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Category B Potential Bioterrorism Agents: Bacteria, Viruses, Toxins, and Foodborne and Waterborne Pathogens

Georgios Pappas, MD[a,*],
Paraskevi Panagopoulou, MD, MPH[a],
Leonidas Christou, MD[b], Nikolaos Akritidis, MD[c]

[a]*Institute for Continuing Medical Education of Ioannina, Velissariou 15-19, 45221, Ioannina, Greece*
[b]*Internal Medicine Department, University Hospital, 45110, Ioannina, Greece*
[c]*Internal Medicine Department, General Hospital "G. Hatzikosta," Makrygianni Avenue, 45500, Ioannina, Greece*

Category B of potential weapons of bioterrorism is by far the broadest, because it includes a wide variety of bacteria, viruses, protozoa, and toxins. Category B agents have been listed both by the Centers for Disease Control and Prevention [1] and the National Institute of Allergy and Infectious Diseases (NIAID) Biodefense Research [2]. The NIAID list is practically an expanded, detailed Centers for Disease Control and Prevention list, and it is used as the basis of this article (Box 1). Inclusion of a certain pathogen in this list implicates either its attractive nature as a potential bioweapon that is accompanied by a low ability to cause massively destructive (and socially deconstructive) consequences, or an ability to induce major public health consequences that is accompanied by certain difficulties in the development and execution of a potential attack (and low probability). Basic aspects of the pathogens included in category B, along with important parameters related to their potential as biological weapons, are discussed.

* Corresponding author.
E-mail address: gpele@otenet.gr (G. Pappas).

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Box 1. Category B potential bioterrorism agents

Bacteria
Brucella species (brucellosis)
Burkholderia pseudomallei (melioidosis) and *B mallei* (glanders)
Chlamydia psittaci (psittacosis)[a]
Coxiella burnetii (Q fever)
Rickettsia prowazekii (typhus fever)

Viruses
Alphaviruses
  Venezuelan equine encephalitis
  Eastern equine encephalitis
  Western equine encephalitis
Bunyaviruses
  LaCrosse
  California encephalitis
Flaviviruses
  West Nile virus
  Japanese encephalitis virus
  Kyasanur Forest virus

Foodborne and waterborne pathogens
Bacteria
  Escherichia coli
  Pathogenic vibrios
  Shigella species
  Salmonella
  Listeria monocytogenes
  Campylobacter jejuni
  Yersinia enterocolitica
Viruses
  Noroviruses
  Hepatitis A virus
Protozoa
  Cryptosporidium parvum
  Cyclospora cayatanensis
  Giardia lamblia
  Entamoeba histolytica
  Toxoplasma
  Microsporidia

Toxins
Epsilon toxin of *Clostridium perfringens*

Ricin toxin (from *Ricinus communis*)
Staphylococcus enterotoxin B

[a] Psittacosis is not included in this list, but is included in the Centers for Disease Control and Prevention list [1].

From the National Institute of Allergy and Infectious Diseases Biodefense Research. NIAID biodefense agenda for CDC category B and C priority pathogens. Available at: http://www3.niaid.nih.gov/Biodefense/Research/categorybandc.pdf. Accessed January 10, 2006.

Brucella

The pathogen

Brucella is a gram-negative zoonotic bacterium, certain species of which are pathogenic to humans [3]. Brucellosis is possibly the commonest zoonosis worldwide, with an increasing number of new disease loci emerging in recent years in various underdeveloped countries [4], and reacquaintance of the developed world with the disease as a result of travel-related infections and through international food trading [5]. Most disease is called by *Brucella melitensis*, whereas other species, such as *Brucella suis*, *Brucella abortus*, and *Brucella canis*, are considered less virulent. The pathogenesis of the disease is unique, as exhibited by the complex immune response evoked by the pathogen and the fact that the bacteria practically hide inside the macrophages, in unique compartments of acidic environment, replicating without affecting cellular viability [6]. The disease is transmitted by direct contact with infected animals (sheep and goats for *B melitensis*, bovine for *B abortus*); consumption of contaminated dairy products or undercooked meat from infected animals; and by inhalation of infective aerosol particles. Person-to-person transmission does not generally occur. Brucellosis in humans causes a febrile disease with nonspecific manifestations. A wide variety of complications have been described in the literature [7], of which spondylitis, central nervous system involvement of various presentations, and endocarditis are the most troublesome. Chronic brucellosis has not been adequately defined. Diagnosis requires isolation of the organism from blood or bone marrow specimens [8], but the reported sensitivity varies significantly in the literature, ranging from 10% to more than 70%. Serodiagnosis (serum agglutination test and similar tests) is widely used, whereas ELISA exhibits better sensitivity and specificity [9]. Polymerase chain reaction (PCR) assays have been developed [10], and real-time PCR assays allow for rapid diagnosis [11]. The traditional treatment options include a combination of doxycycline and rifampin for 6 weeks, or doxycycline for 6 weeks and streptomycin for 2 to 3 weeks [12]. Alternative regimens use other aminoglycosides [13], co-trimoxazole, and quinolones [12]. Relapse rates with accepted regimens are at the level of 10%, and relapse usually presents in the first year posttreatment, related to well-recognized parameters.

Bioterrorism potential

Brucella was one of the first agents to be used in the development of biological weapons, in particular *B suis*, which had been weaponized in the shape of particle-filled bombs, allegedly by the United States, and possibly by other countries more than 50 years ago [14]. The attractiveness of the pathogen lies in its exquisite ability to be transmitted as an inhalational pathogen; laboratory-acquired brucellosis [15] is a common event in nonspecialized laboratories dealing with the agent. The rare respiratory complications of the disease have not been related to inhalational exposure [16]. Reports on attack rates vary, and the mortality of the disease is minimal. The relatively protracted incubation period (varying from 10 days to 3 months) further compromises its potential as a biological weapon to disrupt social structures. Existence of adequate antibiotic options further compromises this pathogen's potential, although development of resistant strains could not be excluded. Moreover, in a situation of deliberate exposure, the use of prophylactic antibiotic administration has not been clarified [17], and universally acceptable human vaccines are not at present available [18]. A final important implication of brucellosis if used as a biological weapon is the effect on animal population of the targeted area, which might be significant for the overall economy of the region, if largely based on animal husbandry.

