Mass burial genomics reveals outbreak of enteric paratyphoid fever in the Late Medieval trade city Lübeck
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INTRODUCTION
In the High and Late Middle Ages, European settlements of all sizes were repeatedly struck by outbreaks of infectious diseases. Some of them reached epidemic proportions such as smallpox (Duggan et al., 2016), leprosy (Krause-Kyora et al., 2018a; Schuenemann et al., 2013, 2018), or the second plague pandemic that started with the Black Death (Bos et al., 2011; Haensch et al., 2010; Spyrou et al., 2016, 2019). Medieval chronicles generally referred to epidemics as “pestis” or “pestilential”, regardless of the causative pathogen responsible (Kahlow 2007). Increasing trade and mobility played an important role in spreading pathogens along cross-country and maritime routes throughout the continent (Yue et al., 2017). One of the most important medieval commercial networks was the Hanseatic League, which dominated the northern European and Baltic maritime trade from the 13th to the 16th century (Dollinger and Krabusch, 1998). At its center was the city of Lübeck in present-day Germany, a wealthy and influential trading hub on the Baltic Sea (Benedictow, 2017; Singman, 1999) (Figure 1A). In 1226 CE, the Heiligen-Geist-Hospital (“Hospital of the Holy Ghost”, HGH) was established there, which was dedicated to the care and welfare of the elderly (Lütgert, 2002). The large building complex still exists today and is currently in use as a retirement home as well as a museum. In 1989, construction works on the southern walls of the hospital revealed a mass burial site nearby (Figure 1B), containing the remains of more than 800 individuals from the Late Middle Ages (Lütgert, 2002; Prechel, 1996, 2002) (Figures 1C and 1D). Based on archaeological evidence and the lack of traumatic lesions on the bones, the dead were assumed to represent victims of an epidemic event, most likely of the Black Death, as the plague was reported to have affected Lübeck successively, first in 1350 CE (Koppmann, 1899) and later again in 1359 CE (Koppmann, 1894). However, these outbreaks were followed by at least four more pestilences of unclear etiology in the 14th century (Koppmann, 1884).

In order to identify the infectious agent that led to the death of the individuals buried next to the HGH in Lübeck, we screened the skeletal remains of 92 individuals from this site for the presence of pathogens. We found evidence of an infection with the bacterium Salmonella enterica subsp. enterica serovar Paratyphi C, suggesting an outbreak of enteric paratyphoid fever. Three reconstructed S. Paratyphi C genomes showed close similarity to a strain from Norway (1200 CE). Radiocarbon dates placed the disease outbreak in Lübeck between 1270 and 1400 cal CE, with historical records indicating 1367 CE as the most probable year. The deceased were of northern and eastern European descent, confirming Lübeck as an important trading center of the Hanseatic League in the Baltic region.
(S. Paratyphi C) in eight individuals, suggesting an outbreak of enteric paratyphoid fever that afflicted the city of Lübeck in the late 14th century.

RESULTS
In this study, we analyzed 92 individuals excavated from the HGH mass burial site in Lübeck. The remains were obtained from four archaeological contexts, i.e., from two mass graves (4528, n = 29 and 4529, n = 11) and two smaller pits (4562, n = 13 and 4571, n = 39; Figures 1C and 1D). We performed radiocarbon dating which placed all four contexts in the same time range from 1270 to 1400 cal CE (Table S1 and Figure S1). However, based on a coin found in situ (4528), the period of use of the mass graves could be narrowed down to 1340–1370 CE. The two smaller pits stratigraphically cut into the mass grave contexts and are therefore considered younger (Prechel, 2002).

We extracted ancient DNA (aDNA) from teeth and/or bones of all 92 individuals following established guidelines for the work with tiny amounts of fragmented DNA (Cooper and Poinar, 2000). All procedures were conducted in dedicated aDNA clean rooms. Blank controls that were included in each experimental step were always negative. Short tandem repeat profiles at seven loci were generated as an additional authentication criterion and to avoid double sampling (Table S2).

Metagenomic screening
First, all metagenomic aDNA extracts were screened for bacterial and viral pathogens using an established in-house pipeline (Krause-Kyora et al., 2018a, 2018b; Susat et al., 2020). Ten data sets were observed to align to the taxonomic node of S. enterica subsp. enterica, based on a threshold of at least 300 reads per sample aligning to S. enterica from an initial sequencing depth of 2–54 million reads per sample (Table S3). The sequencing reads were then competitively mapped to a multi-sequence reference comprising the
complete genomes of 15 different serovars that represent the modern diversity of *S. enterica* (Table S4). In eight of the ten data sets, *S. Paratyphi C* was the reference with the highest number of aligned reads. For two samples (HGH-1458, HGH-1699), the aligned reads were subsequently identified as false positives. The *S. Paratyphi C* classifications in the other eight samples were confirmed through a taxa-specific mapping score (see methods; Table S3) that was developed analogously to the mapping score described in a study of *Yersinia pestis* (*Y. pestis*) (Andrades Valtueña et al., 2017). Additionally, we used the tool MaltExtract (Hübler et al., 2019) to verify the findings (Table S3). Based on these criteria, we identified eight positive samples (HGH-1429, HGH-1510, HGH-1558, HGH-1579, HGH-1599, HGH-1600, HGH-1607, HGH-1638; Table 1) for which additional 185 to 642 million reads were generated to facilitate genome reconstruction. The obtained sequencing reads were then aligned to the reference genome of *S. Paratyphi C* RKS4594 (NC_012125), and consensus sequences with a 2-fold genome-wide coverage of up to 96% were constructed (Table 1). All samples displayed typical damage patterns for both ancient bacterial and human DNA (Figures S2 and S3).

It is noteworthy that the *S. Paratyphi C*-positive results were only observed in human remains from the multiple burial pits 4562 (*n = 2*) and 4571 (*n = 6*) but not in skeletons from the mass graves 4528 and 4529 (Table S3). In previous publications, the plague was assumed to have caused the death of the individuals buried next to the HGH (Prechel, 1996, 2002). However, in our metagenomic screening of the 92 aDNA extracts, there was no evidence of *Y. pestis* reads.

**Phylogenetic reconstruction**

Next, we computed a Bayesian phylogenetic tree based on a multi-variant alignment, including three genomes with high coverage from this study (HGH-1429, HGH-1558, HGH-1600; Table 1) as well as nine ancient (Key et al., 2020; Vågene et al., 2018; Zhou et al., 2018) and 123 modern *S. enterica* subsp. *enterica* genomes (Alikhan et al., 2018; O’Leary et al., 2016; Zhou et al., 2020). The consensus sequences of the remaining five samples from Lübeck were not considered for phylogenetic reconstruction due to low coverage (a 1-fold coverage below 51%; Table 1). All ancient and modern *S. Paratyphi C* strains formed a single clade in the tree, supported by a posterior probability of 1 (Figure 2). The three genomes from Lübeck clustered together. They differed from each other only in up to four single-nucleotide polymorphisms (SNPs); this variation probably reflects the micro-diversity within an outbreak (Table S5). The SNP effect analysis showed that none of the four variants had an influence on virulence or function. Furthermore, the three HGH genomes were similar to two previously published ancient *S. Paratyphi C* genomes, those from Ragna (Norway 1200 CE) (Zhou et al., 2018) and Tepos (Mexico 1545 CE) (Vågene et al., 2018) (Figure S4).

**Population genetic analyses**

In a second approach, we analyzed the 92 metagenomic sequences with respect to the presence of human DNA. Fifty-three data sets were of sufficient quality for population genetic analyses (Table S3). In a principal component analysis, the individuals from medieval Lübeck grouped together and showed genetic similarities to modern populations from Scandinavia and the Baltic region, including northern Germany (Figures 3 and 4).
and S5). Further, F3 statistics revealed affinities between Lübeck and populations from northern and eastern Europe (Figure S6). Mitochondrial (mt) haplogroups could be determined in 52 individuals. The mtDNA distribution in Lübeck was similar to that observed in other central and northern European populations, with haplogroups H and U being the most abundant (Table S3).

**DISCUSSION**

The Late Medieval mass burial site next to the HGH in Lübeck comprised the skeletons of more than 800 individuals that had been interred in two mass graves and two smaller burial pits, probably in the course of
different disease outbreaks. Our pathogen screening revealed *Salmonella enterica* subsp. *enterica* serovar *Paratyphi C* in skeletal remains from the two burial pits, indicating the highly contagious enteric paratyphoid fever as the cause of death. However, we could not detect any traces of *S. enterica* or *Y. pestis* in the two mass graves.

Our radiocarbon dates (1270–1400 cal CE; Table S1) and the coin (1340–1370 CE) place all four contexts in the 14th century. Several consecutive epidemics were recorded to have affected the city of Lübeck in this period. The first in 1350 CE and the second in 1359 CE are usually attributed to the Black Death that was responsible for a very high death toll in all cities on the coast of northern Germany (Koppmann, 1884). The large number of corpses in the mass graves suggests that they were the victims of one or both plague waves (Prechel, 1996, 2002), though we did not find genetic traces of *Y. pestis*.

