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Biological Agents and Terror Medicine

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In the last decade, terror has become an increasingly global problem. More people have become radicalized, the know-how to use weapons of mass destruction (WMD) is easily accessible by Internet and electronic media, and precursors and basic ingredients are easily purchased. Terrorists are innovative and we now face a new era of nonconventional terrorism: chemical, biological, radiological, nuclear (CBRN), as well as cyber terrorism.

The deliberate use of (WMD–CBRN) by hostile states or terrorists and of naturally emerging infectious diseases that have a potential to cause illness on a massive scale could pose a national security threat.[1] Resulting panic and economic damage could paralyze a country.

Terrorists can act as independent groups or individuals, or can be sponsored, directed, and motivated by states. The use of bioagents intended to harm people, environments, or governments may also be deemed a biocrime. Assessing the threat of bioterrorism and its potential outcome was evaluated in tabletop exercises and mathematical modeling. TOPOFF 2 tested the scenario of a chemical attack in New Hampshire, a radiological event in Washington DC, and a biological event (plague) in Denver, Colorado.[2] Dark Winter exercise (2001–2002) tested a scenario of simultaneous spread of smallpox in Pennsylvania, Georgia, and Oklahoma.[3] TOPOFF 3 (April 2005) tested the scenario of a chemical attack in Connecticut, a bioterror event (pneumonic plague) in New Jersey and international involvement in United Kingdom and Canada.[4]

Bioterrorism has been described by the Center for Disease Control and Prevention (CDC) as "the deliberate release of viruses, bacteria, or other germs (agents) used to cause illness or death in people, animals, or plants. These agents are typically found in nature, but they could possibly be changed to increase their ability to cause disease, make them resistant to current medicines, or to increase their ability to be spread into the environment. Biological agents can be spread through the air, through water, or in food. Terrorists may use biological agents because they can be extremely difficult to detect and do not cause illness for several hours to several days. Some bioterrorism agents, like the smallpox virus, can be spread from person to person and some, like anthrax, cannot."[5]

The use of bioagents by military forces in battlefield conflicts has occurred throughout history.[6] It is generally assumed that only state-sponsored terrorists have

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the technical and scientific capability to weaponize biological agents. While it is questionable how effective terrorists can be in manufacturing or producing a bioweapon, we should be aware that the advanced biological warfare (ABW) agents, including classical agents that could be genetically manipulated or engineered, pose a new and complicated challenge.[7] (Table 12.1)

The biological weapons system comprises four components:

1. *The biological material* consisting of the infectious agent or a toxin produced by bacteria, plants, or animals is the payload.
2. *Munitions* are the agents that carry, protect, and maintain the virulence of the payload during delivery.
3. *The delivery system* can range from a missile, a vehicle (aircraft, boat, automobile, or truck), an artillery shell, an expendable soldier, or martyr, to mailed letters, as was the case with the 2001 anthrax incidents.
4. *The dispersion system* ensures dissemination of the payload at and around the target site among susceptible populations.[9–11]Potential methods of dispersion include aerosol sprays, explosives, and food and water contamination. Aerosol sprays are the most likely method to be used in a potential bioterrorism attack because they are the most effective means of widespread dissemination.[12]

The anthrax attack also demonstrated the effectiveness of the postal system as a facilitator of dispersion.[13]

TABLE 12.1. Case definitions of suspected or confirmed cases due to deliberate release

Deliberate release of anthrax
• ≥1 confirmed case of inhalation anthrax.
• ≥1 confirmed case of cutaneous anthrax arising in individuals who do not routinely have contact with animals or animal hides.
• ≥2 suspected cases of anthrax that are linked in time and place, especially geographically related groups of illness following a wind direction pattern.
Deliberate release of smallpox
• A single confirmed case.
Deliberate release of plague
• A single confirmed case in the European Union must be regarded with a high degree of suspicion of deliberate release.
• A confirmed case of plague in a person without history of being outdoors or having contact with animals.
• ≥2 suspected cases of plague that are linked in time and place, especially to a particular pattern.
Deliberate release of tularemia
• Single confirmed case of indigenously acquired tularemia NOT explained by occupational exposure.
Deliberate release of botulism
• Clusters of >2 cases of acute flaccid paralysis with prominent bulbar palsies, especially where there are common geographic factors between cases, but no common dietary exposure or injected drug use.
• Multiple simultaneous outbreaks with no obvious common source.
• Cases of botulism with an unusual toxin type (type C, D, F or G or E not acquired from an aquatic food).

Source: From Bossi et al.,[8] reprinted with permission from Springer Science + Business Media.

The Centers for Disease Control and Prevention places the bioagents into three categories (A, B, and C), depending on how easily they can be spread and the severity of illness or death they cause (Table 12.2). Category A agents are considered the highest risk and Category C agents are those that are considered emerging

TABLE 12.2. Critical biological agent categories for public health preparedness

Biological agent(s)	Disease
Category A	
Variola major	Smallpox
Bacillus anthracis	Anthrax
Yersinia pestis	Plague
Clostridium botulinum (botulinum toxins)	Botulism
Francisella tularensis	Tularemia
Filoviruses and Arenaviruses (e.g., Ebola virus, Lassa virus)	Viral hemorrhagic fevers
Category B	
Coxiella burnetii	Q fever
Brucella spp.	Brucellosis
Burkholderia mallei	Glanders
Burkholderia pseudomallei	Melioidosis
Alphaviruses (VEE, EEE, WEE)	Encephalitis
Rickettsia prowazekii	Typhus fever
Toxins (e.g., Ricin, Staphylococcal enterotoxin B)	Toxic syndromes
Chlamydia psittaci	Psittacosis
Food safety threats (e.g., *Salmonella spp.*, *Escherichia coli* O157:H7)	
Water safety threats (e.g., *Vibrio cholerae*, *Cryptosporidium parvum*)	
Category C	
Emerging threat agents (e.g., *Nipah virus*, hantavirus)	

Venezuelan equine (VEE), Eastern equine (EEE), and Western equine encephalomyelitis (WEE) viruses.[14]

A recent publication by the US Department of Health and Human Services, [1] based on a list of Material Threat Determinations (MTDs) determined by the Department of Homeland security, describes the agents.

Material Threat Determinants (MTDs) and Population Threat Assessments (PTAs) issued to date by the Department of Homeland Security:

Bacillus anthracis (ANTHRAX)
Botulinum toxins (BOTULISM)
Burkholderia mallei (GLANDERS)
Burkholderia pseudomallei (MELIODOSIS)
Ebola virus (HEMORRHAGIC FEVER)
Francisella tularensis (TULAREMIA)
Junin virus (HEMORRHAGIC FEVER)
Marburg virus (HEMORRHAGIC FEVER)
Multidrug resistant B. anthracis (MDR ANTHRAX)
Radiological/nuclear agents
Ricketsia prowazekii (TYPHUS)
Variola virus (SMALLPOX)
Volatile nerve agents (PTA only)
Y. pestis (PLAGUE)

TABLE 12.3. Characteristics of category A agents

• They can be easily spread or transmitted from person to person.
• They result in high death rates and have the potential for major public health impact.
• They might cause public panic and social disruption.
• They require special action for public health preparedness.[5]

threats for disease. In this chapter, we will touch primarily on the medical aspects and main points concerning those agents that are categorized as Category A biological agents with relevance to bioterrorism. Specific attributes of these agents are listed in Table 12.3.

