

1

Potential Agents of Bioterrorism: Historical Perspective and an Overview

Nancy Khardori

1.1

Historical Perspective – How We Got Here

A quote from Hans Zinser, a bacteriologist and historian during the Great Depression in the United States, puts the concept of “terror associated with biological agents” in the best possible perspective [1]. He said “Infectious disease is one of the great tragedies of living things – the struggle for existence between different forms of life ... incessantly the pitiless war goes on, without quarter or armistice – a nationalism of species against species.” What he seemed to convey in this quote is the fact that mankind will never be able to completely protect itself against many of the biological agents coexisting in nature. The interaction between humans and disease-causing pathogens in nature is constant, with one or the other winning at all times and the course of human history has been altered frequently by the capability of infectious agents to spread and cross national borders.

The epidemics and pandemics of infectious diseases caused by communicable agents have swept unchecked across continents claiming more lives and creating more social devastation than wars. Examples include [2]:

1. diseases like smallpox, measles, plague, typhoid, and influenza causing 95 % of deaths in pre-Columbian native American populations;
2. the death of 25 million Europeans (a quarter of the population) caused by plague in the 14th century; and
3. more than 21 million deaths because of the influenza pandemic of 1918 and 1919.

Worldwide, naturally occurring infectious diseases remain the major causes of death. In the United States and Western Europe, the impact of several very virulent microbial agents and/or their toxins has been much reduced because of a very accessible health-care system and the public health infrastructure – although a substantial number of people (approximately 170,000) still die each year from

infectious diseases in the United States [3]. The travel and trade necessary for economic globalization, the continued potential for transmission of infectious agents from animals to humans, and large populations living and working in proximity in urban areas of the world enable infectious disease outbreaks to remain a major threat. Recent outbreaks of severe acute respiratory syndrome (SARS) and avian influenza are excellent examples. Until the discovery of preventive measures and anti-infective therapies, for example vaccines and antimicrobial agents, large disease outbreaks were even more common during war times. Infectious diseases caused far more deaths than battle injuries until World War II. Wars led to changes in both the host population of humans and animals and the pathogen population of infectious agents. Humans and animals became more susceptible to disease because of famine and malnutrition and the pathogens found new and vast breeding grounds in decaying organic matter including human and animal corpses. This resulted in pollution of scarce food and water supplies. In addition, vectors, the disease-transmitting agents, for example mosquitoes and flies, multiplied unchecked causing vector-borne diseases for which no preventive measures existed.

It is not surprising that a connection between “disease”, “contagion”, filth, and foul odor was made much before microbes were discovered. Human ingenuity made use of this association by the crude use of filth, cadavers, and human and animal carcasses as weapons [4]. These avenues of transmitting disease and devastation to armies and civilian populations have been used to contaminate wells, reservoirs, and other water sources since antiquity through the Napoleonic era and into the 20th century. As early as 300 BC, the Greeks polluted the wells and drinking water supplies of their enemies with animal corpses [5]. The same tactics were used later by the Romans and Persians. The bodies of dead soldiers and animals were used to pollute wells during a battle in Italy in 1155. Pollution (poisoning of potable water) was used as an effective and calculated method of gaining advantage in warfare throughout the Classical, Medieval and Renaissance periods. During the Middle Ages military leaders recognized that victims of disease (infections) could themselves become weapons [6]. Gabriel de Mussis, a notary, described how the plague-weakened Tartar forces catapulted victims of plague into the town of Kaffa in 1346 [7]. An epidemic of plague that followed forced a retreat of the Genoese forces. The population under siege may have been at an increased risk of epidemics because of deteriorating sanitation and hygiene. The imported disease continued to spread in Europe. In 1422 bodies of dead soldiers and 2000 cartloads of excrement were hurled into the ranks of the enemy at Carolstein. These two incidents contributed to the 25 million deaths in Europe in the 14th and 15th centuries during the Black Plague. Russian troops battling Swedish forces in Revat resorted to throwing plague victims over the city walls in 1710.

The use of smallpox victims and their fomites as weapons in the new world received similar notoriety. The indigenous people of Central and South America were decimated by measles and smallpox introduced to them by the Spanish conquistadors. They are said to have been presented with smallpox contaminated clothing in the 15th century [6, 8]. Smallpox-laden blankets were provided to the Indians during the French and Indian Wars (1754–1767). This adaptation of the

Trojan Horse use was followed by a smallpox epidemic among native American tribes in the Ohio River Valley. Smallpox epidemics in Native Americans after initial contact with Europeans had, however, been occurring for more than 200 years. Transmission of smallpox by means of respiratory droplets would have been much more efficient than use of fomites. Confederate General Joseph Johnson used the bodies of sheep and pigs in 1863 to pollute drinking water at Vicksburg during the US Civil War. These early attempts (14th to 18th century) at using biological materials to cause disease in the opponent have been referred to as biological warfare even though the nature of the biological agents in these materials was largely unknown. These early incidents also illustrate the complex nature of disease caused by biological agents. Naturally occurring endemic disease is very difficult to differentiate from that caused by deliberate spread of disease. Therefore the concept of “bioterror” should encompass in its spectrum:

1. naturally occurring infectious diseases;
2. acts of biological warfare; and
3. acts of biological terrorism against the civilians in peace and war time.

In any and all of these roles, biological agents have been, and will remain, potential tools of mass casualties.

1.2

Development of Modern Biological Weapons

Bacillus anthracis was the first specific biological agent attributed to human disease when Robert Koch confirmed his own “postulates” concerning this organism in 1877. The subsequent development of the science of bacteriology in the 19th century expanded the scope of biological agents as weapons of mass destruction. This occurred concomitantly with understanding of the pathogenicity of microbes, host–pathogen interactions, and advances in the prevention and treatment of infectious diseases. Modern microbiology intended primarily for diagnosis and treatment of infectious diseases also afforded the capability to isolate and produce stocks of specific pathogens. Germany developed an ambitious biological warfare program during World War I. Covert operations to infect livestock and contaminate animal feed to be exported to the allied forces were conducted in neutral trading partners [9]. *Bacillus anthracis* and *Burkholderia mallei*, causative agents of anthrax and glanders, respectively, were prepared for use to infect Romanian sheep for export to Russia. These cultures were identified at the Bucharest Institute of Bacteriology and Pathology after being confiscated from the German Legation in Romania in 1916. Between 1917 and 1918, livestock in Mesopotamia and Argentina intended for export to Allied Forces were infected with *B. anthracis* and *B. mallei*. During World War I the horror of chemical warfare clearly superceded the impact of biological agents. International diplomatic efforts were directed at limiting the proliferation and use of weapons of mass destruction culminating in the 1925 Geneva Protocol prohibiting the use in war of asphyxiation, poisons, or other gases

and of biological methods of warfare [10]. Many of the parties that ratified the Geneva Protocol began research programs to develop biological weapons after World War I. These included Belgium, Canada, France, Great Britain, Italy, the Netherlands, Poland, and the Soviet Union. The United States began an offensive biological program in 1942. Japan conducted twelve large-scale field trials of biological weapons during World War II. This operation was conducted largely under the auspices of Unit 731, a biological warfare research facility. Pathogens used in these experiments included *B. anthracis*, *Neisseria meningitidis*, *Shigella* spp. *Vibrio cholera*, and *Yersinia pestis* [11]. During the Japanese program between 1932 and 1945 an estimated 10,000 prisoners died as a result of experimental infection or execution after experimentation. Biological agents were used by Japan to attack 11 Chinese cities. The avenues used included contamination of water supplies and food items, tossing of cultures into homes, and spraying of cultures from aircraft. Pure cultures of *B. anthracis*, *V. cholerae*, *Shigella* spp., *Salmonella* spp., and *Y. pestis* were used. Japan was alleged to have used *Y. pestis* as a biological weapon by feeding laboratory bred fleas on plague-infected rats and releasing them over Chinese cities from aircraft. Large numbers of fleas, as many as 15 million, were used per attack to initiate plague epidemics. Rigorous epidemiological and bacteriological data from these experiments are not available. It is estimated that Japan killed 260,000 people in China with biological weapons, primarily plague. Japanese troops suffered approximately 10,000 biological casualties and 1700 deaths, mostly from cholera, in 1941 because they had not been adequately trained or equipped for the hazards of biological weapons. The success of the Japanese attacks attest to the simplicity and diversity with which biological agents can be used to cause death and devastation.

