Yersinia pestis and the Plague

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Key Words: Plague; *Yersinia pestis*; Bioterrorism

DOI: 10.1309/DQM93R8QNQWBFYU8

Abstract

Yersinia pestis *is the cause of plague, an illness that may manifest in bubonic, pneumonic, or septicemic form. Plague has killed an estimated 200 million humans throughout history, and plague is endemic in many areas of the world. Approximately 2,000 cases of plague are reported each year to the World Health Organization, and concern has been raised about the possible use of* Y pestis *as an agent of bioterrorism. The genome of* Y pestis *has been sequenced, including the 3 virulence plasmids, pPst, pLcr, and pFra, and advances have been made in understanding the bacterial pathogenesis of* Y pestis *infection. Advances also have been made in rapid diagnosis, the understanding of immune responses during plague, and vaccine development.*

Bioterrorism is at the forefront of the world's consciousness, especially after the terrorist attacks of September 11, 2001, and subsequent distribution of *Bacillus anthracis* spores through United States mail. While many articles have focused on the potential threat of biologic agents such as anthrax and smallpox in particular, less attention has been given to other potentially dangerous microorganisms. During the first week of November 2002, *Yersinia pestis*, the causative agent of bubonic plague, made headline news when 2 New Mexico residents visited New York City and were diagnosed with the infection, causing many to wonder at the time if this was a second biologic attack. This incident sparked renewed interest in the disease, and so this article reviews the history, bacterial characterization, clinical manifestations, epidemiology-epizoology, pathogenesis, laboratory diagnosis, and treatment of *Y pestis*. We also briefly discuss recent advances in vaccine development and the potential use of *Y pestis* as a bioterrorism agent.

There are fewer than 10 cases of plague a year in the United States, usually occurring in rural areas of western states. However, over the course of history, epidemics of plague have killed hundreds of thousands of people and devastated cities and countries. The destructive potential of the plague is best evidenced by its presentation in the 14th century as the Black Death, which killed one third of the Western European population. Overall, *Y pestis* is estimated to have killed 100 to 200 million individuals throughout history, making it one of the leading infectious disease killers of humans.[1]

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Historic Background, Epidemiology, and Epizoology

There have been 3 pandemics of (probable) plague in recorded history. The first pandemic, the Justinian plague (541-767 AD), was thought to have originated in east or central Africa and spread from Egypt to the Mediterranean basin and may have resulted in the deaths of 40 to 100 million people. The second pandemic, the Black Death (and subsequent epidemics from 1346 to the 1800s), possibly originated in central Asia, spread to the Caspian Sea and then throughout Europe, and resulted in the deaths of one third of the population of Europe—an estimated 25 million people. The third pandemic began in China in the mid-1800s, spread worldwide via marine shipping through Hong Kong, and resulted in the deaths of approximately 12 million people, primarily in India.[1,2]

The infectious source of the disease was not understood until Alexandre Yersin investigated the Hong Kong epidemic in the mid-1890s. Yersin studied the fluid aspirated from enlarged lymph nodes of people who had died of the plague and discovered gram-negative microorganisms. He injected the fluid into guinea pigs, which subsequently died, and found that their organs were infected with the same microorganisms. Putting this information together with the knowledge that dead rats had long been associated with plague epidemics, Yersin discovered the cause of the plague: a microorganism that infects both rats and humans.[3]

The exact route of transmission remained elusive until a few years later, when Paul-Louis Simond, a French colonial Army physician furthering Yersin's research in Indochina, noticed that humans seemed to contract the disease only when they were exposed to recently dead rats that were still warm. Rats that had been dead for some time and were cold did not seem to pose a danger. He postulated that the rat flea (*Xenopsylla cheopis*) was responsible for the transmission, since it leaves the bodies of dead animals before they become cold. He microscopically examined fleas taken from recently expired rats and discovered that the intestines of fleas harbored the same microorganism that Yersin had found in cadavers and guinea pigs. With a series of experiments, Simond demonstrated that fleas from dead rats that had been infected with the microorganism could move to a healthy rat and infect it with the bacteria carried in the flea's intestine. The healthy rat then would die, and the cycle would continue.[3] If a human was unlucky enough to be bitten by a flea carrying the microorganism, the person also would develop plague. In addition to *X cheopis*, approximately 30 species of fleas worldwide are major vectors capable of transmitting *Y pestis*.[1]

Plague is a zoonosis. Although urban and domestic rats, *Rattus rattus* (the black rat) and *Rattus norvegicus* (the brown rat), were the animal reservoirs in the third pandemic, today they are important as animal hosts in only a few areas of the world, such as Madagascar, India, and the Democratic Republic of the Congo. The majority of human plague cases today are the result of cross-infection occurring from wild animal reservoirs, including prairie dogs, squirrels, marmots, and other small rodents or larger mammals who have become infected, such as cats and coyotes.[4] Human outbreaks are, therefore, usually preceded by epizootic outbreaks. Areas of the world endemic for plague include Madagascar, eastern and southern Africa, Southeast Asia, the western United States, Mongolia and northern China, south Asia, Russia, and central Asia. Globally, approximately 2,000 cases of plague are reported to the World Health Organization each year.[5]

Plague was introduced to the United States from China via ship-borne infected rats in 1900. After an initial period (1900-1926) characterized by urban outbreaks (primarily on the West Coast and associated with rats), *Y pestis* became endemic in wild rodent populations in the western United States. Since 1926, cases of plague in the United States usually have been sporadic and associated with exposure to infected rodents or secondarily infected animals, especially cats.

