Yersinia pestis and plague in the 21st century: learning from a distant past

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Plague is an often fulminant and fatal disease caused by the bacterial pathogen *Yersinia pestis*. Three major historical plague pandemics have heavily influenced human societies, and recent evidence demonstrates that plague has also influenced human biology. Plague is still endemic in several continents and remains a potential public health risk. In this article, we examine the current situation of plague around the world and the different players that participate in plague ecosystems. We discuss in particular the recent research that allows us to understand plague in pre-historical times, as well as how past pandemics have modulated the evolution of human immune genes. We also highlight future challenges that await us in the study of this deadly but fascinating disease.

When one pandemic is not enough: the recurrent history of human plague

Lockdown, quarantines, sanitary passes, masks, hand washing...! The Covid-19 pandemic significantly impacted our lifestyle since March 2020, when public health measures were taken worldwide to stop the spread of the severe acute respiratory syndrome coronavirus 2 (a.k.a. SARS-CoV-2). It is, however, interesting to remind that many of these measures had been already implemented in Europe in the Middle Ages to fight another pandemic disease that, still to this day, brings shivering to anyone who hears the infamous word: plague!

Indeed, plague hit Europe between 1347 and 1352, killing between 30% and 50% of the population during an episode that, today, is commonly referred to as the Black Death. Plague then reappeared periodically during the following 400 years. The word quarantine was first used in Ragusa (today Dubrovnik, Croatia) in 1377 to indicate the 40 days that boats had to remain in the sea before arriving to the city port. During the 17th century, plague stones filled with vinegar as a disinfectant for hands (or coins) could be found at the entrance of several cities in England. Across Europe, masks were used by doctors to protect themselves from 'poisonous air'. And sanitary passes were delivered to allow travellers to move between kingdoms!

Before the Black Death, plague had already impacted Mediterranean countries in 541 during the first major plague pandemic in recorded history, known as the Justinian Plague, after the Byzantine Emperor Justinian I, also causing millions of deaths at the time. And more recently, at the end of the 19th century, plague spread around the world during a third pandemic that is still active to this day.

But... what is plague? And where can we find plague today?

Plague is a disease caused by the bacterium *Yersinia pestis*, named after the French/Swiss doctor Alexandre Yersin, who discovered it in Hong Kong in 1894, at the dawn of the third plague pandemic. In nature, the plague bacillus is present in several rodent reservoirs, which include marmots, gerbils or wild mice (Figure 1). However, the disease is not directly transmitted by rodents but instead by the fleas of these animals. Fleas can transmit the disease to peri-urban rodents such as rats, which have played a major role as reservoirs during plague pandemics, and also to humans. Humans have actually contributed to spreading the disease around the world by transporting rats with them, particularly during the expansion of intercontinental maritime trade after the industrial revolution. Today, plague is endemic in the Americas (United States, Peru or Brazil), Asia (China, Mongolia or Kazakhstan) and Africa, where the most important current plague foci are located in the Democratic Republic of Congo and Madagascar (Figure 2). Circulation of *Y. pestis* among rodent reservoirs allows plague to persist in these endemic areas, and perturbation of plague ecosystems allows the disease to reach human populations and induce plague outbreaks.