Although the German offensive biological weapons threat during World War II never materialized [12], experiments with *Rickettsia prowazekii*, *Rickettsia mooseri*, hepatitis A virus and *Plasmodia* spp. were conducted on Nazi concentration camp prisoners to study pathogenesis and to develop vaccines. As the Weil Felix Test using a cross-reaction immunological method (with *Proteus* OX19) became available, it was used by the German army to avoid areas with epidemic typhus. As a defense against deportation of people in occupied areas of Poland, physicians used *Proteus* OX-19 as a vaccine to induce false positivity for typhus. An example of biological weapons being used in a defensive role was created.

The allies developed biological weapon programs for potential retaliatory use in response to German biological attacks. Bomb experiments involving weaponized spores of *B. anthracis* conducted on Gruinard Island near the coast of Scotland, revealed the extensive longevity of viable anthrax spores in the environment. The island was decontaminated with formaldehyde and seawater during 1986 [13]. The United States offensive biological program was begun in 1942 under the direction of a civilian agency, the War Reserve Service [4]. The program weaponized lethal agents such as *B. anthracis*, Botulinum toxin, *Francisella tularensis*, and incapacitating agents such as *Brucella suis*, *Coxiella burnetii*, Staphylococcus enterotoxin B, and Venezuelan equine encephalitis virus. Anticrop agents such as rice blast, rye stem rust, and wheat stem rust were stockpiled but not weaponized. Cities like New York and San Francisco were surreptitiously used as laboratories to test aerosolization

and dispersal methods for simulants. An outbreak of urinary tract infection caused by *Serratia marcescens* occurred at Stanford University Hospital after covert experiments using *S. marcescens* as a stimulant. When the Washington Post reported these covert experiments much later (in 1976) public interest was aroused. The US program was expanded during the Korean War (1950–1953), but the US denied using biological weapons against North Korea and China. The US offensive biological weapons program was terminated after President Nixon's executive orders in 1969 and 1970. Three months later, he extended the ban to include toxins. The US Army Medical Research Institute for Infectious Disease (USAMRIID) at Fort Detrick, Maryland was established to conduct unclassified research on protection against potential agents of bioterrorism.

The origin of the Biological Weapons Program of the former Soviet Union dates back to the statements made by Lenin. Although experimental work was started in the nineteen-twenties, the modern era was ushered in only with the post World War II military building programs [14]. Despite the wide availability of technology for producing and weaponizing biological agents, the direct use of crude fomites against humans continued. One of the examples is the smearing of pungy sticks with excrement by the Vietcong in the early sixties [15]. In 1973 the Soviet Politburo formed the organization known most recently as the Biopreparat to conduct offensive biological weapons programs concealed behind civil biotechnology research [14]. In January 1991 the first ever visit to Biopreparat facilities was undertaken by a joint United Kingdom and United States technical team. By the mid nineteen-nineties substantial changes occurred within the Biopreparat and a concerted effort is in progress to help the Russians civilianize these former biological weapons research and development establishments. The current capability of the old Russian Ministry of Defense sites remains largely unknown. The status of one of Russia's largest and most sophisticated former bioweapons facilities called Vector in Koltsovo, Novosibirsk, is of concern. The facility housed the smallpox virus and work on Ebola, Marburg, and the hemorrhagic fever viruses (e.g. Machupo and Crimean-Congo) [16, 17]. A visit in 1997 found a half-empty facility protected by a handful of guards. No one is clear where the scientists have gone. Confidence is lacking that this is the only storage site for smallpox outside the Centers for Disease Control and Prevention.

Iraq's biological weapons program dates back to at least 1974, started after the Biological and Toxin Weapons Convention had been signed. In 1995, Iraq confirmed that it had produced and deployed bombs, rockets, and aircraft spray tanks containing *Bacillus anthracis* and botulinum toxin [18]. Unfortunately, the number of countries engaged in biological weapons experimentation grew from four in the nineteen-sixties to eleven in the nineties [19]. It is estimated that at least ten nations and possibly seventeen possess biological warfare agents [20]. Of the seven countries listed by the United States Department of State as sponsoring international terrorism, at least five are suspected of having biological warfare programs [21–23]. Nations and dissident groups have the access to skills needed to selectively cultivate some of the most dangerous pathogens and to deploy them as agents of biological terrorism and warfare.

As the technology for cultivating and transporting microorganisms became easier and cheaper, dissident groups and well-financed organizations used biological agents in attacks and threats to accomplish political goals [24, 25]. Some examples of these attempts between 1979 and 2001 are summarized in Table 1.1.

Tab. 1.1

Examples of political attempts at bioterrorism. (Adapted with minor modifications from Ref. [26].)

Year	Group	Attempt	Outcome
1970	Weather Underground	A. US revolutionary group intended to obtain agents from Fort Detrick by blackmail and to temporarily incapacitate US cities to demonstrate the “impotence of the federal government”	Report originated with a US Customs informant. The case later seemed to be apocryphal.
1972	R.I.S.E.	A group of college students influenced by ecoterrorist ideology and 1960s drug culture planned to use agents of typhoid fever, diphtheria, dysentery, and meningitis, initially to target the entire world population but later narrowed the plan to five cities near Chicago	The attack was aborted and cultures were discarded
1978	Unknown	Bulgarian defector Georgi Markov was assassinated in London when a spring-loaded device disguised in an umbrella was used to implant a ricin-filled pellet in his thigh.	A similar device used against a second defector in the same area was unsuccessful.
1979	Accidental	Accidental release of anthrax spores from a bioweapons facility in Sverdlovsk, Russia, caused an epidemic of inhalational anthrax.	At least 77 cases and 60 deaths.
1980	Red Army Faction	Members of a Marxist revolutionary ideology group allegedly cultivated botulinum toxin in a safe-house in Paris and planned attacks against at least nine German officials and civilian leaders	This was probably an erroneous report, later repudiated by the German government.
1984	Rajneeshee Cult	An Indian religious cult headed by Rajneeshee plotted to contaminate a restaurant salad bar in Dalles, Oregon, with <i>Salmonella typhimurium</i> . The motivation was to incapacitate voters, win local elections, and seize political control of the county.	The incident resulted in a large community outbreak of salmonellosis involving 751 patients and at least 45 hospitalizations. The plot was revealed when the cult collapsed and members turned informants.

Year	Group	Attempt	Outcome
1991	Minnesota Patriots Council	A right-wing "Patriot" movement obtained ricin extracted from castor beans by mail order. They planned to deliver ricin through the skin with dimethyl sulfoxide and aloe vera or as dry aerosol against Internal Revenue Service officials, US Deputy Marshals, and local law enforcement officials	The group was infiltrated by Federal Bureau of Investigation informants.
1995	Aum Shinrikyo	A new age doomsday cult seeking to establish a theocratic state in Japan attempted at least ten times to use anthrax spore, botulinum toxin, Q fever agent, and Ebola virus in aerosol form.	Multiple chemical weapon attacks with sarin, Vx, and hydrogen cyanide in Matsumoto, Tokyo, and assassination campaigns were conducted. All attempts to use biological weapons failed. The nerve gas sarin killed 12 and injured 5,500 in a Tokyo subway.
1997	Disgruntled employee in Texas	Intentional contamination of muffins and donuts with laboratory cultures of <i>Shigella dysenteriae</i> .	Caused gastroenteritis in 45 laboratory workers, four of whom were hospitalized.
2001	Unknown	Intentional dissemination of anthrax spores through the US Postal System led to the deaths of five people, infection of 22 others, and contamination of several government buildings.	Investigation into the attacks so far has not reached a conclusion.