The understanding of how the disease is transmitted, the advent of public health policy, and the development of antibiotics have helped transform the plague from a disease that causes pandemics and epidemics and has killed hundreds of millions to a disease that is relatively rare and treatable. However, occasional outbreaks, antibiotic-resistant strains of *Y pestis,* and the possible use of the organism as an agent of bioterrorism maintain the important public health implications of this organism.

Clinical Features

Human infection with *Y pestis* usually manifests as 1 of 3 clinical pictures: bubonic, septicemic, or pneumonic plague. The majority of cases are bubonic or septicemic. If left untreated, bubonic plague is fatal in 40% to 60% of cases, while untreated septicemic and pneumonic cases have a 100% mortality rate.[6]

The clinical symptoms of bubonic plague include fever, chills, weakness, headache, and swollen, tender lymph nodes (buboes). Which lymph nodes are involved depends on the site of exposure; however, buboes more commonly are found in the inguinal and groin femoral regions (*bubon* is Greek for groin).[1] Onset of lymphadenopathy is rapid and impressive. Marked edema, swelling, and inflammation of tissues overlying the buboes is frequent.[4] The buboes usually are extremely tender, and necessitation may occur. Diarrhea, nausea, and vomiting also are frequent manifestations.[7,8] Symptoms begin approximately 2 to 8 days after exposure.[1,4]

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DOI: 10.1309/DQM93R8QNQWBFYU8

Bacteremia or secondary plague septicemia is a frequent occurrence,[1] and the fatality rate is higher in patients with higher colony counts.[9]

Patients with primary septicemic plague have positive blood cultures but lack lymphadenopathy or pneumonia. The incubation period for primary septicemic plague is 1 to 4 days. In the mid to late 20th century, septicemic plague was responsible for approximately 10% of all cases of plague in the United States.[1] This percentage rose to 25% in the 1980s in cases diagnosed in New Mexico.[7] Plague septicemia is clinically similar to other gram-negative bacterial septicemias and has a 30% to 50% mortality rate, even with antibiotic administration.[6,7] Disseminated intravascular coagulopathy, meningitis, and multiorgan failure are common. Secondary septicemic plague occurs commonly during bubonic and primary pneumonic plague.

Primary pneumonic plague is uncommon and results from inhalation of organisms, usually via respiratory droplets from infected individuals or animals or, in rare circumstances, owing to accidental inhalation of organisms in laboratory and research facilities. The infectious dose by inhalation is estimated to be 100 to 500 organisms.[10] The initial symptoms are flu-like, with rapid progression to pneumonia with bloody, watery sputum production. The incubation period is approximately 1 to 3 days.[1] Since the 1924-1925 Los Angeles epidemic, the majority of cases of primary pneumonic plague in the United States have been the result of transmission from infected cats.[1,11-13] Approximately 12% of patients with bubonic or septicemic plague in the United States develop secondary pneumonic plague, a complication that has a high fatality rate.[12] The fact that *Y pestis* can be transmitted via inhalation is one of the reasons the microorganism has potential use as a bioterrorism agent.

Microbiology

The genus *Yersinia* is part of the Enterobacteriaceae family. *Yersinia* species are anaerobic, gram-negative, non–spore-forming bacilli or coccobacilli. There are multiple *Yersinia* species, including the 3 human pathogens *Y pestis*, *Yersinia pseudotuberculosis,* and *Yersinia enterocolitica*. *Y pseudotuberculosis* causes mesenteric adenitis and septicemia, and *Y enterocolitica* causes gastrointestinal disorders that range from acute enteritis to mesenteric lymphadenitis.[14] All species of *Yersinia* are nonencapsulated, with the exception of *Y pestis*, which develops a cellular envelope at temperatures above 33°C.[15] The lipopolysaccharide element of *Y pestis* contains core constituents but lacks extended O-group side chains.[1]