As the two pits were stratigraphically younger than the mass graves (Lu¨tgert, 2002; Prechel, 2002), we hypothesize that the 120 deceased in the two pits had succumbed to one of the later dated pestilences, i.e., between 1360 and 1400 CE. The archaeological context indicates that the pits were refilled quickly, which suggests that all the individuals had died from the same disease, paratyphoid fever, within a short time. This scenario is supported by our detection rate (8 *S. Paratyphi C*-positives in 52 remains from the two pits) that is remarkably high for ancient pathogen diagnostics.

The city chronicles mentioned a third epidemic of unknown cause in 1367 CE, which was limited to Lübeck only (Koppmann, 1899; Ibs, 1994). The clinical manifestation of paratyphoid fever would be consistent with such a localized event. Lübeck was a clean city by the standards of the time and had invested in eliminating health hazards, for example, by introducing separate sewage and drinking water systems (Grabowski and Schmitt, 1993; Lüdecke, 1980). An outbreak of paratyphoid fever therefore seems somewhat surprising. But *S. Paratyphi C* bacteria, which grow in the intestines and blood, are easily spread via water or food contaminated with the feces of an infected individual. In many cases, transmission occurs when active or asymptomatic carriers (super-spreaders) do not wash their hands before preparing or

Figure 3. Population genetic analysis
Principal component analysis (PCA) of HGH individuals (red circles) from Lübeck in the context of 19 modern-day European populations. A PCA with a larger set of reference populations is shown in Figure S5.
handling food (Bhan et al., 2005; Crump and Mintz, 2010; Sánchez-Vargas et al., 2011). Because the dead were buried next to the HGH, these premises appear to be a possible hotspot of infection. However, the HGH was not a hospital in the modern sense of the word, but rather a social institution managed by the city council providing housing, food, and care for the more affluent elderly who bought the prerogative to reside in the HGH. It thus resembled more a present-day retirement or nursing home. About 51% of the skeletons in the two smaller burial pits represented individuals younger than 40 years (Prechel, 1996) who were unlikely to have lived in an old age home. Besides, the HGH maintained its own regular graveyard in the vicinity. It can therefore be assumed that the majority of the dead in the pits, especially the younger ones, were not residents of the HGH. A paratyphoid fever outbreak that claimed 120 lives from all age groups (Prechel, 1996) must have affected many households. Due to the lack of written or archaeological evidence, we do not know from where the paratyphoid fever emanated or how it spread. But as Lübeck was densely populated and there was no gentrification of residential districts (Hammel-Kiesow, 2006), the outbreak probably affected people of all social strata, including wealthier citizens. This scenario is in agreement with the city chronicles which mentioned that a large proportion of the affluent population was killed in the outbreak of 1367 CE (Koppmann, 1884). However, the latter had their own cemeteries outside the city (Koppmann, 1899), so it was unlikely that they would be interred in a mass burial. In contrast, as evidenced by the different mass graves at the HGH, its unconsecrated grounds were apparently repeatedly used for the disposal of corpses which accumulated rapidly during epidemics and must primarily have come from people who were not wealthy enough for a proper burial in the established cemeteries. In any case, the results of our population genetic analyses showed that the dead represented a varied group of people of northern and eastern European descent. This finding is compatible with the role of Lübeck as an important trading center of the Hanseatic League. The city is often referred to as “The Queen of the Hansa”, attracting merchants, sailors, and workers from all over the Baltic region. Due to the Hanseatic trade and the institutionalized trading stations of the Hanseatic Merchant in Bergen (Norway) and Novgorod (Russia), Lübeck was a melting pot of very diverse people (Dollinger and Krabusch, 1998; Graßmann, 2005).

Paratyphi C is one of the several invasive typhoidal Salmonella enterica serovars that cause enteric fever in humans, including S. Typhi (typhoid fever) and S. Paratyphi A, B, and C (paratyphoid fever). Still today, paratyphoid fever affects about five million people a year worldwide, resulting in 54,000 deaths (Crump and Mintz, 2010; World Health Organization, 2018). However, cases of S. Paratyphi C infection are not frequently reported, and the pathogen is no longer endemic in Europe (Achtman et al., 2012; Crump and Mintz, 2010; Liu et al., 2009; Uzzau et al., 2000). In contrast, S. Paratyphi C has been the only Salmonella enterica serovar found so far in specimens from medieval Europe (Zhou et al., 2018) and 16th-century Central America (a strain imported from Europe) (Vágene et al., 2018). Thus, S. Paratyphi C might have been the predominant typhoidal agent at the time.

Our phylogenetic reconstruction showed strong genomic stability of the S. Paratyphi C subgroup over at least one millennium. This is reflected in a small number of variants between the globally distributed ancient and modern genomes within the Paratyph i C clade, with the oldest genome dating to 1200 CE (Zhou et al., 2018) and the most recent to 2015 (Alikhan et al., 2018). Similarly, the three reconstructed genomes from Lübeck differed in only a few SNPs that were identified to have no effect on the phenotype (Table S5).

This aDNA study reports an epidemic outbreak of paratyphoid fever in Lübeck in the 14th century that killed at least 120 people. The infection dynamics were probably less pronounced and more localized than those of the previous plague. Nonetheless, the effects on life and trade must have been clearly felt. The consequences of the recurring disease outbreaks in the second half of the 14th century were severe and long lasting for Lübeck and the surrounding area, leading to collapsing bond and real estate markets, as well as increasing bankruptcy of local businesses. Moreover, the prospect of imminent death drove many citizens to make generous donations to churches and monasteries out of concern for the salvation of their souls (Fouquet and Zeilinger, 2011).

Limitations of the study
The main challenges in aDNA research are the small number of samples usually available for analysis and the poor DNA preservation. Due to a variety of environmental (Bollongino et al., 2008; Collins et al., 2002) and chemical (Warinner et al., 2017) factors, endogenous (human and pathogen) DNA is degraded over
time, rendering the recovery and analysis of any remaining DNA fragments often very difficult, if not impossible. In our study, we examined the skeletal remains of 92 individuals, a relatively large sample by aDNA standards. However, only 53 of them (~57.6%) contained sufficient human DNA for population genetic analyses. S. Paratyphi C DNA was even less abundant than human DNA in the extracts (Tables 1 and S3). In total, we identified S. Paratyphi C reads in eight individuals, and three of the generated data sets allowed for a full genome reconstruction. So far, only two other ancient S. Paratyphi C strains (Norway 1200 CE, Mexico 1545 CE) have been reported. Additional genomes from other historical periods and regions are therefore needed to provide a more comprehensive phylogeography of the pathogen.

Resource availability
Lead contact
Further information, requests, and inquiries should be directed to the lead contact, Ben Krause-Kyora (b.krause-kyora@ikmb.uni-kiel.de).

Materials availability
The study did not generate new unique reagents or materials.

Data and code availability
The accession number for the data reported in this paper is available through the European Nucleotide Archive: PRJEB41353 (https://www.ebi.ac.uk).

METHODS
All methods can be found in the accompanying Transparent methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102419.

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AUTHOR CONTRIBUTIONS
A.N., S.H., and B.K.-K. conceived and designed the study. M.H., K.C., J.S., A.L.F., A.I., A.F., A.H., and A.K. generated and analyzed aDNA data. M.H., J.K., G.F., D.R., A.N., and B.K.-K. interpreted the results. M.H., A.N., B.K.-K. wrote and revised the manuscript with input from all the other authors.

DECLARATION OF INTERESTS
The authors declare no competing interests.

INCLUSION AND DIVERSITY
We worked to ensure diversity in experimental samples through the selection of the genomic data sets. The author list of this paper includes contributors from the location where the research was conducted who participated in the data collection, design, analysis, and/or interpretation of the work.

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REFERENCES

Achtman, M., Wain, J., Weiss, F.X., Nair, S., Zhou, Z., and Sanghel, Y. (2012). Multilocus sequence typing as a replacement for serotyping in Salmonella enterica. PLoS Pathog. 8, e1002776.

Andradas Valtuen˜a, A., Mittnik, A., Key, F.M., Haak, W., Allmäe, R., and Belinski, A. (2017). The stone age plague and its persistence in Eurasia. Curr. Biol. 27, 3683–3691.e8.

Achtman, M., Hurst, D., Achtman, M., et al. (2010). Distinct population structure of Salmonella. PLoS Genet. 14, e1007261.

Alikhan, N.-F., Zhou, Z., Sergeant, M.J., and Sergeant, M.J., et al. (2012). Multilocus sequence typing of Yersinia pestis caused the black death. PLoS Pathog. 6, e1001134.

Benedictow, O.J. (2017). The Black Death, 1346–1353: The Complete History (Boydell Press).

Bhan, M.K., Bahl, R., and Bhatnagar, S. (2005). Typhoid and paratyphoid fever. Lancet 366, 749–762.

Bollongino, R., Tresset, A., and Vigne, J.-D. (2008). Environment and excavation. Pre-lab impacts on ancient DNA analyses. Comptes. Rendus. Palevol. 7, 91–98.

Bo, K.I., Schuenemann, V.J., Golding, G.B., Burbano, H.A., Wakeleer, N., Coomans, B.K., McPhee, J.B., DeWitte, S.N., Meyer, M., Schmedes, S., et al. (2011). A draft genome of the black death. Nature 478, 506–510.

Collins, M.J., Nielsen-March, C.M., Miller, J., Smith, C.I., Roberts, J.P., Plogodich, R.V., Wess, T.J., Casío, J., Millard, A.R., and Turner-Walker, G. (2002). The survival of organic matter in bone: a review. Archaeometry 44, 383–394.

