DHS SCIENCE AND TECHNOLOGY

Master Question List for *Yersinia pestis* (Plague)

09 May 2024

For comments or questions related to the contents of this document, please contact the DHS S&T Hazard Awareness & Characterization Technology Center at HACTechnologyCenter@hq.dhs.gov.

Science and Technology

DHS Science and Technology Directorate | MOBILIZING INNOVATION FOR A SECURE WORLD

CLEARED FOR PUBLIC RELEASE

The Department of Homeland Security Science and Technology Directorate is committed to providing access to our web pages for individuals with disabilities, both members of the public and federal employees. If the format of any elements or content within this document interferes with your ability to access the information, as defined in the Rehabilitation Act, please contact the Hazard Awareness & Characterization Technology Center for assistance by emailing HACTechnologyCenter@hq.dhs.gov. A member of our team will contact you within 5 business days. To enable us to respond in a manner most helpful to you, please indicate the nature of your accessibility problem, the preferred format in which to receive the material, the web address or name of the document of the material (Master Question List for *Yersinia pestis*) with which you are having difficulty, and your contact information.

Yersinia pestis – Master Question List

Table of Contents

Foreword

The following Master Question List (MQL) was developed by the Department of Homeland Security Science and Technology Directorate (DHS S&T) to present the current state of available information to government decision makers regarding *Yersinia pestis*, the causative agent for Plague. This MQL quickly summarizes what is known and what additional information is needed to address fundamental questions such as, "What is the infectious dose?" and "How long does the virus persist in the environment?" The information provided is a succinct summary to allow structured and scientifically guided discussions across the federal government without burdening them with the need to review scientific reports, and to prevent duplication of efforts by highlighting and coordinating research.

Situation Overview

Yersinia pestis is the bacterium that causes plague. It is a gram-negative, non-motile bacillus or coccobacillus that does not produce spores. *Y. pestis* is related to *Yersinia enterocolitica* and *Yersinia pseudotuberculosis,* which are the only other two human-pathogenic *Yersinia* species.[1] *Y. pestis* is typically transmitted by the bite of an infected flea from rodent hosts, which typically causes bubonic and/or septicemic plague.[2] Airborne transmission is a less common route and may result in pneumonic plague.[2] *Y. pestis* is responsible for the "Black Death" which is estimated to have killed 30% to 40% of the European population between 1,347 and 1,351 BC.[3] The bacteria are endemic to Asia, Africa, and the Americas,[4] with Madagascar and the Democratic Republic of Congo accounting for ~80% and ~15% of cases, respectively.[5] Human cases in the U.S. typically are associated with local rodent populations and occur in remote and rural areas in two regions: the Four Corners area in the Southwest, and at the intersection of California, Nevada, and Oregon.[6] In the U.S. seven human cases are reported each year on average (range: 1-17 cases per year). [6] Worldwide, thousands of cases are reported each year.[7] Actors have considered *Y. pestis* as a biological warfare agent. Notably, in 1995, Larry Wayne Harris, a microbiologist from Ohio, was arrested for obtaining *Y. pestis*.[8] The *Y. pestis* was recovered and Harris was convicted of fraud.[8] The

Japanese army released plague-infected fleas into Chinese populations in the 1930s.[9] Both the United States and the Former Soviet Union studied *Y. pestis* as part of their historical biological weapons programs.[10]

The cutoff date for information gathering related to this document was 03/14/2024.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
BACKGROUND	• *Y. pestis* is the causative agent of plague. It is responsible for the "Black Death" which is estimated to have killed 30% to 40% of the European population between 1,347 and 1,351 BC. • It is a bacterium found in the environment in Asia, Africa, and the Americas. A low number of cases occur in the U.S. each year. • *Y. pestis* is mostly spread by the bite of a flea that has fed on an infected rodent. • Several classes of Food and Drug Administration (FDA)-approved antimicrobial drugs are available for treatment and vaccine candidates are under investigation. Vaccines have been available for use in the past. • *Y. pestis* has been studied by some countries in historical biowarfare programs as an aerosol weapon to cause pneumonic plague.
INFECTIOUS DOSE	• The flea bite or subcutaneous infectious dose, typically causing bubonic plague, is unknown. • The aerosol infectious dose, typically causing pneumonic plague, of *Y. pestis* is estimated to be between 100 and 15,000 organisms in humans.
TRANSMISSIBILITY	• The most common mode of transmission is from the bite of an infected flea. • Person-to-person spread occurs via aerosol transmission when an infected person coughs and through exposure to infected fluids and tissues.
HOST RANGE	• *Y. pestis* is a zoonotic disease affecting over 200 mammalian species. • The most important hosts for *Y. pestis* ecology are rodents, lagomorphs, and fleas. • Domestic cats and dogs have spread *Y. pestis* to people. • Non-human primates and rodents are typically used in vaccine challenge studies.
INCUBATION PERIOD	• The incubation period for plague is one to eight days following infection in humans but can vary. • The incubation period can be one to eight days for the bubonic form, one to four days for the septicemic form, one to six days for the pneumonic form, and (in rare cases) two to four days for the ingestion form.
CLINICAL PRESENTATION	• Bubonic plague is the most common presentation and typically presents with enlarged lymph nodes, fever, malaise, chills, chest pain, and headache.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
	• Pneumonic plague presents with rapid respiratory distress, fever, cough with bloody sputum, and chest pain. • Septicemic plague can present with more vague symptoms of low blood pressure, septic shock, abdominal pain, gastrointestinal symptoms, fever, nausea, vomiting, or diarrhea. It has also been associated with cardiovascular, neurological, and renal complications. • *Y. pestis* infection can also present as meningitis. • Pharyngitis can result if *Y. pestis* is ingested.
CLINICAL DIAGNOSIS	• The gold standard for diagnosis is microbial isolation of the organism from patient samples. • Direct fluorescent antibody, polymerase chain reaction (PCR), serological, and rapid diagnostic tests are available. • *Y. pestis* can be identified microscopically by examination of Gram, Wright, Giemsa, or Wayson's-stained smears of patient samples.
FATALITY RATE	• The World Health Organization (WHO) reported that between 2013 and 2018 the reported case fatality rate was 17.5%. • The Centers for Disease Control and Prevention (CDC) estimates the fatality rate to be 11% for all forms. • Without treatment, pneumonic plague is usually fatal. With treatment the recovery rate can vary from 65% to 83%. • The fatality rate for bubonic plague is 50% to 66% without antimicrobial treatment. With antimicrobial treatment, the fatality rate decreases to 13%. • Septicemic plague is estimated to have an 18% to 55% fatality rate with treatment. Untreated septicemic plague is almost always fatal.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
MEDICAL TREATMENT	• Early diagnosis and treatment is key to successful recovery. • FDA-approved antimicrobials for effective treatment are: streptomycin (aminoglycoside class), ciprofloxacin (fluoroquinolone class), levofloxacin (fluoroquinolone class), moxifloxacin (fluoroquinolone class), and doxycycline and other tetracyclines (tetracycline class). • Gentamicin is a common alternative to streptomycin. • Dual therapy with two different classes of antibiotics is recommended in cases of pneumonic plague and septicemic plague, as well as in case of suspected bioterror event. • Some aminoglycoside classes and all classes of chloramphenicol and trimethoprim-sulfamethoxazole are not FDA approved but they have been shown to be effective options in animal models.
VACCINES	• A formalin killed vaccine was available in the U.S. until 1999. Use was discontinued due to adverse reactions and lack of protection. • A live-attenuated vaccine, EV76, is available in foreign countries. It is not used in the U.S. due to high reactogenicity. • There are several protein subunit, DNA, recombinant, and live-attenuated vaccine candidates in development.
ENVIRONMENTAL STABILITY	• *Y. pestis* persistence in the environment and soil is not well understood. It was shown to persist for 40 weeks in one study. • The stability of *Y. pestis* may be related to temperature and/or relative humidity. • *Y. pestis* growth rate at 25°C was 4-folds faster than the growth rate at 10°C. • At 30°C and 52% relative humidity, *Y. pestis* survived for less than 30 minutes but survived for six hours at 22°C and 52% relative humidity.
DECONTAMINATION	• U.S. Environmental Protection Agency's (EPA's) list of registered disinfectants, List A, contains disinfectants that will destroy or irreversibly inactivate *Y. pestis*. • Other disinfectants have been investigated in the literature but are not necessarily registered disinfectants.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
PERSONAL PROTECTIVE EQUIPMENT (PPE)	• The WHO guidelines for people disposing of deceased plague victims is to wear masks, protective clothing, boots, and thick rubber gloves. Antibiotic post-exposure prophylaxis (PEP) is suggested for those in direct contact. An update to the guidelines has been proposed to make the minimum protective equipment a gown, goggles, an N95 mask, and gloves. • The American Society for Microbiology suggests microbiology laboratories use biosafety level (BSL) 2 practices including a biosafety cabinet as the minimum requirement for safe handling of diagnostic samples. • *Y. pestis* is a biosafety level 3 organism and should be studied under biosafety level 3 practices.
GENOMICS	• The three biovars of *Y. pestis* are grouped according to the ability to reduce nitrate and use glycerol. Antiqua biovars are positive for both abilities. Mediaevalis biovars do not reduce nitrate but are positive for glycerol utilization. Orientalis reduce nitrate but do not utilize glycerol. • Frequent genome rearrangement events cause continuous evolution of *Y. pestis.* • Antimicrobial-resistant strains have been documented in Madagascar, Mongolia, and China. A multi-drug resistant strain was isolated from a patient in Madagascar. Self-transmissible plasmids and ribosomal protein S12 (rpsl) gene mutations were shown to be responsible for the antimicrobial resistance in some cases.

Infectious Dose
How much agent will make a healthy individual ill?

What do we know?

There are two typical routes of transmission: airborne and flea bites. Contact with contaminated fluid and tissue can also result in infection. The infectious dose differs between the different routes. Typically, the median infectious dose (ID_{50}) and median lethal dose (LD_{50}) of *Y. pestis* in humans is measured through enumerations in colony forming units (CFUs) of the bacteria.[11] For ID_{50} and LD_{50}, lower values indicate greater infectivity or greater lethality, as less bacteria or CFUs are needed for infecting or causing mortality in an individual. When ID_{50} and LD_{50} are similar values, this typically indicates a near 100% fatality rate.

The inhalation infectious dose (pneumonic plague) is estimated to be between 100 and 15,000 CFU in humans.[8, 12] The estimated infectious dose is impacted by many variables while controlled laboratory studies may present results that are more precise than would be expected in a real-world scenario.

