SECOND EDITION

BIODEFENSE RESEARCH METHODOLOGY AND ANIMAL MODELS
Cover credits: Top two photos courtesy of Dr. Chad J. Roy and Dr. Xavier Alvarez. Bottom two photos courtesy of Dr. Tom Geisbert.
In the world of biodefense research, there exists a cadre of men and women who have dedicated their lives to protecting the world from those who would use infectious biological organisms and toxins for nefarious purposes. The scientific community has banded together across many organizational lines to bring new technology, information, and countermeasures into the biodefense portfolio to better prepare against these threats. In addition to the devoted scientists, I want to acknowledge the people whose critical contributions made these advances possible. These are the professionals who maintain the facilities, make sure the research is done safely, oversee the use of animals and ensure they are used humanely in accordance with regulatory requirements; and the laboratory and veterinary technicians who are the heart and soul of this research. The vigilance and remarkable talents of these teams of professionals are our best defense.
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Preface

The evolution of biodefense research has made significant advances in animal model development since the publication of the first edition of this book in 2006. The Food and Drug Administration’s (FDA) Animal Efficacy Rule (read more about this in Chapter 3) has begun to mature in both understanding by the scientific community and the expectations of the FDA. Like the first edition, this edition continues to span the spectrum from basic research to advanced development of medical countermeasures. The return reader will most likely notice an increase in discussions about the FDA animal efficacy rule as it applies to animal model development and research directions for the various biological agents and toxins. As we all know, redundant efforts often waste more than just time and fiscal resources—they also result in the unnecessary use of animals. Animals have been and will continue to be an invaluable and absolutely necessary part of infectious disease research, but we all have the ethical and moral obligation to ensure that each animal is used in the most humane manner possible and to obtain the maximum benefit in advancing science and human health. It should be understood that much work precedes moving to the use of animal models, and the models presented in this book were developed in conjunction with many in vitro techniques including computer modeling, cell culture systems, hollow fiber systems, and other in vitro laboratory procedures. All of these techniques have replaced or reduced the use of animals for certain purposes, but as questions arise that require an intact, more complex biological system to answer, animal use becomes essential. The primary aims of this edition remain true to the first edition in an effort to share science, to advance science, and to minimize the number of animals required for use by reducing unnecessary duplication of effort in animal model development and use. The participation of all the chapter authors and coauthors is a testament to their belief in these values and dedication to advancing science, and protecting the health of our world’s population.
Dr. James R. Swearengen, following retirement from the U.S. Army after 21 years of service, served for 4 years as the senior director at the Association for Assessment and Accreditation of Laboratory Animal Care International before joining the National Biodefense Analysis and Countermeasures Center as their comparative medicine veterinarian in 2009. Dr. Swearengen obtained his DVM degree from the University of Missouri-Columbia in 1982 and joined the Army after 2 years of private practice. After tours in Texas and Germany, Dr. Swearengen completed a residency in laboratory animal medicine at the Walter Reed Army Institute of Research from 1990 to 1994, during which period he attained board certification in the specialties of both Laboratory Animal Medicine and Veterinary Preventive Medicine and is a past-president of the American College of Laboratory Animal Medicine.

He began working at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in 1994 as the assistant director, and then director, of the Veterinary Medicine Division. He gained extensive experience in providing veterinary and husbandry support to infectious disease animal research at all levels of biocontainment and spent many hours working under biosafety level 3 and 4 conditions. Dr. Swearengen became intimately involved with the existing animal models used in biodefense research, provided veterinary expertise in the development of new models, and coauthored publications utilizing animal models for Ebola virus and monkeypox virus infections. In 1996, he was selected to serve on the United Nations Special Commission (Biological Group) and spent 3 months in Iraq performing monitoring and verification functions of Iraq’s former biological weapons program. Since 2007, Dr. Swearengen has served on the National Academies of Science National Research Council Standing Committee on Biodefense for the U.S. Department of Defense and the National Academies of Science Institute for Laboratory Animal Research Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents.

In 1997, Dr. Swearengen provided part-time support for a Defense Threat Reduction Agency program by evaluating and modernizing animal care and use programs in infectious disease research institutes in the former Soviet Union. His expertise was recognized in 2003 as he was selected as the Laboratory Animal Medicine Consultant to the Surgeon General of the U.S. Army. Dr. Swearengen’s military career culminated in 2003 as he was chosen to serve as the Deputy Commander of USAMRIID, a position he held until his retirement from the U.S. Army in 2005.
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1 History of Biological Agents as Weapons

