ANNEX 3: BIOLOGICAL AGENTS

1. Introduction

Extensive research, development and testing by military establishments have shown that large-scale production of certain infective agents and their incorporation into weapons for atmospheric dispersal of pathogens is feasible in suitably designed facilities with specialized equipment and appropriate precautions to protect the workers and prevent accidental release to the environment. The selection of the agent and strain, its large-scale growth and its further processing present numerous technical problems and require specialized technologies and associated effort in research, development and testing. Several modes of delivery have received attention in military offensive programmes but by far the greatest emphasis has been placed on methods of disseminating biological agents as inhalable aerosols. Numerous additional technical difficulties must be overcome in order to develop munitions or other devices that produce stable 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 inhalability, infectivity and virulence of the agent. Nevertheless, despite the fact that the development of strategic biological weapons within military establishments historically required large-scale efforts over several years, some infective agents could be produced and used as weapons of terror on a smaller scale using relatively simple techniques. Pathogens variously cited as possible agents of biological warfare or terrorism are listed in Table A3.1 below.

This annex presents information about the 11 particular infective agents, all of them listed in Table A3.1, that were selected in Chapter 3 for inclusion in the representative group of agents. 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, and proper nutrition. The only exception is the variola virus, the agent of smallpox, declared by the 1980 World Health Assembly to have been eradicated.

Table A3.1. Biological agents variously cited as possible weapons for use against humans

Biological agent and WHO alphanumeric code for the disease[a] it can cause	United Nations[b] (1969)	WHO[c] (1970)	BWC[d] CBM-F (1992)	Australia Group[e] (1992)	NATO[f] (1996)	CDC[g] category A (2000)	BWC[h] draft Protocol (2001)
BACTERIA (including RICKETTSIA and CHLAMYDIA)							
Bacillus anthracis, A22 (anthrax)	X	X	X	X	X	X	X
Bartonella quintana, A79.0 (trench fever)				X			
Brucella species, A23 (brucellosis)	X	X	X	X	X		X
Burkholderia mallei, A24.0 (glanders)	X	X	X	X			X
Burkholderia pseudomallei, A24 (melioidosis)	X	X	X	X	X		X
Franciscella tularensis, A21 (tularaemia)	X	X	X	X	X	X	X
Salmonella typhi, A01.0 (typhoid fever)	X	X		X	X		
Shigella species, A03 (shigellosis)	X				X		
Vibrio cholerae, A00 (cholera)	X	X		X	X		
Yersinia pestis, A20 (plague)	X	X	X	X	X	X	X
Coxiella burnetii, A78 (Q fever)	X	X	X	X	X		X
Orientia tsutsugamushi, A75.3 (scrub typhus)					X		
Rickettsia prowazekii, A75 (typhus fever)	X	X	X	X	X		X
Rickettsia rickettsii, A77.0 (Rocky Mountain spotted fever)	X	X		X	X		X
Chlamydia psittaci, A70 (psittacosis)	X				X		
FUNGI							
Coccidioides immitis, B38 (coccidioidomycosis)	X	X			X		
VIRUSES							
Hantaan/Korean haemorrhagic fever, etc., A98.5		X		X	X		
Sin nombre, J12.8							X
Crimean-Congo haemorrhagic fever, A98.0		X		X	X		X
Rift Valley fever, A92.4		X		X	X		X
Ebola virus disease, A98.3				X	X	X	X

Junin haemorrhagic fever, A96.0 (Argentine haemorrhagic fever)				X	X	X	X
Machupo haemorrhagic fever, A96.1 (Bolivian haemorrhagic fever)				X	X		X
Lassa fever, A96.2				X	X	X	X
Tick-borne encephalitis/Russian spring–summer encephalitis, A84.0/ A84	X	X		X	X		X
Dengue, A90/91	X	X		X	X		
Yellow fever, A95	X	X		X	X		X
Omsk haemorrhagic fever, A98.1					X		
Japanese encephalitis, A83.0		X		X			
Western equine encephalomyelitis, A83.1		X		X			X
Eastern equine encephalomyelitis, A83.2	X	X		X	X		X
Chikungunya virus disease, A92.0	X	X		X	X		
O'nyong-nyong, A92.1		X					
Venezuelan equine encephalitis, A92.2	X	X	X	X	X		X
Variola major, B03 (smallpox)	X	X		X	X	X	X
Monkeypox, B04				X			X
White pox (a variant of variola virus)				X			
Influenza, J10, 11	X	X			X		
PROTOZOA							
Naeglaeria fowleri, B60.2 (naegleriasis)							X
Toxoplasma gondii, B58 (toxoplasmosis)		X					
Schistosoma species, B65 (schistosomiasis)		X					

Notes

[a] Diseases are identified by the alphanumeric code assigned by the WHO *International Statistical Classification of Diseases and Related Health Problems*, 10th Revision (ICD-10).

[b] United Nations, *Chemical and bacteriological (biological) weapons and the effects of their possible use: Report of the Secretary-General,* New York, 1969.

[c] World Health Organization, *Health aspects of chemical and biological weapons: Report of a WHO group of consultants,* Geneva, 1970.

