ANNEX 1: BIOLOGICAL AGENTS

1.1 INTRODUCTION

Extensive research, development and testing by military establishments has shown that large-scale production of certain infective agents is feasible for weapons purposes in suitably designed biotechnological facilities with appropriate features and with special precautions to protect the workers. The selection of the agent and strain, its large-scale growth and processing, its formulation and weaponization all require specialized technologies and associated effort for their development, testing and application. Numerous technical difficulties must be overcome in order to develop munitions or other devices that produce stable respirable aerosols, and specific delivery and atmospheric conditions must be met if the aerosol is to reach the target population. Throughout all these steps, including that of aerosol cloud travel, special techniques and conditions are required to maintain the respirability, infectivity and virulence of the agent. Nevertheless, despite the fact that the development of strategic biological weapons within military establishments historically required large-scale multi-year effort, it may be that some agents could be produced and used as weapons of terror on a smaller scale using relatively simple techniques. Agents variously cited as possible agents of biological warfare or terrorism have been listed in Table 3.1 in Chapter 3.

This annex presents information about the particular infective agents that have been listed by states parties to the Biological Weapons Convention in declarations regarding their past offensive research and development programmes or that are designated in reference A1.01 as of special concern for possible use in terrorism. All but one continue to cause naturally occurring human disease, especially in endemic regions and among populations without access to adequate sanitation, public health, veterinary and medical systems. The only exception is the variola virus, the agent of smallpox, declared by the 1980 World Health Assembly to have been eradicated.

During the second world war and the cold war, military establishments developed biological weapons -- principally aircraft bombs, aircraft-mounted aerosol generators, missile warheads and devices for clandestine forces -- designed to release infective agents into the atmosphere as aerosols to be inhaled by target personnel. Principles and factors that govern the number of primary cases that might result from a particular biological weapons attack employing aerosol dispersion, and the considerable uncertainties inherent in any quantitative estimate, are discussed in Annex 4.
Person to person transmission of most of the diseases described in this annex is rare or unknown. Two of them, however, smallpox and pneumonic plague, are highly contagious and therefore present a danger of causing spreading epidemics, particularly if the number of persons initially infected is large. Mathematical modelling of the spread of infectious disease from primary cases is discussed in Annex 5.

1.1.1 Recognising deliberate release

Although all of the listed agents are known because of the diseases they cause naturally, there are respects important for response planning in which their effects if used as weapons, particularly as aerosols, are likely to differ from their effects in naturally occurring infections.

- **Suddenness.** Individual exposures in natural outbreaks affecting groups of people caused by animal or insect carriers or by person-to-person contact are usually spread over a period of many days or longer. In contrast, inhalatory exposures to a pathogen contained in an aerosol in a single attack would be essentially confined to the passage or dispersal time of the aerosol. This is because the limited deposition of aerosol particles and the inefficiency of their re-suspension as particles small enough to be respired would generally make subsequent exposures very much less than those from the initial aerosol. The time course of an outbreak following such an attack would therefore be expected to exhibit a more sudden rise and probably, except for contagious disease, a more rapid fall-off than is characteristic of the same disease in a natural outbreak.

- **Severity of disease following inhalatory infection.** Disease initiated by inhalatory infection may follow a course and exhibit symptoms differing from and more severe than those characteristic of other routes of entry. For some diseases that are ordinarily of low lethality for healthy adults, such as Venezuelan equine encephalomyelitis, normally acquired from the bite of infected mosquitoes, it is possible that atypical infection of humans through the respiratory tract, which may by-pass such normal protective mechanisms as local inflammatory processes, would be less susceptible to vaccine protection and/or would have increased virulence and lethality. By analogy with other infections of humans where inhalatory infection is associated with particularly high lethality, such as pneumonic plague and inhalation anthrax, this should be regarded as a strong possibility.
• Number of cases. If a large-scale attack on a population centre were attempted and if the many technical difficulties in its preparation and execution were overcome, large numbers of people could become infected. The unprecedented case load, the sudden nature of the outbreak and the severe and possibly unfamiliar course of the illness could place demands on even a reasonably well-prepared emergency response and health care system beyond its ability to cope. As in ordinary public health matters, therefore, emphasis must be placed on measures for prevention in all its aspects, a subject addressed elsewhere in this report.

1.1.2 Protection

Exposure to aerosolized biological agents can be greatly reduced by a properly fitted military gas mask, by a HEPA type microbiological mask or by a shelter provided with suitably filtered or disinfected air. Although some buildings could be constantly provided with purified air, the effective use of masks and shelters would generally require advance warning of an impending attack and notification of when the inhalation hazard had passed.

For some but by no means all of the agents of concern, vaccines affording various degrees of protection for various periods of time have been approved by WHO or by national regulatory authorities as effective and sufficiently safe for general use against naturally occurring infection. Also, there is evidence from laboratory studies of the likely efficacy of several additional vaccines.

Because individual vaccines are specific for individual pathogens, a decision to engage in widespread vaccination as prophylaxis against biological attack must be based on a judgement that there is a serious risk to a particular population, that the probable identity of the threat agent is known, and that the vaccine would be effective. A further complexity is that naturally occurring strains of a given agent may differ in their susceptibility to vaccine prophylaxis and strains not amenable to vaccine prophylaxis might be produced artificially. Also, depending on the vaccine, its administration may entail immediate or delayed health risks in the form of adverse reactions and may be subject to contraindications for specific population groups.

Post-exposure vaccination for the agents described here (and for the other agents listed in Table 3.1) is of proven value only in the case of smallpox, where its timely administration to persons who may have been exposed or who show early signs of infection would likely be of major importance in helping to halt epidemic spread.
Antibiotics for prophylaxis in cases of anticipated or suspected exposure and for therapy of those already infected can be effective for many bacterial and fungal diseases. Proper choice, procurement and use of the antibiotics most likely to be effective requires timely identification of the agent. As the initial signs of many of the diseases of concern are nondescript, rapid diagnostic procedures should be immediately instituted whenever there is a sudden appearance of numerous cases of unexplained illness. Advance preparations should therefore be made for rapid access to local, regional, national and international reference laboratories, should they be needed. In this regard, encouragement should be given to the adoption, as they become available, of rapid, reliable and highly specific DNA-based and other newer methods of laboratory diagnosis in order to facilitate the timely and effective treatment and prophylaxis of both natural and, should it occur, deliberately caused infectious disease.

The information that follows is intended to provide only a general description of the characteristics, diagnostic procedures and medical and public health measures relevant to each agent. The information includes the following categories:

- **Name of the agent /disease.** The name of the pathogen and the disease it causes. Each disease is also designated by its numeric code assigned by the WHO *International Classification of Diseases, Tenth Revision.*

- **Description of the agent.** Classification and description of the agent.

- **Occurrence.** Places where the disease is prevalent.

- **Reservoirs.** Principal animal and environmental sources of human infection.

- **Mode of transmission.** Principal modes of transmission to humans: vector-borne, person-to-person, water-borne, food-borne, airborne, etc.

