidemics remains crucial to understand the far-reaching implications of such a natural disaster. Even in cases of historical epidemics, such as in the case of Willmott et al. (2020), the identification of *Y. pestis* in a rural location was the first discovery of its kind in Britain during the Black Death period and has implications to consider for small community behavior during a disaster, and urban-rural interactions of the time.

Plague in Prehistoric Times

Plague infection, if not treated as early as possible, causes death within a matter of days. Because of this rapid process, the skeleton of an infected individual is not stimulated like other organ systems and therefore leaves no traces behind externally for the anthropologist. As such, prehistoric evidence of plague outbreaks is difficult to identify. However, significant steps have been made through the synthesis of biomolecular analysis with bioarchaeological analysis. The molecular clock for plague and its introduction to human populations has been stepped back progressively through the Bronze Age into Neolithic with the earliest known origins of virulent *Y. pestis* at least 7,000 years ago (Rasmussen et al., 2015; Valtueña et al., 2017; Spyrou et al., 2018; Rascovan et al., 2019; Susat et al., 2021).

Sampling 101 individuals from across Eurasia, Rasmussen et al. (2015) identified *Y. pestis* in seven individuals and a lineage of *Y. pestis* that at the time of publication was basal to all known *Y. pestis* genetic sequences. The bacterial phylogenetic sequence from RISE505 and RISE509, two Bronze Age individuals from modern day Russia separated geographically, temporally, and culturally, were used to statistically estimate the divergence date of the *Y. pestis* bacterium from its most recent common ancestor to 5,783 years ago. The reconstructed genetic sequences except for one did not include the Yersinia murine toxin (*ymt*) gene that is known to code for the enzyme that enhances bacterial survival in the flea gut but is not required for virulence (Rasmussen et al., 2015). The lack of *ymt* gene in conjunction with the presence, absence, or mutations to other pathogenicity genes led Rasmussen et al. (2015) to suspect that Bronze Age plague were likely unable to cause bubonic plague but were able to convey septicemic and pneumonic infections.

Further validating Rasmussen et al. (2015)'s phylogenetic and molecular clock results, Valtueña et al. (2017) screened 563 skeletal and tooth samples from the Late Neolithic to the Bronze Age (LNBA) and identified five strong positive *Y. pestis* sequences. Two of the five strong positive samples were enriched for further statistical testing. One of these two samples originated from modern day Russia and formed a genetic clade with samples tested by Rasmussen et al. (2015) while the other originated from Croatia and temporally dated to a similar time as the Russian sample with which it was enriched (Valtueña et al., 2017). Virulence and pathogenicity were similarly assessed and resulted as described in Rasmussen et al. (2015). However, the primary discussion of the article was not the presence of or the genetic makeup of *Y. pestis* but how it was introduced into Europe originally. With the available human genetic and archaeological data, Valtueña et al. (2017) proposed two models for plagues introduction to Europe. Ultimately it was hypothesized that plague was introduced once from the central Eurasian Steppe in Siberia through LNBA Eurasian trade networks before disseminating throughout Europe further from a newly established reservoir. Plague was then genetically reintroduced to the Eurasian step during the Bronze Age. Valtueña et al. (2017) supplied an additional discussion of disease transmission and disease dynamics that allows for plague's bubonic form and/or its adaptability to hosts during a period of cultural change.

In an analysis of nine Middle Bronze Age individuals from the Samara region of modern-day Russia, Spyrou et al. (2018) identified two individuals infected with plague. These genomes were demonstrated to be contemporaneous with by phylogenetically distinct from the clades described by Rasmussen et al. (2015) as well as Valtueña et al. (2017) with major distinctions being that this genome is flea adapted with *ymt* and other genes related to pathogenicity present, and it is closely related to existent *Y. pestis*. Prior to Spyrou et al.'s 2018 publication it was believed that these genetic characteristics originated at approximately 3,000 BP. However, the analyses presented in relationship to individuals RT 5 and RT6 would suggest an earlier arthropod adapted *Y. pestis* originating approximately 4,000 BP. The phylogeny produced by the data is interesting in that it demonstrates the presence of multiple lineages that can cause plague in human populations during the Bronze age.

