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Bioterrorism: management of major biological agents

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Abstract. Bioterrorism is defined by the intentional or threatened of microorganisms or toxins derived from living organisms to cause death or diseases in humans, animals or plants on which we depend. The other major point is to generate fear in the population. More than 180 pathogens have been reported to be potential agents for bioterrorism. The following is an overview of several agents that could be involved in a biological attack.

Keywords. Bioterrorism, anthrax, smallpox, botulism, tularaemia.

Introduction

The deliberate use of microorganisms as weapons has been attempted throughout history. One of the first major biological warfare attacks was reported during the 14th century, in 1346, during the siege of Kaffa with *Yersinia pestis* [1]. The attacking Tatar force catapulted the bodies of their plague-infected soldiers over the city walls. This attack initiated the second major outbreak of plague, the Black Death, which decimated one-third of people living in Europe. The most recent biological warfare attack was the deliberate release of *Bacillus anthracis* in the United States shortly after the terrorist attacks of 11 September 2001 [2]. This terrorist act, perpetrated by an unknown criminal group, caused 22 cases of anthrax, including 5 deaths. This deliberate release of anthrax in the United States brought about a radical change in people's perception of the risk of bioterrorism. The impact was not limited to the United States.

These bioterrorist events, unlike others before, had a worldwide impact not only on security and public health but also in other sectors. The need for bioterrorism preparedness and planning for response at multiple levels has been recognized in many countries. Throughout the world, countries have set up new administrative and operational structures and adapted their preparedness and response plans in order to deal with the new threat. On the wider international level, concerted global health security measures to strengthen the public health response to the threat of international biological, chemical and radio-nuclear terrorism has been initiated. Governments and international entities with responsibilities related to maintenance of peace, security, safety and health protection have made it a priority to review urgently their political, economic, diplomatic, military and legal capacity to face up to such attacks and have embarked on major efforts to increase their preparedness.

Bioterrorism is defined by the intentional or threatened use of microorganisms or toxins derived from living organisms to cause death or disease in humans, animals or plants on which we depend. The other major point is to generate fear in the population. Many such agents are zoonotic and could have a considerable impact on agriculture as well as on human health (e.g. *Bacillus anthracis, Yersinia pestis, Coxiella burnetii, Brucella* spp.).

In 1973, the convention known as the Biological and Toxin Weapons Convention or BWC, prohibited the development, production, stockpiling and acquisition of biological and toxin weapons. Despite international agreements to ban such weapons, there is no effective international mechanism for challenging either the development of biological weapons or their use. The number of countries

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known or suspected of having biological weapons capability has reportedly doubled since the convention went into force in 1975. From 1980 to 2000, estimates suggest that 40 deliberate uses of microorganisms as weapons have been perpetrated throughout the world. In the United States alone, during the last decade (1988–1999), intentional use of infectious agents was considered in 6 cases.

More than 180 pathogens have been reported to be potential agents for bioterrorism (Table 1).These agents are classified (CDC) in three different categories according to the infectiousness, virulence, public perception, impact, cost and sophistication of countermeasures. Category A includes agents that can be easily disseminated or transmitted person-to-person, cause high mortality with potential for major public health impact, may cause public panic and social disruption, and require special action for public health preparedness. Category B includes agents that are moderately easy to disseminate, cause moderate morbidity and low mortality, and require specific enhancement of diagnostic capacity and enhanced disease surveillance. Category C includes emerging infectious agents that could be engineered for mass dissemination in the future because of availability and ease of production and dissemination, and potential for high morbidity and mortality and major health impact.

In the event of biological warfare, these agents would most likely be disseminated by aerosols, which are invisible, silent, odourless, tasteless and theoretically easily dispersed without detection. Infective doses for commonly considered biological warfare agents are reported in Table 2. In 1970 the WHO predicted that a city of 500,000 people would be devasted following an aerosol release of 50 kg of biological warfare agent if deployed under ideal meteorological conditions. For example, an aerosol of anthrax spores could incapacitate 125,000, including 95,000 deaths, and an aerosol of plague 85,000, including 19,000 deaths [3] (Table 2).

Table 1. Biological terrorism agents.

Category	Bacteria	Viruses/Toxins
A	*Bacillus anthracis* (anthrax) *Yersinia pestis* (plague) *Francisella tularensis* (tularemia)	Variola virus (smallpox) hemorrhagic fever viruses (Ebola, Marburg, Lassa viruses …) *Clostridium botulinum* toxin
B	*Burkholderia mallei* (glanders) *Brucella species* (brucellosis) *Coxiella burnetii* (Q fever) food and waterborne pathogens	encephalomyelitis viruses (VEE, EEV, WEV) *Ricinus communis* toxin (ricin)
C	multi-drug-resistant *M. tuberculosis* …	encephalomyelitis viruses (TBE …)

Table 2. Infective aerosolized doses for common biological agents [3].

Agents	Infective dose
Bacillus anthracis	8000–50,000 spores
Brucella spp.	10–100 bacteria
Burkholderia mallei	low
Yersinia pestis	100–500
Francisella tularensis	10–50
Coxiella burnetii	1–10
Encephalitis viruses	10–100 viruses
Hemorrhagic fever viruses	1–10
Variola virus	10–100
C. botulinum toxin	0.001μg/kg

Many guidelines have been published on the treatment and prophylaxis of agents used as biological weapons. Prompt recognition of biological warfare-associated diseases can lead to more rapid mobilization of public health and medical assets. The following is an overview of several agents that could be involved in a biological attack.

Anthrax

Historically, human anthrax has been a disease affecting people who have close contact with animals or animal products contaminated with the spore-forming bacterium *Bacillus anthracis*. Anthrax is a zoonosis and is a normal commensal of many species of grazing mammals such as sheep, cattle and goats, which are infected through ingestion of soil contaminated by *B. anthracis* spores. The incidence of anthrax in herbivores has dramatically decreased in developed countries, but it remains an important health problem in developing countries. Wild and domestic animals in Asia, Africa, South and Central America, parts of eastern and southern Europe, the Caribbean and the Middle East are reported to be infected with the bacterium [4]. Human infections are usually the result of contact with infected animals or anthrax-contaminated animal products, or after direct exposure to *B. anthracis*. One of the latest and largest epidemics of human anthrax occurred in Zimbabwe between 1979 and 1985, with 9445 human cases described, including 141 deaths [5]. Most of the cases were cutaneous, some very rare cases of the gastrointestinal form were noted, but 8 cases of inhalational anthrax were also reported [5].

Anthrax and bioterrorism

Anthrax is seen as one of the most likely biological agents for use as a weapon. *B. anthracis* spores can be transmitted by aerosolisation. Inhalation anthrax has a high mortal-

ity rate, and the organism's spores, compared with other potential biological warfare agents, are quite stable in the environment [2, 4, 6]. It has been estimated that 50 kg of *B. anthracis* spores released over an urban population of 5 million would sicken 250,000 and kill 100,000 [3]. The use of anthrax in warfare has been recorded throughout history. During World War I, Germany used *B. anthracis* to infect livestock during transhipment. In December 1941 the British Government began testing the effect of anthrax on sheep on the Scottish island of Gruinard. Due to the durability of anthrax spores, decontamination was unsuccessful, and the island was quarantined until 1986, when a determined effort was made to decontaminate the island using formaldehyde. It was finally declared safe in 1990 [2]. By 1945 the Japanese programme (Unit 731) had stockpiled 400 kg of anthrax spores to be used in bombs. The United States has weaponised anthrax spores, as did other countries in the 1950s and 1960s. This was evidenced by the accidental aerosol release of *B. anthracis* spores from a Soviet military microbiology facility in Sverdlovsk in the former Soviet Union in April 1979 [7]. This was the largest known outbreak of inhalational anthrax in the 20th century: 68 of the 79 patients with inhalational anthrax died [7]. The first victim died after 4 days; the last one died 6 weeks later. Cases have been also reported in animals located more than 50 km from the site. Initially, the Soviet government claimed the deaths were caused by intestinal anthrax from tainted meat. It wasn't until 13 years later, in 1992, that President Boris Yeltsin admitted that the anthrax outbreak was the result of military activity at the facility.