Y pestis exists as an obligate parasite and has a limited ability to survive outside an infected mammal. It is auxotrophic for several amino acids and has additional nutritional requirements, including the vitamins biotin, thiamine, pantothenate, and glutamic acid during growth at 37°C. *Y pestis* lacks glucose-6-phosphate dehydrogenase; therefore, it is unable to use the hexose monophosphate pathway.[16] Alternatively, *Y pestis* synthesizes pentose with transketolase and transaldolase.[17] The transcarboxylic acid cycle is not completely functional, and, thus, *Y pestis* possesses a unique glyoxalate shunt pathway.[18] *Y pestis* lacks a functional alpha-ketoglutarate dehydrogenase and aspartase[19,20] and is positive by *o*-nitrophenyl-β-D-galactopyranoside (ONPG) testing without lactose production.[1]

Y pestis is subdivided into 3 biotypes or biovars (antiqua, orientalis, and medievalis) contingent on the ability to convert nitrate to nitrite and the ability to ferment glycerol. Previous studies have shown that the different biovars do not exhibit different degrees of virulence.[15] Review of historic clinical records and epidemiologic study of the 3 waves of pandemic plague have led to speculation that biovar antiqua, found in Africa, southeastern Russia, and Central Asia, may have descended from the strain that caused the first pandemic. Medievalis, found in the Caspian Sea region and central Asia, seems to have descended from the bacteria that triggered the second pandemic. The biovar responsible for the third pandemic is thought to be orientalis, found in Asia and the Western Hemisphere.[2] While the virulence between biovars seems similar, specific isolates within the different biovars exhibit heterogeneity, suggesting frequent DNA rearrangements.[21] Studies using molecular biology–based techniques for *Y pestis* surveillance, such as a variable-number tandem repeat, ribotyping, pulsed-field gel electrophoresis, and restriction fragment length polymorphism have been reported, but these approaches are not in routine use by clinical laboratories for strain typing.[21-26]

The genome of *Y pestis* (strain CO92*)* consists of a 4.65-megabase chromosome and 3 plasmids of 9.6 kilobases (kb; pesticin, Pst plasmid), 70 kb (low calcium response, Lcr plasmid, encoding gene products activated under low calcium conditions including the V antigen and outer surface proteins [Yops]), and 110 kb (Fra plasmid, encoding primarily fraction 1 [F1] glycoprotein envelope antigen). The 70-kb virulence plasmid pLcr (also called pYV and pCD1) is found in all pathogenic *Yersinia* species.[27] Plasmid pPst (also called pPCP1) encodes the plasminogen activator, Pla, which has been shown to be required for subcutaneous virulence.[28] Pla is a protease that degrades fibrin and other extracellular proteins and facilitates systemic spread from the inoculation site. The Pst plasmid also expresses pesticin, a bacteriocin thought to be important for iron uptake by *Y pestis* in mammalian hosts. Plasmid pFra (also called pMT1 or the Tox plasmid) expresses phospholipase D (PlD; previously called *Yersinia* murine toxin, Ymt) in addition to the F1

DOI: 10.1309/DQM93R8QNQWBFYU8
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capsular protein, both of which have been shown to have a role in the transmission of the plague.[27]

Several studies have shown that chromosomal DNA from wild-type *Y pestis* and *Y pseudotuberculosis* show a high degree of conservation, indicating that they are more closely related to each other than either is to *Y enterocolitica.*[2,29,30] More than 90% of *Y pestis* and *Y pseudotuberculosis* DNA is identical, and the nucleotide sequence of their 16S ribosomal RNA is 99.7% identical.[31-33] Despite these similarities, *Y pseudotuberculosis* is the causative agent of a self-limited enteric disease that is much more benign than disease caused by *Y pestis*. *Y pseudotuberculosis* is food- and water-borne and not transmitted via fleas. Research suggests that *Y pestis* is a recently emerged clone of *Y pseudotuberculosis*, thought to have diverged between 1,500 and 20,000 years ago.[2] It has been suggested that plasmids pPst and pFra may have been acquired by horizontal gene transfer; for instance, the Fra plasmid has similarities to the *Salmonella enterica* serovar Typhi plasmid pHCM2.[34] Despite the similarity between *Y pestis* and *Y pseudotuberculosis*, additional gene rearrangements seem to have a significant role in *Y pestis* virulence.[27] If the 2 plasmids unique to *Y pestis* are introduced into *Y pseudotuberculosis*, the transformed *Y pseudotuberculosis* lacks the virulence and pathogenicity characteristic of *Y pestis.*[35] Elucidating the reasons for the differences between diseases caused by *Y pestis* and *Y pseudotuberculosis* will require in-depth comparative studies.

Pathogenicity and Virulence

After being ingested by a flea from a rodent host, *Y pestis* multiplies in the flea gut and expresses a coagulase that clots ingested blood, occluding the proventriculus (an organ between the stomach and esophagus of the flea), rendering the flea "blocked," that is, unable to move food (blood) from its esophagus to its midgut. Thus, the flea repeatedly attempts to feed, and because it is unable to ingest the blood, it regurgitates the newly infected blood back into the bloodstream of the mammal on which it is feeding, therefore transferring the microorganism from the flea to the mammal.[36] Approximately 25,000 to 100,000 *Y pestis* organisms are inoculated into the skin of the mammal host during this process.[37] *Y pestis* bacteria then are carried in cutaneous lymphatics to regional lymph nodes, resulting in swelling, edema, and hemorrhagic necrosis of lymph nodes and surrounding tissues.