- **Incubation period.** The time between exposure and the first appearance of symptoms. This will vary from individual to individual and for some pathogens is highly variable. Incubation periods also depend on the route of entry and on dose, generally being shorter for higher doses.

- **Clinical features.** Principal signs and symptoms characteristic of the disease. For many of the listed agents the initial symptoms are nondescript, resembling common flu and making early clinical identification difficult.
• Laboratory diagnosis. Citation of laboratory methods for identification of pathogens in clinical specimens.

• Medical management. Isolation requirements, protection of care-givers, and disposal of contaminated materials.

• Prophylaxis and therapy. Vaccines, antibiotics and antisera, where applicable.

• Other information

More detailed information may be found in the specific references given for each agent and also in the more general works cited at the end of this annex.

1.2 BACTERIA

1.2.1 Bacillus anthracis / Anthrax (A22)

The vegetative form of B. anthracis is a non-motile, rod-shaped, gram-positive, aerobic or facultatively anaerobic bacillus measuring 1-1.2 by 3-5 µm. The vegetative bacillus multiplies readily in infected animals and in laboratory media. Under nutrient-limiting conditions in the presence of free oxygen, an ellipsoidal spore forms within the vegetative cell and is released upon lysis. In contrast to the fragile vegetative form, mature anthrax spores are highly resistant to drying, heat, ultraviolet and ionizing radiation and other forms of stress and can remain infective in the environment for years. When introduced into the body of a susceptible host and if not inactivated by host defence mechanisms, the spore may germinate to become a vegetative bacillus, reinitiating the cycle.

Occurrence

Anthrax is mainly a disease of mammals, most commonly encountered in grazing animals. Until the introduction and widespread use of modern veterinary vaccines, it was a major cause of fatal disease in cattle, sheep, goats, camels, horses and pigs throughout the world. Anthrax continues to be reported from many countries in domesticated and wild herbivores, especially where livestock vaccination programs are inadequate or have been disrupted. Human anthrax, acquired from diseased animals and animal products, is most frequent in Africa, the Middle East and in central and southern Asia.

Reservoirs

Anthrax spores are a contaminant of soil where animals have died of the disease. Depending on temperature and soil
conditions, a proportion of the vegetative cells in blood and
other secretions spilt upon the ground from newly dead or
dying animals form spores upon exposure to air, creating foci
of contaminated soil. These may persist for years as a source
of further infection. Additional foci may be created by the
scavenged remains of dead animals. Infective spores can also
persist for long periods in hides, hair and bone meal from
infected animals. A number of large outbreaks in livestock
have been traced to the introduction of animal feed containing
contaminated bonemeal. Vegetative cells remaining within the
carcass of a diseased animal are rapidly destroyed by the
processes of putrefaction.

Mode of transmission

It is the spore rather than the vegetative form that is the agent
by which the disease is transmitted and it is doubtful that the
vegetative form ever proliferates significantly outside the
animal body. Although definitive studies are lacking, infection
of animals is thought mainly to result from entry of ingested
spores through epithelial lesions, with inhalation of
contaminated dust and transmission by biting flies as less
frequent possibilities.

The most common mode of transmission to humans is by the
entry of spores from infected animal products through lesions
of the skin, especially in exposed parts of the body such as
the arms, face and neck. More rarely, infection is by ingestion
of meat of infected animals or by inhalation of spores, as from
contaminated wool, hair or hides. There are no documented
cases of person to person transmission. Animal experiments,
including experiments in non-human primates, suggest that
the introduction of only a few spores through a lesion is likely
to initiate cutaneous or gastrointestinal infection but that a
much larger number of spores is required to produce a high
probability of respiratory infection.

Incubation period

Symptoms of human cutaneous and gastrointestinal anthrax
generally appear within 1 to several days after exposure.
Reported incubation periods for inhalation anthrax range from
1 day to six weeks, with most cases appearing within the first
2 weeks or perhaps sooner in persons receiving high doses.
Prolonged incubation periods for inhalation anthrax are
attributed to spores that remain dormant in the lungs for
considerable periods before germinating and initiating
systemic infection.
Clinical features

Cutaneous infection starts as a painless, non-scarring, pruritic papule progressing over a period of about a week to a black depressed eschar with swelling of adjacent lymph glands and localized oedema, which may become extensive. Although usually self-limiting, untreated cutaneous anthrax can become systemic and is fatal in 5-20 percent of cases. With proper antibiotic therapy, the death rate of cutaneous anthrax is less than one percent.

Inhalation anthrax begins with nondescript or flu-like symptoms that may elude correct diagnosis. These may include fever, fatigue, myalgia, headache, chills, non-productive cough and vomiting, followed after 1-3 days by the sudden development of dyspnea, cyanosis, shock, coma and death. Chest X-rays often show a widened mediastinum and marked pleural effusions and during terminal stages blood levels of vegetative bacilli may reach $10^8$ or more per ml. although late administration of antibiotics may sterilize the blood while not preventing death from the action of anthrax toxin already released. The average time between onset and death is typically 1-7 days, with reported case fatality rates of 80 percent and higher. Meningitis is not uncommon. Pneumonia may be present but is not a regular feature. Despite its name, therefore, inhalation anthrax is not a true respiratory disease, in that the lungs usually remain clear of growing bacteria until late stages.

Gastrointestinal and oropharyngeal anthrax result from the ingestion of contaminated meat. Gastrointestinal anthrax may be accompanied by fever, nausea, vomiting, abdominal pain and bloody stools. Oropharyngeal infection is characterized by oedematous swelling of the neck, often massive and accompanied by fever. Mortality in gastrointestinal anthrax is variable, depending on the outbreak, but in some outbreaks it is reported to approach that of inhalation anthrax.

Laboratory diagnosis

Confirmation of clinical diagnosis may be made by microscopic identification of the bacilli in vesicular fluid or blood or after isolation by inoculation of mice, guinea pigs or rabbits. On blood agar plates, \textit{B. anthracis} forms white or greyish-white, coherent, non-haemolytic or weakly haemolytic colonies in which chains of vegetative bacilli are present together with spore-containing cells. In infected tissues and also under anaerobic conditions in the presence of bicarbonate, but not on ordinary culture plates, the vegetative
cell forms a prominent poly-D-glutamic acid capsule. If the patient has been treated with antibiotics, however, it may be difficult or impossible to demonstrate the bacilli. Methods have been developed for rapid detection of the pathogen based on monoclonal antibodies and on polymerase chain reaction. Seroconversion may be detected by ELISA. Biosafety Level 2 practices, equipment and facilities are recommended for manipulations involving clinical specimens or experimentally infected laboratory rodents. Biosafety Level 3 practices, equipment and facilities are recommended for manipulations involving the concentration of cultures or activities with a high potential for aerosol production.

Medical management
Patient isolation is not required and there are no quarantine requirements. Dressings, discharges from lesions, other contaminated materials and cadavers should be disinfected, preferably by incineration or cremation or by deep burial with quicklime. Sterilization may also be achieved by autoclaving, washing with aqueous formaldehyde, glutaraldehyde, hypochlorite, hydrogen peroxide or peracetic acid or by fumigation with ethylene oxide or formaldehyde vapour.