Burkholderia mallei and *pseudomallei*

The pathogens

Burkholderia mallei and *Burkholderia pseudomallei* are closely related species of the genus *Burkholderia*, until recently [19] categorized as *Pseudomonas mallei* and *Pseudomonas pseudomallei*, respectively. They are both small, gram-negative, strictly aerobic pathogens that cause zoonoses of varying severity in humans, known as "glanders" and "melioidosis," respectively. Their joint discussion is warranted, although criticized in past efforts [20], because of the paucity of current clinical knowledge about the former of the pathogens, which is in part compensated by application of experience with the latter.

Burkholderia mallei is an ancient pathogen, described by Aristotle [21] as an equine disease, and termed in Greek as "malis/melis," a term further used in nomenclature, also naming the clinical syndrome of melioidosis (melis-like). Glanders is even present in the works of Shakespeare (in The Taming of the Shrew [21]). *B pseudomallei* and *B mallei* are morphologically similar, although the latter exhibits motility because of polar flagella [22]. Glanders is an extremely rare disease nowadays, with extremely few human cases reported in the literature in the second half of the twentieth century, most in laboratory workers working with the pathogen [23]. Melioidosis is an important infectious disease of selected areas, especially Northeast Thailand (but also neighboring countries, including China) and North Australia; recently, however, the disease has been reported in Brazil.

The disease usually presents during the rainy seasons, in people who are in direct contact with soil and surface water, and the usual mode of transmission is percutaneous contact through small skin abrasions. Inhalation of infected aerosol particles is another means of transmission. Person-to-person transmission does not generally occur. *B pseudomallei* exhibit ability to survive intracellularly in phagocytes, forming small colony units.

Most patients exhibit underlying illnesses, diabetes mellitus being the commonest (but also renal and hepatic disease, thalassemia, alcohol consumption, and chronic lung disease including cystic fibrosis). Incubation period ranges from 1 to 14 days, 1 to 5 days in cases of septicemia, and possibly even hours in cases of inhalatory exposure [24]. The disease usually presents dramatically as a rapidly fatal septicemia, mortality rates ranging from 19% to 50%, even with adequate antibiotic treatment. Respiratory failure caused by acute necrotizing pneumonia or acute respiratory distress syndrome may develop. Profound weight loss accompanies the clinical picture. Pulmonary disease often exhibits a chronic form resulting in cavitation, causing misdiagnoses of tuberculosis. Neurologic manifestations are rare but important in terms of morbidity. *B pseudomallei* have a tendency for abscess formation, with a particular tropism for the spleen, but also prostate, liver, and soft tissues. In children in Thailand the disease often presents as unilateral suppurative parotitis. A localized form of the infection with ulceration, subcutaneous nodules, and regional lymphadenopathy is more benign (a similar form of *B mallei* infection is called "farcy").

Despite adequate treatment, the disease can relapse, even after a period of decades. Isolation of the organism in cases of septicemia is feasible, and even throat swabs have been useful in certain clinical settings. The use of selective media, such as Ashdown's medium, enhances diagnostic sensitivity, but misidentifications may occur with automated systems [25]. Serologic diagnosis by means of ELISA is useful in nonendemic areas (in endemic areas, nonprotective seroconversion ensues early in life), but no commercial kits are available. Treatment is prolonged and biphasic: intravenous therapy with ceftazidime or carbapenems has proved superior to traditional regimens [26] in recent years, and should be continued until definite clinical improvement ensues. Amoxicillin-clavulanate has been used as a less efficient, but cheaper, alternative. A second oral antibiotic phase for at least 20 weeks uses doxycycline and co-trimoxazole, with the addition of chloramphenicol for 8 weeks. Monotherapy with co-trimoxazole in the second phase in a recent study showed excellent results. For glanders, the experience on treatment is largely based on in vitro data [27] demonstrating a similar antibiotic susceptibility profile to that of *B pseudomallei*, apart from the additional sensitivity to gentamicin for *B mallei*.

Bioterrorism potential

B mallei have been used as biological weapons in World War I, albeit targeting the enemy's cavalry. It has also been reported that during World

War II Japan carried on experiments in humans at the Pinfang Institute in China [28].

The high infectivity of both diseases in their aerosolized form (attack rates in laboratory exposure being up to 46%), and the possible severe pulmonary or septicemic form of pathology exhibited after such an exposure [29], makes them possible biological weapons. Furthermore, both agents are practically unknown, even to specialists, in the Western World, and a prompt diagnosis and response might not be feasible, especially when taking into account the need for specialized, early, and costly, antibiotic administration. Prophylaxis is another issue because of the absence of adequate data, and co-trimoxazole might be an attractive option, although further issues would ensue over who should receive such a prophylactic regimen. There are no vaccines available (natural immunity does not offer protection). As for brucellosis, animal disease in the setting of a biological weapon incident is also a factor to consider: the notorious outbreak in animals of Paris zoos in 1973, ensuing after a Panda suffering from melioidosis was imported as a gift from Mao ZeDong to the French president, is a characteristic incident of unintentional international bioterrorism at the highest level [30].

Chlamydia psittaci

The pathogen

Chlamydia psittaci (alternatively called *Chlamydophila psittaci*) is the etiologic agent of psittacosis (alternatively called ornithosis), a rare cause of atypical pneumonia that mainly presents as an occupational disease in persons exposed to domestic (and rarely free-ranging) birds. The disease is transmitted through the inhalational route, after exposure to contaminated bird droppings or dust generated in environments contaminated by infected bird droppings. *C psittaci* is an environmentally stable pathogen. Incubation period is 1 to 2 weeks, and the disease produced ranges in severity from asymptomatic to severe pneumonia. Case fatality rate is 15% to 20% in untreated patients, but $<1\%$ posttreatment. Prominent headache is reported as characteristic in the clinical presentation. During convalescence, cases of thrombophlebitis and subsequent pulmonary embolism have been reported. Diagnosis is based on serology (complement-fixing antibody, cross-reacts with other *Chlamydia* species). PCR assays have been developed, whereas culture is laborious and hazardous. Treatment with doxycycline (alternatively macrolides) results in prompt response [31].

