Chapter 10

PLAGUE

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INTRODUCTION

Plague, a severe febrile illness caused by the gram-negative bacterium *Yersinia pestis*, is a zoonosis usually transmitted by fleabites. It is foremost a disease of rodents; more than 200 species of mammals have been reported to be infected with *Y pestis*, but maintenance of plague in nature relies almost exclusively on a smaller number of rodent species and their fleas.[1,2] Humans most often become infected by fleabites during an epizootic event; less frequently they are exposed to blood or tissues of infected animals (including ingestion of raw or undercooked meat) or aerosol droplets containing the organism.[1,3] Humans or animals with plague pneumonia, particularly cats, can generate infectious aerosols.[4,5] The resulting primary pneumonic plague is the most severe and frequently fatal form of the disease. Pneumonic plague is of particular concern to the military because it can also be acquired from artificially generated aerosols.

In the 6th, 14th, and 20th centuries *Y pestis* was the cause of three great pandemics of human disease. The bubonic form of the disease in humans is characterized by the abrupt onset of high fever, painful local lymphadenopathy draining the exposure site (ie, a bubo, the inflammatory swelling of one or more lymph nodes, usually in the groin, axillary, or cervical regions; the confluent mass of nodes, if untreated, may suppurate), and bacteremia. Septicemic plague can ensue from untreated bubonic plague or by passage of bacteria directly into the bloodstream bypassing the local lymph nodes. Patients with the bubonic and septicemic forms of the disease may develop secondary pneumonic plague, which can lead to human-to-human spread by the respiratory route. Cervical lymphadenitis has been noted in several human plague cases, including many fatal cases, and is often associated with the septicemic form of the disease. However, it is possible that these patients were exposed by the oral/aerosol route and developed pharyngeal plague that progressed into a systemic infection and cervical lymphadenitis.[1,6–8] According to Dr Kenneth Gage with the Centers for Disease Control and Prevention (CDC) laboratory in Fort Collins, Colorado, in an email in 2006, cervical lymphadenopathy, which is more common in patients from developing countries, may result from fleabites on the neck or face while sleeping on the dirt floors of heavily flea-infested buildings.

During the past 4 millennia, plague has played a role in many military campaigns. During the Vietnam War, plague was endemic among the native population, but US soldiers were relatively unaffected. The protection of troops was largely attributable to the US military's understanding of the rodent reservoirs and flea vectors of disease, the widespread use of a plague vaccine during the war, and prompt treatment of plague victims with effective antibiotics. Mortality from endemic plague continues at low rates throughout the world despite the availability of effective antibiotics. Deaths resulting from plague occur not because the bacilli have become resistant but, most often, because physicians do not include plague in their differential diagnosis, or because treatment is absent or delayed.

To be best prepared to treat plague in soldiers who are affected by endemic disease or a biological agent attack, military healthcare providers must understand the natural mechanisms by which plague spreads between species, the pathophysiology of disease in humans, and the diagnostic information necessary to begin treatment with effective antibiotics. No vaccine is available for plague in the United States, although candidates are undergoing clinical trials.

The US military's concern with plague is both as an endemic disease and as a biological warfare threat. A better understanding of the preventive medicine aspects of the disease will aid in the prompt diagnosis and effective treatment necessary to survive an enemy attack of plague and protect military and civilian personnel in plague endemic areas where military operations are underway.

Key terms in this chapter include enzootic and epizootic. Enzootic refers to when plague is present in a small number of animals; the host, vector, and bacterium live in an apparent equilibrium in which mortality among rodent hosts is difficult to detect and not obviously resulting from plague. During an epizootic, which typically follows a longer period of enzootic maintenance, widespread plague infections frequently lead to death among susceptible host populations (ie, equivalent to an epidemic in a human population), an event that is often noticeable to residents in affected areas. The death of a rodent causes the living fleas to leave that host and seek other mammals, and when those other mammals die in large numbers, they may seek humans. Knowledge of these two concepts of enzootic and epizootic will help to clarify how and when humans may be infected, in endemic or biological warfare scenarios.

HISTORY

The Justinian Plague (The First Pandemic)

Procopius gave us the first identifiable description of epidemic plague in his account of the plague of the Byzantine Empire during the reign of Justinian I (541–542 CE [the common era]), which we now consider to be the first great pandemic of the Common Era.[9] At the height of the epidemic, more than 10,000 people died each day. As many as one hundred million Europeans died, including 40% of Constantinople's population.[10,11] Repeated, smaller epidemics followed this plague into the 8th century.[12] Recently, *Y pestis* was definitively determined to have caused this pandemic based on extraction of plague-specific DNA from the dental pulp of plague victim skeletons in Germany. DNA fingerprinting strongly suggests China as the source of this First Pandemic.[13]

The Black Death (The Second Pandemic)

The second plague pandemic, known as the Black Death, brought the disease into the collective memory of Western civilization.[12] Plague bacilli probably entered Europe via the trans-Asian Silk Road during the early 14th century in fleas on the fur of marmots (a rodent of the genus *Marmota*). When bales of these furs were opened in Astrakhan and Saray, hungry fleas jumped from the fur seeking the first available blood meal, often a human leg.[12,14,15] In 1346, plague arrived in Caffa (modern Feodosiya, Ukraine) on the Black Sea. Caffa's large rat population helped spread the disease as they were carried on ships bound for major European ports such as Pera, a suburb of Constantinople, and Messina, in Sicily. By 1348, plague had already entered Great Britain at Weymouth.[9]

The Black Death probably killed 24 million people between the years 1346 and 1352 and perhaps another 20 million by the end of the 14th century.[10] However, some believe that the plague persisted through 1750, following a final foray into Marseilles in 1720. During the 15th through the 18th centuries, 30% to 60% of the populations of major cities, such as Genoa, Milan, Padua, Lyons, and Venice, died of plague.[15] Debate has raged for decades about the cause of the Black Death. Some believed that a viral hemorrhagic fever or unknown agent caused the Black Death instead of *Y pestis*. *Y pestis* was only recently proven to have caused the Black Death based on finding *Y pestis* DNA and F1 capsular antigen in the dental pulp of plague victims buried in mass graves in England, France, Germany, Italy, and the Netherlands, and later characterization of the genome of a *Y pestis* strain in DNA extracted from the skeletal material in a cemetery where Black Death victims had been buried in England.[16]

Failing to understand the plague's epidemiology and etiology, physicians could offer no effective treatment. Physicians at the University of Paris theorized that a conjunction of the planets Saturn, Mars, and Jupiter at 1:00 PM on March 20, 1345, corrupted the surrounding atmosphere, which led to the plague.[10] Physicians recommended a simple diet; avoidance of excessive sleep, exercise, and emotion; regular enemas; and abstinence from sexual intercourse.[17] Although some people killed cats and dogs because they were thought to carry disease, rats seemed to escape attention.[10] Christians blamed plague on Muslims, Muslims blamed it on Christians, and both Christians and Muslims blamed it on Jews or witches.[12]

In 1666, a church rector in Eyam, Derbyshire, England, persuaded the whole community to quarantine itself when plague erupted, but this was the worst possible solution because the people then remained close to the infected rats and/or fleas. The city experienced virtually a 100% attack rate with 72% mortality. The average mortality for the Black Death was consistently 70% to 80%.[12,18]

Contemporary observers such as Giovanni Boccaccio (1313–1375), who wrote in his *Decameron*, provided accurate clinical descriptions of the Black Death:

> The symptoms were not the same as in the East, where a gush of blood from the nose was a plain sign of inevitable death, but it began both in men and women with certain swellings [buboes] in the groin or under the armpit. They grew to the size of a small apple or an egg, more or less, and were vulgarly called tumours. In a short space of time these tumours spread from the two parts named all over the body. Soon after this, the symptoms changed and black or purple spots appeared on the arms or thighs or any other part of the body, sometimes a few large ones, sometimes many little ones.[19]

Marchione di Coppo Stefano Buonaiuti (1327–1385) wrote in his memoir about the Black Death in Florence:

> In the year of our Lord 1348 there occurred in the city and contado of Florence a great pestilence and such was its fury and violence that in whatever household it took hold, whosoever took care of the sick, all the carers died of the same illness, and almost nobody survived beyond the fourth day, neither doctors nor medicine proving to any avail . . . those symptoms were as follows: either between the thigh and the body, in the groin region, or under the armpit, there

> appeared a lump, and a sudden fever, and when the victim spat, he spat blood mixed with saliva, and none of those who spat blood survived. Such was the terror this caused that seeing it take hold in a household, as soon as it started, nobody remained: everybody abandoned the dwelling in fear, and fled to another; some fled into the city and others into the countryside . . . sons abandoned fathers, husbands wives, wives husbands, one brother the other, one sister the other. The city was reduced to bearing the dead to burial . . ..[20]

Some writers described bizarre neurological disorders (which led to the term "dance of death"), followed by anxiety and terror, resignation, blackening of the skin, and death. The sick emitted a terrible stench: "Their sweat, excrement, spittle, breath, [were] so foetid as to be overpowering" [in addition, their urine was] "turbid, thick, black, or red."[10]

The second great pandemic slowly subsided in Europe by 1750. The pandemic's decline has been attributed to the replacement of the black rat (*Rattus rattus*) in the area by the Norwegian rat (*Rattus norvegicus*), which is a less efficient host; natural vaccination of animals and/or humans by other *Yersinia* species or by less virulent *Y pestis* strains; and other less plausible hypotheses. The theories are all flawed to some extent, and the disappearance of plague from Europe remains one of the great epidemiological mysteries.[3,8,21]

It is not known why plague spread so easily during the First and Second Pandemics and had such a high case-fatality rate. Based on an analysis of *Y pestis* genomes from Black Death victims, no significant differences existed between 14th century and 21st century strains of the bacterium.[22] The environment, vector dynamics, and host susceptibility likely contributed to the disease spread. Although there are legitimate reasons to question the Oriental rat flea's role in parts of northern Europe where it and black rats appeared absent, the human flea's role in transmitting plague to humans in these regions probably has been underemphasized, and could explain the rapid human-to-human spread during the Black Death.[23–25] It also is possible that human lice became vectors during pandemics. Even though lice are not normal vectors, they are capable of transmitting *Y pestis* infection in rabbits, and presumably humans.[26]

The Third Pandemic

The third, or modern, plague pandemic arose in 1894 in China and spread throughout the world as rats and their fleas traveled via modern transportation.[12,18] In 1894, both Shibasaburo Kitasato and Alexandre JE Yersin independently discovered the plague bacillus. However, it was Yersin who was able to satisfy Koch's postulates for the bubonic plague and his plague bacillus fits the characterization of *Y pestis*.[6] The reservoir of plague bacilli in the fleas of the Siberian marmot was likely responsible for the Manchurian pneumonic plague epidemic of 1910 through 1911, which caused 50,000 deaths.[27] The modern pandemic arrived in Bombay in 1898, and during the next 50 years, more than 13 million Indians died of rat-associated plague.[27–29]

The disease officially arrived in the United States in March 1900, when a plague-infected Chinese laborer's lifeless body was discovered in a hotel basement in San Francisco, California. The disease subsequently appeared in New York City and Washington state the same year.[30,31] The disease appeared in New Orleans, Louisiana, in 1924 and 1926.[31] The Texas Gulf Coast and Pensacola, Florida, also saw the influx of plague. Among these numerous events, only the outbreaks in California appear to have spread inland, leading to the establishment of permanent plague foci in native rodent and flea populations in the interior of the western United States.[32,33] Human plague in the United States was initially a result of urban rat and ground squirrel epizootics until 1925. After general rat control and hygiene measures were instituted in various port cities, urban plague vanished—only to spread into rural areas, where virtually all cases in the United States have been acquired since 1925.[34] Rodents throughout the western United States were probably infected from the San Francisco focus.

PLAGUE AND WARFARE

It is an axiom of warfare that battle casualties are much fewer than casualties caused by disease and nonbattle injuries.[35] *Y pestis* can initiate disease both through exposure to natural sources of infection, such as fleabites, and to a biological warfare agent. Medical officers need to distinguish cases likely acquired from natural sources in a plague-endemic region from those occurring following exposure to a biological warfare agent.

Endemic Disease

Plague has also afflicted armies in more recent times. In 1745, Frederick the Great's troops were devastated by plague. Catherine the Great's troops returned from the Balkans with plague in 1769 through 1771. French military operations in Egypt were significantly impeded by plague in 1798, which caused them to abandon their attack on Alexandria. The modern pandemic

began in China when its troops were deployed in an epidemic plague area to suppress a Muslim rebellion. Military traffic is responsible for the rapid plague spread to nearly every country in Asia.[27]

Endemic plague has not been a source of disease and nonbattle injuries for the US military since the mid 20th century. During World War II and the Vietnam War, US military forces were almost free of plague, although civilian populations in certain areas were subjected to plague outbreaks in both of these wars. However, plague remains on and near military bases in the southwestern United States because the local mammal populations are reservoirs of infection, and it may be found in many areas around the world where US military forces are deployed.

World War II

Endemic plague became established in Hawaii (on the islands of Hawaii and Maui) in December 1899. However, no evidence of the disease in either rodents or humans has been found on Oahu or Kauai since the first decade of the 20th century. A "small outbreak" occurred during World War II on the island of Hawaii (in 1943), but it was contained by strict rat control measures, which prevented any plague spread to military personnel during the war in the Pacific.[36] Official policy during World War II was to vaccinate US troops with the whole-cell killed plague vaccine. No troops contracted plague despite serving in known endemic areas.[36,37] Plague has since disappeared from Hawaii.

Vietnam War

Plague entered Vietnam in Nha Trang in 1898 and several pneumonic epidemics have occurred since then.[27,38,39] Cases were reported in Vietnam every year from 1898 to 2002, except during the Japanese occupation during World War II.[27,40] When French forces departed Vietnam after the Indochina War, public health conditions deteriorated and plague flourished. The reported plague incidence increased from eight cases in 1961 to 110 cases in 1963, and to an average of 4,500 cases from 1965 through 1969.[34,41–44] The mortality in clinically diagnosed cases was between 1% and 5%. In untreated individuals, it was much higher (60%–90%).[27,42] However, only eight American troops were affected (one case per 1 million human-years) during the Vietnam War.[44] The low infection rate in the US troops was attributed to insecticide use, vaccination of virtually all troops, and a thorough understanding of plague's epidemiology, which led to the insect repellent use, protective clothing, and rat-proofed dwellings.[27,42] During this period, knowledge of plague grew dramatically, in large part due to the work of two officers of the US Army Medical Service Corps, Lieutenant Colonel Dan C Cavanaugh and Lieutenant Colonel John D Marshall. These scientists studied plague ecology, related plague epidemics to weather, described the effects of high temperatures (>28°C) on the abilities of fleas to transmit plague, developed serologic tests for plague infection, and significantly contributed to the field of plague vaccinology.[27,45]

Disease Threat on US Military Installations

Human exposure to plague on military installations may occur when pets bring home infected rodents or fleas, at recreation areas with sick or dead rodents and their infected fleas, or at field training and bivouac sites. The consequences of plague at a military installation include morbidity and mortality of both humans and pets; loss of training and bivouac sites; large expenditures of money, personnel, and equipment to eliminate the plague risk; and the loss of recreation areas.[34] Plague risk has been identified on and near several US military installations (Exhibit 10-1). For a description of relevant rodent/flea complexes found in the United States, see the Epidemiology section.