Prophylaxis and therapy
Live spore vaccines based on attenuated strains are produced for human use in China and in the Russian Federation. In most other countries, live spore vaccines are restricted to veterinary applications and are not licensed for human use. Cell-free vaccines containing anthrax protective antigen (see below) are produced and licensed for human use in the UK and the USA. They are administered intramuscularly or subcutaneously in a series of shots over a period of several months, followed by annual boosters. The use of such vaccines has been associated with a major reduction of cutaneous anthrax in individuals whose occupations may place them at risk. Immunization with cell-free vaccines containing anthrax protective antigen is effective in protecting laboratory animals (guinea pigs, rabbits, monkeys) against aerosol challenge. However, comparison of a cell-free vaccine with a live spore veterinary vaccine in experimental animals indicates that the live vaccine provides greater protection against some isolates of \textit{B. anthracis}. Evidence regarding the degree and duration of protection which existing vaccines may afford to humans against aerosol challenge is based on extrapolation from animal experiments and on indirect measures of human immunity.
Antibiotic therapy is effective in treating cutaneous anthrax and is likely to be effective against human inhalation anthrax providing it is begun before or very soon after symptoms appear. Once high levels of toxin are produced by anthrax bacilli in the body, antibiotic therapy becomes ineffective. Antibiotic therapy should also be used for prophylaxis in asymptomatic patients with suspected exposure to anthrax spore aerosol. Prolonged treatment is needed to allow time for clearance or inactivation of spores deposited in the lungs, as spores are not affected by antibiotics. For both therapeutic and prophylactic use, it is therefore advisable to continue antibiotic treatment for six weeks or longer. Penicillin is generally effective against human cutaneous anthrax, although a small percentage of strains isolated from nature are resistant and antibiotic resistant strains have been produced in the laboratory. Tests in non-human primates indicate that penicillin, doxycycline and ciprofloxacin are effective for prophylaxis and early treatment of inhalation anthrax. The use of vaccine for post-exposure prophylaxis in combination with antibiotics has been suggested but there is inadequate evidence of its efficacy in this role. If available, specific human gamma globulin may be effective in cases where otherwise lethal levels of anthrax toxin have already accumulated. Future developments may include the use of genetically modified derivatives of protective antigen both as a vaccine and an antitoxin.

Other information

The disease is mediated by the action on mammalian cells of a toxin comprised of three protein components. One of the components, protective antigen (PA), binds to the cell surface where it forms a channel through which the other two components, edema factor (EF) and lethal factor (LF), enter the cell. The other principal virulence factor, in addition to the toxin, is the polypeptide capsule, which imparts resistance to phagacytosis. The symptoms of anthrax infection in experimental animals can be produced by the administration of the purified toxin.

Reported values of the lethal dose for experimental inhalation anthrax in non-human primates vary enormously, from 2500 to 760,000 spores, apparently reflecting differences in the many variables involved in such experiments. It is important to note that doses far less than the LD50 may nevertheless cause a significant percentage of deaths, owing to heterogeneity in individual susceptibility within any particular population. The largest reported outbreak of human inhalation anthrax took place in 1979 in Sverdlovsk (Ekaterinburg),
USSR. Of 66 documented fatal cases, all were more than 23 years in age, suggesting that adults may be more susceptible to inhalation anthrax than younger individuals. The concomitant infection of sheep and cattle as far as 50 kilometres down wind of the apparent source points to the importance of veterinary and public health measures to protect the human population from consuming contaminated meat or coming into contact with contaminated animal products, in case of a release of anthrax spore aerosols.

**Further reading on anthrax**


ANNEX 1

1.2.2 **Brucella abortus, Brucella suis and Brucella melitensis** / Brucellosis (A23)

*Brucella* species, which may also be regarded as different strains of a single species, are non-motile, gram-negative, aerobic, unencapsulated cocci or short rods measuring approximately 0.5-0.7 by 0.6-1.5 µm. The bacteria are able to grow intracellularly in infected hosts. Infective cells can persist in the environment for weeks and dried preparations can retain virulence for years.

**Occurrence**

World-wide.

**Reservoirs**

Diverse mammals, especially cattle, goats, sheep, pigs, camels and buffaloes. Preferred hosts exist for each species: *B. abortus* commonly infects cattle; *B. suis* commonly infects swine and *B. melitensis*, a particularly virulent strain for humans, commonly infects goats, sheep and camels.

**Mode of transmission**

Most human infections result from ingestion of raw animal products, especially milk and milk products. Infection may also result from entry of the bacteria from diseased animals through skin lesions or mucus membranes or from inhalation of contaminated dust or aerosols. Laboratory infection is common, especially by inhalation of aerosols. Inhalation of only a few organisms is sufficient to cause a significant likelihood of infection. Person to person transmission occurs very rarely, if ever. Many countries are now essentially free of bovine brucellosis, owing to vaccination of cattle.

**Incubation period**

Highly variable, usually 5-60 days but can be as long as several months, with shorter periods expected after severe exposure.

**Clinical features**

Onset may be gradual or acute, with variable symptoms, consisting most frequently of undulating fever, chills,
exhaustion, depression, back and leg pains, sweating, headaches and loss of appetite. Cutaneous and soft tissue manifestations may include contact lesions, rash, and soft tissue abscesses. Splenomegaly and hepatomegaly with associated organ tenderness occur in some patients. Without treatment, patients usually recover within 2-3 months but there may be cycles of relapse and remission extending over years, accompanied by liver, spleen, bone, genitourinary, central nervous system and cardiac complications. Fatality among untreated patients is approximately 2 percent or less, although somewhat higher for B. melitensis, and is usually from endocarditis. All age groups are susceptible, although children may be somewhat less so.

**Laboratory diagnosis**

Laboratory identification to the genus level, sufficient for treatment of patients, may be made in acute cases by microbiological and biochemical identification of the pathogen isolated from venous blood, bone marrow, other tissues. Serologic tests, particularly serum agglutination and ELISA, are useful during acute infection, although antibody titres tend to be low in chronic or recurrent cases. Reliable identification of individual strains by PCR with genus-specific primers has been demonstrated. Biosafety Level 2 practices, equipment and facilities are recommended for manipulations involving clinical specimens. Biosafety Level 3 practices, equipment and facilities are recommended for all manipulations of cultures.

**Medical management**

As there is no evidence of person to person transmission, patient isolation is not required. Standard precautions should be observed against infection from splashes or other direct contact with draining lesions and contaminated discharges or other contaminated materials. Exudates and dressings should be disinfected by autoclaving, incineration or treatment with standard disinfectants.

**Prophylaxis and therapy**

Veterinary vaccines protect animals to a substantial but not unlimited extent. No human vaccine is available. A six-week course of oral doxycycline concomitant with either six weeks of oral rifampin or three weeks of intramuscular streptomycin is usually successful if begun early. Even prolonged antibiotic treatment is only moderately effective in cases of chronic infection.
Further reading on brucellosis

REFERENCES TO BE INSERTED HERE

1.2.3 Burkholderia mallei / Glanders (A24.0)
Formerly classified in as Pseudomonas mallei, the organism is a small, aerobic, non-motile, gram-negative rod.

