USAMRIID
MEDICAL MANAGEMENT
OF BIOLOGICAL CASUALTIES
HANDBOOK

Tenth Edition
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U.S. ARMY MEDICAL RESEARCH
INSTITUTE OF INFECTIOUS DISEASES

FORT DETRICK
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Institute of Infectious Diseases (USAMRIID)
Fort Detrick, Maryland 21702-5011

USAMRIID Medical Management of Biological Casualties Handbook

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The exclusion of anyone on this page is purely accidental and in no way lessens the gratitude we feel for contributions extended by all involved in previous editions.

FOREWORD TO THE TENTH EDITION

The *USAMRIID Medical Management of Biological Casualties Handbook*, commonly known as the "Blue Book," was first released in 1993 and has since become a cornerstone resource for military and civilian healthcare providers worldwide. Over the past 30 years, it has been revised regularly, with more than 225,000 copies distributed globally in both physical and electronic formats. This unclassified resource is designed to enhance medical readiness and optimize responses to biological threats, reflecting the enduring commitment to public health and national security.

The 10th edition has been comprehensively updated to incorporate the latest advances in medical management strategies for diseases and syndromes caused by biological threat agents. It expands on previous editions, addressing a broader scope of challenges to better prepare front-line medical providers operating across diverse and dynamic environments. As part of the Department of Defense's strategy, this handbook underscores the critical need for service members and medical teams to be prepared to conduct operations in a Chemical, Biological, Radiological, or Nuclear (CBRN)—contaminated environment. These threats demand an adaptable and informed approach, making this resource an indispensable guide for navigating complex operational contexts.

Our goal remains to provide a concise yet comprehensive reference that supports medical assets across the continuum of care, where actionable guidance is vital. We are dedicated to improving this resource and welcome your feedback to ensure future editions continue to meet the evolving needs of the operational and medical communities. Thank you for your commitment to this vital mission.

Aaron C. Pitney, MD

Colonel, Medical Corps

Commander,USAMRIID

DISCLAIMER

The purpose of this handbook is to provide concise supplemental reading material to assist healthcare providers in the management of biological casualties. Although every effort has been made to make the information in this handbook consistent with official policy and doctrine (see ATP 4-02.84, *MULTISERVICE TACTICS, TECHNIQUES, AND PROCEDURES FOR TREATMENT OF BIOLOGICAL WARFARE AGENT CASUALTIES*, November 2019; and ATP 3-11.32, *MULTI-SERVICE TACTICS, TECHNIQUES, AND PROCEDURES FOR CHEMICAL, BIOLOGICAL, RADIOLOGICAL, AND NUCLEAR PROTECTION*, January 2024), the information contained in this handbook is not official Department of the Army policy or doctrine and should not be construed as such.

Most of the specific therapies and prophylactic regimens found in this handbook are based upon standard treatment guidelines; however, some of the regimens described here may vary from information found in those sources. This is because the clinical presentation of certain diseases caused by a weaponized biological agent (bio-agent) may vary from the natural (endemic) form of the disease. For ethical reasons, human challenge clinical trials can be performed only with a limited number of these agents. Therefore, treatment and prophylaxis regimens may be derived from *in vitro* data, animal models, historical case reports of accidental occupational exposures, and other limited human data. Occasionally you will find Investigational New Drug (IND) products mentioned; these products are not available commercially and can only be given under a specific investigational protocol with informed consent. They are mentioned for scientific completeness and are not to be construed as recommendations for therapy. For information on their use and availability, see Appendix K **(“**Investigational Medical Products [INDs, etc.] & Emergency Use Authorizations [EUAs]”).

The information contained herein is accurate to the best of our knowledge at the time of this writing. The use of either trade or manufacturers’ names in this book does not constitute an official endorsement of any commercial products. This book may not be cited for purposes of advertisement.

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INTRODUCTION TO THE TENTH EDITION

Medical defense against weaponized pathogens and toxins is an unfamiliar yet vital subject at all levels of military and civilian healthcare delivery. The U.S. military has pursued research relevant to the medical defense against biological threat agents since World War II, first in concert with an offensive weapons program—then since 1969—as a purely defensive research program. Since that time, there have been significant advancements in research, technology, medical countermeasures, and the threats at large.

There has been a sustained momentum among healthcare providers and planners to better understand the medical management of high consequence infectious disease and toxin exposures to minimize casualties and reduce impact to the community; most recently from the lessons learned in the COVID-19 pandemic of 2020. Diverse measures to enhance readiness have grown over the years to include improvements in face-to-face and virtual training platforms/content, improved and accessible references (print and electronic), and added emphasis on integration into military and civilian training scenarios on a routine basis. These measures optimize the ability of healthcare personnel (military and civilian) to identify, mitigate, and recover from these threats while maintaining operations in a bio-threat environment.

This handbook has been methodically revised and enhanced to provide the reader with the tools and references necessary to medically manage biological casualties and lay the framework to streamline the broad medical response. Incorporating perspective, capability, and information from across the Department of Defense (DOD) was a focus to move this handbook to be applicable across the services. We welcome feedback and contributions to better expand our collective toolbox to combat these ever-present threats.

The importance of this topic and the relevance of this handbook is self-evident, and it is hoped that through this resource, medical providers/planners will develop a broad understanding of potential biological threats to our military forces and civilian communities, find high yield information for the management of biological casualties, and appreciate the process inherent to a bio-hazard response.

The purpose of this reference is to serve as a concise handbook that can be pulled off the shelf, in a crisis, to guide medical personnel in the prophylaxis, recognition, and management of biological casualties/events. It is designed as a portable handbook and is not intended as a definitive or exhaustive textbook. A more in-depth discussion of the bio-agents covered here may be found in the U.S. Army Surgeon General's Borden Institute *Textbook of Military Medicine*: *Medical Aspects of Biological Warfare* (2018) and in relevant infectious disease, tropical medicine, and disaster medicine textbooks.

HISTORY OF BIOLOGICAL WARFARE & THE CURRENT THREAT

The use of biological agents in warfare has been recorded throughout history.[1] During the 14th century BC, the Hittites were known to have driven diseased animals and people into enemy territory to initiate an epidemic, successfully propagating the disease we know as tularemia.[2] In the 6th century BC, the Assyrians poisoned enemy wells with rye ergot, and the Greek general Solon used the herb hellebore to poison the water source of the city of Kirra during his siege.[3] In 1346, plague broke out in the Tartar army during its siege of Kaffa (currently Feodosia in the Crimea). The attackers hurled the corpses of plague victims over the city walls, and this act is likely the reason for the entry of the "Black Death" into that city.[4] In 1422, at the siege of Karlstejn during the Hussite Wars in Bohemia, Prince Coribut hurled corpses of plague-stricken soldiers at the enemy troops, and Russian forces may have used the same tactic against the Swedes in 1710.

In 1611, at Jamestown Colony in Virginia, a toxic hallucinogenic drug derived from plants was deployed with some success against the English settlers by Chief Powhatan.[5] A century and a half later, smallpox was used as a biological weapon by the British in North America. In 1763, towards the close of the French and Indian War, Sir Jeffery Amherst recommended that a subordinate provide smallpox-laden blankets to the Native Americans who remained loyal to the French. Another subordinate, Captain Simeon Ecuyer, subsequently gave blankets and a handkerchief from a smallpox hospital to these adversaries, after which he wrote: "I hope it will have the desired effect." The subsequent outbreaks cannot be attributed to Ecuyer's actions with certainty, but the intent was entirely clear.[6] General George Washington ordered variolation (a precursor of vaccination, using material obtained from smallpox scabs) for protection of the Continental Army in 1777, in part due to devastation previously rendered on his forces by natural smallpox outbreaks and in part because of his concerns (and those of Franklin and Jefferson) for the purposeful spread of smallpox among the colonials by the British.[7]

In the 20th century, the stakes became much higher as Germ Theory and subsequent scientific discipline of microbiology provided a new level of sophistication in producing bio-agents for war. During World War I, operatives of Imperial Germany inoculated horses and cattle with anthrax and glanders at several ports around the world, including that of Baltimore, before the animals were shipped to France.[8] The French, for their part, began the world's first truly scientific biological weapons program targeted against human combatants under the direction of Auguste Trillat in the early 1920s.[9] In the early 1930s, Imperial Japan began an ambitious bio-warfare program; by 1937, the notorious facility code-named "Unit 731", located 40 miles south of Harbin, in occupied Manchuria, was operational. Studies directed by Japanese General, and physician, Shiro Ishii continued there until it was destroyed by the Allies in

1945. A post-war investigation revealed that the Japanese program researched numerous bio-agents and used prisoners of war as research subjects. About 1,000 human autopsies were carried out at Unit 731, mostly on victims exposed to aerosolized anthrax. Many more prisoners and Chinese nationals may have died in this facility, up to 3,000 in total. Reportedly, Japanese Forces utilized bio-agents in the field. These instances remain history's only examples of the actual use of biological warfare (BW) on a battlefield or against an enemy's civilian population: the aftermath of the Battle of Khalkhin-Gol in August 1939 (where typhus, paratyphus, cholera, and dysentery were deployed as the Japanese troops retreated from the Soviets), at Ning Bo in Zhejiang Province, China, in 1940, where ~1,000 civilians were sickened and perhaps 100 killed (plague fleas, typhoid and cholera in water) and in several cities of Zhejiang in 1942 (where reportedly ~1,000 Japanese troops were also inadvertently killed). The reported overflights by Japanese planes suspected of dropping plague-infected fleas may have caused the plague epidemics that ensued in China and Manchuria, resulting in untold thousands of deaths.[10] This story remains incompletely understood. One scholar has concluded that:

> "… the latest research… shows that in the two bio-war campaigns alone, those in Yunnan Province in southern China and Shandong Province in the north, more than 400,000 people died of cholera. Special army forces waged germ attacks across China, at countless locations under Imperial Japan's heel of occupation, and even in unoccupied regions that were subject to fly-overs by Japanese planes. Plague literally rained down upon people's heads, sprayed from special bio-war air team planes of the military; cholera, typhoid, dysentery, anthrax, paratyphoid, glanders, and other pestilences infected their food, drinking wells, crops, and livestock…. The number of people killed by Japanese germ warfare and human experiments [was estimated] to be approximately 590,000. This is the figure that was…mutually agreed upon at the International Symposium on the Crimes of Bacteriological Warfare… in December 2002 in the city of Changede, Hunan Province…. The number of physicians and scientists involved in these germ attacks and in the human experiments totaled more than 20,000."[11]

By war's end, the Japanese program had also stockpiled 400 kilograms of anthrax to be used in a specially designed fragmentation bomb, although this particular weapon technology was never tested or proven operationally.