In October 2001, 22 cases of bioterrorism-related anthrax were reported in the United States: 11 confirmed inhalational, and 7 confirmed and 4 suspected cutaneous cases [8, 9]. The cult of Aum Shinrikyo in Japan apparently tried on several occasions to disperse anthrax unsuccessfully in Tokyo before the sarin attack [2], but they used an attenuated vaccine strain. No other bioterrorism-related or outbreak after deliberate release has been reported in the literature.

Clinical features

Infection in humans most often involves the skin, and more uncommonly the lungs and the gastrointestinal tract. In scenarios of deliberate release by a bioterrorist attack, the spread of the bacteria is thought to be usually by aerosol, making inhalational anthrax the most common and lethal form of the disease, although the recent events in the United States show that cutaneous anthrax needs to be considered [4, 8–11].

Inhalational anthrax

Aerosolised anthrax spores can be trapped in the upper airways, although spores of 2–3 μm can pass through the bronchi to the alveoli and be transported via the lymphatics to the hilar and mediastinal lymph nodes, where germination to the bacillary form may occur [10, 12, 13].

Spores do not immediately germinate and may continue to vegetate in the host for several weeks after inhalation. Germination has been described to occur up to 98 days later in non-human primates [14]. The very long incubation times described in the Sverdlovsk outbreak are also attributed to late germination [7]. It has been suggested that antibiotics, which are not effective against the non-vegetative or spore form of *B. anthracis*, may prolong the incubation period. Spores germinate and begin replication only after having been taken up by alveolar macrophages. Replicating bacteria release several toxins, leading to haemorrhagic thoracic lymphadenitis and mediastinitis, oedema and necrosis [2]. Typical bronchopneumonia is not found on clinical or postmortem examination. Haemorrhagic meningitis frequently develops and can be observed in up to half of patients. The median incubation period from exposure to the onset of symptoms is approximately 4 days (range 1–7 days), but cases that occurred from 2 to 43 days after exposure have been reported in humans [7, 8, 14]. This period seems to be inversely related to the dose of *B. anthracis* spores. To cause inhalational anthrax, the estimated infectious dose by the respiratory route is 8,000–50,000 spores, although it may be significantly smaller for some individuals [11].

Early diagnosis is very difficult without a clinician's high index of suspicion. The classic clinical presentation is a biphasic illness. The initial symptoms are non-specific, including mild fever, non-productive cough, myalgias, dyspnoea, headache, vomiting, chills, weakness, abdominal pain, and malaise and chest pain [2, 4, 8]. Physical examination is usually unremarkable, but chest examination can reveal bilateral decreased breath sounds, rhonchi and/or inspiratory rales [4]. The illness progresses to the second phase within 2–3 days. In some patients, a brief period of apparent recovery follows, making it even harder to diagnose the disease. Usually, the second phase begins abruptly with sudden fever and chills, acute dyspnoea, retrosternal chest pressure, diaphoresis, cyanosis and shock [8]. At this stage, a chest radiograph most often shows a widened mediastinum consistent with mediastinal lymphadenopathy and haemorrhagic mediastinitis, pleural effusion and progressive bilateral perihilar infiltrates [8]. CT scan of the chest can demonstrate parenchymal infiltrates or consolidation, large bilateral pleural effusions, pericardial effusion and a widened mediastinum with a complete infiltration of the mediastinal fat planes, bronchial mucosal thickening, encasement and compression of the hilar vessels, and haemorrhagic lymph nodes [8]. Treatment may be successful in the early stages, but by the time respiratory symptoms develop, it is too late for it to have any effect, and death usually occurs within 24–72 h in almost 90% of cases, despite aggressive treat-

ment [7, 9]. Death usually occurs 7 days after the onset of symptoms [10]. Person-to-person transmission of inhalation anthrax has never been described.

Cutaneous anthrax

Cutaneous anthrax accounts for 95% of all naturally occurring anthrax infections with an estimated 2000 cases annually worldwide [2]. It occurs following contact with spore-contaminated materials or infected animal products. Infection requires an existing break in the skin such as previous cuts or abrasions. Rare cases through certain species of biting flies have been reported [5, 10]. Bacteraemia is a very rare complication. Direct exposure to secretions from human cutaneous anthrax lesions may result in secondary cutaneous infection, but this is very rare. The most common areas of exposure are the arms, hands, fingers, face and neck. The incubation period is 1–5 days following cutaneous exposure, although the primary lesion may occur up to 12 days later [2]. After the spore germinates in the skin tissues, toxin production results in local oedema. The primary lesion is usually a painless, small, pruritic papule or macule. Subsequently, within 24–36 h, a vesicle occurs and enlarges into a round ulcer. Two to 6 days later a characteristic black eschar develops, surrounded by extensive local oedema and a number of purplish vesicles. The swelling tends to be much greater than would normally be expected for the size of the lesion. The eschar dries, loosens and falls off in the next 1–3 weeks without complications or scarring in 80–90% of cases [4]. Cutaneous anthrax is a non-febrile disease. Fever indicates a secondary infection (*Staphylococcus* or *Streptococcus*) or a systemic infection due to bacteraemia (20% of patients without antibiotics) [10]. Lymphangitis and painful lymphadenopathy can occur with associated systemic symptoms such as malaise and headache. Antibiotic therapy only decreases the likelihood of systemic disease while not changing the progression of the skin lesion itself [2]. With antibiotic treatment, death due to cutaneous anthrax is rare (<1%); without antibiotic treatment, the mortality rate has been reported to be as high as 20% if cutaneous anthrax develops into systemic infection.

Gastrointestinal and oropharyngeal anthrax are very rarely reported and occur following deposition of spores or vegetative bacilli in the upper or lower gastrointestinal tract after ingestion of raw or undercooked contaminated meat [2].

Diagnosis

The clinical diagnosis is easier in the case of cutaneous anthrax, but is difficult in the other forms of the disease where the evolution is extremely rapid. Case definitions of suspected or confirmed cases due to deliberate release are reported in Table 3. Samples should be drawn prior to antibiotic treatments, because sterilisation of cultures is possible after one dose of antibiotics. Gram stain of the vesicular fluid from cutaneous lesions or of the pleural effusion, cerebrospinal fluid (CSF) and ascites fluid should be obtained [4]. If cutaneous anthrax is suspected, punch biopsy may also be performed for immunohistochemistry. The most useful microbiological test remains the standard blood culture. Organisms must be tested for sensitivity to antibiotics for natural existence of resistant species and possible genetic manipulation before deliberate release. The initial diagnosis of anthrax can also be made by positive cultures of CSF, pleural fluid, pleural or bronchial biopsy, or skin lesion. The rate of positive culture of tissues is poor [10]. Sputum culture and Gram stain are unlikely to be diagnostic of inhalational anthrax given the frequent lack of frank pneumonia. Rapid identification of *B. anthracis* can be made by direct fluorescent antibody testing and gamma-phage lysis. Confirmatory diagnostic tests such as polymerase chain reaction (PCR) can also be used and may help in early diagnosis [10]. Antibody testing by enzyme-linked immunosorbent assay (ELISA) may yield positive results in convalescent serum specimens. Therefore, serologic testing is useful only retrospectively. The predictive value of the nasal swab test

Table 3. Case definitions of suspected or confirmed cases due to deliberate release.