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The earliest use of biological weapons in warfare resulted from the use of corpses first to contaminate water sources and subsequently as a terror tactic, hurling bodies over the wall of fortified cities. From these crude beginnings were to develop national programs for biological weapons development, stockpiling, and deployment that would rival all other weapons systems in scope and magnitude as well as potential to cause human harm. Recent unveiling of these programs as well as recognition of the failure of the Biological Weapons Convention to prevent some countries from engaging in biological weapons development has made the public aware, if not frightened, of the possibilities. Ergo, use of biological agents as weapons of warfare, methods of terrorism, or means for engaging in criminal activity has come to the forefront of public attention in recent years. Widespread understanding of the biological threat in terms of biological agents’ historic use is vital for those who endeavor to find ways to protect society from those who intend to use these agents. It is important to have some common agreement of definitions of terminology used in this discussion. Biological agent refers to any living organism or substance produced by an organism that can be used as a weapon to cause harm to humans. Broadly speaking, this includes any living organism or biologically derived substance, but in practical terms (for the classical biological warfare agents), this list is limited to viruses, bacteria, and toxins. Biowarfare in its broadest sense refers to any use of these agents to harm others. However, biowarfare in more common usage ascribes a narrower definition—use in the context of war, that is, it refers to the use of a biological agent by a nation-state as an act of war. Bioterrorism refers to the use of biological agents by a political group, religious group, or cult (group not otherwise recognized as an extension of the government of a state) to achieve some intended political or ideological objective. However, even this definition is fraught with confusion because it does not preclude use by an organization with state sponsorship which can be covert. The term biocrime refers to the use of biological agents in the perpetration of criminal activity in which the perpetrator’s

* The views expressed in this chapter are those of the author and do not reflect the official policy of the Department of the Army, Department of Defense, or the U.S. Government.
motivation appears to be personal in nature, as opposed to some broader ideological, political, or religious objective. Although specific circumstances and events can blur the distinction, it is helpful to keep these three definitions in mind as we review the world's experience with biological agent use.

De Mussis provides a dramatic record of the use of plague victims in an attempt to engage in biological warfare. After war broke out between the Genoese and the Mongols in 1344 over control of access to the lucrative caravan trade route from the eastern shores of the Black Sea to the Orient, the Mongols laid siege to the Genoese port city of Caffa. The plague, which was later to become known as the Black Death, was spreading from the Far East and reached the Crimea in 1346. The Mongols besieging the city were severely affected and had come close to lifting their siege when they changed their tactics and hurled bodies of plague victims over the city wall, probably with the use of a trebuchet. Eventually, plague did spread to the city, though more likely from rats fleeing the Mongol encampment than as a consequence of the spread of the disease by contamination of the city with plague-infected corpses. After plague struck, the residents of Caffa, who had been successfully withstanding the siege, abandoned their defense and fled to ports in Italy, carrying the plague on board the ships with them. As a consequence, the Black Death began its scourge across Europe [1].

Along with contamination of water sources, another ancient tactic was to allow the enemy to take sanctuary in an area endemic for an infectious agent in anticipation that the enemy force would become infected and weakened by the resulting disease. Most prominent examples were the allowance of unimpeded access to malarious areas, where disease transmission was highly likely to occur [2].

The Carthaginian leader Hannibal is credited with the first use of biological toxins in warfare, in the naval battle of Eurymedon in 184 BC. He ordered earthen pots filled with serpents hurled onto the decks of the Pergamene ships, creating panic and chaos. The Carthaginians exploited the situation, with Hannibal defeating King Eumenes of Peragamum in the battle that ensued [2].

Smallpox was particularly devastating to the Native Americans. Cortez's introduction of smallpox to the Aztecs, whether intentional or not, played a major role in allowing for their defeat and subjugation by the Spanish conquistadors. Sir Jeffery Amherst, British commander of forces in the American colonies during the French and Indian War, provided Indians loyal to the French with blankets and other articles contaminated by smallpox. Native American Indians defending Fort Carillon (subsequently named Fort Ticonderoga) experienced an epidemic of smallpox that contributed to their defeat and the loss of the fort to the British. Subsequently, a smallpox epidemic broke out among the Indians in the Ohio River valley [3].

During the American Revolutionary War, successive smallpox epidemics affected major Continental Army campaigns early in the conflict and resulted in the aborted attempt to capture Quebec City early in the war. The British forces, which were immune to the disease because of their exposure to the natural infections endemic in much of Europe, were relatively protected from smallpox, whereas the colonists, living in more rural and isolated settings, were nonimmune. Because of his recognition of the consequences of this disparity of immunity between the two forces, General George Washington ordered the variolation (inoculation with smallpox) of all
nonimmune recruits in 1778. This was a controversial procedure that predated vaccination and carried a potential mortality of 1–3%; it was the first time in world military history that such a measure had been ordered by a commander and it set the precedence for military immunization programs of today [2].

