Chapter 5

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. Plague is foremost a disease of rodents; over 200 species have been reported to be infected with *Y pestis*. 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. Humans or animals with plague pneumonia, particularly cats, can generate infectious aerosols. The resulting primary pneumonic plague is the most severe and most 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* caused three great pandemics of human disease. The bubonic form of *Y pestis* 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; the confluent mass of nodes, if untreated, may suppurate and drain pus); and bacteremia. Septicemic plague can ensue from untreated bubonic plague or without obvious lymphadenopathy after a fleabite. Patients with the bubonic form of *Y pestis* may develop secondary pneumonic plague, which can lead to human-to-human spread by the respiratory route. Cervical lymphadenopathy 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. Cervical lymphadenopathy, which is more common in patients from developing countries, may result from flea bites on the neck or face while sleeping on the dirt floors of heavily flea-infested buildings.

During the past four 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 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 plague is not the differential diagnosis, or treatment is absent or delayed.

The US military’s concern with plague is both as an endemic disease and as a biological warfare threat. To best prepare 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 currently available for plague, although candidates are in clinical trials. A better understanding of the preventive medicine aspects of the disease will aid in the prompt diagnosis and effective treatment necessary to survive a plague attack.

Key terms in this chapter include enzootic and epizootic. These terms refer, respectively, to plague that is normally present in an animal community but occurs in only a small number of animals, and to widespread plague infections leading to death among susceptible host populations (ie, equivalent to an epidemic in a human population). The death of a rodent causes the living fleas to leave that host and seek other mammals, including humans. Knowledge of these two concepts helps to clarify how and when humans may be infected, in either endemic or biological warfare scenarios.

HISTORY

The Justinian Plague (First Pandemic)

Procopius provided 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 is now considered the first great pandemic of the CE. At the height of the epidemic, more than 10,000 people died each day. As many as 100 million Europeans, including 40% of Constantinople’s population, died during this epidemic. Repeated, smaller epidemics followed this plague.

The Black Death (Second Pandemic)

The second plague pandemic, known as the Black Death, brought the disease into the collective memory of Western civilization. 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. 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 entered Great Britain at Weymouth.

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. However, some people believe that the plague persisted through 1720, with a final foray into Marseilles. 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.

Failing to understand the plague’s epidemiology, 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. Physicians recommended a simple diet; avoidance of excessive sleep, exercise, and emotion; regular enemas; and abstinence from sexual intercourse. Although some people killed cats and dogs because they were thought to carry disease, rats seemed to escape attention. Christians blamed plague on Muslims, Muslims blamed it on Christians, and both Christians and Muslims blamed it on Jews or witches.

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

Accurate clinical descriptions of the Black Death were written by contemporary observers such as Giovanni Boccaccio in *Decameron*:

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.

Marchionne di Coppo di 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 of 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....

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.”

The second great pandemic slowly subsided in Europe by 1720. The pandemic’s decline was 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 epidemiology mysteries.

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. In 1894 Alexandre Yersin discovered *Y pestis* and satisfied Robert Koch’s postulates for bubonic plague. 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.\textsuperscript{21} The modern pandemic arrived in Bombay in 1898, and during the next 50 years, more than 13 million Indians died of rat-associated plague.\textsuperscript{21,22}

The disease officially arrived in the United States in March 1900, when the lifeless body of a plague-infected Chinese laborer was discovered in a hotel basement in San Francisco, California. The disease subsequently appeared in New York City and Washington state the same year.\textsuperscript{23,24} The plague appeared in New Orleans, Louisiana, in 1924 and 1926.\textsuperscript{24} The Texas Gulf Coast and Pensacola, Florida, also saw the influx of plague. Before 1925, human plague in the United States was a result of urban rat epizootics. 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.\textsuperscript{25} 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 fewer than casualties caused by disease and nonbattle injuries.\textsuperscript{26} *Y. pestis* can initiate disease both through endemic exposure and as a biological warfare agent. Medical officers need to distinguish likely from unlikely cases of endemic disease and consider the possible biological warfare threat.

**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.\textsuperscript{21}

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 forces were almost free of plague. However, the disease remains on and near military bases in the western United States because the local mammal populations are reservoirs of infection.

**World War II**

Endemic plague became established in Hawaii (on the islands of Hawaii and Maui) in December 1899. No evidence of the disease, however, in either rodents or humans has been found on the islands of Oahu or Kauai since the first decade of the 20th century. During World War II and the Vietnam War, US forces were almost free of plague. However, the disease remains on and near military bases in the western United States because the local mammal populations are reservoirs of infection.

Official policy during World War II was to vaccinate US troops with the whole-cell killed plague vaccine. No troops contracted plague, although they served in known endemic areas.\textsuperscript{27,28} Plague has since disappeared from Hawaii.