Deliberate release of anthrax
• ≥1 confirmed case of inhalation anthrax
• ≥1 confirmed case of cutaneous anthrax arising in individuals who do not routinely have contact with animals or animal hides
• ≥2 suspected cases of anthrax that are linked in time and place, especially geographical related groups of illness following a wind direction pattern
Deliberate release of smallpox
• A single confirmed case
Deliberate release of plague
• A single confirmed case in the European Union must be regarded with a high degree of suspicion of deliberate release
• A confirmed case of plague in a person without history of being outdoors or having contact with animals
• ≥2 suspected cases of plague that are linked in time and place, especially if the suspected cases are geographically related according to a particular wind pattern
Deliberate release of tularaemia
• Single confirmed case of indigenously acquired tularaemia NOT explained by occupational exposure
Deliberate release of botulism
• Clusters of >2 cases of acute flaccid paralysis with prominent bulbar palsies, especially where there are common geographic factors between cases, but no common dietary exposures or injected drug use
• Multiple simultaneous outbreaks with no obvious common source
• Cases of botulism with an unusual toxin type (type C, D, F or G or toxin E not acquired from an aquatic food)

for the diagnosis or following exposure to *B. anthracis* spore is unknown. A negative swab does not indicate that the patient has not been exposed to *B. anthracis*.

Treatment

Many guidelines have been published on treatment and prophylaxis for anthrax [2, 4, 15, 16]. Private room placement for patients with inhalational anthrax is not necessary. In the case of cutaneous anthrax, healthcare workers should use standard precautions with gloves. Historically, penicillin was the drug of choice for anthrax [4]. It is still approved as an alternative first-line treatment of inhalational anthrax infection when the strain is susceptible to this drug [15]. However, naturally resistant strains to penicillin have been reported. Furthermore, it might not be difficult to induce resistance to penicillin through laboratory manipulation of organisms. Naturally occurring *B. anthracis* resistant to multiple antibiotics has been produced in different laboratories (resistance to penicillin, doxycycline, chloramphenicol, macrolides and rifampicin) [11]. Currently, ciprofloxacin is the recommended first-line treatment (Table 4). Alternative antibiotics are amoxicillin and doxycycline after susceptibility has been confirmed. Despite the absence of studies, it is recommended to use one or two additional antibiotics in inhalational anthrax, such as rifampicin, chloramphenicol, clindamycin, clarithromycin, erythromycin, gentamicin, streptomycin or vancomycin [15–17]. *B. anthracis* is resistant to extended-spectrum cephalosporins, cefuroxime, aztreonam and trimethoprim-sulfamethoxazole. For inhalational anthrax, the duration of treatment is 60 days (8 weeks). Duration of treatment for cutaneous anthrax is 7–10 days. The same antibiotics are recommended for post-exposure prophylaxis. Oral ciprofloxacin is also recommended as a first choice for prophylaxis for persons at risk of inhalational anthrax and must be taken for at least 60 days, unless exposure has been excluded. It has been demonstrated that treatment with antibiotics beginning 1 day after exposure to a lethal aerosol can provide significant protection against death. (See paper by Donald Chabot for anthrax vaccine.)

Table 4. Recommendations for treatment and post-exposure prophylaxis of inhalation/intestinal anthrax [15].

		Treatment of suspected or confirmed clinical cases of inhalation/intestinal anthrax (60 days)	Post-exposure prophylaxis (60 days)
Adults Pregnant women	First line	Ciprofloxacin: 400 mg IV bid followed by 500 mg per os bid	Ciprofloxacin: 500 mg per os bid
It is recommended, when possible, to cease breastfeeding	Alternative to ciprofloxacin	– Ofloxacin: 400 mg IV bid followed by 400 mg per os bid – Levofloxacin: 500 mg IV once a day, followed by 500 mg per os once a day	– Ofloxacin: 400 mg per os bid – Levofloxacin: 500 mg per os once a day
	Alternative first-line treatment and follow-up when susceptibility is confirmed Alternative first-line prophylaxis if susceptibility is confirmed	– Doxycycline: 100 mg IV bid followed by 100 mg bid per os – Penicillin G: 2.4–3 million units IV, 6 times daily – Amoxicillin: 1 g IV 3 times daily, followed by 500 mg per os 3 times daily	– Doxycycline: 100 mg bid per os – Amoxicillin: 500 mg per os 3 times daily
Children	First line	Ciprofloxacin: 10–15 mg/kg IV bid followed by 10–15 mg/kg per os bid	Ciprofloxacin: 10–15 mg/kg per os bid
	Alternative first-line treatment and follow-up when susceptibility is confirmed Alternative first-line prophylaxis if susceptibility is confirmed	– Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years: 2.2 mg/kg IV bid followed by 2.2 mg/kg per os bid (max 200 mg/d) – Penicillin G: >12 years: 2.4–3 million units IV, 6 times daily <12 years: 30 mg/kg IV, 4 times daily – Amoxicillin: 80 mg/kg IV daily in 3 divided doses, followed by 80 mg/kg per os daily in 3 divided doses	– Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years: 2.2 mg/kg per os bid (max 200 mg/d) – Amoxicillin: 80 mg/kg per os daily in 3 divided doses

IV, intravenous; bid, twice daily.

Smallpox

Smallpox is a viral infection caused by the variola virus, which belongs to the family of Poxviridae, subfamily Chordopoxvirinae and genus orthopoxvirus, which includes monkeypox virus, vaccinia virus and cowpox virus [18]. It was declared eradicated worldwide by the World Health Organization (WHO) in 1980 following a smallpox eradication campaign with the last case of endemic smallpox occurring in Somalia in 1977 [19]. The last fatal case was due to a laboratory-acquired infection in the United Kingdom in 1978 [20].

Smallpox and bioterrorism

Variola virus is seen as one of the most likely viruses to be used as a biological weapon. Using this virus in warfare is an old concept: it was used with contaminated clothing in the 18th century by the British to create epidemics among the native American tribes. More recently, during World War II, the Japanese military explored the weaponisation of smallpox in Mongolia and China. The variola virus exists legitimately only in two laboratories in the world: one in the Centers for Disease Control and Prevention in Atlanta, Georgia, USA, and the other in the State Research Centre of Virology and Biotechnology in Novosibirsk, Russia [21]. There is no documentation of clandestine stock of the virus. Any new case of smallpox would have to be the result of human accidental or deliberate release. The aerosol infectivity, high mortality, and stability of the variola virus make it a potential and dangerous threat in biological warfare [22, 23]. In addition, animal poxviruses such as monkeypox or a recombinant variant of Variola virus could be developed as biological weapons. However, for the monkeypox virus, data indicate that it has limited potential for person-to-person transmission and furthermore is not able to sustain an epidemic indefinitely in a community by human transmission only [24]. Two different strains of variola virus are known and associated with two varieties of smallpox: variola major and variola minor or alastrim [23].

Clinical features

Person-to-person contact remains the most common route of transmission but requires close contact [18]. Patients are not infectious during the asymptomatic incubation period (4–19 days; mean 10–12 days) before fever occurs. Smallpox is mostly contagious during the first week of rash, corresponding to the period when the lesions of the enanthem are ulcerated. At this stage, aerosol droplets from oropharyngeal lesions increase the likelihood of person-to-person transmission. After aerosol exposure, the virus infects the regional lymph nodes around the respiratory tract where replication occurs followed by viraemia. Multiplication of the virus may occur in other lymphoid tissues, such as the spleen, liver, bone marrow, lung and lymph nodes. After a second viraemia period, the virus localises in small blood vessels of the dermis and in the oral and pharyngeal mucosa, and proceeds to infect adjacent cells. Viruses remain present in the lesions until all scabs have been shed following recovery. At this stage, when viruses are enclosed within hard dry scabs, infectivity is lower than at the initial stage of the disease. Close contact has been demonstrated to result in efficient transmission of smallpox. Historically, it has been estimated that approximately 30% of susceptible household members became infected at the time when smallpox was endemic. The virus is highly stable and remains infective for long periods of time outside the host. It has been estimated that variola can remain viable in certain conditions for up to a year in dust and cloth.

