Clinical Recognition and Management of Patients Exposed to Biological Warfare Agents

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Concern regarding the use of biological agents—bacteria, viruses, or toxins—as tools of warfare or terrorism has led to measures to deter their use or, failing that, to deal with the consequences. Unlike chemical agents, which typically lead to violent disease syndromes within minutes at the site of exposure, diseases resulting from biological agents have incubation periods of days. Therefore, rather than a paramedic, it will likely be a physician who is first faced with evidence of the results of a biological attack. We provide here a primer on 10 classic biological warfare agents to increase the likelihood of their being considered in a differential diagnosis. Although the resultant diseases are rarely seen in many countries today, accepted diagnostic and epidemiologic principles apply; if the cause is identified quickly, appropriate therapy can be initiated and the impact of a terrorist attack greatly reduced.

THE BREAKUP of the Soviet Union, the perceived dominance of the United States as a conventional military world power, and the rise of radical groups focused on destroying what they believe to be evil have raised concern regarding the use of biological warfare (BW) against military forces in combat and even as a new tool of terrorists against civilians.

The potential impact of biological weapons is well illustrated by a 1970 World Health Organization (WHO) publication.1 It is estimated that 50 kg of aerosolized Bacillus anthracis spores, for example, dispensed by an airplane 2 km upwind of a population center of 50 000 unprotected people in ideal meteorological conditions would travel more than 20 km and kill or incapacitate up to 220 000 people, nearly half of those in the path of the biological cloud. If Francisella tularensis were dispensed, the number of dead or incapacitated would be about 155 000. Thus, if properly used as offensive weapons under ideal meteorological conditions, certain biological agents could cause mass casualties.

In addition to their detrimental health effects on the targeted population, the hostile use of BW agents would be likely to cause significant impacts on the health care system. Patients would present in unprecedented numbers, and demands for intensive care might overwhelm medical resources. Special medications or vaccines not generally available in standard pharmaceutical stocks potentially would be required. Health care professionals and laboratory personnel might need added physical protection, and autopsy and interment of remains could present unusual hazards.

The medical response to the threat or use of biological weapons differs depending on whether medical measures are used before exposure or after exposure and whether symptoms are present. If provided before exposure, active immunization or prophylaxis with antibiotics may prevent illness. Active immunization is probably the best modality for future protection of military forces against a wide variety of biological threats. For civilian populations, preexposure medical countermeasures would likely not be used. After exposure, but before symptoms arise, active or passive immunization, as well as pretreatment with therapeutic antibiotics or antiviral drugs, may ameliorate disease symptoms. After onset of illness, only diagnosis of the disease and general supportive care plus specific medical treatment are left to health care providers. Effective vaccines and antitoxins exist for several of the most likely BW agents. Additional vaccines and new therapies are under development.

Information on diagnostics, medical management, and vaccines is available by contacting Commander, USAMRIID, at 301-619-2833 . . .

Bacteria, viruses, or toxins (of microbial, plant, or animal origin) may be used as BW agents. Examples of microbial agents and toxins that could be used as BW agents include B anthracis (anthrax), botulinum toxin, Yersinia pestis (plague), staphylococcal enterotoxin B (SEB), and Venezuelan equine encephalitis (VEE) virus. Despite the very different characteristics of these organisms and toxins, these agents used as weapons share some common characteristics. They can be dispersed in aerosols of particle size approximately 1 to 10 µm, which may remain suspended (in certain weather conditions) for hours and, if inhaled, can penetrate into distal bronchioles and terminal alveoli of the exposed. The aerosols may be delivered by simple technology, including industrial sprayers with nozzle and energy source modi-
<table>
<thead>
<tr>
<th>Agent</th>
<th>Infective Dose (Aerosol)</th>
<th>Incubation Period</th>
<th>Diagnostic Samples (BSL)**</th>
<th>Diagnostic Assay</th>
<th>Patient Isolation Precautions</th>
<th>Chemotherapy (Rx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthrax</td>
<td>8000 to 50,000 spores</td>
<td>1-5 d</td>
<td>Blood (BSL-2)</td>
<td>Gram stain</td>
<td>Standard precautions</td>
<td>Ciprofloxacin 450 mg IV q 12 h</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>Ag ELISA,</td>
<td></td>
<td>Doxycycline 200 mg IV, then 100 mg IV q 8-12 h</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serology:</td>
<td></td>
<td>Penicillin 2 million units IV q 2 h plus streptomycin 30 mg/kg IM qd (or gentamicin)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>agglutination</td>
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<td></td>
<td></td>
<td></td>
<td>Culture</td>
<td></td>
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<tr>
<td>Brucellosis</td>
<td>10-100 organisms</td>
<td>5-60 d (occasionally months)</td>
<td>Blood, bone marrow, acute and convalescent sera (BSL-3)</td>
<td>Serology:</td>
<td>Standard precautions contact isolation if draining lesions present</td>
<td>Doxycycline 200 mg PO plus rifampin 600-900 mg/d PO q 6 wk</td>
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<td></td>
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<td>agglutination</td>
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<td></td>
<td></td>
<td></td>
<td>Culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plague</td>
<td>100-500 organisms</td>
<td>2-3 d</td>
<td>Blood, sputum, lymph node aspirate (BSL-2/3)</td>
<td>Gram or Wright-Giensa Stain</td>
<td>Standard precautions</td>
<td>Streptomycin 30 mg/kg IM qd in 2 divided doses x 10 d (or gentamicin)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Ag-ELISA,</td>
<td></td>
<td>Doxycycline 200 mg IV then 100 mg IV q 12 h x 10-14 d</td>
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<td></td>
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<td></td>
<td>Culture</td>
<td></td>
<td>Chloramphenicol 1 g IV q 6 h x 10-14 d</td>
</tr>
<tr>
<td>Q fever</td>
<td>1-10 organisms</td>
<td>10-40 d</td>
<td>Serum (BSL-2/3)</td>
<td>Serology:</td>
<td>Standard precautions</td>
<td>Tetracycline 500 mg PO q 6 h x 5-7 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ELISA, IFA</td>
<td></td>
<td>Doxycycline 100 mg PO q 12 h x 5-7 d</td>
</tr>
<tr>
<td>Tularemia</td>
<td>10-50 organisms</td>
<td>2-10 d</td>
<td>Blood, sputum, EM of tissue (BSL-2)</td>
<td>Culture</td>
<td>Standard precautions</td>
<td>Streptomycin 30 mg/kg IM qd x 10-14 d</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Serology:</td>
<td></td>
<td>Gentamicin 3-5 mg/kg/d</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>agglutination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smallpox</td>
<td>Assumed low (10-100 organisms)</td>
<td>7-17 d</td>
<td>Pharyngeal swab, scab material (BSL-4)</td>
<td>ELISA, PCR, virus isolation</td>
<td>Airborne precautions</td>
<td>Cidofovir (effective in vitro)</td>
</tr>
<tr>
<td>Viral encephalitides</td>
<td>10-100 organisms</td>
<td>VEE, 2-6 d EEE/EWE, 7-14 d</td>
<td>Serum</td>
<td>VEE (BSL-3)</td>
<td>Standard precautions (mosquito control)</td>
<td>Supportive therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EEE (BSL-2)</td>
<td></td>
<td>analgesics, anticonvulsants as needed</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>WEE (BSL-2)</td>
<td></td>
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<tr>
<td>Viral hemorrhagic fevers</td>
<td>1-10 organisms</td>
<td>4-21 d</td>
<td>Serum, blood</td>
<td>Most viral hemorrhagic fevers (BSL-4)</td>
<td>Viral isolation</td>
<td>Supportive therapy Ribavirin (CCHF/arenaviruses)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RT-PCR, Ag-ELISA RT-PCR</td>
<td>Serology: Ab-ELISA</td>
<td>Ribavirin 30 mg/kg IV initial dose 15 mg/kg IV q 6 h x 4 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>culture</td>
<td></td>
<td>7.5 mg/kg IV q 8 h x 6 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serology:</td>
<td></td>
<td>Antibody passive for AHF: BHF, Lassa fever, and CCHF</td>
</tr>
<tr>
<td>Botulimum</td>
<td>0.001 µg/kg (type A)</td>
<td>1-5 d</td>
<td>Nasal swab (possibly) (BSL-2)</td>
<td>Ag-ELISA, Mouse neutral</td>
<td>Standard precautions</td>
<td>DOD heptavalent antitoxin for (Serotypes A-G) (IND)</td>
</tr>
<tr>
<td>Staphylococcal enterotoxin B</td>
<td>30 ng/person (incapacitating); 1.7 µg/person lethal</td>
<td>1-6 h</td>
<td>Nasal swab, serum, urine (BSL-2)</td>
<td>Ag-ELISA, Serology: Ab-ELISA</td>
<td>Standard precautions</td>
<td>Ventilatory support and supportive care</td>
</tr>
</tbody>
</table>