**Vietnam War**

Plague entered Vietnam in Nha Trang in 1898 and several pneumonic epidemics have occurred since then.\textsuperscript{21,29,30} Cases have been reported in Vietnam every year since 1898, except during the Japanese occupation in World War II.\textsuperscript{21} When French forces departed Vietnam after the Indochina War, public health conditions deteriorated, and plague flourished. The reported plague incidence increased from 8 cases in 1961 to 110 cases in 1963, and to an average of 4,500 cases annually from 1965 through 1969.\textsuperscript{25,31-34} The mortality in clinically diagnosed cases was between 1\% and 5\%. In untreated individuals, it was higher (60\%–90\%).\textsuperscript{21,32} However, only eight American troops were affected (one case per 1 million human-years) during the Vietnam War.\textsuperscript{34} 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 insect repellent use, protective clothing, and rat-proofed dwellings.\textsuperscript{21,32} During this period, two officers of the US Army Medical Service Corps, Lieutenant Colonel Dan C Cavanaugh and Lieutenant Colonel John D Marshall, 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.\textsuperscript{21,35}

**Disease Threat on US Military Installations**

Human exposure to plague on military installations may occur at home when pets bring in 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. Plague risk has been identified on and near several US military installations (Exhibit 5-1).

Plague as a Biological Warfare Agent

The first 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. 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. The 20th-century use of plague as a potential biological warfare weapon is of concern and should be considered, 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 pneumatic 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 by plague because it could create casualties out of

<table>
<thead>
<tr>
<th>EXHIBIT 5-1</th>
<th>PLAGUE RISKS AT US MILITARY INSTALLATIONS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plague-infected animals on the installation; human case reported on post:</td>
<td>Rocky Mountain Arsenal, Colorado</td>
</tr>
<tr>
<td>Fort Hunter Liggett, California</td>
<td>Vandenberg Air Force Base, California</td>
</tr>
<tr>
<td>US Air Force Academy, Colorado†</td>
<td>White Sands Missile Range, New Mexico</td>
</tr>
<tr>
<td>Human case reported in the same county:</td>
<td>Plague-infected animals or fleas in the same county but not on the installation:</td>
</tr>
<tr>
<td>Edwards Air Force Base, Colorado‡</td>
<td>Bridgeport Naval Facility, California</td>
</tr>
<tr>
<td>FE Warren Air Force Base, Wyoming</td>
<td>Camp Roberts, California</td>
</tr>
<tr>
<td>Kirtland Air Force Base, New Mexico§</td>
<td>Dyess Air Force Base, Texas</td>
</tr>
<tr>
<td>Peterson Air Force Base, Colorado</td>
<td>Fort Bliss, Texas</td>
</tr>
<tr>
<td>Plague-infected animals on the installation:</td>
<td>Fort Lewis, Washington</td>
</tr>
<tr>
<td>Dugway Proving Ground, Utah</td>
<td>Sierra Army Depot, California</td>
</tr>
<tr>
<td>Fort Carson, Colorado</td>
<td>Togolele Army Depot, Utah</td>
</tr>
<tr>
<td>Fort Ord, California</td>
<td>Umatilla Army Depot Activity, Oregon</td>
</tr>
<tr>
<td>Fort Wingate Army Depot Activity, New Mexico</td>
<td>Nellis Air Force Base, Nevada</td>
</tr>
<tr>
<td>Marine Corps Mountain Warfare Training Center, Bridgeport, California</td>
<td>No plague-infected animals or fleas on the installation or in the county, but susceptible animals present:</td>
</tr>
<tr>
<td>Navajo Army Depot Activity, Arizona</td>
<td>Fort Huachuca, Arizona</td>
</tr>
<tr>
<td>Pueblo Army Depot Activity, Colorado</td>
<td></td>
</tr>
</tbody>
</table>

*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.
†Fatalities: 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.
proportion to the number of bacteria disseminated, the most dangerous strains could be used to make a very dangerous 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.³⁶

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 too low for safety. However, clay bombs solved these technical difficulties and resulted in an 80% survival rate of fleas.³⁶

At 5:00 AM on a November day 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 was observed falling from the plane. Within 2 weeks, individuals in that area of the city 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 mortality was not noted until months after the human cases. It was also observed that plague usually spreads with rice (because rats infest the grain) along shipping routes, but the nearest epidemic center was 2,000 km away by land or river. Changteh exported, not imported, rice. These unusual circumstances surrounding the plague outbreak suggest that it may have been of human origin.³⁶

In another incident, on October 4, 1940, a Japanese plane dropped rice and wheat grains mixed with fleas over the city of Chuhsien in the 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 the 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 increased mortality was found in the rat population.³⁶

Since World War II

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.³⁷ 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.”³⁷ 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 groups may likely consider obtaining plague for use as a biological weapon.

During the Korean War, allied forces were accused of dropping insects that could spread plague, typhus, malaria, Japanese B encephalitis, and other diseases on North Korea. However, no evidence supports such claims.³⁸

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.³⁹ 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.⁴⁰ This proposal was not well received, primarily because of 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 millennia, during which the enzootic “pestoides” isolates evolved (see Biochemistry below). The pestoides strains appear to have split from Y pseudotuberculosis over 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.41

**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 5-1). Depending on growth conditions, *Y pestis* can exhibit marked pleomorphism with rods, ovoid cells, and short chains present. A gelatinous envelope, known as the F1 capsular antigen, is produced by the vast majority of strains at a growth temperature of 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.39,42

**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 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% CO₂. The round, moist, translucent, or opaque colonies are nonhemolytic on sheep blood agar and exhibit an irregular edge. 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.20,39,42 Strains to be archived should be grown at low temperatures and frozen promptly at –70°C.