The Germans undertook a covert biological campaign in the United States in the first part of World War I, before the United States had entered the war. The Allies had been purchasing draft animals from the United States for use by their military forces. German operatives infected animals awaiting shipment overseas with glanders and anthrax organisms [4]. The Germans also conducted similar operations in Romania, Russia, Norway, Mesopotamia, and Argentina, with varying levels of success. Attempts were also made to infect the grain production in Spain with wheat fungus, but without success [5].

An international protocol, known as the 1925 Geneva Protocol [for the Prohibition of the Use in War of Asphyxiating, Poisonous, or Other Gases, and Bacteriological (Biological) Methods of Warfare], was created in response to the use of chemical agents during World War I. The 1925 Geneva Protocol created by the League of Nations’ Conference for the Supervision of the International Trade in Arms and Ammunition concerned use only between nation-states. It has no verification mechanism and relies on voluntary compliance. Many of the original signatory states held reservations to the protocol for the right to retaliatory use, making it effectively a no-first-use protocol [2]. After the Japanese defeat of Russia in the 1905 Russo-Japanese War, Japan had become the dominant foreign power in Manchuria. The Kwantung Army was created to maintain Japanese economic interests in the region. During the 15 months from September 1931 to the end of 1932, the Japanese military seized full control of all of Manchuria, setting the stage for its complete exploitation. It was in 1932, just as Japan obtained military control, that Major Ishii Shiro, a Japanese Army physician with a confirmed interest in biological agents, came to Harbin to exploit Manchurian human resources in the name of research. He established his initial laboratory in the industrial sector of Harbin known as the Nan Gang District, but he soon came to realize that his more controversial involuntary human research could not be conducted without scrutiny there and moved the human research to a secret facility at Beiyinhe, which was 100 km south of Harbin.

Unobserved by the outside world, Major Ishii began human experimentation on a more dramatic scale. Each victim, once selected for study, continued to be a study subject until his or her death as part of the study—or through live vivisection. There were no survivors among the research study subjects. These studies continued until the occurrence of a prisoner riot and escape, which resulted in closure of the facility in 1937. Not to be deterred, the closure of the Beiyinhe facility was followed by the creation of even larger, more extensive facilities [6].

In August 1936, Lt. Col. Ishii was made Chief of the Kwantung Army Boeki Kyusui Bu (Water Purification Bureau). That autumn, the Japanese appropriated 6 km² of farmland, which encompassed 10 villages located 24 km south of Harbin, displacing 600 families from their ancestral homes. It was here that Ishii built the massive Ping Fan research facility, where 200 prisoners were always on hand to become the expendable subjects of further experimentation. A minimum of 3000 Chinese prisoners were killed and cremated consequent to these experiments, but
most of the evidence was destroyed at the end of the war—in all likelihood the actual number of victims of this ghastly research was much greater [6].

The Unit 100 facility at Changchun was run by an equally ruthless veterinary officer, Major Wakamatsu Yujiro. In 1936, the Japanese appropriated 20 km² of land near Mokotan, a small village just 6 km south of Changchun, the capital of Japanese-occupied Manchuria. Unit 100 was a predominantly veterinary and agricultural biowarfare research unit—a completely independent operation from Unit 731 at Ping Fan. The principal focus of Unit 100 was to develop biological weapons useful in sabotage operations. Although animals and crops were the focus of most of the research, a tremendous number of human studies were also conducted that were very similar in nature to those conducted at Ping Fan by Unit 731 [6].

In April 1939, a third major research facility, known as Unit Ei 1644, was established in an existing Chinese hospital in Nanking under the command of one of Ishii’s lieutenants, Lt. Col. Masuda. On the fourth floor of the hospital were housed prisoners, many of them women and children, who became the subjects of grisly experimentation. The human experimental subjects were cremated after the studies in the camp incinerator, usually late at night. A gas chamber with an observation window was used to conduct chemical warfare experiments. Unit Ei 1644 supported the research efforts of Unit 731, with support responsibilities that included production of bacterial agents as well as cultivation of fleas [6]. At the end of the war, in a move that has now become controversial, Ishii, then a lieutenant general, and his fellow scientists were given amnesty in exchange for providing information derived from their years of biological warfare research [2].

In contradistinction to Japanese efforts during World War II, German interest seemed to be more focused on developing an adequate defense against biological agents. Although German researchers experimentally infected prisoners with infectious agents, there were no legal actions taken after the war, and no German offensive biological warfare program was ever documented. The Germans, however, accused the British of attempting to introduce yellow fever to the southern Asian subcontinent as well as of an Allied introduction of Colorado beetles to destroy the German potato crops. These claims were never substantiated [5].

