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Plague as a Biological Weapon

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Plague [L. *plaga*] A stroke, blow or wound; an affliction, calamity or scourge, especially a visitation of divine justice or anger.

(Anonymous, 1998)

1. HISTORY OF PLAGUE AND ITS POTENTIAL AS A WEAPON OF BIOTERRORISM

1.1. Pandemic History and Epidemic Potential

Three well-documented plague pandemics have occurred in the past two millennia, resulting in more than 200 million deaths and great social and economic chaos (Perry and Fetherston, 1997; Pollitzer, 1954). The Justinian pandemic arose in northern Africa in the mid-6th century, and by the 7th century had spread throughout the Mediterranean and near-eastern regions – severely impacting both the Roman and Byzantine empires. The second pandemic, the Black Death or great pestilence, originated in Central Asia, was carried to Sicily in 1347 via ships from the Crimea, and rapidly swept through medieval Europe. By 1352, it had killed 30% or more of afflicted populations, slowly playing itself out in successive epidemics, including the Great Plague of London in 1665 (Perry and Fetherston, 1997). The third (Modern) pandemic began in southwestern China in the mid-19th, struck Hong Kong in 1894, and was soon carried by rat-infested steamships to port cities on all inhabited continents, including several in the United States (US) (Link, 1955; Pollitzer, 1954). By 1930, the third pandemic had caused more than 26 million cases and 12 million deaths. Plague in these three pandemics was predominantly the bubonic form, emanating

from *Yersinia pestis*-infected rats and fleas, although terrifying outbreaks of the more virulent person-to-person spreading pneumonic form were recorded during the course of each. The explosive contagiousness and severity of pneumonic plague was most completely documented in Manchurian epidemics in the early 20th century, which involved tens of thousands of cases, virtually all of them fatal (Wu, 1926).

Improved sanitation, hygiene, and modern disease control methods have, since the early 20th century, steadily diminished the impact of plague on public health, to the point that an average of 2,500 cases is now reported annually (World Health Organization, 2003). The plague bacillus is, however, entrenched in rodent populations in scattered foci on all inhabited continents except Australia (Gage, 1998; Gratz, 1999b), and eliminating these natural transmission cycles is unfeasible. Furthermore, although treatment with antimicrobials has reduced the case fatality ratio of bubonic plague to 10% or less, the fatality ratio for pneumonic plague remains high. A review of 420 reported plague cases in the US in the period 1949–2000 identified a total of 55 cases of plague pneumonia, of which 22 (40.0%) were fatal (Centers for Disease Control and Prevention, unpublished data); of 7 primary pneumonic cases, the fatality ratio was 57.1% (Centers for Disease Control and Prevention, 1997). Although pandemics are unlikely to recur, plague – including the pneumonic form – holds considerable outbreak potential (Boisier *et al.*, 2002; Campbell and Hughes, 1995; Chanteau *et al.*, 1998, 2000; Gabastou *et al.*, 2000; Ratsitorahina, 2000a). This potential could be exploited for purposes of terrorism or warfare.

1.2. Plague as a Weapon of Biological Warfare

The idea of using plague as a weapon is not new. Anecdotal reports describe catapulting of plague cadavers into enemy fortifications in 14th and 18th century warfare (Derbes, 1996; Gasquet, 1908; Marty, 2001). In World War II, the Japanese military experimented with plague in human subjects at their clandestine biological research facilities in Manchuria, and on several occasions dropped *Y. pestis*-infested fleas from low-flying planes on Chinese civilian populations, causing limited outbreaks of bubonic plague and initiating cycles of infection in rats (Bellamy and Freedman, 2001; Harris, 1992; Kahn, 2002). Biological warfare research programs begun by the Soviet Union (USSR) and the US during the Second World War intensified during the Cold War, and in the 1960s both nations had active programs to “weaponize” *Y. pestis*. In 1970, a World Health Organization (WHO) expert committee on biological warfare warned of the dangers of plague as a weapon, noting that the causative agent was highly infective, that it could be easily grown in large quantities and stored for later use, and that it could be dispersed in a form relatively resistant to desiccation and other adverse environmental conditions (World Health Organization, 1970). Models developed by this expert committee predicted that the intentional release of 50 kg of aerosolized *Y. pestis* over a city of 5 million would, in its primary effects, cause 150,000 cases of pneumonic plague and 36,000 deaths. It was further postulated that, without adequate precautions, an initial outbreak of pneumonic plague involving 50% of a population could result in infection of 90% of the rest of the population in 20–30 days and could cause a case fatality ratio of 60–70%. The work of this committee provided a basis for the 1972 international Biological Weapons and Toxins Convention prohibiting

biological weapons development and maintenance, and that went into effect in 1975 (Marty *et al.*, 2001). It is now known that, despite signing this accord, the USSR continued an aggressive clandestine program of research and development that had begun decades earlier, stockpiling battle-ready plague weapons (Alibek, 1999). The Soviets prepared *Y. pestis* in liquid and dry forms as aerosols to be released by bomblets, and plague was considered by them as one of the most important strategic weapons in their arsenal. They also developed virulent fraction 1 (F1) capsular antigen-deficient and antimicrobial-resistant strains of *Y. pestis* and performed experiments to create an agent that could evade vaccine-induced immunity, be unresponsive to standard antibiotic treatment, and be difficult to identify. Moreover, the USSR was capable through a number of industrial plants to manufacture a plague weapon in hundreds of tons (Alibek, 1999). The US military biowarfare program also recognized that aerosolized *Y. pestis* had the basic attributes suitable for a large-scale attack (Martin and Marty, 2001; Marty, 2001), but US military scientists failed in their attempts to weaponize plague, apparently because they were unable to produce sufficient quantities of *Y. pestis* in stable form. Offensive biological weapons research was halted by the US in 1970, but Soviet efforts continued at least until 1990. Although Russia converted its civilian biological weapons to legitimate ends, it is unclear whether their military program has ceased development work and eliminated all of its stores (Alibek, 1999). Many nations maintain biological weapons defense programs that adhere to the ban on the development of offensive weapons; however, there is an obvious potential offensive value of studies to better understand candidate agents, and their possible modes of delivery, dispersal, and effectiveness under varying conditions.

1.3. US Countermeasures to Plague as a Weapon of Terrorism

No longer is the capacity for biological weapons development limited to the most technologically advanced states, but has expanded to include small rogue states, terrorist groups, cults, and even individuals. Because of the gathering terrorist threat, the US Congress passed the Biological Weapons Act of 1989 and the Chemical and Biological Weapons Control and Warfare Elimination Act of 1991 (Ferguson, 1997). In the wake of terrorist bombings of the World Trade Center in New York and the Alfred P. Murrah Federal Office Building in Oklahoma City, the arrest in the US of a microbiologist for illegally acquiring *Y. pestis* for suspicious purposes, and numerous bioweapons hoaxes, Congress passed the Anti-Terrorism Act of 1996 (Anonymous, 1996; Ferguson, 1997). Under this Act, the federal Centers for Disease Control and Prevention (CDC) was directed to identify dangerous biological agents that could be used by terrorists (select agents), and to establish a regulatory system for governing their acquisition, use, and transfer (U.S. Department of Health and Human Services, 1996). *Y. pestis* was classified by CDC as 1 of 6 Category A select biological agents that posed the highest risk to national security and was placed under these regulations (Centers for Disease Control and Prevention, 2000, 2002; Rotz *et al.*, 2002). Since 1997, federal law (42 FR 72.6) (Anonymous, 1997) has strengthened regulations on the transfer of *Y. pestis* and other restricted agents from one facility to another, requiring that the shipping and receiving facilities each complete an official transfer form prior to shipment. These regulations were supplemented by 42 CFR part 73

(Possession, Use, Transfer of Select Agents and Toxins), as specified in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (effective 2003). 42 CFR part 73 details requirements for laboratories that handle plague and other select agents, including registration, security risk assessments, safety plans, security plans, emergency response plans, training, transfers, record keeping, inspections, and notifications (Centers for Disease Control and Prevention, 2002). These and other regulations have raised questions about protection of freedom of scientific research and civil liberties while combating potential terrorist actions (Annas, 2002; Fidler, 2001).

1.4. Preparedness and Response to a Possible Plague Attack

The CDC has developed a strategic plan to address the deliberate dissemination of plague and other select agents (Centers for Disease Control and Prevention, 2000; Khan *et al.*, 2000; Rotz *et al.*, 2002). The Johns Hopkins University Schools of Medicine and Public Health formed a Working Group on Civilian Biodefense Strategies to draw up consensus recommendations for measures to be taken following use of plague or other select agents as biological weapons against a civilian population (Johns Hopkins Center for Civilian Biodefense Strategies, 2003; Inglesby *et al.*, 2000). A critical advance was the development by the Johns Hopkins group of a Model State Emergency Health Powers Act that provides a model for state officials to follow in assuming extraordinary powers necessary to better detect and contain a potentially catastrophic disease outbreak. These powers range from pre-emergency planning to compensation for private property, and include tracking and reporting of certain diseases, the management of property, and the protection of persons (Johns Hopkins Center for Civilian Biodefense Strategies, 2003; Mair *et al.*, 2002).