The pediatric age group in relation to bioterrorism deserves special attention.[15–17] Children may be more vulnerable than adults to biological agents because of their higher metabolic and respiratory rates, their proximity to the ground, and their frequent hand–mouth contact. In addition, they may act as vectors through nuclear and extended families, day care, and school systems.

Early attribution of a cluster of febrile, respiratory illnesses to an intentional release of a bioagent might be difficult in children because children are prone to respiratory symptoms with common colds more often than adults, and they cannot easily report the subtleties of their own symptoms.[18] Strict isolation is complicated in children. Young patients might need to be sedated, especially if isolated in negative pressure tents or rooms. Parents might need to remain with their children in isolation, which could expose them to the infectious agents.

Anthrax

Anthrax is a spore-forming gram-positive bacillus considered to be one of the most likely biological agents for use as a weapon. *Bacillus anthracis* spores can even be transmitted by aerosolization.

The name has its origin in the Greek word for coal, *anthracis*, after the characteristic black skin lesion it produces.[19] Anthrax is primarily a zoonotic disease of sheep and cattle. Spores can remain viable in the soil and infective to grazing livestock for many decades.[20] Humans get infected through skin contact and ingestion or inhalation of spores, typically from infected animals or animal products. Person-to-person transmission has not been documented.

Once introduced into the body, the spores are ingested by macrophages and travel to draining lymph nodes, where they germinate to their vegetative bacillary forms. The bacillus then produces an antiphagocytic capsule and three proteins: protective antigen, lethal factor, and edema factor. These act in binary combinations to form toxins. The protective antigen binds with the lethal factor to form lethal toxin and binds to the edema factor to form edema toxin, both of which play key roles in the pathogenesis of the disease.[21]

Cutaneous Anthrax

Approximately 95% of naturally occurring human anthrax is cutaneous and occurs when spores encounter openings in skin. Within 1–12 days after exposure to the spores, a susceptible patient develops a pruritic macule or papule. This progresses to a vesicle in 1–2 days and is followed by erosion, leaving a necrotic ulcer with a small painless, depressed black eschar. Diagnosis is often confused by its similarity to insect bites. The patient may also have symptoms of fever, malaise, headache, and lymphadenopathy. The case fatality rate is up to 20% without therapy and less than 1% with antibiotic treatment.[22]

Gastrointestinal Anthrax

This form of anthrax is rare and is the consequence of eating undercooked, contaminated meat. The incubation period varies from 1 to 7 days. Gastrointestinal anthrax is characterized by acute inflammation of the intestinal tract. Symptoms include fever, abdominal pain, anorexia, nausea, and vomiting. Bloody diarrhea and hematemesis frequently accompany the symptoms. The case fatality rate is unclear but is thought to range from 25% to 60%.

Inhalational Anthrax

Deliberately aerosolizing dry spores could induce widespread inhalation anthrax, the most lethal form of the disease. The spread of anthrax through the US mail in 2001 heightened concern about the feasibility of large-scale dispersal of bioagents by terrorist groups. *B. anthracis* was delivered by mail to various recipients and chiefly intended to cause inhalational anthrax disease. Twenty-two cases were identified, 11 of them inhalational associated with 5 fatalities, 7 confirmed as cutaneous, and 4 suspected cutaneous cases.[23]

Aerosolized anthrax spores of 2–3 μm can pass through the bronchi to the alveoli and be transported via the lymphatics to the hilar and mediastinal lymph nodes, where germination to the bacillary form may occur.[8] Spore may not immediately germinate and may continue to vegetate in the host for several weeks after inhalation. After alveolar macrophages incorporate the spores, the spores germinate and begin replication. That replication releases several toxins, leading to hemorrhagic thoracic lymphadenopathy and mediastinitis, edema, and necrosis. Hemorrhagic meningitis frequently develops and can be observed in up to half of patients. The median incubation period from exposure to the onset of symptoms is 4 days (range 1–7 days), but cases that occurred from 2 to 43 days after exposure have been reported in humans. This period seems to be inversely related to the dose of spores. It is assumed that a dose of 8,000–50,000 spores is sufficient to cause inhalational anthrax.[24]

Early diagnosis is essential for saving lives. The clinical presentation of the disease is classically biphasic. It starts with nonspecific symptoms of sore throat,

mild fever, and muscle aches. The patient may also present nonproductive cough, dyspnea, headache, vomiting, chills, weakness, abdominal pain, malaise, and chest pain.[20,8,24] Physical examination is usually unremarkable, but chest examination can reveal bilateral decreased breath sounds, rhonchi, and/or inspiratory rales.

The illness progresses to the second phase within 2–3 days. In some patients, a brief period of clinical improvement follows, making it even harder to diagnose the disease. Usually, the second phase begins abruptly with sudden fever and chills, acute dyspnea, retrosternal chest pressure, diaphoresis, cyanosis, and shock.[25] At this stage, a chest X-ray most often shows a widened mediastium consistent with mediastinal lymphadenopathy and hemorrhagic mediastinitis, pleural effusion, and progressive bilateral perihilar infiltrates. CT scan of the chest can demonstrate parenchymal infiltrates or consolidation, large bilateral pleural effusions, pericardial effusions, and a widened mediastinum with a complete infiltration of the mediastinal fat planes, bronchial mucosal thickening, encasement, and compression of the hilar vessels, and hemorrhagic lymph nodes[25] (Table 12.4).

Treatment may be successful in the early stages, but by the time respiratory symptoms develop it is too late for it to have any effect, and death usually occurs within 24–72 hours in almost 90% of cases despite aggressive treatment.[23,28] Death usually occurs 7 days after the onset of symptoms. (Experience with the 2001 attacks suggests outcomes may be less dire since 6 of the 11 inhalation cases survived, and all had respiratory symptoms before treatment.[13]) Person-to-person transmission of inhalation anthrax has never been reported.

Diagnosis

Given the rarity of anthrax, especially the inhalation type, making a diagnosis can be difficult. Early diagnosis and effective antibiotic treatment are essential and are the only way to reduce mortality. Table 12.1 defines the recommended parameters for case definition.

It is very important to take samples before treatment since even one dose of antibiotics may cause sterilization of cultures. Sterilization would negate the chance to grow the organism in a standard blood culture, a traditional test to confirm the presence of the bacterium. Gram stain of vesicular fluids from cutaneous lesions, pleural effusions, CSF Cerebral Spinal Fluid, and ascites are essential for diagnosis. In case of cutaneous anthrax, punch biopsy may also be performed for immunohistochemistry.