*Information on diagnostics, medical management, and vaccines is available by contacting Commander, USAMRIID, at 301-619-2833 (phone) or 301-619-4625 (fax). Readers are advised to consult product literature before administering drugs or vaccines. BSL indicates biosafety level; Rx, chemotherapy; Px, chemoprophylaxis; Ag, antigen; ELISA, enzyme-linked immunosorbent assay; IV, intravenously; q, every; IM, intramuscular; qd, each day; PO, by mouth; IFA, immunofluorescent assay; IND, Investigational New Drug; SC, subcutaneous; EM, electron microscopy; PCR, polymerase chain reaction; VIG, vaccinia immune globulin; DOD, Department of Defense; VEE, Venezuelan equine encephalitis; EEE, eastern equine encephalitis; WEE, western equine encephalitis; NA, not available; RVF, Rift Valley fever; KHF, Korean hemorrhagic fever; YF, yellow fever; RT-PCR, reverse transcriptase polymerase chain reaction; Ab, antibody; CCHF, Congo-Crimean hemorrhagic fever; AHF, Argentine hemorrhagic fever; BHF, Bolivian hemorrhagic fever; CDC, Centers for Disease Control and Prevention.

fied to generate the smaller particle size. The aerosol could be delivered from a line source, such as an airplane or boat, traveling upwind of the intended target or from a point source, such as a stationary sprayer or missile bomblets, containing agent in an area upwind of the target. The meteorological conditions in the target area are very important in the use of BW agents as aerosols because higher wind speeds and turbulence tend to break up the aerosol cloud. Other possible routes of exposure for BW agents include oral, by intentional contamination of food and water, and percutaneous. In general, these other routes of exposure are considered less important than the respiratory route in the context of strategic use of BW agents. However, terrorists may not be constrained by either the agent characteristics or the route of exposure required on the biological battlefield. Diseases produced by the offensive use of biological agents against military forces or civilians could be disabling or lethal. Because biological agents, inca-
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pacitating or lethal, produce a more pro-

longed period of illness than chemical,

agents, the impact on the health care in-

frastructure could be enormous. Person-
to-person spread could be important for

some agents, and local disease cycles

might occur if a competent vector for a

pathogen is present in the en-

vironment. The following is an overview

of several BW threat agents, the disease

syndromes resulting from exposure to

them, and medical countermeasures

available to clinicians (see Table).

ANTHRAX

History and Significance

Anthrax is caused by B anthracis, a

gram-positive, sporulating bacillus. The

reservoir of B anthracis is the soil; the

organism is distributed worldwide.23

The organism exists in the infected host

as the vegetative bacillus and in the en-

vironment as a spore. Spores do not

form in the infected host unless the body

tissues are exposed to air. Anthrax

spores can survive adverse environmen-
tal conditions and can remain viable for
decades. The spore is the stage of the

bacterial life cycle that is the usual in-

fective form. Animals contract spores

while grazing. Susceptible animals

include cattle, sheep, goats, and horses,

but other animals may develop infection.

Humans contract anthrax via inocula-
tion of minor skin lesions with spores

from contact with infected animals, their

hides, wool, or other products, from

ingesting contaminated meat, from in-
haling spores during the processing of

wool for textiles, or possibly from biting
flies.8

Anthrax spores were weaponized by

the United States in the 1950s and 1960s

before the US offensive program was ter-
mminated. Iraq admitted to a United Na-
tions inspection team in August 1991 that

it had conducted research on the offensive

use of B anthracis before the Persian Gulf

War and, in 1995, admitted to “weaponiz-
ing” anthrax. Other countries have also

been suspected of weaponizing anthrax

spores. The deaths of at least 66 people

after an accidental release of anthrax

spores in the former Soviet Union under-

covers the weapons potential of this

agent.4

Clinical Features

Anthrax has 3 clinical presentations

in humans: cutaneous, gastrointestinal,

and inhalational.5,6 A biological attack

with anthrax spores would most likely

occur by aerosol delivery and would re-

sult in inhalational anthrax. This illness,

known as woolsorter’s disease, occurs in

the textile and tanning industries among

workers handling contaminated wool,

hair, and hides.7 After being inhaled and
deposited in the lower respiratory tract,

spores are phagocytized by tissue macro-

phages and transported to hilar and

mediastinal lymph nodes. The spores

germinate into vegetative bacilli, pro-
ducing a necrotizing hemorrhagic medi-

astinitis.5,8

Inhalation anthrax begins with a pro-
drome featuring fever, malaise, and fa-
tigue. A nonproductive cough and vague

chest discomfort may be present. This

prodrome may be followed by symptom-

tic improvement for 2 to 3 days or may

progress directly to the abrupt onset of

severe respiratory distress with dysp-
nea, stridor, diaphoresis, and cyanosis.

Bacteremia, septic shock, metastatic in-
fec tion (meningitis in approximately
half of cases), and death usually follow

within 24 to 36 hours.6,7 Once symptoms

of inhalational anthrax appear, treat-

<table>
<thead>
<tr>
<th><strong>Chemoprophylaxis (Px)</strong></th>
<th><strong>Vaccine Availability</strong></th>
<th><strong>Comments</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin 500 mg PO</td>
<td>Michigan Biological Products</td>
<td>Vaccine: boost at-risk annually</td>
</tr>
<tr>
<td>bid × 4 wk if unvaccinated, begin initial doses of vaccine</td>
<td>Institute vaccine (licensed): 0.5 mL SC at 0, 2, 4 wk and 6, 12, 18 mo, then annual boosters</td>
<td>Alternates for Rx: gentamicin, erythromycin, and chloramphenicol</td>
</tr>
<tr>
<td>Doxycycline and rifampin for 3 wk in inadvertently inoculated persons</td>
<td>No vaccine available for human use</td>
<td>Trimethoprim-sulfamethoxazole may be substituted for rifampin; however, relapse rate with this drug may be up to 30%</td>
</tr>
<tr>
<td>Tetracycline 500 mg PO</td>
<td>Greer inactivated vaccine</td>
<td>Boost at-risk 12, 18 mo &amp; yearly</td>
</tr>
<tr>
<td>qid × 7 d</td>
<td>(licensed): 1 mL, then 0.2 mL boost at 1-3 and 3-6 mo</td>
<td>Plague vaccine not protective against aerosol in animal studies</td>
</tr>
<tr>
<td>Doxycycline start 8-12 d</td>
<td>Boost at-risk 12, 18 mo &amp; yearly</td>
<td>Alternate Rx: chloramphenicol or trimethoprim-sulfamethoxazole</td>
</tr>
<tr>
<td>postexposure × 5 d</td>
<td>Tetracycline start 8-12 d</td>
<td>Rx: chloramphenicol for plague meningitis</td>
</tr>
<tr>
<td>Doxycycline start 8-12 d</td>
<td>IND 610-inactivated whole cell vaccine given as single 0.5 mL SC</td>
<td>Recommend skin test before vaccination</td>
</tr>
<tr>
<td>postexposure × 5 d</td>
<td>Tetracycline start 8-12 d</td>
<td>文化的 difficult and potentially dangerous</td>
</tr>
<tr>
<td>Doxycycline 100 mg PO</td>
<td>Live attenuated vaccine (IND): scarification</td>
<td></td>
</tr>
<tr>
<td>q 12 h × 14 d</td>
<td>Vaccinia immune globulin 0.6 mL/kg IM</td>
<td></td>
</tr>
<tr>
<td>Tetracycline 2 g/d PO</td>
<td>Wyeth calf lymphp vaccinia vaccine (licensed)</td>
<td></td>
</tr>
<tr>
<td>q1 2 h</td>
<td>DOD cell-culture derived vaccinia vaccine (IND): scarification</td>
<td></td>
</tr>
<tr>
<td>Vaccine used for nonresponders</td>
<td>Preexposure and postexposure vaccination recommended if &gt;3 y since last vaccination</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>VEE DOD TC-83 live attenuated vaccine (IND): 0.5 mL SC</td>
<td></td>
</tr>
<tr>
<td>VEE DOD C-84 (formalin inactivated TC-83)(IND): 0.5 mL SC for up to 3 doses</td>
<td>TC-83 reagentigenic in 20%</td>
<td></td>
</tr>
<tr>
<td>EEE inactivated (IND): 0.5 mL</td>
<td>No seroconversion in 20%</td>
<td></td>
</tr>
<tr>
<td>SC at 0 &amp; 2, 8 &amp; 12 wk, then yearly boosters</td>
<td>Only effective against subtypes 1A, 1B, and 1C</td>
<td></td>
</tr>
<tr>
<td>WEE inactivated (IND): 0.5 mL SC at 0, 7, and 28 d</td>
<td>Vaccine used for nonresponders to TC-83</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>AHH Candid #1 vaccine</td>
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</tr>
<tr>
<td>NA</td>
<td>AHF calf lymphp vaccinia vaccine (licensed)</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>DOD pentavalent Toxoid for serotypes A-6 (IND): SC at 0, 2, &amp; 12 wk, then yearly boosters</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>No vaccine available</td>
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</tbody>
</table>

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ment is almost invariably ineffective, although there are anecdotal reports of patients surviving after early, aggressive therapy.5,7

Diagnosis and Management

Physical findings are usually nonspecific. The chest x-ray film is typically without infiltrates but may reveal a widened mediastinum with pleural effusions, which may be hemorrhagic. Meningitis, often hemorrhagic, has been reported in up to 50% of cases.8 Bacillus anthracis can be visualized by Wright or Gram stain of peripheral blood and isolated by blood cultures but often not until late in the disease course. Vegetative bacilli are present during infection and sporation does not occur in vivo. Animal studies of inhalational anthrax demonstrate that bacilli and toxin appear in the blood late on day 2 or early on day 3 after aerosol challenge. Toxin levels parallel the development of bacteremia. An enzyme-linked immunosorbent assay (ELISA) to detect circulating toxin is available for rapid diagnosis.