Plague as a Biological Warfare Agent

The first known attempt at what is now called "biological warfare" is purported to have occurred at the Crimean port city of Caffa on the Black Sea in 1346 and 1347.[10,27] During the conflict between Christian Genoese sailors and Muslim Tatars, the Tatar army was struck with plague. The Tatar leader catapulted corpses of Tatar plague victims at the Genoese sailors. The Genoese became infected with plague and fled to Italy. However, the disease was most likely spread by the local population of infected rats, not by the corpses, because an infected flea leaves its host as soon as the corpse cools.[10]

The 21st century use of plague as a potential biological warfare weapon is the immediate concern of this chapter. Medical officers need to consider this use of plague, particularly if the disease appears in an unlikely setting.

World War II

During World War II, Japan established a secret biological warfare research unit (Unit 731) in Manchuria, where pneumonic plague epidemics occurred from 1910 through 1911, 1920 through 1921, and 1927; a cholera epidemic also spread in 1919. General Shiro Ishii, the physician leader of Unit 731, was fascinated

EXHIBIT 10-1

PLAGUE RISKS AT US MILITARY INSTALLATIONS*

Plague-infected animals on the installation; human case reported on post:
- Fort Hunter Liggett, California
- US Air Force Academy, Colorado†

Human case reported in the same county:
- Edwards Air Force Base, Colorado‡
- FE Warren Air Force Base, Wyoming
- Kirtland Air Force Base, New Mexico§
- Peterson Air Force Base, Colorado

Plague-infected animals on the installation:
- Dugway Proving Ground, Utah
- Fort Carson, Colorado
- Fort Ord, California
- Fort Wingate Army Depot Activity, New Mexico
- Marine Corps Mountain Warfare Training Center, Bridgeport, California
- Navajo Army Depot Activity, Arizona
- Pueblo Army Depot Activity, Colorado
- Rocky Mountain Arsenal, Colorado
- Vandenberg Air Force Base, California
- White Sands Missile Range, New Mexico

Plague-infected animals or fleas in the same county but not on the installation:
- Bridgeport Naval Facility, California
- Camp Roberts, California
- Dyess Air Force Base, Texas
- Fort Bliss, Texas
- Fort Lewis, Washington
- Sierra Army Depot, California
- Tooele Army Depot, Utah
- Umatilla Army Depot Activity, Oregon
- Nellis Air Force Base, Nevada

No plague-infected animals or fleas on the installation or in the county, but susceptible animals present:
- Fort Huachuca, Arizona

*Does not include military installations near Los Angeles and San Francisco, California, where urban plague cases and deaths were common in the first quarter of the 20th century; no plague cases have occurred in these urban areas since the mid 1920s.
†Fatality: 18-month-old child died of pneumonic plague; rock squirrels and their fleas had taken up residence in the ducts of the child's on-base house.
‡Two human cases in the same county in 1995; animal surveillance on base began in 1996.
§Plague-infected animals in the county in 1995; last human case in the county in 1993; no animal surveillance on base since 1986.
Data sources: (1) Harrison FJ. *Prevention and Control of Plague.* Aurora, CO: US Army Center for Health Promotion and Preventive Medicine, Fitzsimons Army Medical Center; September 1995: 3–8. Technical Guide 103. (2) Data collected from Preventive Medicine Officers on 30 military bases in the United States, March 1996.

by plague because it could create casualties disproportionate to the number of bacteria disseminated, the most dangerous strains could be used to make a lethal weapon, and its origins could be concealed to appear as a natural occurrence. Early experiments, however, demonstrated that aerial bomb dropping of bacteria had little effect because air pressure and high temperatures created by the exploding bombs killed nearly 100% of the bacteria.[46]

One of Ishii's more frightening experiments was his use of the human flea, *Pulex irritans*, as a stratagem to simultaneously protect the bacteria and target humans. This flea is resistant to air drag, naturally targets humans, and can infect a local rat population to prolong an epidemic. Spraying fleas from compressed-air containers was not successful because high-altitude release resulted in too much dispersion and aircraft had to fly low for safety. However, clay bombs solved these technical difficulties and resulted in an 80% survival rate of fleas.[46]

At 5:00 AM on a November morning in 1941, a lone Japanese plane made three low passes over the business center of Changteh, a city in the Hunan province. This area of China was not a plague endemic area. Although no bombs were dropped, a strange mixture of wheat and rice grains, pieces of paper, cotton wadding, and other unidentified particles were observed falling from the plane. Within 2 weeks, individuals in this same area began dying of plague. No individual who contracted plague had recently traveled outside Changteh. Unlike the zoonotic form of the disease that is typically observed, rat mortalities were not noted until months after the human cases. It was also observed that plague usually spreads with rice shipments (because rats infest the grain) along shipping routes, but the nearest plague epizootic was 2,000 km away by land or river. Furthermore, Changteh exported—rather than imported—rice. These unusual circumstances surrounding the plague outbreak suggest that it may have been of deliberate human origin.[46]

In another incident, on October 4, 1940, a Japanese plane dropped rice and wheat grains mixed with fleas over the city of Chuhsien, in Chekiang province. In November, bubonic plague appeared for the first time in the area where the particles had been dropped. Plague caused 21 deaths in 24 days. On October 27, 1940, a Japanese plane was seen releasing similar particles over the city of Ningpo, in Chekiang province. Two days later, bubonic plague occurred for the first time in that city, resulting in 99 deaths in 34 days. No epizootic disease or excessive mortality was found in the rat population.[46]

Since World War II

During the Korean War, allied forces were accused of dropping on North Korea insects that were capable of spreading plague, typhus, malaria, Japanese B encephalitis, and other diseases. However, no evidence exists to support such claims.[47]

In 1999, Dr Ken Alibek (Kanatjan Alibekov), a former Soviet army colonel and scientist, published a book titled *Biohazard* that illuminates the former Soviet Union's extensive biological weapons program.[48] Alibek describes the weaponization of *Y pestis* (including a powdered form) and the development of genetically engineered organisms, one of which was a *Yersinia* strain producing "myelin toxin" that induced both disease and paralysis in animal models. Alibek states that "In the city of Kirov, we maintained a quota of twenty tons of plague in our arsenal every year."[48] Although the accuracy of details presented in the memoir has been debated in some circles, the former Soviet Union had entire institutes devoted to the study of *Y pestis*.

Other state-sponsored or extremist group efforts to obtain *Y pestis* will likely occur. For example, in 1995, a white supremacist and microbiologist fraudulently purchased vials of lyophilized *Y pestis* from the American Type Culture Collection.[49,50] The intended use of these organisms was never determined but it caused alarm and led to legislation requiring that the transfer of disease causing pathogens be reported (CDC Select Agent Program).[49,50]

THE INFECTIOUS AGENT

Taxonomy

Y pestis, the causative agent of plague, is a gram-negative coccobacillus belonging to the family *Enterobacteriaceae*. The genus was named in honor of Alexandre Yersin, the scientist who originally isolated *Y pestis* during a plague outbreak in Hong Kong in 1894; the species name *pestis* is derived from the Latin for plague or pestilence. Previous designations for this species have included *Bacterium pestis*, *Bacillus pestis*, *Pasteurella pestis*, and *Pesticella pestis*.[51] This species is closely related to two other pathogens of the genus *Yersinia*: *Y pseudotuberculosis* and *Y enterocolitica*. The extensive genetic similarity (>90%) between *Y pseudotuberculosis* and *Y pestis* led to a recommendation that *Y pestis* be reclassified as a subspecies of *Y pseudotuberculosis*.[52] This proposal was not well received, primarily because of the fear that this change in nomenclature would increase the potential for laboratory-acquired plague infections. The most recent molecular fingerprinting analysis of *Y pestis* suggests that this pathogen arose from *Y pseudotuberculosis* through microevolution over the past few millennia, during which the enzootic "pestoides" isolates evolved (see Biochemistry on next page). The pestoides strains appear to have split from *Y pseudotuberculosis* more than 10,000 years ago, followed by a binary split approximately 3,500 years later that led to the populations of *Y pestis* more frequently associated with human disease. The isolation of *Y pestis* "pestoides" from both Africa and Asia suggests that *Y pestis* spread globally long before the first documented plague (Justinian) in 784 CE.[53] Recent phylogenetic analyses suggest that among the *Enterobacteriaceae*, *Y pestis* is more closely related to insect and invertebrate-associated genera (such as *Photorhabdus*, *Serratia*, and *Sodalis*) than to vertebrate-associated genera like *Escherichia* and *Salmonella*.[54]

Morphology

The characteristic "safety pin" bipolar staining of this short bacillus (0.5–0.8 μm by 1.0–3.0 μm) is best seen with Wayson's or Giemsa stain (Figure 10-1). Depending on growth conditions, *Y pestis* can exhibit marked pleomorphism with rods, ovoid cells, and short chains present. A gelatinous capsule, known as the F1 antigen, is produced by the vast majority of strains at 37°C. *Y pestis* is nonmotile, unlike the other mammalian pathogens of the genus that produce peritrichous flagella at growth temperatures lower than 30°C.[51,55]

Growth Characteristics

Y pestis can grow at a broad range of temperatures (4°C–40°C) in the laboratory, with an optimal growth temperature of 28°C. Although *Y pestis* grows well on standard laboratory media, such as

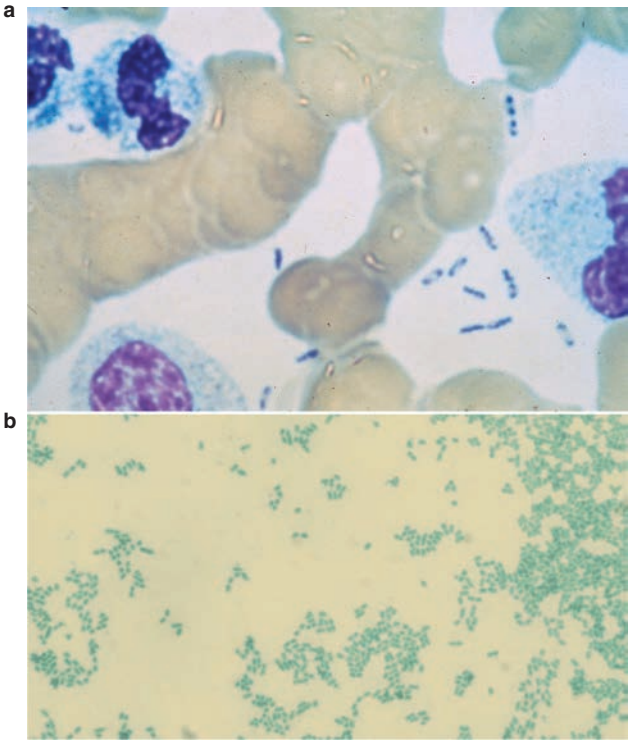

Figure 10-1. (**a**) This Wright-Giemsa stain of a peripheral blood smear from a patient with septicemic plague demonstrates the bipolar, safety-pin staining of *Yersinia pestis*. Gram's and Wayson's stains can also demonstrate this pattern. (**b**) Micrograph of the CO92 strain of *Y pestis* stained with Wayson's stain and examined by microscopy (original magnification × 100).
Photographs: (**a**) Courtesy of Kenneth L Gage, PhD, Centers for Disease Control and Prevention Laboratory, Fort Collins, Colorado. (**b**) Courtesy of Joel Bozue, PhD, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

sheep blood agar, MacConkey agar, or heart infusion agar, growth is slower than that of *Y pseudotuberculosis* or *Y enterocolitica*; more than 24 hours of incubation are required to visualize even pinpoint colonies. Appearance of colonies can be hastened by growth in an environment containing 5% carbon dioxide. The round, moist, translucent, or opaque colonies are nonhemolytic on sheep blood agar and exhibit an irregular edge (Figure 10-2). A fried egg appearance is common in older colonies and is more pronounced in certain strains. Long-term laboratory passage of *Y pestis* or short-term growth under less than optimal conditions is associated with irreversible genetic changes leading to attenuation. These changes include the deletion of a large chromosomal pathogenicity island that encodes factors necessary for growth in both the flea and the mammalian host and the loss of one or more virulence plasmids.[21,51,55] Strains to be archived should be grown at a low temperature and frozen promptly at –70°C.

Biochemistry

Y pestis is a facultative anaerobe, fermenting glucose with the production of acid. It is incapable of a long-term saprophytic existence, partly because of complex nutritional requirements, including a number of amino acids and vitamins. *Y pestis* also lacks certain enzymes of intermediary metabolism that are functional in the closely related but more rapidly growing species such as *Y enterocolitica* or *Y pseudotuberculosis*. *Y pestis* strains have traditionally been separated into three biovars, based on the ability to reduce nitrate (Nit+) and ferment glycerol (Gly+).[21] Some molecular methods of typing, such as ribotyping and restriction fragment-length polymorphisms of insertion sequence locations, support this division of strains.[56,57] Biovar orientalis (Gly^-, Nit^+), which is distributed worldwide and is responsible for the third (modern) plague pandemic, is the only biovar present in North and South America. Biovar antiqua (Gly^+, Nit^+), which is found in Central Asia and Africa, may represent the most ancient of the biovars.[21,53] Biovar mediaevalis (Gly^+/Nit^-) is geographically limited to the region surrounding the

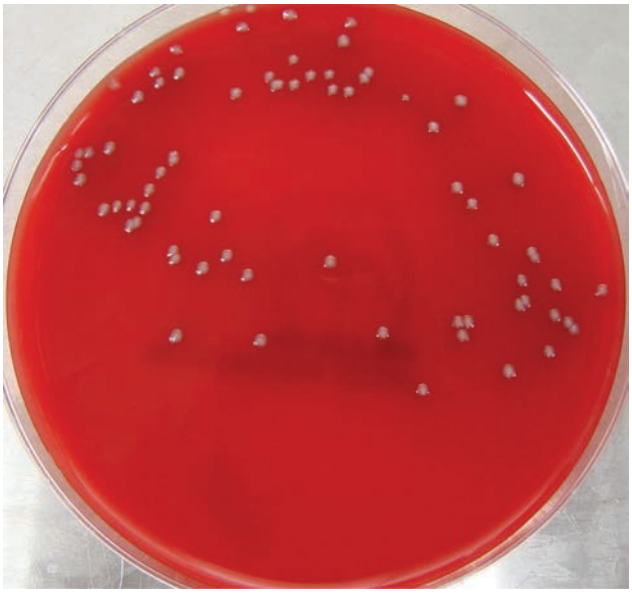

Figure 10-2. Growth of the CO92 strain of *Yersinia pestis* grown on a 5% sheep blood agar plate following 2 days of incubation at 28°C.
Photograph: Courtesy of Joel Bozue, PhD, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

