Chapter 7

BIOLOGICAL AGENTS

Overview

The course of human history has been greatly affected by naturally occurring diseases. AIDS, influenza, malaria, cholera, tuberculosis, plague, and smallpox have killed hundreds of millions of people and profoundly disrupted or destroyed cultures, societies, and civilizations. Since ancient times humans have tried to harness the destructive potential of biological agents to use against enemies. With the advent of the germ theory of disease and worldwide industrialization, our ability to unleash the destructive potential of biological agents on a large scale has grown considerably.

The threat of biological attacks continues today. Technological advances in chemistry, microbiology, and particularly genetic engineering are making it easier for potential terrorists to develop or acquire novel biological agents or highly infectious "superbugs" that are resistant to antibiotics and vaccines. Compared to other types of weapons, biological agents have relatively low cost and high lethality.

To minimize these threats and conserve the fighting strength, military health care providers must be knowledgeable about biological weapons and the medical management of biological casualties, and ensure that appropriate countermeasures are taken.

Characteristics of Biological Agents

Biological weapons are developed from living organisms and viruses capable of causing disease and death in humans, animals, or plants. Weapons associated with high mortality are referred to as lethal agents, and those that usually produce severe illness, but not death, are referred to as incapacitating agents.

Categories of Biological Agents

The three general categories of biological agents are (1) biological toxins, (2) biological modulators, and (3) pathogens. Biological toxins, or biotoxins, are poisons derived from plants, animals, or microorganisms. Like chemical agents, their onset of action is relatively rapid. Examples include staphylococcal enterotoxin B (SEB), botulinum, and ricin. Biological modulators, or response modifiers, are substances that modify immune responses. They can be both endogenous (produced naturally within the body) and exogenous (eg, pharmaceutical drugs). Some of these substances arouse the body's response to an infection, and others can keep the response from becoming excessive.

Pathogens are microorganisms that cause disease (bacteria, rickettsia, and fungi) and viruses, which, while not living organisms, are replicating agents. Pathogens are the classic agents of biological warfare and the focus of this chapter. Pathogens differ from other biological agents and chemical warfare agents in that they can infect a susceptible host and replicate themselves within it, have an incubation period of days to weeks before clinical signs and symptoms manifest, and in the cases of smallpox and plague, may go on to infect subsequent hosts long after the initial exposure.

Portals of Entry

Biological warfare agents can gain access into susceptible hosts through three portals of entry, depending on how they are weaponized:

1. The respiratory tract is susceptible to aerosols (eg, pneumonic plague).
2. The digestive tract is susceptible to infection from intentionally contaminated food and water (eg, cholera).
3. The skin is susceptible when direct contact with pathogens occurs in non-intact areas damaged by cuts, punctures, or abrasions (eg, cutaneous anthrax).

Psychological Manifestations

Biological agents can produce a patient population of the "worried well": patients who have not been exposed but who may be experiencing fear, anxiety, or panic. Expect numerous indirect casualties from psychogenic illnesses when there is an actual or perceived biowarfare threat. Educating and counseling troops about the threat, instituting appropriate countermeasures, and employing stress control teams early on can significantly reduce combat stress reactions and lessen demand for medical services.

Recognition of a Biological Weapons Attack

Biowarfare uses unconventional weapons, and most methods of dissemination are covert and difficult to identify early on. Outdoors, aerosols are most likely to be sprayed during the hours of limited visibility between dusk and dawn. At night, ultraviolet exposure that can kill the organisms is minimal, and temperature inversions help to keep aerosols near ground level with less dispersion. Indoors, bio-aerosols elude the senses by being essentially odorless, tasteless, and invisible. Agents used to contaminate food and water just prior to consumption are very difficult to detect.

Aerosols can be disseminated by jets, missiles, crop dusters, agricultural sprayers, and modified chemical warfare munitions, such as artillery shells, bomblets, or mines. More covert dissemination techniques by individuals may involve the use of hand-pumped sprayers, aerosol spray cans, modified fire extinguishers, small aerosol generators with timers, and sprayers placed on automobiles or boats to look like exhaust emissions.