Historically, penicillin has been the treatment of choice for inhalational anthrax, with 2 million units given intravenously every 2 hours. Some animal studies suggest that addition of streptomycin may have additional benefit. All naturally occurring strains tested to date have been sensitive to erythromycin, chloramphenicol, gentamicin, and ciprofloxacin. In the absence of antibiotic sensitivity data, treatment should be instituted at the earliest signs of disease with intravenous ciprofloxacin (400 mg every 8-12 hours). Supportive therapy for shock, fluid volume deficit, and adequacy of airway may be indicated.

A licensed vaccine, an aluminum hydroxide–adsorbed preparation, is derived from culture fluid supernatant taken from an attenuated strain.9 The vaccination series consists of 6 subcutaneous doses at 0, 2, and 4 weeks, then at 6, 12, and 18 months, followed by annual boosters. There are insufficient data regarding efficacy against inhalational anthrax in humans, although studies in rhesus monkeys indicate it is protective. If there is information indicating that a BW attack is imminent or may have occurred, prophylaxis of unimmunized individuals with ciprofloxacin (500 mg by mouth twice a day), or doxycycline (100 mg by mouth twice a day) is recommended.6 The vaccination series should be initiated for unimmunized individuals. Should an anthrax attack be confirmed, chemoprophylaxis should be continued for at least 4 weeks and until at least 3 doses of vaccine have been received by all those exposed.

BRUCELLOSIS

History and Significance

Brucellae are small, slow-growing, pleomorphic, gram-negative aerobic nontoxigenic, non–spore-forming cocci-bacilli. Although the 6 species of Brucella are closely related,9 they each characteristically infect different animal hosts, in which they usually cause infertility or abortion. Of the 4 species pathogenic for humans, Brucella melitensis usually infects goats, Brucella suis infects swine, Brucella abortus infects cattle, and Brucella canis infects dogs. A pattern of disease severity in humans is as follows: B melitensis > B suis > B abortus > B canis. Most human infections occur by contact with infected animal tissues or ingestion of contaminated raw meat or dairy products. Person-to-person transmission typically does not occur. The bacteria are highly infectious by aerosol and commonly cause infections in laboratory workers.10 Brucellae are susceptible to commonly used disinfectants and heat but may survive for 6 weeks in dust and 10 weeks in soil or water.

The United States weaponized B suis in the 1940s and 1950s but stopped offensive work on the agent in the 1960s. Other countries have or are suspected to have weaponized brucellae.22 The organism could be delivered as a slurry in bomblets or, theoretically, as a dry aerosol.

Clinical Features

Brucellae are facultative intracellular macrophage parasites, and localize in organs (especially the lung, spleen, liver, central nervous system, bone marrow, and synovium) with large numbers of macrophages.24 Disease manifestations reflect this distribution. Symptoms and signs are similar in patients with presumed oral, aerosol, or percutaneous infection. Patients usually have fever, chills, and malaise.14 Respiratory symptoms (cough, pleuritic chest pain) may occur in 20% of patients but do not usually denote pneumonia. Sacroiliitis, large joint infections, and vertebral osteomyelitis are the most common osteo-articular manifestations.6–15 Genitourinary infections and hepatitis may also occur.19 Endocarditis and central nervous system infections are rare, but account for nearly all fatalities, which occur in less than 5% of untreated patients.20 Systemic symptoms may last for weeks or months. Even without antibiotics, most patients recover within a year, but relapses are common.21 Hematological abnormalities, including anemia, neutropenia, and thrombocytopenia, may be present.22

Diagnosis and Management

Symptoms and signs of brucellosis are nonspecific. A serum tube agglutination test is the usual diagnostic method.23 Cultures of blood, bone marrow, and focal sites of infection may be positive.24 The organism grows slowly, but adequately, in conventional blood culture bottles. Cultures must be kept for at least 6 weeks with periodic blind subculturing onto enriched agar plates. A special biphasic culture technique (Cstanela bottle), if available, may facilitate Brucella isolation.25

Patients should be treated with combinations of antibiotics, as treatment with single agents leads to poor response or relapse. A combination of 200 mg/d of doxycycline orally and 600 to 900 mg/d of rifampin orally for 6 weeks is usually the treatment of choice.25–27 Trimethoprim-sulfamethoxazole may be substituted for rifampin. For bone and joint infections, endocarditis, and central nervous system disease, streptomycin or another aminoglycoside should be included, and therapy should be prolonged. Treatment of endocarditis may require valve replacement.28 There is no approved Brucella vaccine for humans.

PLAGUE

History and Significance

Yersinia pestis, the etiologic agent of plague, is a gram-negative bacillus of the family Enterobacteriaceae that is maintained in numerous and diverse rodent reservoirs.29,30 Plague is transmitted via fleas vectors from rodents to humans and by respiratory droplets from animals to humans or humans to humans.29–32

During World War II, Japan investigated the use of plague as a biological weapon. The United States studied Y pestis as a potential BW agent in the 1950s before the offensive BW program was terminated, and other countries have been suspected of weaponizing plague.

Clinical Features

The clinical presentations of plague are bubonic, primary septicemic, and pneumonic disease.29–31 The most likely clinical presentation after a BW attack would be primary pneumonic plague.29,30 After an incubation period of 2 to 3 days, patients present with pneumonia featuring the acute and often fulminant onset of malaise, high fever, chills, headache, myalgia, cough with production of a bloody sputum, and clinical sepsis. The chest x-ray film reveals a patchy or consolidated bronchopneumonia. Pneumonic plague progresses rapidly, resulting in dyspnea, stridor, and cyanosis. The terminal course may feature respiratory failure, shock, and ecchymoses.
Diagnosis and Management

A presumptive diagnosis can be made by identifying a gram-negative coccobacillus and safety-pin bipolar staining organism in gram-stained or Wright–Giemsastained smears from peripheral blood, lymph node needle aspirate, sputum, or other clinical specimens. Immunofluorescent staining for the capsule is diagnostic. The diagnosis can be confirmed by cultivating the organism from blood, sputum, and bubo aspirates. The organism grows slowly at standard incubation temperatures and may be misidentified by automated systems because of delayed biochemical reactions. Most strains of Y pestis produce F1 capsule antigen in vivo, which can be detected in serum samples by immunoassay. A 4-fold rise in antibody titer is also diagnostic.

Streptomycin sulfate, tetracycline, chloramphenicol, and gentamicin sulfate are therapies for bubonic plague, especially if begun within 24 hours of the onset of symptoms.30 Plague pneumonia is almost always fatal if treatment is not initiated within 24 hours of the onset of symptoms. Streptomycin is given intramuscularly in a dose of 30 mg/kg per day in 2 divided doses for 10 days. Gentamicin may be substituted for streptomycin. Chloramphenicol given intravenously is indicated for treating plague meningitis and in cases of circulatory compromise. Intravenous doxycycline (200 mg initially, followed by 100 mg every 12 hours) for 10 to 14 days is also effective. Results obtained from an animal model suggest that quinolones may be effective for treating plague, but they have not been evaluated in humans.31 Supportive therapy includes intravenous crystalloids and hemodynamic monitoring.