In 1942, President Franklin D. Roosevelt initiated a U.S. research program focused on offensive biological agents responding to a perceived German biological warfare threat. This program, based at Camp Detrick, Maryland, produced agents and conducted field tests until 1969 when President Nixon halted all offensive biological and toxin weapon research. Between May 1971 and May 1972, all stockpiled bio-agents and munitions from the now defunct U.S. program were destroyed in the presence of monitors representing the

USDA, the Department of Health, Education, and Welfare, (now the DHHS), and the states of Arkansas, Colorado, and Maryland, where bio-arsenals existed. Included among the bio-agents destroyed were Bacillus anthracis, botulinum toxin, Francisella tularensis, Coxiella burnetii, Venezuelan equine encephalitis virus, Brucella suis, and staphylococcal enterotoxin B. The U.S. Army began a medical defensive program against bio-agents in 1953 which continues today at USAMRIID.[12]

In 1972, the U.S., United Kingdom, and Soviet Union signed the Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological and Toxin Weapons and Their Destruction, commonly called the Biological Weapons Convention (BWC). As of 12 July 2024, 187 countries have added their ratification.[13] This treaty prohibits the stockpiling of bio-agents for offensive military purposes and forbids research on agents for other than peaceful purposes. However, despite this historic agreement among nations, biowarfare research flourished in many countries hostile to the U.S. Moreover, there have been several cases of suspected or actual release of biological weapons. Among the most notorious of these were the "yellow rain" (possible T-2 mycotoxin) incidents in Southeast Asia (1975-78), the use of ricin as an assassination weapon in London in 1978, and the accidental release of weaponized anthrax spores at Sverdlovsk in 1979.

Testimony from the late 1970s indicated that Laos and Kampuchea were attacked by planes and helicopters delivering colored aerosols. After being exposed, people and animals became disoriented and ill, and a small percentage of those stricken died. Some of these clouds may have been comprised of trichothecene toxins (in particular, T2 mycotoxin). These attacks are grouped under the label "yellow rain." There has been controversy about whether these clouds were indeed biowarfare agents. Some have argued that the clouds were nothing more than feces produced by swarms of bees.

In 1978, Georgi Markov, a Bulgarian defector living in the UK, was attacked in London with a device disguised as an umbrella, which injected a tiny pellet filled with ricin toxin into the subcutaneous tissue of his leg. He died several days later. On autopsy, the tiny pellet was found and determined to contain ricin toxin. It was later revealed that the Bulgarian secret service carried out the assassination, and the technology to commit the crime was developed and supplied by the Soviet Union's secret service (KGB).

In April 1979, an incident occurred in Sverdlovsk (now Yekaterinburg) in the Soviet Union, which appeared to be an accidental aerosol release of Bacillus anthracis spores from a Soviet military microbiology facility: Compound 19. At least 77 residents living downwind from this compound developed high fever and had difficulty breathing; at least 66 cases died. The Soviet Ministry of Health blamed the deaths on the consumption of contaminated meat and for years controversy raged in the press over the actual cause of the outbreak. All evidence available to the U.S. Government indicated a release of aerosolized

B. anthracis spores. In the summer of 1992, U.S. intelligence officials were proven correct when the new Russian President, Boris Yeltsin, acknowledged that the Sverdlovsk incident was related to activities at a BW production facility. In 1994, Harvard Professor Mathew Meselson and colleagues published an in-depth analysis of the incident.[14] They documented that all of the cases occurred within a narrow zone extending four kilometers downwind in a southeasterly direction from Compound 19. A more recently reported incident from the Soviet Union revealed that in 1971, a field test of smallpox biological weapon near Aralsk, Kazakhstan caused an outbreak of at least 10 cases and one death. In both Sverdlovsk and Aralsk, a massive intervention by public health authorities greatly helped to lower potential disease spread and deaths.

Since the fall of the Soviet Union in 1991, an understanding of the enormous size of Russia's former BW program, which had its origins in the 1920s, has become apparent.[15,16] By 1960, numerous BW research facilities existed throughout the country; after 1973, they were coordinated by an agency known as Biopreparat. These programs became immense—the largest of any country in history—and at their peak conducted 52 clandestine research and production sites employing over 50,000 people. Annualized production capacity for weaponized smallpox, for example, was 90 to 100 tons. Yeltsin stated that he would put an end to further offensive BW research; however, the degree to which the program was scaled back is not known. Revelations from Colonel Kanatzhan Alibekov (Ken Alibek), a senior biowarfare program manager who defected from Russia in 1992, outlined a still remarkably robust BW program, which included active research into genetic engineering, binary bio-agents and chimeras, and capacity to produce industrial quantities of agents.[17] It is now known that, in the 1980s and '90s, many of these agents were genetically altered to resist heat, cold, and antibiotics. In September 1992, an agreement was signed by Russia with the U.S. and UK promising to end BW programs and convert facilities to benevolent purposes, but compliance with the agreement—and the fate of the former Soviet bio-agents and facilities—is still mostly undocumented. (In a particularly dispiriting development, and despite overwhelming evidence to the contrary, Russian President Vladimir Putin has asserted that the USSR never pursued an offensive BW program in violation of the BWC.[18])

During United Nations (UN) inspections of former BW facilities in Iraq in 1998, it emerged that Iraqi President Saddam Hussein had prisoners tied to stakes and bombarded with anthrax and chemical weapons for experimental purposes. These experiments began in the 1980s during the Iran-Iraq War after initial experiments on sheep and camels. Dozens of prisoners are believed to have died in agony during the program. According to an investigation by the London Sunday Times:

> "Iranian prisoners of war are said to have been tied up and killed by bacteria from a shell detonated nearby. Others were exposed to an aerosol of anthrax sprayed into a chamber while doctors watched behind a glass screen. Two British-trained scientists have been identified as leading figures in the programme… 10 Iranian prisoners of war were taken to a location near Iraq's border with Saudi Arabia. They were lashed to posts and left helpless as an anthrax bomb was exploded by remote control 15 yards away. All died painfully from internal haemorrhaging. In another experiment, 15 Kurdish prisoners were tied up in a field while shells containing camel pox, a mild virus, were dropped from a light aircraft. The results were slower but the test was judged a success; the prisoners fell ill within a week. Iraqi sources say some of the cruellest research has been conducted at an underground facility near Salman Pak, southwest of Baghdad. Here…experiments with biological and chemical agents were carried out first on dogs and cats, then on Iranian prisoners… secured to a bed in a purpose-built chamber, into which lethal agents, including anthrax, were sprayed from a high-velocity device mounted in the ceiling. Medical researchers viewed the results through fortified glass. Details of the experiments were known only to Saddam and an inner circle of senior government officials and Iraqi scientists educated in the West… The facility, which is understood to have been built by German engineers in the 1980s, has been at the centre of Iraq's experiments on "human guinea pigs" for more than 10 years."[19]

In August 1991, following the Gulf War, the United Nations inspected Iraq's biological warfare capabilities. Iraqi officials informed UN Special Commission Team 7 that they had researched the offensive use of Bacillus anthracis, botulinum toxins, Clostridium perfringens, and other biological agents. This admission confirmed U.S. intelligence concerns. Iraq had numerous, extensive research facilities, including at Salman Pak, many of which were destroyed during the war.

In 1995, further information on Iraq's offensive program was made available to UN inspectors. Iraq conducted research and development work on anthrax, botulinum toxins, C. perfringens, aflatoxins, wheat cover smut, and ricin. Field trials were conducted with Bacillus subtilis (a simulant for anthrax), botulinum toxin, and aflatoxin. Bio-agents were tested in various delivery systems, including rockets, aerial bombs, and spray tanks. In December 1990, the Iraqis filled 100 R400 bombs with botulinum toxin, 50 with anthrax, and 16 with aflatoxin. In addition, 13 Al Hussein (Scud) warheads were filled with botulinum toxin, 10 with anthrax, and 2 with aflatoxin. These weapons were deployed in January 1991 to four locations. In all, Iraq produced 19,000 liters of concentrated botulinum toxin (nearly 10,000 liters filled into munitions), 8,500 liters of concentrated anthrax (6,500 liters filled into munitions), and 2,200 liters

of aflatoxin (1,580 liters filled into munitions). It appears that any subsequent BW program in Iraq was limited to research.

The 1990s also saw increasing concern over the possibility of the terrorist use of bio-agents to threaten either military or civilian populations. Extremist groups have tried to obtain microorganisms that could be used as biological weapons. The 1995 sarin nerve agent attack in the Tokyo subway system raised awareness that terrorist organizations could potentially acquire or develop weapons of mass destruction (WMD) for use against civilian populations. Subsequent investigations revealed that, on several occasions, the Aum Shinrikyo cult had attempted to release botulinum toxin (1993 and 1995) and B. anthracis (1995) from trucks and rooftops, efforts that were, fortunately, unsuccessful.[20]

In response, the DOD initially led a federal effort to train the first responders in 120 American cities to be prepared to act in case of a domestic terrorist incident involving WMD. This program was subsequently handed over to the Department of Justice, and then to the Department of Homeland Security (DHS). First responders, public health and medical personnel, and law enforcement agencies have dealt with the exponential increase in biological weapons hoaxes around the country over the past several years. The National Pharmaceutical Stockpile (NPS) was launched in 1999, under direction of the Centers for Disease Control and Prevention (CDC). It became the Strategic National Stockpile (SNS) in 2002 and represents the nation's repository of antibiotics, vaccines, chemical antidotes, antitoxins, and other critical medical equipment and supplies under joint control of CDC and DHS.