Although most such events do not warrant national or international response and security, they can have substantial public health consequences and therefore require resources and preparedness at the local level. Active surveillance and rapid response at the local level are the cornerstones for preparedness against all types of bioterrorism – “think locally, act globally.”

Incidents involving intentional use of microbial agents by small groups or individuals with limited targets are highly likely but the public health consequences are far less. An example is the well publicized arrest on February 18, 1998 of Larry Wayne Harris, a microbiologist who allegedly threatened to release “military grade anthrax” in Las Vegas, Nevada. He had obtained the plague and veterinary vaccine strains of anthrax and reportedly isolated several other bacteria. He made vague threats against US officials on behalf of Christian identity and white supremacists groups. He was arrested when he talked openly about the use of biological agents in terrorist activities. The sensational media coverage appears, however, to have had the unintended effect popularizing anthrax as a potential agent of terrorism among potential perpetrators. The first wave of anthrax hoaxes

followed the report of this event. The ease with which he had obtained the cultures prompted new legislation to ensure legitimate medical and scientific purposes for transfer of biological agents.

1.3

Biological Weapons Systems

Acquisition, storage, and transport of biological weapons is much easier than for chemical and nuclear weapons. A biological weapons system comprises:

1. a payload – the biological material consisting of an infectious agent or a toxin produced by bacteria, plants, or animals;
2. munitions that carry and keep the pathogens virulent during delivery;
3. a delivery system, which can be a missile, a vehicle (aircraft, boat, automobile, or truck), an artillery shell, or even an expendable soldier or martyr or conventional mail;
4. a dispersion system that enables dissemination of the payload, in a virulent form, among the susceptible target population [27, 28].

The dispersion system can be aerosol sprays, explosives, and food and water [29]. Aerosol sprays are the most effective means of widespread dissemination and therefore would be the most likely. The factors that can alter the effectiveness of a given dispersion system include the particle size of the agent, stability of the agent under desiccating conditions and ultra violet light, wind speed, wind direction, and atmospheric stability. Optimum conditions and/or the use of hardy organisms would enable clouds of infectious material to travel several hundred kilometers and be delivered to the terminal airways when inhaled. The natural lag time provided by the organism's incubation period (3 to 7 days for most pathogens) would enable safe escape for terrorists before recognition of the attack. Because heat and physical stress inactivate biological activity, explosives are not very effective in disseminating infectious or toxic materials, although the explosion itself and the threat of biological weapons would still create panic, terror, and civil disruption. Effective contamination of large water supplies would usually require an unrealistically large amount of the biological agent. Potable water would be an ineffective dispersion system unless the agent is introduced into smaller reservoirs or into the water supply after it passes through the purification facility. Contamination of food immediately before consumption is easier and more effective in transmitting infectious agents. Unfortunately, an outbreak associated with intentional contamination of food may be recognized late because of difficulty differentiating it from a naturally occurring event. The use of the US Postal service to disseminate anthrax spores carried on pieces of mail has revealed the potential of novel delivery and dispersal systems. Direct delivery of biological agents as pellets and flechettes has also been used. Biological agents can also be used in combination with conventional weapons to create fear and panic, further increasing the potential of mass casualties.

A successful bioterrorism event depends on several factors. For the most optimum outcome:

1. The microbial agents used should have the specific characteristics required of a bioweapon [30, 31]:
 - Most importantly, they must be suitable for mass production, storage, and “weaponization”. Transforming microbial agents into bioweapons means they must be able to be packaged and distributed in a manner that disseminates them over a broad area without damaging the pathogenicity, and remain stable during dissemination. Covert release in an urban civilian setting may affect individuals in widely dispersed areas. Although they get the same illness, a common source of infection may not be considered early, because of use of different healthcare providers.
 - They should consistently produce the desired effects of disease and death. These outcomes would be magnified by the fact that both lethal and incapacitating agents would have an adverse impact on civilian health care delivery systems. In a military context, the incapacitation agents may better serve the perpetrator’s purpose because the unit will not be able to perform their mission and affected soldiers will consume scarce medical and evacuation resources.
 - They should be highly contagious and infective in low doses. The person-to-person or vector-borne transmission would further increase the number of people affected and enhance the mass casualty effect.
 - They should have a known short and predictable incubation time. This knowledge would favor the terrorists by giving them the lead time and make clinical diagnosis difficult because of multiple possibilities.
 - The disease caused by the agent(s) should be difficult to identify in the target population because of multiplicity of clinical presentation and overlap with common and/or endemic infections. Lack of or low persistence in the environment after delivery would add to the difficulty in determining a “point-source” origin of the disease.
2. The target population should be highly susceptible based on lack of natural or acquired immunity. The lack of herd immunity after infection would lead to ongoing infection as long as the pathogen is around. If no treatment or immunization is available or readily accessible, the disease burden and deaths will increase.
3. The aggressors should have means to protect or treat themselves and their own forces and populations. The presence of partial or full immunity to an agent in the aggressor’s population would also be favorable to them.

Biological weapons used in the form of aerosols are invisible, silent, odorless, tasteless and are dispersed relatively easily [32]. They cost 600 to 2000 times less than other weapons of mass destruction. It is estimated that approximately 0.05 % of the cost of a conventional weapon used for biological agents would produce similar numbers of mass casualties per square kilometer [28]. The economic

impact of a bioterrorism attack has been estimated to be from \$477.7 million per 100 000 persons exposed (brucellosis scenario) to \$26.2 billion per 100 000 persons exposed (anthrax scenario) [33].

1.4

Potential Bioterrorism Agents – Categorization and Prioritization

Many potential biological agents are capable of causing human disease. Although bioterrorism attacks could be caused by virtually any pathogenic microorganism, the list of agents that could cause mass casualties by the aerosol route of exposure is very small. Among the diseases caused by agents capable of being “weaponized” are some that are incapacitating while others cause mass casualties. Examples of the latter include anthrax, plague, and smallpox [26]. A North Atlantic Treaty Organization handbook dealing with biological warfare defense lists 39 agents including bacteria, viruses, rickettsia, and toxins as potential agents [34]. The relationship between aerosol infectivity and toxicity versus quantity of agent determine the potential for equivalent effects and narrows the spectrum of possible agents capable of causing mass casualties [23]. For example, only kilogram quantities of anthrax would be needed to cover a 100-km² area and cause 50% lethality compared with 8 metric tons of a “highly toxic” toxin such as ricin for similar results. The potential impact on a given area can be determined by the effectiveness of an aerosol in producing downward casualties. In a World Health Organization (WHO) model of a hypothetical dissemination of 50 kg of agent along a 2 km line upwind of a large population center, anthrax and tularemia were shown to cause the highest level of disease and death and the greatest downward spread. Before 1969 both the former Soviet Union and the United States spent years determining which pathogens and toxins had strategic and tactical capability. A working group organized by the Johns Hopkins Center for Civilian Biodefense evaluated potential bioterrorism agents to determine which present the greatest risk for a maximum credible event from a public health perspective. A maximum credible event would be one that could cause disruption, panic, and overwhelming of the civilian health-care resources in addition to large loss of life.

Several events in the nineteen-nineties led the US Government to re-embark on a civilian biodefense program [35]. Congress designated Centers for Disease Control and Prevention (CDC) as the lead agency to enhance the nation’s epidemiology and laboratory system. A national pharmaceutical stockpile was also established to assist the National Disaster Medical System to manage mass casualties. In addition to its traditional partners (i.e. local and state health departments and laboratories), CDC added the Department of Defense and law-enforcement agencies as its new partners. A Bioterrorism Preparedness and Response Office was established. For initial preparedness five areas were targeted:

1. planning;
2. improved surveillance and epidemiological capabilities;

3. rapid laboratory diagnostics;
4. enhanced communications; and
5. medical therapeutic stockpiling [36, 37].