A licensed, killed whole-cell vaccine is available for use in those considered to be at risk of exposure.33 While epidemiologic evidence supports the efficacy of the current vaccine against bubonic plague, its efficacy against aerosolized Y pestis is believed to be poor.34

Q FEVER

History and Significance

Q fever, a febrile, zoonotic disease with a worldwide distribution, typically results from exposure to domestic livestock animals (mainly sheep, cattle, and goats). The infection is caused by Coxiella burnetii, an obligate intracellular rickettsialike organism of low virulence but remarkable infectivity.35 Coxiella burnetii produces a sporelike form that may cause infection after indirect exposure to infected animals or animal products, such as can occur in individuals who live or work in the vicinity of infected animals.36-38 In addition, the ability of this sporelike form to withstand heat and drying and to survive on inanimate surfaces allows the organism to persist in the environment for weeks or months after an infected animal has vacated an area and for dissemination by wind with induction of infection at sites miles distant from a source.38

Individuals are at risk for acquisition of Q fever, both in the United States and abroad.39-40 Q fever is currently recognized as a potential BW agent, with a degree of infectivity and casualty production rivalling that of anthrax.1 Coxiella burnetii was studied as a BW agent before the US BW program ended.41

Clinical Features

There is no single syndrome characteristic for acute Q fever, and the infection may be manifested as asymptomatic seroconversion in up to 50% of infections.34-44 The onset of Q fever may be abrupt or insidious, with fever, chills, and headache being the most common symptoms. Diaphoresis, malaise, fatigue, anorexia, and weight loss are also common. Myalgia is a frequent complaint, while arthralgia is less common. Cough tends to appear somewhat late in the illness and may not be a prominent complaint. Chest pain occurs in a minority of patients and may be pleuritic or a vague substernal discomfort. Although nonspecific evanescent skin eruptions have been reported, there is no characteristic rash. Temperature tends to fluctuate, with peaks of 39.4°C to 40.6°C, and approximately 25% of the cases are biphasic. In two thirds of patients with acute disease, the febrile period lasts 13 days or fewer.41 Neurological symptoms are not uncommon and have been observed in up to 23% of acute cases.44

Rales are the most common physical finding; evidence of pleural effusion (including friction rub) and consolidation may also be noted. Although hepatomegaly, splenomegaly, and jaundice have all been reported, they are relatively unusual in acute infection. Reports of abnormalities on chest radiograph vary with locale, but can be identified in 50% to 60% of symptomatic patients and may persist for several months.41 An abnormal chest radiograph may be seen in the absence of pulmonary symptoms, while a normal chest radiograph may be observed in a patient with pulmonary symptoms.38

Laboratory abnormalities associated with acute Q fever usually involve liver function tests, and patients may present with a clinical and laboratory picture consistent with acute hepatitis. Two- to 3-fold elevations of aspartate aminotransferase and/or alanine aminotransferase are observed in 50% to 75% of patients, while elevations of the alkaline phosphatase and/or total bilirubin are observed in only 10% to 15%.34 The white blood cell count is usually normal; mild anemia or thrombocytopenia may also be observed.

The case-fatality rate of acute Q fever is low, even without treatment, and chronic disease, usually manifested by endocarditis, probably develops in less than 1% of acute infections.36,45 Malaise and easy fatigability lasting for months after acute infection have been reported in up to 32% of patients.38

Diagnosis and Management

Diagnosis of Q fever is usually accomplished by serological testing; the most common methods are antibody detection by indirect fluorescent antibody (IFA) or ELISA. Significant antibody titers are not consistently identifiable until 2 to 3 weeks into the illness. Convalescent antibody titers, 2 to 3 months after onset of illness, typically demonstrate a 4-fold increase.46,47 After acute infection, significantly elevated antibody titers may persist for years.48 Chronic infection almost always induces significant antibody titers.49

Treatment of acute Q fever shortens the course of the disease and prevents disease when administered during the incubation period.48 Tetracyclines remain the mainstay of therapy for acute disease. Macrolide antibiotics, such as erythromycin and azithromycin, are also effective. Quinolones, chloramphenicol, and trimethoprim-sulfamethoxazole have also been used to treat Q fever, but clinical experience with these antibiotics is limited. Although an effective vaccine (Q-Vax) is licensed in Australia, all Q fever vaccines used in the United States are investigational.50-52 Individuals already immune to Q fever frequently develop severe local reactions at the site of vaccine injection.53,54 These reactions can be avoided by prior screening with an intradermal skin test to detect presensitized or immune individuals.52

TULAREMIA

History and Significance

Francisella tularensis, the etiologic agent of tularemia, is a small, nonmotile, aerobic, facultative intracellular gram-negative coccobacillus. Tularemia (also known as rabbit fever and deer fly fever) is a zoonotic disease, and humans acquire the disease under natural conditions through inoculation of skin or mucous membranes with blood or tissue fluids of infected animals or bites of infected deerflies, mosquitoes, or ticks. Although less common, inhaling contaminated dusts or ingesting contaminated foods or water may also produce clinical dis-
meat, hides, and for years in frozen rabbit meat.55

Francisella tularensis was weaponized by the United States in the 1950s and 1960s before the US offensive BW program was terminated, and other countries may have weaponized this agent for delivery by aerosol.

Clinical Features

Tularemia may appear in 2 forms in humans depending on the route of inoculation: ulceroglandular or typhoidal. In humans, as few as 10 to 50 organisms will cause disease if inhaled or injected intradermally. The most common ulceroglandular form is usually acquired through inoculation of the skin or mucous membranes with blood or tissue fluids of infected animals. The typhoidal form, which occurs mainly after inhalation of infectious aerosols, accounts for 15% to 25% of naturally occurring cases. Typhoidal or septicemic tularemia manifests as fever, prostration, and weight loss, but without adenopathy.55,57 Respiratory symptoms of substernal discomfort and a nonproductive cough may also be present. Radiological evidence of pneumonic involvement could produce a modified animal poxvirus with enhanced virulence for humans has raised the specter that other poxviruses besides smallpox might constitute serious BW or reemerging public health problems. Mass vaccination of civilian populations is now complicated by the increasing number of immunocompromised patients (eg, those with human immunodeficiency virus infection, organ transplant, and chemotherapy).

Diagnosis and Management

Diagnosis can be established by isolating the organism from blood, sputum, skin, or mucosal membrane lesions, but it is difficult due to unusual growth requirements and/or overgrowth of commensal bacteria. Diagnosis of primary typhoidal tularemia is also difficult because signs and symptoms are nonspecific and frequently there is no suggestive exposure history. The diagnosis can best be established retrospectively by serologic testing.51,52 Streptomycin (30 mg/kg per day intramuscularly in 2 divided doses for 10-14 days) is the treatment of choice.56 Gentamicin (3-5 mg/kg per day parenterally for 10-14 days) also is effective.57-59 Tetracycline and chloramphenicol are effective as well but are associated with significant relapse rates.60 Although laboratory-related infections with this organism are common, human-to-human spread is unusual and respiratory isolation is not required. A live attenuated tularemia vaccine is available as an Investigational New Drug (IND).

SMALLPOX

History and Significance

After the last natural case of variola in Somalia in 1977,51,52 smallpox was declared eradicated in 1980 by the WHO. Natural smallpox outbreaks were contained by rapid vaccination of contacts of the index cases, facilitated by the ease of vaccinia administration. There is no animal reservoir for variola; however, monkeys are susceptible to infection.61 Although a laboratory accident prompted the consolidation of variola virus stocks into 2 WHO-approved repositories at the Centers for Disease Control and Prevention (CDC) in Atlanta and at NPO (Scientific and Production Association) in the Novosibirsk region of Russia, the extent of clandestine stockpiles remains a matter of contention and concern.

The aerosol infectivity, high mortality, and stability of variola make it (and potentially monkeypox virus) a potential threat in BW and terrorism scenarios.62-65,66 Although some have argued that smallpox would have limited potential as a biological weapon,62,63,64 the discontinuation of routine vaccination has rendered civilian and military populations more susceptible to a disease that is infectious by aerosol and infamous for its devastating morbidity and mortality. In 1970, the WHO expressed concerns that smallpox “can easily be produced in large quantities in the laboratory and freeze-dried and its virulence thus preserved for months or years.”67 Other theoretical potential that genetic recombination could produce a modified animal poxvirus with enhanced virulence for humans has raised the specter that other poxviruses besides smallpox might constitute serious BW or reemerging public health problems. Mass vaccination of civilian populations is now complicated by the increasing number of immunocompromised patients (eg, those with human immunodeficiency virus infection, organ transplant, and chemotherapy).

Clinical Features

After aerosol exposure, variola travels from the upper or lower respiratory tract to regional lymph nodes where it replicates and gives rise to viremia followed soon thereafter by rash. During the prodrome before onset of pox lesions, variola virus can be recovered from the blood. The abrupt onset of clinical manifestations is marked by systemic toxicity with prominent malaise, fever, rigors, vomiting, headache, and backache; 15% of patients develop delirium. Approximately 10% of light-skinned patients exhibit an erythematous rash during this phase. Two to 3 days later, an enanthem appears concomitantly with a discrete rash about the face, hands, and forearms. The mucous membrane lesions shed infectious oropharyngeal secretions in the first few days of the eruptive illness.68 These respiratory secretions are the most important but not the sole means of virus transmission to contacts. After eruptions on the lower extremities, the rash spreads centrally to the trunk over the next week. Lesions quickly progress from macules to papules and eventually to pustular vesicles. Lesions are more abundant on the extremities and face, and this centrifugal distribution is an important diagnostic feature. In distinct contrast to varicella, lesions on various segments of the body remain generally synchronous in their stage of development. In the second week after onset, the pustules form scabs that leave depressed depigmented scars on healing. Although variola titers in the throat, conjunctiva, and urine diminish with time,69 virus can readily be recovered from scabs throughout convalescence. Therefore, patients should be isolated and considered infectious until all scabs separate.

