4

Biological Agents

You will be well advised to infect the Indians with sheets upon which smallpox patients have been lying, or by any other means which may serve to exterminate this accursed race.

Jeffery Amherst, 1717–1797
British Commander-in-Chief of North America
Orders to his subordinates in the French and Indian War

For the life of me, I cannot understand why the terrorists have not attacked our food supply, because it is so easy to do.

Tommy Thompson
Former Secretary of Health and Human Services

INTRODUCTION

Biological warfare has been known, and applied to great effect, for millennia. In recent years, however, with enhanced knowledge of microbiology, culturing techniques, and means of dissemination, the threat has become more acute. Terrorist use of bioweapons has occurred and several nations are known to be manufacturing tactical biological weapons. Therefore, awareness of this potential threat by first responders, medical care providers, public health agencies, elected officials, and ultimately the general public, including how to identify such weapons and to respond appropriately, is essential.

CATEGORIES OF BIOTERRORISM AGENTS

Given the events of the past two decades, it is essential that the U.S. public health system and primary health-care providers be prepared to address a wide range of biological agents, including pathogens that rarely occur in the United States. Even before the bioterror attacks of 2001 in which anthrax spores were deliberately released in the U.S. postal system, public health officials were concerned about the potential for such an event. In 1999, the U.S. Centers for Disease Control and Prevention (CDC), one of the key components of the Department of Health and Human Services, devised a classification scheme for major biological agents that terrorists might use to harm civilians (Tables 4.1 and 4.2).

The CDC bioterror lists include those biological agents that pose the greatest threats to national security due to their ease of transmission, high rate of death or serious illness, potential for causing public panic,

Table 4.1 Categories of CDC Biological Agents

Category A Diseases/Agents

High-priority agents include organisms that pose a risk to national security because they

- Can be easily disseminated or transmitted from person to person
- Result in high mortality rates and have the potential for major public health impact
- Might cause public panic and social disruption
- Require special action for public health preparedness

Category B Diseases/Agents

Second highest-priority agents include those that

- Are moderately easy to disseminate
- Result in moderate morbidity rates and low mortality rates
- Require specific enhancements of CDC's diagnostic capacity and enhanced disease surveillance

Category C Diseases/Agents

Third highest-priority agents include emerging pathogens that could be engineered for

- Mass dissemination in the future because of availability
- Ease of production and dissemination
- Potential for high morbidity and mortality rates and major health impact

Source: U.S. Centers for Disease Control and Prevention. No date. Bioterrorism Agents/Diseases. http://www.bt.cdc.gov/agent/agentlist-category.asp.

Table 4.2 Examples of CDC Category A, B, and C Agents

Category A
Anthrax (*Bacillus anthracis*)
Botulism (*Clostridium botulinum* toxin)
Plague (*Yersinia pestis*)
Smallpox (variola major)
Tularemia (*Francisella tularensis*)
Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo])
Category B
Brucellosis (*Brucella* species)
Epsilon toxin of *Clostridium perfringens*
Food safety threats (e.g., *Salmonella* species, *Escherichia coli* O157:H7, *Shigella*)
Glanders (*Burkholderia mallei*)
Melioidosis (*Burkholderia pseudomallei*)
Psittacosis (*Chlamydia psittaci*)
Q fever (*Coxiella burnetii*)
Ricin toxin from *Ricinus communis* (castor beans)
Staphylococcal enterotoxin B
Typhus fever (*Rickettsia prowazekii*)
Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis])
Water safety threats (e.g., *Vibrio cholerae*, *Cryptosporidium parvum*)
Category C
Emerging infectious diseases such as Nipah virus and hantavirus

Source: U.S. Centers for Disease Control and Prevention. No date. Bioterrorism Agents/Diseases. http://www.bt.cdc.gov/agent/agentlist-category.asp.

and special public health measures an epidemic would require. Since the creation of the CDC lists, public health officials and researchers have been planning and preparing intensively for a possible bioterror attack. Following the 2001 anthrax attacks, federal funding for these efforts increased dramatically (NIH, 2007).

HISTORY OF BIOLOGICAL WEAPONS

The use of "biological weapons" has been detected as far back as the pro-to-Neolithic (late Stone Age, approximately 10,000 BCE) hunter-gatherer

societies in Southern Africa. Hunters used poisoned arrows, tipping stone points with venom obtained from scorpions or snakes. Extracts from poisonous plants were also used. The arrow was fired into the target, and the hunter tracked the animal until the poison caused its death.

Documented usage of biological weapons on the battlefield is abundant. In ancient times, many varieties of biological warfare were used, often with startling success; for example, Scythian archers used arrows dipped in decomposing corpses (and also blood and manure) as far back as 400 BCE. The Assyrians poisoned enemy wells with rye ergot, which contains a psychoactive agent. The fungus that causes ergot produces ergotamine, a hallucinogen that causes delusions, paranoia, seizures, and cardiovascular problems that can lead to death. Those affected seemed to go insane, which intensified the terror aspect and demoralized fellow soldiers.

In the 6th century BCE, Solon of Athens used the purgative herb hellebore (skunk cabbage) to poison drinking water supplies during the siege of Krissa. The effects served to render the entrenched enemy unable to conduct battle. The use of decomposing bodies as the carrier of biological agents also proved effective against enemy water supplies. Ancient Persian, Greek, and Roman literature cites examples of the use of dead animals to contaminate wells and other water sources. Barbarossa used this same tactic much later at the battle of Tortona in 1155.

Hannibal, the brilliant general of Carthage, hurled clay pots filled with venomous snakes on to the ships of Pergamus during the battle of Eurymedon in 190 BCE. When the pots broke on the decks, the Pergamene were forced to fight against both snakes and Hannibal's forces (Hannibal's troops were eventually victorious). Roman armies catapulted hives of bees and hornets at their enemies. Some historians claim that such military use was responsible for a shortage of hives and honey during the latter years of the Roman Empire.

One of the best-known historic incidents of biological warfare occurred in 1346 during the siege of Kaffa (now Feodossia, Ukraine), a port city located on the Crimean Peninsula of the Black Sea. The attacking Tatar forces of Kipchak khan Janibeg, backed by Venetian forces, catapulted plague-infected corpses into the city. The ensuing epidemic within the already weakened city forced the defenders to surrender. Some infected citizens who fled Kaffa by ship may have started the Black Death pandemic, which spread throughout Europe. This same stratagem of hurling infected corpses was repeated in 1710 by the Russians besieging Swedish forces at Reval in Estonia.

The Spanish deliberately contaminated wine with the blood of leprosy patients and provided it to the French near Naples in 1495. Another

innovative attempt at biological warfare occurred in 1650 by Kazimierz Siemienowicz, a Polish artillery general, who placed saliva from rabid dogs into hollow projectiles for firing against his enemies.

On the North American continent, smallpox served as a highly effective biological weapon—the disease proved devastating to the Native American population. Pizarro is said to have presented South American natives with variola-contaminated clothing in the 15th century (variola is the causative organism of smallpox). During Pontiac's Rebellion in 1763, Colonel Henry Bouquet, a British officer, suggested giving the Indians at Fort Pitt, Pennsylvania, blankets infected with smallpox. During the French and Indian War of 1754–1767, British forces under Jeffrey Amherst gave blankets that had been used by smallpox victims to the Native Americans in efforts to spread the disease.

During the U.S. Civil War in 1861, Union troops advancing south into Maryland and other border states were warned not to eat or drink anything provided by unknown civilians for fear of being poisoned. Many cases were documented where soldiers thought they had been poisoned after eating or drinking in occupied areas. Confederates retreating in Mississippi in 1863 left dead animals in wells and ponds to deny water sources to the Union troops (Smart, 1997).

World War I

The use of biological weapons during World War I was not nearly as widespread as that of chemical warfare (see Chapter 2). However, it is believed that by 1915 the Germans infected the Allies' horses and cattle with various microbes on both the western and eastern fronts. Also in 1915 the Germans allegedly inoculated pathogenic bacteria into horses and cattle leaving U.S. ports for shipment to the Allies in Europe (Figure 4.1) (Smart, 1997, 1996; Stockholm International Peace Research Institute, 1971). Erich von Steinmetz, a captain in the German navy, entered the United States with cultures of glanders to inoculate horses intended for the western front. His efforts were not successful, however. Dr. Anton Dilger, a German-American physician, developed a microbiology facility in Maryland where he produced large quantities of anthrax and glanders bacteria, using starter cultures provided by the German government. German agents inoculated horses in Baltimore that were awaiting shipment to the Allied forces in Europe; they inoculated 3000 horses, mules, and cattle. Several hundred military personnel were reported to have received secondary infections.

Figure 4.1 Horses awaiting transport to Europe during World War I. (Copyright Unknown, Courtesy of Harry S. Truman Library, Independence, MO.)

Other bioattacks included a reported attempt to spread plague in St. Petersburg, Russia in 1915 (Smart, 1997, 1996; Stockholm International Peace Research Institute, 1971). In 1916 German agents attempted to infect horses with glanders, and cattle with anthrax in Bucharest. In 1917 Germany was accused of poisoning wells in the Somme area with human corpses, and dropping fruit, chocolate, and children's toys infected with pathogenic bacteria into Romanian cities. The authenticity of many of these reports has, however, been called into question and was strongly denied by German officials.