Further exploring the possibility of prehistoric epidemics, Rascovan et al. (2019) re-examined the genetic data available for an early Neolithic mass burial site in western Sweden dating to 5,100-4,900 BP where they found strong evidence of plague infection in two of the 78 buried individuals. Performing phylogenetic, genotyping, and molecular clock analyses, Rascovan et al. (2019) identified a Neolithic strain of *Y. pestis* (Gok2) that was basal to all known *Y. pestis* strains. This new strain, like Rasmussen et al. (2015) and Valtueña et al. (2017), lacked virulence plasmids such as the plasmid that includes the *ymt* gene. Gok2 was also identified as being separate and from the Bronze Age clade. The molecular clock analysis performed with the newly identified Gok2 sequence demonstrated a divergence time between 5,250-6,364 BP while previously the more basal Bronze Age clade diverged between 4,953-5,731 BP (Rascovan et al. 2019). Following their genetic analyses, Rascovan et al. (2019) also did a comparison of human genomes in order to explore the possible role of human migrations during the Neolithic decline. The authors concluded following their analyses was that there was evidence of a prehistoric plague pandemic that contributed to the Neolithic decline of Europe.

Recently, Susat et al. (2021) identified the *Y. pestis* bacterium in a hunter-gatherer in Latvia dated to 5300-5055 cal. BP. This identification makes it the earliest known case of plague in a human population and dates to the beginning of the Neolithic period in Europe. The new molecular clock analysis presented in this study hypothesized the emergence of this Neolithic strain to 7,000 years ago; whereas the division of *Y. pestis* from its most recent common ancestor was 7,400 years ago, which is 1,000 years earlier than previously calculated (Susat et al., 2021). Missing only the *ymt* gene for flea gut adaptation, the genetic analysis of the *Y. pestis* bacterium in this study is interesting when considering the pathogen's evolution and the interactions between the bacterium, non-human, and human host where it was identified. Despite the low transmissibility from human to human, the interface between humans and animals, as well as animals and the environment, warrants further exploration especially considering current climate change.

Additions and refinements to the molecular clock from Rasmussen et al. (2015) and Valtueña et al. (2017) demonstrated the existence of *Y. pestis* in the Bronze Age and Rascovan et al. (2019) traced plague infections of the Eurasian Steppe to the Neolithic. Now Susat et al. (2021) have indicated that there is a correlation to *Y.*

pestis infections dating to the Neolithic at the western edge of the Eurasian Steppe. A study by Yu et al. (2020) further validated the results from Rasmussen et al. (2015), Valtueña et al. (2017), and Rascovan et al. (2019) and additionally demonstrated that *Y. pestis* was at the eastern end of the Eurasian Steppe during the Early Bronze Age. Further, Yu et al. (2020) demonstrated the admixture of populations occurring the Eurasian Steppe from the Upper Paleolithic to the Bronze Age. Given the newest date of *Y. pestis* in the Neolithic Age by Susat et al. (2021) in addition to the findings from Yu et al. (2020) there is a distinct need to explore whether there is earlier evidence of plague outside of Europe, and more explicitly in Asia.