During the Korean conflict, numerous allegations of use of biowarfare by the United States were made by North Korean and Chinese officials. Many of the allegations appear to be based on experiences that the Chinese had in Manchuria with the “field testing” done by Unit 731. Polish medical personnel were sent to China to support the Communist war effort, accompanied by Eastern European correspondents. Numerous allegations based on anecdotal accounts of patients came from these correspondents and other sources. These accounts were not supported with scientific information. Some of the accounts, such as the use of insects for vectors of cholera and the spread of anthrax with infected spiders, had dubious scientific validity [7].

After World War I, Major Leon Fox, Medical Corps, U.S. Army, wrote an extensive report in which he concluded that modern improvements in health and sanitation made use of biological agents unfeasible and ineffective. Some mention was made of the ongoing Japanese offensive biological program in his report, but it was, ironically, his erroneous concerns about German biological weapons’ development that led to serious U.S. interest in the subject. In the autumn of 1941, before U.S. entrance
into World War II, opinions differed as to the validity of biological warfare potential: “Sufficient doubt existed so that reasonable prudence required that a serious evaluation be made to the dangers of a possible attack” [8, p. 1]. As a consequence, the Secretary of War asked the National Academy of Sciences to appoint a committee to study the question. The committee concluded in February 1942 that biowarfare was feasible and that measures were needed to reduce U.S. vulnerability [2].

President Roosevelt established the War Reserve Service, with George W. Merck as director, with the initial task of developing defensive measures to protect against a biological attack. By November 1942 the War Reserve Service asked the Chemical Warfare Service of the Army to assume the responsibility for a secret large-scale research and development program, which included the construction and operation of laboratories and pilot plants. The Army selected the small National Guard airfield at Camp Detrick, Frederick, Maryland, as a site for new facilities in April 1943. By the summer of 1944, the Army had a testing site at Horn Island, Mississippi, which was subsequently moved to Dugway Proving Grounds, Utah, and a production facility in Terre Haute, Indiana, which was soon closed. The War Reserve Service was disbanded and the Research and Development Board established under the War Secretary to supervise the biological research programs. An assessment of the biological warfare situation was provided to the Secretary of War by George Merck in January 1946. The report concluded that the United States clearly needed to have a credible capability to retaliate in kind if ever attacked with biological weapons [7].

Only after the end of World War II did the United States learn of the extent of Japanese biological weapons research. Gradually, in the late 1940s, the scope of the Japanese program became known, along with an awareness of Soviet interest in the program. War broke out on the Korean peninsula in June 1950, adding to concerns about Soviet biological weapons development, and the possibility that the North Koreans, Chinese, or Soviets might resort to biological weapons use in Korea. The Terre Haute, Indiana, production facility, which was closed in 1946, was replaced with a large-scale production facility in Pine Bluff, Arkansas. During the 26 years of biological weapons development, the United States weaponized eight antipersonnel agents and five anticrop agents [9].

Field testing was done in the United States in which the general public and the test subjects themselves were uninformed, and these studies have unfortunately tainted the history of the offensive biological warfare program. The first large-scale aerosol vulnerability testing was the San Francisco Bay study conducted in September 1950. *Bacillus globigii* and *Serratia marcescens* were used as stimulants for biological agents. Unfortunately, a number of *Serratia* infections occurred subsequently in one of the hospitals in the study area, and although none of the infections was ever documented to be the 8UK strain, many people held on to their perceptions that the U.S. Army study had caused the infections [10].

*Serratia marcescens*, then known as *Chromobacter*, was thought to be a non-pathogen at the time. Several controversial studies included environmental tests to see whether African Americans were more susceptible to fungal infections caused by *Aspergillus fumigatus*, as had been observed with *Coccidioides immitis*, including the 1951 exposure of uninformed workers at Norfolk Supply Center, in Norfolk, Virginia, to crates contaminated with *Aspergillus* spores. In 1966, in New York City
subways, the U.S. Army conducted a repeat of studies that had been done by the Germans on the Paris Metro and some of the forts in Maginot Line to highlight the vulnerability of ventilation systems and confined spaces. Light bulbs filled with *Bacillus subtilis* var. *nigeri* were dropped into the ventilator shafts to see how long it would take the organisms to spread through the subway system [11]. The Special Operations Division at Camp Detrick conducted most of the studies on possible methods of covert attack.

After 1954, the newly formed Medical Research Unit conducted medical research separately from the studies done by the Chemical Corps. This research began using human volunteers in 1956 as part of a congressionally approved program known as “Operation Whitecoat.” This use of human volunteers set the standard for ethics and human use in research. The program used army active-duty soldiers with conscientious objector status as volunteers to conduct biological agent-related research. All participation was voluntary and was performed with the written informed consent of each volunteer. The program concluded in 1973 with the end of the draft, which had been the source of conscientious objectors [9]. In July 1969, Great Britain issued a statement to the Conference of the Committee on Disarmament calling for the prohibition of development, production, and stockpiling of bacteriological and toxin weapons [12].