Bioterrorism potential

Psittacosis is included in the Centers for Disease Control and Prevention list [1] but not in the NIAID list [2]. Undoubtedly, as an inhalational disease, it is far less potent than other pathogens of this group in creating major

public health consequences. Moreover, psittacosis is a largely forgotten disease, which implies that knowledge of its pathogenetic properties is limited (a fact that may prove problematic both for weaponization and improvement of response policies). The one intriguing characteristic of the disease in terms of bioterrorism is the ability to be transferred wide distances through infected free-ranging birds. Limited data exist, however, about the ecology of the disease outside captive settings.

Coxiella burnetii

The pathogen

Coxiella burnetii is a gram-negative coccobacillus, usually described in parallel with Rickettsiae, although their phylogenetic differences have been largely outlined in recent years [32]. The disease is a global zoonosis that often runs undetected because of its low mortality. It is an intracellular pathogen, which after infection can survive for prolonged periods in the autophagosomes, leading to chronic infections [33]. It is usually transmitted by inhalation, and the inoculum needed to induce human infection is extremely low, often mentioned as even a sole organism. The disease produced, Q fever, is largely an occupational disease in people in close contact with animal hosts of the pathogen, such as sheep. *C burnetii* exhibits a remarkable environmental stability, and generation of aerosols (dustborne disease) from previously infected areas does not require current presence or contact with infected animal hosts. Person-to-person transmission does not occur.

The clinical syndrome induced 10 to 21 days after exposure is usually mild, in the form of a flulike illness or an atypical pneumonia, with varying radiologic appearances. Mild elevations of serum aminotransferases, indicating hepatitis, are also common. Serious complications are rare, and mortality is minimal, but the disease exhibits a tendency for chronicity, which can manifest in various forms, the most important of which is chronic endocarditis [34]. A chronic fatigue–like syndrome has also been recognized. The diagnosis is usually based on serologic tests, such as ELISA and indirect immunofluorescence assays. PCR detection has gradually become the diagnostic gold standard [35], although its use is still limited to reference laboratories. Q fever is often asymptomatic and self-limited. In detected clinical cases, a short course of doxycycline (7–10 days) is the choice regimen, although alternatives, such as quinolones, erythromycin, and in special populations co-trimoxazole and rifampin, exist [36]. Chronic endocarditis requires protracted administration of doxycycline in combination with hydroxychloroquine, although valve replacement may not be avoided [37].

Bioterrorism potential

The historical military significance of Q fever is characteristically outlined in the large number of outbreaks in military personnel stationed in

Mediterranean countries during World War II [38], although none of these outbreaks was attributed to deliberate release. Q fever exhibits most characteristics of a potential biological weapon, excluding mortality [39]. It is extremely stable in the environment, easily found and aerosolized in large quantities, and has further significance for the animal population and the economy and subsequent living in a targeted area. Various countries have experimented with weaponizing the pathogen in the past, including the United States [40]. In a recent attack scenario [41] targeting a 100,000-person city, massive disruption of societal and health structure was predicted despite an extremely low mortality rate, even without further interventions. Similar results were yielded from historical attack scenarios [42]. Questions regarding response were further raised: doxycycline is an acceptable prophylactic regimen, but whether it should be administered to everyone exposed or only to special populations at risk has not been clarified. Furthermore, the issue of vaccination for *C burnetii* remains underdiscussed: A formalin-activated whole-cell vaccine is extensively used in Australia, and candidate vaccines are under investigation.

Rickettsia prowazekii

The pathogen

Rickettsia prowazekii is the only *Rickettsia* listed as a potential biological weapon, although appeals for the inclusion of other rickettsial species, most notably *R rickettsi*, the causative agent of Rocky Mountain spotted fever, have emerged [43]. Epidemic typhus, a major determinant of significant historical turning points [44], is transmitted to humans by the human body louse, and is related to conditions of poor hygiene and social disruption, as outlined by the characteristics of recent epidemics in Burundi [45] and Russia. Head lice [46] and flying squirrels [47] have been also recently implicated as *R prowazekii* reservoirs. Human-to-human transmission does not occur. Self-inoculation by scratching the bite-site is the usual mode of transmission, although exposure to aerosol particles containing infected lice feces also leads to disease. The typical disease induced by *R prowazekii* 8 to 12 days after exposure is characterized by fever; severe headache; and a subsequent generalized maculopapular, sometimes finally purpuric, rash spreading centrifugally. Mortality is 20% when untreated, significantly higher in older adults and related to gangrene, central nervous system complications, diffuse intravascular coagulation, or severe hypovolemia. Adequate treatment leads to a decline in mortality rates to about 2% to 4%. Establishment of chronic infection can lead to recrudescence (Brill-Zinsser disease) in 15% of the patients, which is usually mild and may appear decades after the typhus episode, often related to external or internal stress. Diagnosis is based on serology (immunofluorescence assays and ELISA), although cross-reactions with other rickettsiae from the typhus group are extensive and

their avoidance subject to newer techniques [48]. Moreover, the need for convalescent samples allows for a retrospective-only diagnosis. When epidemiologic situations related to the disease have been already recognized, the diagnosis is anticipated and can be achieved clinically [45]. Specific PCR techniques for this pathogen have been developed [49], including real-time PCR [50]. Treatment, apart from supportive measures in critically ill patients, relies on the administration of doxycycline, a single dose of which can be life saving [51]. Chloramphenicol is an acceptable alternative regimen.

Bioterrorism potential

An older attack scenario developed by the World Health Organization [42] outlined the grave sequences of an attack with an aerosolized form of *R prowazekii*, with an estimated number of 19,000 deaths. The process of weaponization with *R prowazekii* is not as easy, however, as with *C burnetii*, for example, and one can suppose that such a task could be abandoned in favor of weaponizing a category A pathogen. An alternative approach would use extended spread of infected lice, but in this case, the emerging epidemiologic situation would lead to anticipation of epidemic typhus and rapid response [41]. There are currently no vaccines available for epidemic typhus. The whole-cell vaccine that was successful during World War II in minimizing epidemic typhus among allied troops is not being produced anymore, but projects for new vaccine products are currently under way [52].