**Biochemistry**

*Y pestis* is a facultative anaerobe, fermenting glucose with the production of acid. An obligate pathogen, 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 and ferment glycerol.20 Some molecular methods of typing, such as ribotyping and restriction fragment-length polymorphisms of insertion sequence locations, support this division of strains.43,44 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+), found in Central Asia and Africa and may represent the most ancient
of the biovars.\textsuperscript{20,43} Biovar mediaevalis (Gly\textsuperscript{−}, Nit\textsuperscript{−}) is geographically limited to the region surrounding the Caspian Sea. No apparent differences in pathogenicity exist among the biovars.\textsuperscript{20,44} Recently, three different multilocus molecular methods were used to investigate the microevolution of \textit{Y pestis}. Eight populations were recognized. An evolutionary tree for these populations rooted on \textit{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 \textit{Y pestis}, “pestoides,” which exhibit biochemical characteristics of both \textit{Y pestis} and \textit{Y pseudotuberculosis}.\textsuperscript{46} These isolates represent the most ancient of the \textit{Y pestis} strains characterized to date.\textsuperscript{41}

### 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.\textsuperscript{21} Subsequently, more than 200 species of mammals and 150 species of fleas have been implicated in maintaining \textit{Y pestis} endemic foci throughout the world, although only a relative few species play a significant role in disease transmission.\textsuperscript{23,47,48} \textit{Y pestis} is not capable of blocking (see below) all flea species and there appears to be variability in the ability of various flea species to transmit the organism.\textsuperscript{48}

The oriental rat flea (\textit{Xenopsylla cheopis}) has been largely responsible for spreading \textit{Y pestis} during bubonic plague epidemics. Some researchers think it is the most efficient flea for transmitting plague.\textsuperscript{19} After the flea ingests a blood meal from a bactereemic animal, bacilli multiply and eventually block the flea’s foregut, or proventriculus, with a fibrinoid mass of bacteria as shown in Figure 5-2.\textsuperscript{21} When feeding, the flea ingests approximately 0.03 mL to 0.5 mL of blood. High-level bacteremia is a hallmark of \textit{Y pestis} infection in susceptible hosts. This bacteremia provides a sizeable inoculum for the flea and promotes the subsequent blockade. Blockage limits feeding resulting in repeated attempts by the flea to feed. Because of the blockage, blood carrying \textit{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.\textsuperscript{2} As many as 24,000 organisms may be inoculated into the mammalian host.\textsuperscript{21} 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.\textsuperscript{21,26}

Although the largest plague outbreaks have been associated with \textit{X cheopis}, all fleas should be considered dangerous in plague-endemic areas.\textsuperscript{2,49} During the Black Death, the human flea, \textit{Pulex irritans}, may have aided in human-to-human plague spread; during other epidemics, bedbugs (\textit{Cimex lectularius}), lice, and flies have been found to contain \textit{Y pestis}.\textsuperscript{19} However, the presence of plague bacilli in these latter insects is associated with ingestion of contaminated blood from plague victims, and they apparently had little or no role as vectors for the disease. The most important vector of human plague in the United States appears to be \textit{Oropsylla montana}, the most common flea on rock squirrels and California ground squirrels,\textsuperscript{25} 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.

The black rat, \textit{Rattus rattus}, has been most responsible worldwide for persistence and spread of plague in urban epidemics throughout history. \textit{R rattus} is a nocturnal, climbing animal that does not burrow, but instead nests overhead and lives in close proximity to humans.\textsuperscript{10} In the United Kingdom and much of Europe, the brown rat, \textit{R norvegicus}, a burrowing

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig5-2.png}
\caption{The oriental rat flea (\textit{Xenopsylla cheopis}) has historically been most responsible for the spread of plague to humans. This flea has a blocked proventriculus, 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. \textcopyright Kenneth L Gage, PhD, Centers for Disease Control and Prevention Laboratory, Fort Collins, Colorado.}
\end{figure}
animal that lives under farm buildings and in ditches, has replaced *R. rattus* as the dominant city rat.\(^9\) 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.\(^10\) Most carnivores, except cats, are resistant to plague infection, but animals such as domestic dogs, all rodents, and burrowing owls may transport infected fleas into homes. Mammals that are partially resistant to plague infection are continuous plague reservoirs. Some epidemiologists propose that the true plague hosts are rodent species with populations consisting of both sensitive and resistant individuals, but others have questioned the need for resistant individuals within the species to maintain plague foci.\(^50\) In the United States, prairie dogs (*Cynomys* species) and *Spermophilus* species (rock squirrels and ground squirrels) are most often associated with plague activity. A variety of susceptible mammals, such as chipmunks, tree squirrels, cottontail rabbits, and domestic cats (Figure 5-3), are occasionally infected. Epizootic spread among tree squirrels in Denver recently resulted in the first urban plague case since the 1920s.\(^47\) Although not associated with any human plague cases, the appearance of two infected fox squirrels in Dallas, Texas, in 1993 also caused considerable concern.\(^51\) An increasing

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**Fig. 5-3.** Known mammalian reservoirs of plague in the United States (noninclusive). The common North American marmot (a) and the brown rat (*Rattus norvegicus*) (b), which has largely replaced the black rat, are considered to be reservoirs of plague (ie, hosts to infected fleas). Other reservoirs of plague during enzootics are thought to include the deer mouse (c), the California ground squirrel (d), and the 13-lined ground squirrel (e). Other infective mammals that can spread plague to humans include the chipmunk (f), prairie dogs (g), and the coyote (h). Domestic and nondomestic cats are also reservoirs of plague. This cat (i), which died of pneumonic plague, demonstrates a necrotic head.