In September 1969, the Soviet Union unexpectedly recommended a disarmament convention to the United Nations General Assembly. In November 1969, the World Health Organization of the United Nations issued a follow-on to an earlier report by the 18-nation Committee on Disarmament, on biological weapons, describing the unpredictable nature, lack of control once released, and other attendant risks of biological weapons use. Then, President Nixon, in his November 25, 1969, visit to Fort Detrick, announced new U.S. policy on biological warfare, renouncing unilaterally the development, production, and stockpiling of biological weapons, limiting research strictly to the development of vaccines, drugs, and diagnostics as defensive measures. The 1972 Biologic Weapons Convention, which was a follow-on to the 1925 Geneva Protocol, is more properly known as the “1972 Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological (Biological) and Toxin Weapons and their Destruction.” Agreement was reached among 103 cosignatory nations and went into effect in March 1975. “The convention prohibits the development, production, stockpiling or acquisition by other means or retention of microbial or other biological agents toxins whatever their origin or method of production of types and in quantities that have no justification of prophylactic, protective or other peaceful purposes, as well as weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict”[13].

The U.S. Army, in response to the 1969 presidential directive, did not await the creation of the 1972 Biological Warfare Convention or its ratification. By May 1972, all personnel-targeted agents had been destroyed and the production facility at Pine Bluff, Arkansas, converted to a research facility. By February 1973, all agriculture-targeted biological agents had been destroyed. Fort Detrick and other installations involved in the offensive weapons program were redirected, and the U.S. Army Medical Research Institute of Infectious Diseases was created in place of the U.S. Army Medical Unit, with biosafety level 3 and 4 laboratories dedicated strictly to the development of medical defensive countermeasures [2].
Although a signatory to the 1925 Geneva Convention, the Soviet Union began its weapons development program at the Leningrad Military Academy in Moscow under the control of the state security apparatus, the GPU. Work was initially with typhus, with what was apparently human experimentation on political prisoners during the prewar era conducted at Slovetsky Island in the Baltic Sea and nearby concentration camps. This work was subsequently expanded to include work with Q fever, glanders, and melioidosis, as well as possibly tularemia and plague. Outbreaks of Q fever among German troops resting in Crimea and outbreaks of tularemia among the German siege forces of Stalingrad are two suspected but unconfirmed Soviet uses of biological warfare during World War II [14].

During World War II, Stalin was forced to move his biological warfare operations out of the path of advancing German forces. Study facilities were moved to Kirov in eastern European Russia, and testing facilities were eventually established on Vozrozhdeniya Island on the Aral Sea between the Soviet Republics of Kazakhstan and Uzbekistan. At the conclusion of the war, Soviet troops invading Manchuria captured the Japanese at the infamous Unit 731 at Ping Fan. Through captured documents and prisoner interrogations, the troops learned of the extensive human experimentation and field trials conducted by the Japanese. Stalin put KGB chief Lavrenty Beria in charge of a new biowarfare program, emboldened by the Japanese findings. The production facility at Sverdlosk was constructed using Japanese plans. When Stalin died in 1953, a struggle for control of the Soviet Union ensued. Beria was executed during the struggle to seize power, and Khruschev emerged as the Kremlin leader and transferred the biological warfare program to the Fifteenth Directorate of the Red Army. Colonel General Yefim Smirnov, who had been the chief of army medical services during the war, became the director [14].

Smirnov, who had been Stalin’s minister of health, was a strong advocate of biological weapons. By 1956, Defense Minister Marshall Georgi Zhukov announced to the world that Moscow would be capable of deploying biological in addition to chemical weapons in the next war. By 1960, there existed numerous research facilities addressing every aspect of biological warfare scattered across the Soviet Union [14].

The Soviet Union was an active participant in the World Health Organization’s smallpox eradication program, which ran from 1964 to 1979. Soviet physicians participating in the program sent specimens back to Soviet research facilities. For the Soviets, participation in the program presented an opportunity not only to rid the world of smallpox but also obtain, as source material for biological weapons development, virulent strains of smallpox virus that could be used subsequently for the more sinister purpose of releasing it as a weapon of war. In 1980 the World Health Organization announced the eradication of smallpox, and the world rejoiced at the elimination of a disease that had caused more human deaths than any other infection. However, the Soviets had another reason to celebrate: Elimination of natural disease meant that, over time, vaccination programs would terminate, and neither natural nor vaccine-acquired immunity would exist for the majority of the world’s population [2].

In 1969, President Richard Nixon announced unilateral disengagement in biological warfare research [12]. As mentioned previously, research came to an abrupt halt; production facilities and weapon stockpiles were destroyed. The 1972 Biological
Weapons Convention was signed by the Soviet Union. To the Soviets, this may have seemed like an excellent opportunity to obtain a significant advantage over its adversaries in the West. The Soviets even appear to have increased their efforts [2].