- For example, in one study the ID_{50} of aerosolized *Y. pestis* CO92 strain in cynomolgus macaques is estimated to be 66 CFUs.[13]
- In another study estimating the $LD_{50}$of aerosolized *Y. pestis* CO92 strain in cynomolgus macaques, the LD_{50} was found to be similar at 24 CFU.[11]

The human infectious dose for flea bites (bubonic and septicemic plague) is unknown.

- In one study the average bacteria load was determined to be $3.16x10^5$ CFU per flea one day after feeding on blood containing $1x10^{10}$ CFU/mL of *Y. pestis*.[14]

The infectious dose for ingestion has been measured in rodents.

- ID_{50} values ranging from $1.1x10^5$ to $1.8x10^7$ CFU were determined from rodents infected with two different strains of *Y. pestis* (Vietnam and Brazil) via the intragastric route.[15]

What do we need to know?

- What is the infectious dose in humans by flea bites or subcutaneous routes?
- Does infectious dose vary by subpopulation (age, immunocompromised) or strain?
- What is the correlation of animal models to human infection and disease? (Additional studies are needed to develop improved animal models.)

Transmissibility
How does it spread from one host to another? How easily is it spread?

What do we know?

In the U.S., transmission to humans primarily occurs from the bite of a flea that has fed on an infected rodent.[2, 16] It has been proposed that as many as 11,000 to 24,000 CFU are regurgitated by the flea into the mammalian host.[17]

- The most commonly identified vector for *Y. pestis* is fleas, but it has been isolated from lice and ticks also.[16] Transmission to mammals was demonstrated from lice to rabbits[18] but not demonstrated with experimentally infected ticks, however.[16, 19]
- Temperature affects the dynamics of *Y. pestis* flea-borne transmission[20-21]; recent evidence suggests temperature could change the efficiency of flea transmission in regions that host endemic plague foci.[20] In one study lower temperatures, 23°C vs 30°C, were associated with increased transmission from fleas to mice. Fleas held at 10°C were able to transmit *Y. pestis*, but the bacterial load and survival of fleas was decreased.[21]

- Domestic cats are very susceptible to *Y. pestis* and have directly infected humans (18 cases) with 28% of patients presenting with primary pneumonic plague.[2, 16]
- Transmission of pneumonic plague from a dog to four people occurred in the U.S. in 2014.[22]
- The basic reproductive number (R_0, the average number of secondary cases per case) for the 2015 bubonic plague outbreak in Zambia was estimated to be 1.75.[23]
- Analysis of historical records from London, United Kingdom suggest that the R_0 and epidemic spread increased between the notable Black Death of 1348 and the Great Plague of 1665, with R_0 values estimated to range from 1.06 to 1.69, respectively.[24]

Person-to-Person transmission:

- Humans and other animals usually get infected by the bite from an infected flea but contact with contaminated fluid or tissues can result in bubonic or septicemic plague and the inhalation of infectious droplets can result in pneumonic plague.[2]
- While primary bubonic and septicemic plague are not contagious,[10] the spread of bacteria within the host during bubonic and septicemic cases can lead to secondary pneumonic plague in 5% to15% of cases.[9, 25-26]
- Pneumonic plague is contagious.[26-27] Person-to-person spread can occur from inhaling aerosolized bacteria when a person with pneumonic plague coughs.[28] The CDC has recommended standard and droplet precautions for 48 hours after the start of antibiotics for infection control.[28]
- A review of surveillance data from 1900 to 2009 estimated R_0 for pneumonic plague in the U.S. to be 1.18.[26] Assessment of data from outbreaks in several countries from 1907 to 1997 resulted in an estimated R_0 of 1.3.[29]
- The basic reproductive number for an outbreak of 14 people with pneumonic plague in Madagascar in 2015 was found to be 1.44.[30] Traditional burial practices and poor healthcare systems were implicated in promoting the outbreak.[30] The R_0 for a 2017 outbreak in Madagascar has been estimated from 1.12 to 1.73.[31]

What do we need to know?

- Are there any correlates between the development of secondary pneumonic plague from either bubonic or septicemic plague?
- How many organisms are shed by a coughing person?

Host Range
How many species does it infect? Can it transfer from species to species?

What do we know?

The most important hosts for disease spreading are enzootic rodents. Although some are moderately resistant to infection, their low mortality rate is linked to transmission because they survive as reservoirs which infect more fleas and continue the cycle.[16]

- Over 200 rodents, lagomorphs and mammalian species have been reported to be infected with *Y. pestis.*[16, 32] Specifically, voles,[16] gerbils,[16] marmots,[16] tarabagans,[16] prairie dogs,[10, 16] squirrels,[10, 16] rats,[10, 16] rabbits, [16] goats, [16] camels, [16] dogs,[22] cats,[16] mice,[16] cynomolgus macaques,[11] rhesus macaques,[11] African green monkeys,[11, 33] and humans[16] can show signs of infection. 80 subspecies of fleas have been connected to

plague epidemiology.[32, 34]

- Climate change, deforestation, and urbanization are all environmental factors that can induce a change in the flea and rodent populations leading to an emergence of new vectors, reservoirs, and new genotypes for *Y. pestis.*[35-36]
- Researchers built a machine learning detection model that determined the combination of a changing climate and rodent communities establishing at higher elevations increases the likelihood of creating a *Y. pestis* reservoir in these areas. In some areas, the likeliness of this event increased up to 40%.[37]
- Cynomolgus macaques and African green monkeys have been shown to be good animal models for pneumonic plague in humans as they show similar symptoms and disease pathology.[11, 33]
- Domestic dogs and cats develop disease and have been shown to directly transmit *Y. pestis* to humans.[2, 22]

What do we need to know?

- Which animal hosts (including new animal reservoirs) are capable of harboring disease and what is the ease of transmission?
- How rapidly will climate change alter the endemic range? Further research is needed to fully understand the impact of ongoing climate changes on plague ecology.[35-37]

Incubation Period
How long after infection do symptoms appear? Are people infectious during this time?

What do we know?

The typical incubation period is one to eight days following infection.[7]

Bubonic: one to eight days[10, 16, 38-39]
Septicemic: likely days, but is not well defined[7]

- A minority of patients bitten by a flea develop primary septicemic plague without the development of bubos.[10] Secondary septicemic and pneumonic plague can develop from bubonic plague.[7, 10]

Pneumonic: one to six days[7, 16, 40-41]

- Patients with pneumonic plague typically experience symptoms within one to three days of infection.[16, 26, 42] During the initial infection of the respiratory system, usually around 24 hours, patients are noninfectious.[43] However, once the disease progresses, patients with the pneumonic form can spread *Y. pestis* via respiratory transmission.[25]

Ingestion (rare): two to four days[44-45]

- Two individuals consumed contaminated meat and reported symptoms one to four days after exposure.[46]

The incubation period reported from a 2013 outbreak of pneumonic plague that was a streptomycin resistant *Y. pestis* strain in Madagascar ranged from one to seven days.[47]

What do we need to know?

- What is the degree of infectivity of the host after the onset of pneumonic plague?
- How long does the progression to secondary pneumonic plague typically take?
- What precautions are taken to prevent the spread of pneumonic plague in healthcare?

Clinical Presentation
What are the signs and symptoms of the infected person?

What do we know?

Most cases of *Y. pestis* are divided into three clinical presentations – bubonic plague, pneumonic plague, and septicemic plague. While bubonic plague presents with characteristic buboes, primary septicemic and pneumonic plague can develop without the presence of buboes and can be fatal without early antibiotic treatment. Uncommonly, plague can present itself as meningitis or pharyngitis (ingestion route of infection).[44, 48-49]

Bubonic plague is the most common clinical presentation of plague which is categorized by the painful swelling of lymph nodes (bubos).[26]

- Following an infected flea bite of infection through a skin lesion, *Y. pestis* manifests and reproduces in lymph nodes and can progress into secondary pneumonic, or septicemic plague.[11, 25-26, 42, 50-51]
- Bubos range from 1 to 10 cm as the bacteria multiply and cause necrosis in the lymph node.[25] Symptoms of bubonic plague include fever, malaise, chills, chest pain, and headache.[18, 31, 37, 47-49]

Primary pneumonic plague can occur following inhalation of *Y. pestis* from an infected animal or human and leads to rapid acute respiratory distress.[26]

- Initial infection of the respiratory system can present with rapid pulse and fever, and bacteria are generally undetectable in the sputum for the first 24 hours.[43]
- The disease rapidly progresses following bacterial replication, resulting in severe edema in the lungs, necrotizing pneumonia, and an infectious cough.[43] Clinical presentation includes fever, chest pain, dyspnea, and coughing with bloody sputum.[25, 42-43] If treatment is not initiated within 24 hours of symptom onset, pneumonic plague is almost always fatal.[7, 11, 26, 50, 52]

Septicemic plague, occurring in a minority of cases, is classified by a *Y. pestis* infection in the bloodstream without noticeable lymphadenopathic, pneumonic, or other localizing signs.[25-26, 42]

- Clinical presentation includes low blood pressure, septic shock, abdominal pain, gastrointestinal symptoms, fever, nausea, vomiting, or diarrhea.[17, 18, 37, 43] Septicemic plague is associated with cardiovascular, neurological, and renal complications.[42] Diagnosis is often delayed in primary septicemic plague as the clinical presentation can be non-specific. This results in a higher fatality rate than bubonic plague.[26]

What do we need to know?

- What steps are taken to distinguish or rule out septicemic or pneumonic plague from similarly presenting diseases?
- Given *Y. pestis* is difficult to detect in the first 24 hours of infection, what diagnostic tools can be used to identify the disease upon initial infection?

Clinical Diagnosis
Are there tools to diagnose infected individuals? When during infection are they effective?

What do we know?