During this past century, the prototype disease variola major caused mortality of 3% and 30% in the vaccinated and unvaccinated, respectively.62,63 Other clinical forms associated with variola major, flat-type and hemorrhagic-type smallpox, were notable for severe mortality. A naturally occurring relative of variola, monkeypox, occurs in Africa and is clinically indistinguishable from smallpox except for a notable enlargement of cervical and inguinal lymph nodes. Secondary bacterial pneumonia is associated with greater than 50% mortality.60 Concern has been raised whether monkeypox could be weaponized like variola. Although previous evidence suggested that monkeypox had limited potential for person-to-person transmission,70 recent reports indicate greater potential for sustained interhuman transmission,71 perhaps owing to declining vaccinia immunity of the populace.

Diagnosis and Management

Given the eradication of endemic smallpox, it requires an astute clinician to distinguish the forme fruste of this disease from other similar exanthems, such as chickenpox, erythema multiforme with bullae, or allergic contact dermatitis. Many exposed persons may shed virus
from the oropharynx without ever mani-
festing disease. Some close contacts may
harbor virus in their throats without de-
veloping disease and hence may serve as
a means of secondary transmission.72
Rapid and definitive diagnostic mea-
sures are urgently needed to provide
effective quarantine and countermea-
sures to avert panic.

The appearance of characteristic viri-
on electron microscopy or Guardneri
bodies under light microscopy26 is useful
but does not discriminate variola from
vaccinia, monkeypox, or cowpox. The
traditional method of isolating virus on
chorioallantoic membrane is antiquated.
Polymerase chain reaction diagnostic
techniques promise more accurate and
less cumbersome methods of discrimi-
nating between variola and other ortho-
poxviruses.74

Clinicians must be prepared to recog-
nize a vesicular exanthem in possible
BW theaters as potential variola and to
initiate appropriate countermeasures.
Any confirmed case of smallpox should
be considered an international emer-
gency with immediate report made to
public health authorities. Strict quaran-
tine with respiratory isolation should be
applied for 17 days to all persons in di-
rect contact with the index case, espe-
cially the unvaccinated. Immediate vac-
cination should be undertaken for all per-
sons exposed to either weaponized va-
riola virus or a clinical case of smallpox.
Nosocomial transmission of variola gen-
erally requires close person-to-person
proximity, but there is a potential for
airborne spread.75-77 Patients with small-
pox are infectious from the time of onset
of their eruptive exanthem, most com-
monly from days 3 to 6 after onset of
fever. Infectivity is markedly enhanced
if the patient manifests a cough. Indirect
transmission via contaminated bedding
or other fomites is infrequent.78

Although the antiviral drug methisa-
zone was licensed for the prophylaxis of
susceptible contacts of smallpox in the
1960s,79-82 its efficacy was controversial,
gastrointestinal intolerance limited its
use, and it is no longer available. Al-
though there is no chemotherapeutic
agent proven effective against small-
pox, cidofovir demonstrates broad in
vitro and in vivo activity against Pox-
viridae83 and might be useful as a thera-
petic agent. The US Army Medical Re-
search Institute of Infectious Diseases
(USAMRIID) is evaluating classes of
drugs that target 6 different functions
involved in poxvirus replication. Four
drugs (cidofovir dipivoxil, cidofovir
acicidofovir, and ribavirin) in advanced
clinical testing for other viral infections
showed significant in vitro antiviral ac-
tivity and may be candidates for the
treatment of systemic disease from a
poxvirus (J. W. Huggins, written com-
munication, July 1997).

Of the smallpox vaccines used dur-
ing the WHO smallpox eradication cam-
paign, only calf lymph vaccine (Dryvax,
Wyeth) is still available in the United
States. A replacement vaccine prepared
in cell culture by the Department of De-
fense is currently an IND product. Dur-
ing the WHO smallpox eradication cam-
paign,42,43,111-113 vaccination with a veri-
cified clinical “take” within the past 3
years was considered solid immunity to
smallpox. With longer intervals between
vaccination and subsequent variola ex-
posure, protection is reduced. Given the
potential for breakthrough against par-
tial immunity following high-dose aero-
sol exposure, routine revaccination of all
potentially exposed individuals would
seem prudent in a BW scenario. If vac-
cination is accomplished within a few
days after exposure, protection is also
possible,42 approaching complete protec-
tion in those who have had their primary
vaccination previously.84

VIRAL ENCEPHALITIDES
History and Significance

Venezuelan, eastern, and western
equine encephalitis viruses (VEE, EEE,
and WEE, respectively) are members of
the Alphavirus genus of the family To-
viridae. Several characteristics of the
encephalitis viruses lend them-
selves to weaponization.85 Although
naturally transmitted by mosquitoes,
the encephalitic alphaviruses are also
highly infectious by aerosol.86 These
viruses can be produced in large amounts
in inexpensive and unsophisticated sys-
tems and are relatively stable during
storage and manipulations. Readily
available strains may produce incapac-
tating or lethal infection. The alphavi-
ruses are amenable to genetic manipu-
lation by modern recombinant DNA
technology. This capability may be used
to develop safer and more effective vac-
cines.87

VIE has 11 distinct subtypes: IA, IB,
and IC subtypes are pathogenic for
horses and have the capacity for explo-
sopepidemics with epidemic human dis-
ease. The enzootic strains (subtypes ID,
IE, IF, II, III, IV, V, and VI) are not
virulent for equines but have transmis-
sion cycles involving rodents and Culex
mosquitoes of the genus Melanooco-
nium.88 Both EEE and WEE viruses are
classified into 2 distinct geographic
complexes.

The epidemiology of the equine en-
cephalitides in humans is closely tied to
the ecology of these viruses in naturally
occurring endemic foci.89 Evidence of
widespread human VEE infections out-
side known endemic areas, in the ab-
sence of mosquito vectors or equine dis-
cease, should be viewed with suspicion
and could indicate an unnatural release
of virus into the environment.

Clinical Features

The 3 equine encephalitis virus com-
plexes within the Alphavirus genus are
recognized for their potential for neuro-
invasion and encephalitis in humans,
sometimes in epidemic proportions.
However, the majority of infections
caused by these viruses are manifested
as systemic, viral febrile syndromes con-
sisting of fever, headache, and myalgia.

Therefore, in a potential BW scenario,
alphaviruses should be considered in the
differential diagnosis whenever epidemic
febrile illness occurs, especially with pro-
gression to neurological disease. Sick or
dying equines in the vicinity of an epi-
demic febrile disease should also suggest
the possibility of large-scale alphavirus
exposure. These alphaviruses vary mark-
edly in their neurological sequelae. De-
dpending on the virus producing it, the
general syndrome of alphavirus encepha-
ritis presents with a varying combination
of fever, headache, confusion, obtunda-
tion, dysphasia, seizures, paresis, ataxia,
myoclonus, and/or cranial nerve palsy.

An important characteristic of VEE as
a biological warfare weapon is that
especially all human infections are
symptomatic.90 Both epizootic and enzo-
otic VEE strains cause similar disease
syndromes. Patients develop a prostrat-
ing syndrome of chills, high fever (38°C
to 40.5°C), headache, and malaise. Pho-
tophobia, sore throat, myalgia, and vom-
iting also are common symptoms. How-
ever, only a small percentage of VEE
infections progress to neurological in-
volvement (0.5% to 4%).91 For those who
survive encephalitic involvement of
VEE, neurological recovery is usually
complete.92

Clinical presentation of EEE and
WEE infection is similar. Adults typi-
ally exhibit a febrile prodrum for up to
11 days before the onset of neurological
disease.91 Symptoms usually begin with
malaise, headache, and fever, followed
by nausea and vomiting. Viremia is de-
tectable during this febrile prodrome.94
Over the next few days, the symptoms
intensify as somnolence or delirium may
progress into coma. The magnitude of
morbidity and mortality from aerosol ex-
posure is unknown.

For all 3 equine encephalitis viruses,
patients demonstrate leukopenia early
during the course of their febrile illness,
followed later by a leukocytosis. Elevated
serum aspartate aminotransferase levels
are common in VEE infections. For those
patients with central nervous system involvement, a lymphocytic pleocytosis with a cell count of up to 500×10^6/L will be observed in the cerebrospinal fluid (CSF). Patients with EEE commonly have an elevated opening pressure following lumbar puncture, and in children especially, the CSF pleocytosis may reach a cell count of 2×10^6/L. Of the arboviral encephalitides, EEE is the most severe. High mortality rates and severe neurological sequelae are seen among patients with EEE infection. Case-fatality rates are estimated at 50% to 75%, but asymptomatic infections and milder clinical illness are certainly underreported. Up to 30% of survivors are left with neurological sequelae, such as seizures, spastic paralysis, and cranial neuropathies. Like VEE, WEE is less virulent for adult humans than it is for equines and children, with lower rates of fatalities and neurological sequelae. As with EEE, infants and the elderly with WEE are especially susceptible to severe clinical illness and neurological sequelae, with case-fatality rates of about 10%. Some patients are left with permanent residua of motor weakness, cognitive deficits, or a seizure disorder, with children having a higher incidence of neurological sequelae in inverse proportion to their age.