[d] UN Office of Disarmament Affairs, compilation of declarations of information by BWC States Parties in accordance with the extended confidence-building measures agreed at the Third Review Conference, DDA/4-92/ BW3 plus Add.1, Add.2 and Add.3, data from Section 2, *Past offensive biological R&D programmes*, of Form F as filed by Canada, France, Russian Federation, UK, and USA in 1992.

[e] Australia Group document AG/Dec92/BW/Chair/30 dated June 1992.

[f] *NATO Handbook on the Medical Aspects of NBC Defensive Operations*, AmedP-6(B), Part II – Biological, 1996.

[g] Centers for Disease Control and Prevention: Biological and Chemical Terrorism: Strategic Plan for Preparedness and Response. Recommendations of the CDC Strategic Planning Workgroup. *Morbidity and Mortality Weekly Report*, 2000; 49 (No.RR-4):1–14.

[h] Ad Hoc Group of the States Parties to the Convention on the Prohibition, Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction, document BWC/AD HOC GROUP/56-2, at pp 465-466, which is in Annex A of the Chairman's Composite Text for the BWC Protocol.

1.1 Recognizing deliberate release

Although all of the listed agents are known because of the diseases they cause naturally, there are aspects 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 transmission 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 mainly confined to the passage or dispersal time of the aerosol. This is because the limited deposition of aerosol particles and the inefficiency of their resuspension as particles small enough to be inhaled would generally make subsequent exposures 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. It is also possible, however, that deliberate release could be spread out over time, as would be the case for repeated attacks.

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 encephalitis, normally acquired from the bite of infected mosquitoes, it is possible that atypical infection of humans through the respiratory tract, which may bypass 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 inhalational 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.

Unusual geographical or demographic distribution. An unusual geographical distribution of persons or animals at the time of their probable exposure could point to deliberate use. Aerosol release resulting in an airborne cloud, for example, would give a distribution consistent with meteorological conditions at the time. Other unusual distributions or association with suspicious objects or activities may also be indicative of deliberate use.

Rareness. Although natural or inadvertent introduction of an exotic pathogen is not an uncommon occurrence, the unexplained appearance of an infectious disease of humans or animals that is ordinarily very rare or absent in a region may indicate deliberate use.

1.2 Prevention, protection and therapy

The unprecedented case-load, the sudden nature of the outbreak and the severe and possibly unfamiliar course of the illness resulting from a biological attack 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 in Chapters 4 and 5.

Exposure to aerosolized biological agents can be greatly reduced by a properly fitted military gas mask, by a high-efficiency particulate arresting (HEPA)-type microbiological mask or by a shelter or building provided with suitably filtered or disinfected air. The safe and effective use of masks requires training in their use. Timely masking and unmasking or entry and exit from shelters depend on advance warning of an impending attack and notification of when the inhalation hazard has passed. Some aspects of protection are discussed in Chapters 3 and 4.

Vaccines affording various degrees of protection for various periods of time against a few of the agents of concern have been approved by national regulatory authorities as effective and sufficiently safe for general use against naturally occurring infection. WHO will post information on a number of vaccines and their sources of supply on its web site.

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 against it. 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, the cost and resources required by any large-scale vaccination programme must be balanced against other needs and, depending on the vaccine, its administration may entail health risks in the form of adverse reactions and may be subject to contraindications for specific population groups. Finally, for most of the agents of concern, vaccines approved for general use do not exist.

Post-exposure vaccination for the agents described here is of proven value only in the case of smallpox, where its timely administration to persons who may have been exposed would probably be of major importance in helping to halt epidemic spread.

Antimicrobial drugs 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 antimicrobials most likely to be effective requires timely identification of the agent and its sensitivity to specific antimicrobials. 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 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 specific DNA-based, immunobased 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.

1.3 Specific agents

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 listed agent. Additional information may be found in the specific references given at the end of

the section for each agent and in the more general works cited at the end of this annex. The information given below 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 alphanumeric code assigned by ICD-10.
- *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, waterborne, foodborne, 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 those of influenza and making early clinical identification difficult.
- *Laboratory diagnosis.* Laboratory methods for identification of pathogens in clinical and environmental specimens. Biosafety recommendations for laboratory workers.
- *Medical management and public health measures.* Isolation requirements, protection of caregivers, disposal of contaminated materials and, where applicable, quarantine and hygienic measures.
- *Prophylaxis and therapy.* Vaccines, antimicrobials and antisera, where applicable.
- *Other information.*
- *Selected references.*

2. Bacteria

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 µm x 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 egg-shaped 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, restarting the cycle.

Occurrence

Anthrax is mainly a disease of mammals, most commonly encountered in grazing animals. Until the introduction and widespread use of effective 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 programmes are inadequate or have been disrupted. Human anthrax, acquired from diseased animals and animal products, is most frequent in Africa, the Middle East and 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, vegetative cells in blood and other secretions spilt on 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 bonemeal 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, however, are rapidly destroyed by putrefaction.