Variola major (classical smallpox)

The most virulent strain of variola virus causes variola major. Five clinical forms of variola major, which differ in prognosis are described [18, 23, 25].

Ordinary-type smallpox is the most common form and occurs in 90% of cases. The prodromal phase (2–3 days) has an abrupt onset and is characterised by severe and generalised headache, fever (>40 °C), extreme prostration, intense, ill-defined pain in the back, chest or joints, intense anxiety and sometimes abdominal pain. Children may have convulsions, and some adults are delirious. The fever subsides over a period of 2–3 days. Then enanthem in the form of minute red spots appears, a day before the exanthematous rash, over the tongue, palate, mouth and oropharynx. At this time lesions can also occur in the respiratory tract. The exanthema begins as a small reddish maculopapular rash on the face and forearms and spreads gradually with a centrifugal distribution to the trunk and legs and then to all parts of the body within 24 h, including the palms of the hands and the soles of the feet. Within 1–2 days, the rash becomes vesicular, with a vesicle diameter of 2–5 mm, and later pustular. Pustules that are round (4–6 mm in diameter), tense and deeply embedded in the dermis remain for 5–8 days, followed by umbilication and crusting. The number of pustules can vary from a few to several thousand. These lesions can be confluent, semiconfluent or discrete. A second, less-pronounced, temperature spike might be noted 5–8 days after the onset of the rash. Lesions are generally synchronous in their stage of development, in distinct contrast to varicella. This characteristic also provides the main distinguishing feature from monkeypox. Monkeypox virus is also an orthopoxvirus, found in Africa. It is clinically indistinguishable from smallpox, with the exception of notable enlargement of cervical and inguinal lymph nodes. The disease occurs mainly in monkeys, but

sporadic transmission to humans has been reported, as has limited human-to-human transmission.
During the disease, the fever can remain elevated if secondary pyogenic infection of the skin occurs. Panopthalmitis and blindness, keratitis and corneal ulcers, osteomyelitis, arthritis, orchitis and encephalitis are possible complications (1–5%). Bronchitis, pneumonitis, pulmonary oedema and associated bacterial pneumonia are not rare. Death may occur in the first 48 h, before any feature of smallpox has appeared. Most fulminant cases die by the 4th or 5th day; many other malignant cases die between the 8th and 15th day. Death is ascribed to toxaemia, associated with immune complexes and to hypotension. The mortality rate is 30% in unvaccinated and 3% in vaccinated individuals.

Haemorrhagic-type smallpox is the most virulent form and occurs in 3% of patients. It is characterised by haemorrhages into the skin and/or mucous membranes early in the course of the illness and intense toxaemia. It causes death in 96% of unvaccinated and 94% of vaccinated patients, usually before the occurrence of the lesions.

The modified-type smallpox or milder type is more common in previously vaccinated populations (25%) but can be noted in unvaccinated persons (2%) The onset of the prodromal phase is also abrupt. Usually the lesions are fewer, smaller and more superficial, and evolve more rapidly. Frequently, the pustular stage is absent.

Flat-type smallpox is defined by lesions that evolve more slowly than those of variola major and are coalescent. It is very rare in vaccinated subjects. It occurs in 2–5% of patients and is associated with severe systemic toxic affects. The enanthem is extensive and confluent. Vesicles contain very little fluid, are not multiloculated and do not show umbilication. Respiratory and abdominal complications are frequent. The mortality rate is 95% in unvaccinated and 66% in vaccinated individuals.

Variola *sine eruptione* occurs in previously vaccinated contacts or in infants with maternal antibodies. Patients are asymptomatic or have influenza-like symptoms with or without conjunctivitis, which can be the only clinical manifestation. No rash develops. Usually, diagnosis is performed retrospectively, and serological confirmation is required.

Variola Minor
The strain of variola virus that is associated with variola minor is less virulent than those of variola major. The severity and the mortality rate (<1%) are lower. The onset of the illness can be abrupt with fever (>40 °C), headache and backache. Toxaemia rarely occurs. The sequence of appearance, the distribution and the nature of the lesions are similar to those reported for variola major. But the evolution is usually more rapid. The skin lesions are smaller than those of variola major, are not confluent or umbilicated.

Diagnosis
Case definitions of suspected or confirmed cases due to deliberate release are reported in Table 3. A clinical diagnosis must be suspected in all cases even if many eruptive illnesses can be misdiagnosed as smallpox (e.g. chickenpox, monkeypox) [18]. For differentiation between the various orthopoxes, electron microscopy examination of vesicular or pustular fluid or scabs can be used. Poxviruses cannot be readily distinguished from one another except by PCR assay. Definitive identification of strains is performed by PCR and/or restriction fragment-length polymorphism (RFLP) [18, 21]. Definitive characterisation of the variola virus is made by culture in eggs and cell monolayers.

Treatment
Patients with smallpox must be isolated and managed, if possible, in a negative-pressure room until death or until all scabs have been shed (about 3 weeks) [16, 18, 21, 23]. There is no established antiviral treatment for smallpox. Cidofovir, an antiviral drug, is active *in vitro* on isolates of variola virus [26]. Obviously, no data are available for humans. Antibiotics may be useful in the case of secondary bacterial infection. The most effective prevention is vaccination before exposure [27, 28]. The frequency of complications is low, but is higher than the most commonly used vaccines. Vaccination can modify the course of the disease and reduce mortality if given immediately after exposure (mortality can be reduced by up to 100%) and up to 4 days after (mortality can be reduced by up to 50%).
The vaccine used to eradicate smallpox was highly efficacious; it was prepared using live animal skin as a substrate. During the WHO smallpox eradication programme (1977–1988) many countries produced and used this ‘first-generation’ product despite its known reactogenicity, as it was accepted that the benefits outweighed the risks. Nowadays, quality requirements for vaccines have become much more stringent, leading to the development and testing of so-called second-generation vaccines. These are produced using the same vaccine strains as in the first-generation vaccines on tissue culture substrate, allowing better production consistency and quality control testing (e.g. for adventitious agents). For Europe, the European Medicine Evaluation Agency (EMEA) has developed guidelines for the development and production of second-generation vaccines [29]. In 2002, many countries implemented a preparedness programme in

which smallpox vaccine was administered to volunteers. In the United States, 665,000 persons were vaccinated against smallpox during the 2002–2004 smallpox vaccination campaign [30, 31]. Serious adverse events such as neurologic disorders, myocarditis and/or pericarditis, and generalized vaccinia were unfrequent [30, 31]. There is a need therefore to develop a new (third) generation of smallpox vaccines, with an acceptable safety profile [32]. This could be achieved by attenuating or genetically engineering (disabling) vaccinia vaccine strains, while retaining their immunising properties. (See also the paper by Behazine Combadiere in this issue.)

Plague

Plague is an acute bacterial infection caused by *Yersinia pestis.* Historically, three plague pandemics have killed more than 200 million people, including the Black Death epidemic in 14th-century Europe [1]. This disease, primarily the bubonic form, is still endemic in many countries in Africa, in the former Soviet Union, Asia, South America and rural southwestern parts of the United States [33]. There is currently no plague in Europe. The last reported cases occurred after World War II. Worldwide, it is estimated that 1000 to 6000 cases occur each year (mean: 1500 cases/year) [34]. The total number of human plague cases reported to the WHO by nine countries in 2003 was 2118, of which 182 were fatal. 98.7% of those cases and 98.9% of those deaths were reported from Africa [34a]. It remains an enzootic infection of rats and other rodents. Plague occurs in sylvatic rats, which may then spread among more domestic rat species and finally among humans. Bacteria are usually passed to humans through the bite of a flea that has previously fed on an infected rat. In nature, at least 200 species of mammals and 80 species of fleas serve as reservoirs [35]. Infection may also occur through direct contact with infected tissues or fluids from sick or dead plague-infected animals, by exposure of humans to respiratory droplets from infected animals, especially cats with plague pneumonia, or by laboratory exposure to plague bacteria.

