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Emerging Natural Threats and the Deliberate use of Biological Agents

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The development of vaccines, public health-based disease control measures, and the understanding of germ theory in the eighteenth and nineteenth centuries, as well as the development of effective antibiotics in the twentieth century, lulled many health professionals, politicians, and the public at large into the false belief that infectious diseases have been conquered. As a result we let our guard down and dropped important training programs and projects as being unnecessary. Despite those premature assessments of victory, the past 2 decades have provided a rude awakening. Such issues as new and re-emerging diseases, antibiotic-resistant strains of microbes, and heightened concerns about biological warfare and terrorism have guaranteed that infectious diseases will continue to attract serious attention, particularly in light of their potential to cause extensive morbidity and mortality in humans. Concerns regarding the potential for the terrorist use of biological weapons, in particular, have revitalized long inadequate funding streams into the public health and laboratory infrastructures in the United States. Fortunately, many of the defensive measures that we put into place for bioterrorism, such as improving surveillance systems, building public health laboratory infrastructure, ensuring appropriate laboratory containment and transport regulations, and raising the index of suspicion of health care providers, also have benefits for preventing emerging infectious diseases and discouraging their use by bioterrorists.

In the twentieth century, mankind fashioned massive and diverse conventional weapons, and developed or refined three terrifying unconventional weapons: nuclear weapons, chemical agents, and biological agents (so-called

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"weapons of mass destruction"). Nuclear weapons and chemical agents have been used in war, and terrorists have occasionally used, or have attempted to use, chemical or biological agents as a means to advance their causes. United States intelligence agencies report a growing availability of technologies, materials, information, and expertise in these areas by various nations and terrorist groups, and several nations are suspected of acquiring and developing these agents as weapons [1,2].

Many countries developed offensive biological weapons programs in the twentieth century, including the former Soviet Union, the United States, Japan, Iraq, and the United Kingdom. The Japanese Imperial Army performed a biological program that was particularly brutal in its abuse of healthy, unwilling humans as experimental subjects [3]. Most countries, including the United States, have eliminated offensive biological weapons programs and now concentrate on defensive measures. These defensive measures include as vaccines, diagnostic modalities, and therapeutic drugs and treatment modalities against biological weapons. Special emphasis has focused on ways to detect a biological agent release on the battlefield as well as in American cities and communities. Unfortunately, at least 17 nations are suspected of maintaining an ongoing offensive biological weapons research program despite some having been signatories to the Biological and Toxin Weapons Convention (BWC) of 1972, which entered into force in 1975 [4]. Fortunately, weapons of mass destruction were not used during the Cold War and the impact of the weapon build-up was mainly economic, because nations devoted resources to defend against these weapons systems. The end of the Cold War has highlighted certain problems concerning these weapons. Some former Soviet scientists, disaffected by the lack of financial means to continue working in post-Soviet Russia, are reported to have found employment in some of those nations with aggressive bioweapons programs. In addition, it has been alleged that researchers deliberately have developed chimeric agents with hyperpathogenicity, and vaccine- and antibiotic-resistant organisms. The potential for misusing biotechnology to create pathogens with enhanced virulence, greater antibiotic and antiviral resistance, and greater environmental stability is a source of increasing concern [5]. It also is conceivable that more than one type of weapon could be used simultaneously. The potential confusion that these eventualities could cause to the United States at large and to physicians, veterinarians, and laboratory workers is enormous and demands a high level of awareness and preparedness.

The suspected continued existence of biowarfare programs in other nations and nonstate-sponsored terrorist and other extremist organizations raises additional concerns. The potential for accidental release and spread of the agents that might be produced and stockpiled by these entities is ever present. Even under the stringent controls that are used by established state-sponsored programs, serious accidents have occurred. The best known was in the town of Sverdlovsk (now Ekaterniberg, Russia) in 1979 when

anthrax spores were released accidentally from Compound 19, a Soviet military biological research facility. A minimum of 77 people became ill with 68 deaths reported as a result of this release of anthrax [6–8]. One explanation for this incident is that a filter for an exhaust pipe was removed and not replaced before restarting an anthrax powder production machine [25].