It is assumed that a terrorist attack would most likely use a *Y. pestis* aerosol, possibly resulting in large numbers of severe and fatal primary and secondary pneumonic plague cases. Especially given plague's notoriety, even a limited event would likely cause public panic, create large numbers of the "worried-well," foster irrational evasive behavior, and quickly place an overwhelming stress on medical and other emergency response elements working to save lives and bring about control of its spread (Glass and Schoch-Spana, 2002; Osterholm and Schwartz, 2000; O'Toole and Inglesby, 2001). This was the situation that arose in the large industrial city of Surat, India, during the plague emergency there in 1994 (Dennis, 1994; Ramalingaswami, 2001).

In the US, emergency preparedness to counter bioterrorism includes the stockpiling of drugs, vaccines, and medical equipment for rapid deployment; the establishment of emergency operations centers with advanced communications systems; and a permanently staffed terrorism response unit established at CDC that works closely with the Department of Homeland Security (Centers for Disease Control and Prevention, 2000; Centers for Disease Control and Prevention, Bioterrorism Program, 2003; Rotz *et al.*, 2002). Several simulations of a plague attack have been conducted in the US as learning exercises leading to strengthened national and local preparedness and response; these have involved all levels of government, numerous agencies, and a wide range of first responders, including public safety and law enforcement personnel, hazardous materials teams, emergency medical and public health staff, and information specialists. Two of these, TOPOFF I (Denver, 2001)

and TOPOFF II (Chicago, 2003), so-named because they involved top government officials, were based on coordinated national and local responses to simulated plague attacks. During these simulations, critical deficiencies in emergency response became obvious, including the following: problems in leadership, authority, and decision-making; difficulties in prioritization and distribution of scarce resources; failures to share information; and overwhelmed health care facilities and staff. The need to formulate in advance sound principles of disease containment, and the administrative and legal authority to carry them out without creating confusing new government procedures were glaringly obvious (Block, 2003; Hoffman, 2003; Hoffman and Norton, 2000; Khan and Ashford, 2001; Inglesby *et al.*, 2001).

2. PLAGUE MICROBIOLOGY AND PATHOGENESIS

2.1. The Agent

2.1.1. General Characteristics

Y. pestis is a Gram-negative, microaerophilic, pleomorphic coccobacillus (1.0–2.0 μm \times 0.5 μm) belonging to the family Enterobacteriaceae (Perry and Fetherston, 1997). In direct smears, *Y. pestis* presents as single cells or short chains of cells, characteristically appearing as plump bacilli exhibiting a bipolar staining (closed safety-pin) appearance when treated with Wayson, Giemsa, or Wright stains. The bacillus is nonmotile and nonsporulating. It does not ferment lactose and is catalase-positive, and urease-, oxidase-, and indole-negative. *Y. pestis* is relatively nonreactive, and automated biochemical systems may lead to misidentifications unless correctly programmed (Wilmoth *et al.*, 1996). Growth occurs in a variety of media at a wide range of temperatures (4–40°C; optimal 28–37°C) and pH values (5.0–9.6; optimal 6.8–7.6). However, *Y. pestis* grown at 28–30°C and with pH over 7.2 is more stable under natural conditions and in an aerosol form (K. Alibek, personal communication). *Y. pestis* is relatively slow growing in culture, with pinpoint colonies usually visible after 24 hours of growth at 28°C on sheep blood agar and later on MacConkey agar. The colonies are raised and opalescent in appearance, developing a hammered copper-appearing surface and irregular borders as they grow larger. In broth culture, a stalactite pattern of growth occurs along the sides of the vessel and settles to the bottom in clumps if disturbed. Almost all naturally occurring *Y. pestis* strains have been found to be susceptible in vitro to tetracyclines, chloramphenicol, sulfonamides, aminoglycosides, and fluoroquinolones (Frean *et al.*, 1996, 2003; Smith *et al.*, 1995). Rarely, isolates from several areas of the world have shown incomplete susceptibility to one or more antimicrobials recommended for treating plague (Rasoamanana *et al.*, 1989); these have occurred in isolated instances, have not been followed by a recognized wider emergence of these strains, and have not required modifications of standard protocols for treatment and control. However, of greater concern was the identification in 1995 of a strain of *Y. pestis* from a patient in Madagascar who was multiply resistant to the principal recommended antimicrobials used in treating plague, and the resistance was plasmid-mediated and transferable (Galimand *et al.*, 1997). This finding elicited calls for intensified surveillance of patients

and the environment (Dennis and Hughes, 1997), which fortunately have not led to identification of other such strains in Madagascar or elsewhere.

2.1.2. Molecular Genetics

Gene sequencing comparisons of multiple strains of *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* show that *Y. pestis*, a blood-borne organism, only recently (1,500–20,000 years ago) evolved from *Y. pseudotuberculosis*, an enteric pathogen (Achtman *et al.*, 1999). Decoding of the entire genome of *Y. pestis* (consisting of 4.65 Mb chromosome and three plasmids of 96.2 kb, 70.3 kb, and 9.6 kb) disclosed that the evolution of *Y. pestis* was made possible by the acquisition of virulence determinants suitable for systemic invasion of mammalian hosts and replication in the flea, and by the inactivation of genes required for enteric survival (Parkhill *et al.*, 2001). These genomic studies suggest that *Y. pestis* is a pathogen that has undergone large-scale genetic flux and provide a unique insight into how new and highly virulent pathogens evolve. Three classic biovars of *Y. pestis* have been identified, including biovar Antiqua, Medievalis, and Orientalis, linked respectively to the three historical pandemics. Results of typing by restriction fragment-length polymorphism analysis of rRNA genes (ribotyping) support these distinctions and have shown chromosomal rearrangements in the Orientalis biotype that occurred following its spread around the world about 100 years ago (Guiyoule *et al.*, 1994). Further studies of polymorphisms show considerable genome plasticity, even within strains from one geographic area (Guiyoule *et al.*, 1997; Radnedge *et al.*, 2002).

2.2. Pathogenicity of *Y. pestis*

2.2.1. Virulence Factors

Y. pestis is among the most pathogenic bacteria known. Although it is a facultative intracellular pathogen that normally grows in extracellular environments, virulence is in part dependent on invasion and multiplication within cells, including phagocytes that transport the bacterium in the initial phases of infection (Hinnebusch, 1997; Perry and Fetherston, 1997). Both chromosomal and plasmid-encoded gene products are associated with adaptability to its various hosts and to virulence (Carniel, 2003; Hinnebusch, 1997; Hinnebusch *et al.*, 2002; Koornhof *et al.*, 1999; Perry and Fetherston, 1997; Smego *et al.*, 1999).

Chromosomal genes of *Y. pestis* express a potent lipopolysaccharide endotoxin and a factor that controls the absorption of exogenous iron. Of the three major plasmids, the pesticin (*pst*) plasmid (~9.5 kb) has genes that encode for a plasminogen activator (Pla) and a bacteriocin or pesticin (Pst). The low calcium response or *Lcr* plasmid (~70 kb), which is shared with other yersiniae, encodes products that activate the V and W antigens and outer surface proteins (Yops) under low calcium conditions. The *caf* operon of the *pFra* (Tox) plasmid (~110 kb) encodes the F1 glycoprotein envelope antigen (Caf1) and a murine toxin (Ymt) unique to *Y. pestis*. F1 antigen is produced only when *Y. pestis* grows at 30°C or greater; strains expressing F1 antigen are able to resist phagocytosis in the absence of

opsonizing antibodies. In summary, *Y. pestis* virulence factors are thought to mediate the following responses between invading organism and the human host:

- The lipopolysaccharide endotoxin activates complement and triggers the release of kinins and other proinflammatory mediators;
- The chromosomally mediated hemin storage molecule (Hms) enhances *Y. pestis* survival in phagocytes and facilitates uptake of the bacillus into eukaryotic cells;
- Another chromosomally encoded product, the pH 6 antigen (Psa), inhibits *Y. pestis* phagocytosis;
- The plasminogen activator (Pla) is a single surface protease that degrades fibrin and other extracellular proteins, facilitating systemic spread of *Y. pestis*;
- The V and W antigens block phagocytosis of *Y. pestis*, and the V antigen promotes survival of *Y. pestis* in macrophages;
- Yops expressed by the 70-kb plasmid inhibit phagocytosis and platelet aggregation, and block an effective inflammatory response;
- The 110-kb plasmid-associated 17-kDa polypeptide F1 antigen (produced optimally at 37°C) is antiphagocytic and also elicits a strong humoral immune response.

Several factors have been identified that are selectively expressed by *Y. pestis* in the gut of fleas. For example, hemin storage locus (*hms*) products expressed at <30°C enable the bacteria to form blockages of the flea gut necessary for efficient transmission. Expression in the midgut of murine toxin (recently identified as phospholipidase D) protects *Y. pestis* from cytotoxic digestion by blood plasma products (Perry, 2003).

2.2.2. Pathology of Infection

The virulence of *Y. pestis* is expressed in a wide spectrum of disease that reflects the portal of pathogen entry and the organ systems targeted (Butler, 1972, 1983; Crook and Tempest, 1992; Dennis and Gage, 2004; Dennis and Meier, 1997; Hull *et al.*, 1987; Welty *et al.*, 1985; Wu, 1926). Plague organisms inoculated through the skin or mucous membranes typically are transported within lymphatic vessels to afferent, regional nodes, where they multiply. In the early stages of infection, affected nodes show vascular congestion, edema, and minimal inflammatory infiltrates or vascular injury; later, however, nodes may contain enormous numbers of infectious plague organisms and demonstrate vascular breaks, hemorrhagic necrosis, and infiltration by neutrophilic leukocytes. These affected nodes (buboes) are typically surrounded by a collection of serous fluid, often blood-tinged. When several adjacent lymph nodes are involved, a boggy, edematous mass can result. In later stages, abscess formation and spontaneous rupture of buboes may occur.