Epsilon toxin of *Clostridium perfringens*

The toxin

Epsilon toxin (ETX) is a major toxin produced by the type B and D strains of *Clostridium perfringens*, an anaerobic bacterium the other types of which cause various human diseases as gas gangrene and necrotizing enteritis. There is a paucity of knowledge over the effect of ETX on humans, because the types of *C perfringens* carrying it are not human pathogens [53] and data are largely based on experience from animal disease and mice models. It is known that ETX exhibits an exquisite neurotropism, indicated by the severe neurologic sequelae of certain *C perfringens* type D infections in sheep [54], and the rapid accumulation of ETX in mice brain, resulting in death, after intravenous administration of minimal quantities [55]. This neurotropism may be related to recognition of specific receptors on brain cells. ETX seems to act through alteration of the permeability of the cellular membrane, with formation of a heptameric pore [56]. Increasing knowledge about protective humoral immunity in infected animals may allow further understanding of the pathophysiology of ETX and further extrapolation on human risk.

Bioterrorism potential

ETX is an aberration in category B pathogen list, because practically nothing is known about its possible consequences in humans. To deliver a large-scale biological attack, it has to be used in the form of an aerosol, or through poisoning of water or food supplies. Yet, because nothing is known about ETX's interaction with human gastrointestinal and respiratory mucosa, no safe projections could be made over its lethality and its consequences [56]. For example, it is known that goats infected by *C perfringens* type D develop enterocolitis and a wasting syndrome that is compatible with the mechanism of action of ETX, yet apart from expecting that the cleavage of the protoxin secreted by *C* perfringens by trypsin and chymotrypsin definitely ensues in human gastrointestinal tract as well, no other projections can be made. Attempts to develop a recombinant vaccine against ETX are currently under way [57], although a formalin-inactivated vaccine exists for susceptible animals. Given these facts, one can support that ETX is more suitably placed in category C pathogens, because its potential for biological weapon use is still largely unexplored and unproved.

Ricin

The toxin

Ricin is a toxin derived from the bean of castor plant *Ricinus communis*, and has drawn medical interest since the nineteenth century, Ehrlich being among the scientists experimenting with it [53], but still attractive in the field of oncologic pharmacology. Its importance lies in its ability to act on the ribosomes and halt the procedure of protein synthesis, leading to cellular death. Its crystal structure has been outlined: it is a heterodimer, consisting of two glycoprotein chains, chain B facilitating anchoring to the targeted cell, allowing for chain A to intrude and attack the 28S ribosomal subunit. There are limited data over its effects on human subjects: workers exposed to castor dusts exhibited allergic symptoms [58], but the inhalational dose was presumably low. Mice and monkeys exposed to infected aerosols, however, developed rapidly fatal necrotizing airway pathology and alveolar flooding [59,60]. The inverse relationship between particle diameter and clinical severity has also been experimentally proved. When administered orally, based on the experience drawn from isolated cases of involuntary ingestion of castor beans [61] or from experimental data [62], gastrointestinal symptoms, including bloody diarrhea, predominated and significant histologic pathology was exhibited. There are currently no available methods of diagnosing ricin poisoning: detection of ricin from blood and other fluids is difficult because of its propensity for rapid and subtotal protein binding. Various immunologic methods have been described, but none has been field-tested. Treatment is largely supportive, and although numerous agents

have been screened in vitro, none is currently advocated in the treatment of ricin poisoning. Antitoxins are currently also being evaluated [63]. In cases of gastrointestinal exposure, activated charcoal is recommended, although its interaction with ricin has not been ascertained.

Bioterrorism potential

Ricin exhibits various properties that suit a potential biological weapon: it is abundant worldwide, it is relatively environmentally stable in its aerosolized form, and it is susceptible to mass-production without the need of specialized technology. The toxin has been tested as a biological weapon by various countries in the past and it has been implicated in the murder of a Bulgarian dissident, Georgi Markov, in Great Britain in 1978 [64]. Furthermore, its recent isolation from White House mail facilities, a US senator's office [61], and after a London police raid [65] renewed interest in its potential. It could be distributed either through aerosolization, or through contamination of food or water sources, the former means of distribution being potentially more threatening to public health. In such a situation, preparedness is an issue, because a huge burden of patients with severe respiratory pathology should be handled by health authorities, the suitability of the various existing vaccines (including the ones in production) is a significant issue, and the absence of a definite diagnostic method and the difficulty in differential diagnosis from other respiratory pathogens would further slow the response. Its use through food or water poisoning seems more difficult, taking into account the rather large (compared with other toxins) lethal dose extrapolated for humans, in the range of 5 to 10 mg/kg.

A number of vaccines have been tested, and others are under evaluation: a formalin-treated toxoid was successful in protecting mice in a subsequent aerosol challenge, yet the golden equilibrium between attenuation and immunogenicity is hard to achieve [66], and postexposure prophylaxis has not been ascertained. A deglycosylated ricin chain A vaccine enhanced the toxicity, presumably through delayed clearance [53]. A number of recombinant vaccines have been developed or are under development, excellently summarized by Mantis [53], including mucosal vaccines that take advantage of the protective role of secretory IgA against the toxin, and the first recombinant vaccine to enter phase I trials was recently reported [67]. On the basis of all these factors, one could advocate the inclusion of ricin in category A pathogens in the future.

