Yersinia pestis, a problem of the past and a re-emerging threat

REVIEW ARTICLE

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ABSTRACT

Yersinia pestis is the bacteria that causes plague, one of the deadliest diseases in human history. Three major plague pandemics (The Justinian Plague, the Black Death and the Modern Plague) have been recorded. Each caused massive fatalities and has become defining events in the time periods in places that were affected. The presence of natural plague foci in rodents across the world is one of the risk factors for human plague. While plague is a relatively rare problem for most countries, more than 90% of plague cases in the world still occur in Africa. This article discusses the threat of *Yersinia pestis* in the modern world by considering its prevalence and severity of illness it causes, transmission, antibiotic resistance, and its potential as a bioweapon.

Keywords: *humans, plague, Yersinia pestis, bioterrorism, pandemics, drug resistance, microbial, Africa*

1. Introduction

Plague is an infectious disease caused by *Yersinia pestis*, a gram-negative, nonmotile, non-spore-forming coccobacillus. This bacteria appears as bipolar staining with Giemsa, Wright's, or Wayson staining and is also visible by Gram staining. It survives within a wide range of temperatures with the optimum being 28ºC to 30ºC with pH extremes of 5 and 9.6. *Y. pestis* dies very rapidly if exposed to a UV light, temperatures exceeding 40°C or when exposed to intensive desiccation [1].

Yersinia pestis evolved from the closely related zoonotic enterobacterium *Y. pseudotuberculosis* [2]. *Yersinia pestis* strain CO92 was amongst the first bacteria to be sequenced [3]. Other strains have since been sequenced [4–6,6–8]. The fluid nature of its genome and molecular mechanisms contribute to its resilience, virulence and ability to persist even after the death of its host [3]. For instance, the bacteria evolved to acquire plasmids which enables it to transition into its rodent-flea environments and enhance virulence [9]. The bacteria also has specific genes involved in host virulence, host colonisation and core cellular function [10]. *Yersinia pestis* uses bacterial strategies to take over host innate immune responses and prolong its own survival. Its virulence is determined by specific proteins such as the protease Pla and the *Yersinia* outermembrane proteins (Yops) [11].

Y. pestis has three different biovars in which each causes a distinct pandemic. The three distinct biovars are Antiqua, Mediavalis and Orientalis. Antiqua is found in Africa and central Asia whereas Medievalis is limited only to central Asia and Orientalis is almost worldwide in its distribution [12]. Recently, researchers reported a new biovar called Microtus, found in China [13]. The differences between the biovars can be seen on the basis of conversion of nitrate to nitrite and fermentation of glycerol. The first

strain Antiqua has both characteristics. Orientalis converts nitrates to nitrites but does not ferment glycerol. Medievalis does not possess either of the characteristics to convert nitrates to nitrites or fermenting glycerol [10]. The Microtus strain shows similar characteristics with those of Mediavalis and does not utilise arabinose [13].

1.1 Transmission

The life cycle of *Yersinia pestis* involves rodents and its transmission from hematophagous adult fleas to animal hosts and between hosts, including humans. Infected animals and their fleas can become long-term reservoir hosts for the bacteria. Infections can also occur in other animals such as lagomorphs, artiodactyls, carnivores, hyracoids, insects, marsupials and primates. Birds also have the tendency to spread fleas that carry *Y. pestis* while predators catch prey that may carry the bacteria, which in turn spreads the bacteria around. Thus, *Yersinia pestis* is maintained in nature by animal populations making them accountable for this bacteria being endemic in many parts of the world [14]. Plague infection can occur from person to person through infectious droplets from the coughs of infected people and contact with contaminated bodily fluid or tissue [15]. Besides bites from infected fleas, people working with animals such as farming, butchery or animal husbandry are at risk of plague infection [16].

1.2 Detection

There are a number of ways in which *Yersinia pestis* can be detected. Most target the fraction 1 (F1) capsular antigen which represents the most immunogenic specific protein of the bacteria [17,18]. The most widely used diagnostic tests are the passive hemagglutination test and hemagglutination inhibition test. Dipsticks are the most frequently used detection methods in the field [19]. Point of care testing of *Y. pestis* during field work or visits to affected areas allows detection in the field without having to access a laboratory [20]. Detection by Polymerase Chain Reaction (PCR) may be performed by medical staff in the field by using portable real-time quantitative PCR thermocyclers [15]. Other detection methods such as biosensors [21,22], serodiagnosis [23], lateral-flow immunoassay and up-converting phosphor technology-based biosensors [24] have since been introduced and successfully evaluated.

In compliance with the European policy (Decision no 082/2013/EU), the EMERGE Coordination and the Bundeswehr Institute of Microbiology recommend detecting plague by confirming a first positive/negative test result with a second test. As an example, a positive PCR amplification confirmed by a culture or immunological test conducted in parallel. EMERGE also recommends that additional laboratory investigations be considered in the event of obtaining negative results when evaluating an early stage of disease when there is suspected plague and progression of clinical symptoms [25].

1.3 Treatment

Treatment for plague should administered as soon as possible [26]. The current treatment for plague includes the use of antibiotics, dispense of oxygen, intravenous fluids and respiratory support [27]. Three main antibiotics recommended to treat

plague are streptomycin, tetracycline and chloramphenicol [27]. Other antibiotics that can be used to treat plague are as gentamicin, levofloxacin, ciprofloxacin, doxycycline, moxifloxacin and chloramphenicol [28].

Alternative methods of treatment in the literature comprise serum therapy, phage therapy and bacteriocin therapy. Historically, serum therapy to treat humans with a horse serum was conducted by Alexandre Yersin in 1897 [29]. Serum therapy however, did induce side effects such as serum sickness and anaphylactic shock. Despite this, a significant difference in death rates was found amongst people suffering bubonic plague, for which 13% was reported for those treated with serum therapy and 64% for those untreated. At that time, reduction in death rates was not much different between people suffering from septicaemic or pneumonic plague [26,30].