In part due to the need of more paleodemographic and paleogenomic work in Asia, we have turned our attention to the Neolithic Hamin settlement that has approximately been dated to 5,100-5,600 years ago and was abandoned likely due to an epidemic disaster (Zheng et al., 2014; Zhu et al., 2014; Zhou et al., 2022). At the Hamin site at least 181 individuals were buried in a short timeframe in dwellings, and of which at least 97 individuals were interred in a single structure. There is no evidence of physical violence or geological disaster, but the abandonment of the site is evident from excavated cultural remains or lack thereof in the 54 pit houses. This large settlement (relative to other Neolithic period settlements) relied on hunter-gatherer subsistence practices with limited farming (Chen et al., 2016). Dietary animal remnants consist of rodents, birds, and mussels in large proportion with a smaller proportion of roe deer, horses, pigs and rabbits (Chen et al., 2016). Additionally, rodent burrow remnants are exhibited throughout the cultural layers of the site (Tang et al., 2016). Located geographically in the northeastern Eurasian Steppe that is part of even today's natural reservoir for *Y. pestis,* the Hamin Site may potentially have collapsed from plague. However, given the mobile lifeways of the Neolithic period in conjunction with the proximity of both humans and other animals, another zoonotic infection may be possible.

Conclusions

Bioarchaeological studies of plague continue to have great potential across research disciplines and lend themselves to multidiscipline research. In this brief review we broadly discussed the recent and current trends in the study of plague as a disease caused by the bacterium *Y. pestis*. Understanding the ecology of the pathogen

is strongly linked to the environment it resides in and its ability to adapt and persist in different environments as it inevitably spills over from its endemic populations. Future studies in the ecology of the bacterium are warranted to detect and monitor the pathogen safely within its endemic niches. Continued clinical work should be continued in order to better address infections and responses to infections as the disease regularly enters human populations. Additionally, plague and biomolecular analyses complement the growing field of evolutionary medicine. By utilizing bioarchaeological models of past populations that lived before, during, and after plague infections, population structures and histories can be used to identify meaningful patterns, how they were developed, and their trajectories that can in turn be integrated cross-disciplinarily to generate positive changes in living populations and for the future. In the future it is essential to gather samples of *Y. pestis* genomes from outside of Europe to better understand past evolutionary trends and predict future evolutionary trajectories that may also be applied to other pathogens.

Acknowledgements

Funding from NSF #2040388 to Q.W. and S.D.

Conflicts of Interest

The authors declare no potential conflict of interest.

References

Achtman, M., Morelli, G., Zhu, P., Wirth, T., Diehl, I., Kusecek, B., Vogler, A.J., Wagner, D.M., Allender, C.J., Easterday, W.R., Chenal-Francisque, V., Worsham, P., Thomson, N.R., Parkhill, J., Lindler, L.E., Carniel, E., Keim, P., 2004. Microevolution and history of the plague bacillus, Yersinia pestis. Proceedings of the National Academy of Sciences 101, 17837–17842.. doi:10.1073/pnas.0408026101.

Barbieri R., Drancourt M., and Raoult D., 2021. The role of louse-transmitted diseases in historical plague pandemics. The Lancet Infectious Diseases 21(2), 17-25.

Barbieri R., Signoli M., Chevé D., Costedoat C., Tzortzis S., Aboudharam G., Raoult D., and Drancourt M., 2020. *Yersinia pestis*: the Natural History of Plague. Clinical Microbiology Reviews 34, e00044-00019.

Bos, K.I., Schuenemann, V.J., Golding, G.B., Burbano, H.A., Waglechner, N., Coombes, B.K., Mcphee, J.B., Dewitte, S.N., Meyer, M., Schmedes, S., Wood, J., Earn, D.J.D., Herring, D.A., Bauer, P., Poinar, H.N., Krause, J., 2011. A draft genome of Yersinia pestis from victims of the Black Death. Nature 478, 506–510.. doi:10.1038/nature10549.

Carlson, C.J., Bevins, S.N., Schmid, B.V., 2022. Plague risk in the western United States over seven decades of environmental change. Global Change Biology 28, 753–769.. doi:10.1111/gcb.15966.

Castex, D., Kacki, S., 2016. Demographic Patterns Distinctive of Epidemic Cemeteries in Archaeological Samples. Microbiology Spectrum 4, 1–11.. doi:10.1128/microbiolspec.poh-0015-2015.