Appearance of Weaponized Agents

Unlike many chemical agents, liquid biowarfare agents are non-oily and are typically translucent and slightly more viscous than milk. Unless dyed, bacterial agents in the form of a liquid or powder will likely have a light brown or amber appearance. Only sophisticated production and purification methods will

produce a white bacterial powder. Dried and liquid viral agents can be one of several colors depending on their growth medium: off-white, yellow, brown, or pinkish-red.

Detection

Limited real-time aerosol detection in the field is technically possible with the Biological Integrated Detection System, which a small number of units may have access to (these systems are not widely available because of cost and limitations such as being affected by wind and terrain). For efficient environmental detection, teamwork is necessary. Rapid identification of biowarfare agents is also possible if samples from suspicious ordinance, spraying devices, residues, or powders can be examined. Chemical, biological, radiological, and nuclear reconnaissance teams collect aerosol samples. Preventive medicine personnel collect water samples suspected to be contaminated. Veterinary personnel collect suspect food samples and animal specimens. Medical personnel collect patient specimens. Supporting laboratories evaluate samples for evidence of pathogenic organisms or biotoxins.

Sample Processing

The human respiratory system acts like a suction and filtration system for air, making the nares, cheeks, and hairy portions of the face of people exposed to an aerosol good locations to find the organisms. Taking early postexposure samples with synthetic swabs made out of rayon is preferable, but field expedient sampling with cotton swabs will also work.

Quick tests can be performed in the field to help rule in or out the possibility of a biological exposure. Samples can be tested with litmus paper or a pH meter. If the pH is near neutrality (7.0), viable organisms could be present. The greater the acidity or alkalinity, the less likely it is that any human pathogens will be found. Qualitative ninhydrin tests may show the presence of amino acids. If pH and protein tests are consistent with biological agents, samples may be sent to a lab for further testing. If the sample is unlikely to be a biological agent, it should still be evaluated as a potential chemical agent.

Clothing and equipment are unlikely to reveal significant contamination from an aerosol, but if suspicious powders or liquids are found, place samples of the contaminated material in double zip-locked plastic bags for further testing. All samples should be sent out as soon as the tactical situation permits, and thorough documentation should include person, place, time, and circumstances of possible exposure.

Soon after a biological or chemical attack, consider taking baseline serum samples of all personnel who may have been exposed. Serial serum samples may show changes over time, which helps with the identification of biological and chemical agent exposure. The results may be useful later to epidemiologists evaluating postdeployment syndromes, and assist casualties applying for service-related disability claims.

To take serum samples, draw 20 mL of blood into a tiger-top tube (if these are unavailable, use red-top tubes) and centrifuge for 10 minutes. If clinically indicated, throat swabs, aerobic and anaerobic cultures, sputum, urine, tissue, feces, scrapings, and other specimens should be submitted to a reference lab, where they may undergo various confirmatory tests such as ELISA (enzyme-linked immunosorbent assay), polymerase chain reaction, toxin assays, and microscopic examination.

Epidemiology

Medical intelligence sources may indicate that a biowarfare attack has occurred, prompting a unit to initiate education, vaccination, chemoprophylaxis, treatment, use of protective equipment, or other countermeasures to mitigate risk. In the absence of direct evidence of a biowarfare attack or solid medical intelligence indicating that an attack has occurred, recognition of biological agents must be part of an outbreak investigation seeking a common source of exposure.

The first indication that a biowarfare attack has occurred may be the appearance of a large number of personnel at sick-call presenting with similar signs and symptoms. Soldiers with increased susceptibility, or who were exposed to higher doses of the pathogens, become ill first and act as sentinel cases (or "canaries"). If medics maintain a high index of suspicion, early

diagnosis and treatment may save many lives. Animal illnesses and deaths can also precede human outbreaks and provide clues of a biowarfare attack. Consult with veterinary personnel if signs of suspicious animal deaths and illness occur. Epidemiologic clues of a possible biowarfare attack include the following:

- tight cluster of casualties,
- high infection rate,
- unusual geography,
- apparent aerosol route of infection,
- infection with more than one biowarfare agent,
- unusual clinical presentation,
- unusual munitions,
- animal epizootics,
- sentinel dead animals of multiple species, and
- lower attack rates among the protected.