Organisms must be tested for sensitivity to antibiotics for natural existence or resistant species and possible genetic manipulation before the deliberate release. Sputum culture and Gram stain are unlikely to be diagnostic of inhalational anthrax given the frequent lack of frank pneumonia.

Rapid identification of *B. anthracis* can be made by direct fluorescent antibody testing and gamma-phage lysis. Confirmatory diagnostic tests such as polymerase chain reaction (PCR) can also be used and may help in early diagnosis.[24] Antibody testing by enzyme-linked immunosorbent assay (ELISA) may yield positive results in convalescent serum specimens. Therefore, serologic testing is useful only retrospectively.

TABLE 12.4. Inhalation/intestinal anthrax

Postexposure prophylaxis (60 days)	Treatment of suspected or confirmed clinical cases of inhalation/intestinal anthrax (60 days)	Target population	
– Ciprofloxacin: 500 Mg per os bid	– Ciprofloxacin: 400 mg IV bid followed by 500 mg os bid	First line	Adults (including pregnant women)
– Ofloxacin: 400 mg per os bid	– Ofloxacin: 400 mg IV bid followed by 400 mg per os bid	Alternative to ciprofloxacin	It is recommended, when possible, to cease breastfeeding
– Levofloxacin: 500 mg per os once a day	– Levofloxacin: 500 mg IV once a day, followed by 500 mg per os once a day		
– Doxycycline: 100 mg per os bid	– Doxycycline: 100 mg IV bid followed by 100 mg bid per os	Alternative first-line treatment and follow-up when susceptibility is confirmed	
-Amoxicillin: 500 mg per os 3 times daily	– Penicillin G: 2.4–3 million units IV, 6 times daily – Amoxicillin: 1 g IV 3 times daily, followed by 500 mg per os 3 times daily	Alternative first-line prophylaxis if susceptibility is confirmed	
– Ciprofloxacin: 10–15 mg/kg per os bid	– Ciprofloxacin: 10–15 mg/kg IV bid followed by 10–15 mg/kg per os bid	First line	Children
– Doxycycline >8 years and >45 kg or >8 years 2.2 mg/kg per os bid (max 200 mg/d)	– Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years 2.2 mg/kg IV bid followed by 2.2 mg/kg per os bid (max 200 mg/d)	Alternative first-line treatment and follow-up when susceptibility is confirmed	
	– Penicillin G: >12 years: 2.4–3 million units IV, 6 times daily <12 years: 30 mg/kg IV, 4 times daily	Alternative first-line prophylaxis if susceptibility is confirmed	
– Amoxicillin: 80 mg/kg per os daily in 3 divided doses	– Amoxicillin: 80 mg/kg daily in 3 divided doses, followed by 80 mg/kg per os daily in 3 divided doses		

Source: Data from: Bossi et al.,[8] Jernigan et al.,[24] the European Agency for the Evaluation of Medicinal Products,[26] and the Centers for Disease Control and Prevention[27]
IV intravenous, *bid* twice daily

The predictive value of nasal swab test for the diagnosis following exposure to *B. anthracis* spore is unknown. A negative result does not indicate that the patient has not been exposed to *B. anthracis*.

In inhalational anthrax, postmortem findings are thoracic hemorrhagic necrotizing lymphadenitis and mediastinitis, pleural effusions and, in 50% of cases, hemorrhagic meningitis. Usually, there are no signs of pneumonia.

Treatment

Many guidelines have been published on treatment and prophylaxis for anthrax.[20,23,24,26] There is no need to isolate patients with inhalational anthrax. In the case of cutaneous anthrax, health care workers should apply standard precautions with gloves. In the past, penicillin was the drug of choice for inhalational anthrax. It can still be considered as an option only if the strain is susceptible to this drug. Since there have been reports of resistant strains and it is not complicated to induce resistance to penicillin, doxycycline, chloramphenicol, macrolides, and rifampicine,[29] ciprofloxacin is currently the recommended first-line treatment as described in Table 12.4. Moreover, recommendations include administering one or two additional antibiotics in the case of inhalational anthrax, for example, rifampicin, chloramphenicol, clindamycin, or vancomycin.[23,26,30] For inhalation anthrax, the duration of treatment is at least 60 days. For cutaneous anthrax, duration of treatment is 7–10 days. The same antibiotics are recommended for postexposure prophylaxis as for treatment of the disease. Oral ciprofloxacin is also recommended as a first choice for prophylaxis for those who are at risk, and must be taken for at least 60 days. Starting antibiotic treatment within a day after exposure to a bacterial aerosol can provide protection against infection.

Vaccines for use against anthrax are licensed in the UK and the US.[31,32] These are considered to be first generation, and clinical trials on the safety and efficacy of a new recombinant protective antigen (rPA)-based anthrax vaccine have recently been initiated in the US.[33] The US vaccine is administered in a series of six subcutaneous injections: after the initial dose, injections are given at 2 weeks, 4 weeks, 6 months, 12 months, and 18 months, respectively. The UK vaccine is given in a series of four intramuscular injections at 0, 3, 6 weeks and a fourth booster at 6 months after the third dose, followed by annual boosters.

Smallpox

Smallpox is a viral infection caused by the variola virus, which belongs to the family of Poxviridae, which includes monkeypox virus, vaccinia virus, and cowpox virus.[34] It is a single, linear, double-stranded DNA virus. The disease was eradicated worldwide by the World Health Organization (WHO) in 1980 and last endemic case was reported in Somalia in 1977.[35,36] The last fatal reported case was in 1978 due to a laboratory-acquired infection in the UK. Variola virus is seen as one of the most likely viruses to be used as a biological weapon because of the properties of

the virus: aerosol infectivity, high mortality, and stability.[34,37] Two different strains of variola virus are known and associated with smallpox: variola major and variola minor.

Clinical Features

Person-to-person transmission is the most common route of transmission but requires close contact.[35] Patients are not infectious during the asymptomatic incubation period (4–19 days; mean 10–12 days) before fever occurs. Smallpox is mostly contagious during the first week of rash, corresponding to the period when the lesions of the enanthem are ulcerated. At this stage, aerosol droplets from oropharyngeal lesions increase the likelihood of person-to-person transmission.

