NATO HANDBOOK ON THE MEDICAL ASPECTS OF NBC DEFENSIVE OPERATIONS
AMedP-6(B)

PART II - BIOLOGICAL

ANNEX B

CLINICAL DATA SHEETS FOR SELECTED BIOLOGICAL AGENTS

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ANNEX B

CLINICAL DATA SHEETS FOR SELECTED BIOLOGICAL AGENTS

B.01. Introduction.

a. The following information provides clinical information to assist in the recognition, diagnosis and management of selected diseases, well recognized for their potential as biological weapons. It is not intended to be comprehensive, nor should it be interpreted as a sanctioned "threat list." Likely agents are:

(1) Anthrax.
(2) Botulinum Toxins.
(3) Brucellosis.
(4) Cholera.
(5) Clostridium Perfringens Toxins.
(6) Crimean-Congo Hemorrhagic Fever.
(7) Melioidosis.
(8) Plague.
(9) Q Fever.
(10) Ricin.
(11) Rift Valley Fever.
(12) Saxitoxin.
(13) Smallpox.
(14) Staphylococcal Enterotoxin B.
(15) Trichothecene Mycotoxins.
(16) Tularemia.
(17) Venezuelan Equine Encephalitis.

b. Many products referenced in this annex are currently considered investigational new drugs (IND). This indicates that the product (drug, vaccine, antitoxin, etc.) has been shown to be safe and effective in animal studies and has been approved for limited use as an investigational product in humans. In general, IND products must be obtained through official channels from the government of the producing nation and administered under a research protocol approved by a recognized institutional review board.

B.02. Anthrax.

a. *Clinical Syndrome.*

(1) *Characteristics.* Anthrax is a zoonotic disease caused by *Bacillus anthracis.* Under natural conditions, humans become infected by contact with infected animals or contaminated animal products. Human anthrax is usually manifested by cutaneous lesions. A biological warfare attack with anthrax spores delivered by aerosol would cause inhalation anthrax, an extraordinarily rare form of the naturally occurring disease.

(2) *Clinical Features. The* disease begins after an incubation period varying from 1-6 days, presumably dependent upon the dose of inhaled organisms. Onset is gradual and nonspecific, with fever, malaise, and fatigue, sometimes in association with a nonproductive cough and mild chest discomfort. In some cases, there may be a short period of improvement. The initial symptoms are followed in 2-3 days by the abrupt development of severe respiratory distress with dyspnea, diaphoresis, strider, and cyanosis. Physical findings may include evidence of pleural effusions, edema of the chest wall, and meningitis. Chest x-ray reveals a dramatically widened mediastinum, often with pleural effusions, but typically without infiltrates. Shock and death usually follow within 24-36 hours of respiratory distress onset.

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* Laboratory evaluation will reveal a neutrophilic leucocytosis. Pleural and cerebrospinal fluids may be hemorrhagic.

(2) *Deferential Diagnosis.* An epidemic of inhalation anthrax in its early stage with nonspecific symptoms could be confused with a wide variety of viral, bacterial, and fungal infections. Progression over 2-3 days with the sudden development of severe respiratory distress followed by shock and death in 24-36 hours in essentially all untreated cases eliminates diagnoses other than inhalation anthrax. The presence of a widened mediastinum on chest x-ray, in particular, should alert one to the diagnosis. Other suggestive findings include chest-wall edema, hemorrhagic pleural effusions, and hemorrhagic meningitis. Other diagnoses to consider include aerosol exposure to SEB; but in this case onset would be more rapid after exposure (if known), and no prodrome would be evident prior to onset of severe respiratory symptoms. Mediastinal widening on chest x-ray will also be absent. Patients with plague or tularemia pneumonia will have pulmonary infiltrates and clinical signs of pneumonia (usually absent in anthrax).

(3) *Specific Laboratory Diagnosis. Bacillus anthracis* will be readily detectable by blood culture with routine media. Smears and cultures of pleural fluid and abnormal cerebrospinal fluid may also be positive. Impression smears of mediastinal lymph nodes and spleen from fatal cases should be positive. Toxemia is sufficient to permit anthrax toxin detection in blood by immunoassay.

c. *Therapy*. Almost all cases of inhalation anthrax in which treatment was begun after patients were symptomatic have been fatal, regardless of treatment. Historically, penicillin has been regarded as the treatment of choice, with 2 million units given intravenously every 2 hours. Tetracycline and erythromycin have been recommended in penicillin-sensitive patients. The vast majority of anthrax strains are sensitive in vitro to penicillin. However, penicillin-resistant strains exist naturally, and one has been recovered from a fatal human case. Moreover, it is not difficult to induce resistance to penicillin, tetracycline, erythromycin, and many other antibiotics through laboratory manipulation of organisms. All naturally occurring strains tested to date have been sensitive to erythromycin,

chloramphenicol, gentamicin, and ciprofloxacin. In the absence of information concerning antibiotic sensitivity, treatment should be instituted at the earliest signs of disease with oral ciprofloxacin (1000 mg initially, followed by 750 mg po (orally) bid (twice daily)) or intravenous doxycycline (200 mg initially, followed by 100 mg q (every) 12 hrs). Supportive therapy for shock, fluid volume deficit, and adequacy of airway may all be needed.

d. *Prophylaxis.*

(1) *Vaccine.* A licensed, alum-precipitated preparation of purified *B. anthracis* protective antigen (PA) has been shown to be effective in preventing or significantly reducing the incidence of inhalation anthrax. Limited human data suggest that after completion of the first three doses of the recommended six-dose primary series (0, 2, 4 weeks, then 6, 12, 18 months), protection against both cutaneous and inhalation anthrax is afforded. Studies in rhesus monkeys indicate that good protection is afforded after two doses (10-16 days apart) for up to 2 years. It is likely that two doses in humans is protective as well, but there is too little information to draw firm conclusions. As with all vaccines, the degree of protection depends upon the magnitude of the challenge dose; vaccine-induced protection is undoubtedly overwhelmed by extremely high spore challenge. At least three doses of the vaccine (at 0, 2, and 4 weeks) are recommended for prophylaxis against inhalation anthrax. Contraindications for use are sensitivity to vaccine components (formalin, alum, benzethonium chloride) and/or history of clinical anthrax. Reactogenicity is mild to moderate: up to 6% of recipients will experience mild discomfort at the inoculation site for up to 72 hours (tenderness, erythema, edema, pruritus), while a smaller proportion (<1%) will experience more severe local reactions (potentially limiting use of the extremity for 1-2 days); modest systemic reactions (myalgia, malaise, low-grade fever) are uncommon, and severe systemic reactions (anaphylaxis, which precludes additional vaccination) are rare. The vaccine should be stored at refrigerator temperature (not frozen).

(2) *Antibiotics.* Choice of antibiotics for prophylaxis is guided by the same principles as that for treatment; i.e., it is relatively easy to produce a penicillin-resistant organism in the laboratory, and possible, albeit somewhat more difficult, to induce tetracycline resistance. Therefore, if there is information indicating that a biological weapon attack is imminent, prophylaxis with ciprofloxacin (500 mg po bid), or doxycycline (100 mg po bid) is recommended. If unvaccinated, a single 0.5 ml dose of vaccine should also be given subcutaneously. Should the attack be confirmed as anthrax, antibiotics should be continued for at least 4 weeks in all exposed. In addition, two 0.5 ml doses of vaccine should be given 2 weeks apart in the unvaccinated; those previously vaccinated with fewer than three doses should receive a single 0.5 ml booster, while vaccination probably is not necessary for those who have received the initial three doses within the previous 6 months (primary series). Upon discontinuation of antibiotics, patients should be closely observed; if clinical signs of anthrax occur, patients should be

treated as indicated above. If vaccine is not available, antibiotics should be continued beyond 4 weeks until the patient can be closely observed upon discontinuation of therapy.

B.03. Botulinum Toxins.

a. *Clinical Syndrome.*

(1) *Characteristics.* Botulism is caused by intoxication with the any of the seven distinct neurotoxins produced by the bacillus, *Clostridium botudinum.* The toxins are proteins with molecular weights of approximately 150,000, which bind to the presynaptic membrane of neurons at peripheral cholinergic synapses to prevent release of acetylcholine and block neurotransmission. The blockade is most evident clinically in the cholinergic autonomic nervous system and at the neuromuscular junction. A biological warfare attack with botulinum toxin delivered by aerosol would be expected to cause symptoms similar in most respects to those observed with food-borne botulism.