Human-to-human transmission can occur through infectious respiratory droplets from pneumonic cases of plague [25, 35–39]. Primary pneumonia appears as a consequence of this transmission. Bubonic and other forms of plague in humans, without secondary pneumonia, are not considered to be infectious. It has been reported that inhalation from contaminated clothes could be also a route of transmission. Propagation of a human plague outbreak depends upon the number and susceptibility of rodent populations, the flea-carrying capacity of the rodent species, the number and infectivity of the flea species, the degree of contact of people with infected rat fleas, climatic conditions, and the number and living conditions of susceptible people [39].

Plague and bioterrorism

Y. pestis appears to be a good candidate agent for a bioterrorist attack. The use of an aerosolised form of this agent could an explosive outbreak of primary plague pneumonia in the exposed population; alternatively, the bacteria could be used to infect the rodent population and precipitate a secondary outbreak in humans living in poor conditions [38, 39]. Intentional aerosol release should be suspected in patients presenting with plague pneumonia in non-endemic areas or in patients without risk factors. *Y. pestis* is a relatively fragile organism that remains viable only an hour after an aerosol release. Nevertheless, as few as 1–10 bacteria are sufficient to infect rodents via the oral, intradermal, subcutaneous or intravenous routes [40]. Estimates of human infectivity by the respiratory route vary from 100–20,000 organisms [1, 34]. The Tatars were the first to use plague as a biological weapon, in 1347, during the siege of the Genoese-controlled Black Sea port of Kaffa: they hurled the bodies of their plague victims over the city walls. It has been reported that the Japanese army dropped plague-infected fleas in China during World War II: as many as 15 million fleas were used per attack to infect the population with plague [1]. It has also been reported that cases of plague occurred among Japanese soldiers shortly after a biological attack on Changteh in 1941: 1700 deaths were reported in the Japanese army. In 1970, it was reported that if 50 kg of *Y. pestis* were released over a city of 5 million, plague pneumonia could occur in as many as 150,000 persons, including 36,000 deaths [3]. In May 2000, during the virtual exercise TOPOFF in the United States (for 'top officials'), simulation of a deliberate release of an aerosol of *Y. pestis* at a performing arts centre estimated that 3700–4000 cases of plague pneumonia and between 950 to over 2000 deaths might have resulted [38].

Clinical features

The most usual clinical presentations of plague are bubonic, primary septicaemic and pneumonic disease [1, 25, 33, 36]. In a biological attack, the most likely clinical presentation would be primary pneumonic plague, as aerosol is considered the most likely way of dispersing the agent [34, 38].

Pneumonic plague may occur by primary respiratory infection, or as a complication of the bubonic and septicaemic forms of the disease (secondary pneumonia). In the case of primary pneumonia, the incubation period is 1–6 days. It begins abruptly with intense headache

and malaise, high fever, vomiting, abdominal pain, diarrhoea and marked prostration. Chest pain, cough, dyspnoea and haemoptysis develop thereafter [33]. Chest radiographs show evidence of multilobar consolidation, cavities or bronchopneumonia. Respiratory failure develops quickly with septic shock. Without antibiotics, the disease is fatal in almost all patients within 2 or 3 days. Plague pneumonia is highly contagious to other humans by droplet transmission; patients are still contagious up to 3 days after starting appropriate antibiotic treatment. With the prompt use of antibiotics, the fatality rate decreases below 10%.

Bubonic plague is the most common clinical form of naturally occurring plague (75–97% of all cases). After an incubation period of 2–8 days, there is sudden onset of fever (38.5–40 °C), chills, headache, nausea, vomiting, malaise or prostration and weakness; 6–8 h after the onset of symptoms a bubo develops. It is characterised by severe pain, swelling and marked tenderness. It develops in the area of an infected bite, most commonly in the nodes of the groin (with femoral more frequent than inguinal lymph nodes affected), axilla and neck. It becomes visible after 24 h, and its size varies from 1 to 10 cm in diameter. There is surrounding oedema, and the overlying skin is warm, erythematous and adherent. Pustules, vesicles, eschars, papules or skin ulcerations may occur at the site of the flea bite, which is usually in the lower extremities. Rarely, the bubo may become fluctuant and suppurate. Without specific treatment, complications are common and include primary (without discernible bubo) or secondary septicaemia, secondary pneumonia and meningitis. The mortality rate or case fatality rate for untreated bubonic plague is 60%, which becomes less than 5% with appropriate antibiotic treatment [25]. This form is unlikely to occur in a bioterrorist attack, except if fleas were used as a vector [34, 36].

Septicaemic plague may occur as a complication of untreated bubonic plague or pneumonic plague and can develop in the absence of obvious signs of primary disease. It includes septic shock and disseminated intravascular coagulation with vasculitis, livid cyanotic petechiae, purpura and large ecchymoses that can mimic meningococcaemia. Gangrene of acral regions, like the tip of the nose or the fingers and toes, due to small artery thrombosis may appear in advanced stages of the disease (Black Death). Left untreated, mortality approaches 100%.

Plague meningitis is rare but may occur as a complication of inadequately treated infection elsewhere. **Pharyngeal plague** is very rare and possibly a result of ingestion or inhalation of the organism.

Diagnosis

Case definitions of suspected or confirmed cases due to deliberate release are reported in Table 3. *Y. pestis* can be cultured from blood, sputum, bubo aspirate and CSF samples. Specimens for culture should be taken before initiation of antibiotic treatment. Smears may be stained with Gram, Giemsa or Wayson's stains to demonstrate bipolar staining coccobacilli [34]. The diagnosis of plague is then confirmed by culture. Antimicrobial susceptibility tests must be set up as early as possible. Serological diagnosis is possible, but antibodies may not be detectable when the patient first presents. Detection of anti-capsular antibodies as either a ≥4 fold rise in titres from acute to convalescent serum or a single titre of > 1:128 in patients not previously vaccinated confirms the diagnosis [4]. Other tests include direct immunofluorescence for F1 antigen, specific phage lysis and PCR for the plasminogen activator gene.

Treatment

Treatment should be initiated as soon as the diagnosis is suspected (Table 5). Many antibiotics are active against *Y. pestis* (streptomycin, gentamicin, doxycycline, ciprofloxacin, chloramphenicol, sulfadiazine, trimethoprim-sulfamethoxazole) [1, 16, 36]. Most of the therapeutic guidelines suggest using gentamicin or streptomycin as first-line therapy, with ciprofloxacin as an alternative [1, 16, 36]. Chloramphenicol should be used for the treatment of meningitis. Persons who come in contact (< 2 m) with patients with pneumonic plague should receive antibiotic prophylaxis with doxycycline or ciprofloxacin for 7 days. Other antibiotics (chloramphenicol, sulfadiazine, trimethoprim-sulfamethoxazol) could also be used. Prevention of human-to-human transmission from patients with plague pneumonia can be achieved by implementing standard isolation procedures until at least 4 days of antibiotic treatment have been administered. For the other clinical types of the disease, patients should be isolated for the first 48 h after the initiation of treatment [1, 16, 36].

A live attenuated vaccine is available in the United States, but it retains some virulence and is therefore not considered suitable for human use in most countries [41]. Recent vaccine research in Europe is focused on development of a sub-unit vaccine containing F1 antigens and recombinant V antigens, which proved to be efficacious against pneumonic plague in mice [41, 42].

Tularaemia

Tularaemia is a bacterial zoonosis caused by *Francisella tularensis*. This agent is one of the most infectious pathogenic bacteria known, requiring inoculation or inhalation

Table 5. Recommendations for treatment and post-exposure prophylaxis of plague [15].