Syndromic surveillance is the cornerstone of epidemiology. Medics contribute by accurately documenting patient care and disease-and-nonbattle-injury data, and assisting with predeployment and postdeployment health assessments. Many biological agents cause victims to present with nonspecific flu-like signs and symptoms, but some presentations will narrow the differential diagnosis:

- nonspecific symptoms: may indicate tularemia, brucellosis, Q-fever, or viral equine encephalitis (VEE)
- pneumonia: may indicate tularemia, plague, or SEB
- neuromuscular symptoms: may indicate botulism or VEE
- bleeding: may indicate ricin, plague, or viral hemorrhagic fever (VHF)
- dermatologic symptoms: may indicate smallpox, plague, VHF, or T-2 mycotoxin

Responding to a Biological Agent Attack

The immediate response to a suspected biowarfare aerosol should be the same as for a chemical agent exposure. An enemy may deploy chemicals in conjunction with biological agents or use multiple biological agents simultaneously. Mission-oriented

protective posture (MOPP) gear provides excellent protection against all biological agent aerosols. Because the primary routes of entry are through the mouth and nose, masking alone may be sufficient to prevent illness. Effective field expedient respiratory filters can be improvised by breathing through two or more layers of a T-shirt or several layers of tissue paper.

Decontamination

Bio-aerosols with small particles behave like gases and are readily inhaled into the lungs. Fortunately, these aerosolized pathogens are also very unlikely to adhere to people, clothing, or equipment. This reduces the need for extensive decontamination. Larger particles are much less infectious via the inhalation route and quickly fall to the earth, where they are more likely to adhere to surfaces and significantly reduce the risk of re-aerosolization or secondary contamination.

Whether in the air or on the ground, biowarfare agents undergo biological decay from environmental stresses such as ultraviolet light, temperature extremes, and desiccation. Winds of more than 30 mph promote rapid dilution, reducing the agents' effectiveness. With the exception of anthrax spores and the Q-fever agent, most biowarfare agents pose little threat of infection after approximately 24 hours of outdoor environmental exposure. These agents are considered "nonpersistent."

Decontaminate victims of chemical and biological agent exposure quickly and as close to the areas where they were contaminated as possible. Patients must be decontaminated prior to entering a clean treatment area at a medical treatment facility or being medically evacuated.

Field-expedient mass decontamination of biological casualties can be done in uncontaminated lakes and streams, large stationary or small portable swimming pools, and "water buffaloes" (trailer tanks) with hyperchlorinated water. Consider mass showering in fixed facilities, with fire and water trucks or garden hoses. When possible, use hot water if outdoor temperatures are cold.

Soap and water is sufficient for patient decontamination of most biowarfare agents. A 0.5% hypochlorite (bleach) solution is even more effective. Disinfect wounds with betadine or iodine if

available. Sterilize material as indicated with full-strength bleach, steam, boiling water, or dry heat. Thoroughly wash clothing and if possible allow it to be dried and decontaminated by the sun and wind.

Triage and Evacuation

Triage of biological casualties may differ significantly from that of chemical casualties. With chemical agents, expect a large number of casualties presenting at about the same time. With biological agents, the variable incubation period of pathogens and the delayed onset of action of many biotoxins mean that casualties may present over hours, days, or weeks. Therefore, most biological casualties will be triaged as "delayed" or "minimal" (see Chapter 9, Field Management and Triage of Contaminated Casualties on the Integrated Battlefield).

The evacuation category will depend on the patient's current status and prognosis. When calling in a 9-line medevac request during wartime, use brevity code "B" in line 9 for biological contamination. Continue to monitor casualties and change their triage category as needed. Provide reassurance and psychological support while the patient is awaiting further treatment or evacuation to a higher level of medical care.