(2) *Clinical Features.* Symptoms of inhalation botulism may begin as early as 24-36 hours following exposure or as late as several days. Initial signs and symptoms include ptosis, generalized weakness, lassitude, and dizziness. Diminished salivation with extreme dryness of the mouth and throat may cause complaints of a sore throat. Urinary retention or ileus may also occur. Motor symptoms usually are present early in the disease; cranial nerves are affected first with blurred vision, diplopia, ptosis, and photophobia. Bulbar nerve dysfunction causes dysarthria, dysphonia, and dysphagia. This is followed by a symmetrical, descending, progressive weakness of the extremities along with weakness of the respiratory muscles. Development of respiratory failure may be abrupt. On physical examination, the patient is alert, oriented, and afebrile. Postural hypotension may be present. Ocular findings may include ptosis, extracelluar muscle paralysis, and fixed and dilated pupils. Mucous membranes of the mouth may be dry and crusted. Neurological examination shows flaccid muscle weakness of the palate, tongue, larynx, respiratory muscles, and extremities. Deep tendon reflexes vary from intact to absent. No pathologic reflexes are present, and the sensory examination generally is normal (although reports suggest that obtundation or sensory involvement may sometimes occur).

b. *Diagnosis.*

(1) *Routine Findings.* Routine laboratory findings are of no value in diagnosis. The cerebrospinal fluid is normal.

(2) *Differential Diagnosis.* The occurrence of an epidemic with large numbers of afebrile patients with progressive ocular, pharyngeal, respiratory, and muscular weakness and paralysis hints strongly at the diagnosis. Single cases may be confused with various neuromuscular disorders such as atypical Guillain-Barrè syndrome, myasthenia gravis, or tick paralysis. The edrophonium (tensilon) test may be transiently positive in botulism. Other considerations include enteroviral infections; but in these patients, fever is

present, paralysis is often asymmetrical, and the cerebrospinal fluid is abnormal. It may be necessary to distinguish nerve-agent and atropine poisoning from botulinum intoxication. Briefly, organophosphate nerve agent poisoning results in miotic pupils and copious secretions. In atropine poisoning, the pupils are dilated and mucous membranes are dry, but central nervous system excitation with hallucinations and delirium is present. (See Annex D for a more comprehensive differential.)

(3) *Specific Laboratory Findings.* Detection of toxin in serum or gastric contents from cases of food-borne botulism is often feasible by mouse inoculation. Toxin has also been detected in serum following inhalation exposure in experimental animals. Serum should be obtained from representative cases for such attempts. Survivors probably will not develop an antibody response due to the small amount of toxin necessary to cause death.

c. *Therapy*.

(1) Respiratory failure secondary to paralysis of respiratory muscles is the most serious complication and, generally, the cause of death. Reported cases of botulism prior to 1950 had a mortality of 60%. With tracheotomy and ventilator assistance, fatalities should be <5%. Intensive and prolonged nursing care may be required for recovery (which may take several weeks or even months).

(2) In isolated cases of food-borne botulism, circulating toxin is usually present, perhaps due to continued absorption through the gut wall. Equine antitoxin has been used in these circumstances and is probably helpful. After aerosol exposure, antitoxin can be effective, sometimes even after onset of signs of intoxication. Administration of antitoxin is reasonable if disease has not progressed to a stable state.

(3) There is no prospect for additional human antitoxin to be produced. A "despeciated" equine heptavalent antitoxin (*vs* types A, B, C, D, E, F, and G) has been prepared by cleaving the Fc fragments from horse immunoglobulin G (IgG) molecules, leaving F(ab)2 fragments. Its efficacy is inferred from its performance in animal studies. Use requires pretesting for sensitivity to horse serum (and desensitization for those allergic). Disadvantages include rapid clearance by immune elimination, as well as a theoretical risk of serum sickness.

d. *Prophylaxis.*

(1) A pentavalent toxoid of *Clostridium botulinum* types A, B, C, D, and E is available under IND status. This product has been administered to several thousand volunteers and occupationally at-risk workers and induces serum antitoxin levels that correspond to protective levels in experimental animal systems. The currently recommended schedule (0, 2, and 12 weeks, then a 1 year booster) induces solidly protective antitoxin levels in greater than 90 percent of those vaccinated after 1 year. Transient antitoxin levels are induced after three injections. Contraindications include sensitivity to alum, formaldehyde, and thimerosal, or hypersensitivity to a previous dose. Reactogenicity is mild, with 2-4% of vaccines reporting erythema, edema, or

induration which peaks at 24-48 hours then dissipates. The frequency of local reactions increases with each subsequent inoculation; after the second and third doses, 7-10% will have local reactions, with higher incidence (up to 20% or so) after boosters. Severe local reactions are rare, consisting of more extensive edema or induration. Systemic reactions are reported in up to 3%, consisting of fever, malaise, headache, and myalgia. Incapacitating reactions (local or systemic) are uncommon. The vaccine should be stored at refrigerator temperatures (not frozen).

(2) Three or more vaccine doses (0, 2, and 12 weeks, then 1 year, if possible, by deep subcutaneous injection) are recommended only to selected individuals or groups judged at high risk for exposure to botulinum toxin aerosols. There is no indication at present for use of antitoxin as a prophylactic modality except under extremely specialized circumstances (for example, known impending exposure of small numbers of individuals).

B.04. Brucellosis.

a. *Clinical Syndrome.*

(1) *Characteristics.* Brucellosis is a systemic zoonotic disease caused by one of four species of bacteria: *Brucella melitensis, B. abortus, B. suis,* and *B. canis;* virulence for humans decreases somewhat in the order given. These bacteria are small gram-negative, aerobic, non-motile coccobacilli that grow within monocytes and macrophages. They reside quiescently in tissue and bone-marrow, and are extremely difficult to eradicate even with antibiotic therapy. Their natural reservoir is domestic animals, such as goats, sheep, and camels *(B. melitensis);* cattle *(B. abortus);* and pigs *(B. suis). Brucella canis is* primarily a pathogen of dogs, and only occasionally causes disease in humans. Humans are infected when they inhale contaminated aerosols, ingest raw (unpasteurized) infected milk or meat, or have abraded skin or conjunctival surfaces that come in contact with the bacteria. Laboratory infections are quite common, but there appears to be no human-to-human transmission; isolation of infected patients is, therefore, not required. *Brucella* species long have been considered potential candidates for use in biological warfare. The organisms are readily lyophilized, perhaps enhancing their infectivity. Under selected environmental conditions (for example, darkness, cool temperatures, high $C0_2$), persistence for up to 2 years has been documented. When used as a biological warfare agent, *Brucellae* would most likely be delivered by the aerosol route; the resulting infection would be expected to mimic natural disease.

(2) *Clinical Features.* Brucellosis presents after an incubation period normally ranging from 3-4 weeks, but may be as short as 1 week or as long as several months. Clinical disease presents typically as an acute, non-specific febrile illness with chills, sweats, headache, fatigue, myalgias, arthralgias, and anorexia. Cough occurs in 15-25%, but the chest x-ray usually is normal. Complications include sacroiliitis, arthritis, vertebral osteomyelitis,

epididymo-orchitis, and rarely endocarditis. Physical findings include lymphadenopathy in 10-20% and splenomegaly in 20-30% of cases. Untreated disease can persist for months to years, often with relapses and remissions. Disability may be pronounced. Lethality may approach 6% following infection with *B. melitensis*, but the disease is rarely fatal (0.5% or less) after infection with other serotypes (usually after endocarditis develops).

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* Noncontributory.

(2) *Differential Diagnosis.* The initial symptoms of brucellosis are usually nonspecific. The differential diagnosis is therefore very broad and includes bacterial, viral, and mycoplasmal infections. The systemic symptoms of viral and mycoplasmal illnesses, however, are usually present for only a few days, while they persist for prolonged periods in brucellosis. Brucellosis may be indistinguishable clinically from the typhoidal form of tularemia or from typhoid fever itself.

(3) *Specific Laboratory Diagnosis.* Serology by agglutination or enzyme-linked immunasorbant assay may suggest the diagnosis. A definitive diagnosis of brucellosis is established by culture of blood or bone marrow, which maybe positive in up to *70%* and 90% of cases, respectively.

c. *Therapy*. The recommended treatment is doxycycline (200 mg/day) plus rifampin (900 mg/day) for 6 weeks. Alternative effective treatment consists of doxycycline (200 mg/day) for 6 weeks plus streptomycin (1 gm/day) for 3 weeks. Trimethoprim-sulfamethoxazole given for 4-6 weeks is less effective. In 5-10% of cases, there may be relapse or treatment failure. Laboratory infections with brucellosis are quite common, but there is no human-to-human transmission and isolation is not required.

d. *Prophylaxis.* Killed and live attenuated human vaccines have been available in many countries but are of unproven efficacy. There is no information on the use of antibiotics for prophylaxis against human brucellosis.

B.05. Cholera.

a. *Clinical Syndrome.*

(1) *Characteristics.* Cholera is a diarrheal disease caused by *Vibrio cholera,* a short, curved, gram-negative bacillus. Humans acquire the disease by consuming water or food contaminated with the organism. The organism multiplies in the small intestine and secretes an enterotoxin that causes a secretory diarrhea. When employed as a BW agent, cholera will most likely be used to contaminate water supplies. It is unlikely to be used in aerosol form.