Photographs a, h: Courtesy of Denver Zoological Society, Denver, Colorado. Photographs b-g, i: Courtesy of Centers for Disease Control and Prevention Laboratory, Fort Collins, Colorado.
number of human infections has been associated with domestic cats, usually through bites, contact with tissues, suppurating buboes, or aerosol rather than by transmission of fleas. Cats appear to be particularly efficient at transmitting disease to humans.

Highly susceptible animals amplify both fleas and bacilli and often support the spread of epizootics, especially when these animals occur at high densities. In many developing countries, these epizootics often involve commensal rat species (Rattus). 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 served as sources of infection for humans, who acquired the disease by handling infected prairie dogs. Rock squirrels and California ground squirrels both infect humans via direct contact and fleas. Many other mammals in the United States harbor plague, and a few, including wild carnivores such as bobcats, have served as sources of infection for humans (Exhibit 5-2).

Knowledge of local host species is important, because certain mammal–flea complexes found in the United States are particularly dangerous: these complexes consist of both a susceptible mammal and a flea known to bite humans. More than one host-epizootic complex can occur in a given area. These pairings include the following:

- the rock squirrel (S variegatus) or California ground squirrel (S beechyi) and the flea Oropsylla montana, which is known to readily bite humans;
- the antelope ground squirrel (Ammospermophilus leucurus) and the flea Thrassus bacchi;
- the prairie dog (Cynomys species) and the flea Opisocrostis hirsutus;

<table>
<thead>
<tr>
<th>EXHIBIT 5-2</th>
<th>MAMMALS KNOWN TO HARBOR PLAGUE IN THE UNITED STATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnivores</td>
<td>Black bears, cats (including bobcats and mountain lions), coyotes, dogs, foxes, martens, raccoons, skunks, weasels, wolverines, wolves</td>
</tr>
<tr>
<td>Rodents</td>
<td>Chipmunks, gophers, marmots, mice, prairie dogs, rats, squirrels, voles</td>
</tr>
<tr>
<td>Lagomorphs</td>
<td>Hares, rabbits</td>
</tr>
<tr>
<td>Hooved Stock</td>
<td>Pigs, mule deer, pronghorn antelope</td>
</tr>
</tbody>
</table>


Fig. 5-4. Plague cycles in the United States. This drawing shows the usual, occasional, and rare routes by which plague is known to have spread between various mammals and humans. Reproduced with permission from Poland JD. Plague. In: Hoeprich PD, Jordan MC, eds. Infectious Diseases: A Modern Treatise of Infectious Processes. Philadelphia, Pa: Lippincott; 1989; 1297.
• the Wyoming ground squirrel (Spermophilus richardsoni) or the golden-mantled ground squirrel (S lateralis) and the fleas Opisocrosta labis, Opisocrosta idahoensis, or Thrassus bacchi; and
• various wood rat species (Neotoma sp) and the fleas Orchopeas sexdentatus and Orchopeas neotomae.

Plague exists in one of two states in nature: (1) enzootic or (2) epizootic. An enzootic cycle is a stable rodent–flea infection cycle presumably occurring in a relatively resistant host population and not causing excessive rodent mortality. When the disease is in an enzootic cycle, the fleas have no need to seek less desirable hosts—such as humans. During an epizootic, however, plague bacilli have been introduced into moderately or highly susceptible mammals. High mortality occurs, most conspicuously in larger colonial rodents such as prairie dogs. These epizootics are most likely to occur 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. Humans are accidental hosts in the plague cycle and are not necessary for the persistence of the organism in nature (Figure 5-4).

INCIDENCE

World Health Organization (WHO) member states are required to notify WHO of human plague cases under the International Health Regulations, although the policy on bubonic plague in endemic areas may soon change. Plague may be significantly underreported for several reasons, including the reluctance of some endemic countries to admit public health problems, difficulties in diagnosis, and the absence of laboratory confirmation. From 1989 to 2003, 38,310 cases (with 2,845 deaths) were recorded in 25 countries, with the highest number of human plague cases reported in 1991 and the lowest number in 1989. Generally, the distribution of plague coincides with the geographical distribution of its natural foci.

Plague is endemic in many countries in Africa, the former Soviet Union, the Americas, and Asia. In 2002 human plague was reported in Africa (Democratic Republic of the Congo, Madagascar, Malawi, Mozambique, Uganda, and the United Republic of Tanzania), the Americas (Peru and the United States), and Asia (China, India, Kazakhstan, Mongolia, and Vietnam). The vast majority of these cases (approximately 95%) were in Africa. Since the early 1990s, increasing reports of plague in Africa may represent an increase in disease or an improvement in notification to WHO. Recent resurgence of plague in India, Indonesia, and Algeria during the past decade has occurred after “silent” periods of 30 to 50 years. Worldwide distribution of plague and its epidemiology can be found in the WHO’s Plague Manual available online at http://www.who.int/csr/resources/publications/plague. Recent reports of plague activity and occasional summaries of plague activity can be found at the Web sites for WHO’s Weekly Epidemiological Record (http://www.who.int/CSR/weekly/epi_report) and the Centers for Disease Control and Prevention’s Morbidity and Mortality Weekly Report (http://www.cdc.gov/mmwr). Known foci of plague are shown in Figure 5-5.