PlD, also referred to as Ymt, has been shown to be necessary for the survival of *Y pestis* in the midgut of the rat flea, *X cheopis*. Ymt protects *Y pestis* organisms from a cytotoxic digestion product of ingested blood in the flea gut. In addition, when organisms are inoculated into a mammalian host, PlD/Ymt is released from lysed bacteria and can contribute to hypotension and vascular collapse. It has been postulated that the acquisition of PlD/Ymt by *Y pestis* may have been a determining factor in permitting the bacteria to use an arthropod-borne transmission route.[38]

Although *Y pestis* causes a much more morbid disease and has a different mode of transmission from that of *Y pseudotuberculosis* and *Y enterocolitica*, all 3 species share the Yop virulon. Yop expression is induced under conditions of Ca^2+ depletion, a condition that occurs in extracellular fluid[19,39] and that *Y pestis* encounters once transmitted from the flea to the mammalian host. The Yop virulon includes both intracellular effector proteins and translocator proteins, which enable *Yersinia* to inject effector proteins into the host cell via a well-characterized type III secretion system.[40] The Yop effectors, after being secreted through the host cell's plasma membrane, then alter the host cell cytoskeleton, inhibit phagocytosis, inhibit platelet aggregation, and inhibit production of proinflammatory cytokines, thus inhibiting the host's immune response to the invading bacteria.[40,41] The Yop virulon is an archetype for similar mechanisms of pathogenicity in numerous animal pathogens, including *Salmonella* species, *Shigella* species, enteropathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Chlamydia psittaci,* and *Bordetella* species.[42] An additional type III secretion system, similar to the SPI-2 type III system found in *S enterica* serovar Typhimurium, also is chromosomally encoded in *Y pestis*, although the function of this secondary system is unclear.[27,43]

LcrV (V) is another essential virulence factor expressed on the surface of *Y pestis* and is encoded on the pLcr (pCD1, pYV) plasmid.[44] V is expressed at 37°C. It also is a protective immunogen[45] and seems to have multiple roles in the pathogenesis of *Y pestis*. It causes the organism to become resistant to phagocytosis, inhibits neutrophil chemotaxis, is important for the survival of *Y pestis* within macrophages, and downregulates host cytokine production, especially interferon-γ and tumor necrosis factor α by macrophages.[46,47] V also is important for the function of the type III secretion system, as it is thought to function as a translocator.[48,49] Active immunization with V antigen and passive immunization with anti-LcrV (anti-V) antibodies also have been shown to provide protection against *Y pestis* infection, making the V antigen an attractive target for vaccine development.

Laboratory Diagnosis

Y pestis can be identified in the laboratory by both bacteriologic and serologic methods. Diagnosis can be made from a variety of samples, including blood, aspirates from

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DOI: 10.1309/DQM93R8QNQWBFYU8

involved lymph nodes, skin scrapings, cerebrospinal fluid, urine, and sputum. *Y pestis* appears as a pleomorphic gram-negative rod and may appear as a single cell (1.0-2.0 × 0.5 μm) or in short chains in smears. The organism gives a bipolar "closed-safety pin" appearance on Giemsa, Wright, or Wayson stains (but not on Gram stain). The organism also may be identified via immunohistochemical staining using a monoclonal anti-F1 *Y pestis* antibody on formalin-fixed tissue samples.[50]

The microorganism may be cultured and grows on most routine solid and liquid culture media; however, growth is slow. Colonies are visible on plates after 48 hours, and it is recommended that plates be incubated for a total of 7 days before being discarded.[1,4] Colonies are smooth, opaque, and round but may have irregular edges. Under magnification, colonies can be smooth or finely granular and might have a raised center with a flat periphery ("fried egg" appearance) or a "hammered copper" appearance.[4] Colonies might not be visible on MacConkey or eosin methylene blue agar at 24 hours, and they may be only pinpoint sized after 24 hours on sheep blood agar. Optimal growth is at 28°C. In broth cultures, *Y pestis* may have a stalactite pattern with clumps of cells settling to the bottom when disturbed. Cultures should not be refrigerated, and, if *Y pestis* is suspected, 2 sets of cultures should be established (one at 28°C, the other at 37°C) to evaluate for other bacterial pathogens. *Y pestis* prefers media containing a 2.5-mmol/L concentration of Ca^{2}+ and a 1.5-mmol/L concentration of Mg^{2}+, which are the concentrations found in blood.[19,39] As previously mentioned, virulence factors are induced under conditions of Ca^{2}+ depletion, although low calcium levels impede in vitro growth of *Y pestis*.[19,39]

On biochemical screening, *Y pestis* results in alkaline slant/acid butt (K/A) on triple sugar agar (TSI) without gas or hydrogen sulfide. It is an oxidase-negative, catalase-positive, urea-negative, indole-negative, nonlactose fermenter. Notably, most automated commercial microbiologic identification systems do not include *Y pestis* in their databases, and *Y pestis* might be falsely identified by such systems as *Shigella* species, hydrogen sulfide–negative *Salmonella* species, *Acinetobacter* species, or *Y pseudotuberculosis*.[1,4] Bacteriophage lysis may be used for culture confirmation of *Y pestis*.