Phage therapy through bacteriophages was first attempted by d'Herelle in 1925 to treat four cases of bubonic plague by using highly virulent anti-plague phage that had been isolated in rat faeces. Within several hours after the injection into the buboes the patients began to feel better and had 2ºC average fall in temperature and decreased pain in the buboes. All four patients made a recovery. However, when trying to confirm the efficacy of the treatment using animal models led to conflicting results. This was due to a number of factors such as a poor understanding of the mechanisms of phage–bacterial interactions, poorly designed and executed experiments and clinical trials and the use of undefined phages in the form of non-purified phage preparations [31]. Despite this, phage therapy is gaining renewed interest to combat antibiotic resistant bacteria [32].

Bacteriocin therapy through bacteriocins, antimicrobial peptides produced by bacteria was used by McGeachie in 1970 to treat bacterial infections. Purified colicins V and K were found to show similar inhibitory activities as kanamycin, streptomycin and oxytetracycline [33]. Another bacteriocin called Enterocoliticin was tested against *Yersinia enterocolitica* which is a close relative of *Y. pestis*. The treatment was proven to be effective in the early phase of infection [34].

1.4 Preventive measures

Plague is classified as a quarantinable disease. The first preventive measure is to stop the spread of plague by enhancing surveillance for the disease especially at points of entry into countries to ensure that it does not cross borders into other non-infected countries. This covers persons, baggage, cargo, containers, conveyances goods [35]. The WHO International Health Regulations also recommend that inspection and supervision of vector surveillance should be carried out on products that enter the country. Monitoring of the rodent populations within a country should be conducted. For example, if there is a drastic decrease in rat populations, there is a risk of fleas feeding on other animals which may bring upon a human epidemic [36].

Measures to increase public awareness of plague[37] should be undertaken [35]. Contingency planning within all health sector partners in countries at risk of plague spread should be carried out [38] In some countries such as Hong Kong, plague is a statutory notifiable disease. As such, the Department of Health is authorised to control

the spread of the plague including source finding and contact tracing during which the contacts will also be given post-exposure prophylaxis [35].

Vaccination is a preventive measure for the plague [39–41]. Live attenuated vaccines are used in some countries such as Russia [15]. There is also the killed whole cell vaccine in use produced by the US army [42]. Due to many reported challenges related to vaccination for plague, vaccines are not common in use today. For instance, safety concerns have been raised in the use of live attenuated vaccines [43,44]. Research on new types of vaccines for the plague is vast. Some of those in development include subunit vaccines which use different antigens. Whilst the F1 antigen is not ideal because some *Yersinia pestis* strains do not produce this antigen, the V antigen may be selected to compliment the F1 antigen [42]. There is a possibility that genetically modified vaccines using a strain called Kimberley 53 that produces ten to hundred-fold higher antibody titres to F1 and V could attenuated and used as a live-attenuated vaccine [45]. Furthermore, DNA vaccines could incorporate the use F1 or V antigen DNA [46,47]. This type of vaccine is expected to be useful as part of a prime-boost strategy [45]. While different types of plague vaccines have been in development, no proven effective vaccine for plague prevention is currently available [15]. There is however, a possibility of combining the use of vaccines with antibiotic treatment. Recently, researchers have reported a synergistic protective effect in using a live vaccine upon exposure to the virus with second-line antibiotic treatment [48].

2. Types of plague

There are three main types of plague and a few rare variants of the disease. The first and most common is the bubonic plague. After a bite by an infected flea, *Yersinia pestis* enters the lymphatic system and replicates itself at the nearest lymph node, causing it to swell and become inflamed. The bacteria then incubates between one to ten days [49]. During the later stages of infection, the bubo can develop open sores filled with pus [50]. At this stage, infection may also spread to the lungs and progress into more severe clinical symptoms. The symptoms linked with bubonic plague are fever, headache, chills, weakness, malaise, myalgia other less common symptoms are dizziness, nausea and vomiting [49]. If bubonic plague is left untreated, the fatality rate is between 40% to 70% fatality rate. This accounts for 80% to 95% of cases of plague worldwide. When treated, the case fatality rate is 5% to 15% [49].

The next type of plague is pneumonic plague where the lungs are infected and can be a progression of bubonic plague to this stage or a progression by an infection from an individual affected with pneumonic plague. This is the most virulent form of the plague and can have the quickest onset with the incubation time sometimes being as short as 24 hours or up to 4 days. The symptoms of pneumonic plague are fever, headache, weakness, and a rapidly developing pneumonia with shortness of breath, chest pain, cough, and sometimes presents with bloody or watery mucous [28]. Pneumonic plague is a more severe infection compared to bubonic plague. If pneumonic plague is left untreated, it is almost always fatal but most people can survive the infection with immediate treatment upon clinical manifestation of the symptoms. Due to the short time frame to obtain effective treatment within 24 hours, the case fatality ratio is greater than 50% [49].

The third type of plague is septicaemic plague where blood is infected. Primary septicaemic plague makes up about 10% to 15% of all cases of plague [51]. Septicaemia may also arise as a secondary effect from bubonic plague. It can affect people of all ages, but the elderly are more at risk of developing this type of plague [52]. It affects the body by causing a self-perpetuating immunological cascade due the rapidly replication of *Yersinia pestis* [28]. This causes a wide array of symptoms such as the minor ones like fever, chills, extreme weakness, abdominal pain and shock followed by more severe symptoms such as disseminated intravascular coagulopathy, multiple organ failure, acute respiratory distress syndrome, haemorrhage in skin and serosal surfaces and gangrenous necrosis of acral regions [51]. Septicaemic plague is fatal if left untreated. With treatment, the case fatality ratio lies within the range of 30% to 50% [53].