Recently, 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 for two cases of the septicaemic form, were reported as pneumonic plague. No evidence of bubonic disease was observed. Multidisciplinary health teams from WHO investigated the potential outbreak, but no report has been issued since March 2005. The prevalence of pneumonic disease in the 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 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. In the first quarter of this century, virtually all 432 cases and 284 deaths (65.7% mortality) in the United States occurred in urban port cities. Epidemics occurred in San Francisco, California, during 1900 through 1904 (118 deaths) and 1907 through 1908 (78 deaths). The last time plague was transmitted between humans in the United States was during the 1924 through 1925 pneumonic plague epidemic in Los Angeles, California. Eighty percent of cases since 1925 have been sylvatic, involving contact with wild rodent habitats. Most cases (58%) are in men and occur within a 1-mile radius of home, and half of the victims in the United States have been younger than 20 years old.

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; it then decreased to 9 per year since 1990. 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. Most human plague cases are reported from New Mexico, Arizona, Colorado, and California.

Epizootic cycles occur approximately every 5 years; the last extremely widespread epizootic with a large die-off of rodents over multiple states (in 1982–1984) was accompanied by the highest number of humans infected with plague since the urban epidemics of the first quarter of the 20th century.

**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 different hosts. To maintain the transmission cycle, *Y. pestis* must 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 that of the midgut of the flea, 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 body temperature of this host; presumably additional factors also cue the organism to recognize this environment and respond.
appropriately. Likewise, genes required for replication in the mammalian host are expressed at highest levels at 37°C; and the synthesis of some proteins, thought to be induced in the phagolysosome, is also regulated by pH. In the laboratory, growth temperature and calcium concentration control both the synthesis and secretion of certain essential virulence factors; the induction of these proteins has been termed the low calcium response (LCR).6,20,62,63

Recent genetic analyses of Y pestis and the other pathogenic Yersinia 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: (1) pMT (or pFra), which encodes the F1 capsule, and (2) pPCP, which carries the gene for the virulence factor plasminogen activator. The third plasmid is common to the human pathogenic Yersinia and is known as pCD (calcium dependence), pYV (Yersinia virulence), or pLcr (low calcium response). This plasmid, which is responsible for the synthesis of a number of antihost factors, is an absolute requirement for virulence.20

Type III Secretion System

Like a number of other gram-negative pathogens, the human pathogenic Yersinia 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.6,65 In the case of the Yersinia species, this system is encoded on the pYV plasmid, which encodes the components of the LCR. Toxic activities of the LCR effector proteins, designated Yops (Yersinia outer protein), 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.66 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 antiinflammatory role by inhibiting inflammatory cytokine production and inducing apoptosis in macrophages.63-65,67 Overall, the effect is that of paralyzing professional phagocytes, and it is clear why the pathogen–host interaction mediated by the type III secretion system has been designated the “Yersinia Deadly Kiss.”68

LcrV (historically known as V [or yirulence] an-
tigen), 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: (a) as regulator of Yop transcription, (b) for translocation of Yops into the host cell, and (c) as a virulence factor in its own right.20,63 LcrV appears to stimulate production of the immunosuppressive cytokine interleukin 10 through interactions with Toll-like receptor 2 and CD14 signaling. These effects appear to be mediated by the N-terminal portion of LcrV.69,70

The secretion mechanism includes an “injectisome” that can be visualized as a needle-like structure by electron microscopy. Another group of proteins promotes the secretion process by forming pores in the mammalian cell membrane. 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 a secretion channel that allows the Yops to be translocated through the membrane and into the host cell.64,65

F1 Capsular Antigen

The F1 capsule, 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. This gelatinous envelope is generally thought to protect the organism from host phagocytic cells by interfering at the level of receptor interaction in the phagocytosis process,71 and 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 F1 capsular antigen, F1-negative strains have been isolated from rodent hosts and reportedly from one human case.72-75 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, although mutations leading to loss of the capsular antigen increase time to death in the mouse.72,76 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, the studies indicate 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 F1, or other gene products encoded by this plasmid, may play an

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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*. It is the major antigen recognized in convalescent sera of humans and rodents,\(^{77,78}\) and is also a highly effective protective immunogen.

**Other Virulence Factors in the Mammalian Host**

The virulence factor plasminogen activator (Pla) is encoded by 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.\(^{20,79}\) Pla is necessary for full virulence in some \( Y \) *pestis* strains. However, a few strains that are Pla-negative and appear to be fully virulent have been identified among natural isolates or generated in the laboratory.\(^{20,80,81}\) Presumably, these isolates synthesize other proteins that substitute for Pla function.

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 and pH range of 5.0 to 6.7, which 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.\(^{82}\) More recent data, however, suggest that the pH 6 antigen does not enhance adhesion to mouse macrophages but rather promotes resistance to phagocytosis.\(^{83}\)

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 many bacteria, \( Y \) *pestis* possesses a high-affinity iron uptake system that is capable of procuring this essential nutrient from the host. Strains that do not produce yersiniabactin, a low-molecular-weight iron chelator, or those unable to transport yersiniabactin are not capable of growth in mammals.\(^{20,84}\) The genes encoding this iron transport system are situated on a chromosomal pathogenicity island with the Hms locus (see below).