The only bioagent studied by the United States during World War I was ricin toxin, which was intended for retaliatory purposes. Ricin, which is extracted from the castor bean, was studied for dissemination via either attachment to shrapnel bullets in an artillery shell, or via generation of a ricin dust cloud. Both methods were tested in the laboratory; however, neither was optimized for use during the war (Hunt, 1918).

The Interwar Years

In 1928, Shiro Ishii (Figure 4.2), a Japanese military officer, toured foreign research laboratories and came to the conclusion that several of the world

Figure 4.2 General Shiro Ishii of the Japanese military, ca. 1933. (http://en.wikipedia.org/wiki/File:Shiro-ishii.jpg.)

powers were secretly researching biological warfare (his conclusion was at least accurate for the Soviet Union). In 1929, the Soviets were reported to have established a biological warfare facility north of the Caspian Sea (Robertson and Robertson, 1995; Smart, 1997; Stockholm International Peace Research Institute, 1971; Williams and Wallace, 1989). In 1933, Germany began military training in offensive biological warfare and was reported to have secretly tested *Serratia marcescens*, considered a biological weapon simulant, in the Paris Metro ventilation shafts and near several French forts (Smart, 1997). Soon afterward the Germans conducted experiments on livestock infections with foot-and-mouth disease (FMD). The German Military Bacteriological Institute in Berlin began developing anthrax as a biological weapon, while the Agricultural Hochschule in Bonn examined spraying of crops with bacteria (Robertson and Robertson, 1995; Smart, 1996).

By 1936 France had established a large-scale biological warfare research program that investigated microbial viability while in storage and during detonation of explosives. Canada also initiated biological warfare research, studying anthrax, botulinum toxin, plague, and psittacosis. By 1940, Britain instituted a biological warfare laboratory at Porton Down.

In 1933 Japan, under the direction of General Ishii, set up an offensive biological warfare laboratory near Harbin in occupied Manchuria. The laboratory complex, code-named Unit 731, was devoted to research on the effects of biological agents on various organisms and also prisoners of war (Figure 4.3). About 1000 human autopsies were carried out at Unit 731, mostly on victims exposed to anthrax. Many more prisoners and Chinese nationals may have died in this facility, however. The facility developed and tested a biological bomb within 3 years. Additional biological warfare facilities were established in 1939.

Some key U.S. military officials considered it highly unlikely that biological agents would ever be suited for warfare, particularly due to concerns about the safety of those individuals loading the weapons, and also due to the technical difficulties inherent in cell culturing and preservation. As a result, U.S. weapons research during the interwar years did not place significant emphasis on biological warfare agents.

World War II

By 1940 Japan had developed and tested several different biological devices in the field; more than 1600 bombs were constructed. Some are known to have been filled with a mixture of shrapnel and anthrax spores. By 1945, Ishii's program had stockpiled 400 kg of anthrax to be carried in specially designed fragmentation bombs (Smart, 1997). The Japanese had apparently used cholera, dysentery, typhoid, plague, anthrax, and

Figure 4.3 Japanese Unit 731 complex in Manchuria. (http://en.wikipedia.org/wiki/File:Harbin_maj_enh_731_1.JPG, © 2002 Markus Källander.)

paratyphoid on Chinese troops. Hundreds of Chinese citizens are known to have died from plague epidemics. By the start of World War II the Japanese labs devoted much of their attention to the use of vectors such as the common flea to carry biological agents (Smart, 1996; U.S. Army, 1945; Williams and Wallace, 1989).

In 1943, the U.S. military began research into the use of biological agents for offensive purposes. This work was conducted at Camp Detrick, the Chemical Corps Biological Warfare Center (now Fort Detrick). During the war anthrax became the highest-priority agent for development for the U.S. military arsenal (Smart, 1997).

Post–World War II

During the 1950s the biological warfare programs of many nations were geared toward standardizing and weaponizing selected biological agents. Highest priority was placed on antipersonnel agents. In the United States, Major General Bullene, Chief Chemical Officer, continued to give utmost priority to the development of anthrax (Smart, 1997).

Biological agents were produced at several sites in the United States until 1969 when President Nixon halted all offensive biological weapon research and production by Executive Order. Between 1971 and 1972 all stockpiles of biological agents from the U.S. program were destroyed. The agents eliminated included *Bacillus anthracis, Francisella tularensis, Coxiella burnetii,* Venezuelan equine encephalitis virus, *Brucella suis,* Staphylococcal enterotoxin B and botulinum toxin. The United States created a medical defensive program in 1953 that continues today at the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID) at Fort Detrick, MD.

In 1972 the United States, United Kingdom, and USSR signed the "Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction," also known as the Biological Weapons Convention (BWC). This treaty prohibits the stockpiling of biological agents for offensive military purposes and also forbids research into offensive deployment of biological agents. More than 140 countries have since ratified this convention. Despite this promising international agreement, however, biological warfare research continued in many countries. In addition, several cases of suspected or actual use of biological weapons have been reported. For example, during the Vietnam conflict, Laos and Kampuchea (formerly Cambodia) were attacked by planes delivering aerosols of varying color

(later dubbed "yellow rain"). Some exposed villagers and livestock became ill, and a small percentage died. Some of these clouds were thought to be composed of trichothecene toxins (e.g., T2 mycotoxin).

In Holland and Germany, in 1978, a dozen children were hospitalized after citrus fruit from Israel was found to have been intentionally contaminated with mercury (Khan et al., 2001).

In April 1979, public attention became focused upon an incident that occurred in Sverdlovsk (now Yekaterinburg) in the former Soviet Union. Spores of *B. anthracis* were accidentally released from the Soviet Military's Compound 19, a microbiology laboratory. Workers at a ceramic plant across the street fell ill during next few days, and almost all died within a week. Some residents living downwind from the facility also became ill and died within days of the event. All cases occurred within a narrow zone extending 4 km downwind in a southerly direction from the facility (Figure 4.4). The Soviet Ministry of Health claimed that the deaths were the result of consumption of contaminated meat; however, controversy as to the actual cause continued for years. The death toll from the incident totaled at least 105 but the exact number is unknown as all hospital records and other evidence were destroyed by the KGB, according to

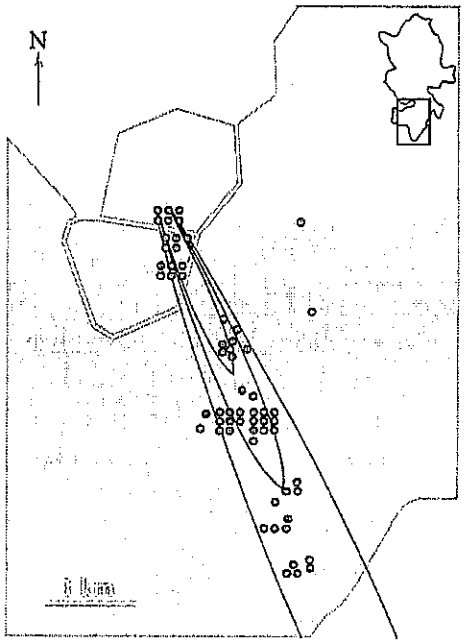

Figure 4.4 Sverdlovsk, USSR, showing direction of *Bacillus anthracis* spore plume, 1979. (From American Association for the Advancement of Science.)

former Biopreparat deputy director Ken Alibek (Alibek and Handelman, 1999). Livestock downwind from the site also succumbed to the disease.

In 1992, the new Russian President Boris Yeltsin acknowledged that the Sverdlovsk incident was in fact related to military-related biological programs underway at Compound 19. A detailed review of the Sverdlovsk incident was published shortly afterward (Meselson et al., 1994). Among the findings was that the accident was caused by failure to replace a filter in an exhaust system at the facility, thus allowing anthrax spores to escape.

Following the accident and the closing of the Sverdlovsk facility, and in order to continue biological weapons production, a new biological warfare R&D facility was created in Stepnogorsk in Kazakhstan. An even more virulent strain of anthrax was produced at the new location, one that was three times as lethal as that produced in Sverdlovsk. The Stepnogorsk operation is recorded to have had a production capacity estimated at 300 tons of anthrax spores in 220 days (Miller and Broad, 2001). Efforts to clean up the Soviet biological warfare installations continues today, as does the effort to determine if any technical expertise or the products of their operations fell into the hands of terrorist organizations (NSA, 2001).

In August 1991, in the aftermath of the Gulf War, Iraq's biological weapons program was inspected by the United Nations. Representatives of the Iraqi government declared to the U.N. Special Commission that research had been ongoing into the offensive use of *B. anthracis*, botulinum toxins, and *Clostridium perfringens*. By 1995 it was determined that Iraq's offensive program conducted research and development activities on anthrax, botulinum toxin, *Clostridium perfringens*, aflatoxins, wheat cover smut, and ricin. Field trials were conducted with *B. subtilis* (an organism that simulates the behavior of anthrax), botulinum, and aflatoxin. Biological agents were tested in numerous delivery systems, including rockets, aerial bombs, and spray tanks. In December, 1990, the Iraqis loaded 100 bombs with botulinum toxin, 50 with anthrax, and 16 with aflatoxin. In addition, 13 al-Hussein (SCUD) warheads were filled with botulinum toxin, ten with anthrax, and two with aflatoxin. These weapons were deployed in 1991 to four locations (USAMRIID, 2005).

