

Emergence of human-adapted *Salmonella enterica* is linked to the Neolithization process

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., Lankapalli A.K., Vågene Å.J., Meyer M., Nagel S., Tukhbatova R., Khokhlov A., Chizhevsky A., Hansen S., Belinsky A.B., Kalmykov A., Kantorovich A.R., Maslov V.E., Stockhammer P.W., Vai S., Zavattaro M., Riga A., Caramelli D., Skeates R., Beckett J., Gradoli M.G., Steuri N., Hafner A., Ramstein M., Siebke I., Lösch S., Erdal Y.S., Alikhan N.F., Zhou Z., Achtman M., Bos K., Reinhold S., Haak W., Kühnert D., Herbig A., Krause J.

Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2020, The Author(s), under exclusive licence to Springer Nature Limited. It has been hypothesized that the Neolithic transition towards an agricultural and pastoralist economy facilitated the emergence of human-adapted pathogens. Here, we recovered eight *Salmonella enterica* subsp. *enterica* genomes from human skeletons of transitional foragers, pastoralists and agropastoralists in western Eurasia that were up to 6,500 yr old. Despite the high genetic diversity of *S. enterica*, all ancient bacterial genomes clustered in a single previously uncharacterized branch that contains *S. enterica* adapted to multiple mammalian species. All ancient bacterial genomes from prehistoric (agro-)pastoralists fall within a part of this branch that also includes the human-specific *S. enterica* Paratyphi C, illustrating the evolution of a human pathogen over a period of 5,000 yr. Bacterial genomic comparisons suggest that the earlier ancient strains were not host specific, differed in pathogenic potential and experienced convergent pseudogenization that accompanied their downstream host adaptation. These observations support the concept that the emergence of human-adapted *S. enterica* is linked to human cultural transformations.

<http://dx.doi.org/10.1038/s41559-020-1106-9>

References

- [1] Fowler, C., Harding, J. & Hofmann, D. *The Oxford Handbook of Neolithic Europe* (OUP, 2015).
- [2] Cockburn, T. A. Infectious diseases in ancient populations. *Curr. Anthropol.* 12, 45-62 (1971).
- [3] Armelagos, G. J. & Cohen, M. N. *Paleopathology at the Origins of Agriculture* (Academic Press, 1984).
- [4] Larsen, C. S. et al. Bioarchaeology of Neolithic Çatalhöyük reveals fundamental transitions in health, mobility, and lifestyle in early farmers. *Proc. Natl Acad. Sci. USA* 116, 12615-12623 (2019).
- [5] Barrett, R., Kuzawa, C. W., McDade, T. & Armelagos, G. J. Emerging and re-emerging infectious diseases: the third epidemiologic transition. *Annu. Rev. Anthropol.* 27, 247-271 (1998).
- [6] Spyrou, M. A., Bos, K. I., Herbig, A. & Krause, J. Ancient pathogen genomics as an emerging tool for infectious disease research. *Nat. Rev. Genet.* 20, 323-340 (2019).
- [7] Key, F. M., Posth, C., Krause, J., Herbig, A. & Bos, K. I. Mining metagenomic data sets for ancient DNA: recommended protocols for authentication. *Trends Genet.* 33, 508-520 (2017).