(2) *Clinical Features.* Cholera may present as mild diarrhea or as a fulminant disease characterized by profuse watery diarrhea with fluid losses exceeding 5 to 10 liters or more per day. Without treatment, death may result from severe dehydration, hypovolemia and shock. Vomiting is often present early in the illness and may complicate oral replacement of fluid losses. There is little or no fever or abdominal pain.

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* On microscopic examination of stool samples there are few or no red cells or white cells. Serum electrolytes may demonstrate hypokalemia or if inappropriate fluid replacement has been given, may show hypernatremia or hyponatremia. Acidosis and renal failure may accompany severe dehydration.

(2) *Differential Diagnosis.* Watery diarrhea can also be caused by enterotoxigenic *E. coli,* rotavirus or other viruses, noncholera *vibrios,* or food poisoning due to ingestion of preformed toxins such as those of *Clostridium perfringens, Bacillus cereus,* or *Staphylococcus aureus.*

(3) *Specific Laboratory Diagnosis. Vibrios* can be identified in stool by darkfield or phase contrast microscopy, and *Vibrio cholera* can be grown on a variety of culture media. Bacteriologic diagnosis is not necessary to treat cholera or related watery diarrheas.

c. *Therapy*. Treatment of cholera depends primarily on replacement of fluid and electrolyte losses. This is best accomplished using oral dehydration therapy with the World Health Organization solution (3.5 g NaCL, 2.5 g NaHC03, 1.5 g KCl and 20 g glucose per liter). Intravenous fluid replacement is occasionally needed when vomiting is severe, when the volume of stool output exceeds 7 liters / day, or when severe dehydration with shock has developed. Antibiotics will shorten the duration of diarrhea and thereby reduce fluid losses. Tetracycline (250 mg every 6 hr for 3-5 days) or doxycycline (200 mg initially followed by 100 mg every 12 hr for 3-5 days) is generally adequate. Other effective drugs include ampicillin (250 mg every 6 hr for 5 days) and trimethoprim sulfamethoxazole (one tablet every 12 hr for 3-5 days).

d. *Prophylaxis.* Improved oral cholera vaccines are presently being tested. Vaccination with the currently available killed suspension of *V. cholera* provides about 50% protection that lasts for no more than 6 months. The initial dose is two injections given at least 1 week apart with booster doses every 6 months.

B.06. Clostridium Perfringens Toxins.

a. *Clinical Syndrome.*

(1) *Characteristics. Clostridium perfringens* is a common anaerobic bacterium associated with three distinct disease syndromes; gas gangrene or clostridial myonecrosis; enteritis necroticans (pig-bel); and clostridium food poisoning. Each of these syndromes has very specific requirements for delivering inocula of *C. perfringens* to specific sites to induce disease, and it is difficult to imagine a general scenario in which the spores or vegetative organisms could be used as a biological warfare agent. There are, however, at least 12 protein toxins elaborated, and one or more of these could be produced, concentrated, and used as a weapon. Waterborne disease is conceivable, but unlikely. The alpha toxin would be lethal by aerosol. This is a well-characterized, highly toxic phospholipase *C.* Other toxins from the organism might be co-weaponized and enhance effectiveness. For example, the epsilon

toxin is neurotoxic in laboratory animals.

(2) *Clinical Features.* The clinical picture of aerosolized *C. perfringens* alpha toxin would be expected to be that of a serious acute pulmonary insult. Absorbed alpha toxin could produce vascular leak, hemolysis, thrombocytopenia, and liver damage. Other toxins admixed could modify the illness. There is insufficient information available to speculate on a clinical syndrome produced by other *C. Perfringens* toxins.

b. *Diagnosis.*

(1) *Routine Findings.* Clinical laboratory findings might include anemia (due to intravascular hemolysis), thrombocytopenia, elevated serum transaminases, and hypoxia.

(2) *Differential Diagnosis.* Pulmonary findings might lead to confusion with staphylococcal enterotoxin B (SEB) initially. Liver damage, hemolytic anemia, and thrombocytopenia are not associated with SEB and the pulmonary findings should be reversible in SEB.

(3) *Specific Laboratory Diagnosis.* Acute serum and tissue samples should be collected and rapidly transported to a reference laboratory. Specific immunoassay are available; however, their utility in diagnosis of human disease is unproven. The enterotoxin can be detected in fecal samples from human food poisoning cases, and bacteria are readily cultured from clinical samples.

c. *Therapy*. No specific treatment is available for *C. pefringens* intoxication. The organism itself is sensitive to penicillin, and consequently, this is the current drug of choice. Recent data indicate that clindamycin or rifampin may suppress toxin production and provide superior results in animal models.

d. *Prophylaxis.* There is no available prophylaxis against most *C. perfringens* toxins. Toxoids are being used to prevent enteritis necroticans in humans, and veterinary toxoids are in wide use.

B.07. Crimean-Congo Hemorrhagic Fever.

a. *Clinical Syndrome.*

(1) *Characteristics.* Crimean-Congo hemorrhagic fever (CCHF) is a viral disease caused by CCHF virus. The virus is transmitted by ticks, principally of the genus Hyalomma, with intermediate vertebrate hosts varying with the tick species. The disease was first recognized in the Crimea, but occurs over most of Africa, the Middle East, the Balkans, the former USSR, and eastern China. Little is known about variations in the virus properties over the huge geographic area involved. Humans become infected through tick bites, crushing an infected tick, or at the slaughter of viremic livestock. (Domestic animals become infected but do not have significant disease.) The spread of disease within hospitals has been documented with this virus and poses a potentially significant problem. Even in epidemics, cases do not show narrow clustering and person-to-person spread is rare. CCHF would probably be

delivered by aerosol if used as a BW agent.

(2) *Clinical Features.*

(a) Typical cases present with sudden onset of fever and chills 3-12 days after tick exposure. Flushing, conjunctival injection, and mild hypotension may be present. After 2-3 days, perhaps with a temporary remission of fever, the patient develops bleeding manifestations such as petechiae, ecchymoses, oozing from puncture sites, melena, hematuria, and gastrointestinal (GI) hemorrhage. Crimean-Congo hemorrhagic fever may cause quite severe ecchymoses and extensive GI bleeding. There is severe headache, lumbar pain, nausea and vomiting, delirium, and prostration. Fatal cases are associated with extensive hemorrhage, coma, and shock. Other common physical findings are epigastric tenderness, modest hepatomegaly, and less frequently icterus.

(b) Mortality among cases recognized as hemorrhagic fever is 15-30%. Convalescence in survivors is prolonged with asthenia, dizziness, and often hair loss. Milder clinical disease occurs in an unknown proportion of infections. There may be geographic variations, possibly related to viral strain differences.

b. *Diagnosis.*

(1) *Differential Diagnosis.* Thrombocytopenia and elevated aspartate aminotransferase (AST) may provide a clue to suggest CCHF in the febrile patient seen early in the course of infection. Other viral hemorrhagic fevers, meningococcemia, rickettsial diseases, and similar conditions may resemble full-blown CCHF. Particular care should be taken in the case of massive GI bleeding not to confuse CCHF with surgical conditions.

(2) *Routine Laboratory Findings.* Leukopenia, thrombocytopenia, and elevated AST are all seen early. Abnormal coagulation tests are common and usually indicate disseminated intravascular coagulation (DIC). Platelets $\leq$ 20,000/ml, APT $\geq$ 260 sec, or AST $\geq$ 200U/ml carry a poor prognosis.

(3) *Specific Laboratory Diagnosis.* Most fatal cases and half the others will have detectable antigen by rapid enzyme-linked immunosorbant assay (ELISA) testing of acute serum samples. IgM ELISA antibodies occur early in recovery. IgG ELISA and fluorescent antibodies also show rising titers. Virus isolation in suckling mice is usually successful from acute sera.

c. *Therapy*.

(1) Supportive therapy with replacement of clotting factors is indicated. Crimean-Congo hemorrhagic fever virus is sensitive to ribavirin *in vitro* and clinicians have been favorably impressed in uncontrolled trials. Patients should be treated with intravenous ribavirin (30 mg/kg followed by 15 mg/kg q 6 h for 4 days and 7.5 mg/kg q 8 h for 6 days). Mild reversible anemia may occur. Immune globulin has also been recommended but is available only in Bulgaria.

(2) Because of several well-defined outbreaks within hospitals, protective measures for medical personnel are an issue. The weight of evidence points

to large droplets or fomites as the mediators of transmission and so strict barrier nursing is indicated and probably sufficient for the care of naturally acquired disease. The virus is aerosol-infectious and additional precautions (for example, respirators) might be considered in a biological warfare setting.

d. *Prophylaxis.*

(1) Although there is little field experience and no definitive data on efficacy, the sensitivity of the virus to ribavirin and the severity of disease suggests that prophylaxis of high-risk exposures is indicated. Persons with percutaneous exposure to contaminated needles or instruments and those exposed directly to fresh blood from CCHF patients should receive 400 mg ribavirin po tid (three times daily) for one day and then continue with 400 mg po tid for 7 days after the last exposure. If more than 48 hours have elapsed after the first such exposure, 30 mg/kg should be given intravenous (IV) followed by three IV doses of 15 mg/kg at 8 hourly intervals; then continue with 400 mg po q 8 hours. If there is GI intolerance, the 400 mg oral dose can be substituted with 180 mg IV. Monitoring for anemia is suggested.