The Current Threat

Biological threats related to both warfare and terrorism continue today. There is increasing concern regarding possible terrorist use of biological agents to threaten either military or civilian populations

(USAMRIID, 2005). In addition, a number of countries, some known to be hostile to the United States are thought to be developing biological agents for offensive purposes. It was reported in January 1998 that Iraq had sent scientists involved in bioweapons research to Libya to help that country develop a biological warfare complex disguised as a medical facility in the Tripoli area. In a report issued in 1997, Secretary of Defense William Cohen identified Libya, Iraq, Iran, and Syria as countries "aggressively seeking" chemical, biological, and nuclear weapons (USAMRIID, 2005).

Recent revelations from Russia have revealed an intensive ongoing biological warfare program that includes active research into genetic engineering, binary biological formulations, and industrial capacity to produce bioagents. Furthermore, there is concern that the smallpox virus, known to be stored in only two laboratories at the CDC in Atlanta and the Institute for Viral Precautions in Moscow, may actually occur in other nations as well.

In August 1984 the Bhagwan Shree Rajneeshee cult, living in eastern Oregon, served water laced with *Salmonella typhimurium* to two county commissioners. Both became ill and one was hospitalized. Later that year several cult members contaminated salad bars in restaurants of nearby communities with *S. typhimurium*. More than 750 were poisoned and 40 hospitalized. The cult was trying to influence a local election by making people too sick to vote. The deliberate food poisoning event was only discovered a year later when members of the cult turned informants. There is evidence that the cult had considered using *S. typhi*, the causative agent of typhoid fever; however, that organism was rejected because of the risks of having it traced back to the cult.

In 1991, the Minnesota Patriots Council extracted ricin from castor beans obtained through a mail order. They planned to disseminate the agent as an aerosol but were arrested after the FBI had infiltrated the group and learned of their plan. Two members of the group, Doug Baker and Charles Wheeler, were the first individuals to be indicted and convicted under the Biological Weapons Anti-Terrorism Act of 1989.

In October 1992 Shoko Asahara, leader of the Aum Shinrikyo cult, with 40 followers, traveled to Zaire reportedly to assist victims of an outbreak of Ebola hemorrhagic fever. According to a report by the U.S. Senate's Permanent Subcommittee on Investigations, however, the cult's true motive was to obtain virus samples to be used for biological attacks. By 1995, it was reported that on at least ten occasions Aum Shinrikyo attempted to disperse anthrax, botulinum toxin, Q fever, and Ebola against civilian populations and government figures in Japan. Some of

their actions included

- Outfitting a car to disperse botulinum toxin through an exhaust system and driving the car around a parliament building.
- Attempting to disrupt the wedding of Prince Naruhito by spreading botulinum in downtown Tokyo via an auto.
- Spreading anthrax in Tokyo via a sprayer system from the roof of a building (this was carried out over 4 days).
- Planting three briefcases designed to release botulinum in a Tokyo subway. The attack was averted when a cult member substituted a nontoxic agent instead.

No infections or illnesses were reported from the above attempts.

In 1995 a group called The Covenant and the Sword smuggled ricin into the United States from Canada. The ricin was baked into cakes that were to be given to the local Internal Revenue Office at Christmas. The perpetrator hanged himself shortly after his arrest. Also in 1995 a Kansas City oncologist, Deborah Green, attempted to murder her husband by contaminating his food with ricin.

In May 1995 a laboratory technician from Ohio (Larry Wayne Harris), ordered plague bacterium (*Yersinia pestis*) from a Maryland biomedical supply firm using a credit card and a false letterhead. He received three vials of the *Y. pestis*. As a result of his suspicious behavior, the supplier contacted federal authorities. An investigation revealed that he was a member of a white supremacist organization. Harris was arrested after he threatened to release anthrax in Las Vegas. The strain in his possession, however, was a harmless veterinary vaccine strain.

In China in 2001, 120 people became ill after food products were laced with rat poison by makers of competing products. In 2002, a similar rat poisoning incident killed at least 38 people and made more than 300 seriously ill (BBC, 2002; CNN, 2002). In 2003 it was discovered that 200 pounds of ground beef was contaminated with an insecticide containing nicotine by a disgruntled supermarket employee in Michigan. This episode resulted in 111 becoming ill, including 40 children (U.S. CDC, 2003).

BACTERIAL DISEASES

Bacteria are one-celled organisms that can survive in a wide variety of environments. The majority of bacteria are either beneficial or do no particular harm to other organisms. Only a minority are **pathogenic**, that is, disease-causing, in animals and humans. Because of the differences in

biochemical processes between bacteria and their hosts, it is possible to treat most bacterial diseases by using antibiotics.

Two pathogenic bacterial agents, *B. anthracis* and *Coxiella burnetii*, have the capability of converting to a highly resistant form, that is, a spore, when the present environment becomes hostile to the organism's survival (too dry, too cold, extremes of pH, etc.). The spore is capable of remaining viable for extended periods and will subsequently revert to the vegetative, disease-producing form when favorable environmental conditions return. The following section will describe a spore-forming bacterial species having a long history of adverse public health impacts.

Anthrax

Anthrax is an acute infectious disease caused by the bacterium *B. anthracis*. The CDC has classified *B. anthracis* as a Category A bioterrorism agent. Anthrax is thought to be the "fifth plague" of Egypt as chronicled in the book of Exodus. The disease is probably best known for its role in the 2001 bioterrorist attacks in the United States in which lethal anthrax spores were sent via U.S. mail to offices in the U.S. Senate and several news media companies (Box 4.1). Twenty-two people became ill and five

BOX 4.1

In October 2001, two letters contaminated with *B. anthracis* spores were processed at the U.S. Postal Service Brentwood Mail Processing and Distribution Center in Washington, DC. Four postal workers became ill with what was eventually diagnosed as inhalational anthrax, and two died. The facility was closed, and postexposure prophylaxis was recommended for approximately 2500 workers and business visitors.

The subsequent investigation disclosed that both letters contained the same strain of anthrax, which was isolated and sent to the Army's Biodefense Labs at Fort Detrick, Maryland. All the letters were postmarked in Trenton, New Jersey.

The anthrax episodes occurred as follows:

- Palm Beach County, FL—October 3, 2001
- New York City—October 12, 2001
- Washington, DC—October 15, 2001
- Trenton, NJ—October 17, 2001
- Oxford, CT—November 20, 2001

died as a result of the attacks. The perpetrator is believed to have been Dr. Bruce Ivins, a biodefense scientist who was working on a vaccine for anthrax at the U.S. Army Biodefense Labs at Fort Detrick, MD. In 2009 he committed suicide during the FBI investigation of his possible role in the attacks.

There were two waves of letters containing anthrax spores sent from the unknown perpetrator. The targets were news media and government officials. The anthrax spores caused pulmonary anthrax (lung infection) and cutaneous anthrax among victims. As many as 30,000 people were placed on medication to prevent illness.

All affected sites were subjected to simultaneous investigations, including public health (case finding) and medical response (treatment); forensics (crime investigation); environmental (worker safety/clean up); and tactical (operations and intelligence). Multiple state and local jurisdictions were involved, as well as multiple federal agencies.

Lessons learned and benefits provided from these episodes included the following:

- The value of integrated emergency response to the incidents
- Dramatic expansion of health information about anthrax and its behavior
- Improved risk communications to media and the public

Anthrax is primarily a disease of ruminant animals, most commonly affecting wild and domestic mammals (cattle, sheep, goats, camels, antelope, and other herbivores), but it can also occur in humans when they are exposed to infected animals or their tissue (U.S. CDC, 2008). Naturally occurring anthrax infections occur worldwide, but the disease is most often a risk in countries with inadequate public health programs. Common locations of infections include South and Central America, Southern and Eastern Europe, Asia, Africa, the Caribbean, and the Middle East.

Human exposure to anthrax is usually caused by occupational exposure to infected animals or their products. Anthrax most commonly occurs in rural/agricultural regions; those who work on farms, ranches, or in slaughterhouses and handle animal hides, hair, or wool may be at risk of contracting the disease. Anthrax in wild livestock has occurred in the United States (CDC, 2008). Cases occur annually, with outbreaks occurring most frequently in the central United States from North Dakota to Texas.

The Agent

The causative agent of anthrax, *B. anthracis,* is a relatively large (approximately 1 by 6 microns, μm) Gram-positive, spore-forming, rod-shaped bacterium. Anthrax was the first bacterium ever to be documented to cause disease, by Robert Koch in 1877. *B. anthracis* lives in soil and the vegetative form has evolved a survival tactic (i.e., spore formation) that allows it to withstand harsh conditions for decades. The spore, which measures approximately 1 μm by 0.5 μm, serves as a resilient resting phase that can tolerate extreme heat, cold, and desiccation. When environmental conditions eventually become favorable, the spores germinate into active bacteria.