Cooper, A., and Poirier, H. (2000). Ancient DNA. Do it right or not at all. Science 289, 5482.

Crum, J.A., and Mintz, E.D. (2010). Global trends in typhoid and paratyphoid fever. Clin. Infect. Dis. 50, 241–246.

Dollinger, P., and Krabusch, M. (1998). Die Hanse (Krämer).

Duggan, A.T., Perdomo, M.F., Piombino-Mascali, D., Marcianni, S., Poirier, D., Emery, M.V., Bucimabh, J.P., Duchêne, S., Janakauski, R., Humphreys, M., et al. (2016). 17th century Variola virus reveals the recent history of smallpox. Curr. Biol. 26, 3407–3412.

Fouquet, G., and Zeiling, G. (2011). Katastrophen im Spätmittelalter (Philipp von Zabern).

Grabowski, M., and Schmitt, G. (1993). Und das Wasser fließt in Röhren. Wasserversorgung und Wasserkunst in Lübeck. In Die Archäologie des Mittelalters und Bauforschung im Hanseraum, M. Schröder, ed. (Rudolf Habelt), pp. 245–286.

Graßmann, A. (2005). Das Hansische Kontor zu Lübeck. In Archa¨ ologie und Kulturgeschichte 26, M. Gla ¨ ser, ed. (Rudolf Habelt), pp. 139–243.

Hammel-Kiesow, R. (2006). Ha¨ user und Ho¨ fe in Lübeck. Wasserversorgung und Grabowski, M., and Schmitt, G. (1993). Und das Wasser fließt in Röhren. Wasserversorgung und Wasserkunst in Lübeck. In Die Archäologie des Mittelalters und Bauforschung im Hanseraum, M. Schröder, ed. (Rudolf Habelt), pp. 139–243.

Hübner, R., Key, F.M., Warinner, C., Bos, K.I., Krause, J., and Herbig, A. (2019). HOPS: automated detection and authentication of pathogen DNA in archaeological remains. Genome Biol. 20, 290.

Ibs, J.H. (1994). Die Pest in Schleswig-Holstein von 1350 bis 1547/48: Eine sozialgeschichtliche Studie über eine große Epidemie mit katastrophalem Ergebnis. In Kiefer Werkstücke: Reihe A, Beiträge zur schleswig-holsteinischen und skandinavischen Geschichte 12, E. Hoffmann, ed. (Peter Lang).

Kahlow, S. (2007). The Pest as an Interpretationsproblem mittelalterlicher und frühneuzeitlicher Massengräber. Bulletin der Schweizerischen Gesellschaft für Anthropologie 13, 97–104.

Key, F.M., Posth, C., Escuviel-Gomez, L.R., Hübner, R., Spyrou, M.A., Neumann, G.U., Furtwängler, A., Sabin, S., Burri, M., Wissgott, A., et al. (2020). Emergence of human-adapted Salmonella enterica is linked to the Neolithization process. Nat. Ecol. Evol. 4, 324–333.

Koppmann, K. (1884). In Die Chroniken der niedersächsischen Städte. Lübeck. Band 1 (S. Hirzel).

Koppmann, K. (1899). In Die Chroniken der niedersächsischen Städte. Lübeck. Band 2 (S. Hirzel).

Krause-Kyora, B., Ja¨ ger, G., Bos, K.I., Herbig, A., Economou, C., Benjak, A., Busso, P., et al. (2013). Genome-wide comparison of medieval and modern Mycobacterium leprae. Science 341, 179–183.

Krause-Kyora, B., Avanz, C., Krause-Kyora, B., Seitz, A., Herbig, A., Insink, S., Bonazzi, M., Reiter, E., Urban, C., Dangvard Pedersen, D., et al. (2018). Ancient genomes reveal a high diversity of Mycobacterium leprae in medieval Europe. PLoS Pathog. 14, e1006997.

Singman, J.L. (1999). Daily Life in Medieval Europe (Greenwood Press).

Spyrou, M.A., Tukhabatova, R.I., Feldman, M., Drath, J., Kacki, S., Beltrán de Heredia, J., Arnold, S., Sitdikov, A.G., Castex, D., Wahl, J., et al. (2016). Historical Y. pestis genomes reveal the European black death as the source of ancient and modern plague pandemics. Cell Host Microbe 19, 874–881.

Susat, J., Keller, M., Tukhabatova, R.I., Scheib, C.L., Nelson, E.A., Andradas Valtuenha, A., Neumann, G.U., Walker, D., Alterauge, A., Carty, N., et al. (2019). Phylogeography of the second plague pandemic revealed through analysis of historical Yersinia pestis genomes. Nat. Commun. 10, 4470.

Susat, J., Bonzarowzka, J.H., Petersen-Goedina, E., Immel, A., Nebel, A., Gerhards, G., and Krause-Kyora, B. (2020). Yersinia pestis strains from Latvia show depletion of the pla virulence gene at the end of the second plague pandemic. Sci. Rep. 10, 14628.

Uzzau, S., Brown, D.J., Wallis, T., Rubino, S., Leori, G., and Bernard, S. (2000). Host adapted Mycobacterium leprae. Science 284, 263–277.

Vägène, Å.J., Herbig, A., Campana, M.G., Robles García, N.M., Warinner, C., Sabin, S., Spyrou, M.A., Andradas Valtueña, A., Huson, D., Tuross, N., et al. (2018). Salmonella enterica genomes from victims of a major sixteenth-
century epidemic in Mexico. Nat. Ecol. Evol. 2, 520–528.

Warinner, C., Herbig, A., Mann, A., Fellows Yates, J.A., Weiß, C.L., Burbano, H.A., Orlando, L., and Krause, J. (2017). A robust framework for microbial archaeology. Annu. Rev. Genomics Hum. Genet. 31, 321–356.

World Health Organization. (2018). Typhoid Vaccines: WHO Position Paper – March 2018, Weekly Epidemiological Record No. 13, 93, pp. 153–172. https://apps.who.int/iris/bitstream/handle/10665/272272/WER9313.pdf.

Yue, R.P.H., Lee, H.F., and Wu, C.Y.H. (2017). Trade routes and plague transmission in pre-industrial Europe. Sci. Rep. 7, 12973.

Zhou, Z., Lundstrøm, I., Tran-Dien, A., Duchène, S., Alikhan, N.-F., Sergeant, M.J., Langridge, G., Fotakis, A.K., Nair, S., Stenøien, H.K., et al. (2018). Pan-genome analysis of ancient and modern Salmonella enterica demonstrates genomic stability of the invasive para C lineage for millennia. Curr. Biol. 28, 2420–2428.e10.

Zhou, Z., Alikhan, N.-F., Mohamed, K., Fan, Y., and Achtman, M. (2020). The EnteroBase user’s guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny, and Escherichia core genomic diversity. Genome Res. 30, 138–152.
Supplemental information

Mass burial genomics reveals outbreak
of enteric paratyphoid fever
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Figure S1. Radiocarbon dates on bone collagen. Atmospheric curves and radiocarbon date determination of the samples HGH-924, HGH-950, HGH-1429, HGH-1579 and HGH-1600. The analyses were performed by the Leibniz Laboratory for Radiometric Dating and Stable Isotope Research at Kiel University. (Related to Figure 1 and Table 1 of main text and Table S1).
Figure S2. *S. Paratyphi C* aDNA damage patterns. Damage plots of the eight HGH samples with reads mapping to *Salmonella enterica* in the metagenomic analysis. Plots were generated with mapDamage after alignment to the *S. Paratyphi C* RKS4594 (NC_012125.1) reference genome. (Related to Table 1 of main text).
Figure S3. Human aDNA damage patterns. Damage plots of the eight HGH samples with reads mapping to *Salmonella enterica* in the metagenomic analysis. Plots were generated with mapDamage after alignment to the *Homo sapiens* hg19 reference genome. (Related to Table 1 of main text).
Figure S4. Phylogenetic relationship of ancient and modern *S. Paratyphi C* genomes. Bayesian phylogenetic tree of three genomes from the HGH mass burial in Lübeck (red), the ancient *S. Paratyphi C* strains Ragna (Norway 1200 CE) and Tepos_14 (Mexico 1545 CE) (blue) and the reference genome of modern *S. Paratyphi C* (RKS4594) (black). The tree is based on a minimal SNP coverage of 5x and 236,622 variant positions. Nodes are labeled with the posterior probability value assigned by MrBayes. Branch lengths correspond to number of substitutions per site. (Related to Figure 2 of main text and Table S5).
Figure S5. Population genetic analysis. Principal component analysis (PCA) of HGH individuals (red circles) from Lübeck in the context of 59 modern-day West-Eurasian populations. A PCA with a smaller set of reference populations is shown in Figure 3. (Related to Figure 3 of main text and Figure S6).
Figure S6. Population genetic analysis. F3-statistics of HGH individuals from Lübeck in the context of 59 modern-day West-Eurasian populations. Genetic distances were calculated with respect to the HGH population. F3-statistics reveal affinities between HGH individuals and those from northern Britain, eastern Europe and the Balkans. (Related to Figure 3 of main text and Figure S5).
**SUPPLEMENTARY TABLES**