Occurrence
The disease in humans is rare or absent in most parts of the world. Enzootic foci exist in Asia, some eastern Mediterranean countries and parts of the Middle East and Central and South America.

Reservoirs
Primarily a disease of equines, including horses, donkeys and mules, for which it is highly contagious.

Mode of transmission
The disease is acquired by humans by direct contact with infected animals or contaminated animal tissue, the agent entering the body through skin lesions or through conjunctival, oral or nasal mucous membranes. The disease is not considered to be very contagious from person to person. It is likely to be infectious by aerosol exposure.

Incubation period
Variable. Although most human cases appear 1-14 days after exposure, the disease can remain latent for many years.

Clinical features
Glanders infection can present in several forms, depending on the route of entry and the site of infection. Initial symptoms may include fever, malaise, myalgia, and headache. Localized infection may become apparent a few days after exposure, with pus-forming ulcerations on the skin that may spread over most of the body, or as purulent ulcerations of the mucosa of the nose, trachea, pharynx and lungs. Pulmonary infection is associated with pneumonia, pulmonary abscesses and pleural effusion. Localized infection in the lobes of the lungs may be apparent in chest X-rays. Bloodstream infections are usually fatal within a few days, even with antibiotic therapy. Chronic infections are associated with multiple abscesses in the muscles of the arms and legs, or in the spleen or liver. Subclinical infections are sometimes detected at autopsy.
Laboratory diagnosis
Identification may be made by isolation of the micro-organism from skin lesions, pus, sputum or blood, followed by direct fluorescent antibody staining or by polymerase chain reaction. Serological tests include complement fixation and agglutination tests. Biosafety Level 2 practices, equipment and facilities are recommended for manipulations involving clinical specimens or experimentally infected laboratory rodents. Biosafety Level 3 practices, equipment and facilities are recommended for manipulations involving the concentration of cultures or activities with a high potential for aerosol production.

Medical management
Standard precautions should be observed against infection from splashes or other direct contact with draining lesions, blood and contaminated discharges or other contaminated materials. Exudates and dressings should be disinfected by autoclaving, incineration or treatment with standard disinfectants. The organism is not highly resistant to environmental conditions.

Prophylaxis and therapy
No vaccine is available. Owing to the rareness of the disease, the medical literature regarding its therapy is sparse. Sulfadiazine and ceftazidine are recommended for therapeutic use. The organism is also sensitive to tetracyclines, ciprofloxacin, streptomycin, novobiocin, gentamycin, imipenem, and sulfonamides. There may be relapses even after prolonged antibiotic therapy.

Further reading on glanders

1.2.4 Burkholderia pseudomallei / Melioidosis (A24)
Formerly classified in as Pseudomonas pseudomallei, the organism is a small, aerobic, motile, gram-negative rod.

Occurrence
The disease is prevalent in Southeast Asia, particularly in wet rice-growing areas, and, less commonly, in northern Australia. A number of cases have also been reported from central and south America.
Reservoir

*B. pseudomallei* is found in soil and water in tropical and subtropical regions and infects many species of mammals, including marine mammals.

Mode of transmission

Humans become infected through skin lesions as a result of contact with contaminated soil or water. Infection can also occur by aspiration or ingestion of contaminated water or by inhalation of contaminated dust. Person to person transmission may occasionally occur but is rare.

Incubation Period

The incubation period may range from a few days to years.

Clinical features

Clinical features resemble those of glanders and are highly variable. Cutaneous infection may give rise to subcutaneous infected nodules with acute lymphangitis and regional lymphadenitis, generally with fever. Inhalation or ingestion or hematogenous spread from cutaneous lesions may result in internal involvement, with chronically infected suppurating abscesses in lungs, liver, spleen, lymph nodes, bone or joints. Pulmonary involvement is associated with consolidation and necrotizing pneumonia, and may vary from mild to fulminant. The disease can resemble tuberculosis or typhoid fever. A fulminant septicemia with shock may occur and is probably invariably fatal. Asymptomatic infection has been detected serologically and may cause disease long after exposure.

Laboratory Diagnosis

Identification may be made by isolation of the organism from sputum or purulent exudates, followed by microbiological identification. Serological testing may be done by ELISA. Biosafety Level 2 practices, equipment and facilities are recommended for manipulations involving clinical specimens. Biosafety Level 3 practices, equipment and facilities are recommended for manipulations involving the concentration of cultures or activities with a high potential for aerosol production.

Medical management

Standard precautions should be observed against infection from splashes or other direct contact with draining lesions, blood and contaminated discharges or other contaminated materials. Exudates and dressings should be disinfected by autoclaving, incineration or treatment with standard
disinfectants. The organism is not highly resistant to
environmental conditions.

Prophylaxis and therapy
No vaccine is available. Ceftazidine and tetracycline have
been used successfully.

Further reading on melioidosis

REFERENCES TO BE INSERTED HERE

1.2.5 Francisella tularensis / Tularaemia (A21)
The organism is a small, non-motile, gram-negative, aerobic
coccobacillus, measuring 0.2 by 0.3-0.7 µm. Of the several strains
that have been described, *F. tularensis* tularensis or type A is more
virulent than *F. tularensis palaeartica* or type B. The organism can
survive for up to several weeks in the natural environment.

Occurrence
*F. tularensis* tularensis is found in North America, while *F.
tularensis palaeartica* occurs in Asia, Europe and North
America.

Reservoirs
Many wild animals, especially rabbits, hares, voles, muskrats
and beavers, also some hard ticks. The disease has been
reported in many other animals, including various rodents,
birds, reptiles, amphibians and marine mammals. It is also
found in soil and water.

Mode of transmission
Tularaemia is primarily a disease of a wide variety of wild
mammals and birds. The natural cycle of infection also
involves ticks, mosquitoes, flies and fleas. Humans become
infected mainly through the bite of certain arthropods,
particularly certain species of hard ticks, and, through the skin
conjunctival sac or nasopharyngeal mucosa, by direct contact
with infected animals or animal materials and by ingestion of
contaminated food or water or inhalation of contaminated
dust. *F. tularensis* is easily transmitted by aerosol and
inhalation of only a few organisms is sufficient to cause a
significant likelihood of infection. Person to person
transmission has not been documented.
**Incubation period**

The incubation period varies from 1 to 14 days, averaging 3-5. Its duration depends upon the strain, the dose and the portal of entry. Infection through the skin and conjunctiva gives longer incubation periods than infection via the respiratory and alimentary systems.

**Clinical features**

Clinical manifestations depend on the route of entry and the virulence of the agent. Infection through the skin or conjunctiva usually produces an ulceroglandular form, with an indolent ulcer at the site of entry and painful swelling of local lymph glands, which may suppurate. In some cases the site of entry is inconspicuous, there being only local lymph gland involvement. Infection resulting from ingestion is characterized by a painful pharyngitis, with abdominal pain, diarrhoea and vomiting. Both forms are usually accompanied by an abrupt onset of fever, accompanied by chills, malaise and joint and muscle pain. Ulceroglandular tularaemia caused by virulent strains, if untreated, has a case fatality rate of about 5 percent and lasts 2 to 4 weeks, with a convalescent period of up to three months.