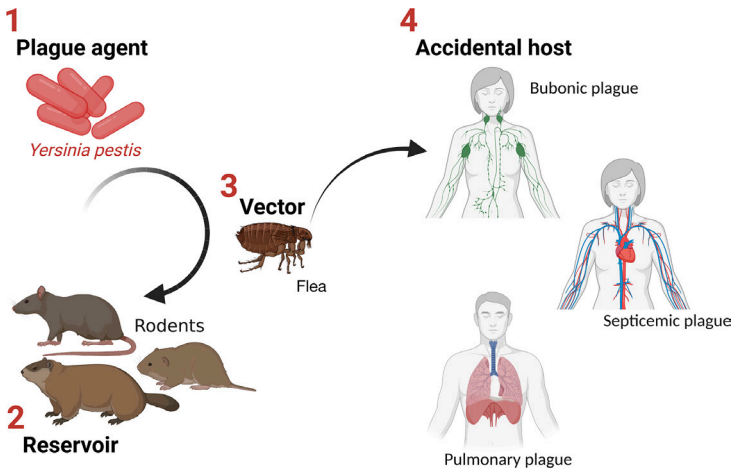

Figure 1. Main players of the plague ecosystem: the etiologic agent of the disease (the Gram-negative bacterium *Y. pestis*), the natural reservoirs of the disease (rodents), the vector (fleas) that allows transmission of the disease between rodents and also to humans which are accidental hosts and which may develop three major forms of the disease (bubonic, pulmonary and septicaemic plague).

In humans, plague is often characterized by the swelling of infected lymph nodes (called 'buboes'), which have drained bacteria inoculated in the skin by contaminated fleas. Bubonic plague can lead to pulmonary plague if the bacteria reach the blood and then the lungs. This form of the disease is particularly dangerous because plague can then be transmitted from person to person via aerosols, exactly as Covid-19. And if the bacteria proliferate in the blood, there is a third clinical form of the disease known as septicaemic plague. In the absence of antibiotics, all forms of the disease are often fatal. Early diagnosis with prompt antibiotic treatment (e.g., streptomycin, gentamicin or fluoroquinolones) is effective against all forms of plague. However, *Y. pestis* strains resistant to antibiotics have been identified in Madagascar, Mongolia and China, highlighting a major potential risk for public health.

Tracking the evolution of a deadly pathogen, hidden in buried victims of past plagues

Our understanding of plague epidemics/pandemics has greatly expanded thanks to the recent advances in genomics or, more precisely, the analysis of the ancient DNA present in remains of human plague victims (Figure 3). Knowing the obsession of microbiologists to classify pathogens and their variants, and that of historians to reconstruct past events, DNA sequences from ancient *Y. pestis* represent sweet evidence to delight the appetite of both types of researchers, leading to the recent emergence of the field of ancient pathogen genomics. Without needing to revive strains from the past, such studies have not only confirmed that

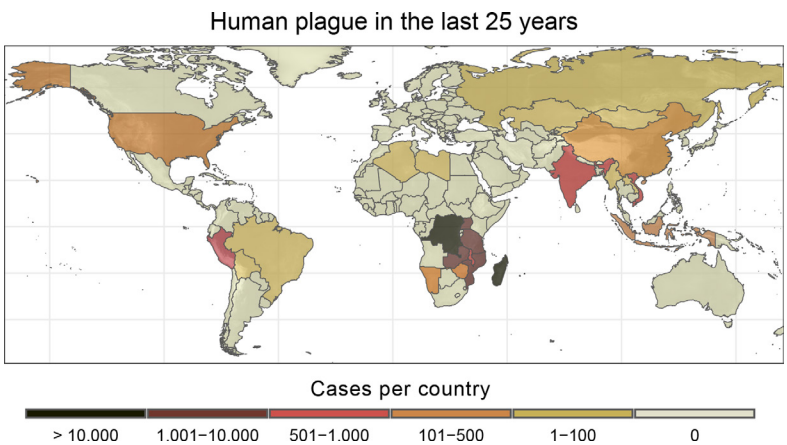

Figure 2. World map indicating the countries which have declared plague cases to the World Health Organization between 1994 and 2018.

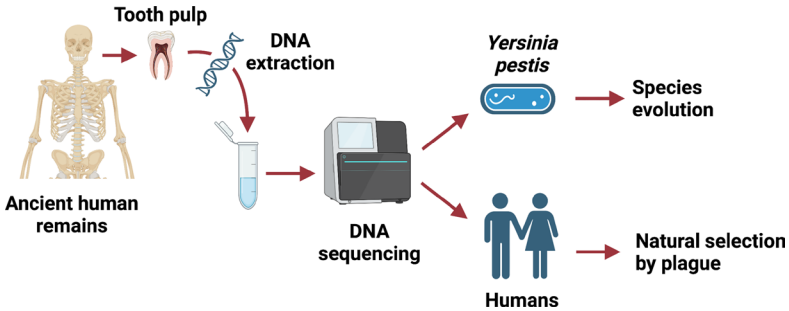

Figure 3. The study of ancient DNA from humans or from *Y. pestis* (isolated from the dental pulp of ancient human remains) has allowed major advances in the understanding of the evolution of the plague bacillus and also in discovering that past plague pandemics have shaped our repertoire of immune genes.