Major Biological Agent Threats

Inhalational Anthrax (*Bacillus anthracis*)

Anthrax is an acute bacterial infection of the skin, lungs, or gastrointestinal (GI) tract. The primary threat is from an aerosol causing the inhalational (lung) form.

Characteristics. Lethal agent. Aerobic, spore-forming, rod-shaped, gram-positive bacterium. Case fatality rate (CFR) if untreated: 5% cutaneous, 30% GI, and 90% inhalational. Incubation periods are typically 1 to 6 days, with an average of 48 hours. Spores can survive in the environment and remain viable for many years.

Pathogenesis. Aerosolized agent enters the lungs and is engulfed by macrophages, in which spores germinate,

reproduce, and release toxins that cause cellular necrosis, edema, and hemorrhage in the lungs and mediastinal lymph nodes. Frequently spreads to the meninges. Death results from sepsis, hemorrhagic shock, or respiratory failure.

Symptoms. Malaise, fatigue, myalgia, headache, dyspnea, shortness of breath, chest pain.

Signs. Fever, cough, tachypnea, hypotension, meningismus, stridor, diaphoresis, cyanosis. Pathognomic widened mediastinum on chest x-ray secondary to hilar adenopathy and hemorrhagic mediastinitis.

Differential diagnosis. Pneumonia, pneumonic plague, tularemia, gram-negative sepsis, SEB inhalation.

Chemoprophylaxis. Oral ciprofloxacin or doxycycline for known or imminent exposure.

Chemotherapy. Ciprofloxacin 400 mg intravenously (IV) every 12 hours, or doxycycline 200 mg IV loading dose followed by 100 mg IV every 12 hours. Three or four different antibiotics simultaneously will be needed for definitive (hospital) care. Use oral ciprofloxacin, doxycycline, amoxicillin, or penicillin as tolerated if IV medications are not available in the field.

Precautions. Standard precautions only (no person-to-person transmission).

Prevention. Bio Thrax (Emergent Biosolutions, Gaithersburg, MD). Five-shot series given over an 18-month period, followed by yearly boosters. After a known exposure, start the series in unimmunized personnel and give booster shots to personnel who are not current in the series.

Cholera (*Vibrio cholerae*)

Cholera is an acute bacterial infection of the GI tract. The primary biowarfare threat is through sabotage of food and water supplies.

Characteristics. Incapacitating agent. Gram-negative, crescent-shaped, motile rod. Incubation period of 4 hours to 5 days (average, 2 to 3 days). Diarrheal illness usually lasts 3 to 5 days. Most illnesses are subclinical and the CFR is only 1% if casualties are treated appropriately. Without treatment, severe diarrhea can cause death within hours, and the CFR can be as high as 50%.

Pathogenesis. Agent enters the GI tract after consumption of contaminated food or water. Organisms adhere to the intestinal mucosa and secrete an enterotoxin, which elicits a secretory diarrhea that can lead to severe dehydration, electrolyte imbalances, hypovolemic shock, and death.

Symptoms. Painless diarrhea, abdominal discomfort, nausea, malaise, thirst, weakness.

Signs. Profuse "rice-water" stools, emesis, increased (or decreased) bowel sounds, no abdominal pain with palpation, fever, hypotension, dehydration, hypovolemic shock.

Differential diagnosis. *Shigella, Escherichia coli,* salmonellosis, *Campylobacter*, norovirus.

Chemoprophylaxis. Reserve antibiotics for symptomatic personnel.

Chemotherapy. Aggressive rehydration with oral rehydration solution or IV Ringer lactate. Doxycycline 100 mg by mouth twice a day for 3 days. Alternatives: sulfamethoxazole/trimethoprim (TMP-SMX), tetracycline, ciprofloxacin.

Precautions. Enteric precautions (person-to-person transmission unlikely).

Prevention. Secure approved food and water sources from sabotage. Enforce proper field sanitation and hygiene. Cholera vaccines have limited efficacy, and none are currently sold in the United States.