A direct immunofluorescence assay using an antibody against the capsular antigen F1 can be used for the laboratory diagnosis of *Y pestis*. However, this antigen is expressed only at 37°C; therefore, samples that have been refrigerated for more than 30 hours, cultures incubated at temperatures less than 37°C, or samples isolated from fleas will be negative.[1]

Serologic tests also may be used in the diagnosis of plague.[4,50] Hemagglutinating antibodies directed against the F1 antigen of *Y pestis* usually appear toward the end of the first week of illness.[4] It is best to obtain paired serum samples, either acute and convalescent or convalescent and postconvalescent, but a single sample may be satisfactory. A 4-fold change in the titer of paired samples is confirmatory for *Y pestis*, and a titer greater than 1:10 in someone not previously infected or vaccinated is evidence of a recent infection in a single sample.[1]

Enzyme-linked immunosorbent assays also have been developed to detect *Y pestis* and can measure F1 antigen levels or levels of serum antibodies to F1 itself. Patients who are positive for the F1 antigen often are negative for serum antibody, and vice versa. Thus, both assays should be performed on each patient.[1] Polymerase chain reaction analysis, using structural genes for the F1 antigen as targets, also is available for diagnosing *Y pestis* infection.[1,51,52]

Treatment

Plague has a high mortality rate if untreated. Therefore, effective antibiotic treatment is critical. Historically, the antibiotics most commonly used have included streptomycin, gentamicin, tetracycline or doxycycline, and chloramphenicol.[1,4] Fluoroquinolones, such as ciprofloxacin, also are quite effective in animal studies and in vitro, and many experts think that fluoroquinolones may have a role in the treatment of people with plague. Of these antibiotics, the most data are available on the efficacy of streptomycin for the treatment of people with plague; unfortunately, this agent often is not commercially available in the United States. (If available, streptomycin should never be administered during pregnancy because irreversible deafness has occurred in children exposed to streptomycin in utero.) An acceptable and preferred alternative to streptomycin is gentamicin. Alternative therapies for adults and children (weighing the risk of a life-threatening illness against the possibility of an often non–life-threatening complication) include doxycycline, ciprofloxacin, and chloramphenicol. Antibiotic therapy should be continued for 10 days. Some experts favor the use of chloramphenicol for the treatment of people with plague meningitis. Many experts would treat people seriously ill with plague with more than one antibiotic, such as streptomycin or gentamicin with tetracycline or doxycycline. Antibiotics preferably should be administered intravenously, unless a mass casualty or outbreak situation results in the overwhelming of health care facilities, in which case orally administered tetracycline, doxycycline, or ciprofloxacin is considered an acceptable alternative.[53,54]

A 7-day course of tetracycline, doxycycline, or ciprofloxacin also may be used for prophylaxis of people exposed to patients with pneumonic plague (defined as coming within 6 feet of a person with pneumonic plague

DOI: 10.1309/DQM93R8QNQWBFYU8
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before that person has received 48 hours of appropriate antibiotics). A few experts think that trimethoprim-sulfamethoxazole also may be used for prophylaxis or treatment when first-line antibiotics are relatively contraindicated (such as in pregnancy or childhood), although data on this point are limited, and most experts would administer tetracycline, doxycycline, or ciprofloxacin, irrespective of age or pregnancy status.[53,54]

If there is a clinical suspicion of plague before laboratory confirmation, droplet and contact precautions should be used until 2 days after the start of antibiotic therapy, to prevent spread of the disease if the patient develops secondary pneumonic plague.[1]

Vaccine Development

Although antibiotics have been used for plague prophylaxis, they are useful only in the setting of a known case of plague. Killed whole vaccines have been used since the 1890s and have been shown to protect against bubonic plague.[55] The whole-cell killed vaccine previously was available for people at possible high risk of exposure, such as military or laboratory personnel. The vaccine's protective efficacy was thought to be associated primarily with its ability to induce anti-F1 antibodies. Side effects were common, and multiple boosters were necessary. It also was unclear how well this vaccine protected against the pneumonic form of plague.[4] Therefore, production of the vaccine was discontinued by the manufacturer in 1999.[54] A live attenuated vaccine, EV76, also was in use in humans in some areas of the world, but it also is not commercially available at present.[55]