Chen, S., Yang, K., Li, B., Zhu, Y., and Ji, P., 2016. A study of the stone tools from the Haminmangha site. Acta Anthropologica Sinica. 35:522-536.

Damgaard, P.D.B., Marchi, N., Rasmussen, S., Peyrot, M., Renaud, G., Korneliussen, T., Moreno-Mayar, J.V., Pedersen, M.W., Goldberg, A., Usmanova, E., Baimukhanov, N., Loman, V., Hedeager, L., Pedersen, A.G., Nielsen, K., Afanasiev, G., Akmatov, K., Aldashev, A., Alpaslan, A., Baimbetov, G., Bazaliiskii, V.I., Beisenov, A., Boldbaatar, B., Boldgiv, B., Dorzhu, C., Ellingvag, S., Erdenebaatar, D., Dajani, R., Dmitriev, E., Evdokimov, V., Frei, K.M., Gromov, A., Goryachev, A., Hakonarson, H., Hegay, T., Khachatryan, Z., Khaskhanov, R., Kitov, E., Kolbina, A., Kubatbek, T., Kukushkin, A., Kukushkin, I., Lau, N., Margaryan, A., Merkyte, I., Mertz, I.V., Mertz, V.K., Mijiddorj, E., Moiyesev, V., Mukhtarova, G., Nurmukhanbetov, B., Orozbekova, Z., Panyushkina, I., Pieta, K., Smrčka, V., Shevnina, I., Logvin, A., Sjögren, K.-G., Štolcová, T., Taravella, A.M., Tashbaeva, K., Tkachev, A., Tulegenov, T., Voyakin, D., Yepiskoposyan, L., Undrakhbold, S., Varfolomeev, V., Weber, A., Wilson Sayres, M.A., Kradin, N., Allentoft, M.E., Orlando, L., Nielsen, R., Sikora, M., Heyer, E., Kristiansen, K., Willerslev, E., 2018. 137 ancient human genomes from across the Eurasian steppes. Nature 557, 369–374.. doi:10.1038/s41586-018-0094-2.

Dean, K.R., Krauer, F., Walløe, L., Lingjærde, O.C., Bramanti, B., Stenseth, N.C., Schmid, B.V., 2018. Human ectoparasites and the spread of plague in Europe during the Second Pandemic. Proceedings of the National Academy of Sciences 115, 1304–1309.. doi:10.1073/pnas.1715640115.

Dewitte, S.N., 2009. The effect of sex on risk of mortality during the Black Death in London, A.D. 1349-1350. American Journal of Physical Anthropology 139, 222–234.. doi:10.1002/ajpa.20974.

Dewitte, S.N., 2010. Sex differentials in frailty in medieval England. American Journal of Physical Anthropology 143, 285–297.. doi:10.1002/ajpa.21316.

Dewitte, S.N., 2018. Stress, sex, and plague: Patterns of developmental stress and survival in pre- and post-Black Death London. American Journal of Human Biology 30, e23073.. doi:10.1002/ajhb.23073.

Dewitte, S.N., Lewis, M., 2021. Medieval menarche: Changes in pubertal timing before and after the Black Death. American Journal of Human Biology 33.. doi:10.1002/ajhb.23439.

DeWitte S.N., Wissler A., 2022. Demographic and evolutionary consequences of pandemic diseases. Bioarchaeology International, 6(1-2): 108-132.

Dewitte, S.N., Wood, J.W., 2008. Selectivity of Black Death mortality with respect to preexisting health. Proceedings of the National Academy of Sciences 105, 1436–1441.. doi:10.1073/pnas.0705460105.

Dewitte, S.N., Hughes-Morey, G., Bekvalac, J., Karsten, J., 2016. Wealth, health and frailty in industrial-era London. Annals of Human Biology 43, 241–254.. doi:10.3109/03014460.2015.1020873.

Drancourt, M., Signoli, M., Dang, L.V., Bizot, B., Roux, V., Tzortzis, S., Raoult, D., 2007. Yersinia pestisOrientalis in Remains of Ancient Plague Patients. Emerging Infectious Diseases 13, 332–333.. doi:10.3201/eid1302.060197.