Diagnosis is made by isolating the organism from blood, pus from a bubo, or sputum.[53] It is recommended that diagnostic testing be performed at a minimum in BSL-2 laboratories using

a biosafety cabinet.[54] In the U.S., local and state health departments should be notified immediately if plague is suspected.[55]

- Among the biological diagnostic tests, microbial isolation of *Y. pestis* remains the gold standard. Using selective agar supplemented with cefsulodin–irgasan–novobiocin (CIN) favors the isolation of the bacterium in polymicrobial samples such as sputum.[56] After two- or three-day incubation at 28 °C, suspected colonies on CIN agar may be identified by biochemical tests, PCR, and *Y. pestis*-specific phage lysis.[56]
- In cases where *Y. pestis* is not culturable, lymphoid, spleen, lung, liver tissue, or bone marrow samples may yield evidence of *Y. pestis* by direct detection methods such as direct fluorescent antibody or PCR.[55] Conventional PCR targeting the pla, caf1, inv, and yopM genes reduces the delay of diagnostics to three to four hours, while real-time PCR can be performed in only two hours.[56]
- Sputum must be tested by multiplex real-time PCR, targeting additional genes due to the presence of additional microbial flora.[56-57]
- *Y. pestis* may be identified microscopically by examination of Gram, Wright, Giemsa, or Wayson's-stained smears of peripheral blood, lymph node specimen, or sputum.[55] (*Y. pestis* is gram-negative.)
- Automated biochemical identification systems and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identification systems may misidentify the cultured organism.[55]
- If cultures are negative and plague is still suspected, serologic testing is possible to confirm the diagnosis. One serum specimen should be taken as early in the illness as possible, followed by a convalescent sample four to six weeks after disease onset.[55]

Pneumonic Plague

- Diagnosis of pneumonic plague relies on the quality of the sample. Pneumonic plague is a lower respiratory tract infection, so deep respiratory secretions are required for biological tests, not saliva or spit.[56] When using the rapid diagnostic test,[58] false-negative and false-positive results may be observed with sticky expectorations, due to absence or incomplete sample migration along the dipstick. False-negative results may also occur with saliva.[56, 59-60]
- Clinically pneumonic plague-suspected patients must be treated without considering the results from rapid diagnostic testing[58] based on the detection of the F1 antigen of *Y. pestis.*[56]

Bubonic Plague

- In contrast to pneumonic plague, rapid diagnostic testing[58] is a practical method that can be implemented providing results within 15 minutes and has been validated for diagnosis of bubonic plague.[56, 61]

Antimicrobial Resistant Strains

- Microbial susceptibility testing including disk diffusion, E test (test strip impregnated with antimicrobials is added to agar plate)[62], and broth microdilution are used to determine if isolates are susceptible or resistant to one or more antimicrobial drugs.[63] The broth microdilution method is the Clinical and Laboratory Standards Institute referenced method.[63] An isolate is considered resistant to an antimicrobial if it grows in the presence

of that antimicrobial and susceptible if it does not.

- Susceptibility testing can take several days to complete[63] and may not be known in time to start or alter effective treatment.[47]

What do we need to know?

- How quickly does a patient become PCR positive after exposure?
- In resource-limited settings, how can tests detect without requiring isolation or amplification be improved?
- How can the antimicrobial resistance testing timeline be shortened?

Fatality Rate
How likely is it that some individuals will die from *Y. pestis*?

What do we know?

In the pre-antibiotic era (1900 through 1941), mortality among those infected with *Y. pestis* in the U.S. was 66%-93% for all forms of plague.[26] Antibiotics greatly reduced mortality, and by 1990-2010 the overall mortality had decreased to 11%.[7]

- Plague is estimated to have killed 30% to 40% of the European population from the 14th century to the 19th century, including the Black Death period from 1347 to 1351 BC.[3]
- Between 2013 and 2018, 2,886 cases and 504 deaths were reported to the WHO (a fatality rate of 17.5%).[3, 27] 95% of cases derive from sub-Saharan Africa, mainly in Madagascar and the Ituri region of the Democratic Republic of Congo.[3, 27]
- From 1998 to 2016, the case fatality rate was 18% in Madagascar for confirmed and probable cases (15% for the bubonic form and 25% for the pneumonic form).[64]
- It was reported that the case fatality rate for all forms of plague in humans in Yunnan Province, China, was 18.39% between 1950 and 2020.[65]

The fatality rate of bubonic plague is 50% to 66% without antimicrobial treatment. With antimicrobial treatment, the fatality rate decreases to 13%.[11, 26, 42, 66] Septicemic plague can have a higher fatality rate than bubonic and is estimated to be 18% to 55% with treatment.[67-68]

- The fatality rate is estimated to be higher for septicemic plague than bubonic plague due to delayed diagnosis and treatment.[7, 67] Untreated septicemic plague is almost always fatal; however, the overall rate is not well defined because it is not typically diagnosed outside of resourced areas where treatment is available.[26, 67]
- In Madagascar the mortality rate of bubonic plague can be as high as 40% to 70% without treatment.[69]

Pneumonic plague is of particular concern as it is fatal if antibiotic treatment is delayed.[40, 53, 70] The time-course of the disease from the start of symptoms to death when untreated is two to nine days.[71]

- Pneumonic plague, if not diagnosed and treated early, is fatal. [53] However, recovery rates are high if detected and treated in time.[26, 53] A review study published that 82% of patients treated with tetracycline, 83% of patients treated with aminoglycosides, and 82% of patients treated with fluoroquinolones, either alone or in combination with other antimicrobials, survived.[26, 68] Patients who received chloramphenicol and sulfonamides, alone or in combination with other antimicrobials, survived in 78% and 65% of cases,

respectively.[68]

The time to death reported from a case study of two individuals who consumed contaminated meat was between three and nine days.[46]

- Two people consumed raw contaminated meat over the course of four days. The first individual died three to six days after exposure at which point the second individual began treatment with ciprofloxacin. The second individual died three days later.[46]

Antimicrobial Resistant Strains

- Antimicrobial susceptibility testing may not be known when treatment needs to be initiated and may lead to treatment with ineffective antimicrobial drugs and potentially preventable loss of life.[47]
- The probable index case for the 2013 Madagascar outbreak of a streptomycin resistant strain reported a time between symptom onset and death of seven days.[47]

What do we need to know?

- Are there demographic subpopulations which are more likely to have fatal outcomes, and if so, which groups are they?
- Do previous or existing medical issues increase the likelihood of *Y. pestis* mortality?

Medical Treatment
Are there effective treatments?

What do we know?

The current FDA-approved antimicrobials for effective treatment include: streptomycin (aminoglycoside class), ciprofloxacin (fluoroquinolone class), levofloxacin (fluoroquinolone class), moxifloxacin (fluoroquinolone class), and doxycycline and other tetracyclines (tetracycline class).[26, 72]

- Treatment should commence during the first 24 hours after the onset of symptoms for best results.[73-74] The effective dose, duration, and administration route for adults, children, and pregnant women with pneumonic or bubonic plague for each antimicrobial is posted by the CDC.[55] The same treatments that are recommended for adults are recommended for elderly and immunocompromised populations.[26]
- Dual therapy with two different classes of antibiotics is recommended in cases of pneumonic and septicemic plague.[26]
- Streptomycin has historically been the most effective, but availability may be limited. Gentamicin is a common alternative aminoglycoside.[75-77]
- Ciprofloxacin and levofloxacin are acceptable substitutes if aminoglycosides are unavailable.[1, 26, 33, 76, 78]
- Doxycycline is as effective as gentamicin, can be given orally, lacks nephrotoxicity, and there is no need to monitor blood concentrations.[67] Oral doxycycline and ciprofloxacin would be most practical in a mass outbreak setting.[25, 79]
- If a bioterrorism event with *Y. pestis* was suspected, health care providers would be advised to treat patients with two distinct classes of antimicrobials until susceptibility profiles were known due to multi-drug resistance concerns.[26]

Other Antimicrobials

- Some classes of aminoglycosides and all classes of chloramphenicol and trimethoprim-sulfamethoxazole are not FDA approved for plague; however, they are effective options based on prior clinical experience and animal data.[26]
- Amphenicols and sulfonamides are other classes of antibiotics that have demonstrated success in treating patients with plague.[26, 80]
- Other classes of antimicrobials are minimally effective or ineffective in treating plague.[80] Penicillins, cephalosporins, macrolides, and vancomycin antibiotics have been unsuccessful at effectively treating plague patients.[80]

Alternative Treatments

- A recent *in vivo* study found a combination phage therapy and second-line ceftriaxone treatment led to the increased survival of all infected animals.[81]
- A recent study found 17 FDA-approved drugs, not classified as antibiotics, to be effective in preventing cytotoxicity from *Y. pestis* infection. Of the 17, three drugs, doxapram (DXP), amoxapine (AXPN), and trifluoperazine (TFP), increased animal survivability in a murine model of pneumonic plague.[82]
- Molecules that inhibit uridine diphosphate-3-O-(R-3-hydroxymyristoyl)-N-acetyl-D-glucosamine deacetylase (LpxC), the enzyme that catalyzes the first step in lipid A biosynthesis, are being studied as novel antibiotics.[5, 83]
- Other proposed treatments include: cationic antimicrobial peptides,[84] drugs that target the type three secretion system,[85] drugs structurally similar to Yersiniabactin (allows for iron uptake),[86] predatory bacteria,[87] monoclonal antibodies,[88] molecules targeting host mechanisms and channels,[82] anti-inflammatories,[89] and compounds that have been found to inhibit adhesion to respiratory cell lines.[5, 90]

Prophylaxis

- In situations where standard and droplet precautions cannot be sustained, pre-exposure prophylaxis might be warranted if sufficient supplies of antimicrobials are available. Currently there is no data on the duration or efficacy of pre-exposure prophylaxis.[26]
- Any persons not wearing sufficient personal protective equipment (PPE) who had sustained contact with a plague patient, infectious materials, or infected animals should seek post exposure prophylaxis treatment.[26]
- Post exposure prophylaxis treatment should include an effective antimicrobial regime of at least seven days.[26, 91]

What do we need to know?

- Is pre-exposure prophylaxis with antimicrobials effective at preventing the spread of pneumonic plague?

Vaccines
Are there effective vaccines?

What do we know?

