aflatoxin in their arsenal, as well as scud-missiles designed to disperse biological agents.

Modern-Day Bioterrorism

Several notable aspects of modern-day society continue to make bioterrorism a worldwide concern, including but not limited to: the availability of biologic agents; scientific advances in the composition and dispersion of biologic agents; ease of transportation both of persons and goods; increasing urbanization and crowding; increasing polarization of cultural or religious beliefs; fractured and/or corrupt governments and rising numbers of extremist groups; and the greed for world power.

Recent examples exemplify the reality of bioterrorism as a threat to our society and the challenges with identifying an outbreak of disease as deliberate act. In 1984, cult followers of the Bagwan Shree Rajneesh contaminated salad bars with Salmonella Typhimurium, a bacterium, to prevent citizens from voting against a land ordinance in their county (3). In all, 751 persons suffered from food poisoning and 45 persons were hospitalized; over one year materialized before the outbreak was identified as intentional. Just one week after the terrorist attacks on the United States in September 2001, a letter containing

anthrax spores was sent to a television broadcaster. Subsequently over the following weeks, 2 other letters containing spores were sent to a major newspaper and a US senator's office. By the end of 2001, 18 total persons had been infected with anthrax, and 5 persons were killed. To date, the persons responsible remain at large.

International Policies

Several international policies have been created to address bioterrorism. The Geneva Protocol, signed into effect by 128 countries on June 17, 1925, prohibits the use of chemical and biologic agents (4, 5). Though they signed it near the time it was put into effect, the United States did not ratify the Geneva Protocol until 1975. The Biologic and Toxin Weapons Convention of 1972 bars possession of deadly biological agents except for those necessary for defensive research (6). This Convention has been signed by over 100 nations, including the United States. Many countries have had known bioweapons programs in the past, namely: Algeria, Canada, China, Egypt, France, Germany, India, Iran, Iraq, Israel, Japan, Libya, North Korea, Pakistan, Russia, South Africa, Syria, Taiwan, the United Kingdom, and the United States. Canada, France, Germany, Japan, South Africa, and the United Kingdom have

all terminated their programs and destroyed their stockpiles; knowledge on the current status of bioweapons programs in other countries varies greatly.

Bioterrorism Agents (2, 7)

Bioterrorism agents are classified into categories based on three properties: 1) the potential health impact, 2) the ease of dissemination, and 3) the need for special public health preparedness to handle the particular disease or agent (see Figure 1). Category A is considered the most critical class of agents, with high potential mortality and morbidity and likely widespread panic and social disruption, ease of dissemination and transmission person-to-person, and special training necessary to deal with agents in this class. Key agents in Category A include: Anthrax, Botulism, Plague, Smallpox, Tularemia, and the Viral Hemorrhagic Fevers including Filoviruses (Ebola, Marburg) and Arenaviruses (Lassa, Junin). Category B agents have low mortality and cause some morbidity, are moderately easy to disperse, and may require additional training for diagnostics and enhanced disease surveillance. Q Fever, Brucellosis, Glanders, Alphaviruses (Venezuelan, Western and Eastern Equine Encephalitis), Ricin toxin, Epsilon toxin, *Staphylococcus* enterotoxin B, and food- or waterborne agents including *Salmonella* species, *Shigella dysenteriae,*

Escherichia coli O157:H7, *Vibrio cholerae,* and *Cryptosporidium parvum* are all considered Category B agents. Category C agents are less well demarcated and include any emerging pathogen that may be a potential threat based on availability, ease of production and dissemination, and potential for high morbidity and mortality. A few agents classified as Category C include: Nipah virus, hantaviruses, tickborne hemorrhagic fever viruses, tickborne encephalitis viruses, yellow fever, and multidrug-resistant tuberculosis.

Anthrax

Anthrax, *bacillus anthracis*, is a spore-forming, gram positive rod; the cells have characteristic

squared ends and the endospores are ellipsoidal shaped and located centrally in the sporangium. The spores are highly refractive to light and are resistant to staining. There are 3 primary types, or manifestations, of anthrax infection: cutaneous, inhalation, and gastrointestinal, based on the route of exposure. Cutaneous anthrax occurs via contact of anthrax spores with the skin. The incubation time, the time from exposure to onset of symptoms, averages 3 to 5 days. The infection begins as a small, painless pruritic papule that develops into a lesion; the lesion becomes a vesicle (blister-like) (~day 2) that becomes necrotic in the center, leaving a characteristic black eschar (~day 4)

surrounded by edema and purplish vesicles. Lesions generally resolve without scarring, and about 80-90% will resolve without treatment. However, mortality from cutaneous anthrax can reach as high as 20%, so it is important to treat as soon as the lesions are discovered. Anthrax is diagnosed by gram stain, polymerase chain reaction (PCR), or culture of vesicular fluid, exudate, or the eschar. A blood culture can reveal anthrax infection if the person is experiencing systemic symptoms. Differential diagnosis includes distinguishing cutaneous anthrax infection from: a spider bite, ecthyma gangrenosum, ulceroglandular tularemia, plague, staphylococcal or streptococcal cellulitis, and herpes simplex virus.

