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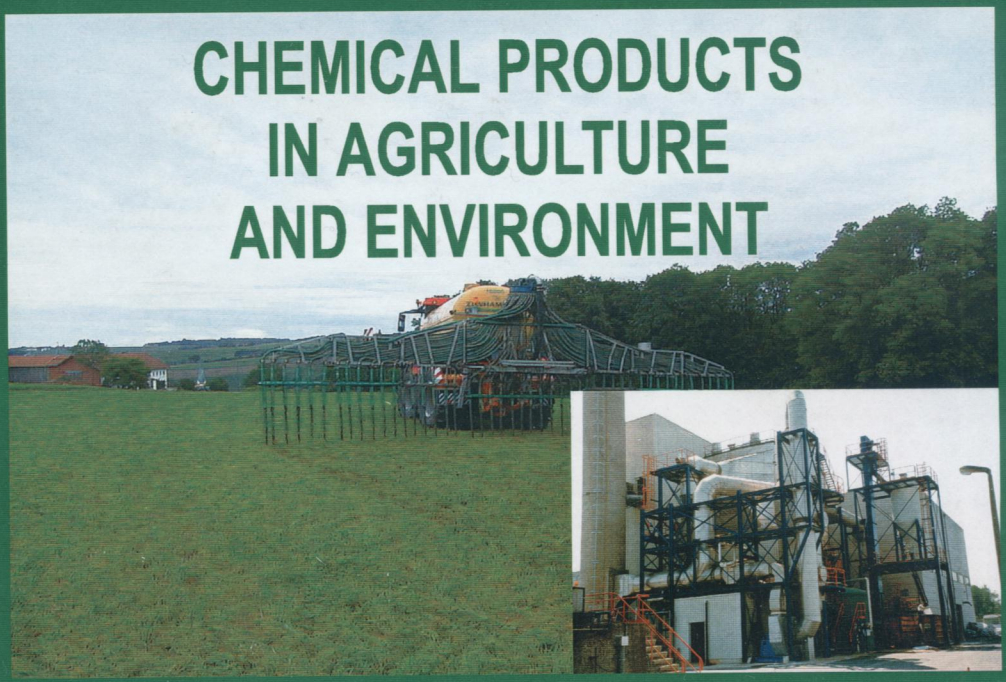
CHEMICAL PRODUCTS IN AGRICULTURE AND ENVIRONMENT



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bioterrorism
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"Germ terrorism... is the single most dangerous threat to our national security in the foreseeable future."
R. James Woolsey, Director of Central Intelligence Agency 1993-95

ZOONOTIC DISEASES AS THE AGENTS IN BIOTERRORISM

Bioterrorism means the unlawful use, or threatened use, of microorganisms or toxins derived from living organisms to produce death or disease in humans, animals, or plants. The act is intended to create fear and/or intimidate governments or societies in the pursuit of political, religious, or ideological goals.

It is important that recognition of and preparation for a biological attack is similar to that for any disease outbreak, but the surveillance, response, and other demands on resources would likely be of an unparalleled intensity. A strong public health infrastructure with epidemiologic investigation capability, practical training programs, and preparedness plans are essential to prevent and control disease outbreaks, whether they are naturally occurring or otherwise.

As steep epidemic curves can be seen in natural point-source exposures, additional characteristics of the outbreak should be investigated in determining whether it is the result of a biological attack [1,2].

None of the following clues alone constitute proof of intentional use of a biological agent, but together they can assist greatly in determining if further investigation is warranted.

1) The presence of a large epidemic, with greater case loads than expected, especially in a discrete population.

2) More severe disease than expected for a given pathogen, as well as unusual routes of exposure, such as a preponderance of inhalational disease as was seen in Sverdlovsk after the accidental release of aerosolized *Bacillus anthracis* spores [3].

3) A disease that is unusual for a given geographic area, is found outside the normal transmission season, or is impossible to transmit naturally in the absence of the normal vector for transmission.

4) Multiple simultaneous epidemics of different diseases.

5) A disease outbreak with zoonotic as well as human consequences, as many of the potential threat agents are pathogenic to animals.

6) Unusual strains or variants of organisms or antimicrobial resistance patterns disparate from those circulating.

7) Higher attack rates in those exposed in certain areas, such as inside a building if the agent was released indoors, or lower rates in those inside a sealed building if an aerosol was released outdoors.

8) Intelligence that an adversary has access to a particular agent or agents.

9) Claims by a terrorist of the release of a biologic agent.

10) Direct evidence of the release of an agent, with findings of equipment, munitions, or tampering.

There are some biological agents which can be used in the terrorists acts [4].

BACTERIAL AGENTS

Bacteria are unicellular organisms. They vary in shape and size from spherical cells - cocci - with a diameter of 0.5-1.0 μm (micrometer), to long rod-shaped organisms - bacilli - which may be from 1-5 μm in size. Chains of bacilli may exceed 50 μm in length.