After aerosol exposure, the virus infects regional lymph nodes around the respiratory tract and in other lymphoid tissues such as the spleen, liver, bone marrow, lung, and other lymph nodes and causes the first wave of viremia. After a second viremia period, the virus localizes in small blood vessels of the dermis and in the oral and pharyngeal mucosa and proceeds to infect adjacent cells. Viruses remain present in the lesions until all scabs have been shed following recovery. At this stage, while viruses are enclosed within hard dry scabs, infectivity is lower than in the initial stage of the disease. Historically, it has been estimated that 30% of susceptible household members became infected when smallpox was endemic. Variola virus is stable and it has been estimated that it can be viable in certain conditions for up to a year in dust and cloth.[35]

Variola Major (Classical Smallpox)

The most virulent strain of variola virus causes variola major. Five clinical forms of variola major which differ in prognosis are described.[27,35]

Ordinary-Type Smallpox

This is the most common form and occurs in 90% of cases. The prodromal phase (2–3 days) has an abrupt onset and is characterized by severe and generalized headache, fever (>40°C), extreme prostration, intense, ill-defined pain in the back, chest or joints, intense anxiety, and occasionally abdominal pain. Children may have convulsions, and some adults are delirious. The fever subsides over a period of 2–3 days. Then, enanthem appears over the tongue, palate, mouth, and oropharynx. Usually, a day after, exanthema begins as a small reddish maculopapular rash on the face and forearms and spreads gradually with a centrifugal distribution within 24 hours to the trunk and legs and then to all parts of the body, including the palms of the hands and the soles of the feet. Within 1–2 days, the rash becomes vesicular (diameter of vesicle 2–5 mm), and then pustular.

The pustules, which are round, tense, and deeply embedded in the dermis, remain for 5–8 days, followed by umbilication and crusting. The lesions may vary in number and can be confluent or discrete. A second, less prominent spike

of fever can be noted 5–8 days after the onset of the rash. Lesions are generally synchronous in their stage of development, not as in varicella. This characteristic also provides the main distinguishing feature from monkeypox. In the case of monkeypox, there is also remarkable enlargement of inguinal and cervical lymph nodes. Secondary pyogenic infection of the skin may occur, and other complications like panophtalmitis, keratitis, osteomyelitis, arthritis, orchitis, pneumonitis, pulmonary edema, and so on.

Death may occur in the first 48 hours, before any feature of smallpox has appeared. Most fulminant cases of death occur by the 4th or 5th day, and many others die between the 8th and 15th day. Mortality rate is 30% in unvaccinated and 3% in vaccinated individuals.

Hemorrhagic-Type Smallpox

This is the most virulent form of the disease. It occurs in 3% of the patients and is characterized by hemorrhages into the skin and/or the mucous membranes and toxemia. Death rate is 96% in unvaccinated and 94% in vaccinated individuals, usually before the occurrence of the lesions.

Other Types

The modified-type smallpox or milder-type, the flat-type smallpox, variola sine eruptione, and variola minor are other less virulent types of smallpox.

Diagnosis

Case definition of suspected or confirmed cases is described in Table 12.1. Differentiation between smallpox and other orthopoxes can be done by electron microscopy and by PCR assay and/or restriction fragment length polymorphism (RFLP).[27,36] Definitive characterization of the variola virus is made by culture in eggs and cell monolayers.

Treatment

Patients with smallpox must be isolated and managed, preferably if possible, in negative pressure rooms until death or for about 3 weeks until all scabs have been shed. There is no proof of any antiviral drug being effective in clinical cases. Antibiotics may be useful in secondary infections. The most effective measure of prevention is vaccination before exposure. Side effects due to vaccination are low but higher than with other vaccines. The more severe complications noted were postvaccinial encephalitis, progressive vaccinia, eczema vaccinatum, and generalized vaccinia.[35] However, vaccination can also modify the course of the disease and reduce mortality by 100% if given immediately after exposure, and by up to 50% if given within 4 days after exposure. Currently, a second generation of vaccine is being developed, and there is a need for developing a third-generation

vaccine with an acceptable safety profile by attenuating or genetically engineering (disabling) vaccinia vaccine strains, while retaining their immunizing properties.

Plague

Plague is an acute bacterial infection caused by *Yersinia pestis*, a Gram-negative bacillus. Historically, three plague pandemics caused the death of more then 200 million people.[38] The disease, primarily the bubonic form, is still endemic in some parts of the world, mainly in Africa and in the former Soviet Union. Each year about 1,500 cases are reported. Plague is an enzootic infection of rodents, prairie dogs, and squirrels. Human transmission occurs via flea vectors from rodents and by respiratory droplets from animals to humans or humans to humans.

Clinical Features

The three clinical syndromes of plague are bubonic, secondary pneumonic, and primary pneumonic. The last one is the most likely in the event of a bioterror attack, being dispersed by aerosol.[39] Incubation period varies from 2 to 8 days. Bubonic plague is the most common naturally occurring form of the disease. Patients often present with a sudden onset of fever (38.5°C to 40°C) and fatigue and development of a bubo, an acutely tender lymph node. A small fraction of patients develop primary septicemic plague, which is remarkable for the absence of buboes. Secondary pneumonic plague develops via hematogeneous spread of the bacilli to the lungs and manifests as dyspnea, chest pain, hemoptysis, and/or severe bronchopneumonia. Primary pneumonic plague, plague meningitis, and plague pharyngitis are some of the other clinical syndromes associated with plague (Table 12.5).

In primary pneumonic plague, the patient presents with symptoms suggestive of pneumonia, with early onset of high fever, myalgia, malaise, headache, and hemoptysis, often progressing rapidly to sepsis and respiratory failure with signs of dyspnea, stridor, and cyanosis. Patients may also progress to shock and have extensive ecchymosis. Plague pneumonia is highly contagious to other humans by droplet transmission, and patients remain contagious up to 3 days after starting antibiotic treatment. But with prompt use of antibiotics the fatality rate decreases below 10%.

Diagnosis

Case definition of suspected cases or confirmed cases is described in Table 12.6. *Y. pestis* can be cultured from blood, sputum, bubo aspiration, and cerebrospinal fluid. Specimens should be taken before initiating antibiotic treatment. Smears can be stained with Gram, Giemsa, or Wayson's stains to demonstrate the bipolar coccobacilli. Serological tests and direct immunofluorescence for F1 antigen,

TABLE 12.5. Summary of clinical and biological characteristics of plague

Clinical description
– Incubation period: 1–6 days
Pneumonic plague
– Abrupt onset of intense headache and malaise, high fever, vomiting, abdominal pain, diarrhea and marked prostration, chest pain, cough, dyspnea, and hemoptysis
– Chest X-ray: multilobar consolidation, cavities, or bronchopneumonia
– Respiratory failure develops quickly with septicemic/shock
Bubonic plague
– Fever (38.5–40°C), chills, headache, weakness, and bubo
– Surrounding edema and the overlying skin is warm, erythematous, and adherent
Septicemic plague
– Septic shock, vasculitis, livid cyanotic petechiae, large ecchymoses, gangrene of acral regions, and multiorgan failure
– Meningitis occurs in 5% of cases
Presumptive diagnosis
– Staining of specimens
– ELISA, direct immunofluorescence, PCR
Diagnosis
– Isolation of *Yersinia pestis* from a clinical specimen
– Demonstration of a specific antibody response to *Y. pestis* fraction 1 (F1) antigen
– Elevated serum antibody titers to *Y. pestis* F1 antigen (without documented specific change) in a patient with no history of plague vaccination
– Detection of F1 antigen in a clinical specimen by fluorescent assay
Management of treatment
– If plague pneumonia, isolation in a negative pressure room (if possible)
– Gentamicin or streptomycin as first-line therapy with ciprofloxacin as an alternative (see also Table 12.7)
– Chloramphenicol should be used for the treatment of meningitis
– Persons in contact (<2 m) with pneumonic plague should receive antibiotic prophylaxis with doxycycline or ciprofloxacin for 7 days. Other antibiotics (chloramphenicol, sulfadiazine, trimethoprim-sulfamethoxazole, etc.) could also be used