Mode of transmission

It is the spore rather than the vegetative form that is generally the agent by which the disease is transmitted and it is doubtful that the vegetative form ever proliferates significantly outside the animal body. The vegetative form is infective, however, and is presumed to be responsible at times for infection by fly bites. 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 on exposed parts of the body such as the arms, face and neck. Less frequently, infection is by ingestion of meat of infected animals or by inhalation of spores, as from contaminated wool, hair or hides. The disease is generally regarded as being non-contagious. Records of person-to-person spread exist, but are rare. Evidence from animal experiments, including experiments in non-human primates, suggest that the introduction of only a few spores through a lesion may induce cutaneous or gastrointestinal infection but that a much larger number of spores is required to produce a high probability of infection by inhalation. Nevertheless, the possibility cannot be excluded that inhalation of even a single spore can initiate infection by any of the routes, although with very low probability in the case of inhalation or ingestion anthrax.

Incubation period

Symptoms of human cutaneous and gastrointestinal anthrax generally appear between 1 and several days after exposure. The incubation period for inhalational anthrax, derived from limited data, is reported to range from 1 to 7 days. Longer times, possibly extending up to several weeks, may occur in rare cases. As with other pathogens, average incubation period may be inversely associated with dose.

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% of cases. With proper antimicrobial therapy, the death rate in cutaneous anthrax is less than 1%.

Inhalational anthrax begins with nondescript or influenza-like symptoms that may elude correct diagnosis. These may include fever, fatigue, chills, non-productive cough, vomiting, sweats, myalgia, dyspnoea, confusion, headache, and chest and/or abdominal pain, followed after 1–3 days by the sudden development of cyanosis, shock, coma and death. Nasal congestion and rhinorrhoea, common in influenza and other viral respiratory illnesses, are rare in patients with inhalational anthrax. Chest X-rays usually show a widened mediastinum, marked pleural effusions and mediastinal lymphadenopathy. During terminal stages, blood levels of vegetative bacilli may reach 10^8/ml or more. Late administration of antimicrobials 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 1–4 days. Reported case-fatality rates without treatment are 90% and higher. Meningitis is not uncommon and is a dangerous potential sequel to any of the forms of anthrax. Pneumonia may be present but is not a regular feature, and 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 and lymphoid involvement. Mortality in gastrointestinal anthrax is variable, depending on the outbreak, but in some outbreaks it is reported to approach that of inhalational anthrax. However, in both gastrointestinal and inhalational forms, mild or subclinical infections may occur and be undetected.

Laboratory diagnosis

Confirmation of clinical diagnosis may be made by direct visualization of the vegetative bacilli or by culturing. Microscopic identification of the vegetative bacilli in fresh smears of vesicular fluid or blood may be done using the McFadyean staining method or the indirect immunofluorescence assay. On blood agar plates, *B. anthracis* forms white or

greyish white, coherent, non-haemolytic colonies in which chains of vegetative bacilli are present together with spore-containing cells. In blood and 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-γ-d-glutamic acid capsule. If the patient has been treated with antimicrobials, however, it may be difficult or impossible to demonstrate the bacilli in blood or tissue specimens. For identification in sterile fluids and other sterile samples, methods have been developed for rapid detection based on monoclonal antibodies and on polymerase chain reaction (PCR). Seroconversion may be detected using enzyme-linked immunosorbent assay (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 culturing or activities with a significant potential for aerosol production.

Medical management and public health measures

Patient isolation is not required and there are no quarantine requirements. Cadavers should be cremated. Dressings, discharges from lesions, and other contaminated materials should be disinfected, preferably by incineration or by deep burial with quicklime. Sterilization or disinfection may also be achieved by autoclaving, soaking with aqueous formaldehyde, glutaraldehyde, hypochlorite, hydrogen peroxide or peracetic acid. Fumigation with ethylene oxide, formaldehyde vapour or chlorine dioxide may be employed to inactivate spores in contaminated rooms or buildings.

Prophylaxis and therapy

Live spore vaccines based on attenuated strains are produced for human use in China and in the Russian Federation. In 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 United Kingdom and the USA. The use of such vaccines has been associated with a major reduction of anthrax in individuals whose occupations place them at risk. Owing to limited reliable data concerning the availability of these vaccines, WHO is unable to provide such

information at the time of going to press. It is hoped that it can be published subsequently on the WHO web site.

With respect to protection against infection by the aerosol route, experimental immunization with live spore vaccines and cell-free vaccines containing anthrax-protective antigen has been shown to be capable of protecting laboratory animals (guinea-pigs, rabbits, monkeys) against inhalational anthrax. Evidence regarding the degree and duration of protection that existing vaccines may afford to humans against inhalationally acquired anthrax is based to some extent on epidemiological analyses of at-risk occupations but, for the purpose of bioaggression scenario modelling, is heavily dependent on extrapolation from such animal experiments and on indirect measures of human immune parameters. Efficacy of these vaccines as part of a post-exposure prophylaxis programme has not been determined.

Antimicrobial therapy is effective in treating cutaneous anthrax and is likely to be effective against human inhalational anthrax provided that it is begun before or very soon after symptoms appear. Once high levels of toxin are produced by anthrax bacilli in the body, antimicrobial therapy becomes ineffective. If available, specific human gamma globulin may be effective in cases where otherwise lethal levels of anthrax toxin have already accumulated. Antimicrobial 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 antimicrobials. Because of the possibility of extended incubation periods in rare instances, continuation of antimicrobial treatment for up to 60 days has been recommended in the USA.