Staphylococcal enterotoxin B

The toxin

Staphylococcal enterotoxin B is the most widely studied of the enterotoxins produced by *Staphylococcus aureus*, and belongs to the family of superantigens, acting after transcytosis through the epithelium by binding

to T lymphocytes and major histocompatibility complex class II molecules and triggering a cytokine storm, predominantly through the release of interferon-γ, tumor necrosis factor-α, and interleukin-1, leading to toxic shock syndrome [68]. Staphylococcal enterotoxin B is a prevalent cause of acute diarrheal illness when ingested, causing a self-limited syndrome of undetermined pathogenesis that starts hours after exposure and gradually wanes during the next 72 hours. Its inclusion, however, in category B pathogen list is warranted because of its possible effects when administered in aerosol form, based on limited human data (involving accidental military exposure) and various experimental animal models. Sixteen cases of inhalational exposure have been documented, resulting rapidly (<24 hours) in evolution of a clinical syndrome characterized by fever, malaise, cough, dyspnea, nausea, and vomiting. Ocular exposure in humans can result in purulent conjunctivitis [69]. In animal models exposed to aerosolized form of staphylococcal enterotoxin B initial gastrointestinal symptoms were followed by death caused by pulmonary edema by Day 3 [70]. The estimated minimum dose for evoking an emetic response in human volunteers is 5 μg [71], whereas in aerosolized exposure, the estimated 50% lethal dose (LD_{50}) is 0.02 μg/kg [72]. Immune protection through anti–staphylococcal enterotoxin B antibodies, even with passive administration, has been outlined [73]. Diagnosis is based on sophisticated toxin assays performed at reference laboratories. Treatment is largely supportive, although experimental therapeutic procedures are currently under investigation, aiming at halting the cytokine domino at various points of its activation [68].

Bioterrorism potential

Certain aspects of a potential deliberate release of staphylococcal enterotoxin B in inhalational form should be addressed, one of the most important being the difficulties posed in differential diagnosis, especially in the context of the absence of widely available diagnostic procedures. A wide variety of both formalin-inactivated and recombinant vaccines have been tested, but none has been approved for human use, although new candidates emerge [53].

Alphaviruses

The viruses

Venezuelan equine encephalitis (VEE), Eastern equine encephalitis (EEE), and Western equine encephalitis (WEE) belong to this category. All are mosquito-borne RNA viruses that rarely cause disease in humans but are significant causes of equine morbidity [74]. VEE is the commonest and the most extensively studied pathogen, with a geographic distribution

encompassing Latin America and in certain outbreaks reaching the United States. The virus uses equine species as amplifiers, because equine disease is related to significant viremia (although this is not the case for newer strains implicated in recent epidemics in Mexico, where strains with particular neurotropism might be implicated) [75,76]. Human disease is rare, usually in the form of a self-remitting flulike syndrome. Progression to encephalitis occurs in 1% of the adults and 4% of the pediatric population, with 20% mortality.

EEE is principally located in the United States, east of the Mississippi River, but principally animal disease has also been reported from the Caribbean and Latin America. An average of 4.9 cases is reported annually in the United States, and the attack rates calculated from clusters of cases is 1 per 1000 population, and most detected cases occur in children and older adults. Incubation period is 5 to 7 days. The ratio of self-remitting or nonapparent to severe infections is 40:1 for adults, and 17:1 for the pediatric population [74]. The clinical syndrome is similar to VEE, but mortality is much higher (>50%), with an additional number of survivors exhibiting severe neurologic sequelae.

WEE is distributed in various western territories of the Untied States and Canada, but also in regions of Latin America. Human attack rates in past outbreaks reached up to 1.7 per 1000 population, and mortality is generally estimated at 3% to 4%. The ratio of self-remitting or nonapparent to severe infections is 1150:1 for adults, but only 1:1 for infants. As with EEE, the disease has a seasonal distribution, probably related to the increased outdoor activities during the summer period. The clinical syndrome induced, after an incubation period of 5 to 10 days, is particularly severe in infants, with common neurologic sequelae present in survivors of this age group.

The diagnosis is based on PCR detection of the viruses in cerebrospinal fluid samples (VEE and EEE) [77]; serum and cerebrospinal fluid serology might also be helpful in diagnosis. Treatment is supportive for all agents, although experimental data suggest a role for interferon-γ for VEE [78]. Human-to-human transmission does not occur for any of the agents.

Bioterrorism potential

The attractiveness of VEE as a biological weapon is based on its potential for widespread infection through aerosolization or release of infected mosquitoes, its relatively low infective dose for humans, its ease of production, and its implications for animal populations of the targeted area [79]. Studies on weaponization of VEE have allegedly taken place in the past [80]. A particular disturbing scenario regarding VEE's low attack rates (at least in the form of severe disease) might include genetic manipulation and evolution of highly neurotropic (inducing more severe disease) strains. Prophylaxis through vaccination is not available for civilians: an inactivated vaccine is available as an investigational new drug, and live attenuated vaccines are

in development [81]. The weapon potential of EEE is roughly equal to that of VEE, its higher infective dose being compensated by the relative severity of the clinical syndrome. Pre-exposure prophylaxis through vaccination is not available for the public, but only through the US Army. On the contrary, the pathogenetic characteristics of WEE make it a pediatric biological weapon (and a wide deliberate release would have the same impact as Herod the Great in the targeted area: if WEE was not exclusively located in the Western Hemisphere, the notorious Massacre of the Innocents by Herod the Great could be viewed as an epidemic of WEE). Various experimental recombinant WEE vaccines are under development [82].

Bunyaviruses

The viruses

LaCrosse virus and California encephalitis viruses are the representatives of the Bunyaviridae family in category B, whereas Crimean-Congo hemorrhagic fever is listed in category C. LaCrosse virus remains the most important cause of pediatric encephalitis in the United States [74], with annual cases reported averaging 70, mostly from states from the middle-north, possibly reflecting the natural host (squirrels and chipmunks) residing in areas rich in forests [74]. The ratio of unapparent to apparent infection is huge, and mortality is extremely low (0.3%), whereas long-term neurologic sequelae are also rare (2%), despite LaCrosse virus's epileptogenic properties and initial clinical resemblance to herpetic encephalitis. Hyponatremia is a common finding, related to adverse prognosis [83]. Incubation period is 1 to 6 days. Distinct histologic findings have been described in the rare fatalities (focusing, in contrast to other viral encephalitis lesions, in the cortical gray matter). Diagnosis is based on serology and various PCR assays recently described [84]. Treatment is largely supportive. Other California encephalitis viruses are extremely rare human pathogens.