(2) In the case of a suspected biological attack, ribavirin could be considered for prophylaxis, but there is insufficient information to make a firm recommendation for dosing. Use of 400 mg tid may result in mild to modest anemia in some recipients, GI intolerance in a small proportion, and the drug is embryopathic in rodents; there are unresolved issues of reversible testicular damage in rodents. An inactivated mouse-brain vaccine is used in Bulgaria, but there is no general experience with this product.

B.08. Melioidosis.

a. *Clinical Syndrome.*

(1) *Characteristics.* Melioidosis is an infectious disease of humans and animals caused by *Pseudomonas pseudomallei,* a gram-negative bacillus. It is especially prevalent in Southeast Asia but has been described from many countries around the world. The disease has a variable and inconstant clinical spectrum. A biological warfare attack with this organism would most likely be by the aerosol route.

(2) *Clinical Features.* Infection by inoculation results in a subcutaneous nodule with acute lymphangitis and regional lymphadenitis, generally with fever. Pneumonia may occur after inhalation or hematogenous dissemination of infection. It may vary in intensity from mild to fulminant, usually involves the upper lobes, and often results in cavitation. Pleural effusions are uncommon. An acute fulminant septicemia may occur characterized by rapid appearance of hypotension and shock. A chronic suppurative form may involve virtually any organ in the body.

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* The white blood cell count may range from normal to 20,000 per mm^3, and a mild anemia may develop during the illness.

(2) *Differential Diagnosis.* Melioidosis should be considered in the differential

diagnosis of any febrile illness, especially if multiple pustular skin or subcutaneous lesions develop, if the illness presents with fulminant respiratory failure, or there is a chest x-ray pattern suggestive of tuberculosis but without acid-fast bacilli on smear.

(3) *Specific Laboratory Diagnosis.* Microscopic examination of sputum or purulent exudates will reveal small, gram-negative bacilli with bipolar staining using methylene blue or Wright's stain. *P. pseudomallei* can be cultured on routine media and identified by standard bacteriologic procedures. A number of serological tests are useful in diagnosis when they show a fourfold titer rise in paired sera.

c. Therapy. Antibiotic regimens that have been used successfully include tetracycline, 2-3 g / day; chloramphenicol, 3 g / day; and trimethoprim-sulfamethoxazole, 4 and 20 mg / kg per day. Ceftazidine and piperacillin have enjoyed success in severely ill patients as well. In patients who are toxic, a combination of two antibiotics, given parenterally, is advised. Treatment should be continued with oral drugs for 60-150 days, and adjusted based on *in vitro* sensitivity studies of the organism isolated from the patient.

d. *Prophylaxis.* There are no means of immunization. Vigorous cleansing of abrasions and lacerations may reduce the risk of disease after inoculation of organisms into the skin. There is no information available on the utility of antibiotic prophylaxis after a potential exposure before the onset of clinical symptoms.

B.09. Plague.

a. *Clinical Syndrome.*

(1) *Characteristics.* Plague is a zoonotic disease caused by *Yersinia pestis.* Under natural conditions, humans become infected as a result of contact with rodents, and their fleas. The transmission of the gram-negative coccobacillus is by the bite of the infected flea, *Xenopsylla cheopis,* the oriental rat flea, or *Pulex irritans,* the human flea. Under natural conditions, three syndromes are recognized: bubonic, primary septicemia, or pneumonic. In a biological warfare scenario, the plague bacillus could be delivered via contaminated vectors (fleas) causing the bubonic type or, more likely, via aerosol causing the pneumonic type.

(2) *Clinical Features.* In bubonic plague, the incubation period ranges from 2 to 10 days. The onset is acute and often fulminant with malaise, high fever, and one or more tender lymph nodes. Inguinal lymphadenitis (bubo) predominates, but cervical and axillary lymph nodes can also be involved. The involved nodes are tender, fluctuant, and necrotic. Bubonic plague may progress spontaneously to the septicemia form with organisms spread to the central nervous system, lungs (producing pneumonic disease), and elsewhere. The mortality is 50 percent in untreated patients with the terminal event being circulatory collapse, hemorrhage, and peripheral thrombosis. In primary pneumonic plague, the incubation period is 2 to 3 days. The onset is acute and fulminant with malaise, high fever, chills, headache, myalgia, cough with

production of a bloody sputum, and toxemia. The pneumonia progresses rapidly, resulting in dyspnea, strider, and cyanosis. In untreated patients, the mortality is 100 percent with the terminal event being respiratory failure, circulatory collapse, and a bleeding diathesis.

b. *Diagnosis.*

(1) *Presumptive.* Presumptive diagnosis can be made by identification of the gram-negative coccobacillus with safety-pin bipolar staining organisms in Giemsa or Wayson's stained slides from a lymph node needle aspirate, sputum, or cerebrospinal fluid (CSF) samples. When available, immunofluorescent staining is very useful. Elevated levels of antibody to *Y. pestis* in a nonvaccinated patient may also be useful.

(2) *Definitive. Yersinia pestis* can be readily cultured from blood, sputum, and bubo aspirates. Most naturally occurring strains of *Y. pestis* produce an "Fl" antigen *in vivo* which can be detected in serum samples by immunoassay. A fourfold rise of *Y. pestis* antibody levels in patient serum is also diagnostic.

(3) *Differential.* In cases where bubonic type is suspected, tularemia adenitis, staphylococcal or streptococcal adenitis, meningococcemia, enteric gram-negative sepsis, and rickettsiosis need to be ruled out. In pneumonic plague, tularemia, anthrax, and staphylococcal enterotoxin B (SEB) agents need to be considered. Continued deterioration without stabilization effectively rules out SEB. The presence of a widened mediastinum on chest x-ray should alert one to the diagnosis of anthrax.

c. *Therapy*. Plague may be spread from person to person by droplets. Strict isolation procedures for all cases are indicated. Streptomycin, tetracycline, and chloramphenicol are highly effective if begun early. Significant reduction in morbidity and mortality is possible if antibiotics are given within the first 24 hours after symptoms of pneumonic plague develop. Intravenous doxycycline (200 mg initially, followed by 100 mg every 12 hours), intramuscular streptomycin (1 g every 12 hours), or intravenous chloramphenicol (1 g every 6 hours) for 10-14 days are effective against naturally occurring strains. Supportive management of life-threatening complications from the infection, such as shock, hyperpyrexia, convulsions, and disseminated intravascular coagulation (DIC), need to be initiated as they develop.

d. *Prophylaxis.* A formalin-killed *Y. pestis* vaccine is produced in the United States and has been extensively used. Efficacy against flea-borne plague is inferred from population studies, but the utility of this vaccine against aerosol challenge is unknown. Reactogenicity is moderately high and a measurable immune response is usually attained after a 3-dose primary series: at 0, 1, and 4-7 months. To maintain immunity, boosters every 1-2 years are required. Live-attenuated vaccines are available elsewhere but are highly reactogenic and without proven efficacy against aerosol challenge.

B.10. Q Fever.

a. *Clinical Syndrome.*

(1) *Characteristics.* Q fever is a zoonotic disease caused by a rickettsia, *Coxiella burnetii.* The most common animal reservoirs are sheep, cattle and goats. Humans acquire the disease by inhalation of particles contaminated with the organisms. A biological warfare attack would cause disease similar to that occurring naturally.

(2) *Clinical Features.* Following an incubation period of 10-20 days, Q fever generally occurs as a self-limiting febrile illness lasting 2 days to 2 weeks. Pneumonia occurs frequently, usually manifested only by an abnormal chest x-ray. A nonproductive cough and pleuritic chest pain occur in about one-fourth of patients with Q fever pneumonia. Patients usually recover uneventfully. Uncommon complications include chronic hepatitis, endocarditis, aseptic meningitis, encephalitis, and osteomyelitis.

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* The white blood cell count is elevated in one third of patients. Most patients with Q fever have a mild elevation of hepatic transaminase levels.

(2) *Differential Diagnosis.* Q fever usually presents as an undifferentiated febrile illness, or a primary atypical pneumonia, which must be differentiated from pneumonia caused by mycoplasma, legionnaire's disease, psittacosis or *Chlamydia pneumonia.* More rapidly progressive forms of pneumonia may look like bacterial pneumonias including tularemia or plague.