Penicillin is generally effective against human cutaneous anthrax. Tests in non-human primates indicate that penicillin, doxycycline and ciprofloxacin are effective for prophylaxis and for early treatment of inhalational anthrax. The use of cell-free vaccine for post-exposure prophylaxis in combination with antimicrobials has been suggested on the basis of limited studies on non-human primates.

Other information

The disease is associated with the action on mammalian cells of a toxin composed of three protein components produced by the vegetative

bacillus. One of the components, protective antigen (PA), binds to receptors on the cell surface and mediates the entry into the cell of the other two components, oedema factor (EF) and lethal factor (LF).

The other principal anthrax virulence factor, in addition to the toxin, is the polypeptide capsule of the vegetative bacillus, which affords protection against phagocytosis. The symptoms of anthrax infection in experimental animals can be produced by the administration of the purified toxin.

Reported estimates of the dose required to infect 50% of a population of non-human primates in experimental studies of inhalational anthrax vary enormously, from 2500 to 760000 spores, apparently reflecting differences in the many variables involved in such experiments. While doses lower than the LD_{50} produce correspondingly lower rates of infection, the very large number of experimental animals that would be required makes it impractical to determine doses that would infect only a small percentage of those exposed.

The largest reported outbreak of human inhalational anthrax took place in 1979 in Sverdlovsk (Ekaterinburg), former Soviet Union. Of 66 documented fatal cases, all were more than 23 years in age, suggesting that adults may be more susceptible to inhalational 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 hazard of long-distance aerosol travel of infective spores.

An outbreak of inhalational anthrax and cutaneous anthrax in the United States during October and November 2001 was caused by *B. anthracis* spores intentionally placed in envelopes sent through the post. Of the total of 11 reported inhalational cases, the probable date of exposure could be determined in six, and for these the median incubation period was 4 days (range 4–6 days). Prolonged antimicrobial prophylaxis administered to persons thought to be at greatest risk may have prevented cases from occurring later. All 11 inhalational cases received antimicrobial and supportive therapy and six survived. As in the Sverdlovsk outbreak, there was a lack of young persons among the inhalational cases, whose ages ranged from 43 to 94.

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

Brucella species, which may also be regarded as different strains of *B. melitensis*, are non-motile, Gram-negative, aerobic, unencapsulated cocci or short rods measuring approximately 0.5–0.7 µm x 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

Worldwide.

Reservoirs

Diverse domesticated and wild mammals, especially cattle, goats, sheep, pigs, camels, buffaloes and marine mammals. Preferred hosts exist for each species: *B. abortus* commonly infects cattle; *B. suis* commonly infects pigs; and *B. melitensis* commonly infects goats, sheep and camels. *B. melitensis* and biovars 1 and 3 of *B. suis* are particularly virulent for humans.

Mode of transmission

Most human infections result from ingestion of raw animal products, especially unpasteurized dairy products. Infection may also result from entry of the bacteria from diseased animals through skin lesions or mucous membranes or from inhalation of contaminated dust or aerosols. Inhalation of only a few organisms is sufficient to cause a significant

likelihood of infection. Laboratory infection is common, especially by inhalation of aerosols. Person-to-person transmission occurs very rarely, if ever. Many countries are now essentially free of animal brucellosis, owing to control and eradication programmes based on test-and-slaughter programmes and/or vaccination of cattle, sheep and goats.

Incubation period

The incubation period is 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, genito-urinary, central nervous system and cardiac complications. Fatality among untreated patients is approximately 2% 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 and other tissues. Serological 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 3 practices, equipment and facilities are recommended for manipulations involving clinical specimens and for all manipulations of cultures.

Medical management and public health measures

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 6-week course of oral doxycycline concomitant with either 6 weeks of oral rifampin or 3 weeks of intramuscular streptomycin is usually successful if begun early. Even prolonged antimicrobial treatment is only moderately effective in cases of chronic infection.

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2.3 *Burkholderia mallei* / Glanders (A24.0)

Formerly classified as *Pseudomonas mallei*, the organisms are Gram-negative rods with rounded ends, 1.5–3.0 µm long and 0.3–0.6 µm wide, which often stain irregularly. They have no flagellae and are therefore non-motile. The organism is not highly resistant to environmental conditions.

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

Although most 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. Untreated bloodstream infections are usually fatal within a few days. 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 microorganism from skin lesions, pus, sputum or blood, followed by direct fluorescent antibody staining or by PCR. Serological tests include complement fixation, agglutination tests and 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 and public health measures

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.

Prophylaxis and therapy

No vaccine is available. Owing to the rareness of the disease, the medical literature regarding its therapy is sparse. Sulfadiazine and ceftazidime are recommended for therapeutic use. The organism is also sensitive to tetracyclines, ciprofloxacin, streptomycin, novobiocin, gentamicin, sulfonamides, or a combination of imipenem and doxycycline. There may be relapses even after prolonged antimicrobial therapy.

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2.4 *Burkholderia pseudomallei* /Melioidosis (A24)

Formerly classified as *Pseudomonas pseudomallei*, the organism is an aerobic, motile, Gram-negative rod 1.5 µm x 0.8 µm. The organism is not highly resistant to environmental conditions.

Occurrence

The disease is prevalent in South-East Asia, particularly in wet, rice-growing areas, and 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 haematogenous 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 septicaemia 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 and public health measures

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.