Although the spore stage will allow the bacteria to survive in unfavorable environments, the potency of anthrax as a killer is the ability of the vegetative form to produce toxins. The resilience of the spore and the production of toxins combine to make *B. anthracis* an intimidating bioterrorism agent. *B. anthracis* possesses several virulence factors: a capsule surrounding the vegetative cell, a protective antigen, and two protein exotoxins, termed **lethal factor**, and **edema factor**. The capsule prevents the bacterial cell from being engulfed and killed by **phagocytes** (i.e., specialized white blood cells). The protective antigen binds to the cell of the infected organism, creates a pore, and channels the edema factor and lethal factor inside the cell. Once inside, the edema factor causes fluid to accumulate at the site of infection. The edema factor can contribute to a fatal buildup of fluid in the cavity surrounding the lungs; it also can inhibit some of the body's immune functions. The lethal factor also works inside the cell, disrupting a key molecular process that regulates cell function. The lethal factor can kill infected cells or prevent them from functioning properly (NIH, 2007).

Clinical Manifestations and Symptoms

Three human forms of anthrax are known, depending on the route of exposure: cutaneous, gastrointestinal (GI), and inhalation. Each form produces its own unique symptoms.

Cutaneous Anthrax

Cutaneous anthrax is the most common manifestation of natural *B. anthracis* infection. Individuals with cuts or open sores can succumb to cutaneous anthrax if they come in direct contact with the bacteria or its spores through broken skin, usually on the hands, arms, or face. Handling contaminated animal products is a common source of infection. The spores

germinate within hours after infection and the vegetative cells multiply and produce toxins.

The first obvious sign of cutaneous anthrax is typically is a small reddish **macule** that develops within a few days after infection. The macule progresses to a **papular** and eventually a **pustular** stage (a fluid-filled vesicle) and the surrounding tissue becomes swollen. Secondary vesicles may surround the initial infected site. The final stage involves the formation of an ulcer with a blackened necrotic **eschar** surrounded by a zone of edema (Figure 4.5). The term "anthrax" arises from the Greek word meaning "coal," because of the development of the black lesions in cutaneous victims. The fully developed lesion is painless.

Most victims of the cutaneous form have no fever and often have no systemic symptoms of infection. Natural healing occurs in the majority (80%–90%) of untreated cases but edema may persist for weeks. In untreated patients who do not naturally heal, cutaneous anthrax can progress to a systemic infection (i.e., septicemia) that can be fatal. Cutaneous anthrax responds favorably to antibiotics; if exposed individuals are administered antibiotics promptly, most will recover within 10 days of onset.

No form of anthrax is considered contagious; however, the fluid in the vesicles of cutaneous anthrax contains viable *B. anthracis*. If fluid from

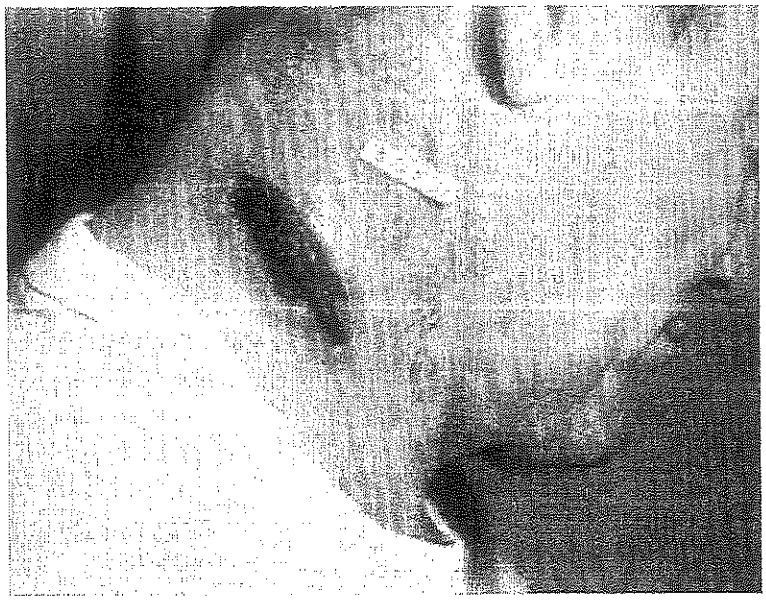

Figure 4.5 Anthrax eschar. (From CDC.gov) (http://www.bt.cdc.gov/agent/anthrax/anthrax-images/cutaneous.asp.)

a victim's vesicles comes into contact with the open wound of another individual, the bacteria can be transferred. The use of gloves and other protective clothing will thus prevent such transfer.

Cutaneous anthrax is rare in the United States. According to the U.S. CDC, only one to two cases in the United States are diagnosed per year. Cutaneous anthrax is not likely to be significant in a bioterrorism event, although it may erupt in conjunction with an aerosol anthrax attack. This occurred in 2001 when 11 of the exposed individuals contracted the cutaneous form.

Gastrointestinal Anthrax

Humans can become infected with GI anthrax from eating undercooked meat contaminated with anthrax bacteria or their spores. GI anthrax is most likely to occur in warm, tropical regions of Asia, Africa, and the Middle East. There have been no confirmed cases of GI anthrax in the United States, although a Minnesota farm family experienced symptoms of the disease in 2000 after eating meat from an anthrax-infected steer (NIH, 2007). The infections were discovered early and treated successfully.

GI anthrax is characterized by an acute inflammation of the intestinal tract. Clinical symptoms begin within days after ingesting spores. If the spores germinate in the upper intestinal tract, ulcers may develop in the mouth or esophagus and cause swelling in the lymph nodes of the neck and surrounding tissues. **Septicemia** may follow. Spores that germinate in the lower intestinal tract will create lesions and may be severe enough to cause intestinal hemorrhage. Early symptoms include loss of appetite, nausea, vomiting, and fever. These symptoms are followed by abdominal pain, vomiting of blood, and severe diarrhea. If left untreated, GI anthrax results in death in 25%–60% of cases (U.S. CDC, 2008). Antibiotic treatment can cure the GI form of anthrax.

Researchers generally report that the cutaneous form of anthrax is much more common than the GI form (Dixon et al., 1999; Jaax et al., 1997). Other researchers (Sirisanthana and Brown, 2002), however, propose that the apparent predominance of cutaneous anthrax is rather due to the difficulty of diagnosis of GI anthrax.

Although there are no reports of anthrax having ever been used in a foodborne bioterrorism attempt, such an act would likely result in severe disease in those individuals consuming the contaminated food.

Inhalation Anthrax

The inhalation form of the disease, also known as Woolsorters disease, is highly lethal, causing a hemorrhagic inflammation of the mediastinum

(generally speaking, the space in the chest between the pleural sacs of the left and right lungs) (Figure 4.6) and often, hemorrhagic meningitis. Fatality rates are very high in untreated cases and may occur in as many as 95% of treated cases if therapy is delayed after the appearance of symptoms.

Spores of *B. anthracis* occurring as airborne particles measure less than 5 μm in diameter and can be deposited directly into the alveoli of the lungs. After spores are inhaled, phagocytes (white blood cells) engulf the spores. The spores are carried by the phagocytes to the mediastinal nodes where they germinate, and release toxins soon afterward. Symptoms usually appear from 1 to 7 days after exposure, but may take as long as 60 days. The time difference is apparently a function of dose received (Fong and Alibek, 2005).

Inhalation anthrax occurs in two stages. The first stage lasts 3–5 days and mimics common flu symptoms; thus, early diagnosis is difficult. The patient exhibits mild, nonspecific upper respiratory symptoms often accompanied by sore throat, mild fever, and myalgia (muscle aches). Nausea and vomiting are among other early symptoms. Later symptoms include cough, chest discomfort, shortness of breath, and fatigue. Near

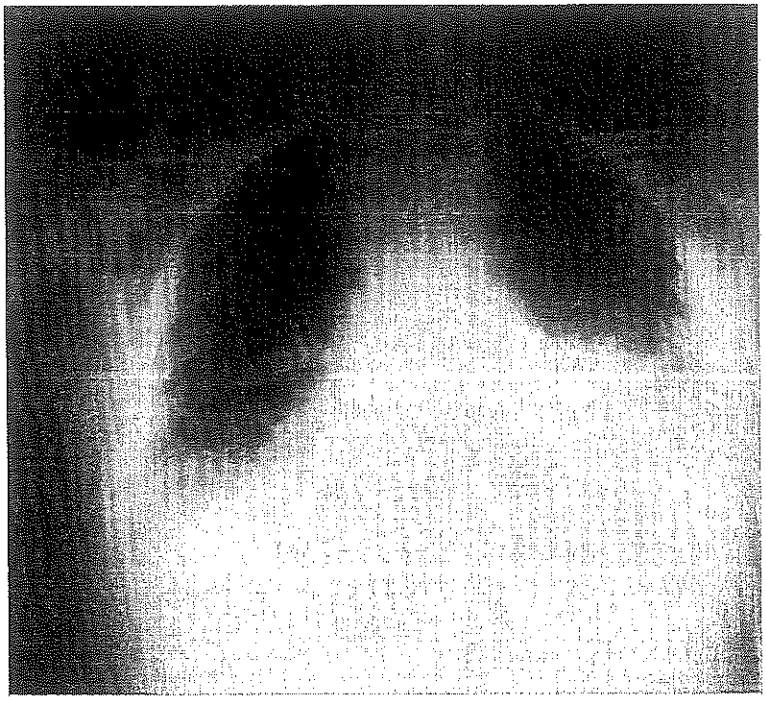

Figure 4.6 Mediastinitis as a result of inhalation anthrax. (From CDC.gov.)

the end of the first stage the patient's condition may appear to improve somewhat. The second stage begins with an abrupt onset of acute respiratory distress, sweating, shock, and **cyanosis** (bluish discoloration of the skin). After 1–3 days, fever increases and **dyspnea, hypoxia,** and **hypotension** occur, usually leading to death within 24 hours. Once this stage is reached, the disease is almost always fatal (NIH, 2007). The second stage is the direct result of **toxemia** (high concentrations of toxins in the blood) and is not treatable with antibiotics.