Human Vaccines:

- A formalin-killed whole-cell vaccine (KWCV) was available in the U.S. until 1999[10] but was removed from use for three reasons: 1) its lack of protection against pneumonic

plague[10, 92] (attributed to lack of the V antigen in the KWCV formulations[93]); 2) caused significant adverse reactions;[94] and 3) it required frequent boosting which is associated with the adverse reactions.[95]

- EV76, a live-attenuated strain, was created by the Soviet Union in 1936 and is still used in some countries.[96-98] It is not used in the U.S. due to high reactogenicity.[96]

Clinical Trials for *Y. pestis* Vaccines:

- Phase II clinical trials conducted with combination F1+recombinant V (F1 antigen, and virulence antigen) vaccine doses showed 100% seroconversion rates of F1 antibodies and no serious adverse events related to the vaccine were observed.[99]

***Y. pestis* vaccines in the research and development phase:**

- Several protein subunit, DNA, recombinant, and live-attenuated vaccine candidates are in development.[96] Many are being tested in mice with some vaccine trials being performed in cynomolgus macaques[11, 13] with a couple in African green monkey,[13, 92] and Rhesus macaques.[13]
- Live attenuated strains of *Y. pestis* or related organisms that contain both F1 and V antigens show protection against both bubonic and pneumonic plague in mice. [100-102]
- *Y. pestis* CO92 delta yopH is a potent live, attenuated plague vaccine eliciting protection from pneumonic and bubonic plague in mice with 100% survival following intranasal and subcutaneous challenge with 10^7 CFU and 10^5 CFU, respectively.[101]
- An attenuated strain of *Y. pseudotuberculosis* was engineered to produce *Y. pestis* F1 antigen and was able to give full protection to mice with a single dose taken orally.[97]
- Subunit vaccines that contain F1 and V antigens have shown strong protection either in combination (F1+V) or genetically fused (F1-V).[93]
- A modified V antigen, LcrV, DNA vaccine with a human tissue plasminogen activator signal sequence was shown to elicit a V-specific antibody response in Balb/C mice and led to protection against lethal intranasal challenge of *Y. pestis*.[103]
  - Antibody-mediated and cell-mediated immune responses are required to achieve full protection. CD8+ T cell epitopes in the LcrV protein were identified and demonstrated to be protective.[104]

What do we need to know?

- Which vaccine provides the best protection and carries the least health burden on the patient?

Environmental Stability
How long does the agent live in the environment?

What do we know?

***Y. pestis* persistence in the environment is poorly understood.[35, 37, 105]**

- One study found *Y. pestis* could remain viable and virulent after 40 weeks in soil.[105-106] The mechanism for how it stays viable in the soil is unclear but could be associated with the saprophytic phase, which would allow *Y. pestis* to survive between epizootics.[105]
- Another possibility suggests *Y. pestis* could act as an endosymbiont with amoebas, thus surviving in the soil where it has the possibility of reinfecting burrowing rodents.[37]

- Infected live rodents and carcasses could provide a reservoir for *Y. pestis* in soils.[35] However, in a study to determine the infectivity of *Y. pestis*-contaminated soil, researchers exposed 104 mice to "burrows" contaminated with highly bacteremic blood (> 10^8 CFU/mL). The mice had an artificial open wound to increase the likelihood of *Y. pestis* infection. Of the 104 animals, only one became infected with *Y. pestis*. Therefore, although it may be possible for *Y. pestis* to infect from soil, it's not likely a major transmission route.[107]

Y. pestis survival in soils suggests a correlation to soil chemistry.[35, 106]

- In a controlled study spanning over four decades, researchers investigated the correlation between trace metals naturally found in soils and plague plots. They determined that concentrations of trace metals in plague plots were up to 20-fold higher or lower than the control plots depending on the metal. Plague was positively associated with manganese and cobalt, while negatively associated with copper, zinc, and molybdenum.[35]
- Another study determined that salts in the soil are one of the factors contributing to the continuation of the plague foci in North Africa and Eurasia. Researchers found that plague foci were concentrated along salt "chotts" around the edges of salt lakes in North Africa. Since very few pathogenic bacteria are halotolerant, the salt tolerance of *Y. pestis* may play a role in maintaining the North African plague foci.[106]

The stability of _Y. pestis_ may be related to temperature and/or relative humidity.[108-109]

- Temperature should be considered a relative parameter when assessing plague outbreaks.[110] Outbreaks have been shown to follow a distinct seasonal and temperature pattern.[32] In the Northern Hemisphere, consistent data demonstrate a temperature-related correlation to the epidemic peak timing and growth rates of plague. The expected outbreak growth rates were positive between 11.7°C and 21.5°C with a maximum around 17.3°C.[110]
- *Y. pestis* can survive and persist in low temperature tap water by entering a viable but non-culturable state. Researchers determined *Y. pestis* became non-culturable after 21 days in low-temperature water but still maintained viable cells.[111]
- One study tested the survivability of conditionally virulent pYV-bearing *Y. pestis* KIM5 on raw ground beef.[108]
  - At 0°C and 4°C, *Y. pestis* KIM5 did not grow but maintained viability on the meat surface.[108]
  - At 10°C and 25°C, *Y. pestis* KIM5 grew to maximum population densities of 8.65, 8.30, and 8.43 $\log_{10}$ CFU/g. The growth rate at 25°C was four-folds faster than the growth rate at 10°C.[108]
  - Raw beef contaminated with *Y. pestis* could cause oro-pharyngeal plague if exposed to temperatures between 10°C and 25°C and not cooked properly.[108]
- Another study found airborne suspensions of *Y. pestis* at 30°C on stainless steel surfaces, survived more than three days at 11% relative humidity but less than two days at 100% relative humidity.[109] Slow drying occurs at high relative humidity and can distort the membrane protein structure.[112]
- *Y. pestis* survived for less than 30 minutes at 30°C and 52% relative humidity, but survived for at least six hours at 22°C and 52% relative humidity.[109]

- *Y. pestis* was shown to persist on paper at 55% relative humidity for five days.[112]

What do we need to know?

- How long is *Y. pestis* viable in samples from patients?
- What is the stability of *Y. pestis* on various common surfaces and food items?

Decontamination
What are effective methods to kill the agent in the environment?

What do we know?

The U.S. EPA's list of registered disinfectants, from both List H and List K,[113-115] can be used against *Y. pestis* on hard, non-porous surfaces. Disinfectants are defined as substances that will destroy or irreversibly inactivate bacteria, viruses, or fungi, but not necessarily bacterial spores. [113]

- The EPA also lists ethylene oxide sterilization, gamma irradiation, and electron beam technologies, and Ultraviolet-C (UVC) light produced with a mercury bulb as possible decontamination methods for items that are sensitive or irreplaceable.[113]

Other literature reviewed for disinfection, inactivation, or decontamination potential on different surfaces include:

- pH-adjusted bleach was made by adding 9.4 parts of sterile water to one part Ultra Clorox® bleach and one part 5% acetic acid.[116] 100 µL of *Y. pestis* CO92 strain at 1 × 10^9 CFU/mL was inactivated in 15 minutes with pH-adjusted bleach on aluminum, glass, and wood.[116]
- Ethanol (70%) inactivated 100 µL of *Y. pestis* CO92 strain at 1 × 10^9 CFU/mL after 15 minutes at room temperature on aluminum and glass.[116] Ethanol (70%) inactivated *Y. pestis* at a lower temperature (10-13°C) after 15 minutes on wood, carpet, and concrete.[116]
- 1 × 10^8 CFU of *Y. pestis* CO92 strain was inactivated by quaternary ammonia after 15 minutes at 10°C -13°C on aluminum, glass, wood, and concrete.[116]
- 1 × 10^8 CFU of *Y. pestis* CO92 strain was inactivated by Pine-sol® after 15 minutes at 10°C -13°C on aluminum, glass, wood, carpet, and concrete.[116]
- A phage cocktail, YPP-100, containing five phages at 10^7 plaque forming units (PFU)/mL or higher decontaminated *Y. pestis* (strains CO92, KIM and 1670G at 1E9) on contaminated glass, gypsum board, and stainless steel in five minutes.[117]
- Hydrogen peroxide vapor fumigation with a two-hour contact time was effective to decontaminate 1.7 × 10^8 CFU of *Y. pestis* CO92.[118]

What do we need to know?

- What is the minimum inhibitory concentration of decontaminating agent and duration of required contact time in samples?

Personal Protective Equipment (PPE)
What PPE is effective and who should be using it?

What do we know?

The 2009 WHO guidelines for people disposing of deceased plague victims are to wear masks, protective clothing, boots, and thick rubber gloves.[4] Antibiotic chemoprophylaxis is suggested for

those in direct contact.[4] An update to the guidelines has been proposed to make the minimum protective equipment a gown, goggles, an N95 mask, and gloves.[4]

- *Y. pestis* is a risk group 3 pathogen and should be handled in BSL 3 laboratories using BSL 3 practices.[119] Laboratory workers should wear protective clothing with a solid front, such as wrap-around gowns, scrub suits, or coveralls, eye and/or face protection, two pairs of gloves, respiratory protection, and shoe covers.[120]
- When performing diagnostic testing of suspected *Y. pestis*-contaminated samples, the American Society for Microbiology suggests microbiology laboratories use BSL 2 practices including a biosafety cabinet as the minimum requirement for safe handling.[54]

What do we need to know?

- What additional precautions are required for the immunosuppressed and populations that may have prolonged contact with an infected individual?

Genomics
How does the disease agent compare to previous strains?

What do we know?

***Y. pestis* has historically been grouped into three biovars according to the ability to reduce nitrate and use glycerol. Antiqua biovars are positive for both abilities. Mediaevalis biovars (CO92 strain) do not reduce nitrate but are positive for glycerol utilization. Orientalis biovars (KIM strain) reduce nitrate but do not utilize glycerol.[121]** Loss of metabolic function created the Mediaevalis and Orientalis biovars from the Antiqua biovar.[121] Orientalis biovars have a deletion in the glpD gene and mediaevalis biovars are associated with two mutations in the napA gene.[121]

- Whole genome sequencing techniques have led to the classification of a five branch population structure: biovar Antiqua, Medievalis, Orientalis, Intermediate, and Pestoides, including Microtus isolates.[56, 122]
- The evolution of *Y. pestis* from *Y. pseudotuberculosis* is associated with the acquisition of plasmids pFra and pPla, which encode virulence factors, and gene loss which impacted adaptation to flea vectors.[56]
- *Yersinia* outer membrane proteins (Yops) have been shown to contain a variety of functions. They down regulate the production of pro-inflammatory cytokines, inhibit Rho GTPases, disrupt the actin cytoskeleton in order to inhibit phagocytosis, and induce cell death by multiple mechanisms.[56]
- *Y. pestis* contains a plasmid, pYV/pCD1, which encodes a type 3 secretion system that helps directly translocate Yops effectors.[56]
- *Y. pestis* subverts the human immune system using the broad-range protease, Pla, an absence of pathogen-associated molecular patterns, and iron capture systems. These virulence determinants can allow unrestricted bacterial replication in lymph nodes and in lungs.[56]
- The capsular antigen, F1, is a target for some diagnostic testing.[56]
- *Y. pestis* has been noted to undergo frequent intra-genomic recombination and have an abnormally fluid genome.[121] Frequent genome rearrangement events cause continuous evolution of *Y. pestis*.[121] Through horizontal gene transfer *Y. pestis* has been able to gain genes and cross species barriers.[121, 123] *Y. pestis* has also evolved to lose genes for better adaptation to flea vectors.[124] Parallel evolution of *Y. pestis* genes may help it

survive in fluctuating environments.[125]