Depending on the position in the respiratory system where infection occurs, inhalation tularaemia may take the form of a primary pneumonia or of tracheitis and bronchitis. The initial manifestation, however, may be influenza-like without evident signs of respiratory involvement. Pleuropulmonary tularaemia with a virulent strain has a high case fatality rate (40-60 percent) if untreated. The organism may also enter the bloodstream, causing toxaemia and tularaemia sepsis, which is almost invariably fatal.

**Laboratory diagnosis**

Direct microscopic examination of clinical specimens showing the characteristic small bacteria and specific staining with fluorescent antibody can provide a rapid diagnosis which may be confirmed by polymerase chain reaction, ELISA or by the appearance of specific serum antibodies that usually appear a week after onset. Biosafety Level 2 practices, equipment and facilities are recommended for manipulations involving clinical specimens from humans or animals. Biosafety Level 3 practices, equipment and facilities are recommended for all manipulations of cultures.

**Medical management**

There is no requirement for quarantine of patients or immunization of contacts. Standard precautions are indicated
where there are open lesions and discharges from ulcers, including autoclaving, incineration or disinfection of discharges and contaminated materials.

Prophylaxis and therapy

Live attenuated vaccines applied intradermally have proved effective in preventing infection in humans following exposure by scarification or inhalation. For antibiotic prophylaxis and post-exposure therapy, streptomycin given intravenously for 7-14 days is highly effective if administered soon after exposure to the agent, and works well even if started within 48 hours. If streptomycin resistance is indicated, doxycycline and ciprofloxacin are recommended. Other antibiotics, including gentamicin, tetracyclines and chloramphenicol, are also effective. Antibiotic therapy must be continued for at least 14 days to prevent relapses.

Further reading on tularemia

REFERENCES TO BE INSERTED HERE

1.2.6 Yersinia pestis / Plague (A20)

Y. pestis is a gram-negative non-motile, non-sporeforming coccobacillus measuring approximately 1.5 by 0.75 µm capable of both aerobic and anaerobic growth. The pathogen can remain viable for days in water or moist soil and can resist drying if protected by mucous or other substances but is killed by a few hours of direct exposure to sunlight.

Occurrence

During the 1990s there were human outbreaks in Africa, Asia, and South America and sporadic cases in many countries, including the USA. Known historically as the Black Death and still a serious problem, it is limited to sporadic cases where adequate surveillance and modern public health measures are practised.

Reservoirs

The pathogen is present in animal reservoirs, particularly in wild rodents, in endemic foci world-wide, with the exception of Australia.

Mode of transmission

Plague is transmitted between rodents and to other animals via fleas, cannibalism or, possibly, contaminated soil. Plague
occurs sporadically among people who come into contact with wild rodents. Outbreaks affecting large numbers of people can occur in cities when plague infects populations of urban rodents, particularly rats of the genus Rattus. The usual form of the disease in humans, bubonic plague, is spread mainly by the bite of fleas regurgitating blood from infected rodents or by entry of the pathogen from infected fleas through a skin lesion. If the lungs become infected, as may occasionally occur in patients with the bubonic form, a much more virulent form, pneumonic plague, ensues and can be transmitted directly from person to person by droplet infection.

**Incubation period**

The incubation period in humans is 1-7 days in bubonic plague and somewhat less for the pneumonic form.

**Clinical features**

Initial symptoms may be nonspecific, with sudden onset of fever, chills, malaise, myalgia, nausea, sore throat and headache. Cases acquired by aerosol inhalation would probably present as primary pneumonitis. Infection spreads from the inoculation site via the lymphatics to regional nodes, which become swollen and painful (buboes). In a minority of cases, the pathogen enters the bloodstream giving rise to plague septicaemia. Haematogenous spread of the pathogen to the lungs causes the pneumonic form of the disease, which then can spread directly from person to person by droplet infection. As the disease progresses, patients experience shock, delirium and coma. Untreated bubonic plague has a case fatality rate of 25-50 percent, while untreated pneumonic plague is almost always fatal. Less common forms are plague meningitis and plague pharyngitis.

**Laboratory diagnosis**

Strong suggestive evidence of *Y. pestis* in sputum, blood or material aspirated from a bubo is provided by observation of gram-negative ovoid bacilli that stain preferentially at their ends with Giemsa or Wayson’s stains, although such bipolar distribution of stain may not always be clearly evident. The bacillus may be identified by direct fluorescent antibody stain for the *Y. pestis* capsular antigen and by lysis by specific bacteriophage. Various serological methods are also available. Biosafety Level 2 practices, equipment and facilities are recommended for all activities involving infective clinical materials and cultures. Biosafety Level 3 should be used for activities in which there is a high potential of aerosol or air droplet production or for work with antibiotic-resistant strains.
**Medical management**

Emphasis must be placed on preventing epidemic spread. For patients with pneumonic plague, strict precautions against airborne droplet spread are essential, including patient isolation and wearing of surgical masks by patients and caregivers. Patients with confirmed pneumonic plague may be placed together in shared rooms if private rooms are not available. For patients with any type of plague, standard precautions must be taken against contamination from discharges and contaminated articles, including hand-washing and the wearing of gloves, gowns and face protection. If there should be an outbreak of the pneumonic form, people should be advised to avoid crowded places, to report any definitely elevated fever or unusual rodent mortality and to institute measures of flea control if indicated.

**Prophylaxis and therapy**

Preventive vaccination with killed or live attenuated *Y. pestis* is moderately effective against bubonic but not against pneumonic plague. With killed vaccine, protection is relatively short-lived (3-12 months) and periodic revaccination is necessary. Vaccination is of little use during a plague outbreak, as at least a month is needed for immunity to build up. As with various other pathogens, massive infection can overcome vaccine-conferred immunity. Persons in close contact with pneumonic plague patients or who are likely to have been exposed to infected fleas, to have had direct contact with body fluids or tissues of an infected mammal, or who for any other reason are suspected to have been exposed to the pathogen should receive antibiotic prophylaxis for a week after the last suspected exposure. Doxycycline and ciprofloxacin are recommended for such use.

Antibiotic therapy is effective if begun early in the disease and continued until 3 days after temperature returns to normal. Streptomycin and gentimycin are known to be effective and ciprofloxacin has proven effective in animal studies. Aggressive supportive management is essential. Multidrug resistance imparted by a transferable plasmid has been reported in a clinical isolate and antibiotic resistant strains have been developed in the laboratory.

Further reading on plague

[REFERENCES TO BE INSERTED HERE]
1.2.7 *Coxiella burnetii* / Q Fever (A78)

*C. burnetii* is a pleomorphic gram-negative spore-forming obligate intracellular coccobacillus measuring 0.2 by 0.7 µm. The spore form, produced in infected host cells, is resistant to drying and environmental influences and can survive for months in water and food. It is extremely infective to humans.

**Occurrence**

World-wide.