Inhalational anthrax is of primary concern as a bioterrorist agent because of the potential for it to be aerosolized, and occurs when anthrax spores enter the respiratory tract. The incubation period is generally 1 to 7 days, but rare instances of periods up to 42 days have been documented. Inhalation anthrax begins as cold or flu-like symptoms, and may include sore throat, fever, and muscle aches. Symptoms with later onset include cough, chest discomfort, shortness of breath, fatigue, and muscle aches, and may progress to shock and respiratory failure, resulting in death. Chest x-rays of persons infected with inhalation anthrax will demonstrate a widened mediastinum, pleural

effusions, infiltrates and pulmonary congestion. Diagnostic specimens for inhalation anthrax may include immunohistochemistry of affected tissue biopsy or pleural fluid cell block, and gram stain, PCR or culture of any fluid taken from a sterile site. Inhalation anthrax must be identified apart from other diseases with similar clinical presentation, including: mycoplasmal pneumonia, viral pneumonia, Legionnaires' disease, psittacosis, tularemia, Q fever, histoplasmosis (fibrous mediastinitis), and coccidioidomycosis.

Gastrointestinal anthrax, the most severe form of anthrax illness, develops as a result of ingestion of anthrax, often as a contaminant of meat products. The incubation averages 2 to 5 days and symptoms are characteristic of food poisoning, with nausea, loss of appetite, vomiting, and fever. Symptoms progress to include severe abdominal pain and diarrhea, and abdominal bleeding that is evident in the stool and in emesis. Mortality may exceed 50% among persons infected with gastrointestinal anthrax, and death may occur as a result of electrolyte balance, blood loss, shock, and abdominal perforation. Often lesions occur in the oropharangeal cavity when anthrax is ingested, and an oropharangeal swab may be used for diagnosis; blood cultures can also elucidate gastrointestinal anthrax infection.

An anthrax vaccine has been developed and used to help protect persons with anticipated exposures to anthrax, namely: laboratory workers, members of the military, and workers who enter and re-enter contaminated areas. The vaccine is not recommended nor is it available for use in civilian populations. Treatment for anthrax includes a 60-day course of antibiotics, and treatment success is dependent on how early treatment begins and the type of anthrax infection.

Botulism

Botulism, *Clostridium botulinum*, is a gram-positive, rod-shaped bacterium. Illness occurs as a result of spore germination and neurotoxin release and can be manifest in 3 forms: foodborne botulism, infant botulism, and wound botulism. Entry of spores by ingestion or inhalation raises concerns about botulism as a bioterrorist agent. Botulism cannot be spread from person to person. Botulism that is ingested or inhaled has an incubation of 12 to 36 hours (range: 6 hrs – 2 weeks). Symptoms include double or blurred vision, drooping eyelids, slurred speech, difficulty swallowing, and dry mouth.

Persons affected by botulism toxin experience muscle weakness that descends throughout body: first the shoulders, then the upper arms, lower arms, respiratory muscles, thighs, and calves. Paralysis of breathing muscles can lead to death. Botulism toxin works by blocking the release of acetylcholine, the neurotransmitter responsible for muscle contraction. Diagnosis of foodborne botulism is made clinically supplemented with toxin assays of stool and serum. A mouse bioassay has also been developed for diagnosis, but is time-intensive. Clinical findings leading to the diagnosis of botulism should be sure to exclude other diseases or syndromes, including: Guillain-Barre syndrome, Myasthenia gravis, tick paralysis, stroke or central nervous system mass lesion, poliomyelitis, paralytic shellfish poisoning or ingestion of puffer fish, other toxicities (hypermagnesemia, organophosphates, nerve gas, carbon monoxide), and other central nervous system conditions. A botulinum antitoxin, a trivalent equine product effective against types A through E is available and is most effective if given early; however, treatment of botulism is often limited to ventilatory assistance and supportive care.