**Diagnosis and Management**

Specific diagnosis of alphavirus encephalitis can be accomplished by virus isolation or serologic testing. During the first few days of symptoms of non-specific febrile illness, virus may be recovered from a patient’s serum. Isoates of VEE and WEE virus also have been recovered from throat washes of acutely ill patients. Although viremia is rarely detectable by the time patients present with encephalitic symptoms, hemagglutination-inhibiting, ELISA, or plaque-reduction neutralization antibodies are generally present by the second week of illness. In acute phase serum samples, IgM antibodies are present. Identifying the VEE subtype of an isolate involved can be accomplished by cross neutralization tests or nucleotide sequence analysis. Four-fold titer rises in convalescent serum samples or isolation of virus are considered diagnostic, but because of serological cross reactions with other alphaviruses, neutralization tests are preferred. Virus may occasionally be isolated from CSF in encephalitis cases and is frequently recovered from postmortem brain tissue of infected patients.

No specific therapy exists for the alphavirus encephalitides, and hence treatment is aimed at management of specific symptoms (eg, anticonvulsant medication or protection of the airway). A special problem occasionally seen among patients infected by WEE virus is an extremely high fever, which may require aggressive antipyretic measures. Observations of both humans and animals indicate that treatment with virus-neutralizing antisera fails to halt progression of disease if brain infection is firmly established. Defense against alphavirus infection, beyond respiratory protection with a high-efficiency particle filter, depends on immunization. Although the requisites for protection against parenteral infection with equine encephalitis viruses are well described, the requirements for protection against infectious aerosols as would be encountered in a BW scenario are certainly more stringent. Although immunity to the homologous serotype after VEE infection is probably lifelong, cross-immunity to heterologous serotypes is weak or nonexistent, and adequate immunization may require polyvalent vaccines. A live attenuated vaccine for VEE (TC-83) has largely eliminated homologous VEE strain infections among at-risk laboratory personnel. However, the TC-83 vaccine is reactive, as more than 20% of vaccine recipients experience fever, malaise, and headache after vaccination, with half of these severe enough to warrant bed rest for 1 to 2 days. Investigational formalin-inactivated vaccines for humans exist for VEE, WEE, and EEE but require multiple injections and are poorly immunogenic. In view of these shortcomings, live attenuated, genetically engineered vaccines have been developed by site-directed mutagenesis of various alphaviruses and show excellent promise with regards to safety and immunogenicity.

**VIRAL HEMORRHAGIC FEVERS**

**History and Significance**

The viral hemorrhagic fever (VHF) syndrome is a useful clinical concept that describes the disease processes associated with infection by a variety of RNA viruses. Viral hemorrhagic fever syndrome is an acute febrile illness characterized by malaise, prostration, generalized signs of vascular permeability, and abnormalities of circulatory regulation. Life-threatening loss of blood volume is rare, although bleeding manifestations often occur as a result of damage to the vascular endothelium. Despite their diverse taxonomy, the VHF agents are all RNA viruses and are typically transmitted to humans by contact with infected animal reservoirs or arthropod vectors whose distributions determine in part the geographic ranges of these diseases. Recent changes in human demographics have increased human exposures to these viruses. In addition to natural disease potential, many of the VHF agents are potential BW threats as well. These viruses are highly infectious by aerosol; are associated with high morbidity and, in some cases, high mortality; and may replicate sufficiently well in cell culture to permit weaponization.

Viruses from the Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae families are associated with VHF. The Arenaviridae includes the viruses of Lassa fever (Lassa virus), and Argentine, Bolivian, Venezuelan, and Brazilian hemorrhagic fevers (Junin, Machupo, Guanarito, and Sabia viruses, respectively). Among the Bunyaviridae, the significant human pathogens include Rift Valley fever (RVF) virus, causative agent of a major disease in Africa frequently associated with unusual increases in mosquito populations. Congo-Crimean hemorrhagic fever (CCHF) virus is carried by ticks and has been associated with sporadic, yet particularly severe VHF in Europe, Africa, and Asia. It has frequently been associated with small, hospital-centered outbreaks. Hantaviruses, unlike other Bunyaviridae, are not transmitted by infected arthropods; rather, they infect humans by contact with infected rodents and their excreta. Hantaviruses are significant infectious disease threats to both military and civilian populations. However, hantaviruses replicate poorly in cell culture and are not considered to be significant BW threats; thus, they are not discussed further here.

Another VHF group with biological warfare potential is the Filoviridae, which includes the agents of Ebola hemorrhagic fever and Marburg disease. Filoviruses have gained notoriety through their association with explosive, although limited, outbreaks. The original Marburg virus outbreak in 1967 was associated with 31 cases and 9 deaths. Ebola was first recognized in association with 2 explosive outbreaks that occurred almost simultaneously in 1976. The original outbreaks of Ebola in Zaire and Sudan were associated with mortality rates of 92% and 53%, respectively. In both cases, transmission was exacerbated through reuse of unsterilized needles and syringes and nosocomial contacts.

Yellow fever is another VHF virus. Despite its high aerosol infectivity, widespread use and availability of licensed vaccines limit the concern regarding its potential as a BW threat.

**Clinical Features**

The dominant clinical features of VHF are usually a consequence of microvas-
cular damage and changes in vascular permeability. Common presenting complaints are fever, myalgia, and prostration. Initial clinical examination may reveal only conjunctival injection, mild hypotension, flushing, and petechial hemorrhages. Full-blown VHF typically evolves to shock and generalized mucous membrane hemorrhage and often is accompanied by evidence of neurological, hematopoietic, or pulmonary involvement. Hepatic involvement is common, but clinical jaundice is a regular event only with yellow fever. Renal failure is proportional to cardiovascular compromise.

Although all VHF cases share common features, certain clinical characteristics predominate and serve to distinguish among the causative agents. For example, hemorrhagic manifestations are not pronounced for Lassa fever patients, and neurological complications are infrequent. For the South American arenaviruses (Junin and Machupo), neurological and hemorrhagic manifestations are much more prominent. For RVF, hemorrhagic fever is seen in only a small proportion of the cases, as the virus is primarily hepatotropic. Unlike other VHF's, retinitis is a frequently reported component of RVF disease. In contrast, hemorrhagic manifestations are predominant with CCHF infection, which causes a profound disseminated intravascular coagulation (DIC). Marburg and Ebola viruses produce prominent maculopapular rashes, and DIC is a major factor in their pathogenesis.

**Diagnosis and Management**

Viral hemorrhagic fever should be suspected in any patient presenting with a severe febrile illness and evidence of vascular involvement who has traveled to an area where the virus is known to occur or when a BW threat is suspected.

Definitive diagnosis in an individual case requires specific virologic diagnosis. Most patients will present with viremia that can be detected by antigen-capture ELISA or reverse transcriptase polymerase chain reaction (RT-PCR). Likewise, early IgM antibody responses to these agents can be detected by ELISA, often during the acute illness. Definitive virus isolation takes longer and requires specialized analysis in a biosafety laboratory (BSL 3 or BSL 4). When the identity of the VHF agent is totally unknown, isolation in cell culture and direct visualization by electron microscopy, followed by immunohistologic identification by immunohistological techniques is often successful. Immunohistological techniques are also useful for retrospective diagnosis of formalin-fixed tissues.

Patients with VHF generally benefit from rapid, nontraumatic hospitalization to prevent unnecessary damage to the fragile capillary bed. Secondary infections are common and should be sought and treated aggressively. Intravenous lines, catheters, and other invasive techniques should be avoided unless clearly indicated in management. The management of bleeding is controversial. In the absence of definitive evidence, it is recommended that mild bleeding manifestations not be treated at all. More severe hemorrhage indicates a need for appropriate replacement therapy. When definite laboratory evidence of DIC develops, heparin therapy should be used if appropriate laboratory control is available.

Management of hypotension and shock is difficult. Patients often are moderately dehydrated, and there are covert losses of intravascular volume through hemorrhage and increased vascular permeability. Nevertheless, these patients often respond poorly to fluid infusions and readily develop pulmonary edema. The diffuse nature of the vascular process may lead to a requirement for support of several organ systems.

Ribavirin, a nonimmunosuppressive nucleoside analog with broad antiviral properties, is of proven value for some of the VHF agents. Ribavirin has been shown to reduce mortality from Lassa fever in high-risk patients and presumably decreases morbidity in all Lassa patients. Treatment is most effective if begun within 7 days of onset. In Argentina, ribavirin has been shown to reduce virologic parameters of Junin infection and is now used routinely as an adjunct to immune plasma. Small studies of ribavirin in treatment of Bolivian hemorrhagic fever (BHF) and CCHF have been promising, as have preclinical studies for RVF. Conversely, preclinical studies predict ribavirin will be ineffective against both the filoviruses and the flaviviruses. No other antiviral compounds are currently available for these infections.

Argentine hemorrhagic fever (AHF) responds to therapy with 2 or more units of convalescent plasma containing adequate amounts of neutralizing antibody provided treatment is initiated within 8 days of onset. Antibody therapy is also beneficial for treating BHF. Efficacy of immune plasma in treatment of Lassa fever and CCHF is limited by low neutralizing antibody titers and the consequent need for careful donor selection. Equine immune globulin against Ebola virus has been proposed for treating this infection, but data obtained from experimentally infected monkeys do not support this recommendation.