- Plasmids,[123] genomic islands,[123] and transposons[124] play a key role in virulence adaptation.[123]

Antimicrobial Resistance

- Five *Y. pestis* strains with unique antimicrobial resistance profiles have been isolated in Madagascar.[47, 124] Another streptomycin-resistant isolate was identified in China.[124, 126]
- A multi-drug resistant isolate from a patient in Madagascar was shown to contain a self-transmissible plasmid, pIP1202, which was responsible for antimicrobial resistance.[127] This plasmid confers resistance to first-line antimicrobials including: streptomycin, tetracycline, chloramphenicol, and sulfonamides.[127-128] *Y. pestis* isolates with this plasmid produce proteins that inactivate antimicrobial drugs.[124]
- The plasmids pIP1203 (self-transferable) and pIP2180H (mobile plasmid), found in separate antimicrobial resistant isolates, encode resistance to streptomycin and doxycycline, respectively.[124, 129-130] *Y. pestis* isolates with the pIP1203 plasmid encode genes for aminoglycoside 3"-O-phosphotransferase and a 6-O-phosphotransferase.[129] The pIP2180H plasmid was found to produce a tetracycline efflux protein and carry regulators for the protein.[130]
- Ribosomal protein S12 (*rpsl*) gene mutations were found to be responsible for the streptomycin resistance of two isolates recovered from Madagascar and one from China.[124] Ribosomal mutations were hypothesized to disrupt the binding of streptomycin to ribosomal RNA binding sites.[124, 126]
- Other antimicrobial resistant isolates have been identified, but the genetic basis or transferability was not known.[124]
- An isolate resistant to gentamicin, tetracycline, doxycycline, trimethoprim-sulfamethoxazole, and chloramphenicol was identified in a Mongolian marmot.[124]
- In Madagascar a tetracycline-resistant isolate and an ampicillin-resistant isolate were identified in a rat and a flea, respectively.[124, 131]

What do we need to know?

- Are there changes due to genomic destabilization and gene loss that may pose a potential threat for accelerated adaptation to humans?
- Could further adaptation to humans occur through gene gain or through nucleotide changes resulting in increased virulence or transmission?

Commonly Used Acronyms and Abbreviations

Acronym/Term	Definition	Description
BSL	Biosafety Level	Level of safety practices and engineering features used to contain pathogenic microorganisms in a laboratory setting
CDC	Centers for Disease Control and Prevention	N/A
CFU	Colony Forming Unit	A unit that estimates the number of microbial cells in a sample that are viable by visualizing growth of a colony of microbes on an agar plate
DHS S&T	U.S. Department of Homeland Security Science and Technology Directorate	N/A
EPA	U.S. Environmental Protection Agency	N/A
FDA	U.S. Food and Drug Administration	N/A
ID_{50}	Median Infectious Dose	The dose required to infect 50% of the population
KWCV	Killed Whole-Cell Vaccine	N/A
LD_{50}	Median Lethal Dose	Dose required to cause a lethal effect in 50% of subjects
MQL	Master Question List	N/A
PCR	Polymerase Chain Reaction	Assay used to determine the number of RNA or DNA molecules representing a specific sequence target are present in a sample
PFU	Plaque Forming Unit	Unit representing a single infectious viral particle derived from viral quantification via plaque assay
PPE	Personal Protective Equipment	Equipment intended to protect individuals against hazardous environments
R_0	Basic Reproductive Number	Average number of new infections that each case is expected to generate in a population where all individuals are susceptible to infection
UVC	Short-Wave Ultraviolet Light	Light with wavelength in the 100-280 nm range
WHO	World Health Organization	N/A
Yops	*Yersinia* outer membrane proteins	N/A

References

1. Barbieri, R.; Signoli, M.; Cheve, D., et al., Yersinia Pestis: The Natural History Of Plague. *Clin Microbiol Rev* **2020,** *34* (1). https://www.ncbi.nlm.nih.gov/pubmed/33298527

2. Centers for Disease Control and Prevention, Ecology And Transmission. https://www.cdc.gov/plague/transmission/index.html (accessed March 4, 2024).

3. Valles, X.; Stenseth, N. C.; Demeure, C., et al., Human Plague: An Old Scourge That Needs New Answers. *PLoS Negl Trop Dis* **2020,** *14* (8), e0008251. https://www.ncbi.nlm.nih.gov/pubmed/32853251

4. World Health Organization, Who Guidelines Approved By The Guidelines Review Committee. https://www.ncbi.nlm.nih.gov/pubmed/34125504 (accessed March 6 2024).

5. Sebbane, F.; Lemaitre, N., Antibiotic Therapy Of Plague: A Review. *Biomolecules* **2021,** *11* (5). https://www.ncbi.nlm.nih.gov/pubmed/34065940

6. Centers for Disease Control and Prevention, Maps And Statistics. https://www.cdc.gov/plague/maps/index.html (accessed March 4, 2024).

7. Centers for Disease Control and Prevention, Frequently Asked Questions. https://www.cdc.gov/plague/faq/index.html (accessed March 3, 2024).

8. Carus, S. W., *Bioterrorism And Biocrimes:The Illicit Use Of Biological Agents Since 1900* Center for Counterproliferation Research: Washington, D.C., (February 2001 Revision. https://irp.fas.org/threat/cbw/carus.pdf

9. Feodorova, V. A.; Corbel, M. J., Prospects For New Plague Vaccines. *Expert Rev Vaccines* **2009,** *8* (12), 1721-38. https://www.ncbi.nlm.nih.gov/pubmed/19943765

10. Inglesby, T. V.; Dennis, D. T.; Henderson, D. A., et al., Plague As A Biological Weapon: Medical And Public Health Management. Working Group On Civilian Biodefense. *JAMA* **2000,** *283* (17), 2281-90. https://www.ncbi.nlm.nih.gov/pubmed/10807389

11. Warren, R.; Lockman, H.; Barnewall, R., et al., Cynomolgus Macaque Model For Pneumonic Plague. *Microb Pathog* **2011,** *50* (1), 12-22. https://www.ncbi.nlm.nih.gov/pubmed/21040776

12. Withers, M. R., *Medical Management Of Biological Casualties Handbook; Eighth Edition*; Sep, 2014, 2014. https://www.hsdl.org/c/view?docid=827685

13. Van Andel, R.; Sherwood, R.; Gennings, C., et al., Clinical And Pathologic Features Of Cynomolgus Macaques (Macaca Fascicularis) Infected With Aerosolized Yersinia Pestis. *Comp Med* **2008,** *58* (1), 68-75. https://www.ncbi.nlm.nih.gov/pubmed/19793459

14. Zhang, Y.; Dai, X.; Wang, Q., et al., Transmission efficiency of the plague pathogen (Y. pestis) by the flea, Xenopsylla skrjabini, to mice and great gerbils. *Parasit Vectors* **2015,** *8*, 256. https://www.ncbi.nlm.nih.gov/pubmed/25928441

15. Butler, T.; Fu, Y. S.; Furman, L., et al., Experimental Yersinia Pestis Infection In Rodents After Intragastric Inoculation And Ingestion Of Bacteria. *Infect Immun* **1982,** *36* (3), 1160-7. https://www.ncbi.nlm.nih.gov/pubmed/7095845

16. Perry, R. D.; Fetherston, J. D., Yersinia Pestis--Etiologic Agent Of Plague. *Clin Microbiol Rev* **1997,** *10* (1), 35-66. https://www.ncbi.nlm.nih.gov/pubmed/8993858

17. Burroughs, A. L., Sylvatic Plague Studies: The Vector Efficiency Of Nine Species Of Fleas Compared With Xenopsylla Cheopis. *J Hyg (Lond)* **1947,** *45* (3), 371-96. https://www.ncbi.nlm.nih.gov/pubmed/20475778

18. Houhamdi, L.; Lepidi, H.; Drancourt, M., et al., Experimental Model To Evaluate The Human Body Louse As A Vector Of Plague. *J Infect Dis* **2006,** *194* (11), 1589-96. https://www.ncbi.nlm.nih.gov/pubmed/17083045

19. Thomas, R. E.; Karstens, R. H.; Schwan, T. G., Experimental Infection Of Ornithodoros Spp. Ticks (Acari: Argasidae) With Yersinia Pestis. *J Med Entomol* **1990,** *27* (4), 720-3. https://www.ncbi.nlm.nih.gov/pubmed/2388251

20. Lemon, A.; Cherzan, N.; Vadyvaloo, V., Influence Of Temperature On Development Of Yersinia Pestis Foregut Blockage In Xenopsylla Cheopis (Siphonaptera: Pulicidae) And Oropsylla Montana (Siphonaptera: Ceratophyllidae). *J Med Entomol* **2020,** *57* (6), 1997-2007. https://www.ncbi.nlm.nih.gov/pubmed/32533162

21. Schotthoefer, A. M.; Bearden, S. W.; Holmes, J. L., et al., Effects Of Temperature On The Transmission Of Yersinia Pestis By The Flea, Xenopsylla Cheopis, In The Late Phase Period. *Parasit Vectors* **2011,** *4*, 191. https://www.ncbi.nlm.nih.gov/pubmed/21958555

22. Runfola, J. K.; House, J.; Miller, L., et al., Outbreak Of Human Pneumonic Plague With Dog-To-Human And Possible Human-To-Human Transmission--Colorado, June-July 2014. *MMWR Morb Mortal Wkly Rep* **2015,** *64* (16), 429-34. https://www.ncbi.nlm.nih.gov/pubmed/25928467

23. Sichone, J.; Simuunza, M. C.; Hang'ombe, B. M., et al., Estimating The Basic Reproduction Number For The 2015 Bubonic Plague Outbreak In Nyimba District Of Eastern Zambia. *PLoS Negl Trop Dis* **2020,** *14* (11), e0008811. https://www.ncbi.nlm.nih.gov/pubmed/33166354

24. Earn, D. J. D.; Ma, J.; Poinar, H., et al., Acceleration Of Plague Outbreaks In The Second Pandemic. *Proc Natl Acad Sci U S A* **2020,** *117* (44), 27703-27711. https://www.ncbi.nlm.nih.gov/pubmed/33077604

25. Weant, K. A.; Bailey, A. M.; Fleishaker, E. L., et al., Being Prepared: Bioterrorism And Mass Prophylaxis: Part I. *Adv Emerg Nurs J* **2014,** *36* (3), 226-38; quiz 239-40. https://www.ncbi.nlm.nih.gov/pubmed/25076398