Another type of plague is the rare pharyngeal plague [54]. People who eat undercooked or raw meat from an animal that has been infected with *Y. pestis* can get infected [55]. Although pharyngeal plague clinical symptoms are similar to those of other plagues, those unique to pharyngeal plague are pharyngitis, dysphagia, tender submandibular lymphadenitis, tonsillar enlargement and occasional abdominal pain [56]. Pharyngeal plague is more likely to occur in Middle East [57,58], North Africa [59,60] and Central Asia [61]. Another rare form of plague is gastrointestinal plague [53]. The source of infection is raw or undercooked contaminated meat. Other infections associated with plague which have been reported are cutaneous plague, meningitis, and endophthalmitis [15].

3. Prevalence and Severity

Natural plague is prevalent in many countries across the world and is endemic in a large area of the world [62]. In fact, the effect of plague across the world may be underrepresented in data due to unrecognized cases and the failure of countries to report plague cases. While plague is still a relatively rare problem for most countries, more than 90% of plague cases in the world occur in Africa [12]. The three most endemic countries in the world are the Democratic Republic of the Congo, Madagascar, and Peru [50]. Madagascar is the most affected country in the modern world with reports of plague outbreaks in the population every year [63]. In fact, the most recent outbreak at the Uganda-Congo border was reported on the 5th of March 2019 [64].

A total of 2,417 confirmed cases were reported in the 2017 plague outbreak in Madagascar [38,65]. The death toll was 209 leading to a case fatality ratio of 8.6% [66]. What stands out about this outbreak is the high amount of pneumonic plague cases totalling 1854, of which 76.7% were pneumonic plague. There were 355 confirmed cases of bubonic plague, comprising 14.7% of cases. There was also a single case of septicaemic plague and 207 cases that have not been classified. This outbreak differed to outbreaks that occurred in Uganda where 7.1% cases of plague were pneumonic [67] and the USA where 8.1% of plague cases were also pneumonic [16]. This suggests that human to human transmission was prevalent in the 2017 Madagascar outbreak. The majority of cases occurred in a very small area of the country. Younger patients were more likely to suffer from plague [68]. Between 1996 and 1998, 60.9% of plague cases in Madagascar occurred in people under the age of 19. When affected individuals up to the ages of 29 were included, the number of cases

totalled 80% of all cases during this time [66]. In Uganda, a similar pattern in age groups was found with the most cases reported in patients under the age of 19 from 2008 to 2016 [67].

From 2000 to 2012, there were sixteen reported cases of plague in the USA [65]. In view of the vast size of the country, a plausible explanation for the low number of plague cases is that the American public may be less likely to come into direct contact rodents or other carriers of the plague as the public in Madagascar [16]. Plague cases that affected eight veterinarians, five people who worked with animals (e.g. wildlife biologist or animal control personnel) and five plague laboratory researchers in the USA may be attributed to occupational hazards [16,69,70]. Pets such as dogs may also facilitate the transfer of infected fleas into homes through close living proximity and interaction with their owners [71].

4. Bioterrorism potential

Yersinia pestis is regarded as a Category A organism by the CDC. Category A organisms are considered to be a high priority agent that poses a risk to national security due to its easy transfer from person to person where infection can result in a high mortality rate and public panic. *Y. pestis* infection also requires special action for public health preparedness [28]. Currently, under natural conditions the plague is fairly well controlled with it still being a rare disease to occur even in most areas of the world in which it is endemic. Nevertheless, one of the biggest future dangers may be the imminent threat of plague used as a biological weapon [72]. The idea of using plague as a biological weapon is not new and has been considered since World War Two.

The history of the research and possible use of *Yersinia pestis* as a bioweapon began with the Japanese military's research on the use of many different biological weapons. The program was led by Shiro Ishii (1932–1942) and Kitano Misaji (1942–1945) and the research was performed by a group known as Unit 731 [73]. The first plague biological weapon was made by allowing laboratory fleas to feed on infected rats. Wheat and rice with infected fleas were scattered over Chinese cities to attempt to start a plague epidemic [74]. One case occurred in Ningpo where 100 people died of plague after the release of fleas onto the city. Although these attacks were carried out to relative success, this did not have much of an effect on the war [75].

After World War Two, *Yersinia pestis* continued to be developed in this role, reportedly in the Soviet Union and the USA [76,77]. The two countries developed a method for introducing *Y. pestis* by aerosolising the plague directly without having to use fleas as carriers [78]. This would make the plague an unpredictable bioweapon and could also enable scientists to determine the type of plague to infect victims with. Whilst the release of *Y. pestis* using fleas caused cases of bubonic plague, the aerosol version would cause pneumonic plague which is more virulent and severe [79,80]. In 1970 the WHO estimated that in the worst-case scenario that if 50kg of *Y. pestis* was released in a city with a population of 5 million, up to 150,000 people could be infected and 36,000 of those people would be expected to die.

Today, most countries have stopped conducting offensive bioweapons programmes. The United Kingdom ceased in the 1950s, followed by the USA in 1969. The Biological and Toxin Weapons Convention was written in 1969 and further drafted by the British

and completed by the Soviet Union although the latter did not disband their bioweapons programme until 1992 [80]. The *Yersinia pestis* strain that the Soviet Union was working on was genetically modified to be multidrug-resistant and fluoroquinolone-resistant [51]. In the event that this bioweapon were to be released, the drugs that would have been used are streptomycin, gentamicin, tetracycline, or fluoroquinolones with doxycycline, ciprofloxacin or chloramphenicol as alternatives to treat the pneumonic plague outbreak [81]. This would have also included giving antibiotic prophylaxis with doxycycline or ciprofloxacin for seven days to anyone who comes into contact with the patients with pneumonic plague [82].

In the event that a biological threat occurs, affected countries would require strategic biosafety management the surveillance, subsequent investigation, treatment and control of infection [83,84]. *Yersinia pestis* is a bioterrorism threat due to a number of factors that make it fit for this purpose [85]. The first is the accessibility of *Y. pestis* due to the amount of countries it is endemic in and the possibility for it to be isolated and cultured in a laboratory. Furthermore, information for its optimum growth conditions are available [86]. Secondly, infection by *Y. pestis* has a high mortality rate in pneumonic plague which can be easily spread between people. Thirdly, access to previous research left behind by former scientists who worked on *Y. pestis* could be exploited with the intent of weaponising the strain [87]. Last but not least, antibiotic resistance in *Y. pestis* strains [88,89] could pose to be challenging for affected countries to control disease and manage the delivery of treatment and healthcare [36,90].