(3) *Specific Laboratory Diagnosis.* Identification of organisms by staining sputum is not helpful. Isolation of the organism is difficult and impractical. The diagnosis can be confirmed serologically.

c. *Therapy*. Tetracycline (250 mg every 6 hr) or doxycycline (100 mg every 12 hr) for 5-7 days is the treatment of choice. A combination of erythromycin (500 mg every 6 hr) plus rifampin (600 mg per day) is also effective.

d. *Prophylaxis.* Vaccination with a single dose of a killed suspension of *C. burnetii* provides complete protection against naturally occurring Q fever and >90% protection against experimental aerosol exposure in human volunteers. Protection lasts for at least 5 years. Administration of this vaccine in immune individuals may cause severe cutaneous reactions including necrosis at the inoculation site. Newer vaccines are under development. Treatment with tetracycline during the incubation period will delay but not prevent the onset of illness.

B. 11. Ricin.

a. *Clinical Syndrome.*

(1) *Characteristics.* Ricin is a glycoprotein toxin (66,000 daltons) from the seed of the castor plant. It blocks protein synthesis by altering the rRNA, thus killing the cell. Ricin's significance as a potential biological warfare agent relates to its availability world wide, its ease of production, and extreme

pulmonary toxicity when inhaled.

(2) *Clinical Features.* Overall, the clinical picture seen depends on the route of exposure. All reported serious or fatal cases of castor bean ingestion have taken approximately the same course: rapid onset of nausea, vomiting, abdominal cramps and severe diarrhea with vascular collapse; death has occurred on the third day or later. Following inhalation, one might expect nonspecific symptoms of weakness, fever, cough, and hypothermia followed by hypotension and cardiovascular collapse. In monkeys, inhalation toxicity is characterized by a dose dependent preclinical period of 24-36 hours followed by anorexia and progressive decrease in physical activity. Death occurs 36-48 hours post challenge. In mice, histopathologic change is characterized by necrotizing, suppurative airways lesions: rhinitis, laryngitis, tracheitis, bronchitis, bronchiolitis, and interstitial pneumonia with perivascular and alveolar edema. Histopathologic change in the airways is seen as early as 3 hours post challenge. The exact cause of death is unknown and probably varies with route of intoxication. High doses by inhalation appear to produce severe enough pulmonary damage to cause death.

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* Laboratory findings are generally nonspecific. Neutrophilic leukocytosis beginning between 12-18 hours was reported in a case of human lethal intramuscular intoxication that was purposely inflicted. Leukocytosis, beginning 12-18 hours after challenge, also occurs following aerosol exposure of laboratory animals.

(2) *Differential Diagnosis.* In oral intoxication, fever, gastrointestinal involvement, and vascular collapse are prominent, the latter differentiating it from infection with enteric pathogens. With regard to inhalation exposure, nonspecific findings of weakness, fever, vomiting, cough, hypothermia, and hypotension in large numbers of patients might suggest several respiratory pathogens. The temporal onset of botulinum intoxication would be similar, but include ptosis and general muscular paralysis with minimal pulmonary effects. Staphylococcal enterotoxin B intoxication would likely have a more rapid onset after exposure and a lower mortality rate but could be difficult to distinguish. Nerve agent intoxication is characterized by acute onset of cholinergic crisis with dyspnea and profuse secretions.

(3) *Specific Laboratory Diagnosis.* Based on animal studies, ELISA (for blood) or immunohistochemical techniques (for direct analysis of tissues) may be useful in confirming ricin intoxication. Postmortem pathologic change is route specific: inhalation results in airways lesions; ingestion causes gastrointestinal hemorrhage with necrosis of liver, spleen, and kidneys; and intramuscular intoxication causes severe local muscle and regional lymph node necrosis with moderate involvement of visceral organs. Ricin is extremely immunogenic; sera should be obtained from survivors for measurement of antibody response.

c. *Therapy*. Management is supportive and should include maintenance of intravascular volume. Standard management for poison ingestion should be employed if

intoxication is by the oral route. There is presently no antitoxin available for treatment.

d. *Prophylaxis.* There is currently no prophylaxis approved for human use. Active immunization and passive antibody prophylaxis are under study, as both are effective in protecting animals from death following exposure by intravenous or respiratory routes. Ricin is not dermally active, therefore, respiratory protection is the most critical means of prevention.

B.12. Rift Valley Fever.

a. *Clinical Syndrome.*

(1) *Characteristics.* Rift Valley Fever (RVF) is a viral disease caused by RVF virus. The virus circulates in sub-Saharan Africa as a mosquito-borne agent. Epizootics occur when susceptible domestic animals are infected, and because of the large amount of virus in their serum, amplify infection to biting arthropods. Deaths and abortions among susceptible species such as cattle and sheep constitute a major economic consequence of these epizootics, as well as providing a diagnostic clue and a method of surveillance. Humans become infected by the bite of mosquitoes or by exposure to virus-laden aerosols or droplets. Although disease may occur during an unexceptional rainy season, outbreaks are typically associated with very high densities of arthropod vector populations that may occur during heavy and prolonged rains or in association with irrigation projects. During epidemics the virus may be transmitted by many species of mosquitoes; its potential for introduction into areas with susceptible livestock and dense mosquito populations is believed to be high, as exemplified by a major epidemic in the Nile valley in 1977-79. The human disease appears to be similar whether acquired by aerosol or by mosquito bite. A biological warfare attack, most likely delivered by aerosol, would be expected to elicit the rather specific spectrum of human clinical manifestations and to cause disease in sheep and cattle in the exposed area. If disease occurred in the absence of heavy vector populations or without domestic animals as amplifiers of mosquito infection, a BW attack would also be a likely cause. Domestic animals are probably susceptible to aerosol infection or could be covertly infected to initiate an epidemic which might propagate itself by the usual means.

(2) *Clinical Features.* The incubation is two to five days and is usually followed by an incapacitating febrile illness of similar duration. The typical physical findings are fever, conjunctival injection, and sometimes abdominal tenderness. A few petechiae or epistaxis may occur. A small proportion of cases (approximately one percent) will progress to a viral hemorrhagic fever syndrome, often with associated hepatitis. These cases may manifest petechiae, mucosal bleeding, icterus, anuria, and shock; mortality in this group is roughly 50 percent. A similar proportion will develop clinically significant ocular changes; macular lesions associated with retinal vasculitis,

hemorrhage, edema, and infarction. Ocular manifestations begin after the patient enters convalescence from acute illness and about half of the patients will have permanent visual defects. A small number of infections will lead to a late encephalitis. After apparent recovery from a typical febrile illness, the patient develops fever, meningeal signs, obtundation, and focal defects. These patients may die or often have serious sequelae.

b. *Diagnosis.*

(1) *Differential Diagnosis.* The clinical syndrome in an individual is not pathognomonic, but the occurrence of an epidemic with febrile disease, hemorrhagic fever, eye lesions, and encephalitis in different patients would be characteristic of RVF.

(2) *Routine Laboratory Findings.* In acute uncomplicated disease, there is often a transient leucopenia, but liver and clotting function tests are normal. In hemorrhagic fever, abnormalities of hepatic and coagulation tests are proportional to severity of disease. Disseminated intravascular coagulation may be present. Patients with encephalitis have up to several hundred cells/mm in CSF, predominantly lymphocytes.

(3) *Specific Laboratory Diagnosis.* Demonstration of viral antigen in blood by ELISA is rapid and successful in a high proportion of acute cases of uncomplicated disease or hemorrhagic fever. IgM antibodies appear with cessation of viremia and are present when ocular or central nervous system (CNS) manifestations are noted. False positive reactions may occasionally be noted in patients with multiple sandfly fever infections. Encephalitis patients have IgM and IgG antibodies in CSF. A proportion of cases should be studied by classical means such as determination of neutralizing antibodies and virus isolation. Wide-scale surveillance is readily accomplished by simultaneous determination of IgG (infection or vaccination at an indeterminate time) and IgM (recent exposure) antibodies in human or domestic animal blood.

c. *Therapy*. In hemorrhagic fever, supportive therapy may be indicated for hepatic and renal failure, as well as replacement of coagulation factors. The virus is sensitive to ribavirin *in vitro* and in rodent models. No studies have been performed in human or the more realistic monkey model to ascertain whether administration to an acutely ill patient would be of benefit. It would be reasonable to treat patients with early signs of hemorrhagic fever with intravenous ribavirin (30 mg/kg followed by 15 mg/kg q 6 hr for 4 days and 7.5 mg/kg q 8 hr for 6 days). This regimen is safe and effective in hemorrhagic fevers caused by some viruses, although a reversible anemia may appear. Therapy may be stopped 2-3 days after improvement begins or antibody appears. Penetration of ribavirin into the CNS is slow and perhaps limited, but in the absence of any other specific therapy, the drug might be used in ocular and encephalitic cases.

d. *Prophylaxis.* Avoidance of mosquitoes and contact with fresh blood from dead domestic animals and respiratory protection from small particle aerosols are the mainstays of prevention. An effective inactivated vaccine is available in limited quantities. The dose is one ml given sc on days 0, 7, and 28; exact timing is not critical. Protective antibodies begin to appear within 10-14 days and last for a year,

at which time a one ml booster should be given. A single injection probably is not protective, but two inoculations may provide marginal short-term protection. Ribavirin prophylaxis (400 mg q 8 hr) of a related sandfly fever virus was successful, but the dose used might be expected to produce anemia and other effects in some recipients. The utility of lower doses has not been determined. Interferon alpha in doses not expected to be reactogenic in humans (5 x 103 - 5 x 104 U / kg daily) is preventive in monkeys and might be considered for post-exposure prophylaxis in humans.