Dubyanskiy, V.M., Yeszhanov, A.B., 2016. Ecology of Yersinia pestis and the Epidemiology of Plague. In: Yang, R. and A. Anisimov (eds.), *Yersina pestis*: Retrospective and Perspective. Springer Science+Media, Dondrecht, pp. 171-192.

Eisen, R.J., Beard, C.B., Gage, K.L., Winters, A.M., Griffith, K.S., Mead, P.S., Enscore, R.E., Borchert, J.N., Schriefer, M.E., Macmillan, K., Acidri, R., Apangu, T., Zielinski-Gutierrez, E., Owor, N., Acayo, S., 2010. Assessing Human Risk of Exposure to Plague Bacteria in Northwestern Uganda Based on Remotely Sensed Predictors. The American Journal of Tropical Medicine and Hygiene 82, 904–911.. doi:10.4269/ajtmh.2010.09-0737.

Godde, K., Pasillas, V., Sanchez, A., 2020. Survival analysis of the Black Death : Social inequality of women and the perils of life and death in Medieval London. American Journal of Physical Anthropology 173, 168–178.. doi:10.1002/ajpa.24081.

Gopalakrishnan, S., Ebenesersdóttir, S.S., Lundstrøm, I.K.C., Turner-Walker, G., Moore, K.H.S., Luisi, P., Margaryan, A., Martin, M.D., Ellegaard, M.R., Magnússon, Ó.Þ., Sigurðsson, Á., Snorradóttir, S., Magnúsdóttir, D.N., Laffoon, J.E., Van Dorp, L., Liu, X., Moltke, I., Ávila-Arcos, M.C., Schraiber, J.G., Rasmussen, S., Juan, D., Gelabert, P., De-Dios, T., Fotakis, A.K., Iraeta-Orbegozo, M., Vågene, Å.J., Denham, S.D., Christophersen, A., Stenøien, H.K., Vieira, F.G., Liu, S., Günther, T., Kivisild, T., Moseng, O.G., Skar, B., Cheung, C., Sandoval-Velasco, M., Wales, N., Schroeder, H., Campos, P.F., Guðmundsdóttir, V.B., Sicheritz-Ponten, T., Petersen, B., Halgunset, J., Gilbert, E., Cavalleri, G.L., Hovig, E., Kockum, I., Olsson, T., Alfredsson, L., Hansen, T.F., Werge, T., Willerslev, E., Balloux, F., Marques-Bonet, T., Lalueza-Fox, C., Nielsen, R., Stefánsson, K., Helgason, A., Gilbert, M.T.P., 2022. The population genomic legacy of the second plague pandemic. Current Biology 32, 4743–4751.e6.. doi:10.1016/j.cub.2022.09.023.

Harbeck, M., Seifert, L., Hänsch, S., Wagner, D.M., Birdsell, D., Parise, K.L., Wiechmann, I., Grupe, G., Thomas, A., Keim, P., Zöller, L., Bramanti, B., Riehm, J.M., Scholz, H.C., 2013. Yersinia pestis DNA from Skeletal Remains from the 6th Century AD Reveals Insights into Justinianic Plague. PLOS Pathogens 9, e1003349.. doi:10.1371/journal.ppat.1003349.

Hudson, P.J., Cattadori I.M., 1999. The Moran effect: a cause of population synchrony. Trends in Ecology & Evolution, 14(1), 1-2.

Jullien, S., De Silva, N.L., Garner, P., 2021. Plague Transmission from Corpses and Carcasses. Emerging Infectious Diseases 27, 2033–2041.. doi:10.3201/eid2708.200136.