The shape of the bacterial cell is determined by the rigid cell wall. The interior of the cell contains the nuclear material (DNA), cytoplasm, and cell membrane, that are necessary for the life of the bacterium. Many bacteria also have glycoproteins on their outer surfaces which aid in bacterial attachment to cell surface receptors. Under special circumstances some types of bacteria can transform into spores. The spore of the bacterial cell is more resistant to cold, heat, drying, chemicals and radiation than the vegetative bacterium itself. Spores are a dormant form of the bacterium and, like the seeds of plants, they can germinate when conditions are favourable.

The term rickettsia generally applies to very small, gram-negative coccobacillary organisms of the genera *Rickettsia* and *Coxiella*. Rickettsiae are unique from classical bacteria in their inability to grow (with rare exceptions) in the absence of a living host cell, but many are susceptible to treatment with antibiotics.

Bacteria generally cause disease in human beings and animals by one of two mechanisms: by invading host tissues, and by producing poisons (toxins). Many pathogenic bacteria utilize both mechanisms. The diseases they produce often respond to specific therapy with antibiotics. It is important to distinguish between the disease-causing organism and the name of the disease it causes (in parentheses below). This manual covers several of the bacteria or rickettsiae considered to be potential BW threat agents: *Bacillus anthracis* (Anthrax), *Brucella* spp. (Brucellosis), *Burkholderia mallei* (Glanders), *Burholderia pseudomallei* (melioidosis), *Yersinia pestis* (Plague), *Francisella tularensis* (Tularemia), and *Coxiella burnetii* (Q Fever).

Antrax

Bacillus anthracis, the causative agent of Anthrax, is a gram-positive, sporulating rod. The spores are the usual infective forms. Anthrax is primarily a zoonotic disease of herbivores, with cattle, sheep, goats, and horses being the usual domesticated animal hosts,

but other animals may be infected. Humans generally contract the disease when handling contaminated hair, wool, hides, flesh, blood and excreta of infected animals and from manufactured products such as bone meal. Infection is introduced through scratches or abrasions of the skin, wounds, inhalation of spores, eating insufficiently cooked infected meat, or by biting flies. The primary concern for intentional infection by this organism is through inhalation after aerosol dissemination of spores. All human populations are susceptible. The spores are very stable and may remain viable for many years in soil and water. They resist sunlight for varying periods.

Brucellosis

Brucellosis is one of the world's most important veterinary diseases, and is caused by infection with one of six species of *Brucellae,* a group of gram-negative cocco-baccillary facultative intracellular pathogens. In animals, brucellosis primarily involves the reproductive tract, causing septic abortion and orchitis, which, in turn, can result in sterility. Consequently, brucellosis is a disease of great potential economic impact in the animal husbandry industry. Four species (*B. abortus, B. melitensis, B. suis*, and, rarely, *B. canis*) are pathogenic in humans. Infections in abattoir and laboratory workers suggest that the *Brucellae* are highly infectious via the aerosol route. It is estimated that inhalation of only 10 to 100 bacteria is sufficient to cause disease in man. Brucellosis has a low mortality rate (5% of untreated cases), with rare deaths caused by endocarditis or meningitis. Also, given that the disease has a relatively long and variable incubation period (5-60 days), and that many naturally occurring infections are asymptomatic, its usefulness as a weapon may be diminished. Large aerosol doses, however, may shorten the incubation period and increase the clinical attack rate, and the disease is relatively prolonged, incapacitating, and disabling in its natural form.

Brucellosis, also known as "undulant fever", typically presents as a nonspecific febrile illness resembling influenza. Fever, headache, myalgias, arthralgias, back pain, sweats, chills, generalized weakness, and malaise are common complaints. Cough and pleuritic chest pain occurs in up to twenty percent of cases, but acute pneumonitis is unusual, and pulmonary symptoms may not correlate with radiographic findings. The chest x-ray is often normal, but may show lung abscesses, single or miliary nodules, bronchopneumonia, enlarged hilar lymph nodes, and pleural effusions. Gastrointestinal symptoms (anorexia, nausea, vomiting, diarrhea and constipation) occur in up to 70 percent of adult cases, but less frequently in children. Ileitis, colitis, and granulomatous or mononuclear infiltrative hepatitis may occur, with hepato- and spleno-megaly present in 45-63 percent of cases.

Glanders and Melioidosis

The causative agents of Glanders and Melioidosis are *Burkholderia mallei* and *Burkholderia pseudomallei,* respectively. Both are gram-negative bacilli with a "safety-pin" appearance on microscopic examination. Both pathogens affect domestic and wild animals, which, like humans, acquire the diseases from inhalation or contaminated injuries.