**Virulence Factors in the Flea**

Researchers have begun to address the factors that allow \( Y \) *pestis* to block the flea and promote vectorborne transmission. Both chromosomal and plasmid-encoded gene products have been found to play roles in flea blockage. One of these loci, Hms, is expressed only at temperatures lower than 28°C; bacteria producing the Hms-encoded outer membrane protein are hydrophobic and form aggregates in vitro. Although Hms mutants are capable of colonizing the flea midgut, they are unable to colonize the proventriculus and, therefore, do not block the flea. Hms-mediated aggregation promotes formation of a biofilm that allows the organism to persist in the proventriculus despite the shearing forces that flush nonaggregating cells into the midgut.\(^{85}\) Hms 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 does not appear to play a role in mammalian plague. 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 is responsible for attenuation.\(^{20}\)

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.\(^{86}\) 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.\(^{86}\) This may be explained by the regulation of toxin synthesis. Like Hms, murine toxin is produced more efficiently at 28°C than at mammalian body temperatures.

**PATHOGENESIS**

As few as 1 to 10 \( Y \) *pestis* organisms are sufficient to cause infection by the oral, intradermal, subcutaneous, or intravenous routes.\(^{84}\) Estimates of infectivity by the respiratory route for nonhuman primates vary from 100 to 20,000 organisms.\(^{76,87,88}\)

After being introduced into the mammalian host by a flea, the organism is thought to be initially susceptible to phagocytosis and killing by neutro-
phil. However, some of the bacteria may grow and proliferate within tissue macrophages. The relative importance of intracellular versus extracellular replication in plague has been extensively debated. Although most of the bacterial multiplication in the mammalian host is extracellular, evidence indicates that \textit{Y. pestis} can survive and multiply in macrophages. As reviewed by Fujol and Bliska, growth inside host cells is likely to be of greatest importance at the early stages of colonization. They suggest that, although considerable attention has been focused on how \textit{Y. pestis} subverts the functions of phagocytes from the outside, there is less understanding of how these bacteria affect macrophage functions from the inside. Once the antiphagocytic gene products are expressed, the bacteria are resistant to phagocytosis and multiply extracellularly.

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

Primary pneumonic plague, the most severe form of disease, arises from inhalation of an infectious aerosol. Primary pneumonic plague is more rapidly fatal than secondary.

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 been reported to occur in contacts of patients with either bubonic or pneumonic plague.

**CLINICAL MANIFESTATIONS**

From 1947 through 1996, 390 cases of plague were reported in the United States, resulting in 60 deaths (15.4%). 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%). Seven cases (1.8%) were unclassified, including one (death 14.3%). If \textit{Y. pestis} was used as a biological warfare agent, the clinical manifestations of plague would be (a) epidemic pneumonia with rapid progression and a high fatality rate if aerosolized bacteria were used or (b) bubonic or septicemic plague—or both, if fleas were used as carriers. Infections via ingestion could also occur.

**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). Six to 8 hours after onset of symptoms, buboes, heralded by severe pain, occur in the groin (femoral and inguinal lymph nodes), axillary, or cervical lymph nodes—depending on the site of bacterial inoculation (Figure 5-6). Buboes, which are 1 cm to 10 cm in diameter, have overlying erythematous skin and are so painful that nearly comatose patients attempt to shield them from trauma and abduct their extremities to decrease pressure. Buboes are often associated with considerable surrounding edema, but lymphangitis is rare. Occasionally, buboes can become fluctuant and suppurate. A small minority of patients bitten by plague-infected fleas develop \textit{Y. pestis} septicemia without a discernible bubo. Other manifestations of bubonic plague include bladder distention, apathy, confusion, fright, anxiety, oliguria, and anuria. Tachycardia, hypotension, leukocytosis, and fever are frequently encountered. Untreated bubonic plague can result in septicemia 2 to 6 days later, which, if left untreated, is virtually 100% fatal. In the United States, approximately 10% to 15% of bubonic plague patients will develop secondary pneumonic plague with the potential for airborne transmission of the organism.

**Septicemic Plague**

Septicemic plague may occur primarily, or secondarily, as a complication of hematogenous dissemination of bubonic plague. 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 5-7), disseminated intravascular coagulation, and acral cyanosis and necrosis, particularly of the extremities (Figure 5-8), 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 plague.
Fig. 5-6. 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.

Fig. 5-7. 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.
which indicates the difficulty of diagnosing septicemic plague. Diagnosis of septicemic plague took longer (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 attributable to hepatosplenomegaly.

Pneumonic Plague

Pneumonic plague may occur primarily, from inhaling aerosols, or secondarily, from hematogenous dissemination. Patients with pneumonic plague typically have symptoms of a severe bronchopneumonia, dyspnea, cough, chest pain, and hemoptysis. The findings on chest roentgenography may be variable, but bilateral alveolar infiltrates appear to be the most common finding (Figure 5-9). The sputum, which may be clear, purulent, or hemorrhagic, contains gram-negative rods. Unless appropriate antimicrobial therapy is begun on the first day of illness, pneumonic plague is rapidly fatal. The time from respiratory exposure to death in humans is reported to have been between 2 and 6 days in epidemics during the preantibiotic era.