Y. pestis can invade and cause disease in almost any organ, and untreated infection usually results in widespread and massive tissue destruction. Diffuse interstitial myocarditis with cardiac dilatation, multifocal necrosis of the liver, diffuse hemorrhagic necrosis of the spleen and involved lymph nodes, and fibrin thrombi in renal glomeruli are commonly found in fatal cases (Butler, 1972; Dennis and Meier, 1997; Finegold, 1968). Pneumonitis, pleuritis, and meningitis occur less frequently. Abscesses may form in affected organs. Disseminated intravascular coagulation (DIC) is associated with generation of microthrombi, thrombocytopenia, necrosis, and bleeding in affected tissues (Butler, 1972). Petechiae and

ecchymoses commonly appear in the skin, and on mucosal and serosal surfaces. Ischemia and gangrene of acral parts, such as fingers and toes, may occur in the late stages of this process (Dennis and Meier, 1997; Pollitzer, 1954; Wu, 1926).

Primary plague pneumonia resulting from inhalation of infective respiratory particles usually begins as a lobular process and then extends by confluence, becoming lobar and then multilobar. Typically, plague organisms are numerous in the alveoli and in pulmonary secretions. Secondary plague pneumonia arising from hematogeneous seeding of the lungs may begin more diffusely as an interstitial process. In untreated cases of both primary and secondary plague pneumonia, disease progresses to diffuse pulmonary congestion, hemorrhagic necrosis of pulmonary parenchyma, and infiltration by neutrophilic leukocytes (Wu, 1926). In advanced untreated stages, the alveolae are filled with fluid containing massive numbers of plague bacilli.

3. CLINICAL SPECTRUM

3.1. Bubonic Plague

Bubonic plague is characterized by the development of one or more swollen, tender, inflamed lymph nodes termed *buboes*, from the Greek *bubon*, meaning groin. Bubonic plague has a usual incubation period of 2–6 days, occasionally longer. Typically, bubonic plague is heralded by the sudden onset of chills, fever that rises within hours to 100.4°F (38°C) or higher, accompanied by headache, myalgias, arthralgias, and a profound lethargy. Soon, usually within a few hours of symptom onset, increasing swelling, tenderness, and pain occur in one or more regional lymph nodes proximal to the portal of entry. The femoral and inguinal groups of nodes are most commonly involved, axillary and cervical nodes less frequently, varying with the site of inoculation. Buboes occur at a single site in about 90% of cases; sometimes, more than one regional gland grouping may be affected, and bacteremic spread can result in a generalized lymphadenopathy. Typically, the patient guards against palpation and limits movement, pressure, and stretching around the bubo. The surrounding tissue often becomes edematous, sometimes markedly; and the overlying skin is typically reddened, warm, tense, and occasionally desquamated. The bubo of plague differs from lymphadenitis of most other causes by its rapid onset, extreme tenderness, surrounding edema, accompanying signs of toxemia, and usual absence of cellulitis or obvious ascending lymphangitis. Inspection of the skin surrounding the bubo or distal to it may reveal the site of bacterial inoculation marked by a small papule, pustule, scab, or ulcer (phlyctenule). A large furuncular lesion at site of entry, resulting in an ulcer that may be covered by an eschar, occurs occasionally (Figure 2.1). Presenting manifestations in a series of 40 Vietnamese bubonic plague patients were as follows (Butler, 1972):

- Fever (100%; mean of 39.4°C in 32 patients)
- Bubo (100%): groin (88%); axilla (15%); cervical (5%); and epitrochlear (3%)
- Headache (85%)



Figure 2.1. Native American showing primary cutaneous plague ulcer and eschar on anterior chest wall at site of *Yersinia pestis* inoculation, ascending lymphangitis, and axillary bubo.

- Prostration (75%)
- Chills (40%)
- Anorexia (33%)
- Vomiting (25%)
- Cough (25%)
- Skin rash, including petechiae, purpura, and papular eruptions (23%)
- Abdominal pain (18%)
- Chest pain (13%)

Altered brain function – manifest as lethargy, confusion, delirium, seizures – was also common in patients in the Vietnam series.

If treated with an appropriate antimicrobial agent, uncomplicated plague responds quickly, with resolution of fever and other systemic manifestations over a 2- to 5-day period. Buboes often remain enlarged and tender for a week or more after treatment has begun, and infrequently become purulent and fluctuant, and may require incision and drainage. Untreated, they may spontaneously rupture and drain. Without effective antimicrobial treatment, bubonic plague patients typically become increasingly toxic, with fever, tachycardia, lethargy leading to prostration, agitation and confusion, and, occasionally, convulsions and delirium. In the preantibiotic era, the case fatality ratio for bubonic plague was greater than 50%, and it is now about 5%. Mild forms of bubonic plague, called *pestis minor*, have been described in South America and elsewhere; in these cases, the patients are ambulatory and only mildly febrile and have subacute buboes (Legters *et al.*, 1970). The epidemiology and pathophysiology of these mild cases are poorly described, and the syndrome has been attributed to immunological tolerance rather than to a lesser virulence of infecting strains. There is some evidence from serological studies in endemic areas that subclinical *Y. pestis* infections do occur in endemic populations (Ratsitorahina *et al.*, 2000a, 2000b).

Differential diagnostic possibilities for bubonic plague include streptococcal or staphylococcal adenitis, tularemia, cat scratch disease, mycobacterial infection, acute filarial lymphadenitis, aspergillosis and other fungal conditions, chancroid and other sexually transmitted diseases that cause regional lymphadenitis, and strangulated inguinal hernia.

3.2. Septicemic Plague

Plague sepsis is manifest as a rapidly progressive, overwhelming endotoxemia (Butler *et al.*, 1976; Hull *et al.*, 1987). Plague sepsis in the absence of signs of localized infection, such as a bubo, is termed primary septic plague. It can result from direct entry of *Y. pestis* through broken skin or mucous membranes, or from the bite of an infective flea. Secondary septic plague can occur in the course of bubonic or pneumonic plague when lymphatic or pulmonary defenses are breached, and the plague bacillus enters and multiplies within the bloodstream. Bacteremia is common in all forms of plague; septicemia is less common and is immediately life threatening. A diagnosis of primary plague sepsis is often not made until results of blood culture are reported by the laboratory, since there is little to clinically distinguish plague sepsis from other causes of sepsis. Occasionally, plague organisms are visible in stained peripheral blood smears, indicating a poor prognosis (Butler *et al.*, 1976; Hull *et al.*, 1987; Mann *et al.*, 1984). The clinical diagnosis of plague sepsis may be obscured by prominent gastrointestinal symptoms, such as nausea, vomiting, diarrhea, and abdominal pain (Hull *et al.*, 1986). If not treated early with appropriate antibiotics and aggressive supportive care, septic plague is usually fulminating and fatal. Petechiae, ecchymoses, bleeding from puncture wounds and orifices, and subsequent ischemia and gangrene of acral parts are some manifestations of DIC (Figure 2.2). Refractory hypotension, renal shut down, stupor, and other signs of shock are preterminal events. Acute respiratory distress syndrome, which can occur at any stage of septic plague, may be confused with other conditions, such as the hantavirus pulmonary syndrome.



Figure 2.2. Petechiae and ecchymoses on lateral thigh of a patient with plague pneumonia, septicemia, and intravascular coagulation. This patient subsequently developed ischemia and gangrene of acral parts.

Presenting manifestations of septicemic plague in a case series of 18 patients in New Mexico (Hull *et al.*, 1987) include:

- Fever (100%); mean temperature 38.5°C, range 35.4–40.4°C
- Any gastrointestinal symptom (72%)
- Chills (61%)
- Vomiting (50%)
- Nausea (44%)
- Headache (44%)
- Diarrhea (39%)
- Abdominal pain (39%)

Because the diagnosis of plague is often made late, the case fatality ratio is 25% or greater among septicemic patients treated in the US (Crook and Tempest, 1992; Dennis and Chow, 2004; Hull *et al.*, 1987) and approaches 100% in those not receiving appropriate antibiotics.

Differential diagnostic possibilities include any other overwhelming systemic infection, including Gram-negative sepsis with agents other than the plague bacterium, meningococemia, and bacterial endocarditis.

3.3. Pneumonic Plague

Pneumonic plague is the most rapidly developing and fatal form of plague (Doll, 1994; Laforce *et al.*, 1971; Meyer, 1961; Ratsitorahina *et al.*, 2000a; Tieh *et al.*, 1948; Wu, 1926; Wu *et al.*, 1922; Wynne-Griffith, 1948). The incubation period for primary pneumonic plague is usually 3–5 days (range 1–7 days) (Wu, 1926; Wu *et al.*, 1922; K. Alibek, personal communication). The onset is typically sudden, with severe headache, chills, fever, tachycardia, body pains, weakness, dizziness, and chest discomfort. Abdominal pain, nausea, and vomiting may also be present. Cough, sputum production, increasing chest pain, tachypnea, and dyspnea typically predominate on day 2 of the illness, and these features may be accompanied by bloody sputum, increasing respiratory distress, cardiopulmonary insufficiency, and circulatory collapse. In primary plague pneumonia, the sputum is most often watery or mucoid, frothy, and blood-tinged, but it may become frankly bloody. Chest signs in primary plague pneumonia may indicate localized pulmonary involvement in the early stage; a rapidly developing segmental consolidation may be seen before bronchopneumonia occurs in other segments and lobes of the same and opposite lung (Figure 2.3). Liquefaction necrosis and cavitation may develop at sites of consolidation and leave significant residual scarring.