B. mallei is primarily noted for producing disease in horses, mules, and donkeys. In the past man has seldom been infected, despite frequent and often close contact with

infected animals. This may be the result of exposure to low concentrations of organisms from infected sites in ill animals and because strains virulent for equids are often less virulent for man. There are four basic forms of disease in horses and man. The acute forms are more common in mules and donkeys, with death typically occurring 3 to 4 weeks after illness onset. The chronic form of the disease is more common in horses and causes generalized lymphadenopathy, multiple skin nodules that ulcerate and drain, and induration, enlargement, and nodularity of regional lymphatics on the extremities and in other areas. The lymphatic thickening and induration has been called farcy. Human cases have occurred primarily in veterinarians, horse and donkey caretakers, and abattoir workers.

B. pseudomallei is widely distributed in many tropical and subtropical regions. The disease is endemic in Southeast Asia and northern Australia. In northeastern Thailand, *B. pseudomallei*, is one of the most common causative agents of community-acquired septicemia. Melioidosis presents in humans in several distinct forms, ranging from a subclinical illness to an overwhelming septicemia, with a 90% mortality rate and death within 24-48 hours after onset. Also, melioidosis can reactivate years after primary infection and result in chronic and life-threatening disease.

These organisms spread to man by invading the nasal, oral, and conjunctival mucous membranes, by inhalation into the lungs, and by invading abraded or lacerated skin. Aerosols from cultures have been observed to be highly infectious to laboratory workers. Biosafety level 3 containment practices are required when working with these organisms in the laboratory. Since aerosol spread is efficient, and there is no available vaccine or reliable therapy, *B. mallei* and *B. pseudomallei* have both been viewed as potential BW agents.

Plague

Yersinia pestis is a rod-shaped, non-motile, non-sporulating, gram-negative bacterium of the family *Enterobacteraceae*. It causes plague, a zoonotic disease of rodents (e.g., rats, mice, ground squirrels). Fleas that live on the rodents can transmit the bacteria to humans, who then suffer from the bubonic form of plague. The bubonic form may progress to the septicemic and/or pneumonic forms. Pneumonic plague would be the predominant form after purposeful aerosol dissemination. All human populations are susceptible. Recovery from the disease is followed by temporary immunity. The organism remains viable in water, moist soil, and grains for several weeks. At near freezing temperatures, it will remain alive from months to years but is killed by 15 minutes of exposure to 55°C. It also remains viable for some time in dry sputum, flea feces, and buried bodies but is killed within several hours of exposure to sunlight.

Q Fever

The endemic form of Q fever is a zoonotic disease caused by the rickettsia, *Coxiella burnetii*. Its natural reservoirs are sheep, cattle, goats, dogs, cats and birds. The organism grows to especially high concentrations in placental tissues. The infected animals do not develop the disease, but do shed large numbers of the organisms in placental tissues and body fluids including milk, urine, and feces. Exposure to infected animals at parturition is an important risk factor for endemic disease. Humans acquire the disease by inhalation of aerosols contaminated with the organisms. Farmers and abattoir workers are at greatest risk

occupationally. A biological warfare attack with Q fever would cause a disease similar to that occurring naturally. Q fever is also a significant hazard in laboratory personnel who are working with the organism.

Tularemia

Francisella tularensis, the causative agent of tularemia, is a small, aerobic non-motile, gram-negative cocco-bacillus. Tularemia (also known as rabbit fever and deer fly fever) is a zoonotic disease that humans typically acquire after skin or mucous membrane contact with tissues or body fluids of infected animals, or from bites of infected ticks, deerflies, or mosquitoes. Less commonly, inhalation of contaminated dusts or ingestion of contaminated foods or water may produce clinical disease. Respiratory exposure by aerosol would typically cause typhoidal or pneumonic tularemia. *F. tularensis* can remain viable for weeks in water, soil, carcasses, hides, and for years in frozen rabbit meat. It is resistant for months to temperatures of freezing and below. It is easily killed by heat and disinfectants.

VIRAL AGENTS

Viruses are the simplest microorganisms and consist of a nucleocapsid protein coat containing genetic material, either RNA or DNA. In some cases, the viral particle is also surrounded by an outer lipid layer. Viruses are much smaller than bacteria and vary in size from 0.02 μm to 0.2 μm (1 μm = 1/1000 mm). Viruses are intracellular parasites and lack a system for their own metabolism; therefore, they are dependent on the synthetic machinery of their host cells. This means that viruses, unlike the bacteria, cannot be cultivated in synthetic nutritive solutions, but require living cells in order to multiply. he host cells can be from humans, animals, plants, or bacteria. Every virus requires its own special type of host cell for multiplication, because a complicated interaction occurs between the cell and virus. Virus-specific host cells can be cultivated in synthetic nutrient solutions and then infected with the virus in question. Another common way of cultivating viruses is to grow them on chorioallantoic membranes (from fertilized eggs). The cultivation of viruses is expensive, demanding, and time-consuming. A virus typically brings about changes in the host cell that eventually lead to cell death. This handbook covers three types of viruses which could potentially be employed as BW agents: smallpox, alphaviruses (eg., VEE), and hemorrhagic fever viruses.