26. Nelson, C. A.; Meaney-Delman, D.; Fleck-Derderian, S., et al., Antimicrobial Treatment And Prophylaxis Of Plague: Recommendations For Naturally Acquired Infections And Bioterrorism Response. *MMWR Recomm Rep* **2021,** *70* (3), 1-27. https://www.ncbi.nlm.nih.gov/pubmed/34264565

27. World Health Organization (WHO), Plague Around The World In 2019. *Weekly Epidemiological Record (WER)* **2019,** *94*, 289-292. https://iris.who.int/handle/10665/325481

28. Dillard, R. L.; Juergens, A. L., Plague. *StatPearls* **2024**. https://www.ncbi.nlm.nih.gov/books/NBK549855/

29. Gani, Epidemiologic Determinants For Modeling Pneumonic Plague Outbreak. *Emerging Infectious Diseases* **2004,** *10* (4), 608-614. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3323083/pdf/03-0509.pdf

30. Ramasindrazana, B.; Andrianaivoarimanana, V.; Rakotondramanga, J. M., et al., Pneumonic Plague Transmission, Moramanga, Madagascar, 2015. *Emerg Infect Dis* **2017,** *23* (3), 521-524. https://www.ncbi.nlm.nih.gov/pubmed/28221119

31. Tsuzuki, S.; Lee, H.; Miura, F., et al., Dynamics Of The Pneumonic Plague Epidemic In Madagascar, August To October 2017. *Euro Surveill* **2017,** *22* (46), 17-00710. https://www.ncbi.nlm.nih.gov/pubmed/29162211

32. Andrianaivoarimanana, V.; Kreppel, K.; Elissa, N., et al., Understanding The Persistence Of Plague Foci In Madagascar. *PLoS Negl Trop Dis* **2013,** *7* (11), e2382. https://www.ncbi.nlm.nih.gov/pubmed/24244760

33. Hewitt, J. A.; Lanning, L. L.; Campbell, J. L., The African Green Monkey Model Of Pneumonic Plague And Us Food And Drug Administration Approval Of Antimicrobials Under The Animal Rule. *Clin Infect Dis* **2020,** *70* (70 Suppl 1), S51-S59. https://www.ncbi.nlm.nih.gov/pubmed/32435803

34. Hinnebusch, B. J., The Evolution Of Flea-Borne Transmission In Yersinia Pestis. *Curr Issues Mol Biol* **2005,** *7* (2), 197-212. https://www.ncbi.nlm.nih.gov/pubmed/16053250

35. Kosoy, M.; Biggins, D., Plague And Trace Metals In Natural Systems. *Int J Environ Res Public Health* **2022,** *19* (16). https://www.ncbi.nlm.nih.gov/pubmed/36011612

36. Duplantier, J. M.; Duchemin, J. B.; Chanteau, S., et al., From The Recent Lessons Of The Malagasy Foci Towards A Global Understanding Of The Factors Involved In Plague Reemergence. *Vet Res* **2005,** *36* (3), 437-53. https://www.ncbi.nlm.nih.gov/pubmed/15845233

37. Carlson, C. J.; Bevins, S. N.; Schmid, B. V., Plague Risk In The Western United States Over Seven Decades Of Environmental Change. *Glob Chang Biol* **2022,** *28* (3), 753-769. https://www.ncbi.nlm.nih.gov/pubmed/34796590

38. Centers for Disease Control and Prevention, Plague. http://www.cdc.gov/plague/ (accessed March 9, 2024).

39. McGovern, T. W.; Friedlander, A. M., Plague. *Medical Aspects of Chemical and Biological Warfare* **1997**, 479-502. https://apps.dtic.mil/sti/pdfs/ADA398241.pdf

https://ke.army.mil/bordeninstitute/published_volumes/chemBio/Ch23.pdf

40. Kool, J. L., Risk Of Person-To-Person Transmission Of Pneumonic Plague. *Clin Infect Dis* **2005,** *40* (8), 1166-72. https://www.ncbi.nlm.nih.gov/pubmed/15791518

41. Burmeister, R. W.; Tigertt, W. D.; Overholt, E. L., Laboratory-Acquired Pneumonic Plague. Report Of A Case And Review Of Previous Cases. *Ann Intern Med* **1962,** *56* (5), 789-800. https://www.ncbi.nlm.nih.gov/pubmed/13874924

42. Galy, A.; Loubet, P.; Peiffer-Smadja, N., et al., [The Plague: An Overview And Hot Topics]. *Rev Med Interne* **2018,** *39* (11), 863-868. https://www.ncbi.nlm.nih.gov/pubmed/29628173

43. Anderson, D. M.; Ciletti, N. A.; Lee-Lewis, H., et al., Pneumonic Plague Pathogenesis And Immunity In Brown Norway Rats. *Am J Pathol* **2009,** *174* (3), 910-21. https://www.ncbi.nlm.nih.gov/pubmed/19164505

44. Arbaji, A.; Kharabsheh, S.; Al-Azab, S., et al., A 12-Case Outbreak Of Pharyngeal Plague Following The Consumption Of Camel Meat, In North-Eastern Jordan. *Ann Trop Med Parasitol* **2005,** *99* (8), 789-93. https://www.ncbi.nlm.nih.gov/pubmed/16297292

45. Bin Saeed, A. A.; Al-Hamdan, N. A.; Fontaine, R. E., Plague From Eating Raw Camel Liver. *Emerg Infect Dis* **2005,** *11* (9), 1456-7. https://www.ncbi.nlm.nih.gov/pubmed/16229781

46. Kehrmann, J.; Popp, W.; Delgermaa, B., et al., Two Fatal Cases Of Plague After Consumption Of Raw Marmot Organs. *Emerg Microbes Infect* **2020,** *9* (1), 1878-1880. https://www.ncbi.nlm.nih.gov/pubmed/32762515

47. Andrianaivoarimanana, V.; Wagner, D. M.; Birdsell, D. N., et al., Transmission Of Antimicrobial Resistant Yersinia Pestis During A Pneumonic Plague Outbreak. *Clin Infect Dis* **2022,** *74* (4), 695-702. https://www.ncbi.nlm.nih.gov/pubmed/34244722

48. Shim, H. K.; Musson, J. A.; Harper, H. M., et al., Mechanisms Of Major Histocompatibility Complex Class Ii-Restricted Processing And Presentation Of The V Antigen Of Yersinia Pestis. *Immunology* **2006,** *119* (3), 385-92. https://www.ncbi.nlm.nih.gov/pubmed/16919002

49. Nikiforov, V. V.; Gao, H.; Zhou, L., et al., Plague: Clinics, Diagnosis And Treatment. *Adv Exp Med Biol* **2016,** *918*, 293-312. https://www.ncbi.nlm.nih.gov/pubmed/27722868

50. Keasey, S. L.; Schmid, K. E.; Lee, M. S., et al., Extensive Antibody Cross-Reactivity Among Infectious Gram-Negative Bacteria Revealed By Proteome Microarray Analysis. *Mol Cell Proteomics* **2009,** *8* (5), 924-35. https://www.ncbi.nlm.nih.gov/pubmed/19112181

51. Schofield, D. A.; Molineux, I. J.; Westwater, C., Diagnostic Bioluminescent Phage For Detection Of Yersinia Pestis. *J Clin Microbiol* **2009,** *47* (12), 3887-94. https://www.ncbi.nlm.nih.gov/pubmed/19828743

52. Leslie, T.; Whitehouse, C. A.; Yingst, S., et al., Outbreak Of Gastroenteritis Caused By Yersinia Pestis In Afghanistan. *Epidemiol Infect* **2011,** *139* (5), 728-35. https://www.ncbi.nlm.nih.gov/pubmed/20663260

53. World Health Organization (WHO), Plague. https://www.who.int/news-room/fact-sheets/detail/plague#:~:text=Diagnosing%20plague&text=The%20best%20practice%20is%20to,A%20specific%20Y (accessed March 3, 2024).

54. American Society for Microbiology, Sentinel Laboratory Guidelines For Suspected Agents Of Bioterrorism Yersinia Pestis. **2005**. https://www.epa.gov/sites/default/files/2015-07/documents/asm-ypestis.pdf

55. Centers for Disease Control and Prevention, Resources For Clinicians. https://www.cdc.gov/plague/healthcare/clinicians.html (accessed March 3, 2024).

56. Demeure, C. E.; Dussurget, O.; Mas Fiol, G., et al., Yersinia Pestis And Plague: An Updated View On Evolution, Virulence Determinants, Immune Subversion, Vaccination, And Diagnostics. *Genes Immun* **2019,** *20* (5), 357-370. https://www.ncbi.nlm.nih.gov/pubmed/30940874

57. Melo, A. C.; Almeida, A. M.; Leal, N. C., Retrospective Study Of A Plague Outbreak By Multiplex-Pcr. *Lett Appl Microbiol* **2003,** *37* (5), 361-4. https://www.ncbi.nlm.nih.gov/pubmed/14633104

58. Chanteau, S.; Rahalison, L.; Ralafiarisoa, L., et al., Development And Testing Of A Rapid Diagnostic Test For Bubonic And Pneumonic Plague. *Lancet* **2003,** *361* (9353), 211-6. https://www.ncbi.nlm.nih.gov/pubmed/12547544

59. Rajerison, M.; Melocco, M.; Andrianaivoarimanana, V., et al., Performance Of Plague Rapid Diagnostic Test Compared To Bacteriology: A Retrospective Analysis Of The Data Collected In Madagascar. *BMC Infect Dis* **2020,** *20* (1), 90. https://www.ncbi.nlm.nih.gov/pubmed/32000692

60. World Health Organization (WHO), Plague - Seychelles. https://www.who.int/emergencies/disease-outbreak-news/item/15-october-2017-plague-seychelles-en (accessed March 3, 2024).