Source: Data from: Bossi et al.[39]
PCR polymerase chain reaction, ELISA enzyme-linked immunosorbent assay

TABLE 12.6. Case definitions of possible, probable, and confirmed cases of plague

Possible case
– Sudden onset of severe, unexplained febrile respiratory illness
– Unexplained death following a short febrile illness
– Sepsis with Gram-negative coccobacilli identified from clinical specimens
Probable case
– A case that clinically fits the criteria for suspected plague, and in addition, positive results are obtained on one or more specimens
Confirmed case
– A clinically compatible case with confirmatory laboratory results
– Culture of *Yersinia pestis* from a clinical specimen and confirmation of identification by phage lysis
– A significant (fourfold) change in antibody titre to F1 antigen in paired serum samples
– A definitive diagnosis, by positive PCR or detection of F1 antigen on suspect isolates, will be available within one working day

Source: Data from the Official Journal of the European Communities[40,41]

Table 12.7. Criteria for suspecting deliberate release of plague

Deliberate release
– A single confirmed case in the European Union must be regarded with a high degree of suspicion of deliberate release[a]
– A confirmed case of plague in a person without history of being outdoors or having contact with animals
– ≥2 suspected cases of plague that are linked in time and place, especially if the suspected cases are geographically related according to a particular wind pattern

[a]Cases that occur in people who have returned from endemic areas should be investigated to ascertain that the illness did not occur with intent to deliberately release *Yersinia pestis*

specific phage lysis, and PCR for the plasminogen activator gene, are all available, preferably to be done at reference laboratories (Table 12.7).

Treatment

Treatment should be initiated as soon as the diagnosis is suspected; see Table 12.8. Many antibiotics are active against *Y. pestis*, and most guidelines suggest using Gentamycin or streptomycin as first-line therapy with ciprofloxacin as an alternative.[25,35,36,38,39,42] Chloramphenicol should be used for the treatment of meningitis. Persons who come in contact (<2 m) with patients with pneumonic plague should receive antibiotic prophylaxis with doxycycline or ciprofloxacin for 7 days. Prevention of human-to-human transmission from patients with pneumonic plague pneumonia can be achieved by implementing standard isolation precautions until at least 4 days of antibiotic treatment have been administered. For the other clinical types of the disease, patients should be isolated for the first 48 hours after the initiation of treatment. Health care workers should wear high-efficiency respirators.

Botulism

Aerosols of botulinum toxin could be used as a biological weapon.[37,43–45] Deliberate release may also involve contamination of food or water supplies with toxin or *Clostridium botulinum* bacteria. Botulinum toxin is extremely lethal and easy to produce.[8] The *C. botulinum* is a large, Gram-positive, strictly anaerobic bacillus that forms a subterminal spore. These spores can be found in soil and marine sediments throughout the world. Four groups of *C. botulinum* are shown in Fig. 12.1.

The toxins are ingested or inhaled and their effect is similar. Botulinum toxin does not penetrate intact skin. Toxins act by binding to the presynaptic nerve terminal at the neuromuscular junction and at cholinergic autonomic sites. This binding prevents release of acetylcholine and interrupts neurotransmission. Human botulism is almost always caused by toxin types A, B, E, and in rare cases F. By inhalation, the LD50 (dose that kills 50% of exposed persons) is 0.003 µg/kg of body weight. This toxin is 100,000 times more toxic than sarin gas.[37]

TABLE 12.8. Recommendations for treatment and postexposure prophylaxis of plague

Target	Population	Treatment of suspected or confirmed clinical cases (10 days)	Postexposure prophylaxis (7 days)
Adults (including pregnant women)	First-line treatment	– Gentamicin: 5 mg/kg IV in 1 or 2 doses daily or – Streptomycin: 1 g IM twice daily	
It is recommended, when possible, to cease breast-feeding	Second-line treatment; First-line prophylaxis	– Ciprofloxacin: 400 mg IV bid followed by 500 mg per os bid or – Ofloxacin: 400 mg IV bid followed by 400 mg per os bid or – Levofloxacin: 500 mg IV once a day, followed by 500 mg per os once a day	– Ciprofloxacin: 500 mg per os bid or – Ofloxacin: 400 mg per os bid or – Levofloxacin: 500 mg per os once a day
	Third-line treatment; Second-line prophylaxis	– Doxycycline: 100 mg IV bid followed by 100 mg bid per os	– Doxycycline: 100 mg bid per os
Children	First-line treatment	– Gentamicin: 2.5 mg/kg IV in 3 doses daily or – Streptomycin: 15 mg/kg IM twice daily (max, 2 g)	
	Second-line treatment; First-line prophylaxis	– Ciprofloxacin: 10–15 mg/kg IV bid followed by 15 mg/kg per os bid	– Ciprofloxacin: 10–15 mg/kg per os bid
	Third-line treatment; Second-line prophylaxis	– Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or >8 years: 2.2 mg/kg IV bid followed by 2.2 mg/kg per os bid (max 200 mg/d)	– Doxycycline: >8 years and >45 kg: adult dose >8 years and< 45 kg or <8 years: 2.2 mg/kg per os bid (max 200 mg/d)

Source: European Agency for the Evaluation of Medicinal Products[26] and Bossi et al.[39]
IV intravenous, *IM* intramuscular

Group I are proteolytic in culture and produce toxin types A, B or F.

Group II are non proteolytic and produce toxins types B, E or F.

Group III produce toxin types C or D.

Group IV toxins produce toxin type G.

FIG. 12.1. Four groups of *Clostridium botulinum.*

Clinical Features

Several forms of botulism are known: three natural forms—food or waterborne, wound, and intestinal (adult and infant)—and a fourth, inhalational botulism, which is a man-made form that results from aerosolized botulinum toxin. The incubation period can be brief, depending on the type and dose of toxin: 12–72 hours (range: 2 hours to 10 days).[46,47] Following aerosol exposure, onset of symptoms may be more rapid, possibly 1 hour after exposure. Person-to-person transmission has never been described.

Regardless of the route of contamination, illness is an acute, afebrile, symmetric, descending flaccid paralysis that begins in the head. Multiple cranial nerve palsies produce diplopia, ptosis, blurred vision, enlarged or sluggishly reactive pupils, photophobia, facial weakness, dysphonia, dysphagia, and dysarthria. This is followed by a symmetrical, descending skeletal muscle paralysis with hypotonia, weakness in the neck and arms, after which respiratory muscles and then distal muscles are affected.[37] There is no loss of sensation, and patients are well oriented. Autonomic signs like postural hypotension, dry mouth, cardiovascular, gastrointestinal, and urinary autonomic dysfunction may also be present. Gag reflex may not be lost. Deep tendon reflexes may be present or absent. Pupils are dilated and fixed. Respiratory paralysis may require mechanical ventilation. Laboratory test results, including analysis of cerebrospinal fluid, are unremarkable.