The events of September 11, 2001, and subsequent anthrax mail attacks brought immediacy to planning for the terrorist use of WMD in the U.S. Anthrax-laden letters placed in the mail caused 23 probable or confirmed cases of anthrax-related illness and five deaths, mostly among postal workers and those handling mail. On October 17, 2001, U.S. lawmakers were directly affected by anthrax contamination leading to closure of the Hart Senate Office Building in Washington, D.C. Terrorist plots to use ricin were uncovered in England in January 2003. Ricin was also found in a South Carolina postal facility in October 2003[21] and the Dirksen Senate Office Building in Washington, D.C. in February 2004. Ricin incidents continue to occur due to the availability of the source material from castor beans. (Most recently, in April 2013, envelopes addressed to the office of U.S. Senator Roger Wicker and to President Barack Obama tested positive for ricin. A Mississippi man was ultimately sentenced to 25 years in prison for the crime.)[22]

The National Strategy for Homeland Security (2002) and the Homeland Security Act of 2002 were developed in response to the terrorist attacks. The Department of Homeland Security, with over 180,000 personnel, was created to unify national security efforts. The Office for Domestic Preparedness (ODP) is the principal component of the DHS responsible for preparing the U.S. for acts of terrorism by providing training, funds for the purchase of equipment, support

for the planning and execution of exercises, technical assistance, and other support to assist states and local jurisdictions to prevent, plan for, and respond to acts of terrorism.

The Public Health Security and Bioterrorism Response Act of 2002 requires drinking water facilities to conduct vulnerability assessments; all universities and laboratories that work with biological material that could pose a public-health threat have to be registered with the DHHS or the USDA; and new steps were imposed to limit access to potential bio-agents. Smallpox preparedness was implemented, including a civilian vaccination program, a vaccine injury compensation program, and aid to the states. Before the March 2003 invasion of Iraq, health departments and hospitals nationwide conducted smallpox vaccinations for healthcare workers and developed bioterrorism response plans. Many experts assert that the threat of biowarfare has increased in recent decades, with several countries actively developing offensive biological weapons. According to a 2008 report by the U.S. Congressional Research Service, nine countries—China, Cuba, Egypt, Iran, Israel, North Korea, Russia, Syria, and Taiwan—were identified, with varying degrees of certainty, as having some capability related to biological warfare. Notably, Iran and Syria have been characterized as countries that are "aggressively seeking" not only biological weapons but also nuclear and chemical weapons. [23]

Even today, biological terrorism and biological warfare continue to be a pervasive threat, due to the significant economic disruption it can cause globally. The threat posed by biological agents to both U.S. military forces and civilians may be more pressing now than at any point in history due to the widespread availability of agents, as well as accessible knowledge about their production methods and potential dissemination devices.[24] There is still an intense concern in the West about the possibility of the proliferation or enhancement of offensive programs due to the potential hiring of expatriate and rogue scientists. There is also growing concern that the smallpox virus, lawfully stored in only two laboratories at the CDC in Atlanta and the Russian State Centre for Research on Virology and Biotechnology (Vektor), may exist in other countries around the globe. More recently, the 2022 National Biodefense Strategy, building upon the 2018 strategy, continues to bring further awareness and concerted effort to understand, prevent, prepare for, respond to, and recover from biological threats. [25] This underscores the importance of ongoing education for government officials, public health personnel, the Department of Defense, and law enforcement to ensure the safety and security of our nation and communities.

DISTINGUISHING BETWEEN NATURAL & INTENTIONAL DISEASE OUTBREAKS

General epidemiological principles are as applicable to a biological attack—whether from bioterrorism or biological warfare on the battlefield—as they are to natural or endemic infectious disease outbreaks. The ability to accurately determine who is at risk, and to make appropriate decisions regarding prophylaxis and other responses after a bio-agent attack, rests upon these essential tools.[1] There are, however, some important special considerations that apply to deliberate outbreaks. Because the use of a biological weapon is a criminal act, it will be very important for the evidence gathered to be usable as evidence in court. Therefore, if criminality is suspected, samples should be handled through a formal chain of custody and there must be good communication and information sharing between public health and law-enforcement authorities. In addition, because the attack may be intentional, one must be prepared for the unexpected—there is always the possibility of multiple outbreaks at different locations, as well as of the use of multiple different agents, including mixed chemical and bio-agents or multiple bio-agents.[2]

Surveillance & Detection

After a successful covert bio-agent attack, the most likely first indicator will be increased numbers of patients presenting to individual healthcare providers or emergency departments with similar clinical features caused by the disseminated disease agent. It is axiomatic that a propagating bio-agent (bacterium or virus) has an incubation period typically lasting days, by which time the unwitting victims may have dispersed from the site of the exposure and may even have travelled significant distances. However, this assumption cannot always be relied upon (e.g., preformed toxins). In the days after an unsuspected bio-attack, the possibility exists that other medical professionals, such as pharmacists or laboratorians, who may receive more than the usual numbers of prescriptions or requests for laboratory tests, respectively, may be the first to recognize that something unusual is occurring. Because animals may be sentinels of disease in humans and many of the high-threat bio-agents discussed in this book are zoonoses, it is possible that veterinarians might recognize an event in animals before it is recognized in humans.[3] Medical examiners, coroners, and non-medical professionals, such as morticians, may also be important sentinel event reporters.

To ensure a prompt and efficient response, public officials must implement and utilize routine biosurveillance systems so that they know the background disease rates and can recognize patterns of non-specific syndromes that could represent early manifestations of a bio-agent attack. The system must be timely, sensitive, specific, and practical. To recognize any unusual changes in disease occurrence, surveillance of background disease activity should be ongoing, and any significant variation should trigger a directed examination of the facts regarding

the change. Many public health agencies have initiated syndrome-based surveillance systems in an attempt to achieve near real-time detection of unusual events. Currently, these systems collectively represent something of a hodge-podge (See the subsequent section on "Biosurveillance"). Some collect data broadly from the U.S. healthcare system (for example, the National Electronic Disease Surveillance System [NEDSS][4]). Other approaches incorporate sophisticated national and international laboratory-based surveillance systems to detect specific circulating pathogens. One such example is the Department of Defense Global Respiratory Pathogen Surveillance Program which performs global, laboratory-based respiratory pathogen surveillance through a system of sentinel sites. Regardless of the existence of these systems, a sudden sharp increase in illness rates, or the diagnosis of a rare or unusual case, may still be first recognized by astute individuals working as clinicians or laboratorians.

Outbreak Investigation

After detection of a potential disease outbreak, whether natural or purposeful, a thorough outbreak investigation will assist medical personnel in identifying the pathogen and lead to the institution of appropriate medical and public health interventions. The identification of the affected population, possible routes of exposure, signs and symptoms of disease, along with the rapid laboratory identification of the causative agent(s) are all essential elements of this effort. Good epidemiologic information can guide the appropriate management of those potentially exposed, as well as assist in risk communication to authorities and in formulating responses to the media.[5]

Many diseases caused by weaponized bio-agents initially present with non-specific clinical features—notably undifferentiated fevers—that may be difficult to diagnose and recognize as a biological attack. Features of the epidemic may be important in distinguishing between a natural and a terrorist or military attack. Epidemiologic clues that may suggest an intentional attack are listed in Table 1. While a helpful guide, it is important to remember that naturally occurring epidemics may have one or more of these characteristics and that a biological attack may have none. That being said, if many of the listed clues are recognized, one's index of suspicion for an intentionally spread outbreak should increase.[6]

Possible Epidemiologic Clues to Intentional Bio-agent Use

- The appearance of a large outbreak of cases of a similar disease or syndrome, especially in a discrete population
- Many cases of unexplained diseases or deaths
- More severe disease than is usually expected for a specific pathogen or failure to respond to standard therapy
- Unusual routes of exposure for a pathogen, such as the inhalational route for diseases that normally occur through other exposure routes (e.g., inhalational anthrax vs. cutaneous anthrax)
- A disease case or cases that are unusual for a given geographic area or transmission season
- Disease normally transmitted by a vector that is not present in the local area
- Multiple simultaneous or serial epidemics of different diseases in the same population
- A single case of disease caused by an uncommon agent (smallpox, some viral hemorrhagic fevers, inhalational anthrax, pneumonic plague)
- A disease that is unusual for an age group
- Unusual strains or variants of organisms or antimicrobial resistance patterns different from those known to be circulating
- A similar or identical genetic type among agents isolated from distinct sources at different times or locations
- Higher attack rates among those exposed in certain areas, such as inside a building if released indoors, or lower rates in those inside a sealed building if released outside
- Outbreaks of the same disease occurring simultaneously in non-contiguous areas
- Zoonotic disease outbreaks
- A zoonotic disease occurring in humans, but not animals
- Intelligence of a potential attack, claims by a terrorist or aggressor of a release, and discovery of munitions, tampering, or other potential vehicle of spread (spray device, contaminated letter)

The first step in an outbreak investigation is to confirm that a disease outbreak has in fact occurred. Because an outbreak is defined as a higher rate of an illness than is normally seen in a given population, it is helpful to have handy background surveillance data to determine if what is being seen constitutes a deviation from the norm.[7] For example, in mid-winter, thousands of cases of influenza may not be considered an outbreak, whereas in the summer, it might be highly unusual. Moreover, even a single case of a very unusual illness, such as inhalational anthrax, might constitute an outbreak and should be viewed with high suspicion. The clinical features seen in the initial cases can be used to construct a case definition to determine the number of cases and the attack rate

(i.e., the population that is ill or meets the case definition divided by the population at risk). The case definition allows investigators who are separated geographically to use the same criteria when evaluating the outbreak. The use of objective criteria in the case definition is critical to determining an accurate case number, as additional cases may be found, and some cases may be excluded. This is especially true as the potential exists for panic and for subjective or routine complaints to be confused with actual infection of concern.

Outbreak Description & Analysis

Once the attack rate has been determined, an outbreak can be described in terms of time, place, and person. These data provide crucial information in determining the potential source of the outbreak. The epidemic curve is calculated based upon cases over time. In a point-source outbreak, which is the most likely type in a biological attack or bio-terrorism situation, individuals are exposed to the disease agent in a fairly short time and in a restricted geographic venue. The early phase of the epidemic curve may be compressed compared to a natural disease outbreak due to highly concentrated, high dose exposures. The peak may occur in days or even hours, especially if a toxin (as opposed to a propagating bio-agent) is used. Later phases of the curve may also help determine if the disease is able to spread from person to person. Determining whether the disease is contagious will be extremely important for crafting effective disease control measures, such as deciding whether isolation, or even quarantine, is justified. If the agent(s) is released at multiple times or sites, additional cases and multiple sequential peaks in the epidemic curve may also occur, something that happened with the mailed anthrax letters in 2001.