Plague

Plague, *Yersenia pestis*, is a bacterium found in rodents and fleas. There are 2 types of plague: bubonic and pneumonic, with pneumonic plague as the primary bioterrorism concern. Pneumonic plague is transmitted via inhalation of an aerosol, from person to person, or through inhaling respiratory droplets from an animal infected with plague. Symptoms manifest on average 1 to 6 days following exposure, and include fever, chills, weakness, and headache, rapid development of pneumonia with shortness of breath, chest pain, and cough with bloody or watery sputum. Nausea, vomiting and abdominal pain may also occur; and illness may progress to respiratory failure, shock, rapid death. Chest x-rays may demonstrate patch infiltrates among persons infected with pneumonic plague, and blood chemistries will reveal increased white blood cell and decreased platelet counts. Diagnosis can be made through the presence of bipolar gram-negative coccobacilli (appearing like safety pins) on sputum gram stain or buffy coat; by blood, sputum, or node aspirate culture; or direct fluorescent antibody staining of a nasal swab specimen (early post-exposure). Acute and convalescent serologies may also be useful for plague diagnosis. Pneumonic plague diagnosis should be distinguished from inhalation anthrax, tularemia, and Staphylococcal Enterotoxin B virus inhalation. Prevention, and management includes droplet precautions (standard + mask)

to mitigate secondary transmission, and prophylaxis with oral antibiotics for 7 days. Antibiotic treatment should be given within the first 24 hours following exposure, and continued for 14 days: oral antibiotic recommendations include tetracyclines (e.g., Doxycline) and florquinolones (e.g., Ciprofloxacin); injection or intravenous antibiotics may include Streptomycin or Gentamycin.

Smallpox

Smallpox, *variola major*, is an orthopox virus that was once endemic in many parts of the world. Fortunately, due to the relentless dedication of thousands of public health workers, the massive global vaccination campaign initiated by the World Health Organization in 1967 culminated in the eradication of smallpox from the world in 1980. Because routine smallpox vaccinations were stopped subsequent to worldwide eradication, much of today's population has never been vaccinated; and persons who received the smallpox vaccine over 10 years ago may also have waning protection against smallpox disease. Lack of vaccine and waning efficacy of previous immunizations leave most of today's population completely susceptible to infection with the smallpox virus. The incubation period of smallpox is generally 12 to 14 days (range 7-17), and early symptoms include high fever, fatigue, head and back aches, severe

abdominal pain, and possible delirium. A characteristic rash on face, arms, and legs develops 2-3 days after the onset of initial symptoms. A principal characteristic of smallpox is that all lesions are in the same stage; they begin as flat red lesions, become pus filled, crust by early week two, and scabs fall off after about 3-4 weeks. The person is no longer contagious once the scabs have fallen off. Almost one-third (30%) of persons infected with smallpox will die from the disease. Laboratory diagnosis of smallpox includes testing skin scrapings for identification of the virus via electron microscopy or polymerase chain reaction, culture of blood or lesions, and serology. Previous prevention efforts against smallpox have largely focused on vaccinations. However, routine vaccination is not currently indicated given the absence of naturally-occurring disease in the world population and the potential for adverse reactions from the live virus vaccine, particularly among immunosuppressed persons. The current strategy focuses on rapid implementation of vaccination post-exposure (within 3 days) as a critical step to attenuate infection among exposed persons. Persons with smallpox infection should be isolated until the scabs fall off (~3 weeks) and healthcare personnel (and all others with exposure to the patient) are advised to follow airborne precautions which include: N 95 respirator or a powered air purifying respirator (PAPR), negative

air pressure rooms, and contact precautions (gown and glove). No treatments for smallpox are widely approved.

Tularemia

Tularemia, *francisella tularensis*, is an aerobic, gram-negative coccobacillus that can survive in low temperature, moist environments for several weeks. A person can be infected through skin contact, entry through mucous membranes, ingestion affecting the gastrointestinal tract, or via inhalation into the lungs. The latter mechanism of entry is referred to as pneumonic tularemia and, like anthrax, botulism, and plague the form of disease that affects the respiratory system and is transmitted through inhalation is of greatest concern as a bioterrorism agent. Pneumonic tularemia has an incubation period averaging 3 to 5 days (range: 1-14 days). Persons who have inhaled *francisella tularensis* often present with atypical pneumonia that is unresponsive to antibiotic therapy. Typical symptoms consist of an abrupt onset fever, nonproductive cough, and low back myalgias, and may include nausea and vomiting. Progression may be rapid or slow over several months,