The biological agents toward which the efforts should be targeted needed to be formally identified and prioritized. A meeting of national experts including academic infectious diseases experts, national public health experts, Department of Health and Human Services representatives, civilian and military intelligence experts and law enforcement officials was convened in June, 1999. Under review were lists of previously identified biological threat agents and potential general criteria for selecting the biological agents that pose the greatest threat to civilians. The lists of potential biological threat agents reviewed included the Select Agent Rule List, Australian Group List for Biological Agents for Export Control, unclassified military list of biological warfare agents, Biological Weapons Convention List and the WHO Biological Weapons List. The general criteria used were:

1. public health impact based on illness and death;
2. delivery potential to large populations based on ability to mass produce and distribute an agent, its stability and potential for person-to-person transmission;
3. public perception of the disease caused by the agent as related to fear and potential civil disruption; and
4. special public health preparedness needs pertaining to stockpiling requirements, diagnostic needs and enhanced surveillance.

Discussions were held to identify agents that were felt to have the potential for high impact based on subjective assessment in these four general categories. After the meeting, CDC personnel tried to identify objective indicators in each category that could be used to further define and prioritize the identified high-impact agents. Rating schemes were used to evaluate agents in each of the general areas according to objective criteria. A risk-matrix analysis process was used to evaluate and categorize potential biological threat agents [37]. The agents were placed in one of three priority categories (A, B, or C) for initial public health preparedness efforts (Table 1.2).

Category A, highest priority agents, include organisms that pose a risk to national security because they:

1. can be easily disseminated or transmitted person-to-person;
2. cause high mortality with potential for major public health impact;
3. might cause public panic and social disruption; and
4. require special action for public health preparedness.

The bacteria, viruses, and toxins listed in CDC Category B are the second highest priority agents; these:

1. are moderately easy to disseminate;
2. cause moderate morbidity and low mortality; and
3. require specific enhancement of CDC's diagnostic capacity and enhanced disease surveillance.

Tab. 1.2

Categorization and prioritization of potential agents of bioterrorism (Adapted with minor modifications from Ref. [26].)

Critical biological agents Category A	Category B	Category C
<i>Variola major</i> (smallpox)	<i>Coxiella burnetii</i> (Q fever)	Nipah virus
<i>Bacillus anthracis</i> (anthrax)	<i>Brucella</i> spp. (brucellosis)	Hantaviruses
<i>Yersinia pestis</i> (plague)	<i>Burkholderia mallei</i> (glanders)	Tickborne hemorrhagic fever viruses
<i>Clostridium botulinum toxin</i> (botulism)	<i>Burkholderia pseudomallei</i> (melioidosis)	Tickborne encephalitis viruses
<i>Francisella tularensis</i> (tularemia)	Alpha viruses Venezuelan encephalomyelitis Eastern and Western equine encephalomyelitis	Yellow fever
Filoviruses Ebola hemorrhagic fever Marburg hemorrhagic fever	Ricin toxin from <i>Ricinus communis</i> (castor beans)	Multidrug-resistant <i>Mycobacterium tuberculosis</i>
Arenaviruses Lassa (Lassa fever) Junin (Argentine hemorrhagic fever) Related viruses	<i>Clostridium perfringens</i> ε toxin Staphylococcal enterotoxin B T2 mycotoxins Food or waterborne pathogens, including but not limited to: <i>Salmonella</i> species <i>Shigella</i> species <i>Escherichia coli</i> 0157:H7 <i>Vibrio cholerae</i> <i>Cryptosporidium parvum</i> <i>Rickettsia prowazekii</i> (typhus fever) <i>Chlamydia psittaci</i> (psittacosis)	

Emerging pathogens that could be engineered for mass dissemination are included in the third highest priority Category C. These are:

1. readily available;
2. can be produced and disseminated easily; and
3. have potential for high morbidity and mortality and major health impact.

Ongoing disease surveillance and outbreak response activities are critical to the recognition of diseases caused by emerging pathogens.

In the critical biological agents list, no priority is assigned within the categories. This list does not rank the probability of deliberate use of an agent. Such risk assessments can only be made by intelligence and law enforcement agencies. Although there are severe limitations in predicting the actions of terrorists, risk assessment is critical to balancing preparedness against overreaction.

All Category A critical biological agents will be discussed in Chapters 4–9. A summary is provided in Table 1.3. The overview in this chapter will discuss salient features of the diseases caused by Category B and C agents.

Tab. 1.3
Summary of Category A critical biological agents.

Disease	Agent (type)	Incubation period (mortality without treatment)	Transmission ^{a]}	Disease type	Prevention in humans	Treatment
Smallpox ^[a]	<i>Variola major</i> (virus)	7–10 days (high)	Direct contact Body fluids Fomites	Rash systemic	Vaccine stringent infection control	None (experimen- tal agents)
Anthrax	<i>Bacillus anthracis</i> (Bacteria)	1–5 days (high)	Contact – spore Aerosol – spore Contaminated meat	Cutaneous inhalational gastrointestinal meningeal	Vaccine antibiotics	Antibiotics
Plague ^[a]	<i>Yersinia pestis</i> (Bacteria)	1–6 days (high)	Injection – Flea vec- tor Aerosol-droplets	Bubonic pneumonic septicemic	Antibiotics vector pro- tection stringent in- fection con- trol	Antibiotics
Botulism	<i>Clostridium botulinum</i> toxin (Bacterial toxin)	6 h–14 days (high)	Ingestion – food/ water Aerosol – toxin	Neurological infantile	None	Antitoxin supportive care
Tularemia	<i>Francisella tularensis</i> (Bacteria)	3–14 days (moderate)	Injection – tick vector Aerosol – bacteria Ingestion food/water	Ultero-glandu- lar systemic	Vaccine vector pro- tection	Antibiotics
Viral Hemorrhagic Fevers ^[a]	Filoviruses Arenaviruses (viruses)	2–21 days (high)	Body fluids Possible animal res- ervoir	Hemorrhagic septicemic	Stringent in- fection con- trol	None

a Person-to-person transmission occurs

1.5

Category B – Bacterial/Rickettsial Agents of Bioterrorism

1.5.1

Brucellosis

Also called undulant fever, Mediterranean fever, or Malta fever.

1.5.1.1 Epidemiology and Microbiology

Brucellosis is a zoonotic disease caused by infection with *Brucella*, a group of facultative intracellular Gram-negative coccobacilli [27, 38]. *Brucella* is now regarded as a monospecific genus, that should be termed *B. melitensis*, and other species are subtypes [39]. The natural reservoir is herbivores like goats, sheep, cattle, and pigs. Four subtypes, *B. melitensis* (goat), *B. abortus* (cattle), *B. suis* (pig), and *B. canis* (dog) are pathogenic in humans. Human infection occurs by ingestion of raw infected meat or milk, inhalation of contaminated aerosols, or through skin contact. Brucellosis is highly infective by the aerosol route, with as few as 10–100 bacteria sufficient to cause disease in humans. *Brucella* sp. are stable to environmental conditions and there is long persistence in wet ground or food. These features favor their use as potential agents of bioterrorism. The disease is relatively prolonged, incapacitating, and disabling in its natural form. Intentional large aerosol doses may shorten the incubation period and increase the clinical attack rate. Mortality rate (5% of untreated cases) is low, however, with rare deaths caused by endocarditis or meningitis. *Brucella suis* became the first agent weaponized by the United States at Pine Bluff Arsenal in 1954, when its biological weapons program was active. Human brucellosis is an uncommon disease in the US. The annual incidence is 0.5 cases per 100,000 population. Most cases occur in abattoir and veterinary workers or are associated with the ingestion of unpasteurized dairy products. The disease is usually seen in Hispanic population and may be related to the illegally imported unpasteurized dairy products from Mexico, where the disease is endemic. The disease is still endemic in many parts of the world (128 cases per 100,000 population in some areas of Kuwait), which makes it a hazard to military personnel in those areas.