Diagnosis

Clinical diagnosis may be difficult without strong clinical suspicion. The first and early cases are commonly misdiagnosed. Case definitions of suspected or confirmed cases due to deliberate release are reported in Table 12.2. Laboratory diagnosis relies on isolation and identification of the neurotoxins from sera or other samples like stool, gastric specimen, vomitus, and suspected food. The aerosolized toxin may be detected by ELISA on nasal mucous membranes or bronchoalveolar lavage for 24 hours after inhalation.

Treatment

Without supportive treatment, death often occurs from respiratory failure. Patients with respiratory failure require long-term mechanical ventilation, from 60 days to 7 months.[44] Trivalent (A, B, E) equine antitoxins must be given to patients as soon as possible after clinical diagnosis by slow intravenous infusion.[47] Patients with botulism who survive may have asthenia and dyspnea for years. Muscle function returns after 3–6 months as the neuromuscular junction regenerates. In the United States, investigational pentavalent (A–E) botulinum toxoid vaccine is used for laboratory workers at high risk of exposure and by military personnel. Immunity is induced slowly by this vaccine, and frequent boosters are required.[45]

Tularemia

Francisella tularensis is a nonmotile, obligatory aerobic, facultative intracellular Gram-negative coccobacillus. One of the most infectious pathogenic bacteria known, ten organisms are sufficient to initiate human infection.[48] Inhalational tularemia following intentional release of a virulent strain of *F. tularensis* would have great impact and cause high morbidity and mortality. Another route of contamination in a deliberate release could be contamination of water.[49]

Clinical Features

The incubation period of tularemia is of 3–5 days (range 1–25 days). Seven clinical forms are known, according to route of inoculation: skin, mucous membranes, gastrointestinal tract, eyes, respiratory tract, glandular. Usually, whatever the clinical form, the onset of symptoms is abrupt with fever, chills, myalgias, arthralgias, headache, coryza, sore throat, and sometimes pulse–temperature dissociation, nausea, vomiting, and diarrhea. Inhalational exposure commonly presents as an acute flu-like illness without prominent signs of respiratory disease (Tables 12.9 and 12.10).

The diagnosis of tularemia due to a deliberate release would be suggested if large numbers of patients present with an atypical pneumonia (Tables 12.11, 12.12). Ulceroglandular tularemia is the most common (75–85%) reported form. Ulcers are usually single lesions of 0.4 to 3.0 cm in diameter. The lesion is associated with tender enlargement of one or more regional lymph nodes, which may become fluctuant and rupture releasing caseous material. Lymphadenopathy may persist for as long as 3 years. Neither severe diseases nor complications are usually noted with this form of tularemia. Tularemia sepsis is potentially severe and fatal. Any form of tularemia can be complicated by sepsis.

Diagnosis

Clinical diagnostic suspicion remains crucial. Case definitions of suspected or confirmed cases, and cases due to deliberate release, are shown in Table 12.11. *F. tularensis* may be identified by direct examination of secretions, exudates, or biopsy specimens using direct fluorescent antibody or immunohistochemical stains. Culture is possible but difficult and poses a significant risk of infection to laboratory workers.

Antigen detection assays, PCR, ELISA techniques may be used to identify *F. tularensis*. A fourfold change in titer between acute and convalescent serum specimens, a single titer of at least 1/160 to tube agglutination or 1/128 for microagglutination is diagnostic for *F. tularensis*.[42,48] Serum antibody titers do not attain diagnostic level until 10–14 days after onset of illness. Serologic testing is useful only retrospectively but confirms the diagnosis. For definitive laboratory confirmation, culture and an increase in specific antibodies in paired sera are required.

TABLE 12.9. Summary of clinical and biological description of tularemia

Clinical features
– Incubation period: 3–5 days
– Tularemia pneumonia (primary and secondary pneumonia)
– Inhalational exposure presents as an acute flu-like illness
– Progression to severe pneumonia with bloody sputum, respiratory failure, and death, if appropriate treatment is not started
– Chest radiography: peribronchial infiltrates, bronchopneumonia, pleura effusions, and hilar lymphadenopathy
– Ulceroglandular tularemia, most common form (75% to 85%)
– Local papule at the site of inoculation associated with fever and aches
– Papule pruritic ≥enlarges to pustule ≥ruptures to painful, indolent ulcer, which may be covered by an eschar
– Tender enlargement of ≥1 regional lymph nodes, which may become fluctuant and rupture releasing caseous material
Glandular tularemia
– Lymphadenopathy and fever
– No ulcer
Oculoglandular tularemia
– Purulent conjunctivitis, chemosis, conjunctival nodules, or ulceration
– Periorbital edema
– Tender preauricular or cervical lymphadenopathy
Oropharyngeal tularemia
– Stomatitis, exudative pharyngitis or tonsillitis + painful mucosal ulceration
– Retropharyngeal abscess or suppuration of regional lymph nodes
Typhoidal tularemia
– Acute flu-like illness
– Diarrhea, vomiting, headache, chills, rigors
– Myalgia, arthralgia, weight loss, prostration
– No indication of inoculation site
– No anatomic localization of infection
Tularemia sepsis
– Nonspecific signs confusion
– Septic shock, disseminated intravascular coagulation and hemorrhage, acute respiratory distress syndrome, organ failure, and coma
Diagnosis
Confirmatory tests for identification of *Francisella tularensis* [40,41]
– Isolation of *F. tularensis* from a clinical specimen
– Demonstration of a specific antibody response in serially obtained sera
For probable case
– A single high titer
– Detection of *F. tularensis* in a clinical specimen by fluorescent assay
Treatment [26,42,48,49]
– Private room placement for patients with pneumonia is NOT necessary
– Treatment of choice: Streptomycin and gentamicin (10 days)
– Quinolones effective alternative (10–14 days)
– Tetracyclines and chloramphenicol are associated with high relapse rate, therapy at least 14–21 days
– Combination of two (aminoglycosides and fluoroquinolones) in severe cases
Postexposure prophylaxis
– Streptomycin, gentamicin, doxycycline, or ciprofloxacin (14 days)
– Vaccination is NOT recommended for postexposure prophylaxis

Source: Data from Bossi et al.[49]

Table 12.10. Case definitions of tularemia

Possible case
– NA
Probable case
– A severe, unexplained febrile illness or febrile death in a previously healthy person
– Severe unexplained respiratory illness in otherwise healthy people
– Severe unexplained sepsis or respiratory failure not due to a predisposing illness
– Severe sepsis with unknown Gram-negative coccobacillary species that fails to grow on standard blood agar, identified in the blood or cerebrospinal fluid
– A clinically compatible case that fulfils the laboratory criteria for a probable case or has an epidemiological link
Confirmed case
– A clinically compatible case with positive confirmatory laboratory tests