Prophylaxis and therapy

No vaccine is available. Current recommendations for therapy of severe melioidosis include intravenous ceftazidime or imipenem for 10 days to 4 weeks, followed by maintenance therapy with oral amoxicillin–clavulanic acid or a combination of trimethoprim–sulfamethoxazole and doxycycline for 10–18 weeks.

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2.5 *Francisella tularensis* / Tularaemia (A21)

The organism is a small, non-motile, Gram-negative, facultatively intracellular, aerobic coccobacillus, measuring 0.2 µm x 0.3–0.7 µm. Within the species, there are two predominant sub-species: *F. tularensis tularensis* or Type A is more virulent than *F. tularensis palaearctica* 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 palaearctica* occurs in Asia, Europe and North America.

Reservoirs

Many wild animals, especially rabbits, hares, voles, muskrats and beavers, as well as 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. Humans become infected mainly through the bite of arthropods, particularly ticks and mosquitoes, and through the skin, conjunctival sac or oropharyngeal mucosa, by direct contact with infected animals or animal materials and by ingestion of contaminated food or water or inhalation of contaminated dust or aerosols. *F. tularensis* is easily transmitted by aerosols and inhalation of only a few organisms is likely to cause infection. Person-to-person transmission has not been documented.

Incubation period

The incubation period varies from 1 to approximately 14 days, averaging 3–5 days.

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 and associated cervical lymphadenitis. Rarely, an intestinal form may develop with infection of the mesenteric nodes, and characterized by 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% and lasts 2–4 weeks, with a convalescent period of up to 3 months.

Depending on the site in the respiratory system at which infection occurs, inhalational 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%) if untreated.

The organism may enter the bloodstream, causing systemic illness, often severe. Untreated sepsis with the more virulent Type A strain is often fatal. Systemic illness without apparent site of primary infection is commonly termed "typhoidal tularaemia".

Laboratory diagnosis

Direct microscopic examination of clinical specimens showing small, poorly staining Gram-negative bacteria may suggest the diagnosis, and be supported by direct fluorescent antibody staining. Other supportive tests include PCR and antigen-capture ELISA methods. Confirmation is obtained by culturing the organism in cysteine-rich media, such as cysteine-enriched broth, thioglycolate broth, or cysteine heart blood agar, and by detecting diagnostic antibody titres to *F. tularensis*. Confirmatory tests, however, do not provide rapid results, and treatment should not be delayed if the diagnosis is clinically suspected.

The organism is extremely infectious and poses a substantial risk of laboratory-acquired infection unless handled according to stringent safety guidelines. Biosafety Level 2 practices, equipment and facilities are recommended for routine handling of clinical specimens from humans or animals. Biosafety Level 3 practices, equipment and facilities, including the use of a negative pressure biosafety cabinet, are recommended for all manipulations of cultures and any procedures posing a risk of aerosolization, such as centrifugation.

Medical management and public health measures

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 or attenuating infection by the cutaneous and inhalatory

routes. Vaccines of this type have been used to reduce the risk of tularaemia in populations living in endemic regions of the former Soviet Union and, although not approved or available for general use in the USA, among at-risk employees at Fort Detrick, Maryland. At present, tularaemia vaccine supplies are available in the Russian Federation only, but the vaccine may be available outside the Russian Federation in the future, if necessary. Attempts to develop improved vaccines are under way in a number of countries.

For antimicrobial prophylaxis, oral administration of doxycycline or ciprofloxacin is recommended for a 14-day period following the last day of exposure. For therapy, streptomycin is the recommended antimicrobial of choice, and is administered parenterally at 15 mg/kg twice daily for 10 days, but not to exceed 2 g per day. Parenteral gentamicin, doxycycline or ciprofloxacin are recommended alternatives to streptomycin. Patients beginning treatment with parenteral doxycycline or ciprofloxacin can switch to oral antimicrobial administration when clinically indicated. Recommended duration of administration of gentamicin or ciprofloxacin for treatment of tularaemia is 10 days. Doxycycline, however, is bacteriostatic, and treatment should continue for 14–21 days to avoid relapse. Chloramphenicol has been used to treat tularaemia, but there is a higher rate of primary treatment failure and relapse with its use than with the antimicrobials noted above.

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2.6 *Yersinia pestis* / Plague (A20)

Yersinia pestis is a Gram-negative non-motile, non-spore-forming coccobacillus measuring approximately 1.5 µm x 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 mucus 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 worldwide, with the exception of Australia.

Mode of transmission

Plague is transmitted between rodents and to other animals via fleas, consumption of infected animal tissues or, possibly, contaminated soil or respiratory droplet exposures. In endemic rural areas, plague typically occurs sporadically among persons who come in contact with wild rodent hosts of *Y. pestis* and their fleas. Outbreaks affecting large numbers of people can occur in cities when plague infects populations of urban rodents, particularly the black rat, *Rattus rattus*, and the brown rat, *Rattus norvegicus*. The most common form of the disease in humans, bubonic plague, is spread mainly by the bite of fleas regurgitating plague bacteria 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 is 2–6 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 pneumonia, possibly accompanied by bloody cough. 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 as high as 60%, 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 ovoidal 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 or specific. The bacillus may be identified by direct fluorescent antibody stain for the *Y. pestis* capsular antigen, by lysis by specific bacteriophage and by PCR. 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 for aerosol or air droplet production or for work with antimicrobial-resistant strains and infected fleas.