Previous experiments in mice revealed that purified F1 antigen was more effective in protecting against plague than the killed whole-cell vaccine.[56] However, attempts to develop a vaccine using only the F1 antigen were unsuccessful. It was found that in mice, F1 mutants maintain virulence, suggesting that other antigens are essential virulence factors and that an effective plague vaccine would induce more than just an anti-F1 response.[56] Promising next-generation vaccines include subunit vaccines containing both F1 and V antigens with the adjuvant alhydrogel, live attenuated mutant strains of *Y pestis*, and a live recombinant vaccine (*S enterica* serovar Typhimurium expressing an F1/V fusion protein).[57-60]

Use of *Y pestis* as a Bioterrorism Agent

Cases of plague resulting from a terrorist attack would have clinical manifestations different from naturally occurring cases. The most likely method of terrorist release would be aerosolized *Y pestis*, which would result in primary pneumonic plague. As previously described, the incubation period for primary pneumonic plague is approximately 1 to 3 days. The first indication of a terrorist attack thus would be a sudden outbreak of severe pneumonia and possibly sepsis a few days after a bioterrorist event.[54] This might call attention to the possibility of plague only if a larger number of people were infected. If a smaller number of people were exposed to the initial release of bacteria, it is possible that their illnesses would be diagnosed as nonspecified pneumonia. The presentation of a larger number of previously healthy individuals with severe pneumonia symptoms, especially with hemoptysis or possibly death, should raise the possibility of pneumonic plague.[54]

Y pestis would not survive long in the environment after an aerosolized release. It is non–spore forming, unlike *B anthracis*, and is sensitive to sunlight and heating.[54] A World Health Organization analysis estimated that a plague aerosol would be infectious for as long as 1 hour in a worst-case scenario.[54] These characteristics would probably keep intentional release of *Y pestis* relatively contained. For these reasons, an expert committee has not recommended widespread empiric use of antibiotics if *Y pestis* is used in a bioterrorist attack, but rather careful clinical follow-up, with institution of appropriate antibiotics at the first sign of clinical illness.[54] As detailed, postexposure prophylaxis would be indicated for people with a known aerosol exposure such as people within 6 feet of an untreated person with pneumonic plague, or people known to be exposed directly during an aerosol release. Although the ability of *Y pestis* to cause panic makes it an attractive agent for bioterrorism, its inability to form spores and inability to survive long-term in the environment limit its usefulness as an agent of bioweaponry.

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Supported by grants AI053442 and AI40725 from the National Institute of Allergy and Infectious Diseases, and International Collaborations in Infectious Disease Research Award No. HD39165 from the National Institute of Child Health and Human Development, Bethesda, MD.

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References

1. Perry RD, Fetherston JD. *Yersinia pestis*: etiologic agent of plague. *Clin Microbiol Rev.* 1997;10:35-66.

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DOI: 10.1309/DQM93R8QNQWBFYU8