Caspian Sea. No apparent differences in pathogenicity exist among the biovars.[21,58] Recently, three different multilocus molecular methods were used to investigate the microevolution of *Y pestis*.[56] Eight populations were recognized. An evolutionary tree for these populations rooted on *Y pseudotuberculosis* was proposed. The eight population groups do not correspond directly to the biovars; thus, it was suggested that future strain groupings be rooted in molecular typing. Four of the groups were made up of transitional strains of *Y pestis*, "pestoides," which exhibit biochemical characteristics of both *Y pestis* and *Y pseudotuberculosis*.[59] These isolates represent the most ancient of the *Y pestis* strains characterized to date.[53]

EPIDEMIOLOGY

During the modern pandemic, WG Liston, a member of the Indian Plague Commission (1898–1914), associated plague with rats and identified the rat flea as a vector.[27] Subsequently, more than 200 species of mammals and 150 species of fleas have been implicated in maintaining *Y pestis* endemic foci throughout the world, although only a relatively few species play a significant role in disease transmission and maintaining plague in nature.[34,60,61] *Y pestis* is not capable of establishing lasting infections in all flea species, and there appears to be variability in the ability of various flea species to transmit the organism.[58]

The oriental rat flea (*Xenopsylla cheopis*) has been largely responsible for spreading *Y pestis* during bubonic plague epidemics. Some researchers think it is the most efficient flea for transmitting plague.[9] However, this long-held belief has recently been challenged. After the flea ingests a blood meal from a bacteremic animal, bacilli can multiply and eventually block the flea's foregut, or proventriculus, with a biofilm mass containing bacteria as shown in Figure 10-3. When feeding, the flea ingests approximately 0.1 μl to 0.3 μl of blood during a blood meal. High-level bacteremia is a hallmark of *Y pestis* infection in susceptible hosts. This bacteremia provides a sizeable inoculum for the flea and promotes the subsequent infection, and in the case of *X cheopis* it likely leads to blockage of the proventriculus. This blockage limits feeding, resulting in repeated desperate attempts by the flea to feed. Because of the blockage, blood carrying *Y pestis* is regurgitated into the bite wounds, thus spreading the disease to new hosts. The blocked flea, also a victim of the disease, eventually starves to death.[2] As many as 24,000 organisms may be inoculated into the mammalian host.[27] This flea species desiccates rapidly in hot and dry weather when away from its hosts, but flourishes at humidity just above 65% and temperatures between 20°C and 26°C; in these conditions it can survive 6 months without a feeding.[27,34]

Interestingly, the belief that feeding by infectious blocked fleas represents the only means by which *Y pestis* can be transmitted efficiently has recently been challenged as a result of experiments with unblocked *Oropsylla* fleas.[24] These fleas rarely become blocked and were shown to transmit *Y pestis* better. The infected *Oropyslla montana* fleas became infectious within a day after feeding and remained infectious for at least 4 days. This timeframe is much shorter than the 2 weeks required for flea blockage to occur and allows *O montana* and certain other fleas to support the rapid plague spread during epizootics, even in the absence of proventricular blockage.

Although the largest plague outbreaks have been associated with *X cheopis*, all fleas should be considered dangerous in plague-endemic areas.[2,60] During the Black Death, the human flea, *Pulex irritans*, may have aided in human-to-human plague spread; during other epidemics, bedbugs (*Cimex lectularius*), lice, and flies were found to contain *Y pestis*.[9,62] However, the presence of plague bacilli in these latter insects is

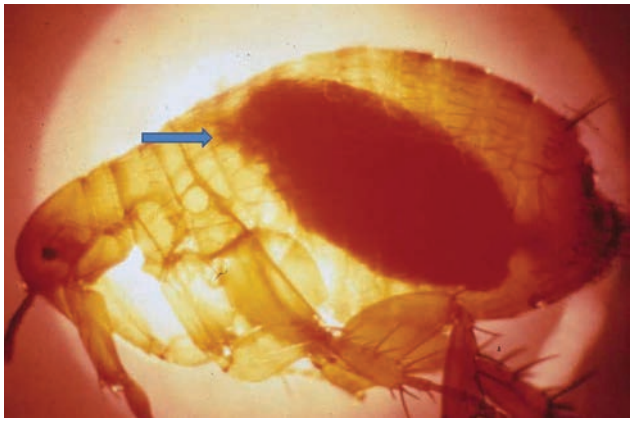

Figure 10-3. The oriental rat flea (*Xenopsylla cheopis*) has historically been most responsible for plague spread to humans. This flea has a blocked proventriculus (indicated at the arrow), the equivalent to a human's gastroesophageal region. In nature, this flea would develop a ravenous hunger because of its inability to digest the fibrinoid mass of blood and bacteria. The ensuing biting of the nearest mammal will clear the proventriculus through regurgitation of thousands of bacteria into the bite wound, thereby inoculating the mammal with the plague bacillus.
Photograph: Courtesy of Kenneth L Gage, PhD, Centers for Disease Control and Prevention Laboratory, Fort Collins, Colorado.

associated with ingestion of contaminated blood from plague victims, and they apparently had little or no role as vectors for the disease, although some have recently suggested lice may be important in some situations.[63] In one laboratory study, bedbugs were able to infect guinea pigs after feeding on a moribund *Y pestis*-infected mouse.[64] The most important vector of human plague in the United States is *O montana*, often the most common flea on rock squirrels and California ground squirrels,[34] although cases have been linked to infectious bites of other flea species, including those found on other ground squirrels, prairie dogs, chipmunks, and wood rats.

Throughout history, the black rat, *Rattus rattus*, has been most responsible worldwide for plague's persistence and spread in urban and most rural village epidemics. *R rattus* is a nocturnal, climbing animal that does not burrow, but instead nests overhead and lives close to humans.[9] In the United Kingdom and much of Europe, the brown rat, *R norvegicus*, has replaced *R rattus* as the dominant city rat.[65] Unlike *R rattus*, *R norvegicus* is essentially a burrowing animal that lives under farm buildings and in ditches. Although often considered less important than *R rattus* as a source of *Y pestis* infection, *R norvegicus* may be involved in both rural and urban plague outbreaks.[9] Most carnivores, except wild and domestic cats, are resistant to plague infection, but animals such as domestic dogs, all rodents, and burrowing owls may transport infected fleas into homes. However, in some instances individuals of normally resistant species, such as dogs, can experience serious illness.[66] For example, in 2009 a pneumonic plague outbreak occurred in China. The index case, which was a herdsman who contracted the disease from a sick dog,[67] was unique because it was the first known pneumonic plague case attributed to an infected dog (generally they are considered naturally resistant). Mammals that are partially resistant to plague infection (ie, consist of a mixture of individuals that are either resistant or susceptible to plague-induced mortality) are continuous plague reservoirs. Some epidemiologists propose that the true plague hosts are rodent species with populations consisting of both sensitive and resistant individuals, while others have questioned the need for resistant individuals to maintain plague foci.[68] In the United States, *Cynomous* species (prairie dogs) and *Spermophilus* species (rock squirrels and ground squirrels) are most often associated with plague activity because of the high mortality they often experience during epizootics. A variety of susceptible mammals, such as chipmunks, tree squirrels, cottontail rabbits, ferrets, and domestic cats, are occasionally infected. Epizootic spread among tree squirrels in Denver, Colorado, in the 1960s resulted in the first urban plague case since the 1920s.[60] A more recent epizootic in Denver, Colorado, also involving tree squirrels, occurred in the summer of 2007.[69]

Although not associated with any human plague cases, the appearance of two infected fox squirrels in Dallas, Texas, in 1993 also caused considerable concern.[69,70] An increasing number of human infections has been associated with domestic cats, usually through bites, contact with tissues, suppurating buboes, or aerosol rather than by flea transmission.[4,5] Cats appear to be particularly efficient at transmitting disease to humans.[4,5]

Highly susceptible animals amplify both flea populations and bacilli within their bloodstreams and often support the spread of epizootics, especially when these animals occur at high densities.[71] In many developing countries, these epizootics often involve commensal rat species (*Rattus*) and potential human exposure to infectious rat fleas. In the United States, such epizootics occur in chipmunks, ground squirrels, and wood rats, but especially in prairie dogs, rock squirrels (*Spermophilus variegatus*), and California ground squirrels (*Spermophilus beechyi*). Although prairie dog fleas rarely bite humans, they have been sources of infection for humans, who can acquire the disease by handling infected prairie dogs. Rock squirrels and California ground squirrels both infect humans via direct contact and fleas.[34,72,73] Many other mammals in the United States harbor plague, and a few, including wild carnivores, have served as infection sources for humans (Exhibit 10-2). In 2007, a National Park Service wildlife biologist died of primary pneumonic plague resulting from *Y pestis*, likely contracted from a necropsy

EXHIBIT 10-2

MAMMALS KNOWN TO HARBOR PLAGUE IN THE UNITED STATES

Carnivores	Black bears, cats (including bobcats and mountain lions), coyotes, dogs, foxes, martens, raccoons, skunks, weasels, wolverines, wolves
Rodents	Chipmunks, gophers, marmots, mice, prairie dogs, rats, squirrels, voles
Lagomorphs	Hares, rabbits
Hooved Stock	Pigs, mule deer, pronghorn antelope

Adapted from Harrison FJ. *Prevention and Control of Plague*. Aurora, CO: US Army Center for Health Promotion and Preventive Medicine, Fitzsimons Army Medical Center; September 1995: 25–28. Technical Guide 103.

performed on a dead mountain lion found within Grand Canyon National Park.[74] Thinking that the mountain lion died from trauma, the biologist did not protect himself while removing the lion's skin and skull while performing the necropsy in his garage.

In the United States, where human plague cases are likely to be associated with exposures to native rodents and their fleas rather than rats and rat fleas, knowledge of local host species is critical because certain mammal-flea complexes are particularly dangerous: these complexes consist of both a susceptible mammal genus or species and one or more associated fleas known to bite humans. More than one host-epizootic complex can occur in a given area. These pairings can include the following[34,61]:

- the rock squirrel (*S variegatus*) of the Southwest and the California ground squirrel (*S beechyi*) of California and bordering regions that both are host to the flea *O montana*, which is known to readily bite humans;
- the antelope ground squirrel (*Ammospermophilus leucurus*) and the flea *Thrassus bacchi* of the Southwest;
- the prairie dogs (*Cynomys species*) of the Great Plains, Rocky Mountains, and Colorado plateau region and the flea *Opisochrostis hirsutus*;
- the Wyoming ground squirrel (*Spermophilus elegans*, formerly known as *S richardsoni*) of Colorado and Wyoming or the golden-mantled ground squirrel (*S lateralis*) of the Rocky Mountains and Sierras, and the fleas *Opisochrostis labis*, *Opisochrostis idahoensis*, or *Thrassus bacchi*; and
- various wood rat species (*Neotoma sp*) found across the West and the fleas *Orchopeas sexdentatus* and *Orchopeas neotomae*.

Plague exists in one of two states in nature: enzootic or epizootic. An enzootic cycle is a stable rodent–flea infection cycle presumably occurring in a relatively resistant host population that experiences low mortality. Enzootic maintenance cycles also appear to occur in some areas in more susceptible populations when the animals occur in separate patches or colonies and transmission among them is delayed to some extent by geographical barriers, seasonal changes, or other means.[68,75] In an enzootic cycle, rodent mortality is limited and the fleas have less need to seek alternative hosts, such as humans. During an epizootic, however, plague bacilli also infect moderately or highly susceptible mammals, and infections spread quickly causing high mortality. High mortality occurs, most conspicuously in larger colonial rodents, such as prairie dogs, but it can occur among animals of the relatively resistant rodent populations presumed to be involved in the enzootic cycle, although this tends to be less noticeable.[1] These epizootics are most common when host populations are dense. Evidence has been presented that epizootics and the frequency of human cases are influenced not only by host density but also by climatic variables.[75] Humans are accidental hosts in the plague cycle and are not necessary for the persistence of the organism in nature (Figure 10-4).

Humans typically acquire plague via infectious bites of fleas whose natural host is another mammal, usually a rodent. Infection via flea feces inoculated into skin with bites may also occur, but this mode of transmission is not considered important compared to direct inoculation of the plague bacilli into the feeding site through the flea's mouthparts. Less common infection sources include infectious human fleas, contact with tissues or body fluids from an infected animal, consumption of infected tissues, handling of contaminated pelts, and respiratory droplet transmission from animals with pneumonic disease.[1,3,27,60,61] Fleas removed by humans during the grooming behavior practiced in some cultures are sometimes killed when the person doing the grooming bites the flea, which can squirt the flea's gut contents and viable *Y pestis* into the mouth and pharynx, an act that has been implicated in some cases of plague.[62] The greatest risk to humans occurs when large concentrations of people live under unsanitary conditions in close proximity to large commensal or wild rodent populations that are infested with fleas that bite both humans and rodents.[27]

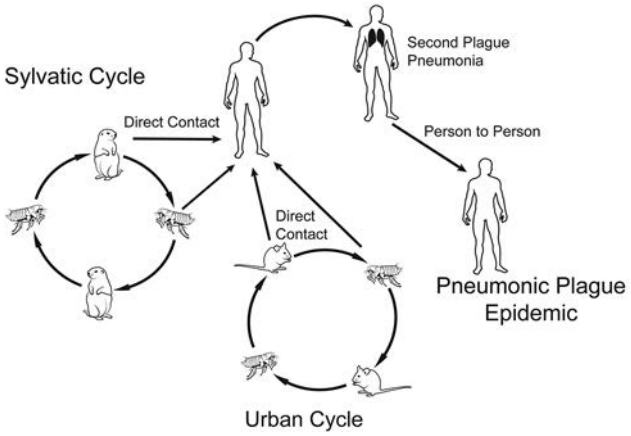

Figure 10-4. This drawing shows the usual, occasional, and rare routes by which plague has spread between various mammals and humans.
Courtesy of William Discher, US Army Medical Research Institute of Infectious Diseases, Visual Information Office, Fort Detrick, Maryland.