**Table S1. Radiocarbon dates on bone collagen.** The analyses were performed by the Leibniz Laboratory for Radiometric Dating and Stable Isotope Research at Kiel University. (Related to Figure 1 and Table 1 of main text and Figure S1).

| Sample      | Context | pMC\(^a\)     | Age (BP) | δ\(^{13}\)C (‰) |
|-------------|---------|---------------|----------|------------------|
| HGH-942     | 4528    | 91.71 ± 0.24  | 696 ± 21 | -20.2 ± 0.1      |
| HGH-950     | 4528    | 91.90 ± 0.27  | 679 ± 24 | -21.1 ± 0.2      |
| HGH-1429    | 4571    | 92.61 ± 0.28  | 617 ± 25 | -19.9 ± 0.1      |
| HGH-1579    | 4571    | 92.00 ± 0.24  | 670 ± 21 | -19.2 ± 0.2      |
| HGH-1600    | 4571    | 92.61 ± 0.33  | 617 ± 29 | -20.0 ± 0.1      |

\(^a\)pMC denotes the percentage of modern carbon, based on the hypothetical value of the atmosphere in 1950, corrected to δ\(^{13}\)C = -25 % using the δ\(^{13}\)C value determined by AMS

**Table S2. STR typing results.** Genetic fingerprinting for HGH samples from Lübeck included in this study. (Related to Table 1 of main text).