**Reservoirs**

The pathogen exists as a zoonosis in a wide range of animal hosts, including domesticated livestock and poultry, dogs, rodents, baboons and wild birds and especially cattle, sheep and goats. The zoonotic cycle includes numerous species of ixodid and argasid ticks, mites and parasitic flies. Arthropod vectors, however, do not play a role in transmission to humans.

**Mode of transmission**

Transmission to humans occurs primarily by inhalation of droplets, dust or aerosols from dried parturient fluids and excreta of infected livestock. Contaminated droplets and dust may also infect the conjunctivae and abraded skin. Inhalation of only a few organisms is sufficient to cause a significant likelihood of infection. Contaminated aerosols released to the atmosphere may cause infection at distances up to several kilometres from their source. Sporadic human infections may also result from ingestion of raw milk. Low temperature vat pasteurization is insufficient to kill the organism. Person to person transmission has been reported but is rare.

**Incubation period**

The incubation period in humans is usually 18-21 days, but can be less if large doses of the organism are inhaled.

**Clinical features**

The onset is sudden, with chills, fever, sweating, headache, loss of appetite, malaise and muscle and chest pains. There may also be nausea, vomiting and diarrhoea. In severe cases the disease progresses to extreme stiffness of the neck and back, disorientation and pneumonia. The fatality rate is usually less than 1 percent, although somewhat higher rates have been reported in some outbreaks. Weakness and fever may continue for months. Long-term complications are uncommon but may include endocarditis. Asymptomatic infections also occur and may be revealed by serology.
Laboratory diagnosis

Isolation and microbiological identification of the organism from blood or other clinical materials is diagnostic but is hazardous to personnel. Specific and relatively rapid identification of the organism in blood or paraffin-embedded tissue may be accomplished by polymerase chain reaction. Serological diagnosis may be performed by microagglutination, complement fixation, indirect immunofluorescent antibody test or ELISA. Biosafety Level 2 practices, equipment and facilities are recommended for activities not involving propagation of the pathogen and involving only limited manipulation of infected materials, such as microscopic and serological examinations. Biosafety Level 3 is recommended for activities involving the handling of infected human or animal tissues or isolation of the pathogen.

Medical management

Patient isolation is not required. Patient materials and contaminated articles should be autoclaved, incinerated or disinfected with solutions containing hypochlorite, peroxide or phenol.

Prophylaxis / treatment

A formalin-inactivated vaccine has been developed for laboratory workers and others at high risk but is not commercially available. Tetracyclines, particularly doxycycline are effective if given early and may abort the infection if administered before symptoms appear.

Further reading on Q fever

[REFERENCES TO BE INSERTED HERE]

1.2.8 Rickettsia prowazekii / Typhus Fever (A75)

*R. prowazekii* is a small obligately intracellular gram-negative bacterium measuring approximately 0.4 by 1.5 µm.

Occurrence

The great epidemics of typhus that plagued humans since ancient times ceased shortly after the Second World War with the widespread application of insect control procedures and other hygienic measures. Endemic foci exist in certain regions where louse infestation is common, including parts of Mexico, Central and South America, central and east Africa and
various regions of Asia. Epidemics could reappear during time or war or famine.

Reservoirs
Humans, flying squirrels

Vector (s)
Transmitted from person to person by lice and fleas.

Mode of transmission
The disease is transmitted particularly by the body louse, *Pediculus humanus corporis*. Infection of humans occurs by contact of mucous membranes or abraded skin with the faeces of lice or fleas that have bitten a person with acute typhus fever. Infection probably also occurs by inhalation of dust contaminated with infected insect faeces or body parts. Patients are infective for lice during the febrile phase of the disease and perhaps for 2-3 days afterwards. Direct person to person transmission does not occur.

Incubation period
Usually 1-2 weeks.

Clinical features
The disease has a variable onset, often sudden, with chills, body aches, fever, headache and weakness. During the first week a macular rash appears, initially on the upper trunk and then spreads. The symptoms grow progressively more severe, with the critical period in the second or third week. Stupor and coma may be interrupted by attacks of delirium. Recovery is marked by abrupt cessation of fever, usually in the second febrile week, but, if untreated, mortality ranges from 10 to 40 percent, increasing with age. The disease may reappear years after the initial infection, usually in a milder form.

Laboratory diagnosis
Sera become positive about two weeks after infection, when diagnosis may be obtained by immunofluorescent antibody test. More rapid diagnosis may be obtained by immunohistological identification of the organism or by polymerase chain reaction, using blood collected during the acute phase of the disease. Biosafety Level 2 practices, equipment and facilities are recommended for activities not involving propagation of the pathogen, such as microscopic and serological examinations. Biosafety Level 3 is recommended for activities involving the handling of infected human or animal tissues.
Principles of medical management

Isolation of patients is not necessary. If lice are present, insecticide should be applied to patient clothing, bedding, living quarters and patient contacts in order to prevent spread of the disease. Louse infested individuals likely to have been exposed to typhoid fever should be deloused and placed under quarantine for 15 days after insecticide application and close patient contacts should be kept under fever watch for 2 weeks.

Prophylaxis / treatment

Antibiotics including doxycycline are effective in prophylaxis and treatment and should be given if typhus is suspected.

Further reading on typhus

[REFERENCES TO BE INSERTED HERE]

1.3 FUNGI

1.3.1 Coccidioides immitis / Coccidioidomycosis (B38)

The agent is a dimorphic fungus that propagates as a mycelial mould in soil and as spherules bearing endospores in mammalian tissue. Mature hyphal filaments of the mycelial form develop arthroconidia which detach and may then become wind born. Arthroconidia are light-weight barrel-shaped cells measuring approximately 3 by 6 µm that are stable to drying but are killed by exposure to sunlight.

Occurrence

The fungus occurs in soil, especially in arid and semiarid regions of Southwestern United States, Northern Mexico and Central and South America. A substantial percentage of cattle, swine, sheep, dogs and humans in endemic regions have had asymptomatic infections, as revealed by immunophoresis and skin tests. The fungus has also been reported in the former Soviet Union.

Reservoirs

Soil, especially in arid regions.

Mode of transmission

Infection usually takes place by inhalation of contaminated dust or free arthroconidia. A dust storm originating in an endemic region of California in 1977 caused an elevated incidence of the disease over an area of thousands of square
kilometres. Mammals, including humans, inhaling even small numbers of arthroconidia may become infected, whereupon these may develop into 30-60 µm diameter spherules that contain thousands of 2-3 µm ovoidal endospores which themselves may develop into endospore-bearing spherules, spreading the disease throughout the body.

**Incubation period**
Usually 1-4 weeks.

**Clinical features**
The initial symptoms of the disease resemble those of other upper respiratory infections, including cough, fever, night sweats, chills, chest pain, sputum production and headache. Less often, there may also be various forms of erythema with or without pus formation. The initial form of the disease usually resolves without therapy within several weeks.