5. Antibiotic resistance

Bacterial resistance to antibiotics is a global challenge [81,91,92]. The problem of antibiotic resistance lies in the ability of the bacteria to transfer its antibiotic resistance plasmids via conjugation to other non-resistant strains of *Y. pestis* or to *Escherichia coli* (*E. coli*) [93]. Antimicrobial susceptibility of isolated strains in Madagascar have previously been researched [94]. Recently, two strains of *Yersinia pestis* were found to exhibit antibiotic resistance. *Y. pestis* 17/95 carries 8 antibiotic resistances on a plasmid of 150kb called pIP1202 and is reported to exhibit high-level resistance to eight antimicrobial agents used for treatment and some prophylactic drugs. It is also resistant to some of the typical alternative drugs such as ampicillin, kanamycin, and spectinomycin. While *Y. pestis* 16/95 has only streptomycin resistance on a plasmid of 40 kb called pIP1203, it remains susceptible to other antibiotic treatment [81]. The transfer of the antibiotic resistances to *E. coli* may be important as *E. coli* has a documented history of interbacterial species transmission of antibiotic resistances [95]. Currently, both of these strains are confined to Madagascar. If they reach another country in which the plague is endemic, antibiotic resistant genes could be spread to the endemic plague population. It may also spread to other bacteria in the same way that it does with *E. coli* [96]. Furthermore, if some *E. coli* carry the plasmid containing the resistance genes from *Y. pestis*, antibiotic resistance may be transferred to other bacteria [93].

The rising level of antibiotic resistance in Madagascar may lead to governmental pressure to conduct research into alternative methods of treatment instead of sole reliance on antibiotic treatments. An example of alternative treatment is via

bacteriophage[97] which is expected to eventually become one of the most effective antibacterial alternatives [98,99]. The bacteriophage method is offers more specific than serum therapy and bacteriocin treatment as the bacteriophages only destroy the targeted pathogenic bacteria [100]. Another advantage of the bacteriophage is that phage mutation is significantly higher than that of bacteria mutation so it responds quickly to phage-resistant bacterial mutants. Bacteriophages are also cheaper to develop than new antibiotic development. In addition, few side-effects from bacteriophage treatment have been reported [100]. Nevertheless, the development of bacteriophage treatment may face some challenges such as toxin encoding amongst phages, a lack of pharmacokinetic data and neutralisation of the phase by the host immune system leading to the failure of the treatment [100].

6. Trade routes and human travel

The routes that support the spread of *Y. pestis* strains from Madagascar to other parts of the world are similar to those of historical trade routes. Today, these trade routes via air and sea are as vast as ever. Wildlife trade can potentially bring zoonotic diseases to different countries [101]. Madagascar has a large amount of exports that go around the world. The main export is vanilla which goes to many different countries with top importers such as France, the USA, Germany, China and Japan [102]. Therefore, it is possible for *Y. pestis* to be moved across these trade routes to other countries. The spread of *Y. pestis* can happen through human travel via tourism if people travel to Madagascar during the seasons of the year when plague is most prevalent. Tourists may get infected and return to their own country before symptoms appear. During the 2017 outbreak in Madagascar, nine countries and overseas territories were listed as priority countries for plague preparedness due to the trade and travel links of the countries to Madagascar. These countries and overseas territories were Comoros, Ethiopia, Kenya, Mauritius, Mozambique, La Réunion (France), Seychelles, South Africa, and Tanzania [38].

7. Economic consequences

The effect of plague is detrimental in countries during and after outbreaks. The consequences of affected locations are deteriorating local businesses and loss of income to the public places that had to be shut down. For instance, the outbreak in Western India in 1994 affected food vendors, restaurants, and public gatherings [103]. This plague was a relatively small with 700 cases of plague. However, due to the fear of plague infection, many restrictions were placed upon air and sea travel and trade of India following recommendations from health authorities. This caused the collapse of trade and the tourism industry which caused an estimated 1.8 billion dollars damage to the economy. The consequence of this was a definite impact on public life and the economy of India at that time [103,104].

8. Concluding remarks

This article discussed the threat of *Yersinia pestis* in the modern world. The plague has the potential to cause many fatalities across a global scale if it is not controlled or used as a weapon. The classification of plague as a re-emerging disease with the high number of cases across Madagascar suggests that *Y. pestis* can cause serious problems to a country if left unchecked or if the environmental conditions are suitable

for it [1]. The threat of antibiotic resistance in the emergence of the two resistant natural stains of *Y. pestis* may become a real problem in the future if other antibiotics or alternative treatments are not discovered [81]. The threat of bioterrorism and bioweapons by way of *Y. pestis* has the potential to cause thousands of deaths in a heavily populated area [80,87]. Exercises in bioterrorism awareness and preparedness should be made available to the general public [105]. Further research is needed to find alternatives to antibiotic treatment to combat the risk of plague. Cost-effective and sustainable preventive and management strategies should be deployed against plague, a re-emerging threat to the modern world.

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REFERENCES

[1] R.C. Ngeleja, L.S. Luboobi, Y. Nkansah-Gyekye, The Effect of Seasonal Weather Variation on the Dynamics of the Plague Disease, International Journal of Mathematics and Mathematical Sciences. 2017 (2017).

[2] M. Achtman, K. Zurth, G. Morelli, G. Torrea, A. Guiyoule, E. Carniel, Yersinia pestis, the cause of plague, is a recently emerged clone of Yersinia pseudotuberculosis, Proceedings of the National Academy of Sciences. 96 (1999) 14043–14048.