		Treatment of suspected or confirmed clinical cases (10 days)	Post-exposure prophylaxis (7 days)
Adults Pregnant women	First-line treatment	– Gentamicin: 5 mg/kg IV in 1 or 2 doses daily or – Streptomycin: 1 g IM bid	
It is recommended, when possible, to cease breastfeeding.	Second-line treatment; first-line prophylaxis	– Ciprofloxacin: 400 mg IV bid followed by 500 mg per os bid or – Ofloxacin: 400 mg IV bid followed by 400 mg per os bid or – Levofloxacin: 500 mg IV once a day, followed by 500 mg per os once a day	– Ciprofloxacin: 500 mg per os bid or – Ofloxacin: 400 mg per os bid or – Levofloxacin: 500 mg per os once a day
	Third-line treatment; second-line prophylaxis	– Doxycycline: 100 mg IV bid followed by 100 mg bid per os	– Doxycycline: 100 mg bid per os
Children	First-line treatment	– Gentamicin: 2.5 mg/kg IV in 3 doses daily or – Streptomycin: 15 mg/kg IM bid (max 2 g)	
	Second-line treatment; first-line prophylaxis	– Ciprofloxacin: 10–15 mg/kg IV bid followed by 10–15 mg/kg per os bid	– Ciprofloxacin: 10–15 mg/kg per os bid
	Third-line treatment; second-line prophylaxis	– Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years: 2.2 mg/kg IV bid followed by 2.2 mg/kg per os bid (max 200 mg/d)	– Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years: 2.2 mg/kg per os bid (max 200 mg/d)

IM, intramuscularly.

of as few as 10 organisms to initiate human infection [43, 44]. Tularaemia is distributed worldwide but occurs especially in the northern hemisphere, in Europe, North America, the Middle East, the former Soviet Union, China and Japan. Outbreaks have been commonly reported in some areas of Europe, for example Sweden, Finland, Spain and Kosovo [45–47]. In 2000, 270 cases in Sweden and 327 cases in Kosovo have been reported [46, 47]. During the last decade of the 20th century, 1368 cases were reported in the United States (< 200/year) [48]. In some endemic regions, outbreaks occur frequently, whereas adjacent parts of the same country may be completely free of the disease. Usually, cases are reported during the summer, from June to September, when arthropod-borne transmission is more common. *F. tularensis* may be found in contaminated water or soil, infected ticks or deerflies, wild animals and occasionally certain domestic animals [49]. A variety of small animals are probably natural reservoirs of infection. They acquire infection through bites by ticks, flies and mosquitoes, or by contact with contaminated environments. Humans become infected by various modes, including arthropod bites (ticks, deerflies, mosquitoes), which represent a major route of contamination, handling infectious animal tissues or fluids, direct contact with or ingestion of contaminated water, food, or soil, and inhalation of infective aerosols (e.g. aerosolisation by using a lawn mower or a brush cutter) [43, 40].

Tularaemia and bioterrorism

Inhalational tularaemia following intentional release of a virulent strain of *F. tularensis* would have the greatest adverse human consequence because of its very high infectivity after aerosolisation. Outbreaks of pneumonic tularaemia, particularly in low-incidence areas, should prompt consideration of bioterrorism [43, 44]. It has been estimated that an aerosol dispersal of 50 kg of virulent *F. tularensis* over a metropolitan area with 5 million inhabitants would result in 250,000 incapacitating casualties, including 19,000 deaths [3]. An outbreak of tularaemia reported in Soviet and German soldiers during the Second World War may have been the result of intentional release [43]. *F. tularensis* has been studied, weaponised and stockpiled by many countries, including Japan and the United States [43]. Another route of contamination in a deliberate release could be contamination of water. Transmission from person to person has never been reported.

Clinical features

After an incubation period of 3–5 days (range 1–25 days), seven clinical forms, according to route of inoculation (skin, mucous membranes, gastrointestinal tract, eyes, respiratory tract), dose of the inoculum and virulence of the organism (types A or B) are identified [43–45]. Usually, whatever the clinical form, the onset of tularaemia is abrupt with fever, chills, myalgia, arthralgia, headache, coryza, sore throat and sometimes pulse-temperature dissociation, nausea, vomiting and diarrhoea.

Respiratory tularaemia usually results from the direct inhalation of contaminated aerosols (primary pneumonia or inhalational tularaemia) or follows secondary haematogenous spread from a distal site (secondary pneumonia) [49]. In the United States, approximately 10–20% of patients with tularaemia present with pneumonia [50, 51]. In Sweden, during the 2000 tularaemia outbreak, more than 5% of patients were reported to have pneumonia [46]. Inhalational exposure commonly presents as an acute flulike illness without prominent signs of respiratory disease. Features include fever, chills, headache, muscle aches, joint pain, non-productive cough, pharyngitis and pleuritic chest pain. Signs from the respiratory system may, however, be minimal or absent. Chest radiography frequently shows peribronchial infiltrates, typically progressing to bronchopneumonia, pleural effusions and hilar lymphadenopathy. Progression to severe pneumonia with breathing difficulty, bloody sputum, respiratory failure, systemic forms and death may occur if appropriate treatment is not started.

Ulceroglandular tularaemia (75–85%) is the most common form reported in patients with tularaemia. It arises from handling a contaminated carcass or following an arthropod bite. Typically a local papule appears at the site of inoculation associated with symptoms, including fever and aches. The lesion may be pruritic and enlarges to form a pustule, which ruptures and develops into a painful, indolent ulcer, possibly covered by an eschar. Ulcers are usually single lesions of 0.4–3.0 cm in diameter. A localised vesiculopapular eruption may also occur. Lesions acquired from mammalian vectors are usually located on the upper extremities, whereas lesions acquired from arthropod vectors are usually located on the lower extremities. The lesion is associated with tender enlargement of one or more regional lymph nodes, which may become fluctuant and rupture releasing caseous material. Local disease often continues to progress despite appropriate antibiotic therapy. Neither severe disease nor complications are usually noted with this form of the disease. Lymphadenopathy may persist for as long as 3 years.

Glandular tularaemia (5–10%) presents with lymphadenopathy and fever but without ulcer.

Oculoglandular tularaemia (1–2%) follows airborne exposure, autoinoculation or after cleaning infected animal carcasses. Ulceration of the cornea produces purulent conjunctivitis, chemosis, periorbital oedema, conjunctival nodules or ulceration, pain and is accompanied by tender preauricular or cervical lymphadenopathy.

Oropharyngeal tularaemia (25%) is acquired by drinking contaminated water or ingesting contaminated food, direct inoculation from the hands to the mouth and sometimes by inhaling contaminated droplets or aerosols. Affected persons may develop stomatitis, but more commonly exudative pharyngitis or tonsillitis ensues with or without painful mucosal ulceration. A retropharyngeal abscess or suppuration of regional lymph nodes may occur.

Typhoidal tularaemia is used to define a non-specific acute flu-like illness, often with diarrhoea and vomiting, headache, chills, rigor, myalgia and arthralgia, prostration and weight loss. There are no clinical signs indicating either site of inoculation or anatomic localisation of infection. Typhoidal tularaemia may follow ingestion or inhalation of *F. tularensis*. Pneumonia, mucocutaneous lesions and regional lymphadenopathy are usually absent.

Tularaemia sepsis is potentially severe and fatal. Any form of tularaemia may be complicated by sepsis. Nonspecific signs such as fever, abdominal pain, diarrhoea, and vomiting may be prominent early in the course of illness. Pulse-temperature dissociation occurs in less than 50% of cases. Then patients typically appear toxic and may progress to septic shock, disseminated intravascular coagulation, haemorrhage, acute respiratory distress syndrome, confusion, organ failure and coma.

Pericarditis can complicate both syndromes [45]. Mild hepatitis is common. Occasionally, erythema nodosum, enteritis, appendicitis, peritonitis and meningitis have been reported.