61. Sabhnani, L.; Rao, D. N., Identification Of Immunodominant Epitope Of F1 Antigen Of Yersinia Pestis. *FEMS Immunol Med Microbiol* **2000,** *27* (2), 155-62. https://www.ncbi.nlm.nih.gov/pubmed/10640611

62. Lonsway, D. R.; Urich, S. K.; Heine, H. S., et al., Comparison Of Etest Method With Reference Broth Microdilution Method For Antimicrobial Susceptibility Testing Of Yersinia Pestis. *J Clin Microbiol* **2011,** *49* (5), 1956-60. https://www.ncbi.nlm.nih.gov/pubmed/21411569

63. Guo, J., Antibiotic Resistance Evaluation Of Yersinia Pestis. *Yersinia Pestis Protocols* **2018**, 237-242. https://doi.org/10.1007/978-981-10-7947-4_27

64. Andrianaivoarimanana, V.; Piola, P.; Wagner, D. M., et al., Trends Of Human Plague, Madagascar, 1998-2016. *Emerg Infect Dis* **2019,** *25* (2), 220-228. https://www.ncbi.nlm.nih.gov/pubmed/30666930

65. Han, H.; Liang, Y.; Song, Z., et al., Epidemiological Characteristics Of Human And Animal Plague In Yunnan Province, China, 1950 To 2020. *Microbiol Spectr* **2022,** *10* (6), e0166222. https://www.ncbi.nlm.nih.gov/pubmed/36219109

66. Kugeler, K. J.; Staples, J. E.; Hinckley, A. F., et al., Epidemiology Of Human Plague In The United States, 1900-2012. *Emerg Infect Dis* **2015,** *21* (1), 16-22. https://www.ncbi.nlm.nih.gov/pubmed/25529546

67. Butler, T., Plague Gives Surprises In The First Decade Of The 21st Century In The United States And Worldwide. *Am J Trop Med Hyg* **2013,** *89* (4), 788-93. https://www.ncbi.nlm.nih.gov/pubmed/24043686

68. Nelson, C. A.; Fleck-Derderian, S.; Cooley, K. M., et al., Antimicrobial Treatment Of Human Plague: A Systematic Review Of The Literature On Individual Cases, 1937-2019. *Clin Infect Dis* **2020,** *70* (70 Suppl 1), S3-S10. https://www.ncbi.nlm.nih.gov/pubmed/32435802

69. Agrawal, R.; Murmu, J.; Pattnaik, S., et al., One Health: Navigating Plague In Madagascar Amidst Covid-19. *Infect Dis Poverty* **2023,** *12* (1), 50. https://www.ncbi.nlm.nih.gov/pubmed/37189153

70. Richard, V.; Riehm, J. M.; Herindrainy, P., et al., Pneumonic Plague Outbreak, Northern Madagascar, 2011. *Emerg Infect Dis* **2015,** *21* (1), 8-15. https://www.ncbi.nlm.nih.gov/pubmed/25530466

71. Sun, W.; Sanapala, S.; Rahav, H., et al., Oral Administration Of A Recombinant Attenuated Yersinia Pseudotuberculosis Strain Elicits Protective Immunity Against Plague. *Vaccine* **2015,** *33* (48), 6727-35. https://www.ncbi.nlm.nih.gov/pubmed/26514425

72. U.S. Food & Drug Administration, Products Approved For Other Bioterrorism Emergencies. https://www.fda.gov/drugs/bioterrorism-and-drug-preparedness/products-approved-other-bioterrorism-emergencies (accessed March 8, 2024).

73. Rosario-Acevedo, R.; Biryukov, S. S.; Bozue, J. A., et al., Plague Prevention And Therapy: Perspectives On Current And Future Strategies. *Biomedicines* **2021,** *9* (10). https://www.ncbi.nlm.nih.gov/pubmed/34680537

74. Zhou, H.; Guo, S., Two Cases Of Imported Pneumonic Plague In Beijing, China. *Medicine (Baltimore)* **2020,** *99* (44), e22932. https://www.ncbi.nlm.nih.gov/pubmed/33126357

75. D'Ortenzio, E.; Lemaitre, N.; Brouat, C., et al., Plague: Bridging Gaps Towards Better Disease Control. *Med Mal Infect* **2018,** *48* (5), 307-317. https://www.ncbi.nlm.nih.gov/pubmed/29773334

76. Mussap, C. J., The Plague Doctor Of Venice. *Intern Med J* **2019,** *49* (5), 671-676. https://www.ncbi.nlm.nih.gov/pubmed/31083805

77. Zhu, M.; Zhang, D.; Zhang, L., et al., Spray-Dried Inhalable Powder Formulations Of Gentamicin Designed For Pneumonic Plague Therapy In A Mouse Model. *Pharmaceutics* **2022,** *14* (12). https://www.ncbi.nlm.nih.gov/pubmed/36559140

78. Campbell, J. L.; Fay, M. P.; Lanning, L. L., et al., Effect Of Delaying Treatment On Efficacy Of Ciprofloxacin And Levofloxacin In The African Green Monkey Model Of Pneumonic Plague. *Clin Infect Dis* **2020,** *70* (70 Suppl 1), S60-S65. https://www.ncbi.nlm.nih.gov/pubmed/32435805

79. Yang, R., Plague: Recognition, Treatment, And Prevention. *J Clin Microbiol* **2018,** *56* (1). https://www.ncbi.nlm.nih.gov/pubmed/29070654

80. Godfred-Cato, S.; Cooley, K. M.; Fleck-Derderian, S., et al., Treatment Of Human Plague: A Systematic Review Of Published Aggregate Data On Antimicrobial Efficacy, 1939-2019. *Clin Infect Dis* **2020,** *70* (70 Suppl 1), S11-S19. https://www.ncbi.nlm.nih.gov/pubmed/32435800

81. Vagima, Y.; Gur, D.; Aftalion, M., et al., Phage Therapy Potentiates Second-Line Antibiotic Treatment Against Pneumonic Plague. *Viruses* **2022,** *14* (4). https://www.ncbi.nlm.nih.gov/pubmed/35458417

82. Andersson, J. A.; Fitts, E. C.; Kirtley, M. L., et al., New Role For Fda-Approved Drugs In Combating Antibiotic-Resistant Bacteria. *Antimicrob Agents Chemother* **2016,** *60* (6), 3717-29. https://www.ncbi.nlm.nih.gov/pubmed/27067323

83. Tomaras, A. P.; McPherson, C. J.; Kuhn, M., et al., Lpxc Inhibitors As New Antibacterial Agents And Tools For Studying Regulation Of Lipid A Biosynthesis In Gram-Negative Pathogens. *mBio* **2014,** *5* (5), e01551-14. https://www.ncbi.nlm.nih.gov/pubmed/25271285

84. Abdelbaqi, S.; Deslouches, B.; Steckbeck, J., et al., Novel Engineered Cationic Antimicrobial Peptides Display Broad-Spectrum Activity Against Francisella Tularensis, Yersinia Pestis And Burkholderia Pseudomallei. *J Med Microbiol* **2016,** *65* (2), 188-194. https://www.ncbi.nlm.nih.gov/pubmed/26673248

85. Morgan, J. M.; Lam, H. N.; Delgado, J., et al., An Experimental Pipeline For Initial Characterization Of Bacterial Type Iii Secretion System Inhibitor Mode Of Action Using Enteropathogenic Yersinia. *Front Cell Infect Microbiol* **2018,** *8*, 404. https://www.ncbi.nlm.nih.gov/pubmed/30524970

86. Ferreras, J. A.; Gupta, A.; Amin, N. D., et al., Chemical Scaffolds With Structural Similarities To Siderophores Of Nonribosomal Peptide-Polyketide Origin As Novel Antimicrobials Against Mycobacterium Tuberculosis And Yersinia Pestis. *Bioorg Med Chem Lett* **2011,** *21* (21), 6533-7. https://www.ncbi.nlm.nih.gov/pubmed/21940166

87. Russo, R.; Kolesnikova, I.; Kim, T., et al., Susceptibility Of Virulent Yersinia Pestis Bacteria To Predator Bacteria In The Lungs Of Mice. *Microorganisms* **2018,** *7* (1). https://www.ncbi.nlm.nih.gov/pubmed/30577606

88. Xiao, X.; Zhu, Z.; Dankmeyer, J. L., et al., Human Anti-Plague Monoclonal Antibodies Protect Mice From Yersinia Pestis In A Bubonic Plague Model. *PLoS One* **2010,** *5* (10), e13047. https://www.ncbi.nlm.nih.gov/pubmed/20976274

89. Levy, Y.; Vagima, Y.; Tidhar, A., et al., Adjunctive Corticosteroid Treatment Against Yersinia Pestis Improves Bacterial Clearance, Immunopathology, And Survival In The Mouse Model Of Bubonic Plague. *J Infect Dis* **2016,** *214* (6), 970-7. https://www.ncbi.nlm.nih.gov/pubmed/27402776

90. Thomas, R.; Brooks, T., Attachment Of Yersinia Pestis To Human Respiratory Cell Lines Is Inhibited By Certain Oligosaccharides. *J Med Microbiol* **2006,** *55* (Pt 3), 309-315. https://www.ncbi.nlm.nih.gov/pubmed/16476795

91. Urich, S. K.; Chalcraft, L.; Schriefer, M. E., et al., Lack Of Antimicrobial Resistance In Yersinia Pestis Isolates From 17 Countries In The Americas, Africa, And Asia. *Antimicrob Agents Chemother* **2012,** *56* (1), 555-8. https://www.ncbi.nlm.nih.gov/pubmed/22024826

92. Welkos, S.; Pitt, M. L.; Martinez, M., et al., Determination Of The Virulence Of The Pigmentation-Deficient And Pigmentation-/Plasminogen Activator-Deficient Strains Of Yersinia Pestis In Non-Human Primate And Mouse Models Of Pneumonic Plague. *Vaccine* **2002,** *20* (17-18), 2206-14. https://www.ncbi.nlm.nih.gov/pubmed/12009274

93. Williamson, E. D.; Oyston, P. C. F., The Natural History And Incidence Of Yersinia Pestis And Prospects For Vaccination. *J Med Microbiol* **2012,** *61* (Pt 7), 911-918. https://www.ncbi.nlm.nih.gov/pubmed/22442294

94. Sun, W.; Six, D.; Kuang, X., et al., A Live Attenuated Strain Of Yersinia Pestis Kim As A Vaccine Against Plague. *Vaccine* **2011,** *29* (16), 2986-98. https://www.ncbi.nlm.nih.gov/pubmed/21320544

95. Smiley, S. T., Current Challenges In The Development Of Vaccines For Pneumonic Plague. *Expert Rev Vaccines* **2008,** *7* (2), 209-21. https://www.ncbi.nlm.nih.gov/pubmed/18324890

96. Rosenzweig, J. A.; Hendrix, E. K.; Chopra, A. K., Plague Vaccines: New Developments In An Ongoing Search. *Appl Microbiol Biotechnol* **2021,** *105* (12), 4931-4941. https://www.ncbi.nlm.nih.gov/pubmed/34142207