Keller, M., Spyrou, M.A., Scheib, C.L., Neumann, G.U., Kröpelin, A., Haas-Gebhard, B., Päffgen, B., Haberstroh, J., Ribera I Lacomba, A., Raynaud, C., Cessford, C., Durand, R., Stadler, P., Nägele, K., Bates, J.S., Trautmann, B., Inskip, S.A., Peters, J., Robb, J.E., Kivisild, T., Castex, D., Mccormick, M., Bos, K.I., Harbeck, M., Herbig, A., Krause, J., 2019. Ancient Yersinia pestis genomes from across Western Europe reveal early diversification during the First Pandemic (541–750). Proceedings of the National Academy of Sciences 116, 12363–12372.. doi:10.1073/pnas.1820447116.

Klunk, J., Vilgalys, T.P., Demeure, C.E., Cheng, X., Shiratori, M., Madej, J., Beau, R., Elli, D., Patino, M.I., Redfern, R., Dewitte, S.N., Gamble, J.A., Boldsen, J.L., Carmichael, A., Varlik, N., Eaton, K., Grenier, J.-C., Golding, G.B., Devault, A., Rouillard, J.-M., Yotova, V., Sindeaux, R., Ye, C.J., Bikaran, M., Dumaine, A., Brinkworth, J.F., Missiakas, D., Rouleau, G.A., Steinrücken, M., Pizarro-Cerdá, J., Poinar, H.N., Barreiro, L.B., 2022. Evolution of immune genes is associated with the Black Death. Nature 611, 312–319.. doi:10.1038/s41586-022-05349-x.

Kugeler, K.J., Staples, J.E., Hinckley, A.F., Gage, K.L., Mead, P.S., 2015. Epidemiology of Human Plague in the United States, 1900–2012. Emerging Infectious Diseases 21, 16–22.. doi:10.3201/eid2101.140564.

Mahmoudi, A., Kryštufek, B., Sludsky, A., Schmid, B.V., De Almeida, A.M.P., Lei, X., Ramasindrazana, B., Bertherat, E., Yeszhanov, A., Stenseth, N.C., Mostafavi, E., 2021. Plague reservoir species throughout the world. Integrative Zoology 16, 820–833.. doi:10.1111/1749-4877.12511.

Mühlemann, B., Vinner, L., Margaryan, A., Wilhelmson, H., De La Fuente Castro, C., Allentoft, M.E., De Barros Damgaard, P., Hansen, A.J., Holtsmark Nielsen, S., Strand, L.M., Bill, J., Buzhilova, A., Pushkina, T., Falys, C., Khartanovich, V., Moiseyev, V., Jørkov, M.L.S., Østergaard Sørensen, P., Magnusson, Y., Gustin, I., Schroeder, H., Sutter, G., Smith, G.L., Drosten, C., Fouchier, R.A.M., Smith, D.J., Willerslev, E., Jones, T.C., Sikora, M., 2020. Diverse variola virus (smallpox) strains were widespread in northern Europe in the Viking Age. Science 369, eaaw8977., doi:10.1126/science.aaw8977.

Nguyen V.K., Parra-Rojas C., and Hernandez-Vargas E.A., 2018. The 2017 plague outbreak in Madagascar: Data descriptions and epidemic modelling. Epidemics, 25, 20-25.

Pedersen, M.W., Antunes, C., De Cahsan, B., Moreno-Mayar, J.V., Sikora, M., Vinner, L., Mann, D., Klimov, P.B., Black, S., Michieli, C.T., Braig, H.R., Perotti, M.A., 2021. Ancient Human Genomes and Environmental DNA from the Cement Attaching 2,000-Year-Old Head Lice Nits. Molecular biology and evolution, 39(2), DOI: msab351.

Prentice, M.B., Rahalison, L., 2007. Plague. The Lancet, 369(9568), 7-13.

Raoult, D., Aboudharam, G., Crubézy, E., Larrouy, G., Ludes, B., Drancourt, M., 2000. Molecular identification by “suicide PCR” of Yersinia pestis as the agent of Medieval Black Death. Proceedings of the National Academy of Sciences 97, 12800–12803.. doi:10.1073/pnas.220225197.