[3] J. Parkhill, B. Wren, N. Thomson, R. Titball, M. Holden, M. Prentice, M. Sebaihia, K. James, C. Churcher, K. Mungall, Genome sequence of Yersinia pestis, the causative agent of plague, Nature. 413 (2001) 523.

[4] W. Deng, V. Burland, G. Plunkett III, A. Boutin, G.F. Mayhew, P. Liss, N.T. Perna, D.J. Rose, B. Mau, S. Zhou, Genome sequence of Yersinia pestis KIM, Journal of Bacteriology. 184 (2002) 4601–4611.

[5] Y. Song, Z. Tong, J. Wang, L. Wang, Z. Guo, Y. Han, J. Zhang, D. Pei, D. Zhou, H. Qin, Complete genome sequence of Yersinia pestis strain 91001, an isolate avirulent to humans, DNA Research. 11 (2004) 179–197.

[6] P.S. Chain, P. Hu, S.A. Malfatti, L. Radnedge, F. Larimer, L.M. Vergez, P. Worsham, M.C. Chu, G.L. Andersen, Complete genome sequence of Yersinia pestis strains Antiqua and Nepal516: evidence of gene reduction in an emerging pathogen, Journal of Bacteriology. 188 (2006) 4453–4463.

[7] N.R. Thomson, S. Howard, B.W. Wren, M.T. Holden, L. Crossman, G.L. Challis, C. Churcher, K. Mungall, K. Brooks, T. Chillingworth, The complete genome sequence and comparative genome analysis of the high pathogenicity Yersinia enterocolitica strain 8081, PLoS Genetics. 2 (2006) e206.

[8] V.V. Kutyrev, G.A. Eroshenko, V.L. Motin, N.Y. Nosov, J.M. Krasnov, L.M. Kukleva, K.A. Nikiforov, Z.V. Al'khova, E.G. Oglodin, N.P. Guseva, Phylogeny and Classification of Yersinia pestis through the Lens of Strains from the

Plague Foci of Commonwealth of Independent States, Frontiers in Microbiology. 9 (2018).
[9] F. Sebbane, C.O. Jarrett, D. Gardner, D. Long, B.J. Hinnebusch, Role of the Yersinia pestis plasminogen activator in the incidence of distinct septicemic and bubonic forms of flea-borne plague, Proceedings of the National Academy of Sciences. 103 (2006) 5526–5530.
[10] R.D. Perry, J.D. Fetherston, Yersinia pestis--etiologic agent of plague., Clinical Microbiology Reviews. 10 (1997) 35–66.
[11] C.E. Demeure, O. Dussurget, G.M. Fiol, A.-S. Le Guern, C. Savin, J. Pizarro-Cerdá, Yersinia pestis and plague: an updated view on evolution, virulence determinants, immune subversion, vaccination, and diagnostics, Genes & Immunity. (2019) 1.
[12] N.C. Stenseth, B.B. Atshabar, M. Begon, S.R. Belmain, E. Bertherat, E. Carniel, K.L. Gage, H. Leirs, L. Rahalison, Plague: past, present, and future, PLoS Medicine. 5 (2008) e3.
[13] D. Zhou, Z. Tong, Y. Song, Y. Han, D. Pei, X. Pang, J. Zhai, M. Li, B. Cui, Z. Qi, L. Jin, R. Dai, Z. Du, J. Wang, Z. Guo, J. Wang, P. Huang, R. Yang, Genetics of Metabolic Variations between Yersinia pestis Biovars and the Proposal of a New Biovar, microtus, Journal of Bacteriology. 186 (2004) 5147–5152. doi:10.1128/JB.186.15.5147-5152.2004.
[14] K.L. Gage, M.Y. Kosoy, Natural history of plague: perspectives from more than a century of research, Annu. Rev. Entomol. 50 (2005) 505–528.
[15] R. Yang, Plague: recognition, treatment, and prevention, Journal of Clinical Microbiology. 56 (2018) e01519-17.
[16] K.J. Kugeler, J.E. Staples, A.F. Hinckley, K.L. Gage, P.S. Mead, Epidemiology of human plague in the United States, 1900–2012, Emerging Infectious Diseases. 21 (2015) 16.
[17] W.J. Simpson, R.E. Thomas, T.G. Schwan, Recombinant capsular antigen (fraction 1) from Yersinia pestis induces a protective antibody response in BALB/c mice, The American Journal of Tropical Medicine and Hygiene. 43 (1990) 389–396.
[18] G.E. Benner, G.P. Andrews, W.R. Byrne, S.D. Strachan, A.K. Sample, D.G. Heath, A.M. Friedlander, Immune response to Yersinia outer proteins and other Yersinia pestis antigens after experimental plague infection in mice, Infection and Immunity. 67 (1999) 1922–1928.
[19] S. Simon, C. Demeure, P. Lamourette, S. Filali, M. Plaisance, C. Créminon, H. Volland, E. Carniel, Fast and simple detection of Yersinia pestis applicable to field investigation of plague foci, PloS One. 8 (2013) e54947.
[20] NCEZID, Innovative technologies, (2013). https://www.cdc.gov/ncezid/pdf/innovative-technologies-2013.pdf (accessed May 29, 2019).
[21] L.K. Cao, G.P. Anderson, F.S. Ligler, J. Ezzell, Detection of Yersinia pestis fraction 1 antigen with a fiber optic biosensor., Journal of Clinical Microbiology. 33 (1995) 336–341.
[22] M.H. Meyer, M. Stehr, S. Bhuju, H.-J. Krause, M. Hartmann, P. Miethe, M. Singh, M. Keusgen, Magnetic biosensor for the detection of Yersinia pestis, Journal of Microbiological Methods. 68 (2007) 218–224.
[23] W. Splettstoesser, R. Grunow, L. Rahalison, T. Brooks, S. Chanteau, H. Neubauer, Serodiagnosis of human plague by a combination of