Human-to-human plague transmission can occur from patients with pulmonary infection and cough. However, the understanding of pneumonic plague is incomplete. Most large pneumonic epidemics have occurred in cool climates with moderate humidity and close contact between susceptible individuals. Pneumonic plague outbreaks have been rare in tropical climates even during bubonic disease. The role of particle size in efficiency of transmission is unknown, although it may occur more efficiently via larger respiratory droplets or fomites rather than via small-particle aerosols.[76]

Only the pneumonic form of plague can spread between humans. The risk of person-to-person plague transmission via infectious respiratory droplets is lower than once believed. A pneumonic plague outbreak in Madagascar resulting from an index case with secondary pneumonic plague infected 18 individuals and killed 8 of them.[77] However, once the outbreak's cause was determined and appropriate measures were taken, including avoidance of severely ill persons with cough, no person developed infection. Of 154 contacts of these patients who understood the risk, only 8.4% (13/154) developed antibodies to F1 antigen; few were symptomatic and then only had pharyngitis.[77]

Pneumonic plague patients typically transmit disease only several hours before death when they cough up copious amounts of bloody sputum full of bacteria, and then only to individuals who approach them within 1 to 2 meters.[78] The initial pneumonic plague cough is dry. During the Manchurian pneumonic plague epidemics in the first half of the 20th century, prolonged and close contact with end-stage patients were necessary to transmit disease; layered cotton and gauze masks were effective transmission barriers.[78]

A physician with 20 years of experience who cared for 400 to 500 patients with pneumonic plague reportedly has never seen a healthcare worker develop plague from these patients.[78] This record has been attributed to maintaining well-ventilated wards, having patients cough away from healthcare workers during examinations, and limiting time spent close to patients. Most workers in this situation did not have protective masks.

No human-to-human plague transmission cases have been documented after exposure to droplet nuclei (particles <10 microns), which linger for minutes to hours after coughing. All person-to-person transmission seems to be caused by airborne droplets (>10 microns) released immediately during a cough; these droplets rapidly fall to the ground.[77–79] High concentrations of aerosolized droplet nuclei that can transmit plague are used in the laboratory to infect experimental animals.[80] Such small particle aerosols are of particular concern from a biological defense perspective.

INCIDENCE

Under the 2007 revised International Health Regulations, the World Health Organization (WHO) member states are required to report human plague cases in suspected cases in areas not known to be endemic.[81] The cases are then verified, which involves consulting an expert committee to confirm plague based on evidence and additional laboratory testing. Plague may be significantly underreported for several reasons, including the reluctance of some endemic countries to admit to public health problems, difficulties in diagnosis, and the absence of laboratory confirmation. Generally, the distribution of human plague coincides with the geographical distribution of its natural foci.[82,83] In the first decade of the 21st century, 21,725 cases were reported worldwide; 1,612 patients died (7.4%), and 97% of cases were in Africa.[40,84]

Plague is endemic in many countries in Africa, the former Soviet Union, the Americas, and Asia. From recent reports the Democratic Republic of the Congo had 10,581 human plague cases followed by Madagascar (7,182), Zambia (1,309), and Uganda (972). The United States placed 11th with 57 cases, but at least one was reported every year of the decade.[40,82,84]

Since the early 1990s, there have been increasing reports of plague in Africa. It is not clear whether this represents an increase of disease or an improvement of notification to WHO. However, for the Congo, the increase in human plague cases is attributed to civil wars, breakdown in health services, and a greater association of humans with rats.[85–87] Recent plague resurgence in India, Indonesia, and Algeria during the past decade occurred after "silent" periods of years.[82,84] Worldwide distribution of plague and its epidemiology can be found in the *WHO's Plague Manual* (http://www.who.int/csr/resources/publications/plague/). Recent reports of plague activity and occasional summaries of plague activity can be found at the websites for WHO's Weekly Epidemiological Record (http://www.who.int/wer/en/) and the CDC Morbidity and Mortality Weekly Report (http://www.cdc.gov/mmwr/). Known foci of plague are shown in Figure 10-5.

WHO reported 57 deaths among 130 suspected plague cases in the Democratic Republic of the Congo based on a retrospective analysis of cases since December 2004. The victims were employed as miners in a diamond mine at the time of the outbreak. All cases, except

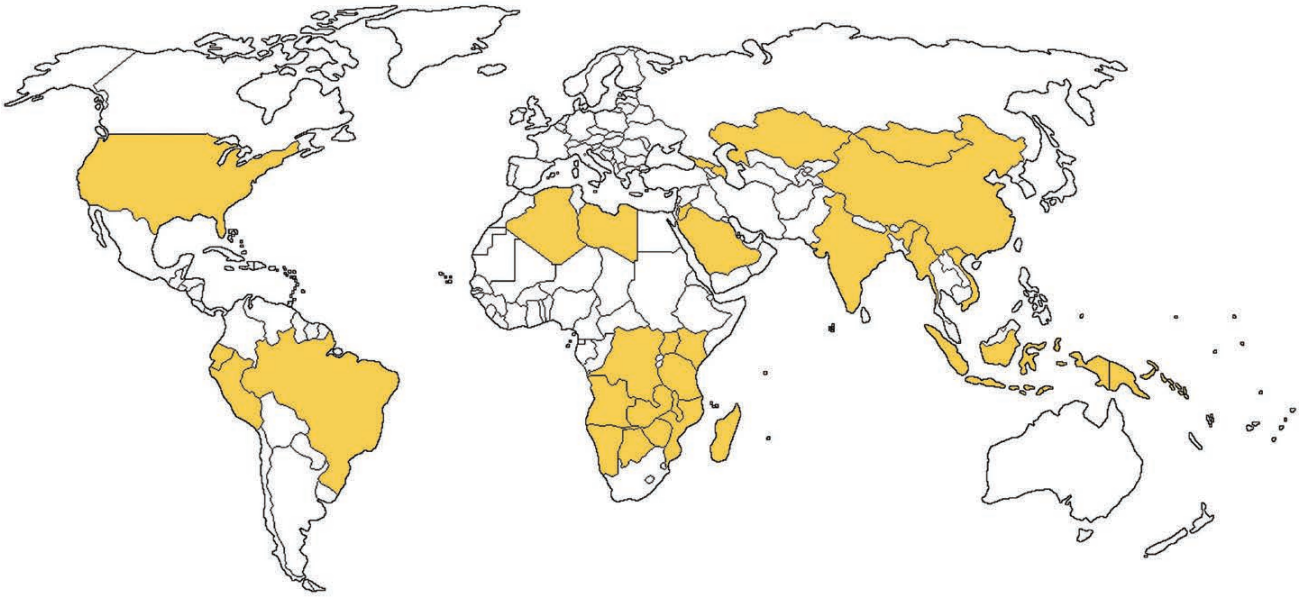

Figure 10-5. Known worldwide foci of human plague infection as indicated by yellow.
Data source: Epidemic Readiness and Interventions, Department of Epidemic and Pandemic Alert and Response, World Health Organization, Geneva, Switzerland.

for two cases of the septicemic form, were reported as pneumonic plague. No evidence of bubonic disease was observed. WHO sent multidisciplinary health teams to investigate the potential outbreak, but no report has been issued since March 2005.[88] The prevalence of pneumonic disease in this group of cases (assuming that this was plague) has not yet been explained.

Plague has been endemic in the continental United States since at least 1900 and now is permanently established from the High Plains on the eastern slope of the Rocky Mountains westward—especially in pine–oak or piñon–juniper woodland habitats at altitudes of 5,000 to 9,000 feet, or on lower, dry grassland or desert scrub areas.[2,30,34,61] In the first quarter of the 20th century, virtually all 432 cases and 284 deaths (65.7% mortality) in the United States occurred in urban port cities.[34] Epidemics occurred in San Francisco, California, from 1900 through 1904 (118 deaths) and from 1907 through 1908 (78 deaths).[34] The last time plague was transmitted between humans in the United States was during the 1924–1925 pneumonic plague epidemic in Los Angeles, California. Eighty percent of cases since 1925 have been sylvatic, involving contact with wild-rodent habitats.[31] Most cases (58%) are in men and occur within a 1-mile radius of home, and half of the US victims have been younger than 20 years old.[31,34]

Between 1926 and 1960, the United States averaged only one plague case per year. This number steadily rose to 3 per year during the 1960s, 11 during the 1970s, and 18 during the 1980s; then it decreased to 9 per year since 1990.[72,73] Between 2000 and 2010, 57 cases were reported; the most active year was 2006 (17 cases).[40,89] The number of states reporting human plague cases has steadily increased over the past 5 decades, most likely because increasing encroachment of humans on previously wild areas brings people closer to infected animals and their fleas.[34] Most human plague cases are reported from New Mexico, Arizona, Colorado, and California.[70,90] In 2002, a couple from New Mexico travelled to New York City and subsequently became ill with plague. However, the couple most likely acquired the infection in an endemic area because *Y pestis* was identified in the dead wood rats and fleas on their property. The bacterial strains recovered from the rats and fleas were indistinguishable from those of the infected couple.[91] In addition, in 2010 two bubonic plague cases from the same household were reported in Oregon, the first in this state since 1995. Most likely, a pet dog's fleas infected the patients. The dog was found to be seropositive.[92]

Epizootic cycles occur approximately every 5 years; the last extremely widespread epizootic with a large die-off of rodents over multiple states (1982–1984) was accompanied by the highest number of humans infected with plague since the urban epidemics of the first quarter of the century.[72,73] In 2009, a fatal laboratory-acquired plague infection occurred by an unknown route with an attenuated

strain of *Y pestis* (KIM D27), which contains defects in the ability to acquire iron.[93,94] However, the scientist was diagnosed portmortem with hemochromatosis. The possibility exists that the excess iron resulting from the condition may have compensated for the iron limitations of the attenuated strain and led to the septicemic infection.[95] This hypothesis was further supported by a recent study that demonstrated virulence can be restored to a *pgm*-strain of *Y pestis* in a mouse model of hereditary hemochromatosis.[95] Before the 2009 case, the last known laboratory-acquired infection in the United States was a pneumonic plague case that occurred in 1959 with a virulent strain of *Y pestis*.[96]

VIRULENCE DETERMINANTS

The persistence of plague in endemic areas requires cyclic transmission between rodents and fleas; thus, *Y pestis* has evolved to survive and replicate in two very different hosts. To maintain the transmission cycle, *Y pestis* must either be transmitted within the few days of the early phase period or multiply within the flea sufficiently to cause blockage and promote the infection of a new mammalian host. Equally critical is the ability to establish an infection and induce a sufficient bacteremia in the mammal to infect fleas during the blood meal. The milieu of the mammalian host is radically different from the flea's midgut, yet, clearly, the organism successfully adapts to each host to complete its life cycle. The adaptation occurs through environmental regulation of virulence factors. For example, gene products necessary for growth in the flea are expressed most efficiently at the flea's body temperature. Likewise, genes required for replication in the mammalian host are expressed at highest levels at 37°C, the internal body temperature of these animals; and the synthesis of some proteins, thought to be induced in the phagolysosome, is also regulated by pH. In the laboratory, the synthesis and secretion of certain essential virulence factors are controlled by both growth temperature and calcium concentration; the induction of these proteins has been termed the low calcium response.[2,21,97,98]

Recent genetic analyses of *Y pestis* and the other pathogenic *Yersiniae* have begun to unravel the unique qualities that make *Y pestis* a successful pathogen in both the flea and the mammalian host. Most strains of *Y pestis* carry three plasmids, two of which are unique to this species:

- pMT (or pFra), which encodes the F1 antigen "capsule"; and
- pPCP, which carries the gene for the virulence factor plasminogen activator.

The third plasmid is common to the human pathogenic *Yersiniae* and is known as pCD (calcium dependence), pYV (*Yersinia* virulence), or pLcr (low calcium response). This plasmid, which is responsible for the synthesis of many antihost factors, is an absolute requirement for virulence.[21]

Type III Secretion System

Like a number of other gram-negative pathogens, the human pathogenic *Yersinae* possess a type III secretion system that enables an organism in close contact to host cells to deliver toxic proteins directly into the eukaryotic cell cytosol.[97,99] In the case of the *Yersinia* species, this system is encoded on the pYV plasmid, which encodes the components of the low calcium response. Toxic activities of the low calcium response effector proteins, designated Yops (***Y****ersinia* **o**uter **p**rotein), include disruption of the cytoskeleton, interference with phagocytic activity, prevention of proinflammatory cytokine synthesis, inhibition of the oxidative burst, and induction of programmed cell death (apoptosis). Yop delivery is necessary for growth of *Y pestis* in the liver and spleen.[100] Specifically, YopM appears to induce a global depletion of natural killer cells. YopH, a protein tyrosine phosphatase, inhibits host cell phagocytosis by dephosphorylating several focal adhesive proteins and inhibiting calcium signaling in neutrophils. YopE, YpkA, and YopT are also antiphagocytic; these toxins inhibit cytoskeletal mobilization. YopJ plays an immunosuppressive role by inhibiting inflammatory cytokine production and inducing apoptosis in macrophages.[97,101,102] Overall, the effect is that of paralyzing professional phagocytes. It is clear why the pathogen-host interaction mediated by the type III secretion system has been designated the "*Yersinia* Deadly Kiss."[102]

LcrV (historically known as V [or "virulence"] antigen), another virulence factor associated with the type III secretion system, is an important protective immunogen in new-generation plague vaccines. This protein serves many roles for the pathogen:

- as regulator of Yop transcription;
- for translocation of Yops into the host cell; and
- as a virulence factor in its own right.[21,101]

LcrV appears to stimulate production of the antiinflammatory cytokine interleukin 10 through interactions with Toll-like receptors 2 and 6 as well as CD14

signaling. These effects appear to be mediated by the N-terminal portion of LcrV.[102,103] Repression of proinflammatory cytokines is presumed to be a result of the interleukin 10 induction. In addition, LcrV released from the cell appears to interact directly with IFN-γ and may contribute to immunosuppression through this binding.[104]

The secretion mechanism includes an "injectisome" that can be visualized as a needle-like structure using electron microscopy. The type III secretion injectisome consists of a cylindrical basal structure spanning the two bacterial membranes and the peptidoglycan, connected to a hollow "needle."[105,106] The needle is tipped by a structure that allows formation of pores in the host cell membrane, and the length of the needle is governed by a protein deemed the "molecular ruler." At body temperature, the secretion apparatus is synthesized on the outer surface of the bacterial cell. Contact with the host cell induces transcription of the Yops and opens this secretion channel that allows the Yops to be translocated through the membrane and into the host cell.[105,106] YopK (also called YopQ) controls the rate of Yop injection from within the host cell.[107] Under certain environmental conditions, proteins with adhesin activity (Ail, Pla, Psa) appear to facilitate Yop delivery.[108]

F1 Antigen

The F1 antigen, encoded by the largest plasmid of *Y pestis* (pMT), is produced in large quantities by *Y pestis* in vivo and when cultured in the laboratory at 37°C. The F1 antigen structure has been described as both capsular- and fimbrial-like because it is composed of fibers that can be shed from the bacteria.[109–112] This capsule-like polymer is generally thought to protect the organism from host phagocytic cells by interfering at the level of receptor interaction in the phagocytosis process.[113] It likely acts in concert with the type III secretion system to provide *Y pestis* with protection from phagocytes. Although the vast majority of natural isolates produce the antigen, F1-negative strains have been isolated from rodent hosts and reportedly from one human case.[110,114–116] In the laboratory, spontaneous mutants defective in F1 production have been obtained from immune animals, cultures treated with antiserum containing F1 antibody, and chronically infected rodents.[114–116] Examination of isogenic F1-positive/-negative strain pairs revealed that F1 is not an absolute requirement for virulence in the mouse and the African green monkey models, including aerosol models, although mutations leading to loss of the capsular antigen increase time to death in the mouse.[114,117] However, a recent study demonstrated a F1 mutant in *Y pestis* was attenuated by bubonic and pneumonic (intranasal) models of infection depending on the strain of mouse.[118] Older studies suggesting a role of F1 in the infection of guinea pigs and rats used F1-negative strains that were not genetically defined and, thus, are more difficult to interpret. However, these studies suggest that the importance of F1 in pathogenesis may vary with the species of the host. The fact that F1-negative strains are relatively rare among natural isolates suggests that the capsular antigen, or other gene products encoded by this plasmid, may play an important role in the maintenance of the disease in animal reservoirs. Historically, F1 has been important as a diagnostic reagent because it is specific to *Y pestis*.[110,119] It is the major antigen recognized in convalescent sera of humans and rodents,[120,121] and also a highly effective protective immunogen.