Medical management and public health measures

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 indicated, flea control measures should be instituted.

Prophylaxis and therapy

Plague vaccines are available worldwide but are not recommended for immediate protection in outbreak situations. Vaccination is recommended only for high-risk groups, e.g. health workers and laboratory personnel who are constantly exposed to the risk of contamination.

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 and recommendations for administration of killed bacteria vaccines include an initial injection and two booster injections over a period of 6 months. 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, have had direct contact with body fluids or tissues of an infected mammal, or for any other reason are suspected to have been exposed to the pathogen should receive antimicrobial prophylaxis for a week after the last suspected exposure. Doxycycline and ciprofloxacin are recommended for such use.

Antimicrobial therapy is effective if begun early in the disease and continued for at least 3 days after body temperature returns to normal. Streptomycin is the historical drug of choice but is not immediately available everywhere. Gentamicin is considered to be an acceptable alternative to streptomycin, based on in vitro and animal experiments, and on limited clinical observations in humans. Tetracyclines are effective against plague, and are widely used for treatment and prophylaxis. Doxycycline, administered twice daily, is preferred for oral treatment because of its ready gastrointestinal absorption. Chloramphenicol has been used to treat various forms of plague, including plague pneumonia, and is recommended for treatment of plague meningitis because of its ability to cross the blood–brain barrier. Fluoroquinolones have demonstrated efficacy in treating plague in animal experiments. Ciprofloxacin was observed to be at least as efficacious as aminoglycosides and tetracyclines in studies of mice with pneumonic plague. In vitro studies show activity of several fluoroquinolones to be equivalent to or greater than that of the aminoglycosides or tetracyclines. Several sulfonamides (sulfathiazole, sulfadiazine, sulfamerazine and trimethoprim–sulfamethoxazole) have been used successfully for the treatment and prophylaxis of plague. Data indicate, however, that sulfonamides are less effective than streptomycin or tetracycline, particularly for pneumonic plague. Sulfisoxazole should not be used because of its rapid renal excretion. Penicillins, macrolides and cefalosporins are thought not to be clinically efficacious and are not recommended for treatment of plague. Multidrug resistance imparted by a transferable plasmid has been reported in a single clinical isolate, as has plasmid-mediated streptomycin resistance. Antimicrobial-resistant strains have been developed in the laboratory.

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2.7 *Coxiella burnetii* / Q Fever (A78)

Coxiella burnetii is a pleomorphic, Gram-negative obligate intracellular coccobacillus measuring approximately 0.2 µm x 0.7 µm. The spore-like 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

Worldwide.

Reservoirs

The zoonotic pathogen exists in a wide range of animal hosts, including domesticated livestock (especially cattle, sheep, goats) cats, dogs, rodents, baboons and wild birds. The enzootic cycle includes numerous species of ixodid and argasid ticks. Arthropod vectors, however, do not play a significant role in transmission to humans.

Mode of transmission

Transmission to humans occurs primarily by inhalation of dust, droplets or aerosols from 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 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 unpas-

teurized dairy products. High-temperature pasteurization is sufficient to kill the organism. Person-to-person transmission has been reported but is rare.

Incubation period

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

Clinical features

The onset may be 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%, 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 routinely occur and may be revealed by serology.

Laboratory diagnosis

Isolation and microbiological identification of the organism from blood or other clinical materials is a valid diagnostic test but is hazardous to personnel. Specific and relatively rapid identification of the organism in blood or paraffin-embedded tissue may be accomplished by PCR assays. Serological diagnosis may be performed by 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 and public health measures

Patient isolation is not required. Patient materials and contaminated articles should be autoclaved, incinerated or disinfected with solutions containing hypochlorite, peroxide, 70% ethanol, phenol or a quatenary ammonium compound.

Prophylaxis and therapy

A formalin-inactivated vaccine, commercially available in Australia, has been developed for laboratory workers and others at high risk.

Tetracyclines, particularly doxycycline, are effective if given early and may abort the infection if administered before symptoms appear.

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2.8 *Rickettsia prowazekii* / Epidemic typhus (A75)

Rickettsia prowazeki is a small obligately intracellular Gram-negative bacterium measuring approximately 0.4 µm x 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 may reappear during times of war or famine.

Reservoirs

Humans, flying squirrels (United States only).

Vectors

Transmitted from person to person by lice; fleas may play a role in transmission of flying-squirrel-associated typhus.

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

The incubation period is 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%, increasing with age. The disease may reappear years after the initial infection, usually in a milder form known as Brill-Zinsser disease.

Laboratory diagnosis

Specific antibodies appear about 2 weeks after infection, when diagnosis may be obtained by immunofluorescent antibody test. More rapid diagnosis may be obtained by immunohistological demonstration of the organism or by PCR, 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.

Medical management and public health measures

Isolation of patients is not necessary. If lice are present, insecticide should be applied to clothing, bedding, living quarters and patient contacts in order to prevent spread of the disease. Louse-infested individuals likely to have been exposed to typhus 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. Reapplication of insecticide may be needed as previously laid eggs hatch.