Once the disease agent is recognized, appropriate prophylaxis, treatment, and other measures to decrease disease spread, can be instituted. These may need to be modified as additional data on the agent (strain, anti-infective susceptibilities, etc.) come to light. In fact, evidence of resistance to front line therapeutics may provide further evidence of the intentional nature of the outbreak. The ultimate test of whether control measures are effective will be simply careful observation to see if they reduce ongoing illness or spread of disease.

Finally, it is important to understand that although the recognition of and preparation for a biological attack will be similar to that for any infectious disease outbreak, the surveillance, response, and other demands on resources will likely be of an unparalleled intensity, with potential for further added complexity from law enforcement investigation. Public anxiety will be greater after an intentionally caused event; therefore, a sound risk-communication plan that involves public health authorities will be vital to an effective response and to allay the fears of the public. A strong public-health infrastructure—with an effective epidemiological investigation capability, practical training programs, and preparedness plans—is essential to the prevention and control of disease outbreaks, whether they are naturally-occurring or purposeful.

BIOSURVEILLANCE

Syndromic Surveillance

The need to rapidly detect an intentional disease outbreak has prompted a search for faster and more reliable methods for disease surveillance. Syndromic surveillance typically refers to the automated analysis of routinely collected health data that are available even before specific diagnoses are made. The rapid expansion of such surveillance systems in recent years can be attributed to 1) increasingly available and timely electronic data entered into accessible databases, 2) advances in informatics and statistics for data extraction, normalization, and detection of aberrations in temporal or spatial data, and 3) recognition of the threat of epidemics and pandemics in light of the COVID-19 pandemic, and growing concerns of bio-terrorism and biowarfare. In many situations, syndromic surveillance systems may not detect outbreaks faster than traditional epidemiological surveillance methods. However, these systems may be able to provide information that can assist with the outbreak investigation, situational awareness, tracing the spread of outbreaks and the effectiveness of countermeasures.

Data that arise from an interaction with the health care system, but do not include confirmed or definitive diagnoses, can include early, non-specific diagnoses, such as gastroenteritis, or procedures from initial encounters, such as stool culture. They can be recorded as text in an electronic record, or through codes such as the International Classification of Diseases (ICD) or Current Procedural Terminology (CPT). A chief complaint such as cough can be entered in an Emergency Department electronic medical record, or “rash, unknown etiology” entered in a billing database. These data can also include initial impressions from emergency medical personnel on ambulance runs or calls to nurse advice lines or doctor’s offices for information. Pre-encounter information obtained about the health of a population before presentation to a health care provider includes over-the-counter pharmacy sales for items such as cough syrup or anti-diarrheal medication. Behavioral changes can be detected in school or work absenteeism rates or internet queries. In general, the closer the data source is to a medical encounter (chief complaints, provider initial impressions, laboratory test orders), the more reliable the information.

Indicator health events must be grouped into syndromes to be analyzed for anomalies and compared to expected illness rates. Most data types, including pharmacy sales and prescriptions, laboratory tests, ambulance runs, chief complaints and diagnostic codes can be grouped into syndromes. Common syndrome groups include respiratory, gastrointestinal, rash, neurological, and febrile illnesses. A syndrome grouping schema based on diagnosis codes, with an emphasis on bio-terrorism detection, is available from the CDC.[1]

The most commonly promoted use of syndromic surveillance in a bioterrorism or biological warfare context is for early detection of an attack. Timely

awareness of an increase in disease incidence can assist in mobilizing resources and potentially decrease associated morbidity and mortality. There are many examples of retrospective studies showing that syndromic surveillance can provide early warning of large community-wide disease outbreaks when compared to traditional disease reporting. Furthermore, it is assumed that such an alert could affect earlier etiologic diagnoses, and early implementation of control measures such as vaccination and antibiotic prophylaxis, as well as prioritization of these measures to affected communities in time to reduce morbidity and mortality.

The characteristics of an outbreak that make it most likely to be detected by syndromic surveillance are 1) narrow distribution of the incubation period, 2) longer prodrome, 3) absence of a pathognomonic clinical sign that would speed diagnosis, and 4) diagnosis that is dependent on the use of specialized tests that are unlikely to be ordered. Not all biowarfare or terrorism-caused outbreaks will have these characteristics. In addition, early detection may or may not assist with determining whether the outbreak is the result of an intentional biological attack. Any disease outbreak must be investigated by appropriate public health officials. Law enforcement will generally only be involved if evidence arises suggesting a deliberate nature of the outbreak. Early detection alone does not ensure recognition of a biological attack, but data in a syndromic system may help find clues that suggest an intentional event.

Besides early detection, syndromic surveillance systems can assist with the evaluation of the effectiveness of countermeasures and provide support to epidemiological investigations by finding potential cases that have recently presented and have the same syndromic presentation as those already identified. It can also be used for situational awareness, providing reassurance during periods of high concern such as large public events or when bio-agents have been used on a small scale (e.g., anthrax-laced letters in the Amerithrax incident). Ideally, syndromic surveillance systems can be used in conjunction with other surveillance methodologies to enhance overall surveillance capabilities. An example would include the use of environmental sensors for bio-terrorism detection in large metropolitan areas, where potential alerts can be shared with public health officials who can then carefully monitor syndromic data in the same geographic area.

National Strategy for Biosurveillance

Protecting the health and safety of the American people through a well-integrated national biosurveillance (BSV) enterprise has become a top national security priority. This requires a focus on core functions if progress is to be made. It also necessitates an embrace of an “all-of-Nation” approach, and indeed a global health security intent, since the effects of any deliberate CBRN (chemical, biological, radiological, or nuclear) attack or accident, or emerging infectious disease, can easily transcend national borders. There exists an imperative that the Federal Government expand its efforts to detect, rapidly, a

potential incident of national significance affecting human, animal, or plant health, whether resulting from a bio-terror attack or other CBRN threat, an emerging infectious disease, pandemic, or a food-borne illness. Rapid detection is critical to save lives and improve incident outcomes, and the United States serves as a key participant and leader in an international network of BSV centers operating across the globe.[2-5]

A National Strategy for Biosurveillance (NSB)[6], initiated by the Obama Administration in the summer of 2012, seeks to leverage existing capabilities across the nation, yet emphasizes a discrete focus on specified core functions. It articulates that essential information can be derived from a specific set of questions to speed the detection of a deliberate or accidental CBRN incident or naturally occurring disease outbreak. This strategy further articulates that when the collection and sharing of this essential information is prioritized, decision making can be expedited at all levels of government and beyond. While other activities are integral to everyday local BSV efforts that can and should continue, the NSB calls for a national focus on fewer issues so that more can be achieved collectively. This approach also seeks to inspire new thinking and revised methodologies to forecast that which we cannot yet prove, so that timely decisions can be made to save lives and reduce impacts during an emergency incident. It is a national plan of action to protect the health, well-being, and safety of the American people as part of the greater global community.

The NSB, defines biosurveillance as "the process of gathering, integrating, interpreting, and communicating essential information related to all-hazard threats or disease activities affecting human, animal, or plant health to achieve early detection and warning; contribute to overall situational awareness of the health aspects of an incident; and enable better decision-making at all levels." The NSB specifies the U.S. Government's approach to strengthening our national BSV enterprise. It describes a core set of functions critical to success as:

(1) Scan and discern the environment;
(2) Identify and integrate essential information;
(3) Inform and alert decision-makers; and
(4) Forecast and advise potential impacts.

The approach builds on existing BSV concepts and capabilities to enable more rapid detection, knowledge, and characterization of human, animal, and plant disease activities to enhance situational awareness. The NSB is consistent with the *National Strategy for Countering Biological Threats*[7], which emphasizes information sharing among Federal departments and agencies to identify biological threats.

In the context of the 2012 launch of the NSB, the Office of Management and Budget (OMB) asked the DOD to review its BSV programs, prioritize missions and desired outcomes, evaluate how DOD programs contribute to

these, and assess the appropriateness and stability of the Department's funding system for biosurveillance. In support of this strategy, the DOD is endevouring to strengthen its BSV capabilities to enhance all-hazards incident management by providing essential information for timely decision-making at all levels, whether an incident is deliberate, accidental, or naturally occurring.

The Deputy Secretary of Defense (DEPSECDEF) published interim DOD guidance for implementing the NSB[8] in the summer of 2013. The DOD defines biosurveillance the same way as does the NSB. The interim DOD guidance states that Combatant Commands (CCMDs) will continue updating directed plans and corresponding capability gaps for improved analysis of data and reporting generated by ongoing BSV-related activities. CCMDs will also identify requirements and gaps for improved integration of data and reporting generated by ongoing BSV-related activities. For the Military Services, it states that Secretaries of the Military Departments, in coordination with their Surgeons General (SGs), will make their BSV-related data, reporting and analyses available for integration at the tactical, operational, and strategic levels. DOD BSV activities include the areas of Comprehensive Health Surveillance (CHS); Force Health Protection (FHP), food protection and zoonotic disease surveillance; CBRN detection and monitoring; intelligence; law enforcement; and installation environmental and wildlife monitoring.

In 2018, the Trump Administration released a broader National Biodefense Strategy, incorporating biosurveillance as a key objective in one of the five goals of the strategy, to "Enable risk awareness to inform decision-making across the biodefense enterprise."[9] The Biden Administration further refined the National Biodefense Strategy incorporating advances and lessons learned during the COVID-19 pandemic in the 2022 National Biodefense Strategy and Implementation Plan.[10] Further in 2024, the Biden Administration released the U.S. Global Health Security Strategy which focuses on a global approach in, "working with countries around the world to ensure they are better able to prevent, detect, and respond to global health security threats."[11]

Implementation, and refinement of BSV programs are ongoing, multiple programs and projects are currently contributing to national BSV efforts, including the Laboratory Response Network, the Real-time outbreak and disease surveillance system (RODS), and a DOD syndromic surveillance system; the Electronic Surveillance System for the Early Notification of Community-based Epidemics (ESSENCE). The Federal Government seeks to galvanize jurisdictions across the nation to further extend and integrate a distributed national biodefense enterprise. The National Biodefense Strategy and Implementation plan and Global Health Security Strategy embrace the need to engage in surveillance for a broad range of human, animal, and plant health threats, including bioterrorism, biowarfare, emerging infectious diseases, pandemics, agricultural threats, and food-borne illnesses.