Rascovan, N., Sjögren, K-G., Kristiansen, K., Nielsen, R., Willerslev, E., Desnues, C., Rasmussen, S., 2019. Emergence and Spread of Basal Lineages of Yersinia pestis during the Neolithic Decline. Cell 176, 295-305.

Rasmussen, S., Morten, Nielsen, K., Orlando, L., Sikora, M., Sjögren, K.-G., Anders, Schubert, M., Alex, Christian, Henrik, Brunak, S., Avetisyan, P., Epimakhov, A., Mikhail, Gnuni, A., Kriiska, A., Lasak, I., Metspalu, M., Moiseyev, V., Gromov, A., Pokutta, D., Saag, L., Varul, L., Yepiskoposyan, L., Sicheritz-Pontén, T., Robert, Marta, Nielsen, R., Kristiansen, K., Willerslev, E., 2015. Early Divergent Strains of Yersinia pestis in Eurasia 5,000 Years Ago. Cell 163, 571–582.. doi:10.1016/j.cell.2015.10.009.

Schmid, B.V., Büntgen, U., Easterday, W.R., Ginzler, C., Walløe, L., Bramanti, B., Stenseth, N.C., 2015. Climate-driven introduction of the Black Death and successive plague reintroductions into Europe. Proceedings of the National Academy of Sciences 112, 3020–3025.. doi:10.1073/pnas.1412887112.

Schuenemann, V.J., Bos, K., Dewitte, S., Schmedes, S., Jamieson, J., Mittnik, A., Forrest, S., Coombes, B.K., Wood, J.W., Earn, D.J.D., White, W., Krause, J., Poinar, H.N., 2011. Targeted enrichment of ancient pathogens yielding the pPCP1 plasmid of Yersinia pestis from victims of the Black Death. Proceedings of the National Academy of Sciences 108, E746–E752.. doi:10.1073/pnas.1105107108.

Spyrou, M.A., Musralina, L., Gnecchi Ruscone, G.A., Kocher, A., Borbone, P.-G., Khartanovich, V.I., Buzhilova, A., Djansugurova, L., Bos, K.I., Kühnert, D., Haak, W., Slavin, P., Krause, J., 2022. The source of the Black Death in fourteenth-century central Eurasia. Nature 606, 718–724.. doi:10.1038/s41586-022-04800-3.

Spyrou, M.A., Tukhbatova, R.I., Wang, C.-C., Valtueña, A.A., Lankapalli, A.K., Kondrashin, V.V., Tsybin, V.A., Khokhlov, A., Kühnert, D., Herbig, A., Bos, K.I., Krause, J., 2018. Analysis of 3800-year-old Yersinia pestis genomes suggests Bronze Age origin for bubonic plague. Nature Communications 9.. doi:10.1038/s41467-018-04550-9.

Stenseth, N.C., Atshabar, B.B., Begon, M., Belmain, S.R., Bertherat, E., Carniel, E., Gage, K.L., Leirs, H., Rahalison, L., 2008. Plague: Past, Present, and Future. PLOS Medicine 5, e3.. doi:10.1371/journal.pmed.0050003.

Stenseth, N.C., Tao, Y., Zhang, C., Bramanti, B., Büntgen, U., Cong, X., Cui, Y., Zhou, H., Dawson, L.A., Mooney, S.J., Li, D., Fell, H.G., Cohn, S., Sebbane, F., Slavin, P., Liang, W., Tong, H., Yang, R., Xu, L., 2022. No evidence for persistent natural plague reservoirs in historical and modern Europe. Proceedings of the National Academy of Sciences 119.. doi:10.1073/pnas.2209816119.