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.
Pharyngeal Plague

Asymptomatic transient pharyngeal carriage has been reported to occur in healthy contacts of bubonic plague cases. Rarely, pharyngitis resembling tonsillitis and associated with cervical lymphadenopathy has been reported. A plague syndrome of cervical buboes, peritonsillar abscesses, and fulminant pneumonia has also been reported. A similar syndrome may have also occurred in Vietnam. Consuming meat from infected camels and goats is also implicated in the development of disease.

Cutaneous Manifestations

Approximately 4% to 10% of plague patients have an ulcer or pustule at the inoculation site (Figure 5-10). The flea typically bites the lower extremities; therefore, femoral and inguinal buboes are the most common. Infection arising from skinning plague-infected animals typically produces axillary buboes. Buboes may point and drain spontaneously or, rarely, they may require incision and drainage because of pronounced necrosis. Petechiae and ecchymoses may occur during hematogenous spread to such an extent that the signs mimic severe meningococcemia, with similar lesions. The pathogenesis of these lesions is probably that of a generalized Shwartzman reaction (disseminated intravascular coagulation secondary to the *Y pestis* endotoxin). When purpura and acral gangrene occur, possibly resulting from the activities of the plasminogen activator, the prognosis is poor. Patients in the terminal stages of pneumonic and septicemic plague often develop large ecchymoses on the back. Lesions like these are likely to have led to the medieval epithet “the Black Death.” Ecthyma gangrenosum has been reported in several patients. A sample from a case of ecthyma gangrenosum grew *Y pestis*, which suggests that the skin lesions resulted from septicemic seeding of the organism.
Plague

UNIT 30

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 healthcare provider is unfamiliar with plague or if the patient presents in a nonendemic area or without a bubo, the diagnosis may be delayed or missed. When a bubo is present, the differential diagnosis should include tularemia, cat scratch disease, lymphogranuloma venereum, chancroid, tuberculosis, streptococcal adenitis, and scrub typhus (Figure 5-11). In both tularemia and cat scratch disease, the inoculation site is typically more evident and the patient is usually not 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 have a recent history of sexual contact and

Fig. 5-11. (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.
genital lesions. Those with the latter disease may be as sick as patients with plague. Streptococcal adenitis may be difficult to distinguish from plague initially, but the patient is not usually septic, and the node is more tender when plague is present.

Implications of the absence of a bubo were discussed in a review of 27 plague cases seen in New Mexico. In this study, there were 8 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 development was associated with a delay in the diagnosis of plague and increased mortality.100

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 lead to 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 Centers for Disease Control and Prevention Web site (http://www.bt.cdc.gov/agent/plague/index.asp).100 The World Health Organization offers its Plague Manual online at http://www.who.int/emc-documents/plague/whocdcsredc992c.html. A recent review of the methodology for isolating and identifying Y pestis from clinical samples and animals is available. Standard bacterial methodologies include staining and microscopic analysis of the organism, isolation on culture medium, and biochemical tests. Laboratories experienced in the identification of Y pestis with the appropriate containment facilities should perform diagnostic tests for plague. Care should be taken to avoid aerosols; in this regard, fixing slides with methanol rather than heat fixing is preferred.

Reference laboratories, such as those found in major county or state health departments, have additional tests to confirm the diagnosis of Y pestis. These include direct fluorescent antibody tests to detect the F1 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. Serologic tests 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 Centers for Disease Control and Prevention, Fort Collins, Colorado; and the US Army Medical Research Institute of Infectious Diseases at Fort Detrick, Maryland.104 Serologic assays measuring the immune response to plague infection are mainly of value retrospectively because patients present clinically before they develop a significant antibody response.

When using the fluorescent antibody test to detect the plague-specific F1 capsular antigen, it is important to realize that F1 is produced only at temperatures greater than 33°C. Thus, this method requires a relatively fresh sample from the patient or 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-negative.42 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 test detected concentrations of F1 antigen as low as 0.5 mg/mL in as little as 15 minutes and had a shelf life of 21 days at 60°C. It had 100% sensitivity and specificity against laboratory isolates of Y pestis, and the agreement between field-testing and reference laboratory testing was 89.9%. The test demonstrated positive and negative predictive values of 90.6% and 86.7%, respectively.105 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 the rapid diagnosis of plague 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 the surveillance of rats and could be adapted to help diagnose human infection.106 The use of Pla primers for simulated detection of Y pestis in sputum was reported recently to have a sensitivity of 10⁴ colony forming unit/mL and a 5-hour turnaround.107 When trying to determine whether Y pestis has been used as a biological weapon, it should be kept in mind that F1 or Pla are not necessary for virulence in animal models,72,76,80 and strains lacking these important diagnostic targets could be seen.

Cultures of blood, bubo aspirate, sputum, and cerebrospinal fluid (if indicated) should be performed. Tiny, 1- to 3-mm “beaten-copper” colonies 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. 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 remembered that the characteristic bipolar staining is not specific for \textit{Y pestis}, nor is it always observed. If available, a direct fluorescent antibody stain of bubo aspirate for the presence of \textit{Y pestis} capsular antigen should be performed; a positive direct fluorescent antibody result is more specific for \textit{Y pestis} than are the other listed stains (Figure 5-12).\textsuperscript{58}

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. When disseminated intravascular coagulation is present, fibrin degradation products are elevated. Because of liver involvement, alanine aminotransferase, aspartate aminotransferase, and bilirubin levels are often increased.