TEN STEPS IN THE MANAGEMENT OF POTENTIAL BIOLOGICAL CASUALTIES

Military medical personnel require a firm understanding of key elements of biological defense in order to manage, effectively, the consequences of a biological attack amid the confusion of the modern battlefield. Civilian providers who might be called upon to respond to a terrorist attack employing biological agents require a similar understanding. Familiarity with the symptomatology, pathogenesis, transmissibility, and available diagnostic and treatment options for each of the potential bio-agents thus becomes imperative. Acquiring such an understanding is relatively straightforward once the identity of the agent is known; many references[1], including this handbook, exist to assist medical personnel in pathogen-specific therapy. A larger problem occurs, however, when the identity of a causative agent is unknown. In some cases, an attack may be threatened, but it may remain unclear whether such an attack has actually occurred. Similarly, it may be unclear whether casualties that present are suffering from the intentional release of a biological or chemical agent, or due to a naturally occurring infectious disease outbreak or a toxic industrial exposure. We recommend a ten-step process to guide medical personnel in the evaluation and management of outbreaks of unknown origin and etiology. Such an algorithmic approach—which incorporates elements of the Advanced Trauma Life Support (ATLS) course sponsored by the American College of Surgeons[2]—is desirable when dealing with the unknown, especially under austere conditions or amid the chaos of the battlefield. The development of this approach has been detailed elsewhere[3-5] and a greatly expanded version is available in the *Textbook of Military Medicine* (TMM).[6]

1. Maintain an Index of Suspicion

In the case of chemical or conventional warfare and terrorism, the sinister nature of an attack may be immediately obvious. Victims would likely succumb in close temporal and geographic proximity to a dispersal or explosive device, clustered in time and space. Complicating the discovery of a biological attack, however, is the fact that bio-agents possess inherent incubation periods, typically days to even weeks in length, which permit the wide dispersal of victims (both spatially and temporally) after exposure and infection. Moreover, they make it likely that the first responder to a biological attack would not be a traditional first responder (fireman, policeman, paramedic), but rather fixed facility medics, or primary care providers (physicians, physicians assistants, nurses), emergency department personnel, and public health officials. In such circumstances, the maintenance of a pre-existing index of suspicion is essential if a timely diagnosis is to be made and prompt therapy instituted.[7] This is especially the case at lower echelons of care, remote from diagnostic and consultative resources.

For many of the diseases typically regarded as potential bio-weapons, very early intervention is mandatory if an optimal outcome is to be achieved. Anthrax, botulism, plague, and smallpox are readily preventable if patients are provided proper anti-infectives, antisera, or vaccination following exposure. Conversely, all of these diseases may prove fatal if prophylaxis or therapy is delayed until symptoms develop. Unfortunately, symptoms in the prodromal phase of these illnesses are non-specific, making diagnosis difficult. Furthermore, many bio-agent-induced diseases, such as brucellosis, Q-fever, and Venezuelan equine encephalitis (VEE), often present simply as undifferentiated fevers. In such cases, epidemiologic clues might prove helpful in placing the available information in the proper context. (See the section on "Distinguishing Between Natural & Intentional Disease Outbreaks"). On the battlefield, the M1135 Stryker NBC Reconnaissance Vehicle possesses a robust suite of sensors and diagnostic systems which might provide early warning of CBRN attack.

2. Protect Yourself

Before medical personnel approach a potential CBRN casualty, they must first take steps to protect themselves. These may involve a combination of physical, chemical, and immunologic modalities. On the battlefield, *physical protection* typically includes a protective mask. Designed primarily with chemical vapor hazards in mind, the M-40/42, M-45, and M-50/51/53 series masks certainly provide adequate protection against all aerosolized BW threats. In fact, a HEPA-filter (or even a simple surgical) mask will often afford adequate protection against bio-agents, although not against chemical threats. *Chemical protection* refers, in general, to the pre-exposure or post-exposure administration of antibiotics; such strategies are discussed on an agent-specific basis in the relevant sections of this book. *Immunologic protection* involves active vaccination and applies mainly to protection against anthrax, smallpox, and possibly Ebola. Specific vaccination strategies are discussed throughout this book. Obviously, not all of these protective strategies would be applicable in every situation.

3. Assess the Patient

This initial assessment is somewhat analogous to the primary survey and ABCDE algorithm of ATLS management. As such, airway adequacy should be assessed, and breathing and circulation problems addressed, before attention is given to specific management. This initial assessment is conducted before decontamination is accomplished and should thus be brief, but the need for decon and for the administration of antidotes for rapid-acting chemical agents should be determined at this time.[8]

4. Decontaminate as Appropriate

Decontamination plays an important role in the approach to chemical casualty management. Conversely, the incubation period of biological agents makes it unlikely that victims of a biological attack will present for medical care until days after exposure. At such a late point—given that the victim has likely bathed and changed clothing several times, effectively accomplishing self-decontamination—the need for further intervention in this regard is minimal or non-existent. In those exceptional cases where decontamination is warranted, simple soap and water bathing will usually suffice. Certainly, standard military decontamination solutions, typically employed in cases of chemical agent contamination, will be effective against all biological agents. In fact, even 0.1% bleach reliably kills anthrax spores, the hardiest of bio-agents; however, the use of caustic substances, especially on human skin, is rarely warranted following a biological exposure. More information on decontamination for bio-agents (and on the management of scenarios involving announced threats, empty letters, suspicious packages, and delivery devices) is included in the section on Decontamination in this book as well as in the manuals at Reference 1.
It should also be kept in mind that a biological attack constitutes a criminal act and that hasty or ill-considered decontamination risks destroying valuable forensic evidence.

5. Establish a Diagnosis

With decontamination (where warranted) accomplished, a more thorough attempt to establish a diagnosis can be conducted. This attempt, somewhat analogous to the secondary survey of ATLS, should involve a combination of clinical, epidemiological, and laboratory examinations. The ATLS "A.M.P.L.E." mnemonic provides a helpful approach to obtaining a medical history. Information about illnesses among other unit members or co-workers, the presence of unusual munitions or dispersal devices, food and water procurement sources, vector exposure, vaccination history, travel history, occupational duties, and MOPP (or other PPE) status may all be relevant. Physical exam at this point should concentrate on the pulmonary and neuromuscular systems, as well as unusual dermatologic manifestations.

Resources available to a clinician vary by echelon of care. At higher echelons, a full range of lab capabilities might enable prompt definitive diagnoses. At lower echelons, every attempt should be made to obtain diagnostic specimens from representative patients; these should be forwarded through lab channels. Nasal swabs (important for culture and PCR, even if the clinician is unsure *which* organisms are present), blood cultures, serum, sputum cultures, blood and urine for toxin analysis, throat swabs, and environmental samples might be considered. In no case, however, should the performance (or unavailability) of lab studies delay expeditious empiric diagnosis and therapy.

Diagnostic Matrix: Chemical & Biological Casualties

	Rapid-Onset	**Delayed-Onset**
Respiratory Casualties	Cyanide High-Dose Mustard High-Dose Lewisite High-Dose Phosgene High-Dose SEB Inhalation	Inhalational Anthrax Pneumonic Plague Pneumonic Tularemia Q-Fever Ricin Inhalation Low-Dose Mustard Low-Dose Lewisite Low-Dose Phosgene Low-Dose SEB Inhalation
Neurologic Casualties	Nerve Agents	Botulism (Peripheral Symptoms) VEE (Central Symptoms)

While awaiting lab confirmation, a physician should attempt to make a clinical (presumptive) diagnosis. Access to infectious disease, preventive medicine, and other specialists, can assist in this process. At lower echelons, where such consultation may be unavailable, the clinician should, at the very least, be familiar with the concept of syndromic diagnosis. Chemical and bio-agent diseases can be divided into those that present almost immediately, with little or no incubation period (principally the chemical agents) and those with a considerable delay in presentation (principally the biological agents). Moreover, bio-agent-induced diseases are likely to present as one of a limited number of clinical syndromes. For example, plague, tularemia, and SEB may present as pneumonia. Botulism and VEE may present with peripheral and central neuromuscular findings, respectively. This knowledge lends itself to the construction of a simple diagnostic matrix as shown in Table 1. Even basic syndromic diagnosis, however, is complicated by the fact that the incapacitating bio-agents (VEE, Q-fever, brucellosis) present simply as undifferentiated febrile illnesses, and may remain as such, whereas the lethal bio-agents (anthrax, plague, tularemia, smallpox) present as undifferentiated febrile prodromes initially, but then progress, sometimes dramatically.

6. Render Prompt Treatment

Unfortunately, it is precisely in the prodromal phase of many diseases that therapy is most likely to be effective. For this reason, empiric therapy of pneumonia or undifferentiated febrile illness on the battlefield, or in a potential bio-terrorism scenario, might be indicated under certain circumstances. Table 2 was constructed by eliminating from consideration those diseases which definitive therapy is not warranted, not available, or not essential. Empiric treatment of respiratory casualties might then be entertained, given that

prodromal anthrax, plague and tularemia would be managed similarly. Doxycycline, for example, is effective against most strains of *Bacillus anthracis, Yersinia pestis,* and *Francisella tularensis,* as well as against *Coxiella burnetii*, and the *Brucellae*. Other tetracyclines and fluoroquinolones might also be considered. Similarly, rapid-onset respiratory casualties might be treated empirically using a cyanide antidote kit, while rapid-onset neurological casualties might warrant prompt empiric therapy with a nerve agent autoinjector. It should be noted that empiric therapy is not a substitute for a careful and thorough diagnostic evaluation, when the environment is permissive of such.