B.13. Saxitoxin.

a. *Clinical Syndrome.*

(1) *Characteristics.*

(a) Saxitoxin is the parent compound of a family of chemically related neurotoxins. In nature they are predominantly produced by marine dinoflagellates, although they have also been identified in association with such diverse organisms as blue-green algae, crabs, and the blue-ringed octopus. Human intoxications are principally due to ingestion of bivalve molluscs which have accumulated dinoflagellates during filter feeding. The resulting intoxication, known as paralytic shellfish poisoning (PSP), is known throughout the world as a severe, life-threatening illness requiring immediate medical intervention.

(b) Saxitoxin and its derivatives are water-soluble compounds that bind to the voltage-sensitive sodium channel, blocking propagation of nerve-muscle action potentials. Consistent with this mechanism of action, victims typically present with neurological symptoms and in severe cases, death results from respiratory paralysis.

(c) The natural route of exposure to these toxins is oral. In a BW scenario, the most likely route of delivery is by inhalation or toxic projectile. In addition, saxitoxin could be used in a confined area to contaminate water supplies.

(2) *Clinical Features.* After oral exposure, absorption of toxins from the gastrointestinal tract is rapid. Onset of symptoms typically begins 10-60 minutes after exposure, but may be delayed several hours depending upon the dose and individual idiosyncrasy. Initial symptoms are numbness or tingling of the lips, tongue and fingertips, followed by numbness of the neck and extremities and general muscular incoordination. Nausea and vomiting may be present, but typically occur in a minority of cases. Other symptoms may include a feeling of light headedness, or floating, dizziness, weakness, aphasia, incoherence, visual disturbances, memory loss and headache. Cranial nerves are often involved, especially those responsible for ocular movements, speech, and swallowing. Induced reflexes are normal and the patient remains conscious. Respiratory distress and flaccid muscular paralysis are the terminal stages and can occur 2-12 hours after intoxication. Death results from respiratory paralysis. Clearance of the toxin is rapid and

survivors for 12-24 hours will usually recover. Complete recovery may require 7-14 days. There are no known cases of inhalation exposure to saxitoxin in the medical literature, but data from animal experiments suggest the entire syndrome is compressed and death may occur in minutes.

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* Routine laboratory evaluation is not particularly helpful. Cardiac conduction defects may develop. Elevation of serum creatine kinase levels in some patients has been reported.

(2) *Differential Diagnosis.* Exposure to tetrodotoxin or the ciguatera toxins can manifest very similar signs and symptoms. Ciguatoxins (by oral exposure) typically demonstrate a much greater degree of gastrointestinal involvement, and can also be differentiated by a history of eating finfish rather than shellfish. Tetrodotoxin intoxication is nearly identical to that caused by the saxitoxins except that hypotension typically plays a greater role in severe intoxication. Differential diagnosis may require toxin detection. Gas chromatographic analysis of food or stomach contents can rule out pesticide exposure.

(3) *Specific Laboratory Tests.* Diagnosis is confirmed by detection of toxin in the food, water, stomach contents or environmental samples. Saxitoxin, neosaxitoxin, and several other derivatives can be detected by ELISA or by mouse bioassay. Specific toxins can be differentiated by high pressure liquid chromatography (HPLC). The Association of Official Analytical Chemists has adopted an official method for mouse bioassay for the analysis of seafood.

c. *Therapy.* Management is supportive and standard management of poison ingestion should be employed if intoxication is by the oral route. Toxins are rapidly cleared and excreted in the urine, so diuresis may increase elimination. Charcoal hemoperfusion has been advocated, but remains unproven in its utility. Incubation and mechanical respiratory support may be required in severe intoxication. Timely resuscitation would be imperative, albeit very difficult, after inhalation exposure on the battlefield. Specific antitoxin therapy has been successful in animal models, but is untested in humans.

d. *Prophylaxis.* No vaccine against saxitoxin exposure has been developed for human use.

B.14. Smallpox.

a. *Clinical Syndrome.*

(1) *Characteristics.* Smallpox virus, an orthopoxvirus with a narrow host range confined to humans, was an important cause of morbidity and mortality in the developing world until recent times. Eradication of the natural disease was completed in 1977 and the last human cases (laboratory infections) occurred in 1978. The virus exists today in only 2 laboratory repositories in the U.S. and Russia. Appearance of human cases outside the laboratory would signal use of the virus as a biological weapon. Under natural conditions, the virus

is transmitted by direct (face-to face) contact with an infected case, by fomites, and occasionally by aerosols. Smallpox virus is highly stable and retains infectivity for long periods outside of the host. A related virus, monkeypox, clinically resembles smallpox and causes sporadic human disease in West and Central Africa.

(2) *Clinical Features.* The incubation period is typically 12 days (range, 10-17 days). The illness begins with a prodrome lasting 2-3 days, with generalized malaise, fever, rigors, headache, and backache. This is followed by defervescence and the appearance of a typical skin eruption characterized by progression over 7-10 days of lesions through successive stages, from macules to papules to vesicles to pustules. The latter finally form crusts and, upon healing, leave depressed depigmented scars. The distribution of lesions is centrifugal (more numerous on face and extremities than on the trunk). Lesions are in the same stage of development at any point in time. Fever may reappear around the 7th day after onset of rash. The case fatality rate is approximately 35% in unvaccinated individuals. A subset of patients develop a hemorrhagic diathesis with disseminated intravascular coagulopathy and have a poor prognosis. Other complications include arthritis, pneumonia, bacterial superinfection of skin lesions, osteomyelitis, and keratitis. Permanent joint deformities and blindness may follow recovery. Vaccine immunity may prevent or modify illness. Fully immune individuals exposed to the virus by the respiratory route may develop fever, sore throat, and conjunctivitis ("contact fever") lasting several days.

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* Leukopenia is frequently present in severe cases of smallpox. The differential count shows granulocytopenia and a relative increase in lymphocytes. In the early hemorrhagic form, with onset of bleeding before the eruption, severe thrombocytopenia, global reduction in clotting factors, and circulating antithrombin are present, as well as a marked increase in immature lymphoid cells in the peripheral blood, sometimes mistaken for acute leukemia.

(2) *Differential Diagnosis.* The eruption of chickenpox (varicella) is typically centripetal in distribution (worse on trunk than face and extremities) and characterized by crops of lesions in different stages on development. Chickenpox papules are soft and superticial, compared to the firm, shotty, and deep papules of smallpox. Chickenpox crusts fall off rapidly and usually leave no scar. Monkeypox cannot be easily distinguished from smallpox clinically, although generalized lymphadenopathy is a more common feature of the disease. Monkeypox occurs only in forested areas of West and Central Africa as a sporadic, zoonotic infection transmitted to humans from wild squirrels. Person-to-person spread is rare and ceases after 1-2 generations. Mortality is 15%. Other diseases that are sometimes confused with smallpox include typhus, secondary syphilis, and malignant measles.

(3) *Specific Laboratory Diagnosis.* Skin samples (scrapings from papules,

vesicular fluid, pus, or scabs) may provide a rapid identification of smallpox by direct electron microscopy, agar gel immunoprecipitation, or immunofluorescence. Virus may be recovered from these samples or blood by inoculation of eggs or cell cultures, but culture techniques require several days. Serological tests may be useful for confirmation, or early presumptive diagnosis.

c. *Therapy*. There is no specific treatment available although some evidence suggests that vaccinia-immune globulin may be of some value in treatment if given early in the course of the illness. The antiviral drug, n-methylisatin ß-thiosemicarbazone (Marboran ®) is not thought to be of any therapeutic value.

d. *Prophylaxis.*

(1) *Vaccines.*

(a) Vaccinia virus is a live poxvirus vaccine that induces strong cross-protection against smallpox for at least 5 years and partial protection for 10 years or more. The vaccine is administered by dermal scarification or intradermal jet injection; appearance of a vesicle or pustule within several days is indication of a "take." Contraindications to vaccination are pregnancy, clinical immunosuppression, eczema, or leukemia/lymphoma. Complications are infrequent, but include: 1) progressive vaccinia in immunosuppressed individuals (case-fatality >75%); 2) eczema vaccinatum in persons with eczema or a history of eczema, or in contacts with eczema (case-fatality 10-15%); 3) postvaccinal encephalitis, almost exclusively seen after primary vaccination, occurring at an incidence of about 1/500,000, with a case-fatality rate of 25%; 4) generalized vaccinia, seen in immunocompetent individuals and having a good prognosis; and 5) autoinnoculation of the eye or genital area, with a secondary lesion.