immunomagnetic separation and flow cytometry, Cytometry Part A: The Journal of the International Society for Analytical Cytology. 53 (2003) 88–96.
[24] Z. Yan, L. Zhou, Y. Zhao, J. Wang, L. Huang, K. Hu, H. Liu, H. Wang, Z. Guo, Y. Song, Rapid quantitative detection of Yersinia pestis by lateral-flow immunoassay and up-converting phosphor technology-based biosensor, Sensors and Actuators B: Chemical. 119 (2006) 656–663.
[25] EMERGE, Plague diagnostic recommendations, (2017). https://www.emerge.rki.eu/Emerge/EN/Content/AboutUs/aboutus_node.html;jsessionid=B00297869FA9DD90366A6977710DFB64.1_cid390 (accessed May 29, 2019).
[26] A.P. Anisimov, K.K. Amoako, Treatment of plague: promising alternatives to antibiotics, Journal of Medical Microbiology. 55 (2006) 1461–1475.
[27] J.D. Poland, D.T. Dennis, Treatment of plague, Plague Manual: Epidemiology, Distribution, Surveillance and Control. (1999) 55–62.
[28] CDC, Plague, (2018). https://www.cdc.gov/plague/index.html (accessed February 4, 2019).
[29] A. Yersin, Sur la peste bubonique (sérothérapie), Ann Inst Pasteur. 11 (1897) 81–93.
[30] T. Butler, Plague history: Yersin's discovery of the causative bacterium in 1894 enabled, in the subsequent century, scientific progress in understanding the disease and the development of treatments and vaccines, Clinical Microbiology and Infection. 20 (2014) 202–209. doi:10.1111/1469-0691.12540.
[31] W.C. Summers, Cholera and plague in India: the bacteriophage inquiry of 1927–1936, Journal of the History of Medicine and Allied Sciences. 48 (1993) 275–301.
[32] K.E. Kortright, B.K. Chan, J.L. Koff, P.E. Turner, Phage therapy: a renewed approach to combat antibiotic-resistant bacteria, Cell Host & Microbe. 25 (2019) 219–232.
[33] J. McGeachie, An in vitro comparison of colicines K and V and some therapeutic antibiotics., Zentralblatt Fur Bakteriologie, Parasitenkunde, Infektionskrankheiten Und Hygiene. 215 (1970) 245–51.
[34] C. Damasko, A. Konietzny, H. Kaspar, B. Appel, P. Dersch, E. Strauch, Studies of the efficacy of enterocoliticin, a phage-tail like bacteriocin, as antimicrobial agent against Yersinia enterocolitica serotype O3 in a cell culture system and in mice, Journal of Veterinary Medicine, Series B. 52 (2005) 171–179.
[35] CHP, Scientific Committee on Vector-borne Diseases Situation of Plague and Prevention Strategies, (2008). https://www.chp.gov.hk/files/pdf/diseases-situation_of_plague_and_prevention_strategie_r.pdf (accessed January 4, 2019).
[36] M. Keeling, C. Gilligan, Metapopulation dynamics of bubonic plague, Nature. 407 (2000) 903.
[37] G. Raza, B. Dutt, S. Singh, Kaleidoscoping public understanding of science on hygiene, health and plague: a survey in the aftermath of a plague epidemic in India, Public Understanding of Science. 6 (1997) 247–268.
[38] WHO, Plague outbreak Madagascar External Situation Report 14, (2017). https://apps.who.int/iris/bitstream/handle/10665/259556/Ex-PlagueMadagascar04122017.pdf;jsessionid=A1CDD22A9195FDC35C3FA3960EBBCCE9?sequence=1 (accessed May 4, 2019).

[39] S. Leary, E.D. Williamson, K.F. Griffin, P. Russell, S.M. Eley, R.W. Titball, Active immunization with recombinant V antigen from Yersinia pestis protects mice against plague., Infection and Immunity. 63 (1995) 2854–2858.
[40] X. Yang, B.J. Hinnebusch, T. Trunkle, C.M. Bosio, Z. Suo, M. Tighe, A. Harmsen, T. Becker, K. Crist, N. Walters, Oral vaccination with Salmonella simultaneously expressing Yersinia pestis F1 and V antigens protects against bubonic and pneumonic plague, The Journal of Immunology. 178 (2007) 1059–1067.
[41] S.S. Bubeck, P.H. Dube, Yersinia pestis CO92ΔyopH is a potent live, attenuated plague vaccine, Clin. Vaccine Immunol. 14 (2007) 1235–1238.
[42] R.W. Titball, E.D. Williamson, Yersinia pestis (plague) vaccines, Expert Opinion on Biological Therapy. 4 (2004) 965–973.
[43] J.B. Whitney, R.M. Ruprecht, Live attenuated HIV vaccines: pitfalls and prospects, Current Opinion in Infectious Diseases. 17 (2004) 17–26.
[44] A.S. Lauring, J.O. Jones, R. Andino, Rationalizing the development of live attenuated virus vaccines, Nature Biotechnology. 28 (2010) 573.
[45] V.A. Feodorova, M.J. Corbel, Prospects for new plague vaccines, Expert Review of Vaccines. 8 (2009) 1721–1738.
[46] H.S. Garmory, D. Freeman, K.A. Brown, R.W. Titball, Protection against plague afforded by immunisation with DNA vaccines optimised for expression of the Yersinia pestis V antigen, Vaccine. 22 (2004) 947–957.
[47] S. Wang, D. Heilman, F. Liu, T. Giehl, S. Joshi, X. Huang, T. Chou, J. Goguen, S. Lu, A DNA vaccine producing LcrV antigen in oligomers is effective in protecting mice from lethal mucosal challenge of plague, Vaccine. 22 (2004) 3348–3357.
[48] A. Zauberman, D. Gur, Y. Levy, M. Aftalion, Y. Vagima, A. Tidhar, T. Chitlaru, E. Mamroud, Post-exposure administration of a Yersinia pestis live vaccine potentiates second-line antibiotic treatment against pneumonic plague, The Journal of Infectious Diseases. (2019).
[49] CFSPH, Plague, (2013). http://www.cfsph.iastate.edu/Factsheets/pdfs/plague.pdf (accessed April 23, 2019).
[50] WHO, Plague fact sheet, (2017). https://www.who.int/news-room/fact-sheets/detail/plague (accessed April 4, 2019).
[51] S. Riedel, Plague: from natural disease to bioterrorism, in: Taylor & Francis, 2005: pp. 116–124.
[52] H.F. Hull, J.M. Montes, J.M. Mann, Septicemic plague in New Mexico, Journal of Infectious Diseases. 155 (1987) 113–118.
[53] L.D. Crook, B. Tempest, Plague: a clinical review of 27 cases, Archives of Internal Medicine. 152 (1992) 1253–1256.
[54] F.M. Burkle, Plague as Seen in South Vietnamese Children: A chronicle of observations and treatment under adverse conditions, Clinical Pediatrics. 12 (1973) 291–298.
[55] CDC, Fatal human plague --Arizona and Colorado, 1996., 1997.
[56] A.A.B. Saeed, N.A. Al-Hamdan, R.E. Fontaine, Plague from eating raw camel liver, Emerging Infectious Diseases. 11 (2005) 1456.
[57] A. Christie, T. Chen, S.S. Elberg, Plague in camels and goats: their role in human epidemics, Journal of Infectious Diseases. 141 (1980) 724–726.
[58] A. Arbaji, S. Kharabsheh, S. Al-Azab, M. Al-Kayed, Z. Amr, M. Abu Baker, M. Chu, A 12-case outbreak of pharyngeal plague following the consumption of