CW & BW Diseases Potentially Requiring Prompt Empiric Therapy

	Rapid-Onset	**Delayed-Onset**
Respiratory Casualties	Cyanide	Inhalational Anthrax Pneumonic Plague Pneumonic Tularemia Q-Fever
Neurologic Casualties	Nerve Agents	Botulism

7. Practice Good Infection Control

Standard Precautions (see Appendix I) provide adequate protection against most infectious diseases, including those potentially employed in a biological attack.[9] Anthrax, tularemia, brucellosis, glanders, melioidosis, Q- fever, the alphaviral encephalitides, and the toxin-mediated diseases are not generally contagious, and victims can be safely managed using standard precautions. Under certain circumstances, however, one of three forms of Transmission-based Precautions (again, see Appendix I) would be warranted. Smallpox patients should, wherever possible, be managed using Airborne (and Contact) Precautions (including, ideally, a HEPA filter mask). Pneumonic plague warrants the use of Droplet Precautions (which include, among other measures, the wearing of a simple surgical mask), and certain viral hemorrhagic fevers (VHFs) mandate the use of Contact Precautions. Owing to the very low infectious dose of certain VHFs (as low as a single virion in the case of Ebola), many recommend enhancement of such contact precautions with full skin coverage, double or triple gloving, and an N-95 or PAPR-type respirator, when such equipment is available. For additional information see Appendix I – Patient Isolation Precautions.

8. Alert the Proper Authorities

In any military context, the command should immediately be notified of casualties potentially resulting from exposure to chemical or biological agents. The relevant clinical laboratory should also be notified. This will enable lab personnel to take proper precautions when handling specimens and will also permit the optimal use of available diagnostic modalities. Chemical Corps and preventive medicine personnel should be contacted to assist in the delineation of contaminated areas and the search for additional victims.

In a civilian context, notification should be made through local health department channels. In the U.S., larger cities often have their own health departments. In most other areas, the county health department represents the lowest echelon public health jurisdiction. In some rural areas, practitioners would access the state health department directly. Once alerted, local and regional health authorities can assist in requesting additional support. Every medical practitioner should have points of contact at such agencies and should be familiar with mechanisms for engaging them *before* a crisis arises.

9. Assist in the Epidemiologic Investigation and Manage the Psychological Consequences

All health care providers should have a basic understanding of epidemiological principles.[11] Even under austere conditions, a rudimentary outbreak investigation may assist in diagnosis and in the discovery of additional victims. Clinicians should educate patients about illness onset and symptoms, potential exposures, ill unit members or co-workers, food/water sources, unusual munitions and vector exposures. Early discovery of additional cases through an expedient outbreak investigation might inform the need for post-exposure prophylaxis (PEP), thereby preventing additional morbidity and mortality. Public health officials would normally conduct more formal and thorough epidemiologic investigations and should be contacted as soon as one suspects the possibility of a biological attack. In a military setting, preventive medicine officers, field sanitation personnel, epidemiology technicians, environmental science officers, and veterinary officers are available to assist the clinician with an epidemiologic investigation.

In addition to implementing specific medical countermeasures and initiating the outbreak investigation, the clinician must be prepared to address the psychological effects of a known, suspected, or feared exposure. Such exposure (or threat of exposure) will likely provoke anxiety, even panic, in a community, and may result in overwhelming numbers of patients seeking urgent medical evaluation. Many of these may have unexplained symptoms and may demand antidotes, antibiotics, or other therapies. Moreover, symptoms due to anxiety and autonomic arousal, as well as the side effects of PEP may suggest prodromal disease due to bio-agent exposure and may pose challenges in differential diagnosis. This behavioral contagion is best preempted by robust, proactive,

risk communication from public health and other governmental authorities.[11] This should include a realistic assessment of the risk of exposure, information about the resulting disease, steps to be taken, and points of contact for those who suspect exposure. It must be timely, accurate, consistent, and well-coordinated.

Effective risk communication is predicated upon the existence of detailed risk communication plans (many are available from the CDC). Proactive planning is also critical in successfully and rapidly deploying resources for the initial evaluation and administration of PEP. (Ideally, this will be decentralized to the unit level on the battlefield or to residential areas in a civilian context.) Plans should be made proactively to implement patient and contact tracing, as well as vaccine screening where appropriate. Finally, responders must be familiar with procedures necessary to access stockpiled vaccines and medications, and to identify and prepare local facilities and health care teams for the management of mass casualties.

10. Maintain Proficiency and Spread the Word

Fortunately, the threats of biological warfare and bioterrorism have, to date, largely remained theoretical. An inability to continually practice casualty management, however, can lead to a rapid loss of knowledge and skills. Medics and corpsmen must maintain proficiency in dealing with this low-probability—but high-consequence—problem. This can be done, in part, by availing oneself of several resources.[12,13] The USAMRIID web site[14] provides a wealth of information, including the full text of this handbook, as well as links to other useful sites. Many additional training aids are also available, and the previously mentioned field manuals[1] and relevant *TMM* volume, summarize bio-agent disease management guidelines. Finally, medical personnel, once cognizant of the threat and how to mitigate it, must ensure that their less informed colleagues receive training as well. It is only through ongoing education that personnel will be prepared for the threat posed by biological weapons. By familiarizing yourself with the contents of this handbook, you will have taken a significant step towards such readiness.

EMERGING THREATS: NOVEL INFECTIOUS DISEASES & EMERGING DISRUPTIVE BIOTECHNOLOGY

Context for the Joint Force

The 2023 Biodefense Posture Review correctly highlights the complexity and changing nature of the modern biothreat landscape. Biological threats, regardless of origin, pose tremendous risks to mission and force, threatening the Department of Defense's ability to execute its national defense objectives. The modern Joint Force faces risks from endemic disease, emerging infectious disease, traditional biological warfare agents, and increasing risks associated with novel biotechnologies that might challenge deployed forces with new and unknown infectious disease or toxin related threats. Additionally, the widespread availability of new biotechnologies like clustered regularly interspaced short palindromic repeats (CRISPR) coupled with the many technical challenges of attribution in biological warfare may be lowering the perceived thresholds for the employment of novel agents by competitors to achieve strategic effects. Although risk is difficult to quantify for biological threats, in this chapter we will briefly address threats with perhaps the most potential for major to extreme consequence, namely emerging infectious diseases with pandemic potential and new emerging disruptive biotechnologies.

Emerging Infectious Diseases

Emerging infectious diseases were defined in a landmark report by the Institute of Medicine in 1992[1] and include those infectious diseases that are: (1) newly recognized as occurring in humans (or animals or plants), (2) newly occurring in a different population or geographic region, (3) affecting greater numbers of individuals, or (4) evolving important new attributes (e.g., antimicrobial drug resistance or increased virulence). Even though some "emerging" diseases have been recognized for more than 30 years (e.g., AIDS, Lyme disease, Ebola virus disease, Legionnaire's disease), their importance has not diminished. Since the last edition was published, there have been several important emerging threats identified, and several worrisome trends with previously preventable and treatable infections. Fungal infection like recently discovered *Candida auris* has caused large outbreaks in medical facilities on multiple continents.[2] Increasingly worrisome outbreaks of vaccine-preventable diseases like measles, mumps and pertussis have been caused not only by waning herd immunity, but political movements against routine immunizations. The wise clinician should remain wary to the possibility of these naturally occurring outbreaks being used to military advantage in unconventional ways. A high index of suspicion should be maintained given new genetic engineering technologies that could render such infections undetectable by existing systems, more virulent and/or resistant to countermeasures.

Many factors contribute to the emergence of new infectious diseases, most notably environmental (including climate) changes, increased global travel and trade, social and political upheaval (including military conflicts), and genetic changes in microbial agents, hosts, or vector populations. Once a new disease is introduced into a susceptible human population, it may spread rapidly and could challenge the medical and public health infrastructures. If the disease is severe, it may lead to social disruption and cause severe economic impact. It should be noted that these effects could be seen not only with a new human disease, but also with diseases of crops and/or food production animals. Outbreaks of novel infectious agents such as Ebola virus, COVID-19, and novel influenza viruses appear to be occurring with increasing frequency and with a greater potential for serious consequences. In addition, there is increasing instances of viruses appearing in new geographic regions, as with the case of West Nile virus in the United States in 1999, chikungunya virus in the Caribbean in 2013, Ebola virus in West Africa in December 2013, and the MPOX outbreaks of 2022 and 2024.

In a 2008 study funded by the National Science Foundation (NSF) and published in *Nature*, about two-thirds of emerging infections were found to be zoonotic (animal in origin) and the majority of those came from wild animals (e.g., monkeypox, coronaviruses, Ebola virus).[3] Important geographic areas of emergence include Sub-Saharan Africa, India, China, and South America. New pathogens may be transmitted directly by hunting or accidental contact with wildlife, while others may be transmitted from wildlife to livestock to people (e.g., Malaysia's Nipah virus or Australia's Hendra virus). Humans have evolved little resistance to zoonotic diseases, so these diseases can be extraordinarily lethal.

About 20% of known emerging infections are caused by multidrug-resistant strains of previously known pathogens, such as *Mycobacterium tuberculosis*. Wealthier nations' increasing dependence on, and misuse of, antibiotics amplifies the proliferation of such dangerous variants of common bacteria. An example is enterotoxigenic *Escherichia coli*, now having spread widely and with great speed because products like raw vegetables are processed in huge, centralized facilities, and hastily packaged for rapid onward shipment and consumption.

Emergence of pandemic influenza, Ebola virus, Marburg virus, novel coronaviruses, anthrax, West Nile virus, prion diseases, multidrug-resistant tuberculosis (MDR-TB), and scores of other new diseases remind clinicians and public health officials to remain ever vigilant for outbreaks of novel or unexplained diseases. These emerging infections have a potential to become future biological threats on a large scale, as indeed some of them already have. Natural emerging disease outbreaks may be difficult to distinguish from the intentional introduction of infectious diseases for nefarious purposes; hence, consideration must also be given to this possibility before any question of etiology is considered settled. Because emerging infectious diseases are so diverse, exotic, and vary enormously according to geographic location, their

complete description is beyond the scope of this handbook. Summaries of a few recent emerging infections follow, but one should be mindful that the most worrisome pathogen may well be the one not yet recognized.