Susat, J., Lübke, H., Immel, A., Brinker, U., Macāne, A., Meadows, J., Steer, B., Tholey, A., Zagorska, I., Gerhards, G., Schmölcke, U., Kalniņš, M., Franke, A., Pētersone-Gordina, E., Teßman, B., Tõrv, M., Schreiber, S., Andree, C., Bērziņš, V., Nebel, A., Krause-Kyora, B., 2021. A 5,000-year-old hunter-gatherer already plagued by Yersinia pestis. Cell Reports 35, 109278.. doi:10.1016/j.celrep.2021.109278.

Tang Z., Zhu Y., Ji P., Zhang S., Han L., Xiao X., An S., Shi H., and Wang F., (2016). Spore-pollen analysis of Haminmangha settlement and initial research on paleoecological environment of Hamin Culture. Bianjiang Kaogu Yanjiu (Research of China's Frontier Archaeology) 19, 341-346.

Andrades Valtueña, A., Mittnik, A., Key, F.M., Haak, W., Allmäe, R., Belinskij, A., Daubaras, M., Feldman, M., Jankauskas, R., Janković, I., Massy, K., Novak, M., Pfrengle, S., Reinhold, S., Šlaus, M., Spyrou, M.A., Szécsényi-Nagy, A., Tõrv, M., Hansen, S., Bos, K.I., Stockhammer, P.W., Herbig, A., Krause, J., 2017. The Stone Age Plague and Its Persistence in Eurasia. Current Biology 27, 3683–3691.e8.. doi:10.1016/j.cub.2017.10.025.

Wagner D.M., Klunk J., Harbeck M., Devault A., Waglechner N., Sahl J.W., Erik J., Birdsell D.N., Kuch M., Lumibao C., and Poinar D., 2014. *Yersinia pestis* and the Plague of Justinian 541-543 AD: a genomic analysis. The Lancet infectious diseases, 14(4), 319-326.

Wiechmann, I., Grupe, G., 2005. Detection ofYersinia pestis DNA in two early medieval skeletal finds from Aschheim (Upper Bavaria, 6th century A.D.). American Journal of Physical Anthropology 126, 48–55.. doi:10.1002/ajpa.10276.

Willmott, H., Townend, P., Swales, D.M., Poinar, H., Eaton, K., Klunk, J., 2020. A Black Death mass grave at Thornton Abbey: the discovery and examination of a fourteenth-century rural catastrophe. Antiquity 94, 179–196.. doi:10.15184/aqy.2019.213.

World Health Organization, 2021. WHO guidelines for plague management: revised recommendations for the use of rapid diagnostic tests, fluoroquinolones for case management and personal protective equipment for prevention of post-mortem transmission. World Health Organization, Geneva.

World Health Organization. Regional Office for South-East Asia, 2010. Operational guidelines on plague surveillance, diagnosis, prevention and control. WHO Regional Office for South-East Asia.

Yu, H., Spyrou, M.A., Karapetian, M., Shnaider, S., Radzevičiūtė, R., Nägele, K., Neumann, G.U., Penske, S., Zech, J., Lucas, M., Leroux, P., Roberts, P., Pavlenok, G., Buzhilova, A., Posth, C., Jeong, C., Krause, J., 2020. Paleolithic to Bronze Age Siberians Reveal Connections with First Americans and across Eurasia. Cell 181, 1232–1245.e20.. doi:10.1016/j.cell.2020.04.037.

Zheng J., Zhu Y., and Ji P., 2014. The study of Haminmangha Culture. Bianjiang Kaogu Yanjiu (Research of China's Frontier Archaeology) 15, 247-263.

Zhou Y., Niu X., Ji P., Zhu Y., Zhu H., and Zhang M., 2022. The Hamin Mangha Site: mass deaths and abandonment of a Late Neolithic settlement in Northeastern China. Asian Perspectives, 61(1), 28-49.

Zhu H., Zhou Y., Zhang Q., and Ji P., 2014. Paleodemography research on human bones in the house site of Hamin Mangha Site: Evidence of forensic anthropology on the cause of prehistoric disaster. Jilin University Journal Social Sciences Edition. 54, 26-33.