this pathogen actually caused the major historically documented plague episodes, but provided evidence for the past geographic burden of the disease. Indeed, by applying molecular evolution methods on the DNA fingerprints (mutations) of these ancient *Y. pestis* strains, we can retrace the history of the pathogen and understand the origin of specific pandemics.

For instance, the recent analysis of DNA from remains in well-dated medieval graves in present-day Kyrgyzstan showed that the strain that caused the infamous Black Death outbreaks in Europe probably emerged in Central Asia, where an animal reservoir has uninterruptedly persisted in the area throughout centuries. If you ever wondered which scourges prehistoric humans suffered from, the presence of *Y. pestis* DNA in tens of bodies from the Neolithic has uncovered the possible plague outbreaks in which different bacterial strains became widespread in prehistoric Eurasia (5,000–3,000 years ago), several millennia before the first recorded pandemic occurred. Besides these findings, the unearthing of *Y. pestis* DNA has also fuelled discussions appealing to many microbiologists concerning the evolutionary steps through which a successful pathogen emerges and is efficiently disseminated by vectors (like fleas, in the case of plague). Just like SARS-Cov-2 had to gain the capacity to infect humans before being able to cause a pandemic, prehistoric plague samples retraced the gain of specific factors allowing *Y. pestis* to expand the range of animal hosts in which it can establish stable animal reservoirs, centuries before he major recorded pandemics occurred.

The plague pandemics of past times still influence our times

Beyond its strong imprint on European demography, societies and memories, plague also contributed to natural selection in human populations, favouring survivors and their offspring with an immune system more efficient against infection. Indeed, we recently participated in an innovative study in which the analysis of ancient DNA from humans who died just before or after the Black Death in London (and also in Denmark) allowed us to identify immune genes potentially associated with resistance to plague. Among them, we identified a strong positive selection signal on a functional copy of the gene *erap2*, which codes for an enzyme of the immune system. By infecting in vitro the macrophages of contemporary humans expressing functional or non-functional copies of *erap2*, we confirmed that the enzyme ERAP2 supported their ability to kill the plague bacillus and regulated the production of several molecules that facilitate the activation of the immune system. However, a final surprise awaited our results: the gene *erap2* has been associated by the method of eQTL (see further reading) with a risk of developing autoimmune diseases, such as Crohn's disease, which is a chronic pathology associated with inflammation of our digestive tract. Thus, having a very active immune system which might turn itself against us may represent the price that human populations paid for surviving plague.

Future challenges

Plague is a fatal disease that requires very early diagnosis and effective treatment. Bubonic plague might be easy to identify and diagnose due to the presence of enlarged buboes, which contain high quantities of the plague bacillus that can be extracted to perform classical microbiological tests or molecular analyses. On the contrary, pulmonary plague is more difficult to diagnose, as lungs are not sterile organs and obtaining good sputum samples (particularly from children) can be challenging. The development of rapid diagnostic tests for pulmonary plague is therefore a major defiance, as evidenced during the pulmonary plague outbreak which took place in Madagascar in 2017.