Pandemic Influenza

The threat for pandemic spread of human influenza is substantial. The pathogenicity of influenza viruses is directly related to their ability to rapidly alter their eight viral RNA segments. New antigenic variation results in the formation of new hemagglutinin (HA) or neuraminidase (NA) surface glycoproteins, which may go unrecognized by an immune system primed against heterologous strains. Influenza typically begins with abrupt onset of fever, chills, headaches and myalgias, often involving the upper and lower respiratory tract with development of cough, dyspnea, and, in severe cases, acute respiratory distress syndrome (ARDS). Laboratory findings may include pancytopenia, lymphopenia, elevated liver enzymes, hypoxia, positive RT-PCR and positive neutralization assay for the specific virus.

Two distinct phenomena contribute to a renewed susceptibility to influenza infection among persons previously infected. Clinically significant variants of influenza A viruses may result from mutations in the HA and NA genes, expressed as minor structural changes in the viral surface proteins. As few as four amino acid substitutions in any two antigenic sites can cause a clinically significant variation. These minor changes result in an altered virus able to circumvent host immunity. Additionally, genetic reassortment between avian and human, or avian and porcine, influenza viruses may lead to major changes in HA or NA surface proteins known as antigenic shift. In contrast to the gradual evolution of strains subject to antigenic drift, antigenic shift occurs when an influenza virus with a completely novel HA or NA formation moves into humans from other host species. Global pandemics such as the one in 2009 have resulted from such antigenic shifts.

Influenza causes more than 30,000 deaths and more than 100,000 hospitalizations annually in the U.S. Pandemic influenza viruses have emerged regularly in 10- to 50-yr cycles for the last several centuries. During the 20th century, influenza pandemics occurred four times. The 1918 influenza pandemic illustrates a worst-case public health scenario: it caused 675,000 deaths in the U.S. and 20-40 million deaths worldwide. Morbidity in most affected communities was between 25 and 40%; case fatality rates (CFRs) averaged about 2.5%, compared with the 0.1% in more typical flu outbreaks (a 25-fold increase). The 1957-58 pandemic caused 66,000 excess deaths, and the 1968 pandemic caused 34,000 excess deaths in the United States. The most recent 2009 California A/H1N1 “swine flu” pandemic began in the U.S. and Mexico but spread rapidly around the globe. Fortunately, the 2009 H1N1 virus had a much lower case fatality rate (0.01–0.03% of those infected), making it considerably less lethal than previous pandemic strains (1918 virus was 100 times more lethal). In contrast to the 1918 virus, the 2009 flu virus contained

genes from five different flu viruses: North American swine influenza, North American avian influenza, human influenza, and two swine influenza viruses typically found in Asia and Europe.[4]

Avian Influenza

Wild aquatic birds are the reservoirs of all subtypes of influenza A virus, where they generally cause no harm. Transmission from aquatic birds to humans was originally hypothesized to require infection of an intermediate, such as a pig, that has both human-specific and avian-specific receptors on its respiratory epithelium. Now scientists understand that influenza A viruses can transmit directly from birds to humans. Pigs remain a natural mixing vessel for flu because they can be infected both by avian and human strains allowing for the reassortment before the microbe moves on. Avian influenza virus usually refers to influenza A viruses primarily found in birds; however, occasional confirmed cases of human infection with several subtypes of avian influenza virus have been reported since 1997. Most human cases of avian flu have resulted from direct contact with infected poultry (e.g., domestic chickens, ducks, and turkeys) or surfaces contaminated with secretion/excretions from these birds. The spread of avian influenza viruses from an ill person to another person has been reported only very rarely, and transmission has been limited, inefficient, and unsustainable.

An epizootic of highly pathogenic avian influenza virus (HPAI H5N1) emerged in Southeast Asia in 2003 before spreading to other continents, mostly in animals (poultry, aquatic birds), but also in humans. By January 2011, over 6,780 animal outbreaks of HPAI H5N1 had been reported in 51 countries. In July 2013, the WHO announced a total of 630 confirmed human cases which resulted in the deaths of 375 people since 2003.[5] Disease caused by another avian flu virus (H7N9) was first reported in March 2013 in China. By May of that year, 37 people had died from the infection. As of April 2014, the virus has infected 419 people, leading to 127 deaths.[6] Additionally, at the time of this writing in 2024, several states experienced outbreaks of H5N1 in livestock with a small number of human cases, mostly from direct contact with livestock. While the risk of human-to-human transmission and overall risk from these avian influenzas appear to have subsided, the development of widespread human transmission would be a true emergency.

There are several drugs available to treat influenza, including oseltamivir, zanamivir and amantadine that may have promise in outbreaks, regardless of influenza strain. However, efficacy is modest, reducing the length of clinical infection by 1-2 days at most. There has been some limited demonstration of reductions in complications including pneumonia in adults, but not otitis media or bronchitis in children. There have been no demonstrations of mortality benefit or reductions in severe disease as might result from a biological attack.[7] A systematic review indicated that neuraminidase inhibitors may be effective against pandemic or novel variant influenza though evidence remains limited.[8]

Influenza vaccines may be highly effective against some strains in an outbreak, but this has been shown to be highly variable with reduced effectiveness against H3N2 subtypes compared to pandemic H1N1pdm09 in one systematic review.[9] Delays in production of strain-specific vaccines may prove a critical rate-limiting step in response to novel variant and pandemic influenza outbreaks.

Novel Coronaviruses (SARS and MERS)

The COVID-19 pandemic originated in Wuhan, China on 1 December 2019. The ensuing pandemic caused by the SARS-CoV-2 virus spread rapidly across the globe, ultimately killing more than 7 million people. Theories of origin vary widely from lab derived viral strains to zoonotic transfer in local meat markets.

While quarantine was deemed by experts as the best way to halt the spread of prior coronavirus outbreaks such as SARS 9 (see below), the large number of subclinical cases and inability to distinguish COVID-19 from other common respiratory infections proved more challenging. Numerous nations and their airlines moved quickly to shut down flights to China and neighboring affected countries. An unprecedented attempt to lock down exodus of Wuhan citizens appears to have been only modestly effective at stemming the spread. The U.S. CDC rapidly developed a real-time PCR molecular diagnostic panel making early detection possible through a network of reference laboratories.[10] The experimental antiviral remdesivir, a nucleoside analogue was provided to treat selected cases under emergency use authorization, and two clinical trials planned in China.[11]

The most recent prior example of zoonotic spread of a new infectious disease was the emergence of severe acute respiratory syndrome (SARS) in Southeast Asia in 2003 due to a novel coronavirus that jumped species from animals to humans, rapidly spreading to 29 countries in less than 90 days. Bats appear to be the natural reservoir of the virus, which ultimately infected a total of 8,273 individuals around the world and killed 775 (CFR = 9.4%).[12] Fortunately, the spread of SARS was fully contained with the last infected human case seen in June 2003 (disregarding a 2004 lab exposure).

In 2012, a new SARS-like illness emerged in Saudi Arabia.[13] A new species of coronavirus was isolated from sputum specimens of the index patient and given the name Middle Eastern respiratory syndrome (MERS)-coronavirus (CoV). Person-to-person transmission of MERS-CoV was confirmed in a cluster of over 30 hospitalized cases in the Al-Hasa governorate of Saudi Arabia, and by the end of 2013, there were 163 confirmed cases of infection resulting in 71 deaths (CFR = 44%).[14] The majority of these cases were from Saudi Arabia, but France, Italy, Jordan, Qatar, Tunisia, the United Kingdom, and the United Arab Emirates have all reported cases as well. Cases outside of the Middle East all had recorded recent travel to the Middle East.

Prior to 2003, only two coronaviruses were known to infect humans, and those caused only mild respiratory disease. Now there are at least six coronaviruses known to infect humans and many more that cause disease in a variety of animals. In addition to bats, serum surveys of livestock in Egypt, Oman, and Spain identified high levels of antibodies to MERS-CoV in dromedary camels.[15,16] Subsequently, MERS-CoV RNA was detected in three camels that had close association with two human cases. Human-human transmission occurs primarily in health care settings. Although MERS-CoV has a higher case fatality rate than SARS, mortality risk is greatest among older men with comorbidities.[17] While several small molecule antiviral and monoclonal antibody based therapies are now available for treating COVID-19 infection, their effectiveness against new variants or emergent strains cannot be predicted *a priori*. Novel coronaviruses, as with many other transmissible respiratory infections capable of causing high morbidity, pose a serious risk to readiness and the DoD's ability to project power. As with any novel infectious disease, approved diagnostics may not be available during the early stages of an outbreak, therefore syndromic triage for infection control mitigation and force health protection should be considered.

Emerging Foodborne Disease – *Escherichia coli* O104:H4

In the summer of 2011, two separate outbreaks of bloody diarrhea and hemolytic-uremic syndrome (HUS) occurred in Europe.[18,19] One was centered in Germany and comprised 3,816 cases of bloody diarrhea, 845 cases of HUS and 54 deaths; whereas the other occurred in France and comprised 15 cases of bloody diarrhea, 9 of which progressed to HUS. These were not caused by *E. coli* O157:H7, the typical bacterial cause of HUS, but a more virulent form of Shiga toxin-producing *E. coli* O104:H4. The outbreak had a much higher frequency of HUS and death than previously seen. An epidemiological investigation determined that contaminated sprouts were the source, with distribution from Egypt to Europe. Rapid whole genome sequencing was used to fully characterize the *E. coli* from the 2011 German outbreak in near real-time, determining that the bacterium was a hybrid of enteroaggregative and enterohemorrhagic *E. coli* containing a prophage encoding a Shiga toxin.

Obviously, this list does not do justice to the size and scope of emerging infectious disease threats, as even a modest listing of that type would take an entire volume. For example, at the time of writing this edition, outbreaks of emerging and re-emerging flavi and orthopox viruses are ongoing. That being said, this hopefully offers a sampling of a few relevant threats and encourages the reader to investigate biosurveillance resources for any area they are operating in. Excellent online resources can be found through the Armed Forces Health Surveillance Division (AFHSD) or the Centers for Disease Control and Prevention (CDC).

Novel Disruptive Biotechnology

The evolving bio-warfare/bio-terrorism threat is becoming more complex because of increased bio-agent variety, the increasing ease of *in vitro* genetic or host based genomic modification, and the convergence of biotechnology with AI. Novel genetic engineering and other advances in biotechnology provide powerful capabilities to modify virtually any bio-agent, affecting characteristics such as enhanced virulence, increased environmental stability, resistance to medical countermeasures, and defeat of physical barriers, bio-detectors, and laboratory diagnostics.