| ID  | Context | Sex \(^b\) | Age\(^a\) | Amelogenin | D13S317 | D21S11 | D18S51 | TH01 | D5S818 | FGA |
|-----|---------|------------|-----------|------------|---------|--------|--------|------|--------|-----|
| 886 | 4528    | F          | 26-32     | -/-        | -/-     | -/-    | -/-    | -/-  | -/-    | -/- |
| 888 | 4528    | F          | 40-60     | -/-        | -/-     | -/-    | -/-    | -/-  | -/-    | -/- |
| 892 | 4528    | M          | 40-50     | -/-        | -/-     | -/-    | -/-    | -/-  | -/-    | -/- |
| 894 | 4528    | M          | 40+       | -/-        | -/-     | -/-    | -/-    | -/-  | -/-    | -/- |
| 909 | 4528    | M          | 30-50     | -/-        | -/-     | -/-    | -/-    | 7/-  | -/-    | -/- |
| 924 | 4528    | M          | 23-35     | -/-        | -/-     | -/-    | -/-    | -/-  | -/-    | -/- |
| 926 | 4528    | M\(^b\)    | 18-21     | X/-        | 12/-    | 28/-   | -/-    | 9/9.3| 12/-   | 22.2-|
| 927 | 4528    | M          | 20-23     | -/-        | -/-     | -/-    | -/-    | -/-  | -/-    | -/- |
| 930 | 4528    | M          | 18-23     | -/-        | -/-     | -/-    | -/-    | -/-  | -/-    | -/- |
| 934 | 4528    | F          | 25-35     | X/X        | 12/-    | 27/29  | 13/20  | 6/9.3| 12/-   | 20/22|
| 942 | 4528    | F          | 28-32     | X/X        | 12/-    | 30/31.2| 15/16  | 6/9  | 11/12  | 21/24|
| 943 | 4528    | M\(^b\)    | 37-41     | X/X        | 8/12    | 30/32.2| 13/16  | (8/9/10)| 12/-  | 20/22|
| 947 | 4528    | M          | 23-40     | -/-        | -/-     | -/-    | -/-    | -/-  | -/-    | -/- |
| 948 | 4528    | M          | 40-46     | Y/-        | -/-     | -/-    | -/-    | 6/-  | -/-    | 22/-|
| 950 | 4528    | M          | 40-55     | X/Y        | 11/12   | 30.2/32| 17/-   | 6/8  | 12/-   | 21/22|
| 1006| 4528    | M          | 30-36     | X/Y        | 12/-    | 28/30.2| 12/18  | 6/-  | 11/13  | 21/21.2|
| 1219| 4528    | M          | 21-25     | (X)/Y      | 9/11    | 29/-   | 13/-   | 6/8  | 12/-   | -/- |
| ID   | DOB  | Gender | Age | DOB-Date | Age-Date | Age-Duration | DOB-M | DOB-F | Age-M | Age-F | Age-Duration |
|------|------|--------|-----|----------|----------|--------------|--------|--------|--------|--------|---------------|
| 1226 | 4528 | M      | 35-45 | X/Y      | 11/12    | (29)(30)(31) | 17/-  | 8/9    | 11/13  | 22/-   |               |
| 1227 | 4528 | M      | 32-37 | X/Y      | 12/-     | 30/(31.2)/32 | 13/14 | 7/9.3  | 11/12  | 24/25  |               |
| 1415 | 4528 | M      | 23-40 | X/Y      | 8/11     | 29/30       | 12/19 | 6/9.3  | 8/9    | 17/20  |               |
| 1440 | 4528 | M      | 62-75 | X/Y      | 9/11     | 29/30       | 12/15 | 9.3/-  | 12/14  | 21/24  |               |
| 1441 | 4528 | M      | 67-75 | X/Y      | 8/12     | 30/-        | 12/15 | 9.3/-  | 13/-   | 22/25  |               |
| 1449 | 4528 | M      | 24-30 | X/Y      | 11/12    | (32.2)/33.2 | -/-   | 9/9.3  | 11/-   | 25/26  |               |
| 1476 | 4528 | M      | 36-45 | X/Y      | 12/-     | 29/31.2     | 12/-  | 7/9.3  | 11/12  | 20/23  |               |
| 1551 | 4528 | M      | 44-49 | X/Y      | 10/11    | 28/29       | 18/19 | 7/-    | 11/12  | 20/22  |               |
| 1699 | 4528 | M      | 40-45 | X/Y      | 11/12    | 28/-        | 12/16 | 7/9.3  | 9/11   | 24/-   |               |
| 1701 | 4528 | F³    | 20-26 | X/Y      | 12/13    | 31.2/-      | 15/18 | 9/9.3  | 11/-   | 23/23.2|               |
| 1702 | 4528 | M      | 20-23 | X/Y      | 10/12    | 30/-        | 12/-  | 7/8    | 12/13  | (21/23/24)|               |
| 1710 | 4528 | M      | 55-60 | X/Y      | 9/12     | 28/(31.2)/32 | 13/15 | 7/9    | 11/12  | 21/23  |               |
| 1133 | 4529 | M      | 28-34 | X/Y      | 12/14    | 29/(32.2)/33 | 12/17 | 7/9.3  | 10/12  | 22/24  |               |
| 1154 | 4529 | M      | 32-36 | X/Y      | 8/12     | 30.2/31     | 15/16 | 6/-    | 13/-   | 21/23  |               |
| 1156 | 4529 | M      | 21-24 | X/Y      | 12/-     | 29/30       | 15/-  | 8/-    | 12/13  | -/-    |               |
| 1172 | 4529 | M      | 49-53 | X/Y      | 12/14    | 28/31       | 14/16 | 7/9.3  | (10/12/13)| 21/24  |               |
| 1234 | 4529 | M      | 20-26 | X/Y      | 8/14     | 27/29       | 14/15 | 7/9.3  | 11/12  | 19/22  |               |
| 1571 | 4529 | M      | 20-26 | Y/-      | 11/-     | -/-         | 12/15 | 7/9.3  | 11/12  | 18/22  |               |
| 1612 | 4529 | M      | 20-26 | X/Y      | 11/13    | 28/-        | 13/15 | 7/9.3  | 10/12  | 20/23  |               |
| 1627 | 4529 | M      | 63-68 | X/Y      | 11/-     | 29/30       | 11/19 | 7/9.3  | 11/12  | 25/-   |               |
| 1664 | 4529 | M      | 59-70 | X/Y      | 10/11    | 28/-        | 16/-  | 9/9.3  | 11/13  | 21/25  |               |
| 1730 | 4529 | M      | 29-35 | X/Y      | 12/-     | -/-         | 15/20 | 6/9    | 12/-   | -/-    |               |
| 1780 | 4529 | M      | 24-29 | X/Y      | 11/12    | 28/-        | 13/14 | 9.3/-  | 11/12  | 21/24  |               |
| 1483 | 4562 | M      | 25-30 | X/Y      | 11/-     | 29/30       | 12/17 | 9.3/-  | 11/12  | 21/22  |               |
| 1525 | 4562 | F      | 60-70 | X/X      | 8/10     | 29/31.2     | 12/13 | 9/9.3  | 11/-   | 22/-   |               |
| 1526 | 4562 | M      | 30-50 | X/Y      | 11/12    | 30/33.2     | 14/-  | 7/8    | 12/13  | 23/25  |               |
| 1575 | 4562 | F      | 49-54 | X/X      | 8/11     | 29/-        | 16/17 | 9.3/-  | 11/12  | 21/24  |               |
| 1577 | 4562 | M      | 65-80 | X/Y      | 9/12     | 28/(31.2)/32 | 15/16 | 6/9.3  | 11/12  | 20/25  |               |
| 1578 | 4562 | M³     | 49-54 | X/X      | 11/12    | 29/30       | 12/17 | 8/9    | 9/11   | 22.2/24|               |
| 1579 | 4562 | M³     | 18-23 | X/X      | 11/14    | 28/30       | 12/-  | 6/7    | 11/13  | 22/-   |               |
| 1580 | 4562 | M      | 32-50 | X/Y      | 12/13    | 31/(32)     | (13/16/18)| 9/9.3  | 11/12  | 19/20  |               |
| 1607 | 4562 | M      | 64-75 | X/Y      | 12/13    | 32.2/-      | 14/16 | 9.3/-  | 12/-   | 21/22  |               |
| 1608 | 4562 | F      | 19-24 | X/X      | 10/12    | 29/(30.2)/31.2 | 14/15 | 9.3/-  | 9/11   | 24/-   |               |
| 1628 | 4562 | F      | 63-70 | X/X      | 12/13    | 30/(31.2)/(32)/33.2 | 12/16 | 7/9.3  | 8/9    | 22/-   |               |
| 1633 | 4562 | F      | 57-62 | X/X      | 13/14    | 30/33.2     | 16/18 | 6/9.3  | 12/-   | 19/20  |               |
| 1662 | 4562 | M      | 36-40 | -/-      | -/-      | -/-         | -/-   | -/-    | -/-    | -/-    |               |
| 1286 | 4571 | F      | 21-24 | X/X      | 8/-      | 28/29       | 17/19 | 8/9.3  | 12/-   | 21/22  |               |
| No. | Code | Gender | Age | Initial | Maturity | Birth | Death | Age | Initial | Maturity | Birth | Death |
|-----|------|--------|-----|---------|----------|-------|-------|-----|---------|----------|-------|-------|
| 1316 | 4571 | M      | 33-38 | X/Y     | 11/15    | 29/30.2 | 17/19 | 9.3/- | 11/- | 22/-     |         |       |       |
| 1319 | 4571 | F      | 67-75 | X/X     | 12/-     | 29/32.2 | 13/17 | 7/9.3 | 11/12 | 19/25    |         |       |       |
| 1391 | 4571 | F      | 45-50 | X/X     | 12/-     | (28)/(29)/30 | 12/18 | 9/9.3 | 11/12 | 20/21    |         |       |       |
| 1392 | 4571 | M      | 22-25 | X/Y     | 11/12    | 28/31.2 | 16/-  | 8/-   | 11/12 | 19/22    |         |       |       |
| 1408 | 4571 | F      | 67-75 | X/X     | 12/-     | 31/-    | 14/-  | 6/8   | 12/-   | 23/25    |         |       |       |
| 1409 | 4571 | Fc     | 23-26 | X/Y     | 12/-     | 30/32.2 | 13    | 7/9.3 | 9/12   | 21/22    |         |       |       |
| 1410 | 4571 | F      | 55-60 | X/X     | 10/11    | 30/-    | 14/17 | 6/9.3 | 12/-   | 24/-     |         |       |       |
| 1426 | 4571 | F      | 62-70 | -/-     | 10/-     | -/-     | 14/-  | 9.3/- | -/-    | 22/-     |         |       |       |
| 1427 | 4571 | Me     | 45-52 | X/X     | 11/12    | 28/29   | 13/16 | 6/7   | 11/-   | 21/24    |         |       |       |
| 1429 | 4571 | M      | 22-25 | X/Y     | 10/11    | -/-     | 13/15 | 6/9.3 | 10/11  | 20/-     |         |       |       |
| 1455 | 4571 | F      | 18-23 | -/-     | -/-     | -/-     | -/-   | -/-   | -/-    | -/-      |         |       |       |
| 1458 | 4571 | F      | 60-80 | X/X     | 12/-     | 28/32   | 15/17 | 8/9.3 | (10)/12 | 20/22    |         |       |       |
| 1459 | 4571 | Me     | 18-23 | X/-     | 12/-     | 29/32.2 | 18/-  | 8/9   | 11/12  | 21/-     |         |       |       |
| 1467 | 4571 | M      | 47-63 | X/Y     | 10/11    | 28/((29))/30 | 14/- | 6/7   | 11/12  | 22/25    |         |       |       |
| 1499 | 4571 | M      | 23-40 | X/Y     | 11/13    | 29/31.2 | 13/16 | 8/-   | 11/13  | 18/20    |         |       |       |
| 1508 | 4571 | F      | 19-26 | -/-     | -/-     | -/-     | -/-   | -/-   | -/-    | -/-      |         |       |       |
| 1510 | 4571 | F      | 60-66 | X/-     | 8/11     | 28/31.2 | 12/14 | 7/-   | 12/-   | -/-      |         |       |       |
| 1511 | 4571 | F      | 64-75 | X/X     | 9/10     | 27/((28))/((29))/30 | 12/15 | 6/9.3 | 9/12   | 23/24    |         |       |       |
| 1512 | 4571 | F      | 30-48 | X/X     | 11/15    | 28/((29))/30 | 15/17 | 9/9.3 | 9/12   | 22/-     |         |       |       |
| 1516 | 4571 | M      | 30-60 | X/-     | 8/12     | 29/((33.2))/34.2 | 12/14 | 8/9.3 | 11/13  | 20/21    |         |       |       |
| 1517 | 4571 | Me     | 40-60 | X/-     | -/-      | 31.2/-  | 13/15 | 9/-   | 11/-   | 19/20    |         |       |       |
| 1518 | 4571 | Me     | 20-26 | X/-     | 11/12    | 32/-    | 12/-  | 7/9   | 11/-   | 20/-     |         |       |       |
| 1556 | 4571 | F      | 23-30 | X/-     | 8/12     | 32.2/-  | 13/15 | 9.3/- | 12/13  | 25/26    |         |       |       |
| 1557 | 4571 | Me     | 51-56 | X/-     | -/-      | 30/-    | 17/-  | 8/-   | -/-    | 24/-     |         |       |       |
| 1558 | 4571 | M      | 66-75 | X/Y     | (9/10)/11| -/-     | (14/18)/19 | 6/9  | 11/-   | -/-      |         |       |       |
| 1559 | 4571 | F      | 20-30 | X/X     | 9/12     | 31.2/32.2 | 13/15 | 6/9.3 | 11/13  | 18/21    |         |       |       |
| 1599 | 4571 | F      | 42-48 | X/X     | 9/11     | 27/((32.2))/33.2 | 11/16 | 8/9   | 13/-   | 22/25    |         |       |       |
| 1600 | 4571 | M      | 40-45 | X/Y     | 11/14    | 29/30.2  | 14/-  | 9/9.3 | 11/12  | 18/24    |         |       |       |
| 1601 | 4571 | M      | 53-58 | X/Y     | 11/13    | 28/29  | 14/17 | 6/9.3 | 10/15  | 21/24    |         |       |       |
| 1602 | 4571 | F      | 18-23 | X/X     | 11/14    | 28/29  | 13/-  | 7/-   | 11/-   | 21/-     |         |       |       |
| 1617 | 4571 | Me     | 52-66 | X/-     | 12/14    | 29/-   | 15/17 | 6/9   | 12/-   | 20/23    |         |       |       |
| 1635 | 4571 | M      | 52-57 | X/Y     | 8/10     | 31/32.2 | 14/16 | 6/9   | 11/12  | 20/22    |         |       |       |
| 1636 | 4571 | Fc     | 52-57 | X/Y     | 11/13    | 29/((30))/((31))/32 | 14/15 | 6/9.3 | 12/14  | 19/22    |         |       |       |
| 1637 | 4571 | M      | 49-55 | X/Y     | 12/-     | 29/((31.2))/32.2 | -/-   | 9.3/- | 10/11  | 21/-     |         |       |       |
| 1638 | 4571 | F      | 53-58 | X/X     | 11/12    | 29/34.2 | 14/21 | 8/9   | 11/12  | 21/23    |         |       |       |
| 1639 | 4571 | F      | 40-80 | X/-     | 8/10     | 30/32.2 | 13/15 | 9/9.3 | 11/13  | 22/25    |         |       |       |
| 1653 | 4571 | M      | 47-52 | X/Y     | 9/(11)/14| 29/((30))/((30.2))/3 | 14/17/18 | 8/9  | 12/-   | 20/21    |         |       |       |
Table S3. Summary Table. Complete list of HGH samples included in this study. (Related to Figure 3 and Table 1 of main text).