A licensed and highly efficacious vaccine for yellow fever is widely available. For AHF, a live attenuated Junin vaccine strain is available as an IND. This vaccine may also provide some cross-protection against BHF. Two IND vaccines for RVF were developed by researchers at USAMRIID; an inactivated vaccine that requires 3 boosters has been in use for 20 years and a live attenuated RVF strain (MP-12) is now in phase 2 efficacy testing.

**BOTULINUM TOXINS**

**History and Significance**

Botulinum toxins are proteins of approximately 150,000 molecular weight and are produced by the anaerobic bacterium *Clostridium botulinum*. There are 7 distinct but related neurotoxins, A through G, produced by different strains of the clostridial bacillus. All 7 types act by a similar mechanism and induce similar effects when inhaled or ingested. Botulinum toxins have caused numerous cases of botulism when improperly prepared or canned foods are ingested. Many deaths have occurred after such incidents. It is feasible to deliver botulinum toxins as a biological weapon, and several countries have weaponized or are suspected to have weaponized 1 or more of this group of toxins. Iraq admitted to a United Nations inspection team in August 1991 that it had performed research on the offensive use of botulinum toxins before the Persian Gulf War. Additional information given in 1995 revealed that Iraq had not only researched but had filled and deployed over 100 munitions containing botulinum toxin. Although an aerosol attack is by far the most likely scenario for the use of botulinum toxins, theoretically, the agent could be used to sabotage food supplies.

**Clinical Features**

Botulinum toxins are the most toxic compounds known, with an estimated toxic dose (serotype A) of only 0.001 µg/kg of body weight. Botulinum toxin is 15,000 times more toxic than the nerve agent VX and 100,000 times more toxic than sarin.

Botulinum toxins act by binding to the presynaptic nerve terminal at the neuromuscular junction and at cholinergic autonomic sites. The toxins then act to prevent the release of acetylcholine presynaptically, and thus block neurotransmission. This interruption of neurotransmission causes both bulbar palsies and the skeletal muscle weakness seen in clinical botulism.

The onset of symptoms of inhalation botulism is dose dependent and may vary from 24 to 36 hours to several days after exposure. Bulbar palsies are prominent early with ocular symptoms, such
as blurred vision due to mydriasis, diplopia, ptosis, and photophobia, in addition to other bulbar signs such as dysarthria, dysphagia, and dyspnea. Skeletal muscle paralysis follows, manifested as a symmetrical, descending, and progressive weakness which may culminate abruptly in respiratory failure. Progression from onset of symptoms to respiratory failure has occurred in as few as 24 hours in cases of foodborne botulism.

Physical examination usually reveals an alert and oriented patient without fever. Postural hypotension may be present. Mucous membranes may be dry and crusted, and the patient may complain of dry mouth or even sore throat. Gag reflex may be absent. Pupils may be dilated and even fixed. Pto sis and extraocular muscle palsies are commonly observed. Variable degrees of skeletal muscle weakness occur, depending on progression of intoxication in an individual patient. Deep tendon reflexes may be present or absent. With severe respiratory muscle paralysis, the patient may become cyanotic or exhibit narcosis from carbon dioxide retention.

**Diagnosis and Management**

The occurrence of an epidemic of cases of a descending and progressive bulbar and skeletal paralysis in afebrile patients typical of classical botulism points to the diagnosis of botulinal intoxication. Individual cases might be confused clinically with other neuromuscular disorders such as Guillain-Barré syndrome, myasthenia gravis, or tick paralysis. The edrophonium (or Tensilon) test may be transiently positive in botulism, so it may not distinguish botulism from intoxication from myasthenia.

Laboratory tests are generally of limited value in the diagnosis of botulism. Studies suggest that aerosolized toxin is usually not identifiable in serum or stool, whereas it is with foodborne botulism. Survivors do not usually develop an antibody response due to the subimmunogenic amount of toxin necessary to produce clinical symptoms. Toxin may be present on nasal mucous membranes and detectable by ELISA for 24 hours after inhalation.

Respiratory failure secondary to paralysis of respiratory muscles is the most serious complication and, generally, the cause of death. With tracheostomy or endotracheal intubation and ventilatory assistance, fatalities should be less than 5%. Intensive and prolonged nursing care may be required for recovery, which may take several weeks or even months. Animal experiments show that aerosol exposure, botulinum antitoxin can be very effective, precluding all signs of intoxication if given before the onset of clinical signs. Administration of antitoxin is reasonable if disease has not progressed to a stable state.

A tachyphylactic equine antitoxin is available from the CDC for cases of foodborne botulism. Adverse effects of this antitoxin include the risks of anaphylaxis and serum sickness. A “despeciated”[F(ab’)] equine heptavalent antitoxin (against types A through G) has been prepared by the US Army. This product is under IND status. Its efficacy in humans is not yet known. Use of either antitoxin requires skin testing for horse serum sensitivity before administration.

Immunized laboratory animals are fully protected from lethal inhalation challenges with serotype A toxin (J. E. Brown, written communication, July 1997). A pentavalent toxoid of *C. botulinum* toxin types A through E is available under IND status.

**STAPHYLOCOCCAL ENTEROTOXIN B**

**History and Significance**

*Staphylococcus aureus* produces a number of exotoxins, one of which is SEB. The toxins are referred to as exotoxins because they are excreted from the organism; however, they normally exert their effects on the gastrointestinal tract and therefore are called enterotoxins. SEB is one of the heat-stable pyrogenic exotoxins that commonly causes food poisoning in humans after the toxin is produced in improperly handled foodstuffs and subsequently ingested. Because of its extreme toxicity as an incapacitant, inhalation exposure to this toxin could render a high percentage of exposed personnel clinically ill, requiring medical care beginning a few hours after exposure. SEB is relatively stable in aerosols. It causes symptoms when inhaled at very low doses in humans; an inhaled dose several logs lower than the estimated lethal dose would be sufficient to incapacitate individuals so exposed. Even though SEB is not generally thought of as a highly lethal agent, it may incapacitate humans many miles downwind from the release point of a weapon. This toxin could also be used, theoretically, in a special forces or terrorist mode to sabotage food or small volume water supplies.

**Clinical Features**

Inhaled SEB can induce extensive pathophysiologic changes to include widespread systemic damage and even septic shock. Many of the effects of this family of toxins are mediated by interactions with the host's own immune system. The mechanisms of toxicity are complex, but are related to the toxin binding directly to the major histocompatibility complex and subsequent stimulation of the proliferation of large numbers of T cells. Because these exotoxins are extremely potent activators of T cells, they are commonly referred to as bacterial superantigens. These superantigens stimulate the production and secretion of various cytokines from immune system cells. Released cytokines are thought to mediate most of the toxic effects of SEB.

Inhalation exposure is projected to cause primarily clinical illness and incapacitation; however, intoxications can be lethal. Intoxication with SEB begins 3 to 12 hours after inhalation of the toxin. Those exposed may experience the sudden onset of fever, headache, chills, myalgia, and a nonproductive cough. More severe cases may develop dyspnea and retrosternal chest pain. Nausea, vomiting, and diarrhea will also occur in many patients due to inadvertently swallowed toxin, and fluid losses can be marked. The fever may last up to 5 days and range from 39.4°C to 41.1°C, with variable degrees of chills and prostration. The cough may persist up to 4 weeks, and patients may not be able to return to normal functions for 2 weeks.

Physical examination in patients with SEB intoxication is often unremarkable. Conjunctival injection may be present, and postural hypotension may develop due to fluid losses. Chest examination is unremarkable except in the unusual case where pulmonary edema develops. The chest x-ray film is also generally normal, but in severe cases, increased interstitial markings, atelectasis, and possibly overt pulmonary edema or an adult respiratory distress syndrome may develop.

**Diagnosis and Management**

As is the case with botulinum toxins, intoxication caused by SEB inhalation is a clinical and epidemiologic diagnosis. Because the symptoms of SEB intoxication may be similar to the symptoms triggered by several respiratory pathogens such as influenza, adenovirus, and mycoplasma, the diagnosis may be unclear initially. All of these might present with fever, nonproductive cough, myalgia, and headache. An SEB attack would cause patients to present in large numbers over a very short period of time, probably within 24 hours, in contrast with naturally occurring pneumonias or influenza with patients presenting over a more prolonged interval. Staphylococcal food poisoning cases would not present with pulmonary symptoms. Intoxication with SEB tends to progress rapidly to a fairly stable clinical state, whereas pulmonary anthrax, tularemia pneumonia, or pneumonic plague would all progress if left untreated. Tularemia and plague, as well as Q fever, would be associated with infiltrates on
CHEST RADIOPHGRAPHS, UNLIKE SEB. NERVE AGENT INTOXICATION WOULD CAUSE FASCICULATIONS AND COPIOUS SECRETIONS, AND SULFUR MUSTARD WOULD CAUSE SKIN LESIONS IN ADDITION TO PULMONARY FINDINGS. THE DYSPEA ASSOCIATED WITH BOTULINUM INTOXICATION IS ASSOCIATED WITH OBVIOUS SIGNS OF MUSCULAR PARALYSIS, BULBAR PALSY, LACK OF FEVER, AND A DRY PULMONARY TREE DUE TO CHOLINERGIC BLOCKADE; RESPIRATORY DIFFICULTIES OCCUR LATE WITH BOTULINUM, IN CONTRAST, THEY OCCUR EARLY WITH SEB INHALATION.