In October 1979, a Russian immigrant newspaper published in Frankfurt, Germany, published a sketchy report of a mysterious anthrax epidemic in the Russian city of Sverdlosk (now known as Yekaterinburg). The military were reported to have moved into the hospitals in Sverdlosk and taken control of the care of reportedly thousands of patients with a highly fatal form of anthrax. Suspicions emerged that there had been an accidental release of anthrax agent into an urban area near the Soviet military installation, Compound 17. The CIA asked the opinion of Harvard biologist, Dr. Matthew Meselson, in what turned out to be a poor choice of experts. He attempted to refute the Soviet weapon release theory—with all, he had been a strong proponent of the Nixon ban on the U.S. biological warfare program. More objective observers reviewing the same evidence have reached different conclusions. Furthermore, satellite imagery of Sverdlosk from the late spring of 1979 showed a flurry of activity at and around the Sverdlosk installation, which was consistent with a massive decontamination effort. The event did, however, raise enough concerns within the Reagan administration and the Department of Defense to seek better military biopreparedness [15].

Debate raged on for the next 12 years, with Meselson testifying before the Senate that the burden of evidence was that the anthrax outbreak was a result of the failure of the Soviets to keep anthrax-infected animals out of the civilian meat supply and not the consequence of an accident at a military weapons facility, as maintained by many U.S. officials. Meselson went on to say that in his opinion the 1972 Biological Weapons Convention had been a total success and that no nation possessed a stockpile of biological weapons. In June 1992, during a brief but open period of detente, Meselson was allowed to take a team of scientists to review autopsy material and other evidence from the Sverdlosk incident. Autopsy specimens for mediastinal tissue represented clear evidence to the team pathologist Dr. David Walker that the disease had been contracted from inhalation of anthrax spores, not from ingestion of tainted meat, as the Soviets had continued to allege. Meselson continued to insist that the evidence was not conclusive that this event was not a natural disease occurrence [15].

Previously, in private conversations with President George H. W. Bush, Russian leader Boris Yeltsin admitted that the KGB and military had lied about the anthrax deaths and that he would uncover the explanation. In the meantime, several Soviet defectors, including Ken Alibek, confirmed not only the Sverdlosk incident as an accidental release of weaponized anthrax but also the extensive nature of the Soviet biological weapons program [11]. Subsequently, in a press release, Yeltsin admitted to the offensive program and the true nature of the Sverdlosk biological weapons accident [15].

The Soviet biological weapons program had been extensive, comprising a range of institutions under different ministries, as well as the commercial facilities collectively known as Biopreparat. The Soviet Politburo had created Biopreparat to carry out offensive research, development, and production under the concealment of legitimate civil biotechnology research. Biopreparat conducted clandestine activities at 52 sites, employing over 50,000 people. Annualized production capacity for weaponized smallpox, for instance, was 90–100 tons [14].
Seth Carus from the National Defense University studied all biological agent use in the twentieth century and found 270 alleged cases involving illicit biological agents; of 180 cases of confirmed agent use, 27 were bioterrorism and 56 were biocrimes. In 97 situations, the purpose or intent of the perpetrator was unknown. Ten fatalities were caused by the criminal use of biological agent [5].

An example of state-sponsored bioterrorism occurred in 1978, when a Bulgarian exile named Georgi Markov was attacked in London with a device concealed in the mechanism of an umbrella. This weapon discharged a tiny pellet into the subcutaneous tissue of his leg. He died mysteriously several days later. At autopsy, the pellet was found; it had been drilled for filling with a toxic material. That material turned out to be ricin [9].

In 1995, Dr. Debra Green pleaded no contest to charges of murder and attempted murder. The murder charges stemmed from the deaths of two of her children in a fire for which she was thought to have been the arsonist. The attempted murder charges stemmed from the poisoning of her estranged spouse with ricin. Green was sentenced to life imprisonment [5].

Another example of criminal activity occurred in 1996, when Diane Thompson deliberately infected 12 coworkers with Shigella dysenteriae. She sent an e-mail to her coworkers, inviting them to partake of pastries she had left in the laboratory break room. Eight of the 12 hospital personnel who became ill tested positive for Shigella dysenteriae type 2, and one of the muffins also grew the same pathogen. During their investigation, police were to learn that a year before this incident, her boyfriend had suffered similar symptoms and had been hospitalized at the same hospital facility and that Thompson had falsified his laboratory test results. Thompson was sentenced to 20 years in prison [5].