| Sample ID | Context | Sex | Age | Skeletal elem. | # Reads total (C&M) | # Reads S. enterica | Score | # Ancient reads | HG | PCA | Endogenous DNA (%) |
|-----------|---------|-----|-----|----------------|---------------------|---------------------|-------|-----------------|----|-----|-------------------|
| 886       | 4528    | F   | 26-32 | Femur            | 13,625,657          | 7                   |       |                 | 0.1|     |                    |
| 888       | 4528    | F   | 40-60 | Femur            | 10,194,752          | 10                  |       |                 | 0.1|     |                    |
| 892       | 4528    | M   | 40-50 | Femur            | 11,862,622          | 25                  |       |                 | 0.2|     |                    |
| 894       | 4528    | M   | 40+   | Metatarsus        | 54,740,829          | 16                  |       |                 | 0.1|     |                    |
| 909       | 4528    | M   | 30-50 | Tooth            | 19,447,917          | 9                   |       |                 | 0.1|     |                    |
| 924m      | 4528    | M   | 23-35 | Tooth            | 4,656,827           | 0                   |       |                 | 0.8|     |                    |
| 926       | 4528    | M   | 18-21 | Tooth            | 25,158,177          | 26                  |       |                 | 1  | 0.2|                    |
| 927       | 4528    | M   | 20-23 | Tooth            | 23,646,240          | 22                  |       |                 | 0.1|     |                    |
| 930       | 4528    | M   | 18-23 | Femur            | 22,125,625          | 18                  |       |                 | 2  | 0.2|                    |
| 934       | 4528    | F   | 25-35 | Tooth            | 9,827,192           | 0                   |       |                 | H1 | X  | 26.9               |
| 942       | 4528    | F   | 28-32 | Tooth            | 6,773,853           | 0                   |       |                 | R0 | X  | 12.4               |
| 943       | 4528    | M   | 37-41 | Tooth            | 18,242,433          | 0                   |       |                 | H1 | X  | 16.7               |
| 947       | 4528    | M   | 23-40 | Petrous bone     | 5,734,946           | 0                   |       |                 | 0.4|     |                    |
| 948       | 4528    | M   | 40-46 | Tooth            | 9,582,406           | 14                  |       |                 | 1  | 0.1|                    |
| 950m      | 4528    | M   | 40-55 | Tooth            | 18,119,081          | 10                  |       |                 | H2 | X  | 24.4               |
| 1006      | 4528    | M   | 30-36 | Tooth            | 7,920,752           | 7                   |       |                 | 2  | X  | 47.1               |
| 1219      | 4528    | M   | 21-25 | Tooth            | 9,883,962           | 24                  |       |                 | 3  | U4 | 2.3                |
| 1226      | 4528    | M   | 35-45 | Tooth            | 8,780,302           | 5                   |       |                 | K1 | X  | 30.8               |
| 1227      | 4528    | M   | 32-37 | Tooth            | 5,095,480           | 2                   |       |                 | H1 | X  | 54.8               |
| 1415      | 4528    | M   | 23-40 | Tooth            | 10,425,777          | 15                  |       |                 | 1  | H1 | 30.4               |
| 1440      | 4528    | M   | 62-75 | Tooth            | 7,608,778           | 16                  |       |                 | 2  | X  | 11.5               |
| 1441 | 4528 | M 67-75 | Tooth | 8,484,255 | 23 | 3 | X | 15.9 |
| 1449 | 4528 | M 24-30 | Tooth | 7,411,379 | 40 | 2 | 1.7 |
| 1476 | 4528 | M 36-45 | Tooth | 9,218,640 | 4 | X | 13.6 |
| 1551 | 4528 | M 44-49 | Tooth | 6,312,828 | 11 | 1 | 3.8 |
| 1699 | 4528 | M 40-45 | Tooth | 6,270,615 | 352 | 682 | -0.041 | HV | X | 36.5 |
| 1701 | 4528 | F2 | Tooth | 4,898,711 | 41 | JT | 5.0 |
| 1702 | 4528 | M 20-23 | Tooth | 6,286,548 | 44 | 5.6 |
| 1710 | 4528 | M 55-60 | Tooth | 6,581,118 | 43 | | 12.8 |
| 1133 | 4529 | M 28-34 | Tooth | 10,077,065 | 10 | 2 | X | 22.0 |
| 1154 | 4529 | M 32-36 | Tooth | 4,842,018 | 2 | 1 | I | X | 48.8 |
| 1156 | 4529 | M 21-24 | Tooth | 6,552,887 | 24 | 7 | 2.0 |
| 1172 | 4529 | M 49-53 | Tooth | 7,980,774 | 4 | 1 | J1 | X | 38.1 |
| 1234 | 4529 | M 20-26 | Tooth | 10,714,325 | 9 | 1 | T2 | X | 66.3 |
| 1571 | 4529 | M 20-26 | Tooth | 10,276,425 | 20 | 3 | 1.3 |
| 1612 | 4529 | M 20-26 | Tooth | 5,785,789 | 33 | HV | 14.6 |
| 1627 | 4529 | M 63-68 | Tooth | 6,131,199 | 43 | | 1.5 |
| 1664 | 4529 | M 59-70 | Tooth | 6,283,524 | 30 | X | 23.2 |
| 1730 | 4529 | M 29-35 | Tooth | 7,100,372 | 36 | H1 | X | 30.6 |
| 1780 | 4529 | M 24-29 | Tooth | 6,032,043 | 35 | H1 | 59.3 |
| 1483 | 4562 | M 25-30 | Tooth | 296,206 | 1 | | 11.8 |
| 1525 | 4562 | F 60-70 | Tooth | 5,042,620 | 2 | K1 | X | 44.3 |
| 1526 | 4562 | M 30-50 | Tooth | 16,374,285 | 8 | H1 | X | 6.4 |
| 1575 | 4562 | F 49-54 | Tooth | 10,312,193 | 3 | X | 26.5 |
| 1577 | 4562 | M 65-80 | Tooth | 14,583,068 | 15 | U5 | X | 62.0 |
| 1578 | 4562 | M2 49-54 | Tooth | 8,591,236 | 3 | H6 | X | 19.7 |
| 1579 | 4562 | M2 18-23 | Tooth | 419,943,997 | 156,323 | 32,287 | 0.007 | 51 | 0.5 |
| 1580 | 4562 | M 32-50 | Tooth | 10,803,343 | 10 | U8 | X | 26.2 |
| 1607 | 4562 | M 64-75 | Tooth | 128,490,993 | 7,500 | 8,354 | 0.003 | 315 | H2 | X | 20.4 |
| 1608 | 4562 | F 19-24 | Tooth | 10,442,770 | 1 | U2 | X | 0.222 |
| 1628 | 4562 | F 63-70 | Tooth | 14,202,741 | 2 | X | 0.523 |
| 1633 | 4562 | F 57-62 | Tooth | 14,511,703 | 9 | H5 | X | 15.7 |
| 1662 | 4562 | M 36-40 | Tooth | 12,632,005 | 27 | 3 | X | 2.3 |
| 1286 | 4571 | F 21-24 | Tooth | 2,725,423 | 8 | | 2.6 |
| 1316 | 4571 | M 33-38 | Tooth | 10,883,827 | 37 | 2 | T1 | X | 8.7 |
| 1319 | 4571 | F 67-75 | Tooth | 6,127,663 | 37 | 2 | U8 | X | 29.9 |
| 1391 | 4571 | F 45-50 | Tooth | 3,777,477 | 3 | H1 | X | 20.6 |
| 1392 | 4571 | M 22-25 | Tooth | 14,584,536 | 95 | 1 | K1 | 2.2 |
| 1408 | 4571 | F 67-75 | Tooth | 7,787,795 | 18 | | 3.3 |
| ID   | Species | Gender | Age | Anatomical Site | Total Reads | Number of Same Reads | Mapping Score |
|------|---------|--------|-----|-----------------|-------------|----------------------|---------------|
| 1409 | 4571    | F      | 23-26 | Tooth           | 18,366,406  | 24                   | 1 X 4.5       |
| 1410 | 4571    | F      | 55-60 | Tooth           | 8,167,491   | 10                   | H1 X 14.2     |
| 1426 | 4571    | F      | 62-70 | Femur           | 7,088,473   | 0                    | 0.8           |
| 1427 | 4571    | M      | 45-52 | Tooth           | 2,428,582   | 0                    | R1 19.0       |
| 1429 | 4571    | M      | 22-25 | Tooth           | 451,266,651 | 504,232 155,502     | 0.039 275 J1 X 1.2 |
| 1455 | 4571    | F      | 18-23 | Humerus         | 10,638,683  | 19                   | 0.1           |
| 1458 | 4571    | F      | 60-80 | Tooth           | 537,979,602 | 197,213 12,241       | -0.022 79 U5 X 1.2 |
| 1459 | 4571    | M      | 18-23 | Tooth           | 5,881,509   | 4                    | 5.0           |
| 1467 | 4571    | M      | 47-63 | Tooth           | 13,521,186  | 0                    | R0 X 5.1      |
| 1499 | 4571    | M      | 23-40 | Tooth           | 3,606,575   | 1                    | H1 X 27.2     |
| 1508 | 4571    | F      | 19-26 | Femur           | 6,367,115   | 8                    | W5 0.2        |
| 1510 | 4571    | F      | 60-66 | Tooth           | 15,763,343  | 407 675 0.001       | 13 X 10.6     |
| 1511 | 4571    | F      | 64-75 | Tooth           | 16,592,652  | 11                   | X 41.0        |
| 1512 | 4571    | F      | 30-48 | Tooth           | 7,378,133   | 0                    | X2 X 53.2     |
| 1516 | 4571    | M      | 30-60 | Tooth           | 4,454,874   | 27                   | 1 T2 X 16.8   |
| 1517 | 4571    | M      | 40-60 | Tooth           | 15,965,894  | 5                    | X 6.4         |
| 1518 | 4571    | M      | 20-26 | Tooth           | 14,591,977  | 7                    | 1.7           |
| 1556 | 4571    | F      | 23-30 | Tooth           | 9,469,187   | 6                    | J1 X 61.0     |
| 1557 | 4571    | M      | 51-56 | Femur           | 13,573,557  | 7                    | 1 H1 2.5      |
| 1558 | 4571    | M      | 66-75 | Tooth           | 430,839,256 | 198,088 113,324      | 0.029 343 K1 X 0.8 |
| 1559 | 4571    | F      | 20-30 | Tooth           | 10,911,613  | 2                    | U5 X 53.9     |
| 1599 | 4571    | F      | 42-48 | Tooth           | 6,435,352   | 464 442 0.011       | 14 X 11.3     |
| 1600 | 4571    | M      | 40-45 | Tooth           | 339,436,728 | 641,231 135,367      | 0.067 668 H2 X 58.8 |
| 1601 | 4571    | M      | 53-58 | Tooth           | 13,385,552  | 13                   | 1 R 3.5       |
| 1602 | 4571    | F      | 18-23 | Tooth           | 7,352,266   | 3                    | H1 4.1        |
| 1617 | 4571    | M      | 52-66 | Tooth           | 9,222,778   | 0                    | 5.2           |
| 1635 | 4571    | M      | 52-57 | Tooth           | 13,479,959  | 2                    | H1 X 32.4     |
| 1636 | 4571    | F      | 52-57 | Tooth           | 10,010,869  | 3                    | X 30.6        |
| 1637 | 4571    | M      | 49-55 | Tooth           | 12,199,289  | 118 685 -0.002      | 5 R0 1.8      |
| 1638 | 4571    | F      | 53-58 | Tooth           | 133,292,730 | 10,034 5,946         | 0.003 510 J1 X 13.4 |
| 1639 | 4571    | F      | 40-80 | Tooth           | 16,468,848  | 1                    | X2 X 61.0     |
| 1653 | 4571    | M      | 47-52 | Tooth           | 12,670,312  | 25                   | 1 2.7         |
| 1655 | 4571    | M      | 29-54 | Tooth           | 16,932,750  | 1                    | W3 X 4.9      |