Diagnosis and Treatment

Anthrax is not contagious; therefore, communicability is not a concern in managing patients with inhalation anthrax (U.S. CDC, 2008). Polymerase chain reaction (PCR) tests combined with microbial culture of body fluids can provide an accurate diagnosis of the disease. To date, there is no successful rapid field diagnostic test for *B. anthracis* or for any other bioterrorism agent.

Untreated inhalation anthrax has an extremely high fatality rate. In the event of inhalation exposure, the immediate administration of antibiotics is critical. Antibiotics are effective if provided early during the course of the disease—antibiotic treatment is not effective if begun more than 24 hours after symptoms appear. Large doses of intravenous and oral antibiotics such as ciproflaxin (Cipro®), penicillin, doxycycline, tetracycline, erythromycin, and others are effective for treatment of anthrax. Antibiotic treatment should ideally begin before the onset of symptoms. It should be noted that some antibiotic-resistant strains of anthrax have been identified.

A safe, effective vaccine for inhalation anthrax exists, derived from a portion of the toxin from a nonvirulent strain. The vaccine is safe and effective and can be used both prophylactically and therapeutically.

If livestock are located in the area of an anthrax release and die, their carcasses should be placed in deep burial trenches. Shallow burial is both ineffective and potentially hazardous because other organisms will scavenge carcasses and subsequently disseminate anthrax spores, thus extending the zone of the disease. Incineration of anthrax-infected carcasses may pose practical problems; if incomplete burning occurs, spores may be released downwind in smoke and particulate emissions.

Animals subsequently introduced into an environment that had been infected should be vaccinated. Animal products from livestock living in anthrax-affected areas should not be consumed or handled by untrained and/or unvaccinated personnel.

Recent Developments

Anthrax toxins are being studied to learn how to block their production and ultimately their action. Researchers have discovered the three-dimensional molecular structure of the anthrax protective antigen protein that is used to enter host cells; they have subsequently been able to block the attachment of protective antigen in laboratory experiments, thereby inhibiting anthrax toxin activity (NIH, 2007). In other studies, The National Institute of Allergy and Infectious Diseases (NAID) have synthesized a molecule that interferes with anthrax toxin in cell culture and in rodents. The molecule blocks the pore formed by anthrax protective antigen, which prevents lethal factor and edema factor toxins from entering cells. This promising discovery may lead to new and effective treatments for anthrax (NIH, 2007).

Biowarfare and Terrorism

Anthrax spores were weaponized by the U.S. military in the 1950s and 1960s before the U.S. offensive program was terminated. Other countries have been or are currently suspected of weaponizing this agent.

The anthrax bacterium is relatively easy to cultivate. The formation of spores facilitates both storage and weaponization of the organism. Anthrax spores are highly resistant to sunlight, heat, and disinfectants—properties that are advantageous when choosing a bacterial weapon.

VIRAL DISEASES

A virus (from the Latin meaning "poison") consists of small units of DNA or RNA; a virus does not constitute a complete cell. Virus particles (**virions**) are much smaller than bacteria—they cannot be seen using conventional light microscopy. Each viral particle is enclosed within a protective protein coat termed a capsid. The capsid shape varies from simple helical and icosahedral forms to more complex structures.

Viruses act by parasitizing selected host cells; they are unable to grow or reproduce outside those cells. Viruses are classified into animal, plant, and bacterial types, depending on the type of host infected. Viral infections in human and animal hosts typically result in disease and an immune response. In many cases an invading virus is completely destroyed and removed by the immune system. A key practical difference between viruses and bacteria is that viruses cannot be treated using antibiotics. However, vaccines are effective in preventing some viral infections or in limiting their effects. In some cases, antiviral drugs have been developed to treat life-threatening infections.

Smallpox

The name "smallpox" is derived from the Latin word for "spotted" and refers to the raised bumps that appear on the face and body of an infected victim. Two clinical forms of smallpox exist; variola major is the severe and most common form of smallpox, characterized by an extensive rash and high fever. Variola minor is less common and a less severe disease, with death rates historically of 1% or less. Variola is a member of the orthopoxvirus genus of poxviruses, which includes many species isolated from mammals.

Humans are the only natural hosts of variola; smallpox is not known to be transmitted by insects or animals. Smallpox is highly contagious, and outbreaks have been recorded for thousands of years. The last case of smallpox in the United States was in 1949. The last known endemic case was recorded in October 1977 in Somalia. After a successful worldwide vaccination program the disease was declared eradicated in 1980. Following this historic event, routine vaccination against smallpox ended. The United States discontinued vaccination for civilians in 1972, and by 1985 the military no longer vaccinated its recruits. The smallpox virus still exists, however—both the United States and the former Soviet Union retain stocks of the virus. It is fairly certain that some clandestine stocks of smallpox virus exist in other nations as well.

Direct and prolonged face-to-face contact (resulting in inhalation of aerosolized virus) is typically required to spread smallpox from one person to another. Smallpox is also spread through direct contact with infected body fluids or contaminated objects such as clothing. Only in rare circumstances is smallpox spread by virus carried in the air in enclosed settings (e.g., inside buildings) (U.S. CDC, 2007a).

Clinical Manifestations/Symptoms

Following entry into the body the virus travels to nearby lymph nodes where it multiplies and causes **viremia** (contamination of the bloodstream with the virus). The virus later spreads to the spleen, liver, and lungs. An incubation period of about 12–14 days follows, during which no symptoms are apparent and the victim may feel well. During this time, infected individuals are not contagious.

The first symptoms of smallpox include fever, malaise, head and body aches, and sometimes vomiting (the **prodrome** phase). The fever is usually high, in the range of 101–104 °F. At this time, victims become incapacitated and cannot carry out routine activities. The prodrome phase may

last for 2–4 days (CDC, 2007a). Following the prodrome phase the first visible lesions occur in the mouth as red spots on the tongue and on the oral and pharyngeal mucosa. These lesions enlarge and ulcerate quickly, releasing large amounts of virus into the saliva. During this phase the victim is most contagious. As the sores in the mouth disappear, a rash appears on the skin, starting on the face and spreading to the arms and legs and then to the hands and feet (Figure 4.7). Usually the rash spreads to all parts of the body within 24 hours. During this time, the lesions progress from macules to papules, then to pustular vesicles (a small round raised area of inflamed skin filled with pus). As the rash appears, the fever usually falls and the victim may start to feel better. Fever often will rise again and remain high until scabs form over the bumps (CDC, 2007a). The scabs begin to fall off, leaving marks on the skin, which eventually become pitted scars. Most scabs have fallen off within three weeks after the rash appears. The infected person is contagious until all scabs have fallen off. Fatalities are usually caused by systemic toxicity and occur more frequently during the second week of illness.

Treatment

At the time of the last endemic smallpox cases in Somalia, supportive care was the only possible treatment. As is the case with all viral diseases, antibiotics have no effect on the progress of the disease.

Smallpox vaccine, a live virus preparation derived from vaccinia virus (another member of the orthopoxvirus genus), is highly effective

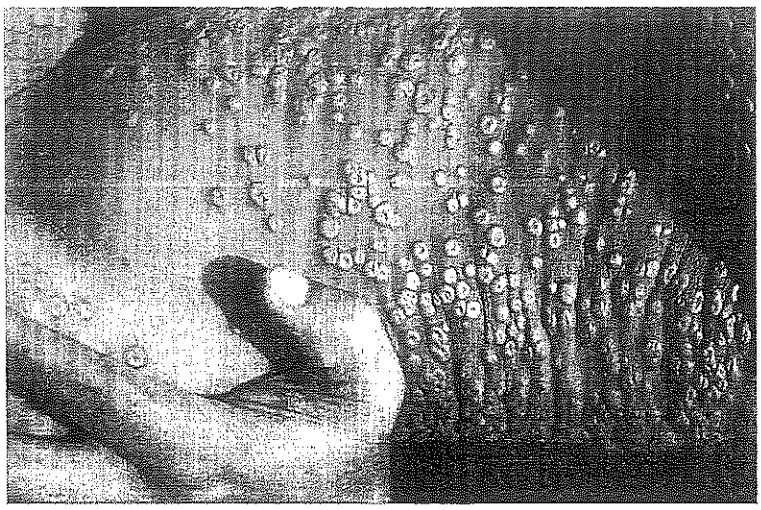

Figure 4.7 Smallpox lesions. Bangladesh, 1973. (From CDC.gov.)

in inducing immunity against the disease before exposure. Dryvax® is the vaccinia-based formulation currently licensed in the United States. Vaccinia preparations do not contain smallpox (variola) virus. The vaccine had previously been prepared using calf lymph tissue; a reformulated vaccine, produced by using cell culture techniques, is now being developed (U.S. CDC, 2001). If administered within 3 days after exposure to smallpox virus the vaccine may prevent disease or decrease the severity of disease and the risk of death (U.S. CDC, 2007b).