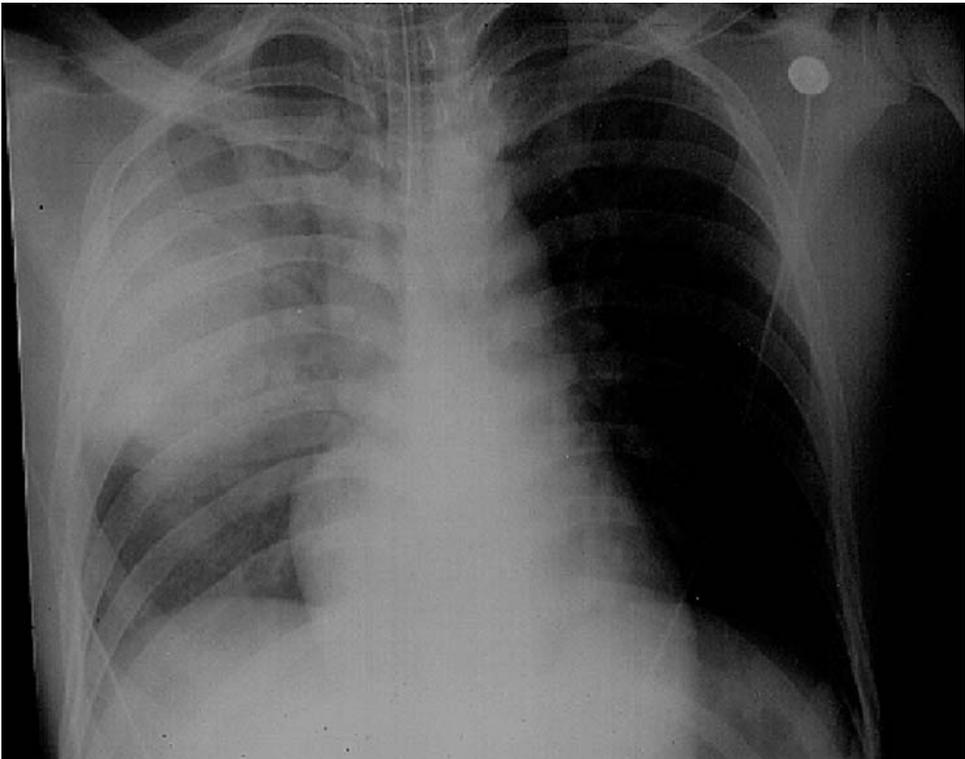


Figure 2.3. Chest radiographs showing rapid progression of primary plague pneumonia on days 3 and 4 of fatal illness.

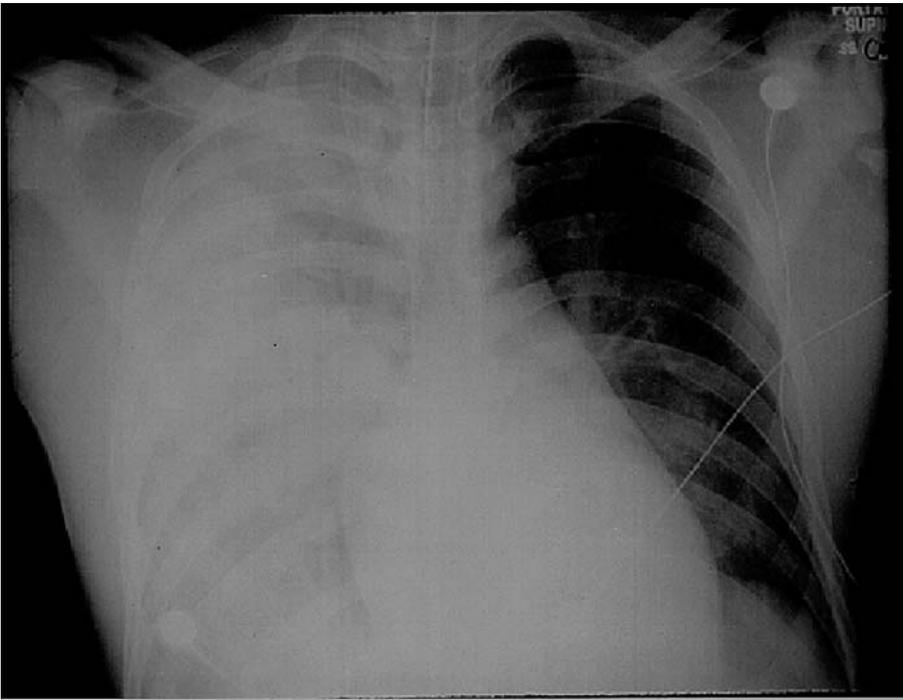
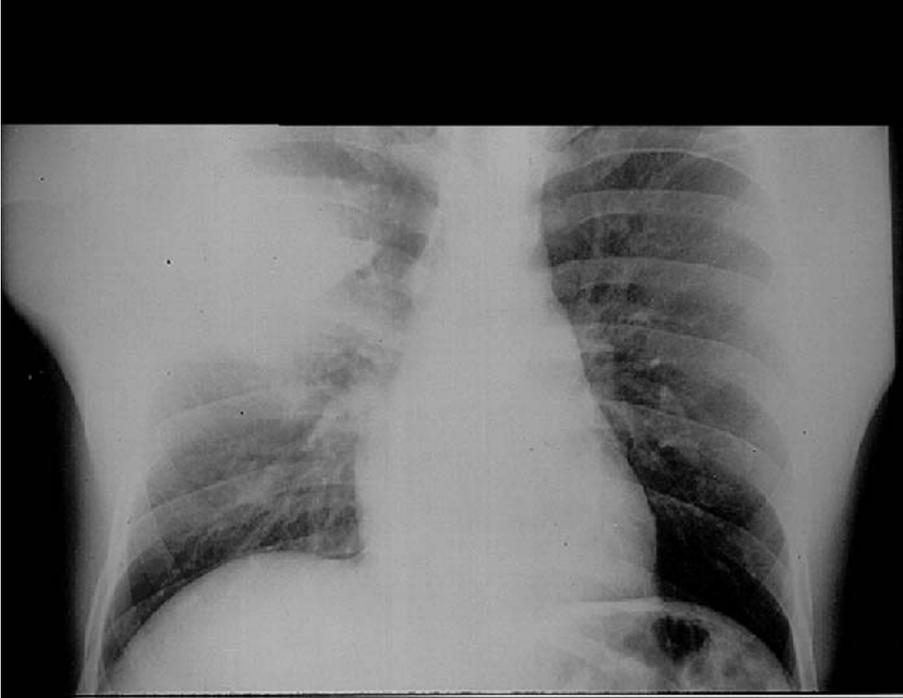


Figure 2.3. Continued

Plague pneumonia arising from metastatic spread is more likely to present in early stages as an interstitial pneumonitis in which sputum production is at first scant. The disease progresses rapidly, however, and chest radiographs described for nine cases of secondary plague pneumonia showed alveolar infiltrates in all cases and pleural effusions in more than half of patients (Alsofrom *et al.*, 1981). Advanced cases often develop refractory pulmonary edema and sepsis syndrome. In the US, there have been no recorded cases of person-to-person transmission of plague since 1924, although more than 50 cases of pneumonic plague have occurred in that time period, with several thousand persons potentially exposed to infection from these patients (Centers for Disease Control, 1984; Centers for Disease Control and Prevention, unpublished data).

Differential diagnostic possibilities include other bacterial pneumonias, such as mycoplasma pneumonia, Legionnaire's disease, staphylococcal or streptococcal pneumonia, tularemia pneumonia, and Q fever. Severe viral pneumonia, including hantavirus pulmonary syndrome and acute respiratory syndrome from coronavirus infection, could be confused with plague.

3.4. Other Clinical Syndromes

Meningitis is an unusual manifestation of plague. In the US, there were 17 (0.4%) cases of meningitis among the total 421 cases of plague reported in the 45-year period from 1947 to 2001, and 13 of these 17 (77%) were in children (Dennis and Chow, 2003). In the majority of these cases, meningitis was a late-arising occurrence in treated bubonic plague and nearly all patients survived (Becker *et al.*, 1987; Centers for Disease Control and Prevention, 1997; Mann *et al.*, 1982). Less commonly, infection of the meninges can occur in the apparent absence of a bubo. The patient typically has fever, meningismus, altered mental status, and a purulent cerebrospinal fluid with early polymorphonuclear cells predominating; endotoxin has been identified within the cerebrospinal fluid of these patients (Butler *et al.*, 1976).