As mentioned earlier, several antibiotics can efficiently kill the plague bacillus, but antibiotic resistances have been identified in *Y. pestis*. Vaccines could help face emerging resistances to antibiotics, but

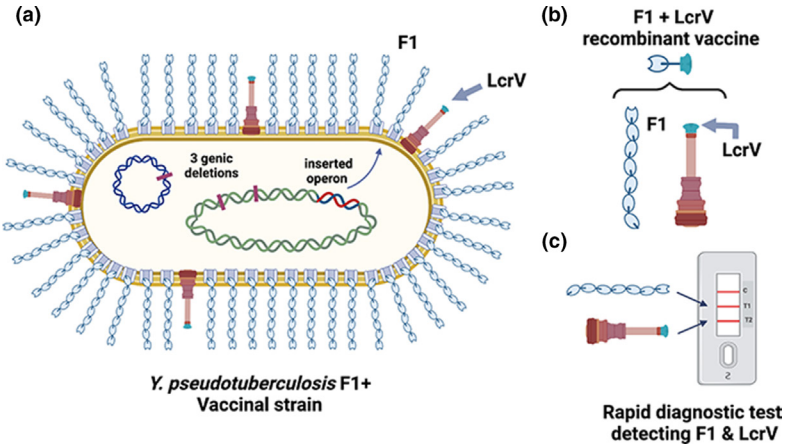

Figure 4. (a) *Y. pseudotuberculosis*, the ancestor of *Y. pestis*, has been modified into an attenuated plague vaccine by inactivating in its genome three genes normally involved in disease development, and by adding an operon from *Y. pestis* that allows production of the protein F1, which forms a capsule at the bacterial surface. Depicted is also LcrV, the tip of a molecular complex that allows pathogenic *Yersinia* to inject molecules into host cells in order to dampen immune responses. (b). Both F1 and LcrV have been used to develop molecular recombinant vaccines. (c). F1 and LcrV are also used as target antigens for the development of rapid diagnostic tests for plague.

no vaccine is currently approved worldwide. Several molecular vaccines based on single *Y. pestis* antigens have been explored, with mixed results until now (Figure 4). We have recently developed a live attenuated oral vaccine against plague using as a backbone the bacterium *Y. pseudotuberculosis*, which is the ancestor of *Y. pestis*, giving excellent results in a rodent model and allowing us to envision its trial in a non-human primate model. RNA-based vaccines, as the ones used against SARS-Cov-2, have not been developed against bacterial pathogens, but they remain an interesting alternative to explore.

Last but not least, *Y. pestis* is one of the most recently emerged bacterial pathogens, and understanding the molecular events that led to the very rapid evolution from its ancestor *Y. pseudotuberculosis* to the current plague bacillus remains a tremendous challenge for biologists, a challenge that might allow to better understand past and current pandemics and to prevent future ones. ■

Further Reading

- A recent general review on plague: Demeure, C.E., Dussurget, O., Mas-Fiol, G., Le Guern, A.S., Savin, C. and Pizarro-Cerda, J. (2019). *Yersinia pestis* and plague: an updated view on evolution, virulence determinants, immune subversion, vaccination, and diagnostics. *Genes Immun.* **20**, 357–370.
- A report on the impact of past plague pandemics on the evolution of human immune genes: 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. and Barreiro, L.B. (2022). Evolution of immune genes is associated with the Black Death. *Nature* **611**, 312–319
- A report on the development of a novel plague vaccine:Derbise, A., Hanada, Y., Khalifé, M., Carniel, E. and C. E. Demeure. (2015). Complete protection against pneumonic and bubonic plague after a single oral vaccination. *PLoS Neglected Tropical Diseases*. **9**,e0004162.
- A recent review on the current advances and challenges on the use of ancient DNA to understand infectious diseases: Spyrou, M.A., Bos, K.I., Herbig, A. and Krause, H. (2019). Ancient pathogen genomics as an emerging tool for infectious disease research. *Nature Rev. Genetics* **20**, 323–340.
- A report on the risk to develop autoimmune diseases associated to ERAP2: Di Narzo, A.F., Peters, L.A., Argmann, C., Stojmirovic, A., Perrigoue, J., Li, K., Telesco, S., Kidd, B., Walker, J., Dudley, J., Cho, J., Schadt, E.E., Kasarkis, A., Curran, M., Dobrin, R. and Hao, K. (2016). Blood and intestine eQTLs from an anti-TNF-resistant Crohn's disease cohort inform IBD genetic association loci. *Clin. Transl. Gastroenterol.* **7**, e177.

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