Smallpox immunity is known to diminish over time. Few in the United States have been vaccinated since 1985; hence, only a small proportion of the population has any residual immunity to the disease. Since the September 11 terrorist attacks only a few thousand individuals have received the vaccine.

In some individuals smallpox vaccination can result in undesirable reactions. The majority of adverse effects caused by the vaccine are mild complications that are resolved on their own. Serious reactions are rare, but can be fatal. Two medications are available to help those with adverse reaction to the smallpox vaccine: vaccinia immune globulin (VIG) and cidofovir. VIG has been extensively used in the past and considered to be quite effective. Cidofovir is considered effective based on studies in animals. These treatments are investigational and may cause side effects (CDC, 2007a).

The potential effects of current antiviral drugs on the smallpox virus are unknown.

Biowarfare and Terrorism

Smallpox virus was researched as a weapon by the Soviet Union during their offensive biological program, and the status of former stockpiles is uncertain. Dr. Ken Alibek, a former official of the Soviet bioweapons program, asserts that Russia continues to research and manufacture biological weapons (Alibek and Handelman, 1999). The Soviet government in 1980 is reported to have begun an ambitious program to produce large volumes of smallpox virus and adapt it for payloads in intercontinental ballistic missiles. The program was known to have an industrial capacity capable of producing tons of smallpox virus annually. It is also reported that the Russian research program continues working to produce more virulent and contagious recombinant strains (Henderson et al., 1999). Many scientists in the Soviet bioweapons industry were put out of work when Russia officially discontinued its offensive biological weapons program in the 1990s, and there are concerns that some may be assisting rogue states working to develop their own biological weapons programs.

A single confirmed case of smallpox would constitute an international public health emergency and an international crime. Given the diminished immunity to the disease mentioned above, there is potential for a global pandemic should the disease become established. An aerosol release of variola virus would disseminate widely due to the stability of the orthopoxvirus in aerosol form (Harper, 1961) and the probability that the infectious dose is very small (Wehrle et al., 1970).

An occurrence of smallpox would require an immediate and coordinated public health, medical, and law enforcement response to control the outbreak and to protect the public from any additional release. Any incidents of smallpox must be reported immediately to public health authorities, local law enforcement, local emergency management officials, and the FBI. Medical personnel must be able to recognize multiple cases of vesicular rash and severe illness as the result of a deliberate attack with smallpox virus. Health care and other emergency response personnel must rapidly establish protocols to prevent the spread of the disease. Anyone exposed to smallpox should be vaccinated immediately, including those individuals in contact with smallpox patients. Smallpox vaccine is available as a component of the Strategic National Stockpile (SNS) (Figure 4.8) (see Box 4.2).

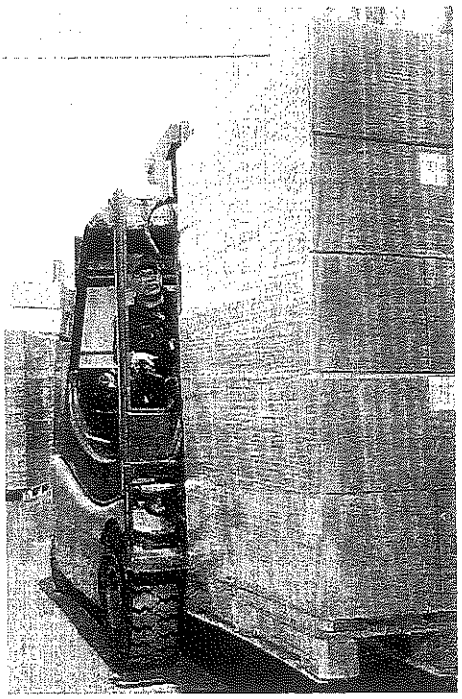

Figure 4.8 Unloading supplies for Strategic National Stockpile. (From Semp.gov.)

BOX 4.2 THE STRATEGIC NATIONAL STOCKPILE

An act of terrorism or a large-scale natural disaster affecting the U.S. population will require rapid access to large quantities of both medicines and medical supplies. Such quantities have been made readily available via the creation of dedicated stockpiles.

CDC's SNS is equipped with large quantities of pharmaceuticals and medical supplies to protect the American public in the event of a public health emergency (terrorist attack, flu outbreak, etc.) severe enough to cause local supplies to become depleted. Once federal and local authorities determine that the SNS is needed, medicines will be delivered to any state in the United States within 12 hours. Each state has procedures to receive and distribute SNS medicine and medical supplies to local communities as quickly as possible (U.S. CDC, 2009).

The SNS contains sufficient medicine to protect citizens in several large cities simultaneously. The medicine is free for all citizens, not just health-care professionals and emergency responders.

Persons who have had direct contact with a smallpox victim must be held in respiratory isolation for 17 days.

In response to the potential use of biological agents against civilians, the federal government has formulated plans for preparedness, readiness, and national defense. The U.S. CDC has been designated as the lead agency for the national public health response to biological terrorism. The *Smallpox Response Plan and Guidelines* (U.S. CDC, 2007b) incorporates and expands many of the approaches that were successfully employed 30–40 years ago to control smallpox outbreaks. These protocols for outbreak containment contributed greatly to the eventual global eradication of smallpox. The *Plan* includes criteria for implementation, notification procedures for suspected cases, CDC and state and local responsibilities and activities (including some that should take place before a smallpox emergency), and CDC vaccine and personnel mobilization. The *Plan* also provides guidelines to assist health officials in implementing the specific actions that are essential for the management of a smallpox emergency (CDC, 2007b).

BIOLOGICAL TOXINS

Toxic substances are produced by numerous bacteria, fungi, protozoa, plants, reptiles, amphibians, fish, mollusks, echinoderma (e.g., certain

urchins and starfish), and insects. Some can be extracted and purified with relative ease, and some can be manufactured in the laboratory. Biological toxins are nonvolatile, are not dermally active (with the exception of mycotoxins), and tend to be more toxic per weight than many chemical agents (USAMRIID, 2005). The LD_{50} values of several biological toxins are shown in Table 4.3.

Some biological toxins have been identified by the intelligence community as biological warfare threats. The most probable routes of toxin entry from a terrorist attack are through the lung by respirable aerosols and through the GI tract by contaminated food or water.

Table 4.3 LD_{50} Values of Selected Biological Toxins

Toxin	LD_{50}*	Toxin	LD_{50}
Abrin	0.7	Diphtheria toxin	0.1
Aerolysin	7.0	Listeriolysin	3–24
Botulinum toxin A	0.0012	Pertussis toxin	15–21
Botulinum toxin B	0.0012	Pseudomonas aeruginosa toxin A	3–14
Botulinum toxin C1	0.0011	Ricin	2.7
Botulinum toxin F	0.0025	Shiga toxin	0.5–20
b-Bungarotoxin	14.0	Shigella dysenteriae neurotoxin A	0.5–1.3
Cholera toxin	260	Staphylococcus enterotoxin B	20–25
Clostridium difficile enterotoxin A	0.5	Staphylococcus enterotoxin F	2–10
Clostridium perfringens lecithinase	3	Tetanus toxin	0.001
Clostridium perfringens enterotoxin	80	Yersinia pestis murine toxin	10
Clostridium perfringens beta toxin	0.3–0.4		

* Oral (μg/kg body weight).

Source: Gill, D.M., *Microbiol. Rev.*, 46, 1, 86–94, 1982; 1993. Sweet, D.V., (ed.). *Registry of the toxic effects of chemical substances, microfiche edition.* National Institute for Occupational Safety and Health, CAELEM Research Corporation, Silver Spring, MD: 1993; ChemCAS. *Free MSDS Search.* Accessed August 18, 2010 at http://www.chemcas.com/.

Ricin

Ricin is a highly toxic glycoprotein that occurs naturally in castor beans (*Ricinis communis*). The ricin molecule consists of two polypeptide chains, the A chain and the B chain, linked by a disulfide bond.

Ricin can be produced easily and inexpensively; it is highly toxic, stable in aerosolized form, and has no approved vaccine. Ricin can be disseminated as an aerosol, by injection, or as a contaminant in food or water. The toxin is not communicable from person to person, however.

One of the more notorious incidents involving ricin intoxication in recent years is the case of Georgi Markov, a Bulgarian dissident. Markov was assassinated in 1978 at a London bus stop. Agents of the Soviet KGB are believed to have used a modified umbrella employing a compressed gas cylinder to fire a tiny ricin pellet into Markov's leg (Figure 4.9). Following the injection he developed severe gastroenteritis and high fever, and died in a hospital a few days later. His body was autopsied and the ricin pellet was discovered. The initial suspects were the Bulgarian secret police; Markov had defected from Bulgaria and written books that were highly critical of the Bulgarian communist regime. Later, however, some high-profile KGB defectors confirmed the involvement of the Soviet KGB in the assassination.