Occasionally, plague presents as pharyngitis, following inhalation of infectious particles or ingestion of *Y. pestis*, such as may occur by eating undercooked, contaminated meat (Christie *et al.*, 1980). Pharyngeal colonization without symptoms also sometimes occurs (Marshall *et al.*, 1967; Tieh *et al.*, 1948). Rare cases of ocular plague have been described, which could arise by inoculation of the conjunctiva or by systemic spread (Poland, 1989). Primary ocular and pharyngeal plague might arise following an intentional release of *Y. pestis*, by direct inoculation of the conjunctivae or inhalation of aerosolized organisms, respectively.

3.5. Pediatric Plague

The clinical presentation of plague in children is similar to that in adults. Based on reported case series (Burkle, 1973; Mann *et al.*, 1982), children with bubonic plague are somewhat more likely than adults to develop complications of systemic spread (septicemia,

pneumonia, and meningitis) and to have a higher case fatality ratio. In a review of 183 childhood cases reported in the US during the period 1947–2001 (Dennis and Chow, 2003), 167 (91%) were classified as bubonic plague; of these, 54 (32%) developed secondary complications, including 41(25%) with sepsis (23 with sepsis alone, 9 combined with meningitis, 7 with pneumonia, and 2 with both pneumonia and meningitis). There were 13 secondary pneumonic cases without mention of sepsis, two of which were complicated by meningitis. In the total series, there were 31 (17%) deaths; mortality was highest in patients with primary sepsis or pneumonia (39%), less in patients with bubonic plague and secondary spread (30%), and least in patients with bubonic plague without recorded spread (9%). Comparing findings with adult cases reported over the same time period, children were more likely to develop complications of dissemination (32% vs. 27%), especially meningitis (13/183 vs. 4/238). Children were also more likely to have a fatal outcome (17% vs. 14%). These findings are similar to those reported in a series of 38 children from New Mexico (Mann *et al.*, 1982). Delay in diagnosis of plague in children is common; in the New Mexico series, the correct diagnosis was considered in less than 10% of patients on first medical encounter and in only about 40% at time of hospital admission. Presenting manifestations of plague in the New Mexico series included:

- Fever (95%)
- Lethargy, malaise, and anorexia (40%)
- Vomiting (50%)
- Chills (29%)
- Headache (29%)
- Abdominal discomfort (26%)
- Diarrhea (8%)

3.6. Plague in Pregnancy

Plague in the pregnant woman can result in infection of the fetus, with stillbirth, abortion, and perinatal infection as outcomes (Mann and Moskowitz, 1977; Welty *et al.*, 1985).

4. DIAGNOSIS

4.1. Laboratory Diagnosis

4.1.1. Laboratory Response Capabilities

Laboratory tests for plague are highly reliable when conducted by persons trained and experienced in the microbiology of *Y. pestis*; until recently, this was limited to a very few reference laboratories. In response to concerns with biological terrorism, the US initiated in 1999 a national Laboratory Response Network to provide upgraded, standardized diagnostic

testing for plague and other select agents (Centers for Disease Control and Prevention, 2000; Morse *et al.*, 2003). This network links state and local public health laboratories with other advanced-capacity laboratories, including those at CDC, the National Institutes of Health, the Food and Drug Administration (FDA), Department of Defense, Federal Bureau of Investigation, and the Department of Agriculture. Member laboratories operate either as sentinel laboratories (Level A) or as reference Levels B–D, representing progressively stringent safety, containment, and technical proficiency capabilities.

Sentinel (Level A) laboratories include hospital and other community clinical laboratories that practice BSL-2 safety procedures and perform initial tests to presumptively identify or rule out *Y. pestis* infection, using such procedures as direct staining, bacterial culture, and biochemical screening tests. Clinical specimens from most victims of a bioterrorist attack would probably be first handled for routine diagnostic procedures in Level A laboratories.

Suspicious isolates and source materials would be forwarded to Level B and C laboratories (federal, state, and local public health laboratories with BSL-2 and BSL-3 capabilities), which are prepared to perform advanced rapid diagnostic tests, to confirm the microbiological identification, and to characterize strain attributes. Level B and C laboratories are also prepared to carry out standardized antimicrobial susceptibility studies, and Level C laboratories routinely perform molecular subtyping tests, such as multiple locus variable number tandem-repeat assay, restriction fragment-length polymorphism, and pulsed-field gel electrophoresis (Henchal *et al.*, 2001; Morse *et al.*, 2003). These latter assays may be useful for tracing epidemiological links and for forensic purposes.

4.1.2. Collection and Processing of Specimens

Clinical Specimens. When plague is suspected, clinical specimens should be obtained promptly for microbiologic studies (Henchal *et al.*, 2001; Miller, 2001), chest radiographs taken, and antimicrobial therapy initiated pending confirmation of diagnosis. Blood and other clinical materials – such as bubo aspirates, sputum, tracheal-bronchial washes, swabs of skin lesions or pharyngeal mucosal, and cerebrospinal fluid – should be inoculated onto suitable media (e.g., brain–heart infusion broth, sheep blood agar, chocolate agar, or MacConkey agar). Blood for culture should be collected prior to administration of antibiotics. Blood culture counts typically range from fewer than 10, to 4×10^7 colony-forming units per mL. Direct bubo aspirates typically yield only small amounts of serosanguinous fluid, and 1–2 mL of saline should be injected into the bubo and withdrawn to ensure adequate aspirate for diagnosis. Smears of each specimen should be stained with Gram stain and a polychromatic stain, such as Wayson or Giemsa stain. Direct fluorescent antibody testing is a useful presumptive diagnostic procedure available at specialized laboratories. An acute-phase serum specimen should be collected for *Y. pestis* antibody testing, followed by a convalescent-phase specimen collected 3–4 weeks later. In the absence of an isolated organism, plague can be diagnosed by demonstrating a four-fold or greater change in serum antibodies to *Y. pestis* antigen using passive hemagglutination testing. A serum antibody titer of 1:128 or greater in a single serum sample from a patient who has a compatible illness and who has not received plague vaccine is also diagnostic. A few plague patients will develop detectable antibodies as soon as 5 days after the onset of illness, most seroconvert 1–2 weeks after onset, a few seroconvert three or more weeks after onset, and a few (<5%) fail to

seroconvert (Butler and Hudson, 1977; Centers for Disease Control and Prevention, unpublished data). Early specific antibiotic treatment may delay seroconversion by several weeks. Positive serologic titers diminish gradually from months to years. Enzyme-linked immunosorbent assays for detecting IgM and IgG antibodies to *Y. pestis* have been found to be useful in identifying antibodies in early infection and in differentiating them from antibodies developed in response to previous vaccination. Presumptive identification of *Y. pestis* can be made by polymerase chain reaction (PCR) or antigen-capture ELISA (Henchal *et al.*, 2001; Loiez *et al.*, 2003; Radnedge *et al.*, 2001; Rahalison *et al.*, 2000). A recently developed rapid immunogold dipstick assay designed to detect *Y. pestis* antigens in patient samples also appears highly promising for rapid presumptive diagnosis at the bedside, even under primitive field conditions (Chanteau *et al.*, 2003). Protocols and algorithms have been developed for clinical laboratories to follow in diagnosing plague and other select agent diseases in the event of a terrorist event (American Society of Microbiology, Biological Weapons Resources Center, 2003; Centers for Disease Control and Prevention, Bioweapons Laboratory Issues, 2001; Henchal *et al.*, 2001).

Nonspecific laboratory findings typically include elevation of various enzymes resulting from damage to the liver, heart, and other organs and tissues, thrombocytopenia, and white cell counts of 10,000–25,000/mm³ with a predominance of early-stage polymorphonuclear leukocytes. Leukemoid reactions, with white cell counts of 50,000/mm³ or higher, sometimes occur (Butler *et al.*, 1974; Welty *et al.*, 1985).

Autopsy Specimens. For diagnosis in fatal cases, tissues, samples of lymph nodes, liver, spleen, lungs, bone marrow, and other affected tissues should be collected at necropsy for culture, fluorescent antibody testing, and histological studies, including possible immunohistochemical staining (Guarner *et al.*, 2002). Cary Blair medium or a similar holding medium can be used to transport *Y. pestis*-infected tissues for later isolation of *Y. pestis*.

4.2. Recognizing a Plague Outbreak Resulting from Intentional Release

The identification of plague resulting from an intentional release must be made quickly to prevent excessive mortality among those persons initially exposed and to interrupt secondary person-to-person transmission. This requires a high level of expertise in clinical, laboratory, and public health services and a smooth integration of response. It is recognized that the most critical components for bioterrorism outbreak detection and reporting are the frontline health care professionals and the local health departments. (Ashford *et al.*, 2003). Principal expected features of an outbreak arising from an aerosol exposure are outlined in Table 2.1.