Source: Data from the Official Journal of the European Communities[40,41]

Table 12.11. Definition of a deliberate release with *Francisella tularensis*

Suspected deliberate release
– Two or more suspected cases of tularemia that are linked in time and place, especially geographically related groups of illness following a wind direction pattern
Deliberate release
– Single confirmed case of indigenous tularemia NOT explained by occupational exposure

Source: Data from Bossi et al.[49]

Treatment

Guidelines have been published for treatment and prophylaxis of tularemia (13.12). Streptomycin and gentamicin are currently considered the treatment of choice for tularemia. Treatment with aminoglycosides should be continued for 10 days. Quinolone may be an effective alternative drug. Despite the absence of large data in patients with tularemia, ciprofloxacin principally, or ofloxacin should be prescribed for 10–14 days. In severe cases, combination of two antibiotics such as aminoglycosides and fluoroquinolones should be considered.[48,49] Use of macrolides in tularemia is not recommended. Usually, the beta-lactamase are considered ineffective. No isolation measures are necessary for patients with pneumonia.

Streptomycin, gentamicin, doxycycline, or ciprofloxacin are recommended for postexposure prophylaxis and must be taken for at least 14 days. An unlicensed live-attenuated vaccine is available. That vaccine appears to offer protection against ulceroglandular and pneumonic tularemia. In the absence of additional data, the vaccine is not recommended for postexposure prophylaxis.

Viral Hemorrhagic Fevers

Viral hemorrhagic fevers (VHFs) are a variety of diseases, associated with fever and bleeding disorders caused by RNA viruses (Table 12.13). These include Arenaviridae, which are composed of the Lassa, Argentine, Bolivian, and Brazilian

TABLE 12.12. Recommendations for treatment and postexposure prophylaxis of tularemia

Target population		Treatment of suspected or confirmed clinical cases (10–21 days)	Postexposure prophylaxis (14 days)
Adults (including pregnant women). It is recommended, when possible, to stop breastfeeding	First-line treatment (10 days)	– Gentamicin: 5 mg/kg IV in 1 or 2 doses daily or – Streptomycin: 1 g IM twice daily	
	Second-line treatment: first-line prophylaxis (14 days)	– Ciprofloxacin: 400 mg IV bid followed by 500 mg per os bid or – Ofloxacin: 400 mg IV bid followed by 400 mg per os bid or – Levofloxacin: 500 mg IV once a day, followed by 500 mg per os once a day	– Ciprofloxacin: 500 mg per os bid or – Ofloxacin: 400 mg per os bid or – Levofloxacin: 500 mg per os once a day
	Third-line treatment; second-line prophylaxis (21 days)	– Doxycycline: 100 mg IV bid followed by 100 mg bid per os	– Doxycycline: 100 mg bid per os
Children	First-line treatment (10 days)	– Gentamicin: 2.5 mg/kg IV 3 times daily or – Streptomycin: 15 mg/kg IM twice daily (max; 2 g)	
	Second-line treatment: first-line prophylaxis (14 days)	– Ciprofloxacin: 10–15 mg/kg IV bid followed by 10–15 mg/kg per os bid	– Ciprofloxacin: 10–15 mg/kg per os bid
	Third-line treatment; second-line prophylaxis (21 days)	– Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years: 2.2 mg/kg IV bid followed by 2.2 mg/kg per os bid (max 200 mg/d)	– Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years: 2.2 mg/kg per os bid (max 200 mg/d)

Source: Data from European Agency for the Evaluation of Medicinal Products[26] and Bossi, P et al.[49]
IV intravenous, IM intramuscular

([Junin, Machupo, Guanarito, Sabia] viruses; Bunyaviridae, which cause Rift Valley fever [RVF] and the Congo-Cremean hemorrhagic fever, [CCHF]); and Filoviridae, which are composed of Ebola, Marburg, and yellow fever viruses.[50,51]

Most of these viruses have zoonotic life cycles independent of humans (dengue and yellow fever partially excepted). These agents are usually transmitted to humans from animals or arthropod reservoirs via mosquitoes, ticks, or infected animal urine or feces. Except for RVF and the *flaviviruses*, person-to-person transmission can occur with close contact but it is not a usual route of transmission.

TABLE 12.13. Hemorrhagic fever viruses (HFVs) that could be involved in biological warfare

Family	Virus	Disease	Vector in nature
Filoviridae	Ebola	Ebola hemorrhagic fever	Unknown
	Marburg	Marburg hemorrhagic fever	Unknown
Arenaviridae	Lassa	Lassa fever	Rodent
	Machupo	Bolivian hemorrhagic fever	Rodent
	Junin	Argentine hemorrhagic fever	Rodent
	Guannarito	Venezuelan hemorrhagic fever	Rodent
	Sabia	Brazilian hemorrhagic fever	Rodent
Bunyaviridae	Rift Valley fever	Rift Valley fever	Mosquito
	Crimean-Congo hemorrhagic fever	Crimean-Congo hemorrhagic fever	Tick
Flaviviridae	Yellow fever	Yellow fever	Mosquito
	Omsk hemorrhagic fever	Omsk hemorrhagic fever	Tick
	Kyasanur Forest disease	Kyasanur Forest disease	Tick

Most of these viruses may be transmitted to humans through aerosolization. Biological weaponization has been proven successfully in nonhuman primates. Hemorrhagic fever viruses (HFVs) are associated with high morbidity, and in some cases high mortality. No specific treatment or vaccines exist for these viruses.

Diseases

Most of the HFVs induce a similar syndrome. The incubation period varies from 1 to 21 days (Table 12.14). Depending on the virus, the disease can progress with respiratory problems, severe bleeding, kidney failure, and shock. All HFVs can induce microvascular damage and capillary leak syndrome. Severity of illness can range from relatively mild to death. Most patients infected with these viruses experience a nonspecific febrile illness, without prominent involvement of a single organ system. A summary of clinical description of HFV is detailed in Table 12.13. Laboratory criteria for diagnosis and case definition are detailed in Table 12.15. Recommendations for treatment and postexposure prophylaxis of VHF are detailed in Table 12.16 and the status of vaccine development is detailed in Table 12.17.