There has been an increasing awareness and interest by the medical community, the media, the United States President, and Congress of the need to defend against biological weapons [9]. It is acknowledged that these bioweapons, along with nuclear and chemical weapons, have potential strategic value. One key component of biological defense requires raising adequate awareness among healthcare providers. Because the early clinical features of many of the high-threat biological agents are nonspecific, and because most American practitioners have little experience dealing with the agents that are considered to be threats, educational efforts that are pointed toward primary caregivers (emergency physicians, family practitioners, pediatricians, and internists) is essential. One key educational component must emphasize the ''soft signs'' that signal an unusual case event, which allow the practitioner to differentiate between the natural occurrence of an unusual disease from the sentinel cases that represent the beginning of widespread disease that is due to the nefarious release of a biological agent. It is clear that the early recognition and management of a biological release may mitigate the potential consequences. Thus, it is incumbent on clinicians, pathologists, and laboratory workers to develop, understand, and use clinical and laboratory techniques that enable them to provide rapid and accurate diagnosis and treatment of casualties. In addition, the use—and even the threatened use—of these agents can have intense psychologic effects on the population at large. Laboratory testing can provide objective data to help distinguish between medically ill patients and patients who have psychosomatic reactions that may mimic physical symptoms and signs that could be associated with biological pathogens.

Certain biological pathogens possess characteristics that make them more useful as weapons. If a terrorist group or nation has the intent to incapacitate or kill large numbers of humans, some desirable properties for a potentially effective pathogen include stability in aerosols for dispersal over large areas, ability to be produced easily in large quantities, a low infective dose, and stability for storage. Certain pathogens that meet some of these criteria, and which have been listed as agents of concern for the military and civilian populations, include *Bacillus anthracis*, *Variola major*, botulinum toxins, *Yersinia pestis*, *Francisella tularensis*, viral hemorrhagic fevers, staphylococcal enterotoxin B, and Venezuelan equine encephalitis virus. Potential terrorist goals are variable, however, and may range from a desire for publicity to actual intent for widespread lethality. For terrorists who desire only publicity, any number of viruses, bacteria, fungi, protozoa, helminths, or toxins (of microbial, plant, or animal origin) may serve their purposes. Terrorists also could target the agricultural industry to produce staggering

economic and medical consequences. The programs of the former Soviet Union and Iraq included specific bioweapons that were intended for use against animals and agricultural crops [4,10,11]. The terrorist use of antiagricultural agents could have a profound and potentially devastating impact on the United States (and world) economy, export markets, and general public health.

The inherently stealthy nature of biological agents makes them attractive weapons for terrorists. During the past century, terrorists and criminals have used, acquired, attempted to acquire, or claimed to possess biological agents in more than 100 instances [12]. Fortunately, these episodes caused few fatalities, and most recent cases have been hoaxes; however, terrorists may learn from past mistakes. We too must learn from their mistakes and improve our ability to recognize and respond rapidly to future attacks.

Legal measures have been instituted to deter the use of biological weapons and to restrict the transfer of dangerous pathogens. The most important is the BWC of 1972. Officially known as the Convention on the Prohibition for the Development and Stockpiling of Bacteriological and Toxin Weapons and on Their Destruction, the BWC was signed by 140 nations and entered into force in 1975. It prohibits the development, possession, use, or transfer of biological weapons. Unfortunately, the BWC does not limit research on biological and toxin agents, and it permits the stockpiling of biological agents for prophylactic, protective, and other peaceful purposes without specific limitation. It also lacks verification provisions and a specific definition of biological weapons and toxins, and (other than a general provision in Article V) fails to specify what national measures are needed to demonstrate compliance. Neither the BWC nor the Chemical Warfare Convention address various newer threat agents that are or could be created by current technologic methods. Several review conferences and an ad hoc group of government experts have attempted to correct these problems, but the issue of verification remains unresolved and controversial.