LABORATORY FINDINGS ARE NOT VERY HELPFUL IN THE DIAGNOSIS OF SEB INTOXICATION. A NONSPECIFIC NEUTROPHILIC LEUKOCYTOSIS AND AN ELEVATED ERTHROCYTE SEDIMENTATION RATE MAY BE SEEN 12 TO 24 HOURS AFTER EXPOSURE, BUT THESE ABNORMALITIES ARE PRESENT IN MANY ILLNESSES. TOXIN IS VERY DIFFICULT TO DETECT IN THE SERUM BY THE TIME SYMPTOMS OCCUR. DATA FROM ANIMAL STUDIES SHOW THAT SEB IN THE SERUM IS TRANSIENT; HOWEVER, SEB OR ITS ANTIGENIC METABOLITES ACUMULATE IN THE URINE AND CAN BE DETECTED FOR SEVERAL HOURS POSTEXPOSURE (C.T. LIOU, USAMRIID, UNPUBLISHED DATA, FEBRUARY 1995). THEREFORE, URINE SAMPLES ALSO SHOULD BE OBTAINED AND TESTED. BECAUSE MOST PATIENTS WILL DEVELOP A SIGNIFICANT ANTIBODY RESPONSE TO THE TOXIN, ACUTE AND CONValesCENT SERUM THAT MAY BE HELPFUL RETROSPECTIVELY IN THE DIAGNOSIS SHOULD BE DRAWN. THE TOXIN MAY BE IDENTIFIED BY ELISA IN NASAL SWABS TAKEN WITHIN 24 HOURS AFTER AEROSOL EXPOSURE.

CURRENTLY, THERAPY IS LIMITED TO SUPPORTIVE CARE. CLOSE ATTENTION TO OXYGENATION AND HYDRATION ARE IMPORTANT, AND IN SEVERE CASES WITH PULMONARY EDEMA, VENTILATION WITH POSITIVE END-EXPIRATORY PRESSURE AND DIURETICS MIGHT BE NECESSARY. THE VALUE OF STEROIDS IS CONTROVERSIAL. MOST PATIENTS WOULD BE EXPECTED TO RECOVER AFTER THE INITIAL ACUTE PHASE OF THEIR ILLNESS, AND MOST WOULD GENERALLY BE ABLE TO RETURN TO NORMAL FUNCTIONS IN 1 TO 2 WEEKS.

ALTHOUGH THERE IS CURRENTLY NO HUMAN VACCINE FOR IMMUNIZATION AGAINST SEB INTOXICATION, SEVERAL VACCINE CANDIDATES ARE IN DEVELOPMENT. PRELIMINARY ANIMAL STUDIES HAVE BEEN ENCOURAGING AND VACCINE CANDIDATES FOR BOTH SEB AND STAPHYLOCOCAL ENTEROTOXIN A, A RELATED TOXIN, ARE NEARING SAFETY AND IMMUNOGENICITY TESTING IN HUMANS. EXPERIMENTALLY, PASSIVE IMMUNOTHERAPY CAN REDUCE MORTALITY IN ANIMAL MODELS, BUT ONLY WHEN GIVEN WITHIN 4 TO 8 HOURS AFTER INHALING SEB.

THE EPIDEMIOLOGY OF A BW OR TERRORIST ATTACK

ALTHOUGH THE LIKELIHOOD OF A BIOLOGICAL ATTACK IS UNKNOWN, AND SIGNIFICANT DEFENSIVE PREPARATIONS ARE UNDERWAY, MANY BELIEVE THE UNITED STATES IS VULNERABLE. THIS IS ESPECIALLY TRUE OF CITIZEN POPULATIONS, WHO OFTEN DO NOT HAVE PROTECTIVE EQUIPMENT OR VACCINES MADE AVAILABLE TO THEM. THE UNFORTUNATE FACT REMAINS THAT HUMANS ARE OFTEN THE MOST SENSITIVE, OR THE ONLY, DETECTOR OF A BIOLOGICAL ATTACK. WITHOUT KNOWLEDGE OF THE ATTACK, AN INCREASED NUMBER OF PATIENTS PRESENTING WITH SIGNS AND SYMPTOMS CAUSED BY THE DISSEMINATED DISEASE AGENT IS THE MOST LIKELY FIRST INDICATOR THAT A BW HAS OCCURRED.

A SOUND EPIDEMIOLOGIC INVESTIGATION OF A DISEASE OUTBREAK, WHETHER NATURAL OR ARTIFICIAL, WILL ASSIST MEDICAL PERSONNEL IN IDENTIFYING THE PATHOGEN, AS WELL AS INSTITUTING THE APPROPRIATE MEDICAL INTERVENTIONS. THE CDC REALIZED THIS AS EARLY AS 1951, WHEN THE EPIDEMIC INTELLIGENCE SERVICE WAS CREATED TO TRAIN EPIDEMIOLOGISTS IN CASE A BW ATTACK SHOULD TAKE PLACE AGAINST THE UNITED STATES DURING THE COLD WAR. DOCUMENTING WHO IS AFfected, POSSIBLE ROUTES OF EXPOSURE, SIGNS AND SYMPTOMS OF DISEASE, AND THE RAPID IDENTIFICATION OF THE CAUSATIVE AGENTS WILL GREATLY INCREASE THE ABILITY TO PLAN AN APPROPRIATE MEDICAL AND PUBLIC HEALTH RESPONSE. GOOD EPIDEMIOLOGIC INFORMATION WILL ALLOW THE APPROPRIATE FOLLOW-UP OF THOSE POTENTIALLY EXPOSED, AS WELL AS HELP DETERMINE PUBLIC INFORMATION GUIDELINES AND RESPONSES TO THE MEDIA.

Many, if not most, diseases caused by weaponized biological agents present with nonspecific signs and symptoms that could be misinterpreted as natural occurrences. The disease pattern that develops is an important factor in differentiating between a natural and a terrorist or warfare attack. In most naturally occurring epidemics, there is a gradual rise in disease incidence, as people are progressively exposed to an increasing number of patients, vectors, or fomites that spread the pathogen. In contrast, those exposed to a BW attack would all come in contact with the agent at approximately the same time. Even taking into account varying incubation periods based on exposure dose and physiological differences, a compressed epidemic curve with a peak in a matter of days, or even hours, would occur. Most point source exposures will present in this fashion, including foodborne outbreaks, which may be natural or possibly intentionally induced. Therefore, further information should be obtained to help establish whether an outbreak is caused by an attack with a BW agent.

The general steps for epidemiologic assessment of any disease can be applied to a BW or terrorist attack. First, public health officials and other health care personnel should formulate a case definition to determine the number of actual cases (verify the epidemic) and from that the approximate attack rate. Is the disease rate greater than the background rate of disease that normally occurs? The potential exists for hysteria to be confused with actual disease; therefore, objective criteria should be used to document the number of people affected. Once a case definition has been determined, description of the epidemic can be done with respect to the timing, place, and other characteristics of those who are ill. The investigation obviously needs to be done expeditiously, but even rudimentary information can be of assistance in determining the source and potential consequences of an outbreak.

The unintended release of anthrax spores from a military compound in the former Soviet Union in 1979 demonstrated some of the epidemiologic indicators of an unnatural epidemic. The location of casualties followed a distinctive downwind pattern from the release site, animals in the same area were affected, and an unusual presentation of the disease, respiratory instead of cutaneous, occurred. Other possible clues to a BW or terrorist attack include high disease rates among exposed individuals, more respiratory cases of disease if the agent is disseminated via aerosol, occurrence of a disease that is unusual in a given geographic area, a naturally vector-borne disease occurring in an area that lacks the appropriate vectors for normal transmission, more than 1 pandemic occurring at the same time, higher morbidity and mortality than normally expected for a disease, lower attack rates in personnel protected from exposure (such as those inside a building), and suspicious activity or discovery of a potential delivery system such as a spray device. If an attack with biological agents is suspected, the proper authorities, whether military or civilian, should be notified immediately.

To minimize the effects of a biological terrorist attack, health care professionals and public health authorities must be aware of the threat of biological warfare and terrorism and have an increased index of suspicion that such an attack can occur. They must have some understanding of the classes of agents that have been and can be weaponized and their effects after inhalation. Surveillance of background disease activity should be ongoing, and any unusual changes in disease occurrence or etiology should be promptly followed up with a directed examination of the facts regarding the increased rates. Through close attention to disease patterns, we can become aware of potential problems in time to institute rapid action that can potentially save many lives, and decrease the impacts of disease, regardless of its origin.
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