Smallpox

Smallpox is caused by the Orthopox virus, variola, which occurs in at least two strains, variola major and the milder disease, variola minor. Despite the global eradication of smallpox and continued availability of a vaccine, the potential weaponization of variola continues to pose a military threat. This threat can be attributed to the aerosol infectivity of the virus, the relative ease of large-scale production, and an increasingly *Orthopoxvirus*-naive populace. Although the fully developed cutaneous eruption of smallpox is unique, earlier stages of the rash could be mistaken for varicella. Secondary spread of infection constitutes a nosocomial hazard from the time of onset of a smallpox patient's exanthem until scabs have separated. Quarantine with respiratory isolation should be applied to secondary contacts for

17 days post-exposure. Vaccinia vaccination and vaccinia immune globulin each possess some efficacy in post-exposure prophylaxis.

Venezuelan Equine Encephalitis

The Venezuelan equine encephalitis (VEE) virus complex is a group of eight mosquito-borne alphaviruses that are endemic in northern South America and Trinidad and causes rare cases of human encephalitis in Central America, Mexico, and Florida. These viruses can cause severe diseases in humans and Equidae (horses, mules, burros and donkeys). Natural infections are acquired by the bites of a wide variety of mosquitoes. Equidae serve as amplifying hosts and source of mosquito infection.

Western and Eastern Equine Encephalitis viruses are similar to the VEE complex, are often difficult to distinguish clinically, and share similar aspects of transmission and epidemiology. The human infective dose for VEE is considered to be 10-100 organisms, which is one of the principal reasons that VEE is considered a militarily effective BW agent. Neither the population density of infected mosquitoes nor the aerosol concentration of virus particles has to be great to allow significant transmission of VEE in a BW attack. There is no evidence of direct human-to-human or horse-to-human transmission. Natural aerosol transmission is not known to occur. VEE particles are not considered stable in the environment, and are thus not as persistent as the bacteria responsible for Q fever, tularemia or anthrax. Heat and standard disinfectants can easily kill the VEE virus complex.

Viral Hemorrhagic Fevers

The viral hemorrhagic fevers are a diverse group of illnesses caused by RNA viruses from four viral families. The *Arenaviridae* include the etiologic agents of Argentine, Bolivian, and Venezuelan hemorrhagic fevers, and Lassa fever. The *Bunyaviridae* include the members of the *Hantavirus* genus, the Congo-Crimean hemorrhagic fever virus from the *Nairovirus* genus, and the Rift Valley fever virus from the *Phlebovirus* genus; the *Filoviridae* include Ebola and Marburg viruses; and the *Flaviviridae* include dengue and yellow fever viruses. These viruses are spread in a variety of ways; some may be transmitted to humans through a respiratory portal of entry. Although evidence for weaponization does not exist for many of these viruses, they are included in this handbook because of their *potential* for aerosol dissemination or weaponization, or likelihood for confusion with similar agents that might be weaponized.

CONCLUSION

The possibility of a terrorist attack using bioweapons would be especially difficult to predict, detect or prevent, and thus, it is among the most feared terrorist scenarios [5].

Biological agents have seldom been dispersed in aerosol form, the exposure mode most likely to inflict widespread disease.

Now the really sobering part—biological warfare agents are very difficult, if not impossible, to detect while they are in the research, production, transit, or employment phases. Normal biological warfare research facilities resemble completely legitimate biotechnical and medical research facilities. The same production facilities that can produce

biological warfare agents may also produce wine and beer, dried milk, food, and agricultural products [6]. The challenge this presents is in distinguishing legitimate production plants from illicit ones.

It becomes nearly impossible to identify the locations and facilities that are actually producing biological warfare weapons. This needs to be done, obviously, in order to confidently highlight a violation of the BWC, or, if necessary, should all peaceful remedies fail, preemptively strike a biological weapons production or storage facility.

In addition, biological warfare agents are virtually undetectable while they are in transit. In other words, if a terrorist wanted to carry the biological agent into the other country in a carry-on bag or checked luggage, there is no mechanism using routine customs, immigration, drug scan, or bomb search procedures to identify the agent [7].

Today 17 nations are suspected of having or trying to acquire biological weapons. Perhaps they want to deter foes. The wild card is that some (Cuba, Iran, Iraq, Libya, North Korea, Syria) are also considered to be involved in one way or another with terrorism. Libya worked hard to join the biological warfare club.

Biological warfare has been a threat for decades if not centuries.

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