1.5.1.2 Diagnosis

The usual incubation period is 8–14 days but may be longer. Brucellosis presents as a nonspecific febrile illness with headache, fatigue, myalgias, chills, sweats, and cough. Lumbar pain and tenderness can occur in up to 60% of cases. Gastrointestinal (GI) symptoms – anorexia, nausea, vomiting, diarrhea and constipation – occur in up to 70% of adult cases, less frequently in children. Hepatosplenomegaly is seen in 45–63% of cases. The significant sequelae include a variety of osteoarticular infections of the axial skeleton, peripheral neuropathy, meningovascular syndrome, optic neuritis, infective endocarditis, anemia, thrombocytopenia, and leukopenia.

Blood cultures are positive in 15–70% of cases and bone marrow cultures in 92% of cases during the acute febrile phase of the illness. A biphasic culture method (Castaneda bottle) may improve the isolation rate from blood. Because it may take longer to grow *Brucella* species, the laboratory must be notified to extend the standard incubation time of 5–7 days. If a community laboratory (Level A) observes tiny, faintly staining Gram-negative coccobacilli with slow-growing, oxidase-positive colonies on sheep blood, all plates and bottles should be placed in a biological safety cabinet. They should be appropriately packaged and shipped to a Level B or C laboratory. Confirmation in these laboratories can be done by biochemical, slide agglutination, or phage lysis tests [30].

The diagnosis of brucellosis is frequently made by serological tests. Acute and convalescent phase serum should be collected 3–4 weeks apart. A serum agglutination test (SAT) is available to detect both IgM and IgG antibodies. A titer of 1:160 or greater in a single specimen is considered indicative of active disease. ELISA and PCR methods are becoming more widely available.

1.5.1.3 Management

The United States military recommends doxycycline (100 mg Q12 h) plus rifampin (900 mg a day) for six weeks. Doxycycline for six weeks plus streptomycin for 2–3 weeks is an acceptable alternative. TMP/SMX for 4–6 weeks is less effective. Long-term therapy with a combination of a tetracycline, rifampin and an aminoglycoside is recommended for patients with meningoencephalitis or endocarditis. Valve replacement and surgical intervention for other forms of localized disease may be needed. Chemoprophylaxis is not generally recommended. For a high risk exposure to veterinary vaccine, inadvertent exposure in a laboratory, or exposure as a result of biological warfare, a 3–6 week course of therapy with the agents used for treatment should be considered for prophylaxis.

Live animal vaccines are used widely and have eliminated brucellosis from most domestic animal herds in the US. No licensed human vaccine is available in the US. A variant of *Brucella abortus*, S19-BA has been used in the former USSR. Efficacy is limited and annual revaccination is needed. A similar vaccine is available in China. Because brucellosis is not usually transmissible from person to person, standard precautions are adequate in managing patients. BSL-3 practices should be used for handling suspected *Brucella* cultures in the laboratory because of the danger of inhalation.

1.5.2

Glanders and Melioidosis

1.5.2.1 Epidemiology and Microbiology

Glanders and melioidosis are caused by *Burkholderia mallei* and *Burkholderia pseudomallei* respectively [38, 40, 41]. Both are Gram-negative bacilli with a “safety pin” appearance on microscopic examination. *Burkholderia mallei*, the causative

agent of glanders, produces disease primarily in horses, mules, and donkeys. Human disease is uncommon despite frequent and/or close contact with infected animals. Low concentrations of the organism and less virulence for humans may be the factors responsible. The acute forms of the disease occur in mules and donkeys resulting in death in 3–4 weeks. The chronic form of the disease is more common in horses with lymphadenopathy, multiple skin nodules that ulcerate and drain, along with induration, enlargement and nodularity of regional lymphatics. The later presentation is called “farcy.” Human cases occur primarily in veterinarians and animal handlers. Infection is acquired from inhalation or contaminated injuries. *B. pseudomallei*, the causative agent of melioidosis is widely distributed in many tropical and subtropical regions. It is endemic in Southeast Asia and Northern Australia. Humans get infected by inhalation or contact with mucous membranes and skin. Melioidosis is one of the most common causes of community acquired septicemia in Northeastern Thailand. This is a hazard to military personnel in those areas. In humans the disease ranges from a subclinical infection to overwhelming septicemia with 90% mortality rate within 24–48 h. Chronic and life-threatening illness can also occur from reactivation of primary illness.

Aerosols from cultures of *B. mallei* and *B. pseudomallei* are highly infectious to laboratory workers making aerosol spread an efficient way of dissemination. A case of glanders in a military research microbiologist was reported recently [42]. Both of these organisms have been viewed as potential biological warfare agents.

During World War I, glanders was spread deliberately by German agents to infect large numbers of Russian horses and mules. This led to an increase in human cases in Russia after World War I. The Japanese infected horses, civilians, and prisoners of war with *B. mallei* at the Pin Fang (China) Institute during World War II. The United States studied both agents as possible biological weapons in 1943–1944 but did not weaponize them. The former Soviet Union is believed to have experimented with *B. mallei* and *B. pseudomallei* as bioweapons.

1.5.2.2 Diagnosis

The incubation period is 10–14 days. In the acute forms, both glanders and melioidosis can present as an acute pulmonary infection or as an acute fulminant, rapidly fatal septicemic illness. These are the forms that would be expected if they are used as bioweapons. Acute infection of the oral, nasal and conjunctival mucosa can cause bloody nasal discharge with septal and turbinate nodules and ulcerations. Systemic invasion can cause a papular or pustular rash that can be mistaken for smallpox, and hepatic, splenic and pulmonary abscesses. Acute forms of the diseases carry a high mortality rate. The chronic form is characterized by cutaneous and intramuscular abscesses on the legs and arms. Osteomyelitis, meningitis, and brain abscesses have also been reported.

Gram stain of the exudates show Gram-negative bacteria with bipolar staining. They stain irregularly with methylene blue or Wright’s stain. The organisms can be cultured and identified with standard bacteriological methods. The cultures

should be maintained under BSL-3 conditions. Agglutination and complement fixation tests are available for serological diagnosis of *B. mallei*. Complement fixation tests are more specific and regarded as positive if the titer exceeds 1:20. For *B. pseudomallei*, a single titer above 1:160 with a compatible illness suggests active infection.

1.5.2.3 Management

For localized disease, oral therapy with amoxicillin/clavulanate, tetracycline or TMP/SMX for 60–150 days is recommended. For severe diseases, parenteral therapy with ceftazidime, imipenem or meropenem, plus TMP/SMX for two weeks then oral therapy for six months is recommended. There are no data on post-exposure chemoprophylaxis which may be tried with TMP/SMX, doxycycline, or ciprofloxacin. No vaccine is available for human use. Standard precautions should be used for infection-control purposes. For patients with skin involvement, contact precautions are indicated.

1.5.3

Psittacosis

1.5.3.1 Epidemiology

Psittacosis, caused by *Chlamydophila psittaci*, is a systemic infection with pneumonia as a frequent presentation [43]. It is common in birds and animals. All birds have the potential to spread the disease – ornithosis. However, the name psittacosis has persisted since its association with parrots was described in Greece in 1892. The carriage rate in bird populations is 5% to 8% and bird nesting can shed the organisms during periods of both illness and health. Most human cases of psittacosis, both outbreaks and sporadic cases, occur as a result of contact with a bird (usually a pet). Turkey-associated psittacosis has the highest attack rate in epidemics. Psittacosis is the most common abattoir-associated pneumonia. The infection is transmitted by respiratory route either by aerosolization of infected discharges or by direct contact.

1.5.3.2 Diagnosis

The incubation period for psittacosis is 5–15 days; this is followed by nonspecific symptoms. The illness may resemble a nonspecific viral illness or present as typhoidal and pulmonary syndromes. Atypical pneumonia is the presentation most suggestive of disease caused by *C. psittaci*, which occasionally progresses to acute respiratory distress syndrome. Less common manifestations includes pericarditis, endocarditis, myocarditis, hepatitis, pancreatitis, and thyroiditis. Because the natural route of infection is aerosol, and lungs are the most commonly affected organ from tissue, it would be very difficult to differentiate natural disease from an act of bioterrorism in the early stages.