(b) Vaccinia-immune human globulin at a dose of 0.3 mg/kg body weight provides $\geq$ 70% protection against naturally occurring smallpox if given during the early incubation period. Administration immediately after or within the first 24 hours of exposure would provide the highest level of protection, especially in unvaccinated persons.

(c) If vaccinia-immune globulin is unavailable, vaccination or revaccination should be performed as early as possible after (and within 24 hours of) exposure, with careful surveillance for signs of illness.

(2) *Antiviral Drug.* The antiviral drug, n-methylisatin ß-thiosemicarbazone (Marboran@) afforded protection in some early trials, but not others, possibly because of noncompliance due to unpleasant gastrointestinal side effects. Critical review of the published literature suggests a possible protective effect among unvaccinated contacts of naturally infected individuals.

(3) *Quarantine, Disinfection.* Patients with smallpox should be treated by vaccinated personnel using universal precautions. Objects in contact with the patient, including bed linens, clothing, ambulance, etc.; require disinfection by fire, steam, or sodium hypochlorite solution.

B.15. Staphylococcal Enterotoxin B.

a. *Clinical Syndrome.*

(1) *Characteristics.* Staphylococcal Enterotoxin B (SEB) is one of several exotoxins produced by *Staphylococcus aureus,* causing food poisoning when ingested. A BW attack with aerosol delivery of SEB to the respiratory tract produces a distinct syndrome causing significant morbidity and potential mortality.

(2) *Clinical Features.* The disease begins 1-6 hours after exposure with the sudden onset of fever, chills, headache, myalgia, and nonproductive cough. In more severe cases, dyspnea and retrosternal chest pain may also be present. Fever, which may reach 103-106° F, has lasted 2-5 days, but cough may persist 1-4 weeks. In many patients nausea, vomiting, and diarrhea will also occur. Physical findings are often unremarkable. Conjunctival injection may be present, and in the most severe cases, signs of pulmonary edema would be expected. The chest x-ray is generally normal, but in severe cases, there will be increased interstitial markings, atelectasis, and possibly overt pulmonary edema. In moderately severe laboratory exposures, lost duty time has been < 2 weeks, but, based upon animal data, it is anticipated that severe exposures will result in fatalities.

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* Laboratory findings are noncontributory except for a neutrophilic leukocytosis and elevated erythrocyte sedimentation rate.

(2) *Differential Diagnosis.*

(a) In foodborne SEB intoxication, fever and respiratory involvement are not seen, and gastrointestinal symptoms are prominent. The nonspecific findings of fever, nonproductive cough, myalgia, and headache occurring in large numbers of patients in an epidemic setting would suggest any of several infectious respiratory pathogens, particularly influenza, adenovirus, or mycoplasma. In a BW attack with SEB, cases would likely have their onset within a single day, while naturally occurring outbreaks would present over a more prolonged interval. Naturally occurring outbreaks of Q fever and tularemia might cause confusion, but would involve much smaller numbers of individuals, and would more likely be accompanied by pulmonary infiltrates.

(b) The dyspnea of botulism is associated with obvious signs of muscular paralysis: its cholinergic blocking effects result in a dry respiratory tree, and patients are afebrile. Inhalation of nerve agent will lead to weakness, dyspnea, and copious secretions. The early clinical manifestations of inhalation anthrax, tularemia, or plague may be similar to those of SEB. However, rapid progression of respiratory signs and symptoms to a stable state distinguishes SEB intoxication. Mustard exposure would have marked vesication of the skin in addition to the pulmonary injury.

(3) *Specific Laboratory Diagnosis.* Toxin is cleared from the serum rapidly and

is difficult to detect by the time of symptom onset. Nevertheless, specific laboratory tests are available to detect SEB, and serum should be collected as early as possible after exposure. In situations where many individuals are symptomatic, sera should be obtained from those not yet showing evidence of clinical disease. Most patients develop a significant antibody response, but this may require 2-4 weeks.

c. *Therapy*. Treatment is limited to supportive care. No specific antitoxin for human use is available.

d. *Prophylaxis*. There currently is no prophylaxis for SEB intoxication. Experimental immunization has protected monkeys, but no vaccine is presently available for human use.

B.16. Trichothecene Mycotoxins.

a. *Clinical Syndrome.*

(1) *Characteristics.*

(a) The trichothecene mycotoxins are a diverse group of more than 40 compounds produced by fungi. They are potent inhibitors of protein synthesis, impair DNA synthesis, alter cell membrane structure and function, and inhibit mitochondrial respiration. Secondary metabolizes of fungi, such as T-2 toxin and others, produce toxic reactions called mycotoxicoses upon inhalation or consumption of contaminated food products by humans or animals. Naturally occurring trichothecenes have been identified in agricultural products and have been implicated in a disease of animals known as moldy corn toxicosis or poisoning.

(b) There are no well-documented cases of clinical exposure of humans to trichothecenes. However, strong circumstantial evidence has associated these toxins with alimentary toxic aleukia (ATA), the fatal epidemic seen in Russia during World War II, and with alleged BW incidents (“yellow rain”) in Cambodia, Laos and Afghanistan.

(2) *Clinical Features.*

(a) Consumption of these mycotoxins results in weight loss, vomiting, skin inflammation, bloody diarrhea, diffuse hemorrhage, and possibly death. Clinical signs in experimental animals (calves) given 0.08-0.64 mg T-2/kg/day for nine days included loss of appetite, weight loss, an increase in prothrombin time, and an increased serum aspartate amino transferase level. The onset of illness following acute exposure to T-2 (IV or inhalation) occurs in hours, resulting in the rapid onset of circulatory shock characterized by reduced cardiac output, arterial hypotension, lactic acidosis and death within 12 hours.

(b) Clinical signs and symptoms of ATA were hemorrhage, leukopenia, ulcerative pharyngitis, and depletion of bone marrow. The purported use of T-2 as a BW agent resulted in an acute exposure via inhalation and/or dermal routes, as well as oral exposure upon consumption of contaminated food products and water. Alleged victims reported painful skin lesions,

lightheadedness, dyspnea, and a rapid onset of hemomhage, incapacitation and death. Survivors developed a radiation-like sickness including fever, nausea, vomiting, diarrhea, leukopenia, bleeding, and sepsis.

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* Hematological alterations in the rodent model (parenteral routes) include marked but transient leukocytosis, characterized by rapid lymphocytosis and a mild neutrophilia. This is followed by a leukopenia that returns to normal values 4-7 days post-exposure. There is a reduced hematocrit with the presence of nucleated erythrocytes. Serum proteins and enzymes are not significantly altered after this acute exposure.

(2) *Differential Diagnosis.* Other diagnoses to consider include radiation toxicity and plant or chemical toxicity.

(3) *Specific Laboratory Diagnosis.* Specific diagnostic modalities are limited to reference laboratories. Gas-liquid chromatography (GC) and high pressure liquid chromatography (HPLC) have been used for detecting T-2 and related trichothecene mycotoxins in plasma and urine. Polyclonal and monoclinal antibodies to trichothecenes are also available for detection in liquid or solid samples after solvent extraction. Because of their long “half-life” the toxin metabolizes can be detected as late as 28 days after exposure. Between 50-75% of the parent toxin and metabolizes are eliminated in urine and feces within 24 hours. Urine should be the biological fluid chosen for diagnostic purposes. A one time urine sample with 0.10CC concentrated hydrochloric acid (HCI) added per 100cc of urine, to kill unwanted bacteria, should be submitted for analysis if the exposure was a recent one. Trichothecene mycotoxins can be detected in the urine out to approximately 14 days after exposure but if several days have elapsed since exposure, a 24 hour urine collection with HCI added should be submitted instead of a one time collection. The urine does not need to be kept refrigerated.

c. *Therapy*. General supportive measures are used to alleviate acute T-2 toxicoses. Prompt (within 5-60 min of exposure) soap and water wash significantly reduces the development of the localized destructive, cutaneous effects of the toxin. After oral exposure management should include standard therapy for poison ingestion. Of note is a superactivated charcoal (such as Superchar™, Gulf Bio Systems, Inc., Dallas, TX). Superchar™ oral may offer an advantage over regular activated charcoal in that one needs to see approximately five times the dose of activated charcoal to gain an equivalent outcome to that if Superchar™ is used. Superactivated charcoal is becoming standard in emergency management of poison ingestion. This substance has an extremely large surface area, two to three times that of regular activated charcoal. Superchar™ oral treatment (1-7 g/kg, po) either immediately or 1 to 3 hours after toxin exposure significantly increases survival times of animals. Some benefit may be derived from giving activated charcoal as late as 5 hours after exposure to T-2 toxins. In animal studies, dexamethasone (1-10 mg/kg, IV) administered as late as 3 hours after exposure to T-2 toxin improved survival and reduced the incidence of massive bloody diarrhea. No antitoxin is presently available for human use.

d. *Prophylaxis.* Ascorbic acid (400-1200 mg/kg, inter-peritoneal (ip)) works to decrease lethality in animal studies, but has not been tested in humans. While not yet available for humans, administration of large doses of monoclinal antibodies directed against T-2 and metabolizes have shown prophylactic and therapeutic efficacy in animal models.