97. Demeure, C. E.; Derbise, A.; Carniel, E., Oral Vaccination Against Plague Using Yersinia Pseudotuberculosis. *Chem Biol Interact* **2017,** *267*, 89-95. https://www.ncbi.nlm.nih.gov/pubmed/27046452

98. Sagiyev, Z.; Berdibekov, A.; Bolger, T., et al., Human Response To Live Plague Vaccine Ev, Almaty Region, Kazakhstan, 2014-2015. *PLoS One* **2019,** *14* (6), e0218366. https://www.ncbi.nlm.nih.gov/pubmed/31199832

99. Hu, J.; Jiao, L.; Hu, Y., et al., One Year Immunogenicity And Safety Of Subunit Plague Vaccine In Chinese Healthy Adults: An Extended Open-Label Study. *Hum Vaccin Immunother* **2018,** *14* (11), 2701-2705. https://www.ncbi.nlm.nih.gov/pubmed/29927704

100. Blisnick, T.; Ave, P.; Huerre, M., et al., Oral Vaccination Against Bubonic Plague Using A Live Avirulent Yersinia Pseudotuberculosis Strain. *Infect Immun* **2008,** *76* (8), 3808-16. https://www.ncbi.nlm.nih.gov/pubmed/18505804

101. Bubeck, S. S.; Dube, P. H., Yersinia Pestis Co92 Delta Yoph Is A Potent Live, Attenuated Plague Vaccine. *Clin Vaccine Immunol* **2007,** *14* (9), 1235-8. https://www.ncbi.nlm.nih.gov/pubmed/17652523

102. Sanapala, S.; Rahav, H.; Patel, H., et al., Multiple Antigens Of Yersinia Pestis Delivered By Live Recombinant Attenuated Salmonella Vaccine Strains Elicit Protective Immunity Against Plague. *Vaccine* **2016,** *34* (21), 2410-2416. https://www.ncbi.nlm.nih.gov/pubmed/27060051

103. Wang, S.; Heilman, D.; Liu, F., et al., A DNA Vaccine Producing Lcrv Antigen In Oligomers Is Effective In Protecting Mice From Lethal Mucosal Challenge Of Plague. *Vaccine* **2004,** *22* (25-26), 3348-57. https://www.ncbi.nlm.nih.gov/pubmed/15308359

104. Wang, S.; Goguen, J. D.; Li, F., et al., Involvement Of Cd8+ T Cell-Mediated Immune Responses In Lcrv DNA Vaccine Induced Protection Against Lethal Yersinia Pestis Challenge. *Vaccine* **2011,** *29* (39), 6802-9. https://www.ncbi.nlm.nih.gov/pubmed/21199697

105. Ayyadurai, S.; Houhamdi, L.; Lepidi, H., et al., Long-Term Persistence Of Virulent Yersinia Pestis In Soil. *Microbiology (Reading)* **2008,** *154* (Pt 9), 2865-2871. https://www.ncbi.nlm.nih.gov/pubmed/18757820

106. Malek, M. A.; Bitam, I.; Levasseur, A., et al., Yersinia Pestis Halotolerance Illuminates Plague Reservoirs. *Sci Rep* **2017,** *7*, 40022. https://www.ncbi.nlm.nih.gov/pubmed/28054667

107. Boegler, K. A.; Graham, C. B.; Montenieri, J. A., et al., Evaluation Of The Infectiousness To Mice Of Soil Contaminated With Yersinia Pestis-Infected Blood. *Vector Borne Zoonotic Dis* **2012,** *12* (11), 948-52. https://www.ncbi.nlm.nih.gov/pubmed/22925020

108. Bhaduri, S., Effect Of Fat In Ground Beef On The Growth And Virulence Plasmid (Pyv) Stability In Yersinia Pestis. *Int J Food Microbiol* **2010,** *136* (3), 372-5. https://www.ncbi.nlm.nih.gov/pubmed/19875186

109. Wilkinson, T. R., Survival Of Bacteria On Metal Surfaces. *Appl Microbiol* **1966,** *14* (3), 303-7. https://www.ncbi.nlm.nih.gov/pubmed/5339359

110. Krauer, F.; Viljugrein, H.; Dean, K. R., The Influence Of Temperature On The Seasonality Of Historical Plague Outbreaks. *Proc Biol Sci* **2021,** *288* (1954), 20202725. https://www.ncbi.nlm.nih.gov/pubmed/34255997

111. Pawlowski, Entry Of Yersinia Pestis Into The Viable But Nonculturable State In A Low-Temperature Tap Water Microcosm. *Plos One* **2011,** *6* (3), E17585. https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0017585&type=printable

112. Rose, L. J.; Donlan, R.; Banerjee, S. N., et al., Survival Of Yersinia Pestis On Environmental Surfaces. *Appl Environ Microbiol* **2003,** *69* (4), 2166-71. https://www.ncbi.nlm.nih.gov/pubmed/12676697

113. EPA, NRT Quick Reference Guide: Yersinia pestis (Plague). https://nrt.response.epa.gov/sites/2/files/NRT%20CBRN%20BIO%20UPDATE%20Plague%20QRG_FINAL%202022%2009%2018.pdf (accessed 08 May 2024).

114. EPA, EPA's Registered Antimicrobial Products Effective Against Methicillin-resistant Staphylococcus aureus (MRSA) and/or Vancomycin-resistant Enterococcus faecalis/faecium (VRE) [List H]. https://www.epa.gov/pesticide-registration/epas-registered-antimicrobial-products-effective-against-methicillin#against (accessed 8 May 2024).

115. EPA, EPA's Registered Antimicrobial Products Effective Against Clostridioides difficile (C. diff) Spores [List K]. https://www.epa.gov/pesticide-registration/epas-registered-antimicrobial-products-effective-against-clostridioides.

116. Calfee, M. W.; Wendling, M., Inactivation Of Vegetative Bacterial Threat Agents On Environmental Surfaces. *Sci Total Environ* **2013,** *443*, 387-96. https://www.ncbi.nlm.nih.gov/pubmed/23208274

117. Rashid, M. H.; Revazishvili, T.; Dean, T., et al., A Yersinia Pestis-Specific, Lytic Phage Preparation Significantly Reduces Viable Y. Pestis On Various Hard Surfaces Experimentally Contaminated With The Bacterium. *Bacteriophage* **2012,** *2* (3), 168-177. https://www.ncbi.nlm.nih.gov/pubmed/23275868

118. Rogers, J. V.; Richter, W. R.; Shaw, M. Q., et al., Vapour-Phase Hydrogen Peroxide Inactivates Yersinia Pestis Dried On Polymers, Steel, And Glass Surfaces. *Lett Appl Microbiol* **2008,** *47* (4), 279-85. https://www.ncbi.nlm.nih.gov/pubmed/19241520

119. Roberts, L. M.; Anderson, R.; Carmody, A., et al., Validation And Application Of A Benchtop Cell Sorter In A Biosafety Level 3 Containment Setting. *Appl Biosaf* **2021,** *26* (4), 205-209. https://www.ncbi.nlm.nih.gov/pubmed/36034097

120. Centers for Disease Control and Prevention, *Biosafety In Microbiological And Biomedical Laboratories. 6th Edition.* U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health: 2020. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

121. Song, Y.; Tong, Z.; Wang, J., et al., Complete Genome Sequence Of Yersinia Pestis Strain 91001, An Isolate Avirulent To Humans. *DNA Res* **2004,** *11* (3), 179-97. https://www.ncbi.nlm.nih.gov/pubmed/15368893

122. Kutyrev, V. V.; Eroshenko, G. A.; Motin, V. L., et al., Phylogeny And Classification Of Yersinia Pestis Through The Lens Of Strains From The Plague Foci Of Commonwealth Of Independent States. *Front Microbiol* **2018,** *9*, 1106. https://www.ncbi.nlm.nih.gov/pubmed/29887859

123. Zhou, D.; Han, Y.; Song, Y., et al., DNA Microarray Analysis Of Genome Dynamics In Yersinia Pestis: Insights Into Bacterial Genome Microevolution And Niche Adaptation. *J Bacteriol* **2004,** *186* (15), 5138-46. https://www.ncbi.nlm.nih.gov/pubmed/15262950

124. Lei, C.; Kumar, S., Yersinia Pestis Antibiotic Resistance: A Systematic Review. *Osong Public Health Res Perspect* **2022,** *13* (1), 24-36. https://www.ncbi.nlm.nih.gov/pubmed/35255676

125. Wu, Y.; Hao, T.; Qian, X., et al., Small Insertions And Deletions Drive Genomic Plasticity During Adaptive Evolution Of Yersinia Pestis. *Microbiol Spectr* **2022,** *10* (3), e0224221. https://www.ncbi.nlm.nih.gov/pubmed/35438532

126. Dai, R.; He, J.; Zha, X., et al., A Novel Mechanism Of Streptomycin Resistance In Yersinia Pestis: Mutation In The Rpsl Gene. *PLoS Negl Trop Dis* **2021,** *15* (4), e0009324. https://www.ncbi.nlm.nih.gov/pubmed/33886558

127. Welch, T. J.; Fricke, W. F.; McDermott, P. F., et al., Multiple Antimicrobial Resistance In Plague: An Emerging Public Health Risk. *PLoS One* **2007,** *2* (3), e309. https://www.ncbi.nlm.nih.gov/pubmed/17375195

128. Galimand, M.; Guiyoule, A.; Gerbaud, G., et al., Multidrug Resistance In Yersinia Pestis Mediated By A Transferable Plasmid. *N Engl J Med* **1997,** *337* (10), 677-80. https://www.ncbi.nlm.nih.gov/pubmed/9278464

129. Guiyoule, A.; Gerbaud, G.; Buchrieser, C., et al., Transferable Plasmid-Mediated Resistance To Streptomycin In A Clinical Isolate Of Yersinia Pestis. *Emerg Infect Dis* **2001,** *7* (1), 43-8. https://www.ncbi.nlm.nih.gov/pubmed/11266293

130. Cabanel, N.; Bouchier, C.; Rajerison, M., et al., Plasmid-Mediated Doxycycline Resistance In A Yersinia Pestis Strain Isolated From A Rat. *Int J Antimicrob Agents* **2018,** *51* (2), 249-254. https://www.ncbi.nlm.nih.gov/pubmed/29030266

131. Chanteau, S.; Ratsitorahina, M.; Rahalison, L., et al., Current Epidemiology Of Human Plague In Madagascar. *Microbes Infect* **2000,** *2* (1), 25-31. https://www.ncbi.nlm.nih.gov/pubmed/10717537