Persistent coccidioidomycosis, seen with increased frequency in AIDS patients and other immunocompromised persons, may appear a few weeks, months or, less often, years after primary disease or asymptomatic infection. It is characterized by progressive destructive pulmonary disease with continuous low-grade fever, weakness, cough with sputum production, cyanosis and dyspnea. In some cases, extrapulmonary dissemination occurs, with abscesses and involvement that may include skin, subcutaneous tissues, bones, joints and the central nervous system. Without treatment, the disseminated form, which may follow a rapid or a prolonged course, has a mortality rate of more than 50 percent, approaching 100 percent if meningitis develops.

In endemic areas, the majority of infections are asymptomatic, but may be detected by skin tests. The percentage of persons residing in endemic areas found to react positively to skin tests ranges from 5 percent to more than 50 percent.

Recovery from clinical disease appears usually to be accompanied by lifelong immunity. It may also be that persons who have had subclinical infections have partial immunity, and that the disease takes a more severe course in individuals with no previous exposure.

**Laboratory diagnosis**
Direct microscopic visualization of spherules and endospores in the presence of 10% potassium hydroxide in biopsy tissue, pus or centrifuged cerebrospinal fluid. Sputum or material from the digestive or urogenital tracts may contain spherule-
like artefacts. Skin tests for hypersensitivity to preparations
derived from the fungal mycelia or from spherules
(coccidioidin or spherulin) are useful for epidemiological
studies but may give false negative results in individual cases,
especially if the disease is advanced. Biosafety Level 2
practices, equipment and facilities are recommended for
activities with clinical specimens. Biosafety Level 3 practices,
equipment and facilities are recommended for activities with
sporulating cultures identified as C. immitis and with soil or
other materials known to be contaminated with the fungus.

Medical management
As the disease is not contagious, quarantine and patient
isolation are not indicated. Manipulation of clinical specimens
should be done under BL2 safety conditions. As the
arthroconidia easily become airborne and are highly infective,
manipulations involving sporulating cultures and soil or other
materials contaminated with infective arthroconidia should be
conducted under BL3 conditions. Contaminated specimens
and materials may be sterilized by autoclaving or by treatment
with iodine or glutaraldehyde based disinfectants.

Prophylaxis and therapy
No vaccine against coccidioidomycosis is available. Prolonged therapy with intravenous Amphotericin B is
moderately effective in persistent cases, unless there is
meningitis, in which case fluconazole is recommended.

Further reading on coccidioidomycosis
[REFERENCES TO BE INSERTED HERE]

1.4 VIRUSES

1.4.1 Venezuelan equine encephalomyelitis (A92.2)
The agent is a member of the genus Alphavirus of the family
Togoviridae. The virion is 60-75 nm in diameter, consisting of a
positive single-stranded RNA enclosed in an icosahedral capsid,
surrounded by a lipid bilayer membrane in which surface
glycoproteins are embedded. Subtypes IA, IB and IC are pathogenic
for equines and are responsible for major outbreaks in humans. Other
variants do not normally cause encephalitis in equids and, although
sometimes encountered in humans, have not been isolated from
major outbreaks.
Occurrence

Epidemics were first registered in the 1930s in the northern part of South America and then spread to Central America, reaching southern states of the USA in the 1970s. The disease is endemic in central and northern South America.

Reservoirs

The virus is maintained in a rodent-mosquito-rodent cycle. During major outbreaks affecting humans, the disease is transmitted in a cycle involving mosquito vectors and horses or other equines as hosts. For this reason, natural outbreaks are normally preceded by equine epizootics. Humans also may develop sufficient viremia to serve as hosts in human-mosquito-human cycles. Epidemic and non-epidemic strains may be distinguished antigenically.

Mode of transmission

Humans become infected from the bite of infected mosquitoes. The major species of mosquito that transmit epidemic VEE are Psorophora confinnis, Aedes sollicitans, Aedes taeniorhynchus, and Deinocerites pseudes. There is no evidence of direct person to person transmission or of direct transmission from horses to humans. Although natural aerogenic transmission is not documented in humans, primary aerosol infection in laboratories is well known and inhalation of only a few organisms is sufficient to cause a significant likelihood of infection.

Incubation period

The incubation period in natural or aerogenic infection is usually 1-6 days.

Clinical features

Clinical manifestations of the naturally occurring disease are influenza-like, with abrupt onset of severe headache, high fever, chills, myalgia in the legs and lumbosacral area and retroorbital pain. There may also be photophobia, sore throat, nausea, diarrhoea and vomiting. Conjunctival and pharyngeal congestion are the only external signs. Most infections are fairly mild, with symptoms usually lasting 3-5 days. The overall case fatality rate in the 1962-63 epidemic in Venezuela, among some 30,000 cases, was approximately 0.6 percent. In some patients there is a second wave of fever and, particularly in children, CNS involvement ranging from somnolence and disorientation to personality change, convulsions, paralysis and death.
The initial symptoms of respiratory infection are like those of insect-born infection but central nervous system involvement appears to be more frequent.

Laboratory diagnosis

The disease exhibits leukopenia during a period usually limited to 1-3 days after onset. During this time, the virus may be sampled from serum or nasopharyngeal swabs and propagated in cell culture or in newborn mice. A variety of serological tests are applicable, including specific IgM ELISA, haemagglutination inhibition, immunofluorescence and complement-fixation. Polymerase chain reaction of can provide definitive identification of the specific strain and may be applied to serum and cerebrospinal fluid without prior propagation of the pathogen. Neutralizing antibodies appear in convalescent sera from the 5th day up to 2 weeks after onset of symptoms. Biosafety Level 3 practices, equipment and facilities are recommended for activities using infective clinical materials, although Biosafety Level 2 may be used for activities in which there is little likelihood of aerosol or air droplet production.

Medical management

Persons caring for infected patients should wear gloves, caps, gowns and surgical masks. Infective virus may be present in fresh or dried blood, exudates, cerebrospinal fluid and urine. Such materials should be decontaminated by heating or by chemical disinfection, as with hypochlorite or chloramine. If mosquito vectors are present, patients should be kept in screened or insecticide-treated rooms to prevent mosquito transmission to healthy persons and general mosquito control measures should be instituted.

Prophylaxis and treatment

Attenuated cell-culture propagated live vaccine TC-83 produced in the USA is moderately effective against both natural infection and aerosol challenge but is somewhat reactogenic and fails to induce a minimum neutralizing antibody response in approximately one-fifth of persons receiving it, presumably leaving them unprotected. Two other attenuated live virus vaccines, strains 15 and 230, reported to offer good protection against aerosol challenge, were developed in Russia. An inactivated vaccine designated C-84, prepared by formalin-inactivation of the TC-83 strain, is currently used to immunize TC-83 non-responders and as a booster for individuals who have declining titres after TC-83 vaccination.
Further reading on VEE

[REFERENCES TO BE INSERTED HERE]

1.4.2 Variola virus/Smallpox (B03)

Variola virus is a member of the genus Orthopoxvirus, subfamily Chordopoxvirinae of the family Poxviridae. Other members of the genus include cowpox, camelpox, ectromelia virus, vaccinia and monkeypox, the pox virus regarded as the cause of the most serious human poxvirus infections since the eradication of variola. The variola virus measures 260 by 150 nm and contains a molecule of double stranded DNA putatively coding for some 200 different proteins, one of the largest viral genomes known. There are at least two epidemiological strains of the virus, the more virulent designated variola major and the milder variola minor or alastrim. The variola virus is relatively stable in the natural environment and, if aerosolized, probably retains its infectivity for at least several hours if not exposed to sunlight or ultraviolet light.