Bioterrorism potential

The weapon potential of LaCrosse virus is certainly lower than that of alphaviruses, because of the lower morbidity and mortality rates exhibited. One may assume that an episode involving deliberate release of a virus would preferentially use an alphavirus as VEE. There are no vaccines available, and development is still at preliminary stages [85].

Flaviviruses

The viruses

Japanese encephalitis virus, West Nile virus (WNV), and Kyasanur Forest virus are included in this genus and listed in category B. Other members

of the genus, such as St. Louis encephalitis virus, could theoretically be included, yet much of what applies for the included viruses could be extrapolated to it. Japanese encephalitis virus is the cause of the most prevalent pediatric viral encephalitis worldwide, with an estimated 50,000 cases diagnosed annually in countries of East and Southeast Asia, although the virus has recently spread to Australia [86]. Pigs and birds serve as the amplifying hosts of this mosquito-borne virus, which causes a similar syndrome to the alphaviruses, with an unapparent to apparent case ratio of 50 to 400:1 and a case fatality rate above 20% (much of which is accredited to the pediatric population, because in adults this rate is 10%). The disease exhibits a peak during summer and the incubation period is 5 to 14 days. Low antibody levels at diagnosis are correlated to an adverse prognosis. The diagnosis is achieved through serology (ELISA) [87] and PCR [88]. Treatment is largely supportive.

WNV is a pathogen at the epicenter of medical interest since its introduction in the New World since 1999 and its subsequent spread to the West and South [89,90] and the evolution of a disease spectrum that encompasses severe neurologic sequelae in the last decade. Birds serve as the amplifying hosts for this virus, and a wide array of arthropods and animals can carry WNV or exhibit pathology [91]. Important issues were raised regarding its transmission through transfusion and transplantation [92], and public health policies had to be adapted quickly to rapidly emerging facts. Unlike other encephalitis viruses, WNV poses a significant risk for older adults, and possibly patients with various forms of immune compromise, especially diabetics [93] (other predisposing factors have been discussed, including hypertension, but are not universally accepted [91]). WNV spreads through the reticuloendothelial system after its entrance to the body by a mosquito bite inoculation, and the ensuing viremia facilitates entrance to the central nervous system (WNV is particularly neurotropic). Incubation period is 5 to 14 days. The clinical syndrome produced may have some characteristic aspects, such as hyponatremia, poliolike acute flaccid paralysis related to poor outcome, and increased dyskinesias in encephalitis cases [94]. Overall mortality reaches 10%, but neurologic sequelae may be observed in more than half of the survivors. Diagnosis can be achieved by real-time PCR [95] or ELISA, although the latter's increased percentage of cross-reactions with other flaviviruses has augmented the development of immunofluorescence assays. Regarding ELISA, persistence of IgM has been reported that may further hamper its diagnostic potential [96]. Experimental data on therapeutic options have offered few hopes until now, with a controversial role for human immunoglobulin [97], and preliminary reports with antisense gene–targeted compounds [98].

The inclusion of Kyasanur Forest virus in this category also seems like an aberration, because most other hemorrhagic fever viruses belong to category A, and Crimean-Congo hemorrhagic fever and Yellow fever belong to category C. Furthermore, the rationale of this classification is not explained in

NIAID documents [2]. Presumably, the propensity for neurologic complications during the second phase of the disease accounts for this joint with viral encephalitides classification. Kyasanur Forest virus is a tick-borne hemorrhagic fever virus found exclusively in Northern India. Incubation period is 3 to 8 days. Case fatality rate is 3% to 5%, and clinical presentation is characterized by a first febrile period during which hemorrhagic manifestations may appear, with bradycardia and hypotension being common findings, and a second febrile period 10 to 21 days after defervescence, which is accompanied by various neurologic symptoms. Diagnosis is based on serology, although the protracted viremia allows for laboratory isolation of the virus. Treatment is supportive [99].

Bioterrorism potential

One important aspect in regard to Japanese encephalitis virus and its bioweapon potential is the current status of vaccine availability: the development of a Food and Drug Administration–approved formalin-inactivated mouse brain vaccine for Japanese encephalitis virus and its wide application in endemic countries in the second half of the twentieth century was a significant weapon against the disease and an important prophylactic component of travel medicine. Its efficacy reached 91% [100]. The vaccine eventually will be replaced by an inactivated cell culture vaccine, or by an attenuated live vaccine similar to the current Chinese approach [101,102].

Regarding WNV, its introduction into the New World raised fears of a bioterrorism act, and such an approach (evolution of a long-term epidemic or endemic has been considered an alternative important aspect of bioterrorism [103]) has been discussed. At present WNV is the subject of significant and rapid scientific progress, which could be used in less benign ways, but still the scientific community remains one step ahead. Development of a vaccine is in process with preliminary reports and phase I trials for DNA vaccines and live attenuated chimeric vaccines [104]. Still, as the complete spectrum of WNV is clarified, its importance as a possible biological weapon may increase, and its inclusion in category A pathogens could be supported.

A formalin-inactivated vaccine for Kyasanur Forest virus has been used in endemic regions [105], but its efficacy is diminished in patients exposed to other flaviviruses.

Foodborne and waterborne pathogens

The bacteria

Most of the known bacterial causes of infectious diarrhea are included in category B pathogen list. Most of them are universally widespread, and responsible for millions of annual cases worldwide [106].

Salmonella species can induce acute infectious diarrhea (usually *Salmonella enterica* serovars *enteritidis* and *typhimurium*) that manifests usually

within 1 day after exposure, demand a low inoculum of almost 100 colony-forming units, and can be treated only with supportive measures. More severe cases may demand the use of co-trimoxazole, quinolones, or ceftriaxone. *Salmonella typhi* is the causative agent of typhoid fever, a protracted systematic illness that, at least in experimental studies, demands a high inoculum [107]. The incubation period ranges from 7 to 14 days, and achlorhydria is one important risk factor. Characteristic (but inconsistently seen) clinical findings include relative bradycardia and truncal rose spots. The disease causes complications in the gastrointestinal tract or the central nervous system in 10% of patients, but the overall case-fatality rate is less than 2%. Blood and bone marrow cultures are the preferential diagnostic methods, the latter reaching a sensitivity of 95%. Quinolones are the treatment of choice, and alternative options include azithromycin (in cases of quinolone resistance); co-trimoxazole; amoxicillin; or ceftriaxone [108].