The first episode of bioterrorism in the United States occurred in 1984. The Rajneeshee cult was founded by an Indian guru named Bhagwan Shree Rajneesh in the 1960s. Rajneesh was a master at manipulating people and was highly successful in attracting followers from the upper-middle classes and accumulating vast amounts of money from donations and proceeds from the sale of books and tapes. Because of the cult’s radical beliefs the ashram became unwelcome in Poona (now Pune), India. Rajneesh acquired the Big Muddy Ranch near The Dalles, Oregon. Here he built a community for his followers, named Rajneeshpuram, which became an incorporated community. Within a few years, the Rajneeshees came into conflict with the local population pertaining to land use and development. To take control of the situation, the Rajneeshees realized that they needed to control the Wasco County government. To accomplish this, they brought in thousands of homeless people from cities around the country through their share-a-home program, counting on their votes in the upcoming elections. The Rajneeshees also plotted to make the local population sick so that they would not participate in the election [5].

The first documented incident of the Rajneeshee use of biological agents involved provision of water contaminated with Salmonella typhimurium. Two of the Wasco County commissioners visiting Rajneeshpuram on August 29, 1984, consumed the contaminated water. Both became sick, and one required hospitalization. In trial runs in the months leading up to the November 1984 elections, several attempts at environmental, public water supply, and supermarket food contamination were
unsuccessful. In September 1984, the Rajneeshees began contaminating food products at local restaurants. A total of 10 restaurants suffered attacks involving pouring slurries of *S. typhinurium* into food products at salad bars, into salad dressing, and into coffee creamer. As a consequence of this attack, much of The Dalles community became sick—there were 751 documented cases of *S. typhimurium* infection, resulting in several hundred hospitalizations. Despite the success of the restaurant contamination, the Rajneeshee cult abandoned its efforts to take over Wasco County. No further attacks were conducted. Interestingly, the Centers for Disease Control and Prevention investigated the outbreak and concluded its cause was poor sanitation and hand-washing practices. Only a year later when several cult members defected and revealed the internal operations of the cult was the sinister nature and cause of the epidemic finally established [5].

In 1995, the Aum Shinrikyo Cult released sarin gas in the Tokyo subway system, resulting in 12 deaths and thousands of persons presenting for emergency medical care. The Aum Shinrikyo Cult, founded by Shoko Asahara, had grown into a massive organization with a membership of approximately 10,000 and financial assets of $300,000,000. Aum Shinrikyo mimicked the organization of the Japanese government, with “ministries and departments.” The department of “Health and Welfare” was headed by Seichi Endo, who had worked in genetic engineering at Kyoto University’s Viral Research Center. “Science and Technology” was headed by Hideo Murai, who had an advanced degree in astrophysics and had worked in research and development for Kobe Steel Corporation. Endo attempted to derive botulinum toxin from environmental isolates of *Clostridium botulinum* at the cult’s Mount Fuji property. There, a production facility was built and horses were stabled for the development of a horse sera antitoxin. It is uncertain whether Endo was able produce potent botulinum toxin successfully [5,15].

In 1993 Aum Shinrikyo built a new research facility on the eighth floor of an office building owned by the cult in eastern Tokyo. At this location, the cult grew *Bacillus anthracis* and installed a large industrial sprayer to disseminate the anthrax. The cult was also believed to have worked with *Coxiella burnetti* and poisonous mushrooms, and they sent a team to Zaire (now Democratic Republic of the Congo) in the midst of an Ebola epidemic to acquire the Ebola virus, which they claimed to have cultivated. According to press accounts from 1990 to 1995, the cult attempted to use aerosolized biological agents against nine targets—three with anthrax and six with botulinum toxin. In April 1990, the cult equipped three vehicles with sprayers targeting (with botulinum toxin) the Japan’s parliamentary Diet Building in central Tokyo, the city of Yokohama and the Yosuka U.S. Navy Base, and Nairta International Airport. In June 1993, the cult targeted the wedding of Japan’s Crown Prince by spraying botulinum toxin from a vehicle in downtown Tokyo. Later that month, the cult spread anthrax using the roof-mounted sprayer on the same eight-story office building used as their research and production facility. In July 1993, the cult targeted the Diet Building in central Tokyo again, this time with a truck spraying anthrax, and later the same month they targeted the Imperial Palace in Tokyo. On March 15, 1995, the cult planted three briefcases designed to release botulinum toxin in the Tokyo subway. None of these numerous attacks were successful; none are known to have produced any casualties from biological weapons. Ultimately, Aum Shinrikyo
gave up on its biological weapons program and released sarin in the Tokyo subway on March 20, 1995, with results that shocked and horrified the world [5].

Reasons given for the cult’s failure to produce effective biological attacks include use of a nontoxin-producing (or low-yield) strain of *C. botulinum*; use of a vaccine strain (low pathogenicity) of *Bacillus anthracis*; use of inappropriate spray equipment, on which nozzles clogged; and perhaps subversion by some cult members reluctant to follow through with the planned operation [5].