but is generally accompanied with weight loss and weakness. Illness can progress to respiratory failure, shock, meningitis, sepsis, and death. Bilateral infiltrates are commonly present on chest x-ray and diagnosis can be made through culture of sputum or respiratory secretions. Serum antibody detection, direct fluorescence antibody stain, polymerase chain reaction, and antigen detection can also be useful for diagnosis. Differential diagnosis includes discriminating from community-acquired bacterial pneumonia, inhalational anthrax, pneumonic plague, Q fever, tuberculosis, and viral pneumonia. A vaccine for tularemia is in development; however, there is no current preventive therapy available. Health workers are advised to use standard precautions when attending to a person infected with pneumonic tularemia; additional precautions are not indicated as it is not known to be transmissible from person to person. An immediate, post-exposure prophylaxis with streptomycin or ciprofloxacin may prevent disease. Antibiotics are recommended for treatment with Streptomycin and Gentamicin recommended as first-line drugs and Doxycyline and Ciprofloxacin as alternatives.

Viral Hemorrhagic Fevers

Viral hemorrhagic fevers describe the group of severe febrile illnesses caused by several different families of single-stranded RNA viruses encapsulated in a

protective lipid envelope. All of these viruses are zoonotic and therefore the survival of these viruses is dependent on the natural animal or insect reservoir; humans are not natural hosts for the viral hemorrhagic fevers. This group of highly-contagious viruses leads to a potentially lethal syndrome of illness characterized by severe impairment of multiple organ systems, most notably the vascular system, and compromise of the body's ability to self-regulate. Hemorrhaging from multiple orifices is also a recurrent symptom of the viral hemorrhagic fevers, including mucous membrane hemorrhaging, bloody diarrhea, ecchymoses, and oozing of blood from puncture sites. The viral hemorrhagic fevers are comprised of four taxonomic families: Arenaviridae (Lassa, New World), Bunyaviridae (Rift Valley), Filoviridae (Ebola, Marburg), and Flaviviridae (Yellow Fever, Kyasanur Forest, Omsk).

Arenaviridae viruses are spread to humans by contact with virus-containing rodent excrement and include Lassa virus and the New World viruses (Machupo, Junin, Guanarito, and Sabia). Lassa fever, present in West Africa since its discovery in 1969 in Lassa, Nigeria, has an incubation of 5 to 16 days, and begins with a gradual development of fever, weakness, and general malaise. After about 3 to 4 days of illness, infected persons may experience arthralgias, back pain, retrosternal pain, and have a nonproductive cough.

Several days later (days 6-8), persons develop severe exudative pharyngitis, severe prostration, and a maculopapular rash may be evident if the person is fair-skinned. As with all hemorrhagic fevers, as the disease progresses hemorrhaging may occur and case fatality rates range from 1-25%. The New World viruses, South African viruses Machupo, Junin, Guanarito, and Sabia, have similar incubation period to Lassa (7 – 16 days) and present as a gradual onset of fever, sore throat, myalgias, and low back and abdominal pain. Vascular and neurologic manifestations, including bleeding develop after 5 to 7 days of illness. The mortality rate among persons infected with the New World viruses ranges from 15 to 30%.

The family Bunyaviridae includes Rift Valley Fever, Crimean-Congo hemorrhagic fever, and several hantaviruses. Rift Valley Fever virus is transmitted to humans and domestic livestock by mosquitos; humans may also contract disease through exposure to blood or body fluids of infected animals. Persons with Rift Valley Fever may remain asymptomatic or have mild symptoms associated with fever and liver abnormalities. If symptoms are present, they usually manifest 2 – 6 days following exposure, and include fever, headache, photophobia, retro-orbital pain, nausea, and vomiting. Later symptoms involve hepatitis, bleeding, encephalitis, and retinitis. About half

(50%) of cases involving hemorrhagic symptoms result in death. For those with less severe symptoms, case fatality rates range from 17 to 33%.

The most well known viral hemorrhagic viruses, Ebola virus and Marburg virus, belong to the family Filoviridae. Since their discovery in 1967 (Marburg) and 1976 (Ebola), these viruses have been responsible for several outbreaks of febrile, rapidly fatal hemorrhagic illnesses in central Africa. The natural reservoir for both Ebola and Marburg virus remains unknown. Ebola virus has an incubation averaging 2 to 21 days and is easily spread from person to person, through contact with infected body fluids, cadavers, or animals. The virus may be aerosolized and the stability of the virus in the environment or on fomites has not been fully elucidated. Early symptoms include fever, severe prostration, headache, myalgias; around day 5 a maculopapular rash may develop on the trunk, accompanied by jaundice and pancreatitis. Classic viral hemorrhagic fever symptoms are common, and the case fatality rate is extremely high, ranging from 50 to 90%. The incubation period for Marburg virus is 2 days to 2 weeks (14 days). The source of transmission and clinical presentation of Marburg virus mimic Ebola virus; persons with Marburg may also have what is referred to as a "clouded consciousness" during the early

stages of illness. Case fatality rates in persons infected with Marburg virus range from 23 to 93%.