4.3. Detection of *Y. pestis* in the Environment

Microbiological culture of *Y. pestis* is the standard method for confirming its presence. However, growth of the bacillus is relatively time-consuming and insensitive, and rapid and

Table 2.1
Diagnosis of Plague following Release of an Aerosol of *Y. pestis*

Epidemiology and Symptoms	Sudden outbreak of geographically linked persons with fever, cough, shortness of breath, hemoptysis, and chest pain; point source exposure pattern, incubation period 1–7 days, peaking 3–5 days postexposure; gastrointestinal symptoms common (e.g., nausea, vomiting, abdominal pain, and diarrhea); rapidly developing toxemia; fulminant and fatal course common.
Clinical Signs	Tachypnea, dyspnea, and cyanosis; pneumonic consolidation on chest examination; septic shock and organ failure; disseminated intravascular coagulation, petechiae, ecchymoses; occasional cases of plague pharyngitis and cervical adenitis, conjunctivitis, possible.
Laboratory Studies	Sputum, throat swab, tracheal-bronchial washes for Gram, and Wayson or Giemsa staining; culture at 28°C and 37°C on suitable media (e.g., sheep's blood agar, McConkey's, chocolate agar, BHI broth); if suspicion high, send above to LRN reference laboratory for DFA, advanced rapid testing; standard blood culture as per institution protocol; if suspicion high, culture subset at 28°C; acute-phase serum specimen to be held at 4°C until plague ruled out; antimicrobial susceptibility testing of <i>Y. pestis</i> isolates; subtyping studies to characterize isolates, e.g., PFGE, PCR, MLVA; virulence testing; chest radiographs.
Pathology	Lobular exudation, bacillary aggregation, hemorrhagic necrosis of pulmonary parenchyma; tissues processed for direct detection (DFA, IHC) and isolation of <i>Y. pestis</i>

BHI, brain–heart infusion; DFA, direct fluorescence antibody testing; IHC, immunohistochemical; LRN, Laboratory Response Network; MLVA, multiple locus variable number tandem-repeat assay; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis.

reliable tests are needed for early warning systems, for rapid identification of a contaminated environment, and for epidemiological and forensic investigations. Gene amplification systems have been developed that use PCR technology to amplify and characterize specific DNA sequences of *Y. pestis*, such as Pst, CafI, YopM, and Pla targets (Henchal *et al.*, 2001; Radnedge *et al.*, 2001; Rahalison *et al.*, 2000). Most such rapid sequencing approaches use fluorogenic 5' nuclease chemistry. PCR assay coupled to probe hydrolysis (e.g., TaqMan[®]) allows “real-time” detection of PCR products. Rapid thermocycling instruments, such as the Lightcycler[™] (Roche Molecular Systems), the Ruggedized Advanced Pathogen Identification Device (RAPID[™], Idaho Technologies), or the SmartCycler[™] (Cepheid) allow identification of the agent in 20–40 minutes after nucleic acid purification (Henchal *et al.*, 2001). In the US, several “sniffing devices” to detect aerosolized microbial pathogens have been developed and tested. The Department of Homeland Security and the Environmental Detection Agency have deployed a PCR-based detection system named BioWatch to continuously monitor filtered air in major cities for *Y. pestis* and other select agents. Other monitoring systems include the Interim Biological Agent Detector System (IBADS[™]) that uses immunoassay to detect particles captured on a flowthrough membrane, and the Biological Integrated Detection System (BIDS[™]) that uses a light addressable potentiometric device as the detector (Henchal *et al.*, 2001). Such technologies, designed by the US military for the battlefield,

have been used experimentally to monitor specific high-profile civilian events and sensitive sites that could be targets of terrorism. In the event of a real or suspected aerosol release by terrorists, immediate environmental sampling could be performed using PCR, hand-held immunochromatographic assay, and cultural isolation techniques to identify the agent used and to determine the extent of contamination. Only cultural isolation determines viability of the agent involved, and allows characterization of important attributes, such as virulence and antimicrobial susceptibility.

In some circumstances, there may be a concern that release of live *Y. pestis* could result in infection of rodents or other susceptible animals, and potentially pose a risk to humans from animal- or flea-borne plague. In this circumstance, field teams would be deployed and standard procedures used to collect and process susceptible animals and their fleas for *Y. pestis* infection, including rapid detection methods and cultural isolation. If required, standard procedures for flea and rodent control would be implemented (Gage, 1998; Gratz, 1999). Special surveillance for plague in domestic cats might be indicated, since these animals are susceptible and can transmit infection – including respiratory infection – to humans (Gage *et al.*, 2000).

5. MEDICAL MANAGEMENT OF PLAGUE PATIENTS

5.1. Antimicrobial Treatment of Acute Illness in Naturally Occurring Plague

Untreated, plague is fatal in more than 50% of bubonic plague patients and in nearly all patients suffering from septic or pneumonic plague. The overall mortality in plague cases in the US in the past 50 years has been approximately 15% (Centers for Disease Control and Prevention, 1997; Craven *et al.*, 1993; Dennis and Campbell, 2004). Fatalities are almost always due to delays in seeking treatment, misdiagnosis and delayed or incorrect treatment (Centers for Disease Control and Prevention, 1997; Crook and Tempest, 1992). Rapid diagnosis and treatment with an efficacious antimicrobial agent are essential, and initiation of treatment within 24 hours of onset of pulmonary symptoms is often cited as the critical period in successful treatment of pneumonic plague (Butler, 1994; Butler and Dennis, 2004). With rare exceptions, antimicrobial susceptibility studies of human and animal strains of *Y. pestis* have shown low minimum inhibitory concentrations for the standard agents recommended for treating plague (i.e., streptomycin, gentamicin, doxycycline, chloramphenicol, and trimethoprim-sulfamethoxazole), as well as for several aminoquinolones, such as ciprofloxacin, ofloxacin, and trovafloxacin (Frean *et al.*, 1996; Smith *et al.*, 1995; Wong *et al.*, 2000). In vitro activity of ceftriaxone has also been found to be high, although clinical experience with cephalosporins has not been favorable. Studies in Russia have shown that doxycycline or ciprofloxacin was not inferior to streptomycin or tetracycline in treating bubonic plague in baboons, and that gentamicin or streptomycin were highly efficacious in treating aerosol-induced pneumonic plague in these animals (Romanov *et al.*, 2001a, 2001b). Antimicrobials that have been shown to have poor or only modest efficacy in experimental pneumonic plague in mice include rifampin, ampicillin,

aztreonam, ceftazidime, cefotetan, and ceftazolin, as compared with high efficacy of streptomycin and gentamicin (Byrne *et al.*, 1998).

Streptomycin has long been considered the drug of choice for treating plague (Butler and Dennis, 2004), and is FDA-approved for this use; however, there is no longer any manufacture of streptomycin in the US, availability is not widespread, and the drug must be obtained by special request. Although not FDA-approved for treating plague, gentamicin has increasingly been used in the US in place of streptomycin because of its ready availability and intravenous administration. Gentamicin has been anecdotally reported to be efficacious in treating plague in the US (Crook and Tempest, 1992; Welty *et al.*, 1985). A recent retrospective analysis of 50 plague patients treated with gentamicin in New Mexico since 1970 revealed that gentamicin – or a combination of gentamicin and doxycycline – is at least as efficacious as streptomycin, and was more often used than streptomycin in treating plague in that state (Boulanger *et al.*, 2004). Tetracyclines or chloramphenicol are suitable alternatives to the aminoglycosides. Doxycycline has – because of its ease of administration, rapid gastrointestinal absorption, and superior ability to achieve peak serum concentrations – become the tetracycline of choice for treating plague. Doxycycline treatment should be initiated with a loading dose, either intravenously or orally, depending on the severity of illness. In adults, a loading dose of 200 mg every 12 hours rapidly achieves a peak serum concentration of $\sim 8 \mu\text{g/mL}$ (Cunha, 2003). Chloramphenicol is indicated for conditions in which high tissue penetration is important, such as plague meningitis, pleuritis, endophthalmitis, or myocarditis. It may be used separately or in combination with an aminoglycoside. Trimethoprim-sulfamethoxazole (co-trimoxazole) has been used successfully to treat bubonic plague but response may be delayed and incomplete, and it is not considered a first-line choice. Penicillins, cephalosporins, and macrolides have a suboptimal clinical effect and are not recommended for use in treating plague. In general, antimicrobial treatment should be continued for 7–10 days or for at least 3 days after the patient has become afebrile and has made a clinical recovery (Butler and Dennis, 2004; Dennis, 2001; Inglesby *et al.*, 2000). Patients begun on intravenous antibiotics may be switched to oral regimens, as indicated by clinical response; in uncomplicated cases, this can usually be made on the 4th or 5th day of treatment. Clear signs of improvement are usually evident 2–3 days from the start of treatment, even though fever of lessened amplitude may continue for several more days. Strains of *Y. pestis* that are resistant to antimicrobials have only rarely been isolated from humans. Such resistant strains have usually involved partial resistance to a single agent only and have not been associated with treatment failures. Fortunately, the recent single plasmid-mediated multidrug-resistant strain of *Y. pestis* isolated from a bubonic plague patient in Madagascar (Galimand *et al.*, 1997) appears to be a unique finding. Antimicrobial guidelines for treating plague in non-bioterrorism settings are given in Table 2.2.