Other Virulence Factors in the Mammalian Host

Plasminogen Activator

The virulence factor plasminogen activator (Pla) is encoded on a 9.5 kb plasmid, pPCP1, unique to *Y pestis*. Inactivation of the *pla* gene leads to a significant attenuation of virulence from a subcutaneous but not an intraperitoneal or intravenous route of infection in mice, suggesting that Pla promotes dissemination of the organism from peripheral sites of infection, and plasminogen-deficient mice are 100-fold more resistant to *Y pestis* than normal mice.[21,121,122] Although Pla is necessary for full virulence in some *Y pestis* strains, a few strains that are Pla^- and appear to be fully virulent have been identified among natural isolates or generated in the laboratory.[61,122] Presumably, these isolates synthesize other proteins that substitute for Pla function.

Fimbrae

The so-called pH 6 antigen is a fimbrial structure on the surface of *Y pestis* that is necessary for full virulence in the mouse model. Researchers have proposed that pH 6 antigen mediates attachment of the organism to host cells via binding to glycosphingolipids. The temperature and pH of the environment tightly control the biosynthesis of these fimbriae; the expression of pH 6 antigen is most efficient in vitro with a growth temperature between 35°C and 41°C and a pH range of 5.0 to 6.7. This situation suggests that, in vivo, the adhesin activity is likely to be expressed only in specific microenvironments, such as the phagolysosome, necrotic tissue, or an abscess. Intracellular association with macrophages in the laboratory induces synthesis of the fimbriae.[123] More recent data, however, suggest

that the pH 6 antigen does not enhance adhesion to mouse macrophages but rather promotes resistance to phagocytosis.[124] Additional data suggest that this protein is not an essential virulence factor in wild type *Y pestis*; the use of laboratory-passaged strains may have influenced the results of previous studies.[125] Alternatively, there may be redundancy of some functions in *Y pestis* as implied by the work of Felek et al.[108]

Iron and Manganese Sequestration

Acquisition of nutrients in the host is an essential part of pathogenesis. In the mammalian host, iron is sequestered from invading pathogens; therefore, the level of free iron in the extracellular milieu is less than that necessary for bacterial growth. Like most bacterial pathogens, *Y pestis* possesses a high-affinity iron uptake system that is capable of procuring this essential nutrient from the host. Strains that do not produce the low-molecular-weight iron chelator, known as yersiniabactin, or those unable to transport yersiniabactin are highly attenuated by the subcutaneous route of infection and somewhat affected in pneumonic models. Such strains are capable, however, of infecting via the intravenous route (septicemic model). The genes encoding this iron transport system are situated on a chromosomal pathogenicity island known as the pigmentation locus (pgm).[126] Manganese transport is also important for full virulence.[127]

Phage Shock Protein Response

The phage shock protein (PSP) response is almost ubiquitous among microbes; homologues are found in numerous gram-positive and gram-negative bacteria, as well as archaebacteria and even chloroplasts. This regulon appears to respond to environmental stressors, including disturbances in the cell envelope and changes in the proton motive force that are induced by impaired inner membrane integrity.[128,129] For pathogens, environmental stressors triggering the PSP regulon likely include environments within the host, and the PSP response is associated with virulence in *Salmonella enterica*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and Y *pestis*.[128,130,131] For *Y pestis*, it is required for virulence by both the aerosol and subcutaneous routes of infection.[131]

Twin Arginine Transport

Gram-negative bacteria have numerous ways to transport molecules across their membranes. One of these mechanisms is the twin arginine transport (Tat) pathway. The Tat pathway secretes folded proteins that are identified by an N-terminal signal peptide containing a twin arginine motif across the inner membrane. The TatA gene product mediates the actual translocation event,[132] and it is an important virulence factor of *Y pestis* in both bubonic and pneumonic models of infection.[133] The *tatA* mutation in *Y pestis* leads to many phenotypic changes, including a defect in the secretion/assembly of the F1 antigen on the cell surface. However, the attenuation of a *Y pestis tatA* mutant cannot be explained by the defect in F1 synthesis; the *tatA* mutant is more attenuated than mutants affected in the capsular synthetic genes per se.[133]

Surface Structures

Bacterial surface structures, such as porins and phage receptors, have been implicated in virulence. OmpA, a major outer membrane porin, was identified as an in vivo-expressed protein and subsequently proven to be essential for virulence.[134] Receptors for *Y pestis*-specific bacteriophage also play an important role in virulence; these tend to be associated with various portions of the lipopolysaccharide inner and outer core.[135]

Small RNAs

Posttranscriptional control of virulence determinant expression by small RNAs was recently documented in *Y pestis*.[136] Expression of the majority of small RNAs in *Y pestis* is dependent on the RNA-binding regulatory protein Hfq. Hfq is necessary for growth of the organism at 37°C and for virulence.[136] Identification of regulons governed by small RNAs may lead to identification of virulence factors previously unknown.[137]

Yersinia Autotransporter Proteins

Bacterial autotransporters proteins are secreted via type V secretion pathway and have three conserved domains necessary for mediating secretion. An aminoterminal signal peptide targets the autotransporter to the general secretory pathway for secretion across the inner membrane. From the periplasm, the proteins are translocated to the outer membrane for tethering to the bacterial surface for release following proteolytic cleavage. Numerous autotransporters have been established to be virulence factors in many bacterial pathogens.[138–140] The genome of *Y pestis* encodes for numerous functional *Yersinia* autotransporter proteins (Yaps).[141–143] Many of these Yap genes have increased expression during infection of mammals,[141] and the proteins are necessary for efficient adherence to host cells and colonization of the mammalian host.[142,144,145]

Virulence and Transmission Factors in the Flea

Researchers have identified many factors that allow *Y pestis* to block the flea and promote vectorborne transmission.[146] *Y pestis* has a natural resistance to antimicrobial peptides at growth temperatures similar to that of the flea gut. Such peptides are an integral part of the insect immune system.

Blockage of the proventriculus occurs as a result of bacterial aggregates embedded in a biofilm synthesized by the bacterium at temperatures lower than 28°C. This allows the organism to persist in the proventriculus despite the shearing forces that flush nonaggregating cells into the midgut.[147] The two-component regulatory system, *phoP-phoQ*, contributes to stable biofilm production in the flea.[148] The chromosomal *hmsHFRS* operon, part of the *Yersinia* pathogenicity island ("pgm"), encodes the polysaccharide extracellular matrix that is essential to biofilm formation. The temperature-dependent biofilm synthesis is posttranscriptionally regulated.[149] Although Hms^- mutants are capable of colonizing the flea midgut, they are unable to colonize the proventriculus and, therefore, do not block the flea. The *hms* operon mediates storage of hemin or Congo red in the outer membrane of *Y pestis* on agar medium containing these compounds. This "pigmentation" phenotype, or Pgm, has been associated with virulence of *Y pestis* in animal models; however, Hms per se does not appear to play a role in mammalian plague other than promoting flea transmission. The spontaneous loss of pigmentation in the laboratory usually results from a large chromosomal deletion affecting not only the genes necessary for the Hms phenotype, but also the genetically linked yersiniabactin uptake system. The absence of the high affinity iron transport system in Pgm strains, rather than the loss of Hms, is responsible for attenuation in animal models.[126]

Studies examining the role of the *Y pestis* plasmids in the flea host indicated that one or more genes on the plasmid pMT are necessary for colonizing the midgut.[150] The so-called murine toxin encoded by this plasmid appears to be one of these colonization factors. Murine toxin has phospholipase D activity, and although toxic to mice and rats in pure form, it is not important for virulence in rodent models.[150] This may be explained by the regulation of toxin synthesis. Like Hms, it is produced more efficiently at 28°C than at mammalian body temperatures. Ymt, the *Yersinia* murine toxin, appears to protect the bacterium from an unidentified antibacterial substance in the midgut.[146]

PATHOGENESIS

As few as 1 to 10 *Y pestis* organisms are sufficient to cause infection by the oral, intradermal, subcutaneous, or intravenous routes.[21] Estimates of infectivity by the respiratory route for nonhuman primates vary from 100 to 20,000 organisms.[110,151,152] After being introduced into the mammalian host by a flea, where it had been at ambient temperature, the organism is thought to be initially susceptible to phagocytosis and killing by neutrophils. In rodent models of bubonic plague, it has been shown that neutrophils are quickly recruited to the area associated with the bacteria. Furthermore, the ability to evade and neutralize neutrophils was necessary for infection.[153] However, some of the bacteria may grow and proliferate within tissue macrophages.[154] A vigorous debate has raged for many years regarding the relative importance of intracellular versus extracellular replication in plague. Although most of the bacterial multiplication in the mammalian host is extracellular, evidence indicates that *Y pestis* can survive and multiply in macrophages. As reviewed by Pujol and Bliska, growth inside host cells is likely to be of greatest importance at the early stages of colonization.[155] They suggest that, although considerable attention has focused on how *Y pestis* subverts the functions of phagocytes from the outside, there is less understanding of how these bacteria affect macrophage functions from the inside.[155] Once the antiphagocytic gene products are expressed, the bacteria are resistant to phagocytosis and multiply extracellularly. However, another recent study demonstrated that a *Y pestis* mutant strain severely defective for intracellular recovery within macrophages was still fully virulent in a murine pneumonic plague challenge.[156] The ability of *Y pestis* to reside and replicate in an intracellular environment may be dependent upon the route of infection (parenteral versus respiratory).

During the incubation phase, the bacilli most commonly spread to regional lymph nodes, where lymphadenitis develops, producing the characteristic bubo. Dissemination from this local site leads to septicemia and seeding of other organs, including the liver, spleen, lung, and (less often) the meninges. The endotoxin of *Y pestis* probably contributes to the development of septic shock, which is similar to the shock state seen in gram-negative sepsis from other causes. The endotoxin may also contribute to the resistance of the organism to the bactericidal activity of serum.[151]

Primary pneumonic plague, the most severe form of disease, arises from inhalation of infectious respiratory droplets or an aerosol. Primary pneumonic plague is more rapidly fatal than secondary.[1] During primary

pneumonic plague, the disease can be divided into two host response phases. During the initial preinflammatory phase, the bacteria are actively replicating in the absence of host innate immune responses. Next, a proinflammatory phase occurs with a neutrophil influx, proinflammatory cytokine storm, and tissue destruction within the lung. Evidence suggests that *Y pestis* suppresses the host immune cells in the lung early during infection. As discussed above, the type III secretion system Yop effectors act upon numerous mammalian cells to elicit antiinflammatory and antiphagocytic effects. During pneumonic infection, the type III secretion system initially targets macrophages and neutrophils.[157]

Primary septicemic plague can occur from direct inoculation of bacilli into the bloodstream, bypassing initial multiplication in the lymph nodes. Asymptomatic pharyngeal carriage of plague has occurred in contacts of patients with either bubonic or pneumonic plague.[77,158,159]

CLINICAL MANIFESTATIONS

From 1947 through 1996, 390 cases of plague were reported in the United States, resulting in 60 (15.4%) deaths.[70,91] Of these deaths, bubonic plague accounted for 327 cases (83.9%) and 44 deaths (13.5%); primary septicemic plague accounted for 49 cases (12.6%) and 11 deaths (22.4%); and primary pneumonic plague accounted for 7 cases (1.8%) and 4 deaths (57.1%).[70,91] Seven cases (1.8%) were unclassified, including 1 death (14.3%).[70,160] If *Y pestis* was used as a biological warfare agent, the clinical manifestations of plague would be (*a*) rapidly progressive, highly fatal epidemic pneumonia if aerosolized bacteria were used, or (*b*) bubonic and septicemic plague if fleas were used as carriers. Infections via ingestion could also occur.[1]

Bubonic Plague

Human symptoms of bubonic plague typically develop 2 to 8 days after being bitten by an infected flea. Presenting symptoms include prostration or severe malaise (75% of cases), headache (20%–85% of cases), vomiting (25%–49% of cases), chills (40% of cases), altered mentation (26%–38% of cases), cough (25% of cases), abdominal pain (18% of cases), and chest pain (13% of cases).[27] In the United States, fleabites typically occur in the lower extremities; therefore, buboes are most common in the femoral and inguinal regions. As noted previously, the proportion of bubonic cases with cervical buboes is often higher in poverty-stricken areas of developing countries because these cases involve persons that frequently sleep on the dirt floors of huts where fleas are likely to bite them on the head and neck. Infection arising from skinning plague-infected animals typically produces axillary buboes due to inoculation of the upper extremities. Six to 8 hours after onset of symptoms, buboes, heralded by severe pain, appear (Figure 10-6). Buboes may drain spontaneously and rarely require incision and drainage because of pronounced necrosis. Within 2 days, a warm, erythematous bubo can grow to the size of an egg and eventually reach 10 cm in diameter as a cluster of infected lymph nodes. Buboes are so intensely painful that even nearly comatose patients attempt to shield them from trauma and will abduct their extremities to decrease pressure. Buboes are often associated with considerable surrounding edema, but lymphangitis is rare. Occasionally, buboes become fluctuant and suppurate. Histologically buboes demonstrate hemorrhagic necrosis, numerous neutrophils, a plethora of extracellular bacteria, and completely destroyed lymph node architecture.[40]

A small minority of patients bitten by plague-infected fleas develop *Y pestis* septicemia without a discernable bubo. Other manifestations of bubonic plague include bladder distention, apathy, confusion, fright, anxiety, oliguria, and anuria. Tachycardia, hypotension, leukocytosis, thrombocytopenia, and fever are frequently encountered. In about half of untreated cases of bubonic plague, septicemia ensues 2 to 6 days later, which is virtually 100% fatal if untreated.[40,161] In the United States, approximately 10% to 15% of bubonic plague patients have developed secondary pneumonic plague with the potential for airborne transmission of the organism.[162]