The United States has enacted specific laws that are designed to deal with these issues, including the Chemical and Biological Weapons Control and Warfare Elimination Act of 1991 (Pub L No. 102-182). This law addresses economic and diplomatic sanctions for countries and international companies that violate international law. Another is the Biological Weapons Antiterrorism Act of 1989 (Pub L No. 101-298), which provides criminal penalties for developing, stockpiling, or possessing any biological agent, toxin, or delivery system that may be used as a biological weapon. It also includes criminal penalties for assisting foreign governments with developing, stockpiling, or possessing any biological agent, toxin, or delivery system that can be used as a biological weapon. It further authorizes the federal government to seize infectious materials and to develop regulatory procedures for the transportation of biological material in the United States. In addition, the law provides law enforcement personnel with broad civil and investigative powers. The Antiterrorism Act and Effective Death Penalty

Act of 1996 (Pub L No. 104-132) gives federal law enforcement personnel expanded investigative, regulatory, and prosecutory powers to deal with weapons of mass destruction, and provides criminal penalties for any person who threatens or attempts to use biological weapons or who uses techniques to develop more virulent pathogens. Additionally, there are two key administrative regulations. The first is 55 FR 51740, the Exportation of Biological Materials (1990), and the second is 42 CFR §72, the List of Select Agents (1996) 61 FR 55,190 (1996). Despite these legal measures, and in part because of the loopholes in these documents, the potential for proliferation of biological agents remains a concern.

Although the existence of biological weapons and the threat of bioterrorism have raised the collective awareness of infectious diseases in the past decade, one might consider bioterrorism as only one facet of the larger realm of new and re-emerging diseases for several reasons. Many of the diseases that are considered as potential bioweapon threats also are re-emerging diseases. For example, anthrax periodically challenges the cattle industry, and thus, can lead secondarily to human exposures, as occurred in Minnesota [13], or actual disease outbreaks, such as the epidemic in Zimbabwe [14]. Viral hemorrhagic fevers also make the high-threat lists [15,16], but they continue to be a concern as emerging diseases, as exemplified in Ebola outbreaks in Uganda and Zaire [17,18]. Fortunately, the ongoing work in biodefense also has beneficial spin-offs for how we respond to new and emerging diseases. The knowledge, response infrastructure, communication methods, surveillance techniques, and diagnostic and sampling devices we design, develop, and prepare for managing a deliberate biological threat incident can help us manage the public health aspects of ongoing natural biological threats.

Infectious diseases cause enormous human suffering, deplete scarce resources, impede social and economic development, and contribute to global instability. In the United States, the direct and indirect costs that are related to infectious diseases exceeds $120 billion [19]. Tourists, military personnel, traders, settlers and immigrants, and travel adventurers may carry new pathogens to unsuspecting and susceptible populations. People, storms, and floods can and have transported arthropods, rodents, snails, birds, and other creatures that bring new infections to previously unaffected areas. Changes in human behavior, technologic devices, the environment, institutional living, and in the lack of availability of nutrition or vitamins can spark new epidemics. Likewise, microorganisms are able to evolve, grow, and metamorphose to take advantage of changes.

In the last 30 years, a multitude of new pathogens that can cause human misery and death has been recognized (Box 1).