camel meat, in north–eastern Jordan, Annals of Tropical Medicine & Parasitology. 99 (2005) 789–793.
[59] E. Bertherat, S. Bekhoucha, S. Chougrani, F. Razik, J.B. Duchemin, L. Houti, L. Deharib, C. Fayolle, B. Makrerougrass, R. Dali-Yahia, Plague reappearance in Algeria after 50 years, 2003, Emerging Infectious Diseases. 13 (2007) 1459.
[60] N. Cabanel, A. Leclercq, V. Chenal-Francisque, B. Annajar, M. Rajerison, S. Bekkhoucha, E. Bertherat, E. Carniel, Plague outbreak in Libya, 2009, unrelated to plague in Algeria, Emerging Infectious Diseases. 19 (2013) 230.
[61] Z. Sagiev, T. Meka-Mechenko, T. Kunitsa, R. Musagalieva, A. Ismailova, M. Kulbaeva, A. Eszhanov, E. Ablaikhanov, S. Umarova, Diseases of Human Plague in 1974-2003 in Kazakhstan, Ekoloji. 28 (2019) 39–48.
[62] B.P. Zietz, H. Dunkelberg, The history of the plague and the research on the causative agent Yersinia pestis, International Journal of Hygiene and Environmental Health. 207 (2004) 165–178.
[63] W.M. Lotfy, Current perspectives on the spread of plague in Africa, Research and Reports in Tropical Medicine. 6 (2015) 21.
[64] Aljazeera, WHO: Deadly plague breaks out on Uganda-Congo border, (2019). https://www.aljazeera.com/news/2019/03/deadly-plague-breaks-uganda-congo-border-190314075949596.html (accessed May 29, 2019).
[65] ECDC, Epidemiological update - Plague in Madagascar, (2017). https://ecdc.europa.eu/en/news-events/epidemiological-update-plague-madagascar (accessed March 4, 2019).
[66] V.K. Nguyen, C. Parra-Rojas, E.A. Hernandez-Vargas, The 2017 plague outbreak in Madagascar: data descriptions and epidemic modelling, Epidemics. 25 (2018) 20–25.
[67] J.D. Forrester, T. Apangu, K. Griffith, S. Acayo, B. Yockey, J. Kaggwa, K.J. Kugeler, M. Schriefer, C. Sexton, C.B. Beard, Patterns of Human Plague in Uganda, 2008–2016, Emerging Infectious Diseases. 23 (2017) 1517.
[68] S. Chanteau, M. Ratsitorahina, L. Rahalison, B. Rasoamanana, F. Chan, P. Boisier, D. Rabeson, J. Roux, Current epidemiology of human plague in Madagascar, Microbes and Infection. 2 (2000) 25–31.
[69] R.M. Pike, S.E. Sulkin, Occupational hazards in microbiology, The Scientific Monthly. 75 (1952) 222–227.
[70] K.A. Sepkowitz, Occupationally acquired infections in health care workers: part I, Annals of Internal Medicine. 125 (1996) 826–834.
[71] L. Hannah Gould, J. Pape, P. Ettestad, K. Griffith, P. Mead, Dog-associated risk factors for human plague, Zoonoses and Public Health. 55 (2008) 448–454.
[72] S.M. Block, The Growing Threat of Biological Weapons: The terrorist threat is very real, and it's about to get worse. Scientists should concern themselves before it's too late, American Scientist. 89 (2001) 28–37.
[73] S. Riedel, Biological warfare and bioterrorism: a historical review, in: Taylor & Francis, 2004: pp. 400–406.
[74] T.J. Johnson, A History of Biological Warfare from 300 BCE to the Present, American Association for Respiratory Care, Irving, TX, 2011. http://www.haadi.ir/Upload/Image/2016/10/Orginal/f8c8c444_e249_404f_abc0_16da4f33acb5.pdf.
[75] J. Guillemin, Crossing the Normative Barrier—Japan's Biological Warfare in China in World War II, in: Biological Threats in the 21st Century: The Politics, People, Science and Historical Roots, World Scientific, 2016: pp. 17–40.