- [8] Vågane, A. J. et al. *Salmonella enterica* genomes from victims of a major sixteenth-century epidemic in Mexico. *Nat. Ecol. Evol.* 2, 520–528 (2018).
- [9] Zhou, Z. et al. 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 (2018).
- [10] Alikhan, N.-F., Zhou, Z., Sergeant, M. J. & Achtman, M. A genomic overview of the population structure of *Salmonella*. *PLoS Genet.* 14, e1007261 (2018).
- [11] Kirk, M. D. et al. World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. *PLoS Med.* 12, e1001921 (2015).
- [12] Kingsley, R. A. & Bäumlner, A. J. Host adaptation and the emergence of infectious disease: the *Salmonella* paradigm. *Mol. Microbiol.* 36, 1006–1014 (2000).
- [13] Kingsley, R. A. et al. Epidemic multiple drug resistant *Salmonella typhimurium* causing invasive disease in sub-Saharan Africa have a distinct genotype. *Genome Res.* 19, 2279–2287 (2009).
- [14] Barrow, P. A. & Methner, U. *Salmonella in Domestic Animals* (CABI, 2013).
- [15] Drancourt, M., Aboudharam, G., Signoli, M., Dutour, O. & Raoult, D. Detection of 400-year-old *Yersinia pestis* DNA in human dental pulp: an approach to the diagnosis of ancient septicemia. *Proc. Natl Acad. Sci. USA* 95, 12637–12640 (1998).
- [16] Anthony, D. W. *The Horse, The Wheel, and Language: How Bronze-Age Riders from the Eurasian Steppes Shaped the Modern World* (Princeton Univ. Press, 2010).
- [17] Schulting, R. J. & Richards, M. P. in *A Bronze Age Landscape in the Russian Steppes. The Samara Valley Project* (eds Anthony, D. W. et al.) 127–149 (Cotsen Institute of Archaeology Press, 2016).
- [18] Didelot, X. et al. Recombination and population structure in *Salmonella enterica*. *PLoS Genet.* 7, e1002191 (2011).
- [19] Haase, J. K. et al. Population genetic structure of 4,12:a:– *Salmonella enterica* strains from harbor porpoises. *Appl. Environ. Microbiol.* 78, 8829–8833 (2012).
- [20] Uzzau, S. et al. Host-adapted serotypes of *Salmonella enterica*. *Epidemiol. Infect.* 125, 229–255 (2000).
- [21] Taylor, J. & Douglas, S. H. *Salmonella birkenhead*: a new *Salmonella* type causing food poisoning in man. *J. Clin. Pathol.* 1, 237–239 (1948).
- [22] Rambaut, A., Lam, T. T., Max Carvalho, L. & Pybus, O. G. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). *Virus Evol.* 2, vew007 (2016).
- [23] Hedge, J. & Wilson, D. J. Bacterial phylogenetic reconstruction from whole genomes is robust to recombination but demographic inference is not. *mBio* 5, e02158–02114 (2014).
- [24] Duchêne, S., Duchêne, D., Holmes, E. C. & Ho, S. Y. The performance of the date-randomization test in phylogenetic analyses of time-structured virus data. *Mol. Biol. Evol.* 32, 1895–1906 (2015).
- [25] Bouckaert, R. et al. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* 10, e1003537 (2014).
- [26] Rhen, M. & Mastroeni, P. *Salmonella: Molecular Biology and Pathogenesis* (Horizon Scientific Press, 2007).
- [27] Guiney, D. G. & Fierer, J. The role of the *spv* genes in *Salmonella* pathogenesis. *Front. Microbiol.* 2, 129 (2011).
- [28] Gulig, P. A. et al. Molecular analysis of *spv* virulence genes of the *salmonella* virulence plasmids. *Mol. Microbiol.* 7, 825–830 (1993).
- [29] Rotger, R. & Casadesús, J. The virulence plasmids of *Salmonella*. *Int. Microbiol.* 2, 177–184 (1999).
- [30] Hackett, J., Wyk, P., Reeves, P. & Mathan, V. Mediation of serum resistance in *Salmonella typhimurium* by an 11-kilodalton polypeptide encoded by the cryptic plasmid. *J. Infectious Dis.* 155, 540–549 (1987).
- [31] Langridge, G. C. et al. Patterns of genome evolution that have accompanied host adaptation in *Salmonella*. *Proc. Natl Acad. Sci. USA* 112, 863–868 (2015).
- [32] Liu, W. Q. et al. *Salmonella paratyphi* C: genetic divergence from *Salmonella choleraesuis* and pathogenic convergence with *Salmonella typhi*. *PLoS ONE* 4, e4510 (2009).
- [33] Thomson, N. R. et al. Comparative genome analysis of *Salmonella enteritidis* PT4 and *Salmonella gallinarum* 287/91 provides insights into evolutionary and host adaptation pathways. *Genome Res.* 18, 1624–1637 (2008).
- [34] Parkhill, J. et al. Complete genome sequence of a multiple drug-resistant *Salmonella enterica* serovar Typhi CT18. *Nature* 413, 848 (2001).
- [35] Lee, S.-J. et al. Identification of a common immune signature in murine and human systemic salmonellosis. *Proc. Natl Acad. Sci. USA* 109, 4998–5003 (2012).
- [36] Seth-Smith, H. M. SPI-7: *Salmonella*'s Vi-encoding pathogenicity island. *J. Infect. Dev. Cries* 2, 267–271 (2008).
- [37] Omran, A. R. The epidemiologic transition: a theory of the epidemiology of population change. *Milbank Q.* 83, 731–757 (2005).
- [38] Pinhasi, R. & Stock, J. T. *Human Bioarchaeology of the Transition to Agriculture* (John Wiley & Sons, 2011).
- [39] Miller, L. & Hurley, K. *Infectious Disease Management in Animal Shelters* (John Wiley & Sons, 2009).