Diagnosis by culturing the organism from respiratory secretion is possible but hazardous to laboratory workers. Serological diagnosis is made by microimmunofluorescence (MIF) by an IgM or IgA titer of 1:16 or by fourfold increase in samples drawn two weeks apart by MIF or complement fixation assay. MIF has higher sensitivity and specificity. Polymerase chain reaction (PCR) with the capability to distinguish *C. psittaci* from other Chlamydia is not routinely available.

1.5.3.3 Management

The antibiotic treatment of choice is tetracycline or doxycycline for 10–21 days. Erythromycin is a less efficacious alternate treatment. Azithromycin, chloramphenicol, and newer quinolones have been reported to have activity *in vitro* and in animals. The US Department of Agriculture (USDA) requires that imported birds be quarantined for 30 days and treated with tetracycline for at least 45 days. These requirements are aimed at preventing the introduction of Newcastle Disease and shedding of *C. psittaci* by the birds.

Standard precautions for patient care are recommended, because human-to-human and nosocomial transmission is rare. Antibiotic prophylaxis of contacts in the natural disease setting is not considered necessary. Because the organism is resistant to drying and can remain viable for months at room temperature, environmental sanitation is important.

1.5.4

Q Fever

First described in Australia in 1935 and called query fever because the causative agent was unknown.

1.5.4.1 Epidemiology and Microbiology

Q fever is caused by Rickettsia, *Coxiella burnetii* is a world wide zoonosis [27, 38, 44]. The most common reservoirs are cattle, sheep, and goats. Other natural reservoirs are dogs, cats, and birds. The infected animals do not develop the disease but shed large numbers of organisms in body fluids (milk, urine, and feces) and especially large numbers in the placenta. Humans acquire the disease by inhalation of contaminated aerosol. Q fever as a febrile illness with an atypical pneumonia can resemble mycoplasma, legionnaire's disease, *Chlamydia pneumoniae*, psittacosis, and hantavirus infection. More rapidly progressive cases may resemble tularemia or plague. The organism is resistant to heat and desiccation and highly infectious by the aerosol route. A single inhaled organism is capable of producing clinical illness. *C. burnetii* has the potential to be used as an incapacitating biological warfare agent and the disease would be similar to that occurring naturally.

1.5.4.2 Diagnosis

The incubation period is 7–21 days, varies according to the number of organisms inhaled. The disease presents as a nonspecific acute febrile illness with headaches, fatigue, and myalgias. Pneumonia, manifested by abnormal chest X-ray occurs in 50% of patients and acute hepatitis develops in 30–60% of patients. Culture negative endocarditis (fewer than 5% of acute cases), chronic hepatitis, aseptic meningitis, encephalitis, and osteomyelitis are uncommon complications of Q fever.

Isolation of the organism is difficult. *Coxiella* grows in living cells only and cell cultures should be performed under BSL-3 precautions. Antibody assays (IFA and ELISA and complement fixation tests) are available at reference laboratories. IgM antibodies may be detected by ELISA as early as the second week of illness and are diagnostic. The complement fixation test, the most commonly available serological test, is relatively insensitive.

1.5.4.3 Management

All suspected cases of Q fever should be treated to reduce the risk of complications. Tetracycline or doxycycline or erythromycin for 14 days are the treatments of choice for acute Q fever. Azithromycin and clarithromycin would be expected to be effective, although they have not been tested. Ciprofloxacin and other quinolones are active *in vitro* and should be used in patients unable to take the other agents. For endocarditis, tetracycline or doxycycline given in combination with TMP/SMX or rifampin for 12 months or longer has occasionally been successful. Valve replacement is often required for a cure.

Chemoprophylaxis with tetracycline or doxycycline for 5–7 days is effective if started 8–12 days post exposure. If given immediately (1–7 days) after exposure, however, chemoprophylaxis is not effective and may only prolong the onset of disease.

A formalin-inactivated whole cell vaccine is licensed in Australia and available for at-risk personnel on an investigational basis in the US. A single dose provides complete protection against naturally occurring Q fever and greater than 95% protection against aerosol exposure within 3 weeks. Protection lasts for at least 5 years. The vaccine may cause local induration, sterile abscess, and even necrosis at the inoculation site in immune individuals. An intradermal skin test using 0.02 mg vaccine is required to detect presensitized or immune individuals. A live attenuated vaccine (strain M44) has been used in the former USSR. There is no person-to-person transmission of Q fever. Standard precautions are recommended for health-care workers taking care of patients with suspicion or diagnosis of Q fever.

1.5.5

Typhus Fever

Rickettsia prowazekii can cause devastating naturally occurring epidemics of louse-borne typhus [45]. Epidemics are associated with conditions of war, poverty, natural

disasters, and lack of hygiene. Typhus has affected the outcome of many wars from the fifteen-hundreds to the 19th century. During World War II, Germany conducted experiments with *R. prowazekii* on Nazi concentration camp prisoners to study pathogenesis and to develop vaccines. *R. prowazekii* is transmitted between patients by the human body louse (*Pediculus humanus corporis*). This vector is strictly adapted to humans, lives in the clothes and becomes infected while taking a blood meal from Rickettsemic patients. *R. prowazekii* is excreted in the louse feces, deposited on the skin or mucous membrane and introduced by scratching the skin or rubbing the mucous membranes. Latent infection and reactivation of typhus with the potential to start another epidemic can occur. Typhus is endemic in the Peruvian Andes, Burundi, and Rwanda. Recent cases have been reported in Russia, Algeria, Senegal, and France. Southern flying squirrels which are distributed from Florida to Maine and westward to Minnesota and east Texas are an extra reservoir of *R. prowazekii*. Infection can be transmitted to humans by flying squirrel fleas and by exposure to the feces of the fleas or squirrel species of lice.

1.5.5.1 Diagnosis

The incubation period is 8–16 days. After a prodrome of 2 days, rash and fever lasting for 10–12 days occur in about 80% of patients. Severe muscle pains, rigors, malaise, and severe headaches are a part of the clinical picture. Before the availability of antibiotics the course was characterized by hemorrhagic rash, delirium, severe cough, gangrene, coma, and death in 13% of patients. In recent outbreaks in Ethiopia and Burundi the fatality rate was lower because of effective antimicrobial therapy.

Because of overlap of symptoms and signs with many other illnesses, the diagnosis of louse-borne typhus early in an outbreak is challenging. The same would be true for aerosol-transmitted typhus in a bioterrorism event. Epidemiological clues should raise the index of suspicion.

Laboratory methods such as PCR and immunohistochemical detection of *R. prowazekii* in blood and tissue can be used to diagnose the disease during the acute stage. PCR can detect the organism in lice also. *Rickettsiae* can be isolated from blood or tissue in shell vial cell cultures. These diagnostic capabilities are available in reference laboratories only.

Generally, laboratory diagnosis of louse-borne typhus is made retrospectively by serological methods. The methods available include indirect immunofluorescence assay and enzyme immunoassays. An IgM titer of 1:32 and IgG titer of 1:128 confirm the diagnosis. A cross-reacting serological test (Weil–Felix reaction) using *Proteus vulgaris* OX-19 agglutination has poor sensitivity and specificity. This may be the only method available in many parts of the world where typhus is endemic or likely.

1.5.5.2 Management

The antibiotic treatment of choice is doxycycline for 7 to 10 days. Chloramphenicol and tetracycline are also effective. New macrolides, fluoroquinolones, and rifampin

have been reported to inhibit the growth of *R. prowazekii* in cell culture. Clinical efficacy has not been proven and treatment failures have been reported with quinolones. The mainstay of prevention of epidemic typhus is the control of body lice. Regular washing of all clothes in hot water stops the outbreak. Insecticides such as lindane powder are useful in delousing. Lice can also be killed by application of 1% permethrin dusting powder to the clothing and bedding every 6 weeks. No vaccine is currently available for prevention of louse-borne typhus.