Prophylaxis and treatment

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

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3. Fungi

3.1 *Coccidioides immitis* and *Coccidioides posadasii* / Coccidioidomycosis (B38)

These agents are species of dimorphic fungi that propagate as mycelial moulds 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 airborne. Arthroconidia are lightweight, barrel-shaped cells measuring approximately 3 µm x 6 µm that are stable to drying.

Occurrence

The fungus occurs in soil, especially in arid and semi-arid regions of south-western United States, northern Mexico and focal areas of central and south America. A substantial percentage of cattle, swine, sheep, dogs and humans in endemic regions have had asymptomatic infections, as revealed by skin testing.

Reservoirs

Soil, in particular arid regions of the western hemisphere.

Mode of transmission

Infection usually takes place by inhalation of 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 a single arthroconidium may become infected. Once within the host, arthroconidia undergo a morphological change into spherules. These are round, segmented structures of 30–60 µm. Within these are hundreds of 2–3 µm ovoidal endospores which themselves may develop into endospore-bearing spherules, spreading the disease throughout the body.

Incubation period

The incubation period is usually 1–3 weeks.

Clinical features

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% to more that 50%.

For those developing clinical disease, the initial symptoms resemble those of other upper respiratory infections, and include cough, fever, night sweats, chills, chest pain, sputum production and headache. Less often, there may also be various skin manifestations, including erythema nodosum or erythema multiforme with or without joint aches. The initial form of the disease usually resolves without therapy within several weeks, although occasional patients have a more protracted convalescence.

Persistent symptomatic coccidioidomycosis of the lungs occurs in a small percentage of patients and is more frequent in patients with diabetes mellitus. It is characterized by progressive destructive pul-

monary disease with continuous low-grade fever, weakness, cough with sputum production, dyspnoea, haemoptysis and pleuritic chest pain. Extrapulmonary dissemination is seen in approximately 1% of all infected persons, and usually becomes evident weeks to months after primary disease. It is characterized by involvement of the skin, subcutaneous tissues, bones, joints and the central nervous system Patients with AIDS or other deficiencies in cellular immunity are especially susceptible to these complications. Without treatment, the disseminated form, which may follow a rapid or a prolonged course, has a mortality rate of more than 50%, approaching 100% if meningitis develops.

Recovery from clinical disease appears usually to be accompanied by lifelong immunity, and most individuals with asymptomatic infection also develop lifelong immunity.

Laboratory diagnosis

Spherules and endospores may be visualized with calcafluor, Papanicolaou, haematoxylin–eosin and Gomori methenamine staining in sputum samples, pus and biopsy tissue. The organism is rarely identified in cerebrospinal fluid. Direct microscopic examination of sputum samples placed in 10% potassium hydroxide reveals spherules and endospores in fewer than 30% of cases and may be complicated by the presence of spherule-like artefacts, such as pollen. Skin tests for hypersensitivity to preparations derived from the fungal mycelia (with coccidioidin) or from spherules (with spherulin) have been useful for epidemiological studies but may give false-negative results in individual cases, especially if the disease is advanced. Skin testing reagents are not currently commercially available.

Medical management and public health measures

As the disease is not contagious, quarantine and patient isolation are not indicated. As the arthroconidia easily become airborne and are highly infective, manipulation of clinical specimens and sporulating cultures should be conducted under Biosafety Level 3 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 at the present time. Recombinant coccidioidal antigens have been identified as protective in experimental infections and efforts are under way to

develop them as vaccine candidates for clinical studies. For serious or persistent cases, prolonged therapy with amphotericin B or oral azole antifungal agents (ketoconazole, fluconazole, itraconazole) is moderately effective. Lifelong administration of fluconazole is recommended for coccidioidal meningitis.

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4. Viruses

4.1 Venezuelan equine encephalitis (A92.2)

The agent is a member of the genus *Alphavirus* of the family Togaviridae. The virion is about 70 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 IAB 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. Sizeable epidemics were registered in Mexico in 1969, in Texas in 1971, and in Venezuela in 1995. The disease is endemic in central and northern parts of south America. Enzootic Venezuelan equine encephalitis (VEE) virus is endemic in Mexico and Florida. The Florida virus is Everglades virus, a distinct species.

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 viraemia 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* (recently revised to *Ochlerotatus 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 infective organisms is sufficient to cause a significant likelihood of infection. The VEE virus can initiate infection via the nasal mucosa and the olfactory epithelium of the upper respiratory tract. Virus-containing airborne droplets too large to penetrate more deeply into the respiratory system can therefore constitute a hazard.

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 1962–1963 epidemic in Venezuela, among some 30 000 cases, was approximately 0.6%. 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-borne infection but CNS 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, immuno-

fluorescence and complement fixation. PCR has been successfully used to distinguish strains. It may be applied to serum and cerebrospinal fluid without prior propagation of the pathogen. Neutralizing antibodies first appear in convalescent sera from the fifth day up to 2 weeks after onset of symptoms.

Biosafety Level 3 practices, equipment and facilities are recommended for activities using infective clinical materials.

Medical management and public health measures

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 autoclaving 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 but not licensed 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 the Russian Federation. 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.