*aCorresponds to the archaeological find and context number; **Sex and Age refer to the results of the osteological analysis; *Skeletal element defines the sample type from which DNA was extracted; Total number of reads after clipping and merging; Number of reads assigned to the taxonomic node of *S. enterica* subsp. *enterica* in the metagenomic analysis by MALT; Number of reads aligned to the reference of *S. Paratyphi C RKS4594 (NC_012125)* in the multi-reference serovar determination; Taxa-specific mapping score for *S. Paratyphi C* (see Transparent Methods); Number of authentic ancient reads identified by MaltExtract; Mitochondrial haplogroups
determined by HaploFind; †Samples included in the population genetic analyses (Figure 3 and Figures S5 - S6); ‡Given as proportion of reads mapped to the human genome relative to the total reads (%); ‡Samples found to be S. Paratyphi C-positive (Table 1); ‡‡Samples used for radiocarbon dating (Figure S1 and Table S1).

Table S4. Multi-reference mapping. List of Salmonella enterica reference genomes used in the multi-reference mapping. (Related to Table 1 of main text).

| S. enterica serovar | Strain             | NCBI Accession Number |
|---------------------|--------------------|-----------------------|
| Bareilly            | CFSAN000191        | CP032622.1            |
| Choleraesuis        | SC-B67             | NC_006905.1           |
| Dublin              | USMARC-69838       | CP032449.1            |
| Enteritidis         | ATCC BAA-708       | CP025554.1            |
| Gaminara            | CFSAN070644        | CP024165.1            |
| Heidelberg          | 5                  | CP031359.1            |
| Inverness           | ATCC 10720         | CP019181.1            |
| Johannesburg        | ST203              | CP019411.1            |
| Kentucky            | PU131              | CP026327.1            |
| Paratyphi A         | AKU_12601          | FM200053.1            |
| Paratyphi B         | SPB7               | CP000886.1            |
| Paratyphi C         | RKS4594            | NC_012125.1           |
| Typhi               | CT18               | NC_003198.1           |
| Typhimurium         | 14028S             | CP001363.1            |
| Weltevreden         | 1655               | CP014996.1            |

Table S5. Variant call statistics. Matrix showing the single-nucleotide polymorphism (SNP) calls among the ancient S. Paratyphi C genomes from Lübeck, Ragna and Tepos_14 relative to the reference genome of Paratyphi C RKS4594, with a minimal coverage of 5x per call. (Related to Figure 2 of main text and Figure S4).

| Strain     | S. Paratyphi C RKS4594 | 1429 | 1558 | 1600 | Ragna | Tepos_14 |
|------------|------------------------|------|------|------|-------|----------|
| 1429       | 461                    | -    | 4    | 2    | 54    | 194      |
| 1558       | 314                    | -    | -    | 2    | 50    | 135      |
| 1600       | 806                    | -    | -    | 112  | 403   |          |
| Ragna      | 858                    | -    | -    | -    | 526   |          |
| Tepos_14   | 625                    | -    | -    | -    | -     |          |
TRANSPARENT METHODS

Archaeological background

The human remains analysed in this study were recovered during archaeological excavations next to the Heiligen-Geist-Hospital in Lübeck in the early 1990s (Lütgert, 2002). The archaeological context consisted of two mass graves (4528 and 4529, \( n=696 \)) and two smaller burial pits (4562 and 4571, \( n=120 \)). According to the archaeological documentation, the pits 4562 and 4571 cut into the contexts 4528 and 4529 and are therefore dated younger (Prechel, 2002). Osteological age and sex as well as genetic sex are listed in Tables S2 and S3, respectively.

Precautions

Extraction and all pre-PCR steps were carried out in two dedicated aDNA clean rooms (at Kiel University and Göttingen University) following the guidelines on contamination control for aDNA studies (Cooper and Poinar, 2000), including cleaning of surfaces and re-usable tools with bleach or ready-to-use solutions for removing nucleic acid contaminants and UV radiation before and after use. The commercially certified DNA/RNA-free consumables were additionally irradiated with UV light before use. Negative controls were taken along during the extraction (one control for every 22 samples, without bone/tooth powder) and the library preparation (an additional control for every 22 samples, using ddH\(_2\)O instead of aDNA extract). Wearing of protective clothing, including disposable coveralls, masks and two layers of gloves were mandatory. As a further means of authentication, STR profiles at seven loci were generated. Pre- and post PCR amplification steps were carried out in separate laboratories, located in independent buildings. Furthermore, no studies on ancient or modern \( S. \) enterica material have been conducted in any of our laboratory facilities before this analysis.

DNA extraction

For the study, teeth and/or bones from 92 individuals (4528, \( n=29 \); 4529, \( n=11 \); 4562, \( n=13 \); 4571, \( n=39 \)) were sampled. DNA extracts were generated at the Department of Historical Anthropology and Human Ecology at the University of Göttingen using a protocol modified from Fehren-Schmitz et al. (2010). To remove possible cellular contamination from the sample surfaces, the tooth roots were exposed to a 6% sodium hypochlorite solution (Aug. Hedinger GmbH & Co. KG, Stuttgart, Germany) and rinsed afterwards with purified water (Barta et al., 2013; Kemp and Smith, 2005). For the bone samples, the outer surface was mechanically removed using an electric drill with a diamond tipped saw blade (K10, KaVo, Germany).
Subsequently, the samples were mechanically pulverized in a ball triturator (MM200, Retsch). For decalcification and lysis, 200 mg pulverized sample material was incubated in 1000 µL EDTA (0.5 M, pH 8.0, Invitrogen™) and 50 µL proteinase K solution in Tris/HCl (pH 7.5, 0.01 mol/L, 600 mAnson-U/mL, Merck) for 18 h at 37 °C under constant inversion. In a second lysis step, another 50 µL proteinase K solution was added to the sample and incubated for further 2 h at 56 °C. 20 µL sodium dodecyl sulphate (10 mg/mL, Sigma-Aldrich®) were added and incubated for 5 min at 65 °C. The lysate was centrifuged for 3 min at 3300 rcf. 1000 µL of the supernatant of each sample was subjected to an automated DNA extraction (BioRobot® EZ1 Advanced, Qiagen) by using a custom-made version of the Large-Volume-Protocol (Qiagen) and the EZ1 DNA Investigator® Kit (Qiagen) following the manufacturer’s instructions. The DNA was eluted in 100 µL TE Buffer. The DNA extracts were stored at -22 °C. In addition, DNA extractions from six teeth were performed at the Institute of Clinical Molecular Biology (IKMB) in Kiel (Krause-Kyora et al., 2018a).

**Library preparation and sequencing**

All DNA extracts were converted into indexed partial Uracil-DNA Glycosylase (UDG) libraries in Kiel using a protocol modified from Rohland et al. (2015) (as described in Krause-Kyora et al., 2018a). Shotgun sequencing was performed on the Illumina HiSeq 4000 (2x75) platform of the IKMB in Kiel.