Shigella species may be the most fearsome foodborne and waterborne pathogen, because it can induce bloody diarrhea with a very low inoculum (on average 200 colony-forming units); can further be transmitted from person to person through the fecal-oral route; and is related to the late development of hemolytic uremic syndrome. Its incubation period is 1 to 7 days, and achlorhydria also predisposes to clinical disease. The importance of Shiga's toxin as a prototype toxin cannot be overemphasized. Treatment is based on co-trimoxazole or quinolones. Azithromycin and ceftriaxone may also be used [109].

Escherichia coli strains can be implicated in acute watery diarrhea (enteropathogenic *E coli*) [110], but also in a low-inoculum-induced, often afebrile (50%), bloody (in 90% of the patients) diarrhea by Shiga's toxin–producing strains [111], the importance of which is paramount, because the disease is also related to the development of hemolytic uremic syndrome in a significant percentage of children. Rapid diagnosis is imperative, because antibiotics may further predispose to hemolytic uremic syndrome development, although other reports suggest the opposite [112], and antimotility drugs are contraindicated. Attack rates for Shiga's toxin–producing strains are estimated at 20%.

Campylobacter jejuni is the most prevalent bacterial cause of infectious diarrhea in adults, with an incubation period of 1 to 7 days, and a very high ratio of subclinical to clinical cases, possibly because of its exquisite susceptibility to hydrochloric acid. It is also related to certain sequelae, as Guillain-Barré syndrome [113]; *Campylobacter* is the syndrome's most common identified cause. Macrolides and azithromycin are first-line therapeutic choices, especially because quinolone resistance to the pathogen has been continuously on the rise [114].

Of the Vibrios, *V cholerae* has been historically responsible for a huge number of epidemics worldwide [115], inducing through an exotoxin a severe watery diarrhea that leads to hypovolemia and death in certain clinical settings. Achlorhydria is also a risk factor for symptomatic disease. The attack

rates are high, however, and treatment, beyond aggressive fluid and electrolyte resuscitation, may be achieved with a single dose of doxycycline or ciprofloxacin (or erythromycin in pregnancy). *V parahemolyticus* is the commonest pathogen implicated in vibrio-related diarrhea in the Western world, causing in less than 24 hours after exposure a usually benign gastroenteritis, although septicemia might develop in immunocompromised patients and patients with underlying liver disease. It requires a high inoculum (>10,000 colony-forming units). Treatment is largely supportive, and in severe cases doxycycline and quinolones can be used [116].

Listeria monocytogenes, the gram-positive pathogen of the group, a facultative anaerobe, is known to induce significant disease in immunocompromised patients, with a spectrum including septicemia and meningitis [117]. Listeriosis is also important for pregnant women, but foremost for the safety of the fetus [118]. In the United States 2500 cases occur annually, with 500 deaths recorded on average (case-fatality ratio 20%). Most cases are noted in newborns, pregnant women, and immunosuppressed patients. Incubation period is 1 day in uncomplicated cases, but may be prolonged in invasive disease. High-dose ampicillin therapy is warranted.

Yersinia enterocolitica is a zoonotic pathogen largely localized to Northern Europe [119], which usually causes acute enteritis, especially in young children. The severity of infection (including septicemia, which even if treated is associated with 50% mortality) is related to host factors, namely iron overload as in thalassemia, underlying liver disease, diabetes mellitus, and old age; the disease may be more common in African Americans and infants in the United States [120]. Isolation of the organism suggests the diagnosis, although serology might be helpful. Treatment is usually suggested for immunocompromised patients and severe infections, using combinations of doxycycline, co-trimoxazole, aminoglycosides, or quinolones.

The viruses

Noroviruses are the commonest cause of acute infectious diarrhea worldwide, responsible for 90% of the outbreaks and 52% of total cases in the United States [121]. The inoculum is low (<100 viral particles), and attack rates exquisitely high, reaching 100% in contained situations. Further human-to-human transmission is feasible, especially in the presence of severe symptoms. Incubation period is hours to 2 days, and supportive measures are usually the only indicated treatment. Diagnosis is based on real-time PCR, although ELISA may also be helpful. One important aspect of Noroviruses is their ability to exist in aerosolized form, and their relative environmental stability, because they are resistant to common disinfectants.

Hepatitis A virus has been related to massive epidemics because of consumption of infected seafood and can be relatively resistant to chlorination, surviving from common hygiene measures of potable water supplies. The inoculum for induction of infection is low (<100 viral particles), and the

incubation period is 3 to 6 weeks. The resulting acute hepatitis is benign (0.2% mortality in patients who developed jaundice) and does not exhibit chronicity. Reported attack rates vary widely. Serology is usually enough in terms for diagnosis [122].

The protozoa

Cryptosporidium parvum is an intracellular waterborne pathogen that has caused few significant epidemics [123], but is of main importance in the pathology of AIDS. Its cysts are environmentally stable, and a small number of cysts (even one to two) are required to induce infection. Incubation period is 1 to 14 days. Immunocompetent patients are usually symptom-free or exhibit limited symptoms, but the disease is more severe in immunocompromised patients, and can be related to significant weight loss. Human-to-human secondary transmission is feasible. Treatment is advocated for severe cases and paromomycin is the agent of choice, although new treatment options are emerging [124].

Cyclospora cayetanensis is a recently recognized coccidian parasite of unknown ecology, which can cause protracted, relapsing diarrheic syndromes, accompanied by fatigue and weight loss, and in 50% of the cases fever. Incubation period is 1 week, and infection has been related to contaminated basil and lettuce, and most notoriously Guatemalan raspberries [125]. Immunologically naive populations may be more susceptible to severe disease. Attack rates are estimated high, but experimental studies failed to induce disease even with high inocula [126]. Diagnosis can be achieved by staining or PCR, or by ultraviolet fluorescent microscopy, using the oocyst's autofluorescence. Co-trimoxazole is used for treatment. No human-to-human transmission occurs [127].