Plague (*Yersinia pestis*)

A bacterial disease known in the Middle Ages as the Black Death, plague has killed hundreds of millions. The primary threat is from an aerosol that causes primary pneumonic plague when inhaled. A secondary threat is from the release of infected fleas that cause bubonic plague, which can spread through the lymphatics and blood stream to cause septicemic plague.

Characteristics. Lethal agent. Bipolar "safety pin" appearance on microscopy. Incubation period 1 to 8 days (average, 2 to 4 days). If untreated, the CFR for bubonic plague is 60%, for pneumonic plague it is over 95% (if treated, the CFR is 50% for both).

Pathogenesis. Aerosolized agent enters the lungs, where virulent antigens can cause a necrotizing pneumonia. Organisms

traveling through the bloodstream can lead to sepsis, spread to the liver, spleen, and central nervous system, and cause further damage. Death results from respiratory failure, circulatory collapse, or internal bleeding.

Flea bites lead to inflamed, hemorrhagic, and extremely painful lymph nodes called "buboes." Organisms travel from the lymphatics to the blood stream, leading to systemic disease.

Symptoms. Fever, chills, malaise, chest pain, swollen and painful lymph nodes (buboes), headache, meningismus.

Signs. High fever, buboes, severe pneumonia, cough, hemoptysis, cyanosis, convulsions, shock, hemorrhagic skin changes and blackening of skin at extremities, disseminated intravascular coagulation (DIC), septic shock. Chest x-ray is variable, but will likely show patchy or consolidated bilateral infiltrates.

Differential diagnosis. Pneumonia, acute respiratory distress syndrome (ARDS), meningitis.

Chemoprophylaxis. Doxycycline 100 mg by mouth twice a day for 7 days. Alternatives: ciprofloxacin, tetracycline (TCN).

Chemotherapy. Streptomycin, IV or intramuscular (IM). Alternatives: 200 mg IV doxycycline once, then 100 IV twice a day for 14 days, gentamicin, ciprofloxacin. If IV/IM medications are not available in the field, use oral medications.

Precautions. Standard precautions for bubonic plague, respiratory droplet precautions for suspected pneumonic plague.

Prevention. No Food and Drug Administration (FDA) vaccine is currently licensed in the United States.

Q-Fever (*Coxiella burnetti*)

Q-fever is an acute and occasionally chronic rickettsial disease that presents as a nonspecific febrile illness or atypical pneumonia. The threat is from an aerosol or the contamination of food.

Characteristics. Incapacitating agents are highly infectious and environmentally persistent rickettsial organisms, with an incubation period of 7 to 41 days (average, 2 to 3 weeks). The acute form is a self-limited febrile illness for 2 to 14 days.

Pathogenesis. Agent enters through the lungs or GI tract, replicates within phagolysosomes, and spreads throughout the

body, eliciting systemic illness and numerous nonspecific signs and symptoms.

Symptoms. Severe headache, chills, myalgia, and fatigue. Less common symptoms are nausea, vomiting, diarrhea, and abdominal and chest pain.

Signs. High fever, dry cough, sweats, and myalgia. Pulmonary embolism of chest is usually normal, but inspiratory rales may be present, and consolidation may be seen on chest x-ray.

Differential diagnosis. Atypical pneumonias, bacterial and viral pneumonias.

Chemoprophylaxis. Doxycycline 100 mg by mouth twice a day for 5 days. Alternative: TCN.

Chemotherapy. Antibiotic therapy should be started 8 to 12 days postexposure if possible. Doxycycline 100 mg by mouth twice a day for 14 to 21 days. Alternatives: TCN, ciprofloxacin, TMP-SMX.

Precautions. Standard precautions. Person-to-person transmission is rare, but secondary aerosols from fomites, such as blankets, can spread the disease.

Prevention. Secure approved food and water sources from sabotage. Enforce proper field sanitation and hygiene. No FDA-approved vaccines are currently available in the United States.

Smallpox (*Variola major*)

Smallpox is a systemic viral illness that killed millions before it was eradicated from nature in 1980. The primary threat is aerosol release of smallpox from covert bio-weapons that may still exist.