- [40] Schuster, C. J. et al. Infectious disease outbreaks related to drinking water in Canada, 1974–2001. *Can. J. Public Health* 96, 254–258 (2005).
- [41] Dabney, J. et al. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl Acad. Sci. USA* 110, 15758–15763 (2013).
- [42] Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B* 370, 20130624 (2015).
- [43] Meyer, M. & Kircher, M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* 2010, pdb.prot5448 (2010).
- [44] Gansauge, M.-T. et al. Single-stranded DNA library preparation from highly degraded DNA using T4 DNA ligase. *Nucleic Acids Res.* 45, e79 (2017).
- [45] Briggs, A. W. et al. Removal of deaminated cytosines and detection of in vivo methylation in ancient DNA. *Nucleic Acids Res.* 38, e87 (2010).
- [46] Haak, W. et al. Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* 522, 207–211 (2015).
- [47] Hübner, R. et al. HOPS: automated detection and authentication of pathogen DNA in archaeological remains. *Genome Biol.* 20, 280 (2019).
- [48] Briggs, A. W. et al. Patterns of damage in genomic DNA sequences from a Neandertal. *Proc. Natl Acad. Sci. USA* 104, 14616–14621 (2007).
- [49] Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 25, 1754–1760 (2009).
- [50] Peltzer, A. et al. EAGER: efficient ancient genome reconstruction. *Genome Biol.* 17, 60 (2016).
- [51] Kircher, M. in *Ancient DNA. Methods in Molecular Biology (Methods and Protocols)* Vol. 480 (eds Shapiro, B. & Hofreiter, M.) 197–228 (Humana Press, 2012).
- [52] Jolley, K. A. et al. Ribosomal multilocus sequence typing: universal characterization of bacteria from domain to strain. *Microbiology* 158, 1005–1015 (2012).
- [53] Bos, K. I. et al. Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis. *Nature* 514, 494–497 (2014).
- [54] Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313 (2014).
- [55] Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2—approximately maximum-likelihood trees for large alignments. *PLoS ONE* 5, e9490 (2010).
- [56] Moran, A. B. & Edwards, P. Three new *Salmonella* types: *S. richmond*, *S. daytona* and *S. tallahassee*. *Proc. Soc. Exp. Biol. Med.* 62, 294–296 (1946).
- [57] Van der Walt, M. L., Huchzermeyer, F. & Steyn, H. C. *Salmonella* isolated from crocodiles and other reptiles during the period 1985–1994 in South Africa. *Onderstepoort J. Vet. Res.* 64, 277–283 (1997).
- [58] Paton, J. & Mirfattahi, M. *Salmonella meningitis* acquired from pet snakes. *Arch. Dis. Child.* 77, 91 (1997).
- [59] Pedersen, K., Sørensen, G., Szabo, I., Hächler, H. & Le Hello, S. Repeated isolation of *Salmonella enterica* Goverdhan, a very rare serovar, from Danish poultry surveillance samples. *Vet. Microbiol.* 174, 596–599 (2014).
- [60] Sharma, V., Rohde, R., Garg, D. & Kumar, A. Toads as natural reservoir of salmonella. *Zentralbl. Bakteriolog. Orig. A* 239, 172–177 (1977).
- [61] Sharma, V. & Singh, C. *Salmonella goverdhan*, a new serotype from sewage. *Int. J. Syst. Evol. Microbiol.* 17, 41–42 (1967).
- [62] Zhou, Z. et al. The Enterobase user's guide, with case studies on *Salmonella* transmissions, *Yersinia pestis* phylogeny, and *Escherichia* core genomic diversity. *Genome Res.* 30, 138–152 (2020).
- [63] Pagel, M. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. R. Soc. Lond. B* 255, 37–45 (1994).
- [64] Zhou, Z. et al. Transient Darwinian selection in *Salmonella enterica* serovar Paratyphi A during 450 years of global spread of enteric fever. *Proc. Natl Acad. Sci. USA* 111, 12199–12204 (2014).
- [65] Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P. L. & Orlando, L. mapDamage2. 0: fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics* 29, 1682–1684 (2013).
- [66] Renaud, G., Slon, V., Duggan, A. T. & Kelso, J. Schmutzi: estimation of contamination and endogenous mitochondrial consensus calling for ancient DNA. *Genome Biol.* 16, 224 (2015).
- [67] Vianello, D. et al. HAPLOFIND: a new method for high-throughput mtDNA Haplogroup assignment. *Hum. Mutat.* 34, 1189–1194 (2013).
- [68] Korneliussen, T. S., Albrechtsen, A. & Nielsen, R. ANGSD: analysis of next generation sequencing data. *BMC Bioinformatics* 15, 356 (2014).
- [69] Lazaridis, I. et al. Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* 513, 409–413 (2014).