Conclusion

Unlike most types of weapons, biological agents come in many forms ranging from bacteria and viruses to plants and toxins. Some agents cause contagious disease while others do not. Some are lethal while others are likely to cause debilitating illness but not death. Nevertheless, all select agents are recognized as potential weapons that could have serious and even devastating effects on victims. Although more than 70 biological agents have been designated by the CDC as possibly useful for hostile purposes, the six agents deemed especially attractive as terrorist weapons are those in Category A. They are responsible for smallpox, anthrax, plague,

TABLE 12.14. Summary of clinical description of hemorrhagic fever viruses (HFV)

Virus	Incubation (days)	Clinical feature	Mortality (%)
Ebola	2–21	Onset abrupt: high fever, chills, asthenia, headache, muscle aches, anorexia, conjunctivitis, abdominal pain, nausea, vomiting, diarrhea, pharyngitis, sore throat, chest pain, and erythematous macular rash. After 3 days, prostration, hemorrhagic manifestations, petechiae, ecchymosis, conjunctival hemorrhage, gingival bleeding, bleeding from injection site, frank bleeding from gastrointestinal tract with melena, vaginal bleeding, hematemesis, and bleeding from other sites such as internal organs. Patients may die of organ failure and shock.	72
Marburg	3–10	Idem Ebola	23
Lassa fever	10–14	Usually asymptomatic or mild illness. The onset of the disease is insidious with fever and general malaise over a 2 - 4 day period. In more severe cases; weakness, retroorbital pain, joint and lumbar pain, myalgia, headache, pharyngitis, cough and conjunctival injection. In the most severe form of the disease; prostration; abdominal pain, facial and neck edema, hemorrhages (conjunctival hemorrhages, mucosal bleeding, melena, hematochezia, hematuria, vaginal bleeding, hematemesis), encephalitis, capillary leak syndrome and shock. Hepatitis is frequent. Pulmonary manifestations can be significant with ARDS. Long-term sequelae of Lassa infection; sensorineural deafness.	15–20
New World Arenaviruses	7–14	Idem Lassa fever Hemorrhage, and neurological signs are more common; hemorrhage along the gingival margins is characteristic. Neurologic signs may include delirium, confusion, encephalopathy, convulsions and coma. Conjunctival injection, facial flushing, petechial and/or vesicular palatal enanthem and skin petechiae, generalized lymphadenopathy and orthostatic hypotension are common.	10–16

TABLE 12.14. (continued)

Virus	Incubation (days)	Clinical feature	Mortality (%)
Rift Valley fever	3–6	The initial clinical manifestations are a biphasic fever, the first bout lasting 4 days. After 1 or 2 days without fever the second fever spike occurs, lasting for 2 to 4 days. Usually the illness is mild and associated with fever and liver abnormalities. In severe cases hemorrhage (< 1%), encephalitis (1%), and retinitis (10%).	1
Crimean-Congo hemorrhagic fever	3–6	Onset of symptoms is abrupt with fever, myalgia, dizziness, neck pain and stiffness, backache, headache, sore eyes and photophobia, nausea, vomiting, diarrhea and abdominal pain. The patient may experience sharp mood swing, and may become confused and aggressive. After 2 to 4 days, sleepiness, depression and lassitude may replace the agitation, and the abdominal pain may localize to the right upper quadrant, with hepatomegaly. Other clinical signs include tachycardia, lymphadenopathy, and a petechial rash or ecchymoses, both on mucosal surfaces and on the skin. Hemorrhagic symptoms include melena, hematuria, epistaxis and bleeding from the gums. A hepatitis is usually present. Multiorgan failure with hepatorenal and pulmonary failures may develop after the fifth day of illness.	30
Yellow fever	1–3	The onset of the illness is abrupt with fever, headache, generalized malaise, weakness, lumbosacral pain, bradycardia, nausea, and vomiting. This period lasts 3 days and is followed by a remission lasting 24 h. Then intoxication, which can progress to death 7–10 days after presentation appears. Symptoms include jaundice, scleral icterus, albuminuria, oliguria, cardiovascular instability, and hemorrhagic manifestations.	25–50
Omsk Hemorrhagic fever	3–8	The onset is abrupt with fever, headache, severe myalgias, diarrhea, vomiting, severe prostration, conjunctival suffusion, photophobia, cervical and axillary adenopathy, and more rarely splenomegaly or hepatosplenomegaly. Papulovesicular lesions involving the soft palate are frequent. Pulmonary manifestations are also frequent during the first stage of the illness. The second stage of the illness is associated with neurological involvement. Hemorrhagic manifestations are those observed with other VHFs.	0.5–10
Kyasanur Forest fever	3–8	Idem Omsk hemorrhagic fever	3–10

Source: Bossi et al.[51]

TABLE 12.15. Laboratory criteria for diagnosis and case definition

Laboratory criteria for diagnosis
Positive virus isolation
Positive skin biopsy (immunohistochemistry for Ebola/Marburg viruses, Lassa fever virus)
Detection of specific viral nucleic acid sequences
Positive serology, which may appear late in the course of the disease
Case definition of suspected and confirmed cases of viral hemorrhagic fever (VHF)
Possible: Not applicable
Probable: A clinically compatible case with an epidemiological link
Confirmed: A clinically compatible case that is laboratory confirmed
Case definition of a suspected deliberate release of VHF
≤1 confirmed case in Europe which is not an imported case

Source: Data from the Official Journal of the European Communities[40,41]

TABLE 12.16. Recommendations for treatment and postexposure prophylaxis of viral hemorrhagic fever (VHF)

	Treatment of suspected or confirmed clinical cases of VHF (10 days)	Postexposure prophylaxis (7 days)
Adults (including pregnant women). It is recommended, when possible, to stop breastfeeding	*Ribavirin IV*: Initial dose of 2 g followed by 1 g every 6 h for 4 days, followed by 0.5 g every 8 h for 6 days or Initial dose of 20 mg/kg followed by 15 mg/kg every 6 h for 4 days, followed by 7.5 mg/kg every 8 h for 6 days or *Ribavirin per (os)*: 2 g orally (loading dose) followed by 4 g/day in 4 divided doses for 4 days followed by 2 g/day for 6 days	Ribavirin: 2 g/day orally in 4 divided doses
Children	No recommendations can be given	No recommendations can be given

Source: Data from: European Agency for the Evaluation of Medicinal Products[26]

botulinum toxin-caused illness, tularemia, and VHFs. As such, these agents and diseases should be of special interest to members of the medical community, who may be called upon to treat victims of their effects.

The threat of bioterrorism poses a challenge for many sectors of society including public health, primary care, hospitals, first responders, as well as agencies beyond the health community. Coping successfully with bioterrorism will depend upon awareness of the threat, a high level of pre-event preparedness, coordination, and practiced performance at both national and local levels.

TABLE 12.17. Status of vaccine development against hemorrhagic fever virus (HFV) in 2002

Virus	Vaccine candidate	Development stage
Ebola	Recombinant subunit	Preclinical
	Replicons	Preclinical
Marburg	Not mentioned	
Lassa fever	Not mentioned	
New World arenaviruses	Not mentioned	
Rift Valley fever	Inactivated Live, attenuated	Phase II
		Phase I
Yellow fever	Live attenuated (17D strain)	Licensed
	Infectious clone	Preclinical
Omsk hemorrhagic fever	Not mentioned	
Kyasanur Forest fever	Not mentioned	

Source: US Department of Health and Human Services[52]

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