Reservoir

The only known host of the virus was humans, facilitating the world-wide eradication campaign conducted by the WHO. The last naturally acquired case occurred in Somalia in 1977 and there was a laboratory acquired case in England in 1978. The global eradication of smallpox was certified by the WHO Assembly in 1980.

Pending its possible ultimate destruction, all stocks and work with variola virus are authorized only in high containment Biosafety Level 4 laboratories at the CDC in Atlanta, Georgia, USA and at VECTOR, Koltsovo, Novosibirsk Region, Russian Federation.

Mode of transmission

The virus gains entry into the body via respiratory or oropharyngeal mucosa. It is transmitted by aerosols and air-droplets from close contacts with infected patients, particularly if the symptoms include coughing, and also by contaminated clothes and bedding.

Incubation period

The first clinical symptoms appear between 7 and 19 days after exposure, commonly 10-14, with rash appearing 2-5 days afterwards. Patients become infectious only after the
appearance of rash and remain so until all scabs have
detached.

Clinical features

Onset is sudden, with influenza-like symptoms including fever,
malaise, headache, prostration, severe back pain, and, less
often, abdominal pain and vomiting. Two to three days later,
the fever may drop and a rash appears, first about the face,
hands and forearms and then after a few days progressing to
the trunk. Such centrifugal distribution of lesions is an
important diagnostic feature. Lesions progress from macules
to papules and to pustular vesicles and all lesions in a given
area progress together through these stages. From 8 to 14
days after onset, the pustules form scabs which leave
depressed depigmented scars upon healing.

Variola major and variola minor are characterized by similar
lesions but variola minor is accompanied by milder symptoms
and a case fatality rate of less than 1 percent, while the fatality
rate of variola major is 20-40 percent.

Variola is sometimes confused with chickenpox, caused by
the varicella-zoster virus, a member of the family
Herpesvirideae. Chickenpox is a world-wide infection
especially of children that is seldom lethal. It is distinguished
from variola by its much more superficial lesions, their
presence more on the trunk than on the face and extremities
and by the development of successive crops of lesions in the
same area.

There are two rare forms of smallpox, haemorrhagic and
malignant. In the former, invariably fatal in both vaccinated
and nonvaccinated patients, the rash is accompanied by
haemorrhage into the mucous membranes and the skin.
Malignant smallpox is characterized by lesions that do not
develop to the pustular stage but remain soft and flat. It is
almost invariably fatal for nonvaccinated patients and often
fatal even for vaccinated ones.

Laboratory diagnosis

Confirmation of clinical diagnosis may be accomplished by
immunofluorescent microscopy or electron microscopic
observation of the virus. Definitive confirmation and
discrimination of variola major from other pox viruses may be
accomplished by sequencing of amplicons from polymerase
chain reaction with viral DNA extracted from clinical
specimens. If virus-containing specimens are not available,
anti-smallpox antibodies may be detected in serum by various
tests, including virus neutralization, hemagglutination inhibition, Western blot or complement fixation. Scabs, vesicular or pustular fluids and other specimens for diagnosis should be collected only by vaccinated persons. Laboratory manipulations with infective materials should be done in high containment facilities at Biosafety Level 4, authorized only at the two WHO designated laboratories in the USA and the Russian Federation.

Medical management

Emphasis must be placed on preventing epidemic spread. In doing so, it should be kept in mind that smallpox patients are not infectious during the early stage of the disease but become so from the first appearance of rash and remain so until all scabs have detached. Also, immunity develops rapidly after vaccination against smallpox, so that even postexposure vaccination can prevent or ameliorate the disease so long as it is done within approximately 4 days after exposure and before rash appears.

Patients diagnosed with smallpox should be physically isolated and all persons who have or will come into close contact with them should be vaccinated. As hospitals have proven to be sites of epidemic magnification during smallpox outbreaks, patient isolation at home is advisable. This also reduces the risk of infecting persons incorrectly diagnosed with smallpox during an outbreak. Patients who developed rash before their isolation should be asked to recount all recent contacts and, if feasible, these should either be vaccinated or placed on daily fever watch for at least two weeks after contact and vaccinated if fever appears. All specimen collectors, care givers and attendants coming into close contact with patients should be vaccinated as soon as smallpox is diagnosed and all other known contacts not previously vaccinated should be placed on daily fever watch and vaccinated if fever appears. If there is a major outbreak, people should be advised to avoid crowded places, to report any definitely elevated fever and to observe hygienic precautions such as frequent hand washing.

Medical caregivers, attendants, and mortuary workers, even if vaccinated, should wear gloves, caps, gowns and surgical masks. All contaminated instruments, excretions, fluids and other materials should be decontaminated chemically or by heat or incineration. Contaminated clothing and bedding, if not incinerated, should be autoclaved or washed in hot water containing hypochlorite bleach. Fumigation of premises may be done with formaldehyde. Cadavers should be cremated.
whenever possible and all persons coming in contact with them should be vaccinated or at least placed on daily fever watch.

Prophylaxis/treatment

Most existing vaccine stocks and the vaccine used in the WHO eradication campaign consist of pulp scraped from vaccinia-infected animal skin, mainly calf or sheep, with phenol added to a concentration sufficient to kill bacteria but not so high as to inactivate the vaccinia virus. This is then freeze dried and sealed in ampoules for later re-suspension in sterile buffer and intradermal inoculation by jet injector or multiple puncture inoculation with a bifurcated needle. A 19\textsuperscript{1} survey conducted by WHO estimated that there may be approximately 60 million doses of vaccine available world-wide, including 500,000 doses held by WHO and 6 or 7 million under the control of the CDC in Atlanta, Georgia, USA.

Vaccination usually prevents smallpox infection for at least ten years and even if symptoms appear, they are milder and mortality is less than in nonvaccinated persons.

Vaccination is contraindicated for certain groups, including pregnant women and persons with immune disorders or under immunosuppression, HIV infection or history of eczema. Nevertheless, if there is danger of epidemic spread it may be advisable to vaccinate such persons and to attempt to limit adverse effects by intramuscular administration of vaccinia immune globulin, if available, from vaccinia-infected sheep or calves. A less reactogenic vaccinia-based vaccine, produced in cell culture, is expected to become available within a few years and there is interest in developing monoclonal anti-viariola antibody for passive immunization of exposed and infected individuals.

A number of compounds are under investigation as chemotherapeutic agents against variola infection. One of these, Cidofovir, a broad-spectrum inhibitor of viral DNA polymerase, appears to protect mice against cowpox and cynomolgous monkeys against monkey pox and inhibits variola virus replication \textit{in vitro}.

\textbf{Further reading on smallpox}

[REFERENCES TO BE INSERTED HERE]
CITED LITERATURE AND GENERAL REFERENCES

Note that these citations, and also those at the end of each agent section above, have yet to be brought into the format and degree of detail expected by WHO