New (and dangerous) subtypes of old agents and new settings in which old agents may cause unusual disease also have been recognized. The authors have become increasingly aware of the role that is played by host factors in the development of infectious disease. Specifically, these include

Box 1. New pathogens of the last 30 years

1973 Rotavirus: major cause of infantile diarrhea worldwide [20]
1975 Parvovirus: B19 fifth disease; aplastic crisis in chronic hemolytic anemia [21]
1976 *Cryptosporidium parvum*: acute enterocolitis [22]
1977 Ebola virus: Ebola hemorrhagic fever [23]
1977 *Legionella pneumophila*: Legionnaires' disease [24]
1977 Hantaan virus: hemorrhagic fever with renal syndrome [17]
1977 *Campylobacter* spp: enteric pathogens distributed globally [9]
1980 Human T cell: T-cell lymphoma leukemia lymphotropic virus-I (HTLV I) [25]
1981 *Staphylococcus*: Toxic shock syndrome associated with toxin tampon use [26]
1982 *Escherichia coli*: Hemorrhagic colitis; O157:H7 hemolytic uremic syndrome [27]
1982 HTLV II: hairy cell leukemia [28]
1982 *Borrelia burgdorferi*: Lyme disease [29]
1983 Human immunodeficiency syndrome: AIDS virus (HIV) [30]
1983 *Helicobacter pylori*: gastric ulcers [31]
1985 *Enterocytozoon bieneusi*: persistent diarrhea
1986 *Cyclospora cayetanensis*: persistent diarrhea
1988 Human herpesvirus 6: roseola subitum [32]
1988 Hepatitis E: enteric non-A, non-B hepatitis
1989 *Ehrlichia chaffeensis*: human ehrlichiosis [33]
1989 Hepatitis C: parenterally transmitted non-A, non-B hepatitis [34]
1991 Guanarito virus: Venezuelan hemorrhagic fever [35]
1991 *Mycoplasma penetrans*: urogenital infection [16]
1991 *Encephalitozoon hellem*: conjunctivitis, disseminated disease
1992 *Vibrio cholerae*: new strain associated with O139 epidemic cholera [36]
1992 *Bartonella henselae*: cat-scratch disease; bacillary angiomatosis [37]
1992 *Tropheryma whippelii*: Whipple's disease [38]
1993 Hantavirus: hantavirus pulmonary syndrome isolates [39]
1994 *Sabia* virus: Brazilian hemorrhagic fever [16]
1994 Human herpesvirus 8: Kaposi's sarcoma–associated herpesvirus [40]
1994 Asian taeniasis: human tapeworm infection [41]
1995 New variant Creutzfeldt-Jakob disease linked to bovine spongiform encephalopathy

1998 TT virus: a transfusion-transmitted hepatitis virus [42]
1998–1999 Nipah virus: encephalitis [43]
1999 *Ehrlichia phagocytophila, E equi, E ewingii* genogroup: human granulocytic ehrlichiosis [44]
1999 *Bordetella holmesii*: Whooping cough–like illness, septicemia
2000 *Helicobacter canadensis*: another cause of human diarrhea [45]
2001 Bacillus anthracis was disseminated by way of the United States postal system [46]

altered human behavior, altered immune competence in the human host, and altered environment. New mechanisms for infection include phenomena such as the transfer of pathogenicity islands between agents. There has been an unexpected resurgence in the incidence of older agents with resistance to antibiotic and antiviral therapy and other control measures.

Essentially, the United States was caught off-guard by the increasing AIDS epidemic that began in the early 1980s. Today, the AIDS epidemic—at the dawning of the twenty-first century—is worse than the worst-case scenarios that were predicted in the early 1990s [40]. Meanwhile, tuberculosis, re-emerged in the United States in the 1980s after decades of decline, and includes newer multidrug-resistant strains. In the 1990s, epidemic cholera reappeared in the Americas and caused nearly 10,000 deaths from 1991 through June of 1994 [47]. The contamination of the water supply in Milwaukee, Wisconsin, in 1993 resulted in an outbreak of cryptosporidiosis that affected more than 400,000 people and caused nearly 4400 hospitalizations [48,49]. The increasing prevalence of antibiotic-resistant strains of streptococci, malaria, gonococci, enterococci, and staphylococci portend of other serious treatment and control failures. The introduction of West Nile virus into New York in 1999, its overwintering in mosquitoes, and spread to at least 12 eastern US states in 2000 has demonstrated critically important lessons regarding our current laboratory detection and response systems. Since that time we have seen the emergence of additional ''new'' diseases, including severe acute respiratory syndrome and avian influenza.