The Flaviviridae family of viruses includes Yellow Fever, Kyasanur Forest virus, and Omsk virus. Kyasanur Forest virus and Omsk virus both have an incubation period of 2 to 9 days. Kyansanur virus begins with a sudden onset fever, myalgias, headache, later (days 3-4) involves diarrhea and vomiting. Systemic involvement among ill persons may include an enanthem with papulovesicular lesions on the soft palate; ocular complications including conjunctival congestion, subconjunctival hemorrhage, retinal and vitreous hemorrhage; cervical axillary lymphadenopathy; and bleeding manifestations, particularly from the nose, gums, and gastrointestinal tract. Case fatality can reach 10%. Symptoms of Omsk virus also may include enanthem on palate, and hyperemia of the skin on the upper body and mucous membranes. Subsequent symptoms include severe fever, generalized lymphadenopathy, splenomegaly, and pneumonia. Case fatality rate for Omsk virus ranges from 0.5 to 10%.

Diagnosis for the viral hemorrhagic fevers is largely based on characteristic clinical presentation: acute onset of fever (less than 3 weeks' duration); severe prostrating or life-threatening illness; at least 2 of the following bleeding

manifestations: hemorrhagic or purpuric rash, petechiae (particularly in nondependent areas), epistaxis, hematemesis, hemoptysis, blood in stool, or other evidence of bleeding; and no predisposing factors for a bleeding diathesis. Although availability and access to laboratories capable of testing diagnostic specimens for the viral hemorrhagic fevers is limited, laboratory diagnostics are available. For the Arenaviruses and Filoviruses, virus can be cultured during the acute febrile illness, and acute (<7 day) and convalescent serologies (≥14 days) may be useful in establishing etiology. Further, the following specimen samples may be useful: soft tissue, semen, or anterior eye fluid for suspected Ebola or Marburg virus infection; and throat swabs, pleural effusions, urine, or cerebral spinal fluid for suspected Lassa virus infection. Acute and convalescent serologies may also be helpful in suspected cases caused by Flaviviruses or Rift Valley fever, and cerebral spinal fluid may show evidence of these viruses if there is central nervous system involvement.

Due to the generalized, mild symptoms present in the early stages of the viral hemorrhagic fevers, it is important to maintain a high index of suspicion. Viral hemorrhagic fevers have been effectively aerosolized, underscoring their importance as potential biological warfare agents. Vaccines for several of the viral hemorrhagic fevers are in development; however, current prevention

efforts are largely focused on controlling the reservoir populations of rodents and mosquito and arthropod vectors. Avoiding close physical contact with infected persons and their body fluids is the most important way to mitigate the spread of disease. Barrier nursing techniques in healthcare settings are warranted, including wearing of protective clothing, such as masks, gloves, gowns, and goggles; the use of infection-control measures, including isolation of the patient, airborne and contact precautions, environmental decontamination and complete sterilization and disposal of instruments and medical devices used on infected patients. If the patient dies, standard precautions should be used for postmortem care and autopsies should be conducted in a negative-pressure room. The body of the infected person should be cremated without embalming. The intent of these techniques is to avoid contact with blood or secretions of any patient while ill, or with the body if an infected person dies. Treatment for the viral hemorrhagic fevers is supportive, and may involve maintenance of fluid and electrolyte balance, mechanical ventilation, dialysis, and appropriate therapy for secondary infections. Anticoagulant therapies and intramuscular injections are contraindicated. Management of viral hemorrhagic patients with other therapies remain controversial, including the use of clotting factor concentrates,

platelets, fresh frozen plasma, and heparin for disseminated intravascular coagulation (DIC): at this time, none of these therapies are widely recommended.

The Role of Public Health

What would a biologic attack look like? Most likely, it would begin as a large aerosol release that is silent, invisible, and odorless. The first signs of illness would not occur until days later, and then there might be an explosion of patients with severe and strange symptoms that few doctors have ever seen before. **Most importantly, the identification of illness caused by an intentional, biologic attack is the same as detection and identification of a naturally-occurring disease outbreak.** The role of public health professionals is therefore comprised of the essential elements of preparedness, surveillance and detection, diagnosis and characterization of agent and source of outbreak, coordinated response, and communication and education. Preparedness should include the development of coordinated preparedness plans and response protocols, and the ongoing education of health professionals to include performance standards assessed through simulations and exercises. Guidelines for detecting, evaluating, and reporting suspected