2. Achtman A, Zurth K, Morelli G, et al. *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. *Proc Natl Acad Sci U S A*. 1999;96:14043-14048.
3. Gross L. How the plague bacillus and its transmission through fleas were discovered: reminiscences from my years at the Pasteur Institute in Paris. *Proc Natl Acad Sci U S A*. 1995;92:7609-7611.
4. Smego RA, Frean J, Koornhof HJ. Yersiniosis I: microbiological and clinicoepidemiological aspects of plague and non-plague *Yersinia* infections. *Eur J Clin Microbiol Infect Dis*. 1999;18:1-15.
5. World Health Organization. Plague. In: *WHO Report on Global Surveillance of Epidemic-Prone Infectious Diseases 2000*. Geneva, Switzerland: World Health Organization; 2000:chap 3. Available at http://www.who.int/emc-documents/surveillance/whocdscsrisr20001c.html. Accessed February 13, 2003.
6. Crook LD, Tempest B. Plague: a clinical review of 27 cases. *Arch Intern Med*. 1992;152:1253-1256.
7. Hull HF, Montes JM, Mann JM. Septicemic plague in New Mexico. *J Infect Dis*. 198;155:113-118.
8. Von Reyn CF, Weber NS, Tempest B, et al. Epidemiologic and clinical features of an outbreak of bubonic plague in New Mexico. *J Infect Dis*. 1977;136:489-494.
9. Butler T, Levin J, Linh NN, et al. *Yersinia pestis* infection in Vietnam, II: quantitative blood cultures and detection of endotoxin in the cerebrospinal fluid of patients with meningitis. *J Infect Dis*. 1976;133:493-499.
10. Franz DR, Jahrling PB, Friedlander AM, et al. Clinical recognition and management of patients exposed to biological warfare agents. *JAMA*. 1997;278:399-411.
11. Craven RB, Maupin GO, Beard ML, et al. Reported cases of human plague infections in the United States, 1970-1991. *J Med Entomol*. 1992;152:1253-1256.
12. Doll JM, Zeitz PS, Ettestad P, et al. Cat-transmitted fatal pneumonic plague in a person who traveled from Colorado to Arizona. *Am J Trop Med Hyg*. 1994;51:109-114.
13. Gasper PW, Barnes AM, Quan TJ. Plague (*Yersinia pestis*) in cats: description of experimentally induced disease. *J Med Entomol*. 1993;30:20-26.
14. Cover TL, Aber RC. *Yersinia enterocolitica*. *N Engl J Med*. 1989;321:16-24.
15. Brubaker RR. The genus *Yersinia*: biochemistry and genetics of virulence. *Curr Top Microbiol Immunol*. 1972;57:111-158.
16. Koornhof HJ, Smego RA, Nicol M. Yersiniosis II: the pathogenesis of *Yersinia* infections. *Eur J Clin Microbiol Infect Dis*. 1999;18:87-112.
17. Mortlock RP, Brubaker RR. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities of *Pasteurella pestis* and *Pasteurella pseudotuberculosis*. *J Bacteriol*. 1962;84:1122-1123.
18. Hillier S, Charnetzky WL. Glyoxalate bypass enzymes in *Yersinia* species and multiple forms of isocitrate lyase in *Yersinia pestis*. *J Bacteriol*. 1982;145:452-458.
19. Brubaker RR. Factors promoting acute and chronic diseases caused by *Yersinia*. *Clin Microbiol Rev*. 1991;4:309-324.
20. Dreyfus LA, Brubaker RR. Consequences of aspartate deficiency in *Yersinia pestis*. *J Bacteriol*. 1978;136:757-764.
21. Guiyoule A, Grimont F, Iteman I, et al. Plague pandemics investigated by ribotyping of *Yersinia pestis* strains. *J Clin Microbiol*. 1994;32:634-641.
22. Adair DM, Worsham PL, Hill KK, et al. Diversity in a variable-number tandem repeat from *Yersinia pestis*. *J Clin Microbiol*. 2000;38:1516-1519.
23. Guiyoule A, Rasoamanana B, Buchreiser P, et al. Recent emergence of new variants of *Yersinia pestis* in Madagascar. *J Clin Microbiol*. 1997;35:2826-2833.
24. Mahillon J, Chandler M. Insertion sequences. *Microbiol Mol Biol Rev*. 1998;62:725-774.
25. Smith CL, Condemine G. New approaches for physical mapping of small genomes. *J Bacteriol*. 1990;172:1167-1172.
26. Huang X, Chu MC, Engelthaler DM, et al. Genotyping of a homogeneous group of *Yersinia pestis* strains isolated in the United States. *J Clin Microbiol*. 2002;40:1164-1173.
27. Parkhill J, Wren BW, Thomson NR, et al. Genome sequence of *Yersinia pestis*, the causative agent of plague. *Nature*. 2001;413;523-527.
28. Kukkonen M, Lahteenmaki K, Suomalainen M, et al. Protein regions important for plasminogen activation and inactivation of α_2-antiplasmin in the surface protease Pla of *Yersinia pestis*. *Mol Microbiol*. 2001;40:1097-1111.
29. Buchrieser C, Rusniok C, Frangeul L, et al. The 102-kilobase *pgm* locus of *Yersinia pestis*: sequence analysis and comparison of selected regions among different *Yersinia pestis* and *Yersinia pseudotuberculosis* strains. *Infect Immun*. 1999;67:4851-4861.
30. McDonough KA, Hare JM. Homology with a repeated *Yersinia pestis* DNA sequence IS100 correlates with pesticin sensitivity in *Yersinia pseudotuberculosis*. *J Bacteriol*. 1997;179:2081-2085.
31. Bercovier H, Mollaret HH, Alonso JM, et al. Intra- and interspecies relatedness of *Yersinia pestis* by DNA hybridization and its relationship to *Y pseudotuberculosis*. *Curr Microbiol*. 1980;4:225-229.
32. Moore RL, Brubaker RR. Hybridization of deoxyribonucleotide sequences of *Yersinia enterocolitica* and other selected members of Enterobacteriaceae. *Int J Syst Bacteriol*. 1975;25:336-339.
33. Ibrahim A, Goebel BM, Liesack W, et al. The phylogeny of the genus *Yersinia* based on 16S rDNA sequences. *FEMS Microbiol Lett*. 1994;114:173-177.
34. Prentice MB, James KD, Parkhill J, et al. *Yersinia pestis* pFra shows biovar-specific differences and recent common ancestry with *Salmonella enterica* serovar Typhi plasmid. *J Bacteriol*. 2001;183:2586-2594.
35. Dawes H. *Yersinia pestis:* sequence sheds light on the plague's past. *Curr Biol*. 2001;11:R949-R951.
36. Hinnebusch BJ. Bubonic plague: a molecular genetic case history of the emergence of an infectious disease. *J Mol Med*. 1997;75:645-652.
37. Reed WP, Palmer DL, Williams RC Jr, et al. Bubonic plague in the Southwestern United States: a review of recent experience. *Medicine (Baltimore)*. 1970;49:465-486.
38. Hinnebusch BJ, Rudolph AE, Cherepanov P, et al. Role of *Yersinia* murine toxin in survival of *Yersinia pestis* in the midgut of the flea vector. *Science*. 2002;296;733-735.
39. Straley SC, Perry RD. Environmental modulation of gene expression and pathogenesis in *Yersinia*. *Trends Microbiol*. 1995;3:310-317.
40. Cornelis GR. *Yersinia* type III secretion: send in the effectors. *J Cell Biol*. 2002;158:401-408.
41. Cornelis GR. Molecular and cell biology aspects of plague. *Proc Natl Acad Sci U S A*. 2000;97:8778-8783.