Septicemic Plague

Septicemic plague may occur primarily—if the bacteria is inoculated by a fleabite or other means, such as a puncture wound caused by a knife used while skinning an animal or bypass regional lymph nodes—or secondarily as a complication of hematogenous dissemination of bubonic plague.[40] Presenting signs and symptoms of primary septicemic plague are essentially the same as those for any gram-negative septicemia: fever, chills, nausea, vomiting, and diarrhea. Purpura (Figure 10-7), disseminated intravascular coagulation, and acral cyanosis and necrosis (Figure 10-8) may be seen later. In New Mexico between 1980 and 1984, plague was suspected in 69% of patients who had bubonic plague, but in only 17% of patients who had the septicemic form. The mortality was 33.3% for septicemic plague versus 11.5% for bubonic, which indicates the difficulty of diagnosing septicemic plague.[162] Diagnosis of septicemic plague took longer

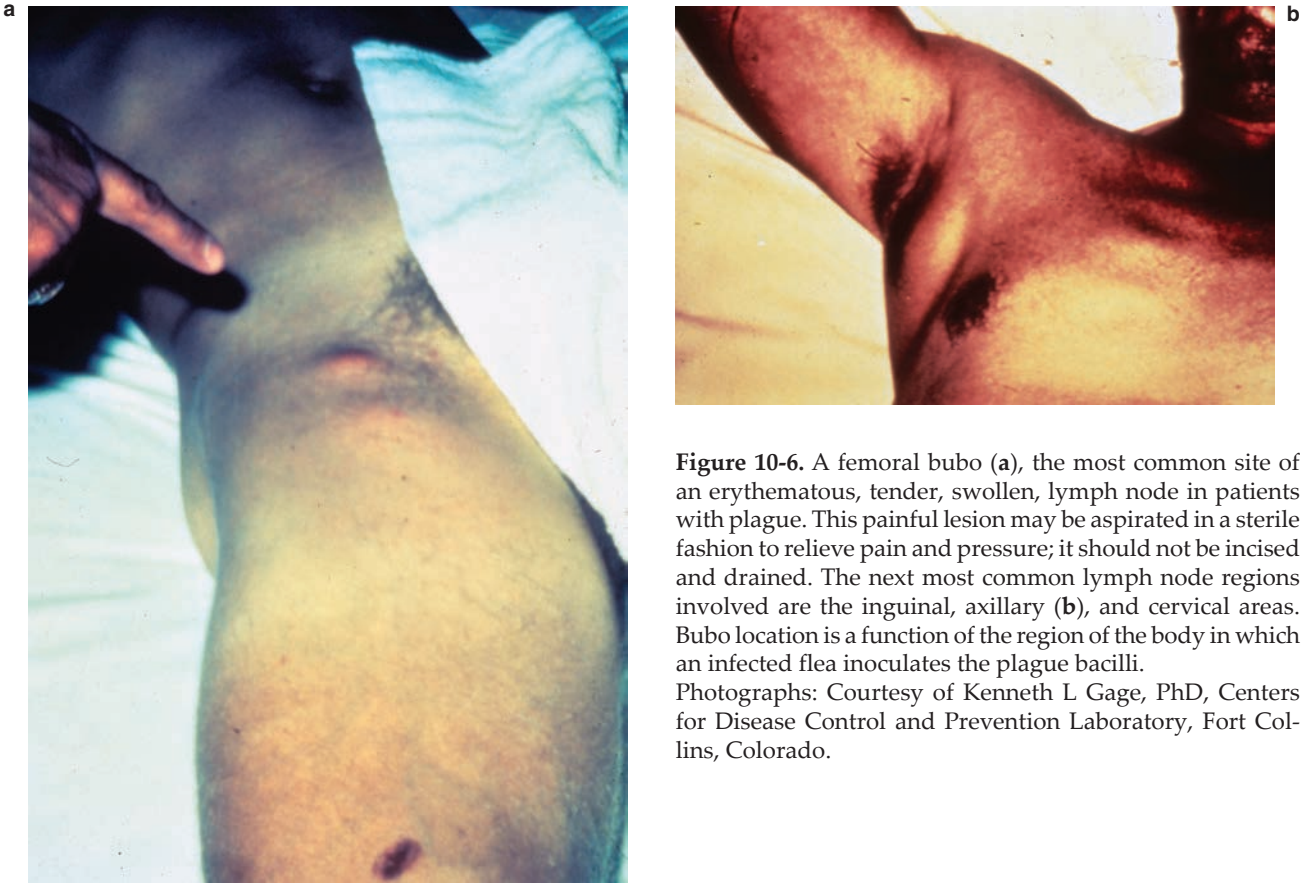

Figure 10-6. A femoral bubo (**a**), the most common site of an erythematous, tender, swollen, lymph node in patients with plague. This painful lesion may be aspirated in a sterile fashion to relieve pain and pressure; it should not be incised and drained. The next most common lymph node regions involved are the inguinal, axillary (**b**), and cervical areas. Bubo location is a function of the region of the body in which an infected flea inoculates the plague bacilli.
Photographs: Courtesy of Kenneth L Gage, PhD, Centers for Disease Control and Prevention Laboratory, Fort Collins, Colorado.

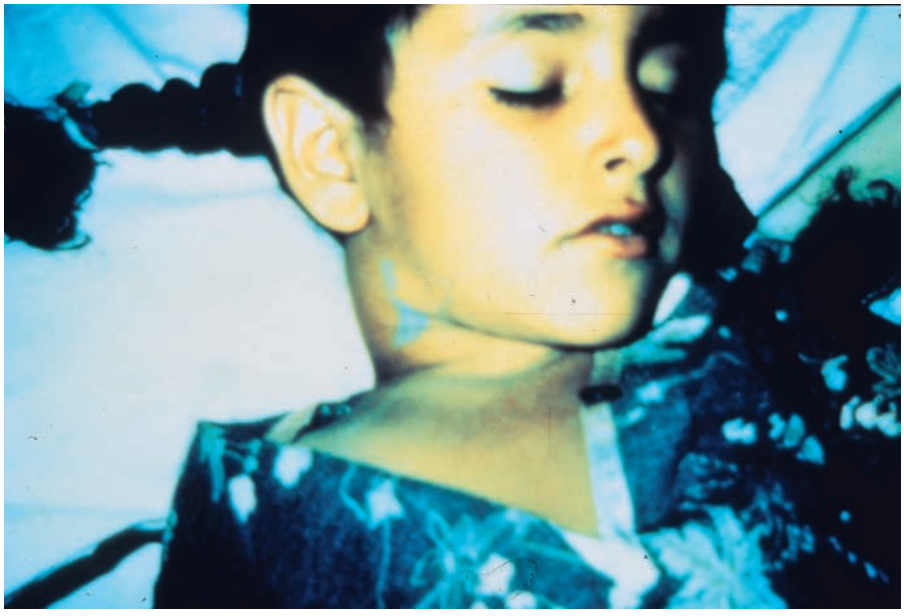

Figure 10-7. Purpuric lesions can be seen on the upper chest of this girl with plague. The bandage on her neck indicates that a bubo has been aspirated.
Photograph: Courtesy of Kenneth L Gage, PhD, Centers for Disease Control and Prevention Laboratory, Fort Collins, Colorado.

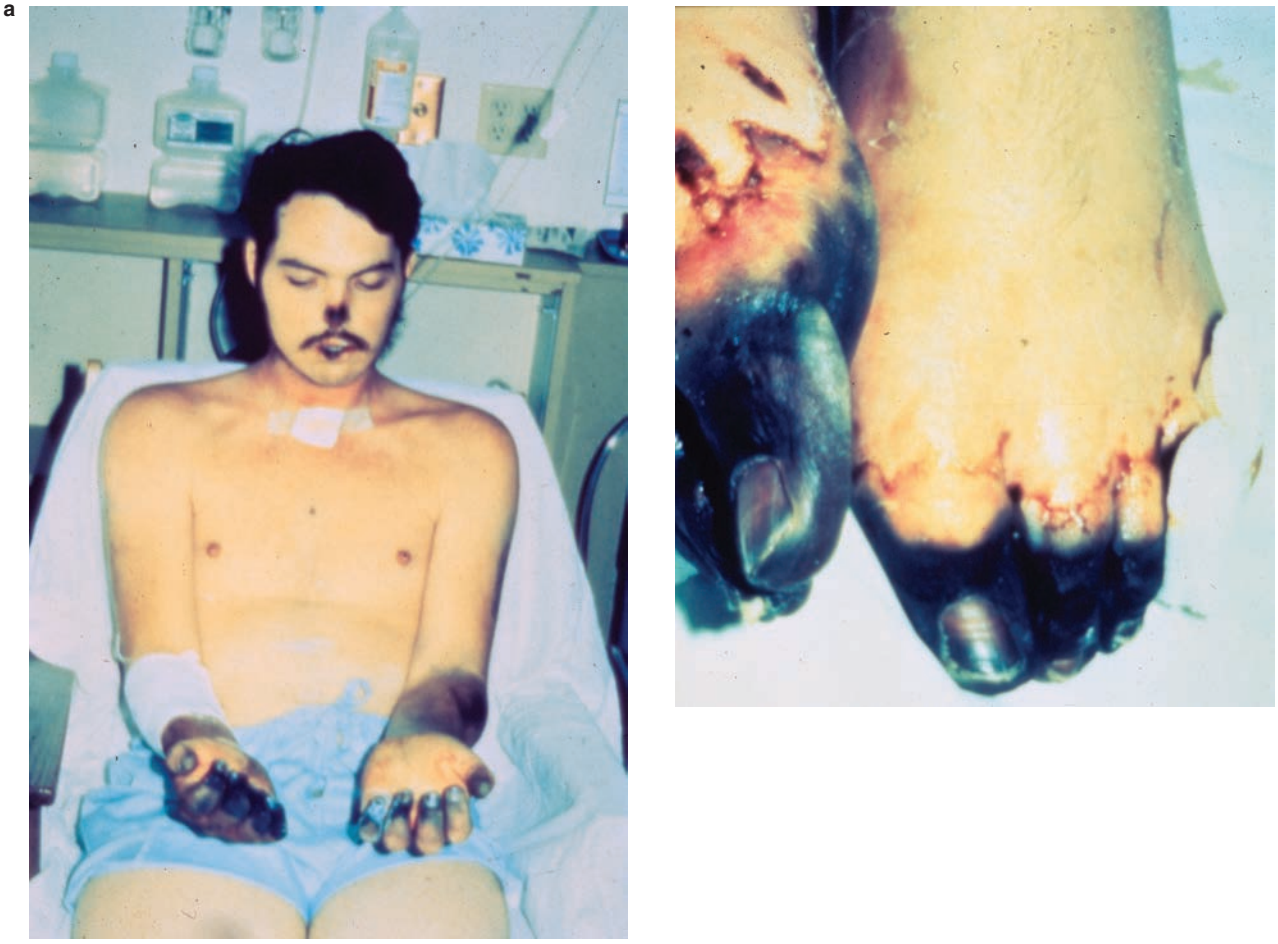

Figure 10-8. This patient is recovering from bubonic plague that disseminated to the blood (septicemic form) and the lungs (pneumonic form). Note the dressing over the tracheostomy site. At one point, the patient's entire body was purpuric. Note the acral necrosis of (**a**) the patient's nose and fingers and (**b**) the toes.
Photographs: Courtesy of Kenneth L Gage, PhD, Centers for Disease Control and Prevention Laboratory, Fort Collins, Colorado.

(5 vs 4 days) after onset, although patients sought care earlier (1.7 vs 2.1 days) and were hospitalized sooner (5.3 vs 6.0 days) than patients with bubonic plague. The only symptom present significantly more frequently in septicemic than in bubonic plague was abdominal pain (40% vs <10%), which was probably caused by hepatosplenomegaly.[162]

Pneumonic Plague

Pneumonic plague may occur primarily, from inhaling infectious respiratory droplets or aerosols, or secondarily, from hematogenous dissemination. It is the only form of plague that can be transmitted from one person to another.[78] Patients with pneumonic plague rapidly develop symptoms of a severe bronchopneumonia, severe headache, chills, malaise, tachypnea, tachycardia, dyspnea, cough, chest pain, and hemoptysis.[78,163,164] Patients initially exhibit a dry cough that progressively becomes productive as sputum concentration of blood and bacilli increases to "almost pure culture" levels at the end.[78] The findings on a chest x-ray may be variable, but bilateral alveolar infiltrates appear to be the most common finding (Figure 10-9).[78,165,166] A chest x-ray with bilateral alveolar infiltrates in a bubonic plague patient sometimes represents adult respiratory distress syndrome and disseminated intravascular coagulation in the absence of plague pneumonia.[164] Depending on the stage of infection, the sputum may be clear, purulent, or hemorrhagic, and contain gram-negative rods. Unless appropriate antimicrobial therapy is begun during the first day of symptoms, pneumonic plague is rapidly fatal.[5,40] The time from respiratory exposure to death in humans is reported to have been between 2 to 6 days (and from symptoms to death 1–3 days) in epidemics during the preantibiotic era.[78,163,167]

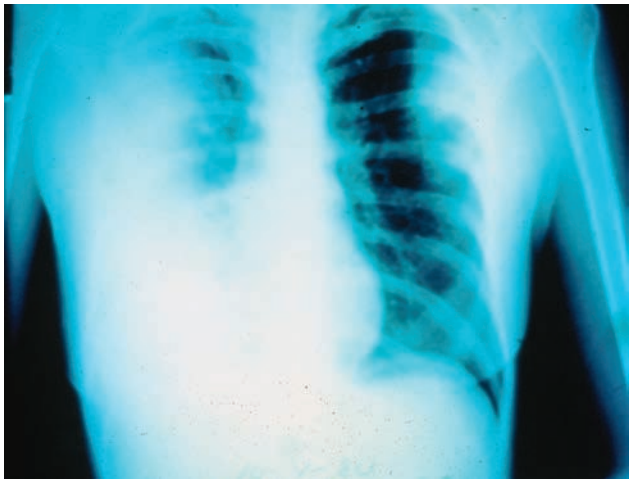

Figure 10-9. This chest roentgenogram shows right middle- and lower-lobe involvement in a patient with pneumonic plague.
Photograph: Courtesy of Kenneth L Gage, PhD, Centers for Disease Control and Prevention Laboratory, Fort Collins, Colorado.

Plague Meningitis

Plague meningitis is seen in 6% to 7% of cases. The condition manifests itself most often in children after 9 to 14 days of ineffective treatment. Symptoms are similar to those of other forms of acute bacterial meningitis.[165]

Pharyngeal and Gastrointestinal Plague

In late December 2007, the first known cases of plague in Afghanistan developed among 83 persons who developed acute gastroenteritis after eating the meat of a slaughtered, sick camel, and 17 of those patients died.[168]

Transient asymptomatic pharyngeal carriage may occur in healthy contacts of bubonic plague patients.[77,158,159,169] Symptomatic pharyngeal plague presents with pharyngitis, fever, and cervical lymphadenopathy after inhalation of plague bacteria or ingestion of meat from infected camels or goats.[1,3,6,161] For example, in early 1997, 12 individuals in Jordan ate raw or cooked meat from the same (infected) camel and all developed pharyngeal plague. One developed pneumonic plague. However, all survived because they were serendipitously treated with gentamicin for the suspected diagnosis of tularemia.[170]

A plague syndrome of cervical buboes, peritonsillar abscesses, and fulminant pneumonia has been reported in Vietnam[161] and among Indians of Ecuador, who are known to catch and kill fleas and lice with their teeth.[62] Endobronchial aspiration from peritonsillar abscesses may lead to fulminant pneumonia.