On October 4, 2001, just two weeks after the United States had been made dramatically aware of its vulnerability to international terrorism with the September 11 attacks on the World Trade Center and the Pentagon, health officials in Florida reported a case of pulmonary anthrax. During the first week of September, American Media, Inc. received a letter addressed to Jennifer Lopez, containing a fan letter and a “powdery substance.” The letter was passed among employees of American Media, Inc., including Robert Stevens. Retrospectively, investigators would consider that it was not this letter, but possibly a subsequent letter, that was the source of his infection [16]. Stevens was admitted to a Palm Beach, Florida, hospital with high fever and disorientation on October 2, 2001. By October 5, 2001, Robert Stevens was dead from inhalational anthrax—the first such case in the United States in over 20 years. An autopsy performed the following day revealed hemorrhagic pleural effusions and mediastinal necrosis. Soon other anthrax mailings and resultant infections became known, first at civilian news media operations in New York City, and then in the Congressional office buildings in Washington, DC, with concurrent contamination of U.S. postal facilities in the national capital area and Trenton, New Jersey [16].

At least five, and theoretically as many as seven, letters (four of which were recovered) containing anthrax spores had been mailed, perhaps in two mailings, on September 18 and the October 9, 2001. A total of 22 people were infected with anthrax, with 11 pulmonary cases resulting in five deaths. Issues of contamination and screening for anthrax exposures resulted in significant disruption of operations at the Congressional office building and U.S. postal facilities, not to mention millions of dollars spent in the cost of decontamination. Probably the most important issue and lesson learned, however, was related to the importance of effective and accurate communication about the nature of the threat and the response efforts to the public [16].

In 2003, at least four ricin-related incidents took place. On January 5, 2003, six Algerians, thought to be part of the Chechen network linked to Al Qaeda and Iraq, Ansar al-Islam, were arrested in North London by British security agencies. They were in the possession of ricin as well as castor seeds and equipment to make ricin. In March 2003, traces of ricin were found by the police in a locker at a railway station in Paris. On October 2003 a container with ricin was discovered at a postal facility in South Carolina, United States. A November 2003 disclosure confirms that traces of ricin were also found in mail bound for the White House [17].

In South Asia, Tamil rebel groups had threatened to use biological materials against the native Sinhalese in the early 1980s and resurfaced again in March 2008. The rebels threatened to spread bilhariasis and yellow fever in the country and allegedly laid out plans to attack rubber plantations and tea gardens using antiplant agents [17]. Feasibility of these threats are at best uncertain, but demonstrate that disparate
terrorist organizations contemplate biological agents, if only as threats, as potential weapons in their campaign of terror.

Al Qaeda’s Abdur Rauf, a Pakistani microbiologist, reportedly has searched Europe to obtain anthrax spores and equipment for Al Qaeda’s biological weapons laboratory in Afghanistan. Menad Benchellali, an Al Qaeda-trained terrorist, engaged in covert activities including weaponization of ricin in his biological and chemical laboratory in Lyon, France, before his arrest in 2004 [17]. This case represents a credible example of handling of biological agents in a small laboratory at the disposal of terrorists confirming the feasibility of Al Qaeda-sponsored bioterrorism.

The use of biological agents has increased dramatically in the last two decades, and the threat of bioterrorism took on dramatically new proportions after the anthrax mail attacks of September–October 2001. Groups with political objectives, religious groups, and apocalyptic cults have become important players in the world of terrorism [18]. Increasingly, these terrorist organizations have taken an interest in biologic agents. One of the more alarming recent trends has been the increased motivation of terrorist groups to inflict mass casualties [19]. The possibility of a major biological agent release as an act of terrorism resulting in massive casualties looms ever more likely, which is all the more reason that medical personnel, public health officials, and government agencies that deal with emergency response must be prepared for such an event.

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   2 3 4 5 -20 0 20 40 60 80 100 120 Heart rate Temperature Temperature Temperature
   -10 -2 3 4 5 6 Day Neurological signs Heart rate Neurological signs 1 2 3 4 5 6 Day
   postexposure -1 0 1 2 3 4 5 -20 0 20 40 60 80
   1 2 3 4 5 6 (a) (b) Temperature Heart rate

   Neurological signs

   FIGURE 12.7 Cynomolgus (a) and rhesus (b) macaques develop fever and encephalitis after aerosol exposure to western equine encephalitis virus (WEEV) (Adapted from Reed, D.S. et al., J. Infect Dis, 2005. 192(7): 1173-1182.). Macaques with telemetry implants for recording body temperature and heart rate were aerosol exposed to WEEV. Temperature and heart rate values shown are the average daily residual values obtained by subtracting predicted from actual values. Neurological signs were assessed daily by the principal investigator, technicians, and animal caretakers and were scored as follows: 5 = normal, 4 = depression,
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