Characteristics. Lethal agent, highly contagious virus with a 30% CFR. Incubation period is 7 to 19 days (average, 12 days). Duration of illness is 4 weeks.

Pathogenesis. Aerosolized agent enters the lungs. Replication within cells can lead to an overwhelming viremia, high levels of circulating immune complexes, with illness and death attributed to toxemia.

Symptoms. Malaise, rigors, headache, backache.

Signs. Macular-papular rash that progresses to characteristic vesicular pustules, which become scabs and scars; high fever; vomiting; prostration; delirium.

Differential diagnosis. Chickenpox, monkeypox, allergic contact dermatitis, erythema multiforme with bullae.

Chemoprophylaxis. None.

Chemotherapy. No effective medications exist, treatment is supportive only.

Precautions. Respiratory precautions, strict quarantine of patients and close contacts.

Prevention. ACAM2000 vaccine (Sanofi Pasteur, Swiftwater, PA) is very effective when given prior to exposure. If given after an exposure, but prior to onset of symptoms, the vaccine can prevent or significantly reduce the severity of disease.

Tularemia (*Francisella tularensis*)

Tularemia is bacterial disease with multiple manifestations depending on the portal of entry. The pneumonic and typhoidal forms can occur after an aerosol exposure and have a CFR of 30% to 60% if untreated.

Characteristics. Lethal agent. Highly infectious, gram-negative coccobacillus. Incubation period is 1 to 21 days (average, 3 to 6 days).

Pathogenesis. Agent can enter and infect through all three portals of entry, causing local lymphadenopathy, ulcerations, and a fulminating sepsis leading to death.

Symptoms. Fever, chills, malaise, myalgia, fatigue, respiratory distress.

Signs. Fever, tachycardia, tachypnea, nonproductive cough, mucous membrane lesions, hypotension, prostration, sepsis. Chest x-ray may show effusions, lobar consolidation, cavitation, or hilar adenopathy.

Differential diagnosis. Pneumonia, gram-negative sepsis, mononucleosis, rickettsial diseases, malaria, ARDS.

Chemoprophylaxis. Ciprofloxacin 500 mg by mouth twice a day for 14 days. Alternatives: doxycycline, TCN.

Chemotherapy. Ciprofloxacin 400 mg IV every 12 hours for 10 days. Alternatives: IV/IM doxycycline, TCN, streptomycin, gentamicin. Use oral ciprofloxacin or doxycycline if the IV/IM form is not available in the field.

Precautions. No person-to-person transmission. Standard precautions (minimum infection prevention practices).

Prevention. No FDA-approved vaccine is available in the United States.

Viral Hemorrhagic Fevers

VHFs include a variety of viruses that cause fever and bleeding of varying severity. Examples are Ebola virus; Marburg virus; Hantavirus; dengue; yellow fever; Lassa fever; Rift Valley fever; Crimean-Congo hemorrhagic fever; and Argentinean, Bolivian, Venezuelan, and Korean hemorrhagic fevers. The threat is that these viruses may be weaponized for aerosol dispersal.

Characteristics. Includes lethal and incapacitating agents. Incubation period can be days to months. CFR ranges from less than 10% (hemorrhagic fever with renal syndrome) to 90% (Ebola). Definitive diagnosis is possible only with sophisticated lab tests not readily available in the field.

Pathogenesis. Poorly understood and varies among the viruses.

Symptoms. Fever, myalgia, malaise, fatigue, prostration.

Signs. Fever, conjunctival injection, petechiae, hypotension. Severe illness may have shock, multiple organ system failure, DIC, and death.

Differential diagnosis. Other viral syndromes.

Chemoprophylaxis. Ribavirin 500 mg by mouth four times a day for 7 days, if available; may be somewhat useful for postexposure prophylaxis with some VHF agents.

Chemotherapy. IV ribavirin, if available, may have some efficacy.

Precautions. Contact isolation and possible quarantine. Droplet precautions include mask and eyewear use and thorough disinfection.

Prevention. The only FDA-licensed VHF vaccine is for yellow fever.