Common complications of delayed treatment include DIC, acute respiratory distress syndrome, and other consequences of bacterial sepsis and endotoxemia. Patients with these disorders require intensive monitoring and close physiologic support, often taxing personnel and facilities resources. In addition to prompt initiation of antibiotics and measures to counter shock, a number of investigational treatments have been proposed for treating sepsis, including recombinant-activated protein C (Dellinger, 2003; Wheeler and Gordon, 1999). A recent report describes the successful treatment of plague sepsis and peripheral gangrene with ciprofloxacin and sympathetic blockade (Kuberski *et al.*, 2003). Abscessed nodes can

Table 2.2
Plague Treatment Guidelines in Usual (Nonbioterrorism) Circumstances

Drug	Dosage	Route of administration
Streptomycin^a		
Adults	1 g q 12 hr	IM
Children	15 mg/kg q 12 hr ^a	IM
Gentamicin^b		
Adults	1–1.5 mg/kg q 8 hr ^c	IV or IM
Children	2.0–2.5 mg/kg q 8 hr ^c	IV or IM
Infants/neonates	2.5 mg/kg q 8 hr ^c	IV or IM
Tetracycline^d		
Adults	0.5 g q 6 hr	PO
Children >8 yr	6.25–12.5 mg/kg q 6 hr	PO
Doxycycline^d		
Adults	100 mg q 12 hr	IV or PO
Children >8 yr and >45 kg	100 mg q 12 hr	IV or PO
Children >8 yr and <45 kg	2.2 mg/kg q 12 hr	IV or PO
Chloramphenicol^d		
Adults	12.5 mg/kg q 6 hr ^e	IV or PO
Children >1 yr	12.5 mg/kg q 6 hr ^e	IV or PO

IM, intramuscularly; IV, intravenously; PO, orally.

^a Not to exceed 2 g/day.

^b Not FDA-approved for use in treating plague.

^c Daily dose should be reduced to 3 mg/kg as soon as clinically indicated.

^d An initial loading dose is usually indicated.

^e Up to 100 mg/kg/day initially. Dosage should be adjusted to maintain plasma concentrations at 5–20 µg/mL. Hematological values should be monitored closely.

be a cause of recurrent fever in patients who have otherwise made a satisfactory recovery, and the cause may be occult if intrathoracic or intraabdominal nodes are involved.

5.2. Postexposure Prophylaxis

Antimicrobial prophylactic treatment of persons having unprotected direct respiratory exposure to a patient with plague pneumonia is recommended as a public health control measure (Centers for Disease Control and Prevention, 1996; Inglesby *et al.*, 2000). Treatment is indicated if exposure has occurred in the previous 7 days, and the recommended duration of treatment is 7 days. Tetracycline, doxycycline, sulfonamides, and chloramphenicol have been recommended for postexposure prophylaxis (Centers for Disease Control and Prevention, 1996; Poland and Dennis, 1999), and tetracycline and doxycycline are approved by FDA for this purpose. Studies in mice have suggested that fluoroquinolones might also be effective in prophylaxis (Russell *et al.*, 1996), and their use has been advocated for use in responding to a plague terrorist event (Inglesby *et al.*, 2000). Short courses of antimicrobial prophylaxis are sometimes recommended for household members of

bubonic plague patients because of the possibility of common rodent flea exposures. Prophylaxis is only rarely warranted for people who visit or reside in an area where plague is occurring. Respiratory plague patients are generally considered to be noncontagious following 48 hours of antibiotic treatment (Anonymous, 2000; Inglesby *et al.*, 2000).

5.3. Treatment of Cases and Case Contacts in a Bioterrorism Event

A plague aerosol attack would require rapid identification and treatment of pneumonic plague cases and case contacts. The Department of Homeland Security is prepared to dispatch immediately shipments of antimicrobials and other materials essential in the management of plague patients, as contained in the National Pharmaceutical Stockpile (NPS). The Johns Hopkins Working Group on Civilian Biodefense has developed consensus-based recommendations for treatment and postexposure prophylaxis of terrorist-caused plague utilizing antimicrobials contained in the NPS (Inglesby *et al.*, 2000). These guidelines consider both a *contained casualty* situation (a situation where a modest number of patients require treatment and can be given maximum individual care, and parenteral antimicrobial treatment is recommended) and a *mass casualty* situation (large numbers of patients require treatment, not allowing full individual care, and oral administration of antimicrobials is recommended). In the contained situation, streptomycin or gentamicin is considered the preferred initial treatment for adults and children, whereas parenteral doxycycline, ciprofloxacin, and chloramphenicol are considered alternatives. Although full courses of treatment are for 10 days, it is recommended that oral therapy should be substituted when the patient's condition improves. In the mass casualty situation, oral doxycycline and ciprofloxacin are the preferred choices, and chloramphenicol is an alternative choice (Table 2.3). The regimen recommended for postexposure prophylaxis is the same as that for persons being treated in the mass casualty situation (i.e., oral doxycycline or ciprofloxacin), except that the duration of recommended treatment is 7 days rather than 10 days (Table 2.4).

Criteria for treatment include the following:

- Potentially exposed persons in an affected community who develop fever of 38.5°C or greater or a new cough should be evaluated and treated for presumptive plague following contained casualty or mass casualty protocols, as appropriate. Young children who develop tachypnea should also be considered for treatment.
- Persons who develop fever or cough while receiving antimicrobial prophylaxis should be evaluated for *Y. pestis* infection and the possibility of antimicrobial resistance or noncompliance with treatment and managed appropriately.

Categories of persons to be considered for postexposure antibiotic prophylaxis within 7 days of an infectious exposure are the following:

- Persons exposed to aerosol or other potentially infective *Y. pestis* release
- Household members of cases with respiratory plague
- Health care workers at facilities that screen or care for suspected plague patients
- Emergency workers who have responded to calls for assistance related to the event
- Persons who have transported suspected plague patients to health care facilities

Table 2.3
Plague Treatment Guidelines Using National Pharmaceutical Stockpile Components

	Initial therapy	Duration
ADULTS	Gentamicin 5 mg/kg IM or IV once daily (or in 3 divided doses) ^{a-c} OR	7–10 days (switch to oral doxycycline when clinically appropriate to complete 10-day therapy)
Parenteral	Doxycycline 100 mg IV twice daily OR	7–10 days
Oral	Doxycycline 100 mg PO twice daily	7–10 days
CHILDREN	Gentamicin 7.5 mg/kg IM or IV once daily (or, in 3 divided doses) ^{a-c} OR	(switch to oral doxycycline when clinically appropriate to complete 10-day therapy)
Parenteral	Doxycycline ^f ≥45 kg: 100 mg IV twice daily ≤45 kg: 2.2 mg/kg IV twice daily OR	
Oral	Doxycycline ^f ≥45 kg: 100 mg PO twice daily ≤45 kg: 2.2 mg/kg PO twice daily	7–10 days
PREGNANCY	Same as for nonpregnant adults ^{g,h}	
IMMUNOCOMPROMISED	Same as for nonimmunocompromised adults and children	

IM, intramuscularly; IV, intravenously; PO, orally.

^a Historic treatment of choice for plague is streptomycin. Streptomycin can be difficult to obtain; therefore, gentamicin is recommended and is included in the National Pharmaceutical Stockpile. Oral therapy should be substituted when clinically indicated.

^b The frequency of administration is left up to the discretion of the clinician. The manufacturers recommend that the daily dose be given in equally divided doses at 8-hr intervals; however, current evidence suggests that once-daily dosing of aminoglycosides is at least as effective as, and may be less toxic than, conventional dosing regimens using multiple daily doses of the drugs.

^c An initial loading dose of 2 mg/kg body weight is standard medical practice when gentamicin is given as three doses per day.

^d Not a US Food and Drug Administration-approved use.

^e Refer to package insert to adjust dose in the event of renal insufficiency.

^f In 1991, the American Academy of Pediatrics amended its recommendation to allow treatment of young children with tetracyclines for serious infections for which doxycycline may be indicated. Doxycycline is preferred for its twice daily dosing and low incidence of gastrointestinal side effects.

^g Aminoglycosides can cause fetal ototoxicity when administered to pregnant women; benefits may, however, outweigh risks when treating serious infections.

^h Tetracyclines can cause damage to fetal teeth and bones when administered to pregnant women.

- Co-workers, friends, and other associates who have had close contact with symptomatic respiratory plague cases
- Persons who have investigated or performed remediation at the release site
- Public health or other personnel who have interviewed patients or investigated sites of possible environmental contamination

Some high-risk categories may also be considered for pre-exposure prophylaxis (effectiveness of this measure is unknown) and the use of close-fitting face masks designed to block respiratory droplets.

Table 2.4
Plague Prophylaxis Guidelines Using National Pharmaceutical Stockpile Components^a

	Therapy	Duration
ADULTS	Doxycycline 100 mg PO twice daily OR Ciprofloxacin 500 mg PO twice daily ^{b,c}	7 days
CHILDREN	Doxycycline ^d ≥45 kg: 100 mg PO twice daily ≤45 kg: 2.2 mg/kg PO twice daily OR Ciprofloxacin 20 mg/kg PO twice daily ^{b,c,e}	7 days
PREGNANCY	Same as for nonpregnant adults ^f	
IMMUNOCOMPROMISED	Same as for nonimmunosuppressed adults and children	

PO, orally.

^aOne antibiotic regimen, appropriate for patient age, should be chosen from among alternatives. The duration of all recommended therapies is 7 days.

^bNot a US Food and Drug Administration-approved use.

^cRefer to package insert to adjust dose in the event of renal insufficiency.

^dIn 1991, the American Academy of Pediatrics amended its recommendation to allow treatment of young children with tetracyclines for serious infections, for which doxycycline may be indicated. Doxycycline is preferred for its twice-a-day dosing and low incidence of gastrointestinal side effects.

^eThe American Academy of Pediatrics states that the use of quinolones in children younger than 18 yr of age may be justified in special circumstances. For the treatment of plague, the assessment of the risks and benefits indicates that administration of ciprofloxacin to pediatric patients is appropriate. Ciprofloxacin dose should not exceed 1 g/day in children.