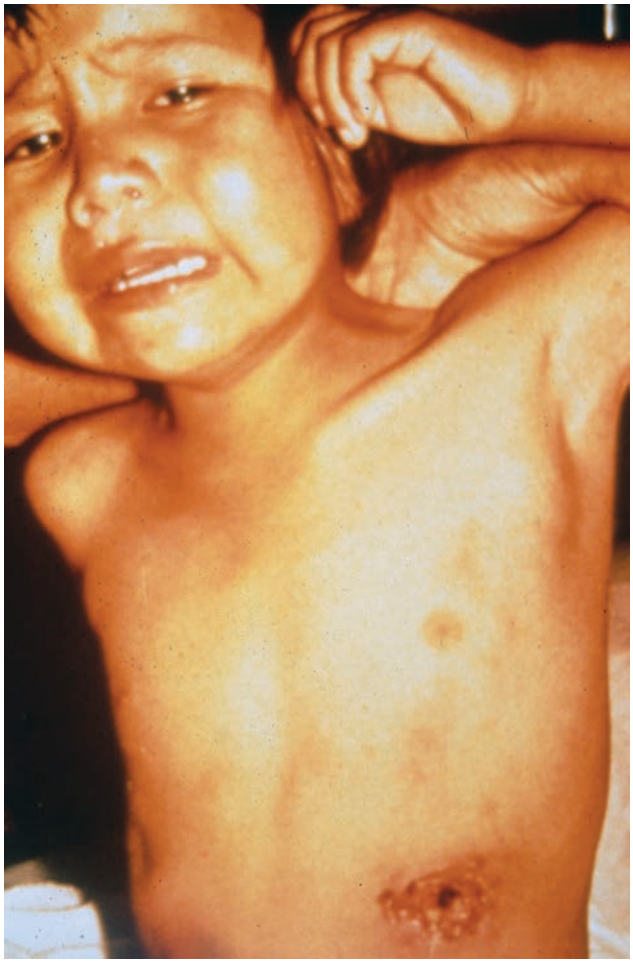

Figure 10-10. This child has left axillary bubonic plague. The erythematous, eroded, crusting, necrotic ulcer on the child's left upper quadrant is located at the presumed primary inoculation site.
Photograph: Courtesy of Kenneth L Gage, PhD, Centers for Disease Control and Prevention Laboratory, Fort Collins, Colorado.

Cutaneous Manifestations

While most plague patients have normal appearing skin (apart from buboes), approximately 4% to 10% of patients develop an inoculation-site pustule, ulcer, eschar, or carbuncle (Figure 10-10).[40,154,165,171–173] A sample from a plague patient eschar (ecthyma gangrenosum lesion) grew *Y pestis*, which suggests that local skin lesions are the result of septicemic seeding of the organism.[173]

Petechiae and ecchymoses may develop during hematogenous spread of bacteria when patients develop disseminated intravascular coagulation secondary to the *Y pestis* endotoxin. When purpura and acral gangrene occur, possibly exacerbated by the tissue plasminogen activator, the prognosis is

poor.[27,40,173] Recently, an American man contracted bubonic plague that progressed to septic shock; he developed ischemic necrosis of his feet requiring bilateral foot amputation.[174] Patients in the terminal stages of pneumonic and septicemic plague often develop large ecchymoses on their backs. Lesions like these are likely to have led to the medieval epithet "the Black Death."

DIAGNOSIS

Signs and Symptoms

The early diagnosis of plague requires a high index of suspicion. Presence of a painful bubo in the setting of fever, prostration, and possible exposure to rodents or fleas in an endemic area should readily suggest the diagnosis of bubonic plague. However, if the health-care provider is not familiar with the disease or does not ask the patient for a travel or exposure history, or if the patient presents in a nonendemic area or without a bubo, then the diagnosis will be difficult to make. For example, in the United States in 1996, fatal plague cases occurred in two young adults who presented for treatment without obvious buboes.[175] The first, an 18-year-old male, presented with left groin swelling and tenderness that was misdiagnosed as a groin muscle strain attributed to a fall 2 days earlier. The second, a 16-year-old female, presented with left arm numbness and left axillary pain that was misdiagnosed as a possible brachial plexus injury related to a fall from a trampoline 3 days earlier. In both cases, the patients were sent home with a pain reliever, and they both experienced rapid progression of their illness within the next day and died.[90]

The wildlife biologist who died of pneumonic plague after necropsy of an infected mountain lion did present to a health clinic with fever, chills, nausea, myalgias, and a cough producing blood-tinged sputum.[74] No chest x-ray was performed. No exposure history to wildlife during his job was elicited.[74] Either the chest x-ray or a job exposure history could have saved his life.

The laboratory plague researcher who died in Chicago of septicemic plague presented to an outpatient clinic 3 days before death.[94] He complained of fever, body aches, and a 3-day history of nonproductive cough. Influenza or other acute respiratory infection was suspected, and he was referred to an emergency department, but the patient did not follow through. Neither at that clinic visit nor at his hospital admission 12 hours before death was his occupation noted.[94]

When a bubo is present, the differential diagnosis should include tularemia, cat scratch disease, lymphogranuloma venereum, chancroid, tuberculosis, streptococcal adenitis, and scrub typhus (Figure 10-11). In both tularemia and cat scratch disease, the inoculation site is typically more evident and the patient will usually not be septic. In chancroid and scrofula, the patient has less local pain, the course is more indolent, and there is no sepsis. Patients with chancroid and lymphogranuloma venereum will have a recent history of sexual contact and genital lesions. Those with the latter disease may be as sick as patients with plague. Streptococcal adenitis may be difficult to distinguish initially, but the patient is usually not septic, and the node is more tender when plague is present.

The implications of the absence of a bubo were demonstrated in a review of 27 plague cases seen in New Mexico.[166] In this study, there were eight cases of septicemic plague and 19 cases of bubonic plague, with six fatalities. Of the patients who died, three had septicemic plague and three had bubonic plague, but all six presented with nonspecific febrile symptoms or symptoms of an upper respiratory tract infection. The authors concluded that the lack of a bubo was associated with a delay in the diagnosis of plague and increased mortality.[166]

The differential diagnosis of septicemic plague also includes meningococcemia, gram-negative sepsis, and the rickettsioses. The patient with pneumonic plague who presents with systemic toxicity, a productive cough, and bloody sputum suggests a large differential diagnosis. However, demonstration of gram-negative rods in the sputum should readily suggest the correct diagnosis, because *Y pestis* is perhaps the only gram-negative bacterium that can cause extensive, fulminant pneumonia with bloody sputum in an otherwise healthy, immunocompetent host.

Laboratory Confirmation

Procedures for the isolation and presumptive identification of *Y pestis* by Level A laboratories can be downloaded from the CDC website (http://www.bt.cdc.gov/agent/plague/index.asp).[172] The World Health Organization offers its Plague Manual online (http://www.who.int/emc-documents/plague/whocdscsredc992c.html). A recent review of the methodology for isolating and identifying *Y pestis* from clinical samples and animals is available.[55] Standard bacterial methodologies include staining and microscopic analysis of the organism, isolation on culture medium, and biochemical tests. Misidentification of *Y pestis* by automated systems used for bacterial identification resulting in the delayed diagnosis of human plague has been reported.[175] Therefore, if plague is suspected, immediate appropriate treatment should be started

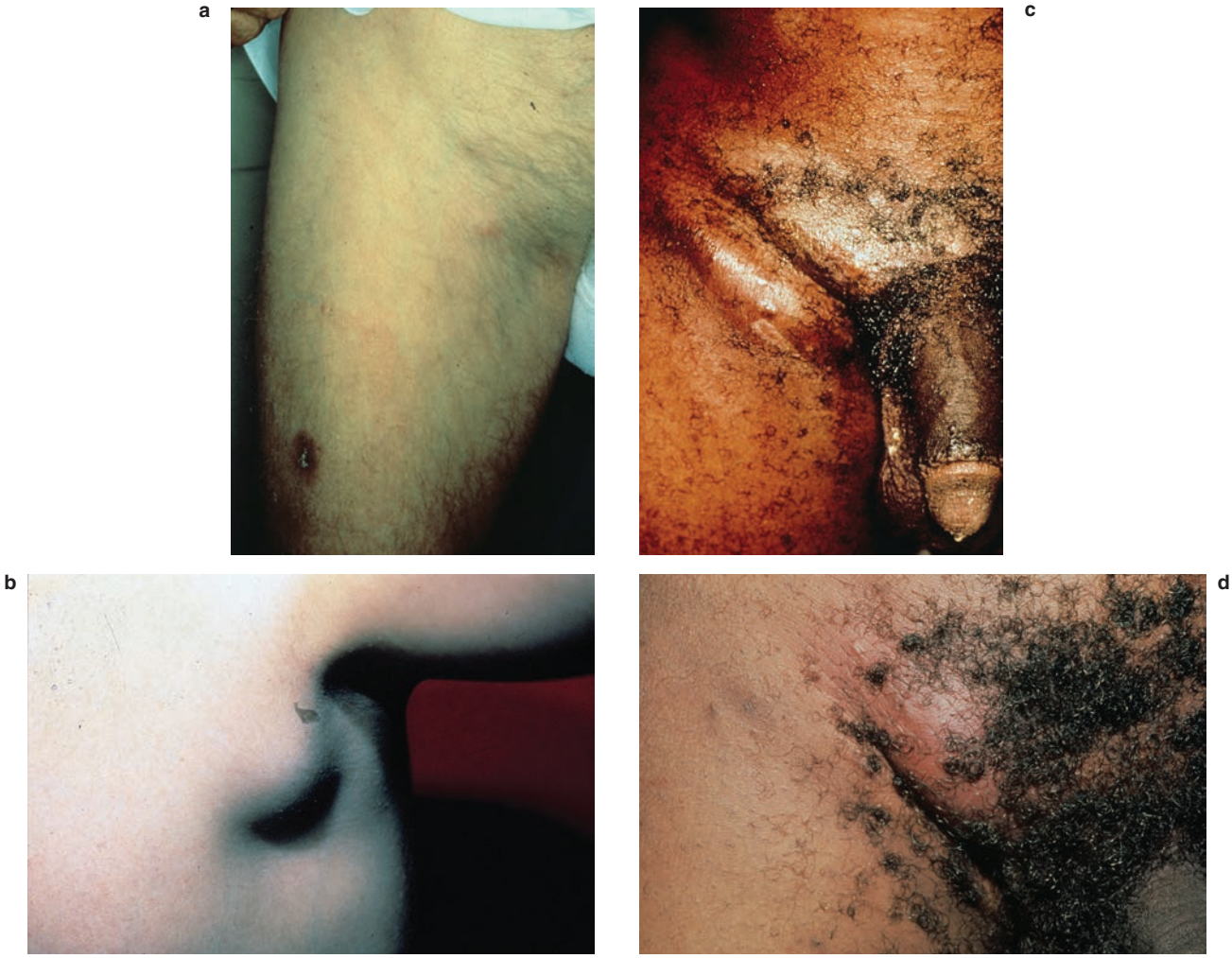

Figure 10-11. (**a**) Small femoral bubo and presumed inoculation site (on the inferior thigh) in a patient with tularemia. This gram-negative bacterial infection (with *Francisella tularensis*) may closely mimic bubonic plague and is successfully treated with the same antibiotics. (**b**) Axillary bubo seen in child with cat scratch disease. (**c**) Greenblatt's sign of ipsilateral femoral and inguinal buboes with intervening depression over the inguinal ligament seen in a patient with lymphogranuloma venereum caused by *Chlamydia trachomatis*. (**d**) Large inguinal bubo seen in a patient with chancroid caused by *Haemophilus ducreyi*. Photographs: Courtesy of Dermatology Service, Fitzsimons Army Medical Center, Aurora, Colorado.

and isolates should be sent to the appropriate laboratories experienced in the identification of *Y pestis*. Care should be taken to avoid aerosols; in this regard, fixing slides with methanol rather than heat fixing is preferred. CDC summarizes diagnosis of plague at its website (http://www.cdc.gov/plague/healthcare/clinicians.html).

Reference laboratories, such as those found in major county or state health departments, have additional tests to confirm the diagnosis of *Y pestis*. These tests include direct fluorescent antibody tests to detect the F1 capsular antigen and polymerase chain reaction based assays, which can be used on isolates or direct clinical samples. Confirmatory testing includes lysis by a species-specific bacteriophage.[1] Serological testing such as passive hemagglutination antibody detection in acute or convalescent-phase plasma or enzyme-linked immunosorbent assay are found at national laboratories such as the CDC at Fort Collins, Colorado, and the US Army Medical Research Institute of Infectious Diseases at Fort Detrick, Maryland.[176] Serological assays measuring the immune response to plague infection are mainly of value, retrospectively, because patients present clinically before they develop a significant antibody response. A four-fold change in antibody between acute and convalescent serum samples is considered confirmatory.

When using the fluorescent antibody test to detect the plague-specific capsular antigen, it is important to recall that F1 antigen is produced only at temperatures

greater than 33°C. Thus, this method requires a relatively fresh sample from the patient/animal or from a laboratory culture incubated at the appropriate temperature. Therefore, flea samples, as well as samples refrigerated for more than 30 hours, are F1 antigen negative.[55] For diagnosing plague in the field, a new rapid diagnostic test with monoclonal antibodies to the F1 antigen has been developed and field tested in Madagascar. The rapid diagnostic test detected concentrations of F1 antigen as low as 0.5 ng/mL in as little as 15 minutes and had a shelf life of 21 days at 60°C. This test had 100% sensitivity and specificity against laboratory isolates of *Y pestis,* and the agreement between field testing and reference laboratory testing was 89.9%. This test demonstrated positive and negative predictive values of 90.6% and 86.7%, respectively.[176] A rapid and reliable test such as the rapid diagnostic test, which healthcare workers can easily perform at the patient's bedside, holds considerable promise for rapid plague diagnosis in endemic countries, but further testing is needed. A polymerase chain reaction test using primers for the plasminogen activator gene (*pla*) can detect as few as 10 *Y pestis* organisms, even from flea tissue. This test may be useful in surveillance of rats and can be adapted to help diagnose human infection.[177] More recently, the use of Pla primers for simulated detection of *Y pestis* in sputum was reported to have a sensitivity of 10^4 CFU/mL and a 5-hour turnaround.[178] In cases where use of *Y pestis* as a biological weapon is a possibility, it should be kept in mind that F1 or Pla are not necessary for virulence in animal models.[59,110,179] Strains lacking these important diagnostic targets may still be threats.