[76] E.M. Eitzen, E.T. Takafuji, Historical overview of biological warfare, Medical Aspects of Chemical and Biological Warfare. (1997) 415–423.
[77] M.G. Kortepeter, G.W. Parker, Potential biological weapons threats., Emerging Infectious Diseases. 5 (1999) 523.
[78] T.V. Inglesby, D.T. Dennis, D.A. Henderson, J.G. Bartlett, M.S. Ascher, E. Eitzen, A.D. Fine, A.M. Friedlander, J. Hauer, J.F. Koerner, Plague as a biological weapon: medical and public health management, Jama. 283 (2000) 2281–2290.
[79] S. Grygorczuk, T. Hermanowska-Szpakowicz, Yersinia pestis as a dangerous biological weapon, Medycyna Pracy. 53 (2002) 343–348.
[80] B.L. Ligon, Plague: a review of its history and potential as a biological weapon, in: Elsevier, 2006: pp. 161–170.
[81] M. Galimand, E. Carniel, P. Courvalin, Resistance of Yersinia pestis to antimicrobial agents, Antimicrobial Agents and Chemotherapy. 50 (2006) 3233–3236.
[82] P. Bossi, A. Tegnell, A. Baka, F. Van Loock, J. Hendriks, A. Werner, H. Maidhof, G. Gouvras, Bichat guidelines for the clinical management of plague and bioterrorism-related plague, Euro Surveill. 9 (2004) 0912–232.
[83] G.F. Gao, For a better world: Biosafety strategies to protect global health, (2019).
[84] G.F. Gao, From “A”IV to “Z”IKV: Attacks from Emerging and Re-emerging Pathogens, Cell. 172 (2018) 1157–1159. doi:10.1016/j.cell.2018.02.025.
[85] R.M. Atlas, The medical threat of biological weapons, Critical Reviews in Microbiology. 24 (1998) 157–168.
[86] R. Smego, J. Frean, H. Koornhof, Yersiniosis I: microbiological and clinicoepidemiological aspects of plague and non-plague Yersinia infections, European Journal of Clinical Microbiology & Infectious Diseases. 18 (1999) 1–15.
[87] M.B. Prentice, L. Rahalison, Plague, The Lancet. 369 (2007) 1196–1207.
[88] A. Guiyoule, G. Gerbaud, C. Buchrieser, M. Galimand, L. Rahalison, S. Chanteau, P. Courvalin, E. Carniel, Transferable plasmid-mediated resistance to streptomycin in a clinical isolate of Yersinia pestis., Emerging Infectious Diseases. 7 (2001) 43.
[89] N. Cabanel, C. Bouchier, M. Rajerison, E. Carniel, Plasmid-mediated doxycycline resistance in a Yersinia pestis strain isolated from a rat, International Journal of Antimicrobial Agents. 51 (2018) 249–254.
[90] P.H. Gilligan, Therapeutic challenges posed by bacterial bioterrorism threats, Current Opinion in Microbiology. 5 (2002) 489–495.
[91] H.C. Neu, The crisis in antibiotic resistance, Science. 257 (1992) 1064–1073.
[92] R.J. Fair, Y. Tor, Antibiotics and bacterial resistance in the 21st century, Perspectives in Medicinal Chemistry. 6 (2014) PMC-S14459.
[93] D.M. Wagner, J. Runberg, A.J. Vogler, J. Lee, E. Driebe, L.B. Price, D.M. Engelthaler, W.F. Fricke, J. Ravel, P. Keim, No resistance plasmid in Yersinia pestis, North America, Emerging Infectious Diseases. 16 (2010) 885.
[94] B. Rasoamanana, P. Coulanges, P. Michel, N. Rasolofonirina, Sensitivity of Yersinia pestis to antibiotics: 277 strains isolated in Madagascar between 1926 and 1989, Archives de l’Institut Pasteur de Madagascar. 56 (1989) 37–53.
[95] H.D. Kalter, R.H. Gilman, L.H. Moulton, A.R. Cullotta, L. Cabrera, B. Velapatiño, Risk factors for antibiotic-resistant Escherichia coli carriage in young children

in Peru: community-based cross-sectional prevalence study, The American Journal of Tropical Medicine and Hygiene. 82 (2010) 879–888.
[96] B.J. Hinnebusch, M. Rosso, T.G. Schwan, E. Carniel, High-frequency conjugative transfer of antibiotic resistance genes to Yersinia pestis in the flea midgut, Molecular Microbiology. 46 (2002) 349–354.
[97] Y. Chen, H. Batra, J. Dong, C. Chen, V.B. Rao, P. Tao, Genetic Engineering of Bacteriophages Against Infectious Diseases, Frontiers in Microbiology. 10 (2019).
[98] A.A. Filippov, K.V. Sergueev, Y. He, X.-Z. Huang, B.T. Gnade, A.J. Mueller, C.M. Fernandez-Prada, M.P. Nikolich, Bacteriophage therapy of experimental bubonic plague in mice, in: Advances in Yersinia Research, Springer, 2012: pp. 337–348.
[99] A.A. Filippov, K.V. Sergueev, M.P. Nikolich, Can phage effectively treat multidrug-resistant plague?, Bacteriophage. 2 (2012) 186–189.
[100] A. Parisien, B. Allain, J. Zhang, R. Mandeville, C. Lan, Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides, Journal of Applied Microbiology. 104 (2008) 1–13.
[101] B.I. Pavlin, L.M. Schloegel, P. Daszak, Risk of importing zoonotic diseases through wildlife trade, United States, Emerging Infectious Diseases. 15 (2009) 1721.
[102] OEC, Madagascar, (2017). https://atlas.media.mit.edu/en/profile/country/mdg/ (accessed March 4, 2019).
[103] N.A. Boire, V.A.A. Riedel, N.M. Parrish, S. Riedel, Lessons learned from historic plague epidemics: the relevance of an ancient disease in modern times, Journal of Ancient Diseases & Preventive Remedies. 2014 (2014).
[104] S. Mangiarotti, Low dimensional chaotic models for the plague epidemic in Bombay (1896–1911), Chaos, Solitons & Fractals. 81 (2015) 184–196.
[105] M. Armstrong, Rehearsing for the plague: citizens, security, and simulation, Canadian Review of American Studies. 42 (2012) 105–120.