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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 virus*, *Camelpox virus*, *Ectromelia virus*, *Vaccinia virus* and *Monkeypox virus*. Since the eradication of variola, the monkeypox virus is regarded as the cause of the most serious poxvirus infections in humans. The vaccinia virus, the best studied poxvirus, measures 370 nm x 270 nm and contains a double-stranded DNA molecule of about 190000 nucleotide pairs, one of the largest viral genomes known, putatively coding for some 200 different proteins. Variola virus has a slightly smaller genome and the size of the virions has not been determined accurately. There are at least two epidemiological strains of variola virus, the more virulent designated variola major and the milder variola minor or alastrim.

Reservoir

The only known host of the virus was humans, facilitating the worldwide eradication campaign conducted by 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 World Health Assembly in 1980.

Pending its possible ultimate destruction, all stocks and work with variola virus are authorized only in maximum containment Biosafety

Level 4 laboratories at the CDC in Atlanta, GA, USA, and at VECTOR, Koltsovo, Novosibirsk Region, Russian Federation.

Mode of transmission

The most frequent mode of transmission is by person-to-person spread from deposit of droplets of saliva or nasal secretion from infected persons onto the oropharyngea. Transmission is mainly by direct face-to-face contact via infective saliva deposited onto the oropharyngeal, nasal or respiratory mucosa of a susceptible person. The virus may also be conveyed to the nose or oropharynx by contaminated fingers or other objects contaminated with infective saliva or nasal exudate. Contaminated clothes, bedding or clothing and other fomites may also present a risk of infection.

Incubation period

The first clinical symptoms appear between 7 and 19 days after exposure, commonly 10–14, with rash appearing 2–5 days afterwards.

Clinical features

Onset is sudden, with a 2–4-day prodromal period with influenza-like symptoms including fever, malaise, headache, prostration, severe back pain, and, less often, abdominal pain and vomiting. Fever may then drop and a maculopapular rash appears, first on the oral mucosa, 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 fall off after 3–4 weeks and 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%, while the fatality rate of variola major is 20–40%.

Variola is sometimes confused with chickenpox, caused by the *varicella-zoster virus* (human(alpha)herpesvirus3), a member of the family Herpesviridae. Chickenpox is a worldwide 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 negative stain electronmicroscopic observation of the virus. Definitive confirmation and discrimination of variola major from other pox viruses may be accomplished by sequencing of amplicons from PCR 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, haemagglutination inhibition, Western blot, ELISA or complement fixation. Scabs, vesicular or pustular fluids and other specimens for diagnosis should be collected only by vaccinated persons. So long as there is no recurrence of smallpox, laboratory manipulations with infective materials must be done in maximum containment facilities at Biosafety Level 4, authorized only at the two WHO-designated laboratories in the USA and the Russian Federation.

Medical management and public health measures

Emphasis must be placed on preventing epidemic spread. It should be kept in mind that smallpox patients are not infectious during the incubation stage of the disease but become so from the onset of rash and remain so until all scabs have detached (approximately 3 weeks). Patients are most infectious during the first week of rash, when lesions in the mouth and pharynx release large amounts of virus into the saliva and nasal exudate. As scabs form, patients become less infectious. Immunity develops rapidly after vaccination against smallpox, so that even post-exposure 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 proved to be major sites of epidemic

magnification during smallpox outbreaks, patient isolation preferably at home or at dedicated facilities with strict limitation of contacts to the essential minimum is advisable. Isolation at home 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 2 weeks after contact and isolated if fever appears. All specimen collectors, caregivers, attendants, family members and others 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 an outbreak of smallpox, people in the surrounding community 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. Cadavers should be cremated whenever possible and all persons coming in contact with them should be vaccinated and placed on daily fever watch. Any presumptive case of smallpox should be regarded as a potential international public health emergency and immediately notified to national health authorities and to WHO.

Prophylaxis and treatment

Most existing vaccine stocks and the vaccine used in the WHO eradication campaign consist of pulp scraped from vaccinia virus-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 resuspension in sterile buffer and intradermal inoculation by jet injector or multiple puncture inoculation with a bifurcated needle. More recent vaccine production is from vaccinia virus-infected human cell-culture or from cultured monkey kidney (Vero) cells.

Vaccination usually prevents smallpox for at least 10 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, with HIV infection or with a 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 vaccinia virus-based vaccine, produced in cell culture, is expected to become available within a few years and there is interest in developing monoclonal anti-variola antibody for passive immunization of exposed and infected individuals.

The smallpox vaccine emergency reserve maintained by WHO currently consists of some 600 000 doses, held in Geneva and regularly tested for potency. WHO conducts surveys to estimate country-held smallpox vaccine stocks for civilian purposes, whether retained from the eradication era or recently produced. WHO has no information on possible additional reserves held for military purposes. At the request of Member States, WHO is increasing the size of the existing emergency reserve of smallpox vaccine as part of global preparedness. The emergency reserve would be used only in response to a smallpox outbreak, confirmed clinically and epidemiologically, and only in cases where the vaccine supplies held by the affected country are inadequate.

A number of antiviral drugs are under investigation as chemotherapeutic agents against variola infections. One of these, cidofovir, a broad-spectrum inhibitor of viral DNA polymerase, appears to protect mice against cowpox and cynomolgous monkeys against monkeypox and inhibits variola virus replication in vitro.

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