Entamoeba histolytica is a parasite with worldwide distribution, usually producing no symptoms (cyst passers), but also able to induce a usually afebrile colitis with ulcer formation, after an incubation period of 3 weeks. Amebomas and hepatic abscesses are known complications. Alternative diagnostic procedures, apart from microscopy of feces, include duodenal biopsy and serology (ELISA). Treatment uses a combination of metronidazole with either iodoquinol or paromomycin [128].

Giardia lamblia is also an intestinal parasite with worldwide distribution correlating with poor hygiene. It can induce a protracted diarrheic syndrome after an incubation period of 12 to 20 days, attack rates varying from 17% to 47%. Severe disease is almost exclusively seen in children and young women. Person-to-person transmission is feasible through the fecal-oral route. Treatment with metronidazole (or alternatively quinacrine or furazolidone) is advocated: 20% of treated patients experience treatment failure or relapse and are usually retreated [129].

Microsporidia have been recently recognized as human pathogens. They are unicellular, obligate intracellular eukaryotes with a characteristic polar tube [130].

Enterocytozoon bieneusi is the commonest human pathogen causing chronic intestinal inflammation in patients with AIDS (but also in other immunocompromised populations). Less than 20 cases have been described in immunocompetent adults, as benign cases of traveler's diarrhea. Diagnosis is based on light microscopy (to the genus level) and PCR. Treatment options are limited, and in general only fumagillin can be suggested [131]. Immune reconstitution through antiretroviral therapy augments clearing of the infection. The pathogen exhibits potential for transmission in aerosolized form, and can be transmitted from person-to-person through the fecal-oral route. *Encephalitozoon intestinalis* has caused similar symptoms in <200 immunocompromised patients reported in the literature, and two immunocompetent travelers. *E intestinalis* is usually sensitive to albendazole. Human infection with *Encephalitozoon hellem* and *Encephalitozoon cuniculi* is very rare.

Finally, *Toxoplasma gondii* is an intracellular foodborne pathogen, which poses significant risks for patients with AIDS, while also adversely affecting the outcome of the fetus when nonimmune pregnant mothers are infected. Rare cases of chorioretinitis are the most significant presentations in immunocompetent adults. The evolution of PCR has augmented accurate diagnosis. Treatment, when needed, is based on combinations of pyrimethamine and sulfadiazine (or clindamycin) [132].

Bioterrorism potential

Foodborne and waterborne bacteria can be implicated in potential bioterrorism events because, besides air, food and water supplies are the other options for accessing massive numbers of candidate patients [133]. Two characteristic episodes of deliberate release have outlined this potential. In 1984, a religious group used *S typhimurium* to contaminate salad bars to influence the outcome of a regional election [134], and an attempt of poisoning using Shiga's toxin was later reported [135]. Category A pathogens can also be implicated in poisoning of the food and water chain [136]. One critical aspect of a potential episode is recognition of intent, because many epidemics occur annually worldwide, often caused by inadvertent contamination of the same sources that are implicated in a voluntary release. For category B pathogens, however, the huge experience gained from naturally occurring outbreaks, the evolution of extended networks for surveillance, and the ability to cooperate in international epidemiologic studies (witness the example of the Guatemalan raspberries [125]), definitely facilitates a prompt, well-organized, and successful response. One problem that may emerge is that, as with naturally occurring outbreaks, a definite diagnosis should be reached quickly to exclude other toxic compounds, and to ensure that empirical decisions are not being made: for example, an outbreak that is ultimately attributed to Shiga's toxin–producing strains should be followed by a guideline for avoidance of antibiotics and antimotility drugs as much as possible (see above). The scale of danger posed by category B foodborne

and waterborne pathogens is not similar: certain pathogens induce secondary medical problems, such as hemolytic uremic syndrome and Guillain-Barré syndrome (or even reactive arthritis), potentiating their overall effect on public health. Certain pathogens have the potential of the creation of a second wave of infection, caused by human-to-human transmission. Other pathogens can facilitate further transmission because of their shedding from nonsymptomatic carriers (Typhoid Mary–like). Certain of these pathogens, such as *V cholerae*, are related to a possibly more extreme public response, because of intrinsic fear of the pathogen [137]. Furthermore, it is obvious that if bioterrorists had the option to choose, they would choose a pathogen with low inoculum and high attack rates. One cannot exclude that certain populations at risk (ie, AIDS patients) might also become the specific target of extremist groups. Certain agents are not that well known, so it is difficult for extremist groups to develop them for dispersion: category B includes both *Shigella*, which might be a primary extremist choice, and cyclospora, scientific knowledge on which is still extremely limited.

Vaccine availability for foodborne and waterborne pathogens is limited at present. Commercially available vaccines exist for typhoid (a polysaccharide vaccine and an oral live attenuated vaccine) [108]; cholera (a killed whole cell vaccine and a live attenuated vaccine, both are not available in the United States) [138]; and hepatitis A virus. Other vaccine candidates for typhoid are emerging [139]. In development (or trials) are also vaccines for enteropathogenic *E coli* [140], *Shigella* [141], *C jejuni*, [142], *Y enterocolitica*, *Entamoeba histolytica* [143], and *Toxoplasma gondii*. Monoclonal antibodies directed against Shiga's toxin are also being studied [144].

Summary

Category B of potential bioterrorism agents is a vast pool of pathogens and toxins, with varying clinical characteristics, ranging from hard to develop as weapons viruses with significant mortality to easily weaponized bacteria that induce clinical syndromes with minimal mortality, to vaguely studied pathogens with unproved risk as weapons. Further stratification of this category is warranted, one that may bring certain agents as ricin, *Shigella*, and West Nile virus closer to category A agents, while simultaneously administering category C status to agents as Kyasanur Forest virus, cyclospora and microsporidia, and epsilon toxin of *C perfringens*. Further stratification of the existing classifications will result in more appropriate definitions of priorities.

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