Clinical Manifestations

Ricin is highly toxic to cells, and acts by inhibiting protein synthesis. The B chain binds to cell surface receptors and the toxin-receptor complex is taken into the cell. The A chain has endonuclease activity and extremely low concentrations will inhibit protein synthesis (USAMRIID, 2005).

The effects of ricin poisoning are a function of route and quantity of exposure. Aerosol exposure results in weakness, fever, and pulmonary

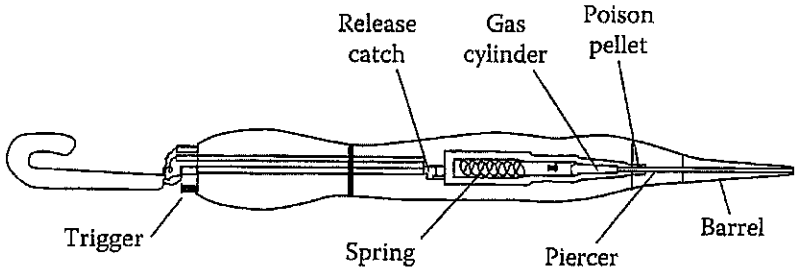

Figure 4.9 Representation of the umbrella-concealed device used to kill Georgi Markov.

edema within 18–24 hours and severe respiratory distress and death within 36–72 hours. Death via inhalation of a lethal dose appears to be caused by hypoxemia resulting from massive pulmonary edema and alveolar flooding (Franz and Jaax, 1996). In rodents aerosol exposure is characterized by necrotizing airway lesions causing tracheitis, bronchitis, bronchiolitis, and interstitial pneumonia with perivascular and alveolar edema (Mirarchi, 2008). The LD_{50} for aerosol exposure to ricin is 2.7 µg/kg. If ingested in sufficient amounts, ricin can cause severe gastroenteritis; GI hemorrhage; and hepatic, splenic, and renal necrosis. The LD_{50} for GI exposure is 30 µg/kg. There is disagreement as to the toxic potential from ingestion of multiple castor bean seeds (Mirarchi, 2008). Puncture (parenteral) exposures can cause severe local necrosis of muscle and regional lymph nodes. Injected ricin is rapidly fatal, with an LD_{50} similar to that of aerosol exposure. Dermal exposure of ricin is a minor concern because the amounts absorbed through the skin tend to be quite low. Dermal exposure probably is unable to achieve toxicity.

Treatment

Treatment of ricin poisoning depends upon route of exposure. Treatment is supportive, and no antidote is currently available for ricin. In the case of aerosol exposure, treatment should be directed toward acute lung injury and pulmonary edema. It is critical to ensure adequate oxygenation and ventilation for the patient. For GI exposure the GI tract should be decontaminated (i.e., lavaged) with superactivated charcoal. For exposure via puncture, the injection site should be excised, if possible, within the shortest amount of time. Antibiotics may help prevent infection. For dermal exposure, a dilute sodium hypochlorite solution or soap and water will serve to decontaminate the skin. Investigations are underway with potential vaccines and ricin inhibitors as antidotes.

Biowarfare and Terrorism

Ricin can be disseminated as an aerosol, by injection, or as a food and water contaminant.

A large quantity of ricin is necessary to produce effects of a WMD, however. For example, the amount of ricin necessary to cover a 100 km^2 area and cause 50% lethality, assuming aerosol toxicity of 3 µg/kg and optimum dispersal conditions, is approximately 4 metric tons, whereas only 1 kg of *B. anthracis* will cause the same extent of damage to populations (Kortepeter and Parker, 1999; Mirarchi, 2008). Ricin would, however,

be effective as a disabling agent. If used as a food and water contaminant ricin could easily incapacitate many and overwhelm local healthcare resources.

The U.S. military investigated the potential of ricin as a weapon during World War I. At that time it was being considered for use either as a toxic dust or as a coating for bullets. The dust cloud method could not be perfected by war's end, and the coated bullet method would have violated The Hague Convention of 1899. During World War II the U.S. military studied the use of ricin in cluster bombs. Though there were plans for mass production and several field trials with different bomb concepts, it was eventually concluded that ricin was no more economical than using phosgene. Ricin was given the military symbol W and later WA. Interest in ricin continued for a short period after World War II but diminished when the U.S. Army Chemical Corps began a program to weaponize sarin. Ricin was never used in battle (Cookson and Nottingham, 1969; Franz and Jaax, 1996).

Whereas ricin is easy to produce it is neither as practical nor likely to cause as many casualties as other agents. Ricin becomes inactivated (i.e., its polypeptide chains become denatured thus becoming less dangerous) much more readily than anthrax spores, which may remain lethal for decades. It is known that al-Qaeda experimented with ricin, which has been interpreted as an inability by their weapons makers to produce and/or weaponize botulinum or anthrax.

AGROTERRORISM

Agricultural terrorism (agroterrorism) is considered by many scientists and policy makers as a subset of biological terrorism. Agroterrorism is defined by U.S. FEMA (2005) as follows:

> The malicious use of plant or animal pathogens to cause devastating disease in the agricultural sector. It may also take the form of hoaxes and threats intended to create public fear of such events.

Acts of agroterrorism may involve contamination of livestock, food crops, and processed foods, and the results can be as catastrophic as other forms of terrorism. The most dangerous mode of agroterrorism is via introduction of pest organisms, which may devastate a commercial source of food. Effects from an attack on crops, livestock, or food supplies would be both short- and long-term and include the following:

- Extensive deaths and illness
- Paralyzes the economy of a nation

- Destroys the livelihood of many people
- The disease may not be detected until it reaches levels that are difficult to control
- Puts food supply at risk, perhaps for long periods

Many consider agriculture to be an ideal target for terrorism because of the size of the industry's revenues and its breadth across a nation. The United States is a global leader in food production, and an attack upon any segment of the food industry would have disastrous effects upon the entire national economy. The magnitude of such damage can be seen from the following data (USDA, 2009):

- The farm sector is the largest contributor to the U.S. trade balance.
- The United States produces nearly 50% of the world's soybeans, more than 40% of its corn, 20% of its cotton, 12% of its wheat, and more than 16% of its meat.
- Approximately 2.2 million farms exist in the United States, totaling more than 920 million acres.

A number of interrelated consequences would contribute to severe financial losses at local to national levels, including (Figure 4.10) (Parker, 2002) the following:

- Direct losses of agricultural commodities to disease
- Decline in sales of agricultural products from diminished consumer confidence
- Costs of diagnosis and surveillance
- Destruction of contaminated crops and animals to contain the disease
- Costs of disposal of animal carcasses
- Losses due to export and trade restrictions
- Disruption of commodity markets
- Insurance costs

Threats

Agricultural diseases have evolved to remain viable in the environment for months to years in spite of exposure to ultraviolet light, heat, lack of moisture, and other stressful conditions. Thus, they are highly resilient in the natural environment and are expected to persist in storage as well. In addition, the supplies and equipment necessary to carry out an agroterror

Figure 4.10 Introduction of a pathogen to an agricultural system could result in severe economic consequences. (From USDA.)

attack are relatively easy to obtain (Kohnen, 2000). Small quantities may be needed, for example, of a specific livestock pathogen, which would be relatively easy to transport to the site of intended use. Infection of a small number of animals may be sufficient to cause a disease outbreak. An epidemic could be caused by placing bird droppings contaminated with Newcastle disease into a feeding trough, or by placing oral scrapings from a FMD-infected animal onto the floor of a cattle barn (U.S. FEMA, 2005).

Animal Diseases

Over many decades a myriad of diseases has been weaponized by the United States, the former Soviet Union, China, Iraq, and other countries. The scientific expertise and technology for developing such weapons on a large scale have been available for over a century. There are approximately 150 diseases of concern within the livestock disease category alone. Animal pathogens known to have been weaponized or pursued for weaponization potential are shown in Table 4.4.

The most successful agriculture biological warfare program was developed by the former Soviet Union, where a wide range of animal pathogens were studied. The Soviets successfully used ticks to transmit FMD and avian ticks to transmit ornithosis to chickens (Ban, 2000). Disease organisms believed to have been weaponized by the former Soviet Union are shown in Table 4.5.

Table. 4.4 Animal Pathogens Pursued for Weaponization Purposes

African swine fever	Ebola virus
Anthrax	Marburg virus
Foot and mouth disease	Rift valley virus
Hog cholera/classical swine fever	*Bacillus anthracis*
Ornithosis/psittacocis	*Burcella melitensis*
Rinderpest	*Burkholderia mallei*
Trypanosomiasis	*Francisella tularensis*
Poxvirus	*Yersinia pestis*

Source: U.S. Centers for Disease Control and Prevention. 2005. Bioterrorism agents/diseases. Emergency preparedness and response. http://www.bt.cdc.gov/agent/agentlist-category.asp5; U.S. Federal Emergency Management Agency. 2005. Appendix E. Agriterrorism. Toolkit for managing the emergency consequences of terrorist incidents. http://fema.gov/pdf/onp/toolkit_app_e.pdf.