Without antibiotics, the overall mortality for type A tularaemia is 8% (range 5–15%); 4% for ulceroglandular and 30–50% for typhoidal, septicaemic and pneumonic types. With appropriate treatment, mortality is reduced to 1%. Type B infections are rarely fatal [43].

Diagnosis

Clinical diagnostic suspicion remains crucial. Nevertheless, within an outbreak, the first case of tularaemia is not always readily diagnosed. Case definitions of suspected or confirmed cases due to deliberate release are shown in Table 3. *F. tularensis* may be identified by direct examination of secretions, exudates or biopsy specimens using direct fluorescent antibody or immunohistochemical stains. Specimens of sputum, pharyngeal washings, fasting gastric

Table 6. Recommendations for treatment and post-exposure prophylaxis of tularaemia [15].

		Treatment of suspected or confirmed clinical cases (10–21 days)	Post-exposure prophylaxis (14 days)
Adults Pregnant women	First-line treatment (10 days)	– Gentamicin: 5 mg/kg IV in 1 or 2 doses daily or – Streptomycin: 1 g IM bid	
It is recommended, when possible, to cease breastfeeding.	Second-line treatment; first-line prophylaxis (14 days)	– Ciprofloxacin: 400 mg IV bid followed by 500 mg per os bid or – Ofloxacin: 400 mg IV bid followed by 400 mg per os bid or – Levofloxacin: 500 mg IV once a day, followed by 500 mg per os once a day	– Ciprofloxacin: 500 mg per os bid or – Ofloxacin: 400 mg per os bid or – Levofloxacin: 500 mg per os once a day
	Third-line treatment; second-line prophylaxis (21 days)	– Doxycycline: 100 mg IV bid followed by 100 mg bid per os	– Doxycycline: 100 mg bid per os
Children	First-line treatment (10 days)	– Gentamicin: 2.5 mg/kg IV 3 times daily or – Streptomycin: 15 mg/kg IM bid (max 2 g)	
	Second-line treatment; first-line prophylaxis (14 days)	– Ciprofloxacin: 10–15 mg/kg IV bid followed by 10–15 mg/kg per os bid	– Ciprofloxacin: 10–15 mg/kg per os bid
	Third-line treatment; second-line prophylaxis (21 days)	– Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years: 2.2 mg/kg IV bid followed by 2.2 mg/kg per os bid (max 200 mg/d)	– Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years: 2.2 mg/kg per os bid (max 200 mg/d)

aspirates, pleural fluid, exudates from cutaneous lesions, biopsies of lymph nodes and blood may be culture positive for *F. tularensis*. It is difficult to culture, and handling this bacterium poses a significant risk of infection to laboratory personnel. Nevertheless, a laboratory experienced in handling *F. tularensis* should perform antibiotic sensitivity. Antigen-detection assays, PCR or ELISA may be used to identify *F. tularensis*. The two last methods have not been adequately evaluated for the diagnosis of pneumonic tularaemia. Nevertheless, a fourfold change in titre between acute and convalescent serum specimens, a single titre of at least 1/160 to tube agglutination or 1/128 for microagglutination is diagnostic for *F. tularensis* [43]. Serum antibody titres do not attain diagnostic level until 10–14 days after onset of illness. Serologic testing is useful only retrospectively but confirms the diagnosis. For definitive laboratory confirmation, culture and an increase in specific antibodies in paired sera are required. The raise in titre is commonly seen 10–14 days after the onset of the disease.

Treatment

Many guidelines have been published for treatment and prophylaxis of tularaemia [15, 43, 44] (Table 6). Streptomycin and gentamicin are currently considered the treatment of choice for tularaemia [43]. Treatment with aminoglycosides should be continued for 10 days. Quinolone may be an effective alternative drug [15, 44]. Despite the absence of large data in patients with tularaemia, ciprofloxacin principally or ofloxacin should be prescribed for 10–14 days [15, 43]. When administered for a short duration, tetracyclines and chloramphenicol are associated with relapses and should be given for at least 14–21 days. In severe cases, combination of two antibiotics, such as aminoglycosides and fluoroquinolones, should be considered. Macrolide antibiotics are not recommended for treating tularaemia [43]. The beta-lactams are usually considered ineffective. No isolation measures for patients with pneumonia are necessary. Streptomycin, gentamicin, doxycycline or ciprofloxacin are recommended for post-exposure prophylaxis and must be taken for at least 14 days. An unlicensed live-attenuated vaccine is available, which does appear to offer protection against ulceroglandular and pneumonic tularaemia. In the absence of larger data, vaccination is not recommended for post-exposure prophylaxis.

Botulism

Botulism is a rare but serious paralytic illness caused by botulinum toxin, which is produced by the *Clostridium botulinum* under anaerobic conditions [52, 53]. This bacterium is a common spore-forming soil contaminant. Botulinum toxin is the most poisonous substance known. Eating or breathing this toxin causes illness in humans [22, 53–55].

Botulism and bioterrorism

Aerosols of botulinum toxin could be used as a biological weapon [22, 53–55]. Deliberate release may also involve contamination of food or water supplies with toxin or *C. botulinum* bacteria. Botulinum toxin is extremely lethal and easy to produce. The Aum Shinrikyo cult in Japan attempted unsuccessfully to release an airborne form of botulinum toxin in Tokyo on three separate occasions in the early 1990s [54]. It is likely that several countries have developed and stockpiled botulinum toxin weapons [54]. It has been estimated that a point-source aerosol release of botulinum toxin could incapacitate or kill 10% of the population. *C. botulinum* is a large, Gram-positive, strictly anaerobic bacillus that forms a subterminal spore. These spores can be found in soil samples and marine sediments throughout the world. Four groups of *C. botulinum* are described. Group I organisms are proteolytic in culture and produce toxin types A, B or F; group II organisms are non-proteolytic and produce toxin types B, E or F; group III produces toxin types C or D, and group IV toxins type G. These toxins are proteins of approximately 150 kD molecular weight and induce similar effects whether inhaled or ingested.

When ingested, toxins are absorbed in the duodenum and jejunum, and enter into the bloodstream, by which they reach peripheral cholinergic synapses. Botulinum toxin does not penetrate intact skin [54]. Toxins act by binding to the presynaptic nerve terminal at the neuromuscular junction and at cholinergic autonomic sites. This binding prevents release of acetylcholine and interrupts neurotransmission [56]. Human botulism is almost always caused by toxin types A, B, E and, in rare cases, F. Types C and D are associated with disease in birds and mammals. Type G is not associated with any disease in humans or animals. It has been estimated that, weight for weight, these toxins are the most toxic compounds known, with an estimated toxic dose, for toxin type A, of only 0.001 μg/kg of body weight when administered intravenously, subcutaneously or intraperitoneally [57]. By inhalation, the dose that would kill 50% of exposed persons (LD50) is 0.003 μg/kg of body weight. This toxin is 100,000 times more toxic than sarin gas [22].

Clinical features

The incubation period is short, depending on the type and dose of toxin: 12–72 h (range: 2 h–10 days) [54, 55]. Following aerosol exposure onset of symptoms may be more rapid, possibly occurring less than 1 h after exposure. Person-to-person transmission has never been described.

Whatever the route of contamination, illness is an acute, afebrile, symmetric, descending flaccid paralysis that begins from the head [22]. Multiple cranial nerve palsies produce diplopia, ptosis, blurred vision, enlarged or sluggishly reactive pupils, photophobia, facial weakness, dysphonia, dysphagia and dysarthria. This is followed by a symmetrical, descending skeletal muscle paralysis with hypotonia, weakness in the neck and arms, after which respiratory muscles and then distal muscles are affected [22]. There is no loss of sensation, and patients are well oriented. There may also be other autonomic signs, including postural hypotension, dry mouth, and cardiovascular, gastrointestinal and urinary autonomic dysfunction. Gag reflex may not be lost. Deep tendon reflexes may be present or absent. Pupils are dilated and fixed. Respiratory paralysis may require ventilatory support. If onset is very rapid, there may be no other symptoms before sudden respiratory paralysis occurs. Nausea, vomiting and diarrhoea followed by constipation are seen in foodborne botulism. Laboratory test results, including analysis of the CSF, are unremarkable [54].