1.5.6

Food and Water Safety Threats

Food and water borne pathogens as potential agents of bioterrorism include, but are not limited to, the subset in Table 1.4 [46]. “Poisoning” of potable water was used as an effective and calculated method of gaining advantage in warfare throughout the Classical, Medieval, and Renaissance periods. Drinking water supplies of the enemies were polluted with human and animal corpses. During World War I Germany used covert operations to infect livestock and contaminate animal feed to be exported to the allied forces. These operations were conducted via neutral trading partners. The organisms used were *B. anthracis* and *B. mallei*. During biological agent attack on eleven Chinese cities (1932–1945), the Japanese contaminated water supplies and food items. Pure cultures of *Salmonella* sp., *Shigella* sp. and *Vibrio cholera* were used. Under current conditions of drinking water treatment and safety, potable water would be an ineffective dispersion system unless the cultures were introduced into smaller reservoirs or into the water supply after it has passed through the purification facility. It is easier and more effective to transmit infectious agents by contaminating food. Such intentional food-borne outbreaks would be difficult to differentiate from naturally occurring events. The spectrum of organisms and of foods causing food-borne disease has expanded in recent years. Many food-borne pathogens, for example salmonella and campylobacter, have become resistant to commonly used antimicrobial agents. In the outbreaks reported to the CDC between 1972 and 2000, the first ten causes of water-borne outbreaks were *Giardia lamblia*, *Shigella* sps., hepatitis A, norovirus, *Campylobacter jejuni*, *Cryptosporidium parvum*, *Salmonella* sps., *E. coli* 0157-H7, *Salmonella typhi*, and *Vibrio cholerae*. The ten most common microbial causes of food-borne outbreaks were *Salmonella* sps, *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium botulinum*, *Shigella* sps, *Escherichia coli*, *Campylobacter jejuni*, *Bacillus cereus*, *Vibrio parahaemolyticus*, and *Listeria monocytogenes*. There is a significant overlap among water and food-borne pathogens. The evaluation of an outbreak suspected to be food-borne may reveal water to be the vehicle. The diagnosis of food-borne disease should be suspected when two or more persons who have shared a meal during the previous week present with an acute illness with gastrointestinal or neurological manifestations. Important clues to the causative agent are provided by the symptom complexes, the incubation period, the type of food probably responsible, and the setting in which it is consumed. The CDC’s

Foodborne Diseases Active Surveillance Network (FoodNet) conducts surveillance for major food-borne pathogens in several states. Prompt reporting by healthcare providers to public health authorities is critical to initiating preventive action including secondary spread of the disease. Because clusters of food-borne disease may arise in geographically different areas in a potential bioterrorism event, intentional contamination of food should be suspected in such instances.

Tab. 1.4
Category B bioterrorism agents.

Bacteria	Viruses	Toxins
1. <i>Brucella</i> species (brucellosis)	Alpha viruses	1. Enterotoxin B (<i>Staphylococcus aureus</i>)
2. <i>Burkholderia mallei</i> (glanders)	– Venezuelan encephalomyelitis	2. Epsilon toxin (<i>Clostridium perfringens</i>)
3. <i>Burkholderia pseudomallei</i> (melioidosis)	– Eastern equine encephalomyelitis	3. Ricin toxin (<i>Ricinus communis</i>)
4. <i>Chlamydia psittaci</i> (psittacosis)	– Western equine encephalomyelitis	4. T2-Mycotoxins ^[a]
5. <i>Coxiella burnetii</i> (Q fever)		
6. <i>Rickettsia prowazekii</i> (typhus fever)		
Food safety threats <i>Salmonella</i> species <i>Shigella</i> species/dysenteriae <i>Escherichia coli</i> O157:H7		Water safety threats <i>Vibrio cholerae</i> <i>Cryptosporidium parvum</i>

a Not listed under CDC Category B agents

1.6 Category B – Viral Agents of Bioterrorism

1.6.1

Alphavirus Encephalomyelitis

1.6.1.1 Epidemiology and Diagnosis

Mosquito-borne alpha viruses cause Venezuelan equine encephalitis (VEE), western equine encephalitis (WEE) virus, and eastern equine encephalitis (EEE) [27, 38, 47]. They are similar, share many aspects of epidemiology and transmission, and are often difficult to distinguish clinically. Natural infections are acquired as a result of bites by a wide variety of mosquitoes. In natural epidemics severe and often fatal encephalitis in horses, mules, and donkeys precedes human cases. In a

biological warfare attack with the virus disseminated as an aerosol, human disease would be a primary event or occur simultaneously with that in equidae. The human infective dose of VEE is 10–100 organisms. VEE is a febrile, relatively mild incapacitating illness. Encephalitis develops in a small percentage of patients. EEE and WEE viruses cause encephalitis predominately.

1.6.1.2 Management

No specific therapy is available. Alpha-interferon and the interferon inducer poly-ICLC have proven highly effective as post-exposure prophylaxis in experimental animals.

A live attenuated vaccine is available as an investigational new drug. A formalin inactivated vaccine is available for boosting antibody titers in those initially receiving the live attenuated vaccine. The viruses can be destroyed by heat (80°C for 30 min) and standard disinfection. There is no evidence of human-to-human or horse-to-human transmission. Standard precautions and vector control while the patient is febrile are adequate hospital infection control procedures.

1.7

Category B – Biological Toxins for Bioterrorism

1.7.1

Enterotoxin B

Staphylococcus aureus produces several exotoxins, some of which normally exert their effect on the GI tract and are called enterotoxins [27, 38]. These toxins are proteins with a molecular weight of 23,000–29,000 kilodaltons. They are also called pyrogenic toxins because they cause fever. Staphylococcus enterotoxin B (SEB) is a pyrogenic toxin that commonly causes food poisoning originating from improperly handled or improperly refrigerated food. The effect of the inhaled SEB is markedly different. Symptoms occur at a very low inhaled dose (less than one-hundredth of the dose causing GI symptoms). The disease begins rapidly 1–12 h after ingestion with sudden onset of fever, chills, headache, myalgia, and a nonproductive cough. Pulmonary edema occurs in severe cases. GI symptoms can occur concomitantly, because of inadvertent swallowing of the toxin after inhalation. The toxin can also be used to contaminate food or small volume water supplies. SEB was one of the seven biological agents the US bioweapons program possessed before its termination in 1969.

There is no specific therapy available. Experimental immunization has been reported. No human vaccine is available. A candidate vaccine is in advanced development. Secondary aerosols are not a hazard and SEB does not pass through intact skin. Standard precautions for healthcare workers are recommended.

1.7.2

Epsilon (Alpha Toxin)

Clostridium perfringens produces twelve toxins [27]. One or more of these could be weaponized. The alpha toxin, a highly toxic phospholipase can be lethal when delivered as an aerosol. The toxin causes vascular leaks and severe respiratory distress. It can also cause thrombocytopenia and liver damage. The toxin can be detected from serum and tissue samples by a specific immunoassay. Bacteria can be cultured easily. *Clostridium perfringens* is sensitive to penicillin, the current antimicrobial agent of choice. Some data show that clindamycin or rifampin may reduce toxin production by *C. perfringens*. Some toxoids are available for enteritis necroticans in humans. Veterinary toxoids are widely used.

1.7.3

Ricin Toxin

Ricin is a protein cytotoxin derived from the beans of the castor plant (*Ricinus communis*). The castor plant is ubiquitous and the toxin is easy to export. It is stable and highly toxic by several routes of exposure including inhalation [27, 38].

After inhalational exposure, acute onset of fever, chest tightness, cough, dyspnea, nausea, and arthralgia occur within 4–8 h. Acute respiratory distress syndrome in 18–24 h is followed by hypoxemia and death in 36–72 h. Ricin antigen can be detected in the serum and respiratory secretions by ELISA. Retrospective diagnosis is provided by antibody testing in acute and convalescent sera.

No specific therapy is available. Gastric lavage and emetics are recommended after ingestion. Because ricin is a large molecule, charcoal is not useful.