We continue to identify new infectious diseases, often with unknown long-term public health impact. We use the term ''emerging'' to include newly recognized agents that prove to be the cause of known diseases or syndromes (eg, rotavirus, parvovirus, human T-cell lymphotropic viruses I and II, *Tropheryma whippelii*: Whipple's disease [38,50], and human herpesvirus type 6) or diseases that recently have been better recognized or defined (eg, Legionnaires' disease, Lyme disease, human ehrlichiosis). In addition, we are finding old agents in new places, such as the recent introduction of the West Nile virus into the United States. Some disease are entirely new, or at least

newly recognized, such as the previously unknown and deadly AIDS, an illness that originated from uncertain sources in Africa and has disseminated globally. One key difference today is the incredible rate of spread internationally that would have been impossible in medieval times. There also has been an increased awareness that certain well-characterized infectious agents can cause new diseases (eg, cysticercoids presenting in extraintestinal sites), particularly in immunocompromised individuals. Another example is lymphocytic choriomeningitis virus, a zoonosis that is acquired from chronically viremic mice or hamsters, which acts as a teratogenic virus. Some diseases that were recognized more than 100 years ago, such as Buruli ulcer, have increased in incidence suddenly. Other diseases, like streptococcal necrotizing fasciitis (flesh-eating bacteria) and methicillin-resistant *Staphylococcus aureus* (MRSA), have produced miniepidemics as well as widespread infection control problems. MRSA has evolved rapidly to become a common cause for hospital-based infections in many communities. Many diseases, such as dengue in Cuba and vivax malaria in Korea, have re-established endemic transmission in areas from which they were once eradicated. Some organisms that we expected to eradicate years ago, such as the parasitic disease dracunculosis, which is caused by *Dracunculus medinensis*, persist. We have begun to understand how microorganisms transfer their pathogenic elements to one another. Specifically, we have recognized the existence of pathogenicity islands [16] (which enable bacteria to gain complex virulence traits in one step) and type III secretion systems (which provide a means for bacteria to target virulence factors directly at host cells). Ultimately, these factors disrupt the host cell and benefit the pathogen.

At, times attempts to provide therapy have led to the unexpected spread of infection, such as AIDS in hemophiliacs or Creutzfeldt-Jakob disease in patients who were treated with growth hormone extracts. Or they have led to new infections becoming more common, such as the use of corticosteroids and other immune-suppressing agents facilitating infections with opportunistic pathogens. The adoption of eating habits from other cultures has brought new illness to unsuspecting populations, such as anasakiasis in the United States. Fortunately, we possess an enormous scientific base, and the rate of acquisition of new information and techniques to diagnose and treat infectious diseases is unprecedented. Clearly, identifying the causative agent for an infectious disease is the key to its eventual control. Several syndromes exist that are caused by infectious pathogens that resist cultivation by standard microbiologic techniques. We are trained to consider common causes for syndromes first—and unless we have a high level of suspicion—we may not realize that we need to apply nonstandard methods to detect many of the agents that a bioterrorist might use.

The rapid identification of a hantavirus as the cause of the outbreak of a severe pulmonary distress syndrome in the southwestern United States demonstrated that applying molecular biology approaches can accelerate the identification of an unknown agent [51]. Extensive nucleic acid and

protein databases are readily available. Isolating and sequencing genomic fragments from tissue secretions or fluids that contain an unknown agent can provide important clues regarding its origin and biological behavior. After a new agent's phylogenetic relationship to other known organisms is established, it is possible to determine its likely source and method of spread. Subsequently, appropriate culture conditions, serologic tests, immunologic stains, in situ hybridization methods, special stains, and preventive and therapeutic strategies can be defined. The use of high technology by sophisticated laboratories may reveal characteristics of an organism that were unrecognized previously. This has been confirmed with *Balamuthia*, *Chlamydia*, *Pneumocystis*, and other infectious agents. After the molecular and other highly sophisticated work is done, standard laboratory and histologic methods can be applied to continue an investigation.