Food or waterborne botulism is caused by ingestion of food containing preformed toxin. A normal healthy adult can consume small numbers of spores with no ill effect. Foodborne botulism has often been caused by home-canned foods with low acid content, such as asparagus, green beans, beets or corn. More unusual sources are chopped garlic in oil, chilli peppers, tomatoes, improperly handled baked potatoes wrapped in aluminium foil, and home-canned or fermented fish.

Wound botulism follows infection of wounds caused by penetrating injuries. *C. botulinum* multiplies and produces its toxin in the contaminated wound. Injecting or sniffing drugs that are contaminated by spores have also been reported as causing botulism. Fever can be present, and reflects wound infection rather than botulism. *C. botulinum* infection may produce abscess formation. Wound botulism can be prevented by promptly seeking medical care for infected wounds.

Infant botulism generally occurs in infants who are under 6 months of age and probably results from the endogenous production of toxin by germinating spores of *C. botulinum* in the intestine after ingestion of contaminated food. Honey, which can contain *C. botulinum* spores, has been a source for infection for children under 12 months old.

Intestinal botulism is caused by colonisation of the gastrointestinal tract by *C. botulinum* with *in vivo* production of toxin.

Inhalation botulism does not occur naturally, but may result from an accidental or a deliberate release of toxin in the form of an aerosol. Few data concerning this route of transmission are reported in humans. An incident involving the accidental exposure of three humans to botulinum toxin occurred in a laboratory in Germany [58]. Clinical features are the same as those observed with the other forms.

Diagnosis

Clinical diagnosis can be problematic without strong clinical suspicion. The first and early cases are commonly misdiagnosed. Case definitions of suspected or confirmed cases due to deliberate release are reported in Table 3.

Laboratory diagnosis relies on isolation and identification of the neurotoxins from sera or other samples (stool, gastric specimen, vomitus and suspect food) [54]. It has been suggested that aerosolised toxin is usually not identifiable in serum or stool [22]. The aerosolised toxin may be detectable by ELISA on nasal mucous membranes or broncho-alveolar lavage for 24 h after inhalation. The standard laboratory diagnostic test remains the mouse bioassay (injection of serum collected before administration of antitoxin). Pus from wounds, biopsy tissues (surgical debridement), and faecal and gastric specimens can also be cultured for *C. botulinum* (anaerobic cultures).

Treatment

Without supportive treatment, death often occurs from respiratory failure. Patients with respiratory failure must be admitted to an intensive care unit and require long-term mechanical ventilation (from 60 days to 7 months) [59]. Trivalent (A, B, E) equine antitoxins must be given to patients as soon as possible after clinical diagnosis by slow intravenous infusion [15, 54]. Heptavalent human (A–G) antitoxins are available in certain countries [54]. Anaerobic antibacterial agents can be used to treat wound infection or abscesses, but these have no effect on botulinum toxin. Antibiotic treatment is not indicated for colonisation, because lysis of intraluminal *C. botulinum* may increase the amount of toxin available for absorption. Patients with botulism who survive may have asthenia and dyspnoea for years, and long-term therapy may be needed to aid recovery. Muscle function returns after 3–6 months as the neuromuscular junction regenerates. In the United States, investigational pentavalent (A–E) botulinum toxoid vaccine is used for laboratory workers at high risk of exposure and by military personnel. Several thousand laboratory workers have been immunised over several decades in many countries. Immunity is induced slowly by this vaccine, and frequent boosters are required [60]. Experimental new vaccine candidates are currently being developed in the United States and in Europe [61, 62].

Other biological agents

Many others bacteria (e.g. *Brucella* spp., *Coxiella burnetii, Burkholderia mallei* and *Burkholderia pseudomallei)* have been reported to be potential agents for bioterrorism [22, 63–65]. Many of such agents are zoonotic and could have a considerable impact on agriculture as well as on human health. Interest in such agents as biological weapons stems from the fact that airborne transmission of these agents is possible. For example, *Brucella* spp. is highly contagious, as it can enter through mucous membranes such as the conjunctiva, oropharynx, respiratory tract and skin abrasions (see also the paper by Pappas in this issue). It has been estimated that only 10–100 organisms are needed to constitute an infectious aerosol dose for humans [66]. For *C. burnetii*, an infectious dose of very few organisms is required to cause infection. It has been estimated that as few as 1–10 organisms could cause disease. *B. mallei* and *B. pseudomallei* infection may be acquired through direct skin contact (abraded or lacerated skin) with contaminated soil or water or through

Table 7. Encephalitis viruses that could be used in a bioterrorist attack.

Family	Genus	Species
Togaviridae	Alphavirus	eastern equine western equine Venezuelan equine
Flaviviridae	Flavivirus	St. Louis Australian encephalitis (Murray valley and Kunjin) West Nile Japanese dengue tickborne complex encephalitis = (Central European and Russian spring-summer) Powassan Rocio Louping ill
Bunyaviridae	Bunyavirus	La Crosse Rift Valley Toscana
Arenaviridae	Arenavirus	lymphocytic choriomeningitis Machupo Junin
Toroviridae		Hendra virus
Others		Herpesvirus simiae (B virus)

Table 8. Haemorrhagic fever viruses that could be involved in biological warfare.

Family	Viruses	Diseases	Vectors
Filoviridae	Ebola	Ebola haemorrhagic fever	unknown
	Marburg	Marburg haemorrhagic fever	unknown
Arenaviridae	Lassa	Lassa fever	rodent
	Machupo	Bolivian haemorrhagic fever	rodent
	Junin	Argentine haemorrhagic fever	rodent
	Guanarito	Venezuelan haemorrhagic fever	rodent
	Sabia	Brazilian haemorrhagic fever	rodent
Bunyaviridae	Rift Valley fever	Rift Valley fever	mosquito
	Crimean-Congo haemorrhagic fever	Crimean-Congo haemorrhagic fever	tick
Flaviviridae	yellow fever	yellow fever	mosquito
	Omsk haemorrhagic fever	Omsk haemorrhagic fever	tick
	Kyanasur forest disease	Kyanasur forest disease	tick

mucosal surfaces of the eyes and nose. Ingestion of contaminated water or dust is another route of transmission. These agents have been studied for weaponisation in several countries in the past. They were used during both the First and Second World Wars [67]. However, all these agents might be used more as incapacitating agents, as the disease they cause is associated with a high morbidity and protracted illness.

Many other viruses are also good candidates for bioterrorist agents: encephalitis-associated viruses and haemorrhagic fever viruses (Tables 7 and 8) could be used by aerosolisation during a bioterrorist attack. Most of these viruses have been weaponised [68, 69].

Conclusion

At the beginning of the 21st century, similar to what has been observed throughout history, among the risks of non-conventional weapons known as nuclear, radiological, biological and chemical weapons, bioterrorism remains a potentially important risk, even if terrorist acts using chemical or radiological weapons or bombs appear to be more plausible.

Technology moves with the times. It thus appears prudent to consider the use of modified infectious agents by not very scrupulous people. Infectious agents are more virulent, have a shorter incubation, are associated with more serious clinical signs, are more easily transmitted human to human, are more difficult to diagnose and would be more resistant to usual treatment. Due to this significant risk, our governments must remain vigilant and, as well as possible, prepare to face such a situation. For clinicians, general practitioners and specialists, preparation consists of better knowledge of the little taught, not-well-known clinical signs generated by infectious agents that are rarely observed in most of our countries. Let us be alert, let us be ready, without being alarmist.

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