**Isolation**

All patients with plague should be isolated for the first 48 hours after the initiation of treatment. Special care must be taken in handling blood and bubo discharge. If pneumonic plague is present, then strict, rigidly enforced respiratory isolation procedures must be followed, including the use of gowns, gloves, and eye protection. Patients with pneumonia must be isolated until they have completed at least 48 hours of antibiotic therapy and have a favorable clinical response. If patients have no pneumonia or draining lesions at 48 hours, they may be taken out of strict isolation. Microbiology laboratory personnel must be alerted when \textit{Y pestis} is suspected (four laboratory-acquired plague cases have been reported in the United States).\textsuperscript{108}

**Antibiotics**

Since 1948 streptomycin has remained the treatment of choice for bubonic, septicemic, and pneumonic plague. Streptomycin is approved by the Food and Drug Administration for treatment of plague. 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.\textsuperscript{110} Although there are no controlled comparative trials, in a recent review of 75 human plague cases in New Mexico researchers concluded that gentamicin alone or in combination with a tetracycline was as efficacious as streptomycin for treating humans infected with plague.\textsuperscript{111} Alternative 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).\textsuperscript{110} Chloramphenicol is indicated for conditions in which high tissue penetration is important, such as plague meningitis, pleuritis, or myocarditis; it can be used separately or combined with an aminoglycoside. In pregnant women, the preferred choice is gentamicin with doxycycline or ciprofloxacin as alternatives. Streptomycin should be avoided in pregnant women if possible.\textsuperscript{110} The treatment of choice for plague in children is streptomycin or gentamicin. Doxycycline, ciprofloxacin, or chloramphenicol are recommended as alternatives by the Working Group on Civilian Biodefense.\textsuperscript{110} Chloramphenicol should not be used on
children less than 2 years old due to the risk of “grey baby syndrome.” 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. In patients 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 the onset of symptoms. Without treatment, mortality is 60% for bubonic plague and 100% for the pneumonic and septicemic forms.

**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. Fleas must always be targeted before rodents, because killing rodents may release massive amounts of infected fleas. 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 through constant preening. Under International Health Regulations, plague must be reported to WHO as an internationally quarantinable disease for which travelers may be detained for up to 6 days. However, because of ongoing revisions in the International Health Regulations, bubonic cases in endemic areas may no longer be subject to mandatory reporting.

**Postexposure Prophylaxis**

Asymptomatic individuals, such as family members, healthcare providers, or others who have had close contact with persons with untreated pneumonic plague should receive antibiotic prophylaxis for 7 days. Close contact is defined as contact with a patient at less than 2 m. Prophylaxis is also recommended for laboratory workers exposed to an accident, which may have created an infectious aerosol. Doxycycline is the preferred antibiotic, given as 100 mg twice daily for 7 days. Ciprofloxacin or chloramphenicol are alternatives. The Working Group for Civilian Biodefense recommends that people who develop fever or cough while receiving postexposure prophylaxis seek prompt medical attention and begin parenteral antibiotic treatment. Hospital personnel who are observing recommended isolation procedures do not require prophylactic therapy, nor do people in contact with patients with bubonic plague. However, people who were in the same environment and who were potentially exposed to the same source of infection as the patients with plague should be given prophylactic antibiotics. The Centers for Disease Control and Prevention 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 known to be 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 was responsible for the multidrug-resistant phenotype of this isolate, suggesting a potential for transfer to other Y pestis strains in nature. Russian scientists have published descriptions of multidrug-resistant plague vaccine strains produced in the laboratory; these techniques also could conceivably be used on virulent strains. Ciprofloxacin-resistant isolates have been obtained in the laboratory from attenuated strains. If Y pestis is used as a biological weapon, antibiotic resistance is a possibility.

**Vaccination**

In 1897 Russian physician Waldemar MW Haffkine developed the first plague vaccine consisting of killed whole cells in India. 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, that 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.

Two new plague vaccine candidates that use the F1 and V antigens of Y pestis have been developed. F1 appears to prevent phagocytosis of plague bacilli,
and 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).\textsuperscript{118} 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.\textsuperscript{119} The separate proteins are then combined, two parts F1 to one part V, to form a subunit vaccine. F1-V vaccine has been shown to protect African green monkeys from pneumonic plague.\textsuperscript{118} Both vaccines are in clinical trials, although the Porton vaccine is somewhat more advanced in the process. After further testing, it is conceivable that one of these vaccine candidates may be selected for further development as a human vaccine candidate that could protect against pneumonic plague.

SUMMARY

Plague is a zoonotic infection caused by the gram-negative bacillus \textit{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. By contrast, in the most likely biological warfare scenario, plague would be spread via aerosol. Person-to-person spread of fulminant pneumonia, characterized by blood-tinged sputum, would then ensue. If, however, an enemy force were to release fleas infected with \textit{Y pestis}, then soldiers would present with classic bubonic plague before a die-off in the local mammalian reservoir occurred.

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 aerosols from an infected human or animal are inhaled.

Diagnosis is established by isolating the organism from blood, sputum, or other fluids or tissues. Rapid diagnosis may be made with fluorescent antibody stains of sputum or tissue specimens. Patients should be isolated and treated with aminoglycosides. Chloramphenicol should be added when meningitis is suspected or shock is present. A licensed, killed, whole-cell vaccine is no longer available. New vaccines that appear to protect against pneumonic plague are being considered for development.

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