DOI: 10.1309/DQM93R8QNQWBFYU8
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42. Cornelis GR, Boland A, Boyd AP, et al. The virulence plasmid of *Yersinia*, an antihost genome. *Microbiol Mol Biol Rev*. 1998;62:1315-1352.
43. Shea JE, Hensel M, Gleeson C, et al. Identification of a virulence locus encoding a second type III secretion system in *Salmonella typhimurium*. *Proc Natl Acad Sci U S A*. 1996;93:2593-2597.
44. Weeks S, Hill J, Friedlander A, et al. Anti-V antigen antibody protects macrophages from *Yersinia pestis*–induced cell death and promotes phagocytosis. *Microb Pathog*. 2002;32:227-237.
45. Cornelis GR. The *Yersinia* deadly kiss. *J Bacteriol*. 1998;180:5495-5504.
46. Nakajima R, Motin VL, Brubaker RR. Suppression of cytokines in mice by protein A–V antigen fusion peptide and restoration of synthesis by active immunization. *Infect Immun*. 1995;63:3021-3029.
47. Welkos S, Friedlander A, McDowell D. V antigen of *Yersinia pestis* inhibits chemotaxis. *Microb Pathog*. 1998;24:185-196.
48. Petterson J, Holmstrom A, Hill J, et al. The V-antigen of *Yersinia* is surface exposed before target cell contact and involved in virulence protein translocation. *Mol Microbiol*. 1999;32:961-976.
49. Fields KA, Nilles ML, Cowen C. Virulence role of V antigen of *Yersinia pestis* at the bacterial surface. *Infect Immun*. 1999;67:5395-5408.
50. Guarner J, Shieh W, Greer PW, et al. Immunohistochemical detection of *Yersinia pestis* in formalin-fixed, paraffin-embedded tissue. *Am J Clin Pathol*. 2002;117:205-209.
51. Raoult D, Aboudharam G, Crubezy E, et al. Molecular identification by "suicide PCR" of *Yersinia pestis* as the agent of medieval black death. *Proc Natl Acad Sci U S A*. 2000;97:12800-12803.
52. Motin VL, Georgescu AM, Elliot JM, et al. Genetic variability of *Yersinia pestis* isolates as predicted by PCR-based IS100 genotyping and analysis of structural genes encoding glycerol-3-phosphate dehydrogenase (*glpD*). *J Bacteriol*. 2002;184:1019-1027.
53. Infectious Diseases Society of America. Plague. Available at http://www.idsociety.org/bt/biotemplate.cfm?template=pl_summary.htm. Accessed March 21, 2003.
54. Inglesby TV, Dennis DT, Henderson DA, et al. Plague as a biological weapon: medical and public health management. *JAMA*. 2000;283:2281-2290.
55. Williamson ED. Plague vaccine and development. *J Appl Microbiol*. 2001;91:606-608.
56. Friedlander AM, Welkos SL, Worsham PL, et al. Relationship between virulence and immunity as revealed in recent studies of the F1 capsule of *Yersinia pestis*. *Clin Infect Dis*. 1995;21(suppl 2):S178-S181.
57. Williamson ED, Eley SM, Stagg AJ, et al. A sub-unit vaccine elicits IgG in serum, spleen cell cultures and bronchial washings and protects immunized animals against pneumonic plague. *Vaccine*. 1997;15:1079-1084.
58. Leary SEC, Griffin KF, Garmory HS, et al. Expression of an F1/V fusion protein in attenuated *Salmonella typhimurium* and protection of mice against plague. *Microb Pathog*. 1997;23:167-179.
59. Titball RW, Williamson ED. Vaccination against bubonic and pneumonic plague. *Vaccine*. 2001;19:4175-4184.
60. Anderson GW Jr, Heath DG, Bolt CR, et al. Short- and long-term efficacy of single-dose subunit vaccines against *Yersinia pestis* in mice. *Am J Trop Med Hyg*. 1998;58:793-799.

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DOI: 10.1309/DQM93R8QNQWBFYU8