**Metagenomic screening**

After using ClipAndMerge v1.7.7 (Peltzer et al., 2016) with default options, the sequencing reads of each sample were screened for the presence of pathogens. MALT v0.4.1 (Vågene et al., 2018) was executed in BlastN mode using a semi-global alignment and a minimum percent identity of 85% to align the samples against a database of 27,730 bacterial and 10,543 viral complete genomes available from the National Center for Biotechnology Information (NCBI) RefSeq database (O’Leary et al., 2016) (January 2019). The results were visualized in MEGAN (Huson et al., 2016). To determine the serovar, reads were aligned to a multi-genome reference including 15 *S. enterica* serovars downloaded from NCBI using BWA v0.7.12 mem (Li, 2013). The number of reads mapping specifically to each genome was retrieved from the BAM files using SAMtools v1.7 idxstats (Li et al., 2009). An endogenous based score was calculated to evaluate the positivity of reads assigned specifically to *S. enterica* and *S. Paratyphi C* using the following formula:
\[
\frac{(SP - SE)}{M} \times 1000
\]

where \(SP\) is the number of reads specifically mapping to \(S.\) Paratyphi C; \(SE\) is the maximum number of reads mapping specifically to any \(S.\) enterica species with the exception of \(S.\) Paratyphi C and \(M\) is the total number of merged reads in the sample. A score > 0 was identified as \(S.\) Paratyphi C-positive. In addition, MaltExtract v1.5 from the software package HOPS (Hübler et al., 2019) was used to screen for ancient \textit{Salmonella enterica} and \textit{Yersinia pestis} reads in all samples from this study.

\section*{Analysis of \(S.\) Paratyphi C genome sequences}

Samples with more than 300 reads mapping to \(S.\) enterica were aligned to the reference genome of \(S.\) Paratyphi C RKS4594 (NC_012125) using BWA mem. DeDup v0.12.2 (Peltzer et al., 2016) was used to remove all duplicate reads from the BAM files and mapDamage v2.0.8 (Jónsson et al., 2013) to evaluate typical aDNA degradation signatures. Genomic variation was identified using Picard Tools v1.139 (http://broadinstitute.github.io/picard) and the Genome Analysis Toolkit (GATK) v3.4.0 (McKenna et al., 2010). The GATK “UnifiedGenotyper” module was used to call reference-based variants from the alignment with default settings and the option “EMIT_ALL_SITES”.

\section*{Variant effect prediction}

VCF files from the three \textit{Salmonella}-positive samples with the highest coverage (HGH-1429, HGH-1558, HGH-1600) were analysed with SnpEff v4.3 (Cingolani et al., 2012) to study effects of variants. The annotated output files were filtered for variants with high impact on the phenotype and researched for their effect.

\section*{Phylogenetic reconstruction}

Homozygous SNPs were called from a complete dataset of twelve ancient (HGH-1429, HGH-1558, HGH-1600, Ragna (Zhou et al., 2018), Tepos_14 (Vågene et al., 2018), ETR001, OBP001, IKI003, IV3002, MUR009, MUR019, SUA004 (Key et al., 2020)) and 123 modern \(S.\) enterica subsp. \textit{enterica} genomes (Alikhan et al., 2018; O’Leary et al., 2016; Zhou et al., 2020) versus the reference genome of \(S.\) Paratyphi C RKS4594 (NC_012125). The MultiVCFAnalyzer v0.87 (Bos et al., 2014) was used with a minimal genotyping quality of 30, minimal base coverage of 5x and minimal allele frequency for homozygous and heterozygous calls of 90%. Variant positions were evaluated in the Integrative Genomics Viewer (IGV) v2.8.2 (Robinson et al., 2011). Positions that
were called in regions of cross-mapping from other organisms and repetitive regions were excluded from the analysis as described previously (Key et al., 2020). The phylogeny was inferred with MrBayes v3.2.7 (Ronquist et al., 2012) using variant-based alignments with a minimal SNP coverage of 5x and 236,622 variant positions. MrBayes was executed with default parameters using the GTR model (lset nst=6 rates=invgamma). The number of simulated generations was increased until the standard deviation of split frequencies was less than 0.01 (mcmc ngen=110,000). Resulting trees were visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree). Mapping, SNP calling and tree reconstruction were repeated for the reference genome of S. Paratyphi A str. AKU_12601 (FM200053.1) to exclude a reference bias and confirm phylogenetic consistency.

**Population genetic analyses**

Human population genetic analyses were performed as described previously (Krause-Kyora et al., 2018b). Mitochondrial haplogroup assignment was conducted with HaploFind (Vianello et al., 2013) using alignments to the human reference genome *Homo sapiens* hg38 mitochondrial DNA (hg38_chrM).
SUPPLEMENTAL REFERENCES

Alikhan, N.-F., Zhou, Z., Sergeant, M.J. and Achtman, M. (2018). A genomic overview of the population structure of Salmonella. PLoS Genet. 14, e1007261.

Barta, J.L., Monroe, C., Kemp, B.M. (2013). Further evaluation of the efficacy of contamination removal from bone surfaces. Forensic Sci. Int. 231, 340–348.

Bos, K.I., Harkins, K.M., Herbig, A., Coscolla, M., Weber, N., Comas, I., Forrest, S.A., Bryant, J.M., Harris, S.R., Schuenemann, V.J., et al. (2014). Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis. Nature 514, 494–497.

Cingolani, P., Platts, A., Le Wang, L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X. and Ruden, D.M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 6, 80–92.

Cooper, A., Poinar, H. (2000). Ancient DNA. Do It Right or Not at All. Science 289, 5482.

Fehren-Schmitz, L., Reindel, M., Cagigao, E.T., Hummel, S. and Herrmann, B. (2010). Pre-Columbian population dynamics in coastal southern Peru: A diachronic investigation of mtDNA patterns in the Palpa region by ancient DNA analysis. Am. J. Phys. Anthropol. 141, 208–221.

Hübler, R., Key, F.M., Warinner, C., Bos, K.I., Krause, J. and Herbig, A. (2019). HOPS: automated detection and authentication of pathogen DNA in archaeological remains. Genome Biol. 20, 280.

Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H.-J. and Tappu, R. (2016). MEGAN Community Edition – Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data. PLoS Comput. Biol. 12, e1004957.

Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P.L.F. and Orlando, L. (2013). mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. Bioinformatics 29, 1682–1684.
Kemp, B.M., Smith, D.G. (2005). Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci. Int. 154, 53–61.

Key, F.M., Posth, C., Esquivel-Gomez, L.R., Hübler, R., Spyrou, M.A., Neumann, G.U., Furtwängler, A., Sabin, S., Burri, M., Wissgott, A., et al. (2020). Emergence of human-adapted Salmonella enterica is linked to the Neolithization process. Nat. Ecol. Evol. 4, 324–333.

Krause-Kyora, B., Nutsua, M., Boehme, L., Pierini, F., Pedersen, D.D., Kornell, S.-C., Drichel, D., Bonazzi, M., Möbus, L., Tarp, P., et al. (2018a). Ancient DNA study reveals HLA susceptibility locus for leprosy in medieval Europeans. Nat. Commun. 9, 1569.

Krause-Kyora, B., Susat, J., Key, F.M., Kühnert, D., Bosse, E., Immel, A., Rinne, C., Kornell, S.-C., Yepes, D., Franzenburg, S., et al. (2018b). Neolithic and medieval virus genomes reveal complex evolution of hepatitis B. Elife 7, e36666.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G. and Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997v1.

Lütgert, S.A. (2002). Archäologische Untersuchungen der Massenbestattungen am Heiligen-Geist-Hospital zu Lübeck: Auswertung der Befunde und Funde. In Lübecker Schriften zu Archäologie und Kulturgeschichte 26, M. Gläser, ed. (Rudolf Habelt), pp. 139–243.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., et al. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20, 1297–1303.

O'Leary, N.A., Wright, M.W., Brister, J.R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., et al. (2016). Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 44, D733–45.
Peltzer, A., Jäger, G., Herbig, A., Seitz, A., Kniep, C., Krause, J. and Nieselt, K. (2016). EAGER: efficient ancient genome reconstruction. Genome Biol. 17, 60.

Prechel, M. (2002). Eine Lübecker Population von 1350 – Krankheiten und Mangelerscheinungen. In Lübecker Schriften zu Archäologie und Kulturgeschichte 26, M. Gläser, ed. (Rudolf Habelt), pp. 245–286.

Robinson, J.T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G. and Mesirov, J.P. (2011). Integrative genomics viewer. Nat. Biotechnol. 29, 24–26.

Rohland, N., Harney, E., Mallick, S., Nordenfelt, S., Reich, D. (2015). Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 370, 20130624.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Vågene, Å.J., Herbig, A., Campana, M.G., Robles García, N.M., Warinner, C., Sabin, S., Spyrou, M.A., Andrades Valtueña, A., Huson, D., Tuross, N., et al. (2018). Salmonella enterica genomes from victims of a major sixteenth-century epidemic in Mexico. Nat. Ecol. Evol. 2, 520–528.

Vianello, D., Sevini, F., Castellani, G., Lomartire, L., Capri, M. and Franceschi, C. (2013). HAPLOFIND: a new method for high-throughput mtDNA haplogroup assignment. Hum. Mutat. 34, 1189–1194.

Zhou, Z., Lundstrom, I., Tran-Dien, A., Duchêne, S., Alikhan, N.-F., Sergeant, M.J., Langridge, G., Fotakis, A.K., Nair, S., Stenøien, H.K., et al. (2018). Pan-genome Analysis of Ancient and Modern Salmonella enterica Demonstrates Genomic Stability of the Invasive Para C Lineage for Millennia. Curr. Biol. 28, 2420–2428.e10.

Zhou, Z., Alikhan, N.-F., Mohamed, K., Fan, Y. and Achtman, M. (2020). The EnteroBase user's guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny, and Escherichia core genomic diversity. Genome Res. 30, 138–152.