Cultures of blood, bubo aspirate, sputum, and bronchial/tracheal washings, and/or cerebrospinal fluid (if indicated) should be performed based on the clinical presentation. Tiny 1- to 3-mm "beaten-copper" colonies will appear on blood agar by 48 hours, but *Y pestis* is slow growing and cultures may appear negative at 24 hours. In one study, 24 of 25 blood cultures (96%) of patients with bubonic plague were positive on standard supplemented peptone broth.[6] In patients with lymphadenopathy, a bubo aspirate should be obtained by inserting a 20-gauge needle attached to a 10-mL syringe containing 1 mL of sterile saline. Saline is injected and withdrawn several times until it is tinged with blood. Repeated, sterile bubo aspiration may also be done to decompress buboes and relieve pain. Drops of the aspirate should be air-dried on a slide and methanol-fixed for staining. When evaluating stained material, it should be considered that the characteristic bipolar staining is not specific for *Y pestis* nor is it always observed. If available, a direct fluorescent antibody stain of bubo aspirate for the presence of *Y pestis* capsular antigen should be performed; a positive direct fluorescent antibody result is more specific for *Y pestis* than are the other listed stains (Figure 10-12).[180]

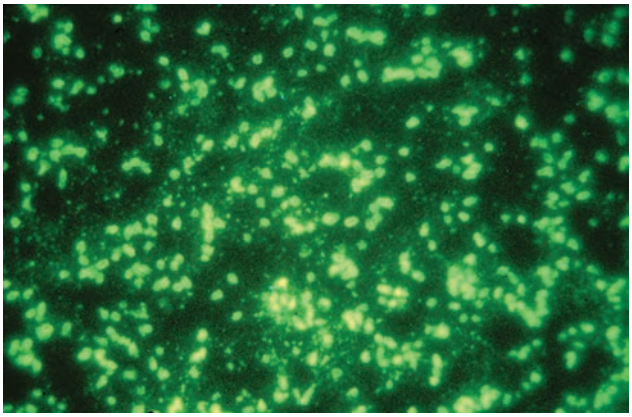

Figure 10-12. These *Yersinia pestis* fluorescent cells are from an infected mouse spleen. Notice how the outlines of the coccobacilli "light up" in this direct fluorescent antibody (DFA) test. The DFA test is specific and therefore better than the other stains discussed in this chapter (original magnification × 1,000).
Photograph: Courtesy of MC Chu, Centers for Disease Control and Prevention Laboratory, Fort Collins, Colorado.

In patients with plague, complete blood counts often reveal leukocytosis with a left shift. Leukemoid reactions with up to 100,000 white blood cells/μL may be seen, especially in children. Platelet counts may be normal or low, and partial thromboplastin times are often increased. Leukocytosis with thrombocytopenia is common in plague, but rare in other conditions.[181] When disseminated intravascular coagulation is present, fibrin degradation products will be elevated. Because of liver involvement, alanine aminotransferase, aspartate aminotransferase, and bilirubin levels are often increased.

TREATMENT

Isolation

Previous editions of this chapter recommended strict isolation until patients had received treatment for 48 hours. However, only standard infection precautions are necessary when caring for bubonic and septicemic plague patients, whereas droplet precautions are still recommended until pneumonic plague patients have received 48 hours of antibiotic therapy. However, such droplet precautions are strictly only necessary when patients are coughing up of bloody sputum. Simply wearing masks, practicing good

hand hygiene, and avoiding close contact (within 2 m) will almost always prevent pneumonic plague transmission.[77,182]

Microbiology laboratory personnel must be alerted when *Y pestis* is suspected because laboratory-acquired plague cases have been reported in the United States.[92,96] Treatment of plague is summarized on the CDC website (http://www.cdc.gov/plague/healthcare/clinicians.html).

Antibiotics

Both because of difficulty acquiring streptomycin and its unfavorable side effect profile, the Working Group on Civilian Biodefense and the CDC now recommend gentamicin as a first-line alternative to streptomycin.[79,183] Gentamicin is given 5 mg/kg intramuscularly (IM) or intravenously (IV) once daily, or 2 mg/kg loading dose followed by 1.7 mg/kg IM or IV three times daily.[79] A recent review of 75 cases of human plague in New Mexico demonstrated that gentamicin alone or in combination with a tetracycline was as efficacious as streptomycin for treating human plague cases.[184] Alternate regimens recommended by the Working Group on Civilian Biodefense include doxycycline (100 mg IV twice daily or 200 mg IV once daily), ciprofloxacin (400 mg IV twice daily) or chloramphenicol (25 mg/kg IV as a loading dose, followed by 60 mg/kg/d in four divided doses).[79]

Recently, a randomized, comparative, open label clinical trial comparing monotherapy with gentamicin or doxycycline for treating plague was conducted in Tanzania.[185] Thirty-five plague patients received gentamicin (2.5 mg/kg IM every 12 hours for 7 days) and 30 patients received doxycycline (100 mg [adults] and 2.2 mg/kg [children] orally every 12 hours for 7 days). Both gentamicin and doxycycline were found to be effective therapies for adult and pediatric plague, resulting in favorable response rates of 94% and 97% for gentamicin and doxycycline, respectively. [185] The three deaths occurred on the first or second day of treatment when disease was thought to be too advanced to respond to antibiotics. However, in this study a paucity of pneumonic plague cases existed, and the authors also recommended the use of a loading dose when using doxycycline (200 mg every 12 hours for 72 hours before completing the therapy with the conventional dose of 100 mg every 12 hours [or 200 mg every 24 hours]) to ensure early attainment of high-tissue concentrations of doxycycline in the face of a life-threatening infection.[186]

Chloramphenicol is indicated for conditions in which high tissue penetration is important, such as plague meningitis, pleuritis, or myocarditis, but it can cause bone marrow suppression. It can be used separately or combined with an aminoglycoside. In pregnant women, the preferred choice is gentamicin with doxycycline or ciprofloxacin as alternatives, and streptomycin should be avoided if possible.[79] The treatment of choice for plague in children is streptomycin or gentamicin. The Working Group on Civilian Biodefense recommended doxycycline, ciprofloxacin, or chloramphenicol as alternatives.[79] Chloramphenicol should not given to children younger than 2 years old because of the risk of grey baby syndrome.

In 2013, the Food and Drug Administration approved the fluoroquinolone antibiotic levofloxacin for treating patients with plague. This approval was based on the agency's Animal Efficacy Rule, which allows studies from animals to be used in situations where it is not feasible or ethical to conduct clinical trials in humans. In this study, 24 African green monkeys who had been challenged with aerosolized *Y pestis* of the CO92 strain received either placebo or levofloxacin at a dose equivalent to 500 mg IV every 24 hours for 10 days. Treatment was begun after the fever developed. Sixteen of 17 levofloxacin treated animals survived as compared with none of the seven control animals, which all died with 5 days of exposure. The one treated animal that died was euthanized because of vomiting and inability to retain food; blood cultures 2 to 4 days before death were negative.[80]

The Working Group on Civilian Biodefense has also proposed recommendations for antibiotic therapy in a mass casualty setting and for postexposure prophylaxis. Because IV or IM therapy may not be possible in these situations, oral therapy preferably with doxycycline or ciprofloxacin is recommended.[79] Levofloxacin, 500 mg once a day for 10 days, is also Food and Drug Administration- approved for postexposure prophylaxis[187] based on the Animal Efficacy Rule. If treated with antibiotics, buboes typically recede in 10 to 14 days and do not require drainage. Patients are unlikely to survive primary pneumonic plague if antibiotic therapy is not initiated within 18 hours of symptom onset. Without treatment, mortality is 60% for bubonic plague and 100% for the pneumonic and septicemic forms.[169]

Prevention

All plague-control measures must include insecticide use, public health education, environmental sanitation to reduce sources of food and shelter for rodents, and perhaps reduction of rodent populations with chemicals such as cholecalciferol.[27,40] Fleas must always be targeted before rodents, because killing rodents may release massive amounts of infected fleas.[161] The

use of insecticides in rodent areas is effective because rodents pick up dust on their feet and carry it back to their nests, where they distribute it over their bodies via constant preening.[27]

Postexposure Prophylaxis

Asymptomatic individuals such as family members, healthcare providers, or other close contacts with persons with untreated pneumonic plague should receive antibiotic prophylaxis for 7 days. Close contact is defined as contact with a patient at distance less than 2 m.[79] Prophylaxis is also recommended for laboratory workers who have had an exposure to *Y pestis*. Doxycycline is the preferred antibiotic, given as 100 mg twice daily for 7 days. Levofloxacin or ciprofloxacin are alternatives. The Working Group for Civilian Biodefense recommends that people who develop fever or cough while receiving prophylaxis should seek prompt medical attention and begin parenteral antibiotic treatment.[79] Hospital personnel who are observing recommended isolation procedures do not require prophylactic therapy, nor do contacts of bubonic plague patients. However, people who were in the same environment and were potentially exposed to the same source of infection as the plague patients should be given prophylactic antibiotics. The CDC also recommends that prophylactic antibiotics be given to persons potentially exposed to the bites of infected fleas (during a plague outbreak, for example) or who have handled animals infected with the plague bacterium. In addition, previously vaccinated individuals should receive prophylactic antibiotics if they have been exposed to plague aerosols.

Natural antibiotic resistance is rare in *Y pestis*; however, a chilling report appeared in 1997 of a human isolate in Madagascar resistant to streptomycin, tetracycline, chloramphenicol, ampicillin, kanamycin, and sulfonamide. A transmissible plasmid, pIP1202, was responsible for the multidrug-resistant phenotype of this isolate, suggesting a potential for transfer to other *Y pestis* strains in nature.[188] This plasmid is closely related to transmissible multidrug-resistant plasmids of *Yersinia ruckeri* and *Salmonella enterica* serotype Newport SL254 identified in the United States.[189] More recently, a multidrug-resistant strain of *Y pestis* was isolated from a rodent in Mongolia.[190] Russian scientists have published descriptions of multidrug-resistant plague vaccine strains produced in the laboratory; these techniques could conceivably be used on virulent strains.[191] Ciprofloxacin-resistant isolates have been obtained in the laboratory from attenuated strains.[192] If *Y pestis* is used as a biological weapon, then antibiotic resistance is a possibility; the stability and transmissibility of the multidrug-resistant pIP1202 plasmid in *Y pestis* suggests that such a strain could be engineered in the laboratory via conjugation without modern molecular technologies.

Vaccination

While working in India in 1897, Ukrainian microbiologist Waldemar MW Haffkine developed the first plague vaccine consisting of killed whole cells. In 1942, Karl F Meyer, DVM, began developing an immunogenic and less reactogenic vaccine for the US Army from an agar-grown, formalin-killed, suspension of virulent plague bacilli. This same procedure (with minor modifications) was used to prepare the licensed vaccine, Plague Vaccine USP, which was routinely given to military personnel stationed in Vietnam and other individuals such as field personnel working in plague-endemic areas with exposure to rats and fleas and laboratory personnel working with *Y pestis*. However, this vaccine was discontinued by its manufacturers in 1999 and is no longer available. Although Plague Vaccine USP was effective in preventing or ameliorating bubonic disease, as seen by the low incidence of plague in US military personnel serving in Vietnam, data from animal studies suggest that this vaccine does not protect against pneumonic plague.[151,152,193–195]

The former Soviet Union and many other nations have traditionally focused on live attenuated vaccines, with tens of millions of humans receiving the live plague vaccine. Many investigators continue to believe that live attenuated vaccines are preferable to subunit vaccines. Live plague vaccines, usually derived from the attenuated EV76 strain, have been used alone and also successfully in a prime-boost strategy with subunit vaccines. Even the most recent reviews on plague vaccination continue to revisit the appeal of live attenuated vaccines.[196,197]

Two new plague vaccine candidates that use the F1 and V antigens of *Y pestis* have been developed. F1, a capsular antigen of *Y pestis*, appears to prevent phagocytosis of plague bacilli, whereas V antigen has a key role in the translocation of the cytotoxic Yops into host cells, as well as stimulating the production of immunosuppressive cytokines. US Army Medical Research Institute of Infectious Diseases scientists developed the first vaccine, F1-V, which consists of a recombinant fusion protein expressing F1 and V antigens (F1-V).[198] Porton Down, the biodefense laboratory in the United Kingdom, developed a similar candidate that is a recombinant protein-based vaccine consisting of two separate proteins, F1 and V.[199] The separate proteins are then combined, two parts F1 to one part V, to form a subunit vaccine.

The F1-V vaccine, which has been shown to protect African green monkeys from pneumonic plague,[198] is currently in advanced development by the Department of Defense's Joint Project Manager Medical Countermeasures, a component of the Joint Program Executive Office for Chemical and Biological Defense. The Joint Project Manager Medical Countermeasures facilitates the advanced development and acquisition of medical countermeasures and systems to enhance the US biodefense response capability.

SUMMARY

Plague is a zoonotic infection caused by the gram-negative bacillus *Y pestis*. Plague is maintained in nature, predominately in urban and sylvatic rodents and flea vectors. Humans are not necessary for the persistence of the organism, and they acquire the disease from animal fleas, contact with infected animals, or, rarely, from other humans via aerosol or direct contact with infected secretions. Healthcare providers must understand the typical way in which humans contract plague in nature to differentiate endemic disease from plague used in biological warfare. First, a die-off of the mammalian reservoir that harbors bacteria-infected fleas will occur. Second, troops who have been in close proximity to such infected mammals will become infected and typically develop the bubonic form of the disease. By contrast, in the most likely biological warfare scenario, plague would spread via aerosol and result in primary pneumonic plague cases. Person-to-person spread of fulminant pneumonia, characterized by blood-tinged sputum, would then ensue. If, however, an enemy force released fleas infected with *Y pestis*, the soldiers would present with classic bubonic plague before a die-off in the local mammalian reservoir occurred, although such a die-off may be possible later if the introduced strain of *Y pestis* succeeded in proliferating among local rodent populations.

The most common form of the disease is bubonic plague, characterized by painful lymphadenopathy and severe constitutional symptoms of fever, chills, and headache. Septicemic plague without localized lymphadenopathy occurs less commonly and is difficult to diagnose. Secondary pneumonia may follow either the bubonic or the septicemic form. Primary pneumonic plague is spread by airborne transmission, when infectious respiratory droplets from an infected human or animal are inhaled or a person inhales an aerosol released as the result of biological weapon attack.

Diagnosis is established by isolating the organism from blood or other tissues. Rapid diagnosis may be made with fluorescent antibody stains of sputum or tissue specimens or detection of F1 antigen in serum. Patients should be isolated and treated with aminoglycosides. Chloramphenicol should be added when meningitis is suspected or shock is present. Although the licensed, killed, whole-cell vaccine is no longer available, a new vaccine that appears to protect against pneumonic plague is in advanced development.

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