^fTetracyclines can cause damage to fetal teeth and bones when administered to pregnant women.

6. INFECTION CONTROL

6.1. Hospital Infection Control

Some previous public health authorities have recommended strict isolation of untreated pneumonic plague patients (Anonymous, 2000). However, the current consensus of scientific opinion is that transmission of infection from a pneumonic plague patient is via respiratory droplets (droplets larger than 5 μm in diameter) rather than true airborne transmission (droplet nuclei of suspended evaporated droplets, or dust particles, less than 5 μm in diameter). Respiratory droplets are generated primarily during coughing, sneezing, and talking; and during the performance of certain patient care procedures, such as suctioning and bronchoscopy. Respiratory droplets are typically transmitted across short distances only (<2 meters) from the source patient. Because they do not remain suspended in air, special air handling and filtered ventilation are not required in isolation spaces. Accordingly, in addition to applying Standard Precautions recommended for the care of all patients, patients with suspected pneumonic plague should be isolated in a private room, or cohorted with other suspect plague patients and managed under Respiratory Droplet Precautions as promulgated by the CDC and the Hospital Infection Control Practices Advisory

Committee (Centers for Disease Control and Prevention, Hospital Infections Program, 1997; Garner, 1996). In addition to Respiratory Droplet Precautions, Standard Precautions, including eye protection, use of gloves, mask, and gowns should be followed at all times when working within 2 meters of the contagious patient (Grow and Rubinson, 2003). Patients should be moved from isolation for essential purposes only, avoiding contact with others and, if possible, masked. Environmental control guidelines can be obtained at the CDC website for hospital infection control practices (Centers for Disease Control and Prevention, Hospital Infections Program, 2003).

6.2. The Role of Isolation and Quarantine

Isolation is defined as the separation of a person or group of persons, for the period of communicability, from others to prevent the spread of infection (Last, 1983). All confirmed, probable, or suspect cases of plague pneumonia should be isolated under respiratory droplet precautions during the first 48 hours of antimicrobial treatment and until clinical improvement occurs or the diagnosis of plague is ruled out. Although the isolation of a limited number of confirmed or presumptive plague cases may be initially accomplished in a hospital setting, health authorities should be prepared to use alternative facilities if the capacity is exceeded. These facilities should be secured to prevent unwanted movement of persons in or out; be supported with water, heat, electricity, and sanitation; have provision for adequate medical care; and good communications.

Asymptomatic persons with possible infective exposures must be quickly identified, given antimicrobial prophylaxis, and monitored for the development of fever and cough. Although isolation would not likely be required for asymptomatic contacts receiving prophylactic antimicrobials, these persons should be advised that they might be infected and a potential risk to others in close contact with them, and that it would be prudent to avoid intimate interpersonal contacts with others and to restrict activities that could spread infection, at least for the first 48 hours of prophylactic treatment.

Quarantine is defined as the restriction of activities or limitation of freedom of movement, for a period not longer than the longest incubation period, of well persons exposed to a communicable disease in order to prevent transmission of the disease to others should they become contagious (Last, 1983; World Health Organization, 1983). The term is usually applied to groups of persons or populations. The legal basis for quarantine rests both with state and federal powers. The draft of a model law for state emergency powers that might be required in the event of a release of a biological weapon, including quarantine, was recently made available by under the Model State Emergency Health Powers Act (Johns Hopkins Center for Civilian Biodefense Strategies, 2003; Mair *et al.*, 2002). This Act addresses the legal basis for states to close buildings, take over hospitals, and order quarantine during a biological attack. Some quarantine measures used in response to an intentional release of *Y. pestis* might include the suspension of public gatherings, closing of public places, restriction of travel (air, rail, motor vehicle, pedestrian), and/or *cordon sanitaire* (literally a sanitary cord or line around a quarantined area guarded to prevent spread of disease by restricting passage in and out of an area) (World Health Organization, 1983). There

should be a high threshold for instituting restrictions on travel and imposition of a *cordon sanitaire*, since these are seen by most civilians as a potential infringement on basic rights and may conflict with an individual's actions to protect self or family. These restrictions could also lead to irrational fear and civil disobedience.

7. PREVENTION

7.1. Prevention and Control of Naturally Occurring Plague

7.1.1. General Guidelines

The WHO recommends a four-phased system of plague prevention and control. The first two phases address emergency measures to be implemented whenever a human plague case occurs; Phases 3 and 4 outline the establishment of a surveillance system and development of long-term prevention and control measures (Gage, 1999; World Health Organization, 1980, 1983). The four phases are:

- Case recognition and medical intervention
- Epidemiological/epizootiological investigation and emergency control
- Predictive surveillance and preventive control
- Management

In endemic areas, public health services should provide a continuing system of human and animal plague surveillance, epidemiologic investigations, and control actions. The principal environmental remediation measures during outbreaks of human plague or dangerous epizootics are insecticidal flea control, rodent control, and sanitation to remove food and harborage for rodents. If killing of rodents is considered, flea control should be carried out before or simultaneously with the killing to reduce the chances that infective fleas will feed on humans (Gage, 1998; Gratz, 1999a).

In the event of an outbreak of human plague, measures should be taken to rapidly control spread, as described in international regulations and plague control manuals (Gage, 1999; Gratz, 1999a; World Health Organization, 1983). These measures include:

- Establishing the source
- Defining the geographic limits of activity
- Instituting active surveillance
- Laboratory confirmation of cases and isolation of pneumonic cases
- Rapid treatment of cases and close contacts of infectious pneumonic plague cases
- Control of fleas and rodents in plague-infected areas, with attention to port facilities, ships, and other conveyances
- In the event of a pneumonic plague outbreak, measures may be instituted to screen travelers departing from the epidemic area and to quickly identify and isolate suspect cases in travelers arriving from an outbreak area (Fritz *et al.*, 1996).

7.2. Plague Vaccine

A killed, whole-cell plague vaccine has limited availability and utility. Although no longer manufactured in the US, a comparable killed vaccine is manufactured by the Commonwealth Serum Laboratories (CSL Ltd., 45 Poplar Road, Parkville, 3052, Australia). The vaccine is given subcutaneously at a recommended initial course for adults of two 0.5-mL doses at an interval of 1 to 4 weeks, followed by 6-monthly booster doses (Titball *et al.*, 2004). Recommendations for use of the killed vaccine have been limited to certain groups at high risk, including research laboratory workers handling virulent *Y. pestis* strains, biologists working with susceptible animal populations in plague enzootic areas, and some military personnel. The efficacy of killed plague vaccines has never been evaluated in controlled clinical trials, and evidence for protection has been based on animal experiments, immunogenicity studies in humans, and observations on its use in US servicemen during the Vietnam conflict. Killed vaccines are thought to be protective against flea-borne exposures but to be only partially protective, if at all, against respiratory exposures (Centers for Disease Control and Prevention, 1996; Titball *et al.*, 2004).

Vaccines made of live attenuated strains have been used extensively in the past by the former Soviet Union, India, and former French colonies. These vaccines require only a single immunizing dose and provide early (albeit incomplete) protection against either flea-borne or respiratory routes of transmission. Revaccination is required after a year to maintain protection. Their use is accompanied by a high incidence of adverse reactions, especially fever, aching pain, and malaise in the first several days after administration. Reactions may sometimes be severe. Live vaccines are not commercially available, and the only country currently using a live plague vaccine (EV strain of *Y. pestis*) is Russia, where it is applied as an annual vaccination to persons at high risk (K. Alibek, personal communication).

Research is underway to develop improved plague vaccines that are likely to be protective against airborne routes of exposure (Titball *et al.*, 2004; Williamson, 2001). At present, the most promising candidates are recombinant subunit vaccines that express both the F1 and V antigens of *Y. pestis* (Titball and Williamson, 2003). These recombinant vaccines have been prepared both as combination and fusion products, and appear to protect animals against infective aerosol exposures. Experiments are being conducted to develop vaccine formulations that can be delivered as an inhaled aerosol (Eyles *et al.*, 2000). Interest in developing effective plague vaccines has increased greatly in recent years because of concerns with protection against biological weapons.

8. RESEARCH DIRECTIONS

Preparing for the emergency of a release of plague places a high imperative on new and improved technologies for early detection of the agent in the environment, such as B-cell lines engineered to express bioluminescent protein detectors (Rider *et al.*, 2003) and other technologies to improve performance of biological sniffing devices; new and improved rapid diagnostic markers of infection, such as sensitive, specific, and easy-to-use

hand-held assays (Chanteau *et al.*, 2003); rapid characterization of the organism and its virulence and pathogenic potential through better understanding of the *Y. pestis* genome (Parkhill *et al.*, 2001) and resultant proteomic advances; improved and standardized antimicrobial treatments of infection, using agents that are effective, simple to use, and that circumvent engineered resistance, such as bacteriocidal/permeability increasing proteins (Beamer, 2002); the development of materials that can combat shock and other serious consequences of endotoxemia, such as recombinant-activated protein C (Dellinger, 2003; Wheeler and Gordon, 1999); and the manufacture of recombinant inhalant vaccines (Eyles *et al.*, 2000), DNA vaccines (McDonnell and Askari, 1996), and postexposure immunoprotective agents, such as monoclonal antibodies directed against *Y. pestis* and its major virulence factors (Casadevall, 2002).

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