Given that some of the biological threat agents and emerging infectious diseases can be highly virulent and may lack specific treatments or preventive measures, it is imperative that we have certain standards for working with them in the laboratory setting. Biosafety levels, formerly called "P" levels for "physical containment," are the ratings for laboratory facilities under which agents can be handled. They depend on an agent's potential for causing the disease, level of virulence, level of infectivity, and our ability to prevent and to treat the disease. There are four biosafety levels of increasingly stringent laboratory design that were developed by the National Institutes of Health, World Health Organization, Centers for Disease Control and Prevention, United States Army Medical Research Institute of Infectious Diseases, and other institutions for work with biological agents or genetic engineering.

Level 1: Educational facilities and teaching laboratories. Well-defined and characterized strains not known to cause disease in healthy adults or low-risk genetic engineering experiments. Basic containment. Standard microbiologic practices. No special one or two barriers except handwashing.

Level 2: Clinical, reference, and teaching laboratories. Indigenous moderate-risk agents cause human disease or moderate-risk genetic work. Any human-derived fluids or tissues where presence of infectious agent may be unknown. Open bench permissible if potential for creating aerosols or splashes is low. Careful handling of sharps. Hand-washing and waste decontamination facilities must be available to reduce potential environmental contamination.

Level 3: Clinical, diagnostic, reference, teaching, research, or production facilities. Indigenous or exotic agents with a potential for respiratory transmission that may cause serious and potentially lethal infection; or recombinant DNA molecules and recombinant organisms with similar serious or lethal potential. Primary and secondary barriers to protect personnel in contiguous areas, community, and environment from

exposure to infectious aerosols. All manipulations in a biological safety cabinet or similar enclosed chambers. Secondary barriers include controlled access to laboratory. Specialized ventilation system that minimizes release of infectious aerosols from laboratory.

Level 4: Dangerous and exotic agents that pose a high risk for life-threatening disease, may be transmitted by aerosols, and have no vaccine or therapy. Primary hazards: exposure to infectious aerosols, mucous membrane exposure, and autoinoculation. All manipulations of infectious materials, isolates, and infected animals pose high risk for exposure and infection. Biosafety cabinet or a full-body, air-supplied positive-pressure personnel suit. Facility usually a separate building or completely isolated with its own specialized ventilation and waste management systems to prevent release of viable agents to environment.

These institutions have the capability to provide personnel safety during laboratory diagnosis and research and they provide a critical aspect in our defense against biological weapon and emerging infectious disease pathogens.

History reminds us that all aspects of infectious diseases, including recognition, treatment, containment, and legal aspects of the threat, will continue to challenge us. The possible scenario of an intentional release of an exotic pathogen makes this challenge more problematic. Whether one is dealing with a biological terrorist event or an emerging infectious disease, laboratory diagnosis remains of paramount importance. The early clinical features of many of the biological weapon agents are nonspecific, and if one is dealing with a new disease, the clinical features may not be characterized. A scenario for managing bioterrorism or emerging infectious diseases is not complete without the most critical diagnostic area being addressed: the clinical and anatomic pathology laboratories. It behooves all clinicians and veterinarians to be aware of laboratory issues. They can provide the best samples that are transported under the safest conditions with careful adherence to the chain-of-custody protocol; this enables the pathology laboratory to provide them with a rapid and accurate, specific diagnosis. Likewise, it behooves all pathologists to be intimately familiar with the clinical and pathologic aspects of these agents and to maintain an index of suspicion for unusual pathogens. Training in the clinical recognition and laboratory diagnosis of emerging infectious diseases is of critical importance to the successful management of a potential biological weapon attack [52,53].

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