Table. 4.5 Disease Organisms Believed to Have Been Weaponized by Former Soviet Union

African swine fever	Potato virus
Anthrax	Psittacosis
Avian influenza	Rice blast
Brown grass mosaic	Rinderpest
Brucellosis	Rye blast
Contagious bovine pleuropneumonia	Tobacco mosaic
Contagious ecthyma (sheep)	Venezuelan equine encephalitis
Foot and mouth disease	Vesicular stomatitis
Glanders	Wheat and barley mosaic streak
Maize rust	Wheat stem rust
Newcastle disease	

Source: U.S. Federal Emergency Management Agency. 2005. Appendix E. Agriterrorism. Toolkit for managing the emergency consequences of terrorist incidents. http://fema.gov/pdf/onp/toolkit_app_e.pdf.

Plant Pathogens

Plant pathogens typically occur as fungi and to a lesser extent by viruses and bacteria. Plant pathogens kill or injure a particular crop species;

however, in some unusual cases the disease may produce toxins that can be harmful to humans.

Plant pathogens known to have been weaponized include the following:

- Rice blast (*Mangaporthe grisea*)
- Wheat stem rust (*Puccinia graminis f.sp.triciti*)
- Wheat smut (*Fusarium graminearum*)

Additional plant pathogens that have the potential for weaponization are shown in Table 4.6.

Rice and wheat are two cereal crops that supply the majority of the world's daily intake of calories. A disease event involving either of these crops, whether by natural outbreak or from terrorist activity, would have significant consequences.

Rice blast is an extremely destructive disease of rice as well as a number of other agriculturally important cereals, including wheat, rye, barley, and pearl millet. The disease is particularly destructive to upland rice crops (Figure 4.11) (Knowledgeblast, 2005). A fungus, *Magnaporthe grisea*, is responsible for the disease, which is known to occur in 85 countries worldwide. Rice blast is widespread and causes severe economic

Table 4.6 Selected Plant Pathogens That Have the Potential for Weaponization

Crop	Pathogen
Wheat	Wheat dwarf (*geminivirus*)
	Barley yellow dwarf virus (*Pseudomonas fascovaginaei*)
Corn (maize)	Barley yellow dwarf virus (*Pseudomonas fascovaginaei*)
	Brown stripe mildew (*Sclerophthora rayssiae*)
	Sugarcane downy mildew (*Peronosclerospora sacchari*)
	Java downy mildew (*Peronosclerospora maydis*)
Soybean	Soybean dwarf virus (*Luteovirus*)
	Red leaf blotch (*Pyrenochaeta glycines*)

Source: Parker, H.S., Agricultural Bioterrorism: A Federal Strategy to Meet the Threat, McNair Paper 65, National Defense University, March, 2002. http://www.ndu.edu/inss/McNair/mcnair65/McN_65.pdf; Steele, N., Econoterrorism: U.S. Agricultural Productivity, Concentration, and Vulnerability to Biological Weapons, Unclassified Defense Intelligence Assessment for DOD Futures Intelligence Program, January 14, 2000.

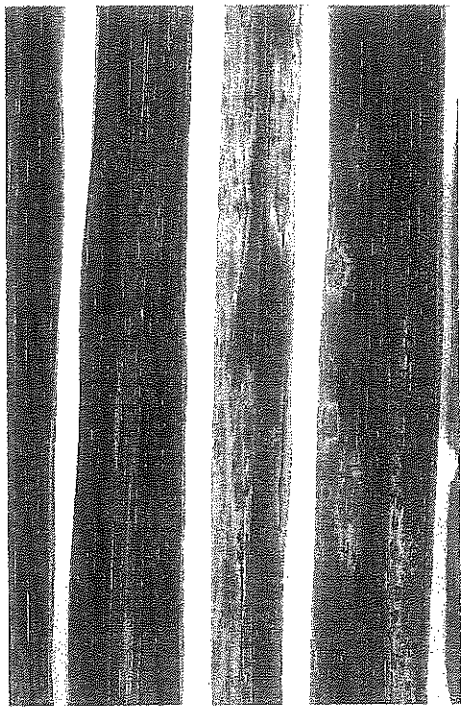

Figure 4.11 Typical eye-shaped lesion of rice blast disease on a U.S. rice cultivar inoculated with *Magnaporthe grisea*. (From USDA.)

losses—each year it is estimated to destroy enough rice to feed more than 50 million people.

During World War II, *M. grisea* spores were prepared as a biological weapon by the United States and the USSR. The U.S. Chemical Warfare Service is known to have researched the agent for use against Japan's rice crop during the war (Croddy and Wirtz, 2005). The United States worked with Canadian and British scientists to weaponize rice blast but it was not ready for use in battle by war's end (Levy, 2000).

Karnal bunt, a fungal disease of wheat, was first discovered in wheat-growing areas of India in 1931. It has subsequently been located in all major wheat-growing areas in India, Pakistan, Iraq, Afghanistan, and South Africa. The disease was introduced to Mexico in 1960, and by 1996 Karnal bunt was found in the United States in Arizona. The disease does not severely impact crop production; however, due to international regulation, an outbreak of Karnal bunt would significantly restrict export of wheat overseas. The U.S. Department of Agriculture conducts an annual national survey to certify the U.S. wheat crop as being free from Karnal bunt (USDA, 2006).

Transmission of Plant Pathogens

Fungi, viruses, and bacteria that cause crop diseases can be transmitted by the following methods (U.S. FEMA, 2005):

- *Airborne*—Most fungal diseases are carried by the wind, possibly over great distances. Bacteria are carried by air currents in certain cases (see the earlier discussion of anthrax spores). Spores also readily attach to surfaces (packaging, farm equipment, clothing, etc.). It is difficult to eradicate spores from infected areas, and once-productive land may have to be abandoned as "useless" for long periods.
- *Vectors*—Both viruses and bacteria can be spread by insect vectors. Those diseases that depend upon vectors spread slowly, because transmission depends on the movement of the insect hosts.
- *Water-borne*—Bacteria typically spread in moist materials and environments.

Zoonotic Diseases

Zoonotic diseases are those transferred from animals to man or from man to animals. Approximately 150 zoonotic diseases are known to exist (University of Minnesota, 2005). Zoonotic diseases are of significant concern to U.S. public health and animal health sectors. The recent emergence of Avian Influenza and West Nile Virus demonstrates the vulnerability of industrialized nations to the spread of zoonotic diseases, and the general inability to contain such diseases once they become established in wild animal populations.

Indicators of an Agroterrorism Event

The indicators of an agroterrorism event might not appear for days or weeks. Certain patterns may appear to alert veterinarians, health-care providers, and other public health professionals of the intentional release of an infectious disease (Moore, 2002):

- Unusual clustering of illness or mortality in a particular region or within a short span of time for large numbers of animals or humans. This may include atypical, unexplained symptoms.
- Symptoms occurring in an area where a particular disease is extremely rare.
- Normally healthy individuals becoming suddenly ill.
- An unusual age distribution for common diseases.
- A disease occurring outside its typical season.

Response to a Disease Event

Response to agroterror events is different from responses to human disease events in a number of ways. The primary intent in responding to an agricultural disease event is to contain and destroy the disease by isolating the infected crops or animals. This usually includes destruction of the crops and euthanasia of the animals involved. Emergency responders may become involved in the containment, eradication, and/or quarantine enforcement to limit the spread of agricultural diseases.

Responders must remain alert to the hazards presented by an agricultural disease event. Many such diseases can affect humans as well as animals. Many of the diseases can be life-threatening, and many can be passed along to other people once contracted, or if proper decontamination procedures are not followed. The following steps in response can be applied to agroterrorism events, as well as other CBRNE events:

- *Recognize*—Recognize the indicators of an agricultural disease event.
- *Avoid*—Practice avoidance measures to prevent the accidental exposure or spread of the disease.
- *Isolate*—Isolation or quarantine procedures are essential in the control and eradication of a disease.
- *Notify*—Know whom to call at the first possible indications of a disease event, so that the identification and response process can begin.

QUESTIONS

1. Suppose it is determined that an act of bioterrorism results in the release of smallpox virus in your area. Does the CDC have the right to force you and your family to receive a vaccination? Why or why not?
2. Would the sealing of windows with duct tape and plastic sheeting help protect you in your home during a bioterrorist attack with anthrax spores? Explain.
3. What regulations or guidance documents exist that would help with investigation and development of safe and effective new products that might be used in countering bioterrorism? Check the FDA.gov web site.
4. What is the most effective route for dispersal of a biological agent to enter a target population?

5. What atmospheric properties would affect migration of an aerosol plume (cloud) of biological agent?
6. Explain the difference between an *infectious* disease and a *contagious* disease.
7. Any identification of smallpox outbreak should be considered an international emergency, and most likely a terrorist event. True or false? Explain.
8. Could biological agents be effective in contaminating large water supplies (e.g., reservoirs)? Discuss.
9. List and discuss two possible indicators of a terrorist biological attack.
10. Define or discuss the following terms: edema; eschar; mediastinitis.

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