B.17. Tularemia.

a. *Clinical Syndrome.*

(1) *Characteristics.* Tularemia is a zoonotic disease caused by *Francisella tularensis,* a gram-negative bacillus. 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. Less commonly, inhalation of contaminated dusts or ingestion of contaminated foods or water may produce clinical disease. A BW attack with *F. tularensis* delivered by aerosol would primarily cause typhoidal tularemia, a syndrome expected to have a case fatality rate which may be higher than the 5-10% seen when disease is acquired naturally.

(2) *Clinical Features.*

(a) A variety of clinical forms of tularemia are seen, depending upon the route of inoculation and virulence of the strain. In humans, as few as 10-50 organisms will cause disease if inhaled or injected intradermally, whereas 10^8 organisms are required with oral challenge. Under natural conditions, ulceroglandular tularemia generally occurs about 3 days after intradermal inoculation (range 2-10 days), and manifests as regional lymphadenopathy, fever, chills, headache, and malaise, with or without a cutaneous ulcer. In those 5-10% of cases with no visible ulcer, the syndrome may be known as glandular tularemia. Primary ulceroglandular disease confined to the throat is referred to as pharyngeal tularemia. Oculoglandular tularemia occurs after inoculation of the conjunctival with a hand or fingers contaminated by tissue fluids from an infected animal. Gastrointestinal tularemia occurs after drinking contaminated ground water, and is characterized by abdominal pain, nausea, vomiting, and diarrhea.

(b) Bacteremia probably is common after primary intradermal, respiratory, or gastrointestinal infection with *F. tularensis* and may result in septicemia or "typhoidal" tularemia. The typhoidal form also may occur as a primary condition in 5-15% of naturally-occurring cases; clinical features include fever, prostration, and weight loss, but without adenopathy. Diagnosis of primary typhoidal tularemia is difficult, as signs and symptoms are non-specific and there frequently is no suggestive exposure history. Pneumonic tularemia is a severe atypical pneumonia that may be fulminant, and can be primary or secondary. Primary pneumonia may follow direct inhalation of infectious aerosols, or may result from aspiration of organisms in cases of pharyngeal tularemia. Pneumonic tularemia causes fever, headache, malaise, substernal discomfort, and a non-productive cough; radiologic evidence of

pneumonia or mediastinal lymphadenopathy may or may not be present.

(c) A biological warfare attack with *F. tularensis* would most likely be delivered by aerosol, causing primarily typhoidal tularemia. Many exposed individuals would develop pneumonic tularemia (primary or secondary), but clinical pneumonia may be absent or non-evident. Case fatality rates may be higher than the 5-10% seen when the disease is acquired naturally.

b. *Diagnosis.*

(1) *Differential Diagnosis. The* clinical presentation of tularemia may be severe, yet nonspecific. Differential diagnoses include typhoidal syndromes (e.g., salmonella, rickettsia, malaria) or pneumonic processes (e.g., plague, mycoplasma, SEB). A clue to the diagnosis of tularemia delivered as a BW agent might be a large number of temporally clustered patients presenting with similar systemic illnesses, a proportion of whom will have a nonproductive pneumonia.

(2) *Specific Laboratory Diagnosis.* Identification of organisms by staining ulcer fluids or sputum is generally not helpful. Routine culture is difficult, due to unusual growth requirements and/or overgrowth of commensal bacteria. The diagnosis can be established retrospectively by serology.

c. *Therapy*. Streptomycin (1 gm q 12 intramuscular (IM) for 10-14 days) is the treatment of choice. Gentamicin also is effective (3-5 mg/kg/day parenterally for 10-14 days). Tetracycline and chloramphenicol treatment are effective as well, but are associated with a significant relapse rate. Although laboratory-related infections with this organism are very common, human-to-human spread is unusual and isolation is not required.

d. *Prophylaxis.* A live, attenuated tularemia vaccine is available as an investigational new drug (IND). This vaccine has been administered to more than 5,000 persons without significant adverse reactions and is of proven effectiveness in preventing laboratory-acquired typhoidal tularemia. Its effectiveness against the concentrated bacterial challenge expected in a BW attack is unproven. The use of antibiotics for prophylaxis against tularemia is controversial.

B.18. Venezuelan Equine Encephalitis.

a. *Clinical Syndrome.*

(1) *Characteristics.* Eight serologically distinct viruses belonging to the Venezuelan equine encephalitis (VEE) complex have been associated with human disease; the most important of these pathogens are designated subtype 1, variants A, B and C. These agents also cause severe disease in horses, mules, and donkeys (Equidae). Natural infections are acquired by the bites of a wide variety of mosquitoes; Equidae serve as the viremic hosts and source of mosquito infection. In natural human epidemics, severe and often fatal encephalitis in Equidae always precedes that in humans. A BW attack with virus disseminated as an aerosol would cause human disease as a primary event. If Equidae were present, disease in these animals would occur

simultaneously with human disease. Secondary spread by person-to-person \contact occurs at a negligible rate. However, a BW attack in a region populated by Equidae and appropriate mosquito vectors could initiate an epizootic/epidemic.

(2) *Clinical Features.* Nearly 100% of those infected suffer an overt illness. After an incubation period of 1-5 days, onset of illness is extremely sudden, with generalized malaise, spiking fever, rigors, severe headache, photophobia, myalgia in the legs and lumbosacral area. Nausea, vomiting, cough, sore throat, and diarrhea may follow. This acute phase lasts 24-72 hours. A prolonged period of aesthenia and lethargy may follow, with full health and activity regained only after 1-2 weeks. Approximately 470 of patients during natural epidemics develop signs of central nervous system infection, with meningismus, convulsions, coma, and paralysis. These necrologic cases are seen almost exclusively in children. The overall case-fatality rate is < 1%, but in children with encephalitis, it may reach 20%. Permanent neurological sequelae are reported in survivors. Aerosol infection does not appear to increase the likelihood of CNS disease. A VEE infection during pregnancy may cause encephalitis in the fetus, placental damage, abortion, or severe congenital neuroanatomical anomalies.

b. *Diagnosis.*

(1) *Routine Laboratory Findings.* The white blood cell count shows a striking leukopenia and lymphopenia. In cases with encephalitis, the cerebrospinal fluid may be under increased pressure and contain up to 1000 white cells/mm^3 (predominantly mononuclear cells) and mildly elevated protein concentration.

(2) *Differential Diagnosis.* An outbreak of VEE may be difficult to distinguish from influenza on clinical grounds. Clues to the diagnosis are the appearance of a small proportion of neurological cases or disease in Equidae, but these might be absent in a BW attack.

(3) *Specific Laboratory Diagnosis.* Viremia during the acute phase of illness is generally high enough to allow detection by antigen-capture enzyme immunoassay. Virus isolation may be made from serum, and in some cases throat swab specimens, by inoculation of cell cultures. A variety of serological tests are applicable, including the IgM ELISA, indirect fluorescent assay (FA), hemagglutination inhibition, complement-fixation, and neutralization. For persons without prior exposure to VEE complex viruses in tropical areas, a presumptive diagnosis may be made by finding antibodies in a single serum sample taken 5-7 days after onset of illness.

c. *Therapy*. There is no specific therapy. Patients with uncomplicated VEE infection may be treated with analgesics to relieve headache and myalgia. Patients who develop encephalitis may require anticonvulsant and intensive supportive care to maintain fluid and electrolyte balance, adequate ventilation, and to avoid complicating secondary bacterial infections.

d. *Prophylaxis.*

(1) *Vaccine.*

(a) An experimental vaccine, designated TC-83 is a live, attenuated cell-culture-propagated vaccine which has been used in several thousand persons to prevent laboratory infections. The vaccine is given as a single 0.5 ml subcutaneous dose. Febrile reactions occur in up to 18% of persons vaccinated, and may be moderate-to-severe in 5%, with fever, myalgia, headache, and prostration. Approximately 10% of vaccinees fail to develop detectable neutralizing antibodies, but it is unknown whether they are susceptible to clinical infection if challenged. Nonresponders may be revaccinated with TC-83. Contraindications for use include an intercurrent viral infection or pregnancy. TC-83 is a licensed vaccine for Equidae.

(b) A second investigational product that has been tested in humans is the C-84 vaccine, prepared by formalin-inactivation of the TC-83 strain. The vaccine is presently not recommended for primary immunization, on the basis of animal studies indicating that it may not protect against aerosol infection. However, it may be useful for aerosol protection for persons not responding to TC-83 (0.5 ml subcutaneously at 2 to 4 week intervals for up to 3 inoculations or until an antibody response is measured.)

(2) *Antiviral Drugs.* In experimental animals, alpha-interferon and the interferon-inducer poly-ICLC (lysine-polyadenosine) have proven highly effective for post-exposure prophylaxis of VEE. There are no clinical data on which to assess efficacy in humans.