- [70] Patterson, N., Price, A. L. & Reich, D. Population structure and eigenanalysis. *PLoS Genet.* 2, e190 (2006).
- [71] Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 19, 1655–1664 (2009).
- [72] Ramsden, C. et al. High rates of molecular evolution in Hantaviruses. *Mol. Biol. Evol.* 25, 1488–1492 (2008).
- [73] Stadler, T., Kühnert, D., Bonhoeffer, S. & Drummond, A. J. Birth-death skyline plot reveals temporal changes of epidemic spread in HIV and hepatitis C virus (HCV). *Proc. Natl Acad. Sci. USA* 110, 228–233 (2013).
- [74] Reimer, P. J. et al. IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP. *Radiocarbon* 55, 1869–1887 (2013).
- [75] Yoon, S. H., Park, Y.-K. & Kim, J. F. PAIDB v2. 0: exploration and analysis of pathogenicity and resistance islands. *Nucleic Acids Res.* 43, D624–D630 (2014).
- [76] Fuentes, J. A., Villagra, N., Castillo-Ruiz, M. & Mora, G. C. The *Salmonella* Typhi hlyE gene plays a role in invasion of cultured epithelial cells and its functional transfer to *S. Typhimurium* promotes deep organ infection in mice. *Res. Microbiol.* 159, 279–287 (2008).
- [77] Vernikos, G. S. & Parkhill, J. Interpolated variable order motifs for identification of horizontally acquired DNA: revisiting the *Salmonella* pathogenicity islands. *Bioinformatics* 22, 2196–2203 (2006).
- [78] Blondel, C. J., Jiménez, J. C., Contreras, I. & Santiviago, C. A. Comparative genomic analysis uncovers 3 novel loci encoding type six secretion systems differentially distributed in *Salmonella* serotypes. *BMC Genomics* 10, 354 (2009).
- [79] Elder, J. R. et al. The *Salmonella* pathogenicity island 13 contributes to pathogenesis in streptomycin pre-treated mice but not in day-old chickens. *Gut Pathog.* 8, 16 (2016).
- [80] Shah, D. H. et al. Identification of *Salmonella gallinarum* virulence genes in a chicken infection model using PCR-based signature-tagged mutagenesis. *Microbiology* 151, 3957–3968 (2005).
- [81] Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. Preprint at <https://arxiv.org/abs/1303.3997> (2013).
- [82] Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26, 841–842 (2010).
- [83] McKenna, A. et al. The genome analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 20, 1297–1303 (2010).
- [84] Fellows Yates, J. A. et al. Central European woolly mammoth population dynamics: insights from Late Pleistocene mitochondrial genomes. *Sci. Rep.* 7, 17714 (2017).