There is no vaccine or prophylactic immunotherapy available for human use. Immunization seems promising in animal models. A protective mask is the best protection against inhalation. Secondary aerosols are not a danger to others and ricin is nonvolatile. Standard precautions are adequate for healthcare workers. Hypochloric solution (0.1% sodium hypochlorite) inactivates ricin.

1.7.4

T-2 Mycotoxins

Trichothecene mycotoxins are a group of more than forty toxins produced by common molds such as *Fusarium*, *Myrothecium*, *Trichoderma*, *Stachybotrys*, and other filamentous fungi [27, 38]. They are extremely stable in the environment and the only class of biological toxins that cause skin damage. Hypochlorite solution does not inactivate these toxins. They retain bioactivity even after autoclaving. Skin exposure causes pain, pruritus, redness, vesicles, necrosis, and sloughing. Contact results in severe irritant effects on the respiratory tract, GI tract, and eyes. Severe intoxication results in shock and death. Diagnosis should be suspected if an aerosol

attack occurs in the form of “yellow rain” with contamination of the clothes and the environment by pigmented oily fluids.

Treatment is supportive only. Washing with soap and water can prevent or significantly reduce dermal toxicity if done within 1–6 h. Superactivated charcoal should be used after oral intoxication. No prophylactic chemotherapy or immunotherapy is available. Exposure during an attack should be prevented by use of masks and protective clothing. Secondary aerosols are not a hazard. Contact with contaminated skin and contaminated clothing can produce secondary dermal exposures. Until decontamination is accomplished, contact precautions are needed. Subsequently, standard precautions are recommended for healthcare workers. Environmental decontamination requires 1% sodium hypochloride with 0.1 m sodium hydroxide with 1 h contact time.

1.8

Other Toxins With Potential for Bioterrorism

Other toxins include:

- tetanus toxin from *C. tetani*
- toxic-shock syndrome toxin (TSST-1) from *S. aureus*
- exfoliative toxins from *S. aureus*
- saxitoxin – a dinoflagellate toxin responsible for paralytic shellfish poisoning
- tetrodotoxin – a potent neurotoxin produced by fish, salamanders, frogs, octopus, starfish, and mollusks
- toxins from blue–green algae

The agents in Category C, the third highest priority, include emerging pathogens that can be engineered for mass dissemination (Table 1.5).

Tab. 1.5

Category C bioterrorism agents.

Viruses	Bacteria
Nipah virus	Multidrug-resistant
Hanta viruses	<i>Mycobacterium tuberculosis</i>
Tickborne hemorrhagic fever viruses	
Tickborne encephalitis viruses	
Yellow fever virus	

1.8.1

Nipah and Hendra Viruses

Nipah and hendra viruses are closely related, were discovered in the last decade, and have been limited to outbreaks in northern Australia, the Malay Peninsula, and more recently Bangladesh [48]. No cases have been documented in the US. Fruit bats (flying foxes) are infected by these viruses but do not show signs of illness. Hendra virus has been reported to cause outbreaks, small clusters, and isolated cases of acute respiratory diseases in humans. There is no evidence of human to human transmission. Horses shed the infectious virus in urine and nasal secretions which may lead to transmission between animals.

Nipha virus caused an outbreak of acute illness in swine and humans between 1998 and 1999 in Malaysia and Singapore. The disease was highly contagious and spread rapidly among swine causing an acute febrile illness, with respiratory symptoms, with a mortality rate of 5 to 15%. The outbreak caused over 1 million deaths in swine. Humans contracted the infection from swine. The illness was more severe in humans and was characterized by fever, headache, myalgia, and encephalitis with 40% mortality rate among the 265 humans. The disease was eradicated from swine in Malaysia with no further outbreaks. The virus is still likely to be present in fruit bats with the potential of reappearing among animals and humans.

Infections caused by hantaviruses and yellow fever virus and the viruses that cause tickborne hemorrhagic fevers are discussed in Chapter 9. Tickborne hemorrhagic fevers include Crimean–Congo hemorrhagic fever, Omsk hemorrhagic fever, and Kyasanur Forest disease [49]. Tickborne complexes of viruses that cause encephalitis in humans include Far Eastern, Central European, Kyasanur forest, Louping ill, Powasan, and probably Negishi [47].

1.9

Emerging Threats and Potential Agents of Bioterrorism

1.9.1

Pandemic Influenza – Human and Avian Influenza Viruses

The threat of global outbreak of influenza is substantial and believed by the WHO and the wider scientific community to be close [50, 51]. The question is whether it will be caused by H5N1, the avian influenza virus strain, or one of the previously known human influenza strains [52]. The recent avian influenza outbreak in east Asia has met two of the three widely recognized prerequisites for a human pandemic:

1. emergence of a new influenza virus (H5N1) against which there is no natural or vaccine-induced immunity; and
2. its transfer to human beings with virulence and remarkably high (72%) mortality.

The virus may even be getting through the final barrier of person-to-person transmission. There is concern that currently circulating H5N1 viruses will evolve into a pandemic strain by adapting to humans by genetic mutation or re-assortment with human influenza strains [53]. These same characteristics favor the use of a microbial agent as a tool for bioterrorism. Avian influenza virus (H5N1) was recently reported to have ocular tropism like the ocular human pathogens adenovirus serotype 37 and enterovirus serotype 70 [54]. Increased surveillance for influenza virus in ocular infections and the use of eye protection when handling avian and zoonotic influenza may reduce bird-to-bird and human-to-human transmission.

1.9.2

Severe Acute Respiratory Syndrome (SARS) – SARS-associated Coronavirus (SARS–COV)

Severe acute respiratory syndrome was first recognized during the outbreak of 2002–2003. It spread from China to more than 50 countries [55, 56]. The pandemic affected over 8000 individuals worldwide and caused over 700 deaths. Soon after identification of this new disease, WHO initiated a global network for collaborative work. A novel coronavirus (SARS–COV) was identified with unprecedented speed and shown to fulfill Koch's postulates. Several epidemiological and public health lessons were learned from the outbreak control measures for SARS in Toronto [57]. The spread of SARS occurred mainly before it was recognized – emphasizing the importance of ongoing surveillance for new and emerging infectious diseases. The importance of good infection-control practices in the healthcare setting, including emergency departments where most cases are seen first, became clear. The onset of a new infection is difficult to detect in the elderly and patients with underlying diseases. The index of suspicion should be higher in the context of an outbreak. Healthcare providers should be aware of the possibility of new disease among returning travelers and their household contacts. The events related to the SARS outbreak emphasize the reality of the times that natural infectious diseases remain a global threat. A global infrastructure for the surveillance of emerging and new pathogens and rapid communications about them is crucial to public health safety.

SARS-COV has several characteristics that make it a concern as a potential tool for bioterrorism. It is highly contagious and lethal; the disease is difficult to differentiate clinically from other respiratory infections, and there is no rapid diagnostic test, treatment or vaccine available. Access to the virus is not heavily restricted as it is for smallpox.

1.9.3

Other Emerging Threats

The recent spread of West Nile virus infection to the US and monkey pox imported via prairie dogs are reminders of what the scientific community has always been

concerned about – an infectious disease anywhere in the world is a threat to anyone everywhere in the world. Healthcare workers and public health officials must remain vigilant toward novel or unexplained diseases. The US anthrax attack in 2001 was recognized as a result of meticulous attention to detail by a single clinician. More than 75% of emerging infectious agents are from zoonotic sources [58]. The history of important environmental factors, e.g. exposure to animals and vectors can be the first clue to a disease outbreak. The magnitude of the zoonotic outbreaks of avian influenza was unprecedented and affected several species of animals [59]. Control of the outbreak has required the implementation of integrated human and veterinary health surveillance and response efforts. These experiences emphasize the value of multidisciplinary approaches to addressing future emerging infectious disease outbreaks, including bioterrorism.

The range of potential weapons of mass destruction and the ways they can be deployed against civilian populations are diverse and extensive [60]. The key elements of protection against biological agents with potential for mass destruction are effective and ongoing surveillance, early detection, and rapid identification. For countermeasures to be effective, they must be deployed before the agent is widely disseminated.

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