The type of situational awareness needed for a global pandemic overlaps with that needed to detect a major bio-terrorist campaign in its early stages. Along with the wider civilian community, the DOD has recognized that emerging infectious diseases could be harnessed for nefarious purposes. During the past 25 years, more than 30 novel lethal pathogens have been identified. In addition to the traditional bio-agents such as anthrax and plague, more familiar reemerging pathogens, such as influenza, represent significant future threats to both military and civilian populations. This is especially true since modern molecular biology techniques allow modified or completely new organisms to be made in the laboratory.

The DOD has placed increased emphasis on non-proliferation and emerging threats and recognize the challenge of developing countermeasures against non-traditional agents. Addressing these novel bio-agents is a central objective of the Homeland Security Presidential Directive (HSPD)-18, *Medical Countermeasures against Weapons of Mass Destruction* (2007), written in coordination with the Executive Office of the President, DOD, and Department of Health and Human Services (DHHS). HSPD-18 framed the biological threat spectrum into four distinct categories, the last three of which concern non-traditional agents.

(a) ***Traditional agents:*** naturally occurring microorganisms or toxins with the potential to be disseminated to cause mass casualties (e.g., *Bacillus anthracis* [anthrax] and *Yersinia pestis* [plague]).

(b) ***Enhanced agents:*** traditional agents that have been modified or selected to enhance their ability to harm human populations or circumvent current countermeasures, such as a bacterium that has been modified to be antibiotic resistant.

(c) ***Emerging agents:*** previously unrecognized pathogens that might be naturally occurring and present a serious risk to human populations (e.g., MERS-coronavirus).

(d) ***Advanced agents:*** novel pathogens or biologicals that have been artificially engineered in the laboratory to bypass traditional medical countermeasures or produce a more severe or otherwise enhanced spectrum of disease.

Bioengineered Threats

Without human intervention, the natural world has produced innumerable microbial threats that continue to emerge and cause new forms of disease. However, recently (in terms of human history), we have acquired the technical capacity to create microbial threats far more deadly than natural evolution could create. Genetic engineering, the intentional molecular manipulation of genes and/or genomes, has proven, like so many technologies, to have the capacity for both good and ill. A few examples from the open scientific literature are mentioned here to illustrate the seriousness of the threat of genetically engineered microorganisms (GEMs).

Antibiotic resistant strains of *B. anthracis* have been derived, not only by biological selection, but also more directly by genetic engineering. Scientifically, the capacity to do so with any bacterial threat is easily available. Similarly, for anyone moderately skilled in microbiology, it is obvious that otherwise harmless bacteria may be engineered to synthesize toxins made by unrelated lethal strains of bacteria. Buffering the threat, unauthorized conduct of most such experimentation has become not only difficult but illegal—subject to fines and incarceration—in many countries including the U.S. In the U.S, federally funded research that may result in knowledge that could be used for nefarious purposes, so called "dual use research of concern," or DURC, is subject to review prior to initiation of research and also at the stage of submission of the data for publication.

Today, viral genomes can quite easily be manipulated in the laboratory and infectious viruses can be generated from plasmid DNA. The progression of this technology with human pathogens began some 20 years ago with the simpler viruses (positive sense, single-strand, small genomes) such as poliovirus, alphaviruses, and flaviviruses. It has grown to include negative-strand viruses (e.g., vesicular stomatitis virus, respiratory syncytial virus, Ebola virus, and Crimean-Congo hemorrhagic fever virus) and segmented viruses (e.g., influenza virus). The relatively large genome of vaccinia virus can be derived from DNA cloned into bacteria. Even the capacity to derive a human pathogenic virus (poliovirus) completely by chemical synthesis was demonstrated.[20] Even more controversial were the efforts to genetically resurrect the 1918 influenza virus that killed some 20 million persons before disappearing and the proposals to genetically manipulate smallpox virus.[21,22] Perhaps the most prominent example of DURC in recent years came in late 2011, when two independent groups prepared to publish research studies in which mutations were introduced into highly pathogenic influenza H5N1 viruses that facilitated efficient transmission of the viruses in the ferret model, and thus presumably in humans as well.[23,24] As a result, research proposals for this type of study submitted for U.S. federal funding are subject to additional layers of review. It is expected that other countries will follow suit if they do not already have such a framework in place. Ultimately, the capacity to create deadly pathogens through genetic engineering is restrained in large part by technical knowledge and opportunity, and in the

final analysis, by intent. That is, what is straightforward for skilled scientists is impossibly difficult for the untrained and unequipped. This threshold is diminishing every day as technology advances and proliferates. Additionally, with the emergence of artificial intelligence, design of relevant modifications may become increasingly available to even those without the normal associated skillsets.

Bioregulators/Biomodulators

Bioregulators, or biomodulators, are biochemical compounds, such as peptides, that occur naturally in organisms. Advances in biotechnology have created the potential for the misuse of bioregulators as biological weapons. As bio-weapons, they could damage the nervous system, alter moods, trigger psychological changes, and kill. The potential weaponized use of bioregulators is somewhat similar to that of toxins. Many bioregulators can be used to cause illness, but only a few can threaten civilian populations on a large scale. If released upon a civilian population in sufficient quantity and concentration, they could pose significant challenges for public health and medical responses.

Biological response modifiers (BRMs) direct the myriad complex interactions of the human immune system. Examples of BRMs include erythropoietins, interferons, interleukins, colony-stimulating factors, stem cell growth factors, monoclonal antibodies, tumor necrosis factor inhibitors, and vaccines. A growing understanding of the structure and function of various BRMs has resulted in many novel compounds including synthetic analgesics, antioxidants, antiviral, and antibacterial substances. For example, BRMs are used to treat debilitating rheumatoid arthritis by targeting cytokines that contribute to the disease process, to reduce symptoms and decrease inflammation. Recently marketed BRM-based medications include etanercept (*Enbrel*) and infliximab (*Remicade*), both of which have been used to target the tumor necrosis factor alpha (TNF-α) cytokine, as well as anakinra (*Kineret*), which targets interleukin-1 (IL-1). More of these new drugs are currently in development. It can be easily imagined that research to develop various BRMs could be subverted to a malicious end. That is, instead of using BRMs to suppress cancer growth or disease susceptibility, such compounds could potentially be developed to have the opposite effect, causing illness and death to those exposed.

Synthetic biology and Emerging Disruptive Biothreats

Genome synthesis is no longer limited to the realm of viruses. In 2008, researchers described the complete chemical synthesis of all 582,970 nucleotides of the *Mycoplasma genitalium* genome.[25] The starting material for the synthesis was short oligonucleotides that can be purchased for $0.10 per base or less. Following closely on the heels of this achievement, the same group in 2010, reported the complete chemical synthesis of the 1.08 megabase-pair genome of *Mycoplasma mycoides*.[26] This genome was synthesized in a manner similar to that of *M. genitalium*, but they went one step further. They transplanted the

synthetic genome into the husk of a *M. capricolum* cell from which the normal genome had been removed. The cellular materials left behind after removing the normal genome were able to accept the new, synthetic genome and kick-start replication of the novel bacterium *Mycoplasma mycoides* JCVI-syn1.0 (named after the J. Craig Venter Institute where the work was performed).

As another example of dual use research of concern, Cappelluti et al recently demonstrated the ability to lower biosynthetic cholesterol levels in mice using a technique called gene silencing.[27] Gene silencing can modify the epigenome, without need of direct editing, by introducing mRNA that encodes for targeted DNA-binding proteins that silence the transcription of genes of interest. While promising as a medical breakthrough in several regards, the obvious dual use nature of these types of technologies create significant challenges in that no detection modalities or countermeasures to reverse these effects exist. With converging advances in genomics and artificial intelligence, it is easy to imagine the design of highly targeted biological threats that mask detection, attribution, and that potentially are tailored to achieve specific tactical, operational, or strategic effects. Figure 1, adapted from the 2023 Biodefense Posture Review, highlights the promise and challenges posed by new technologies in this area.[28]

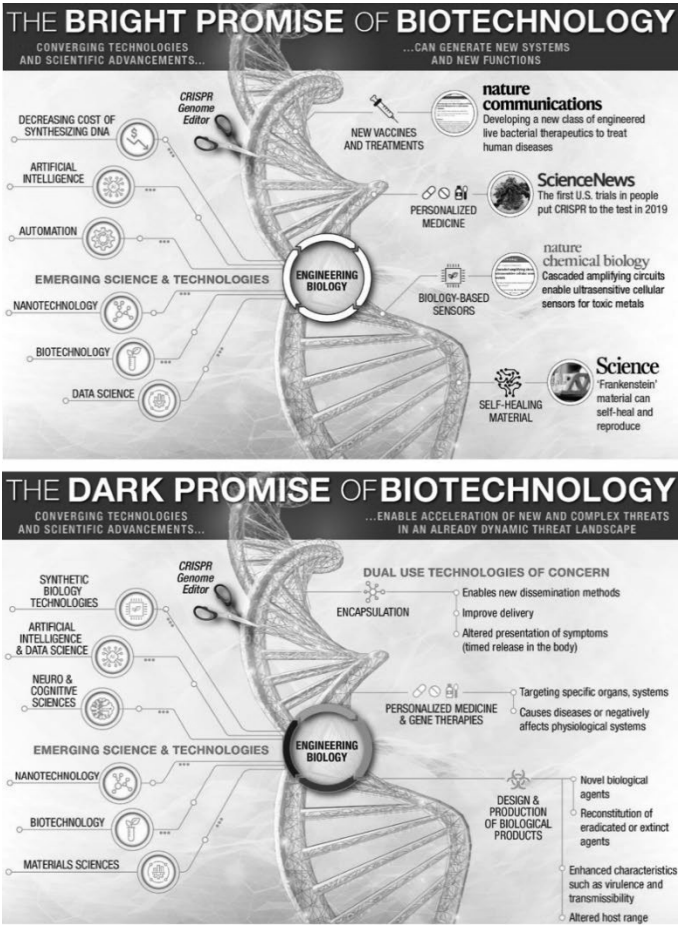


Figure 1: Adapted from the 2023 Biodefense Posture Review. This figure highlights the promise and challenges posed by rapid advances in biotechnology.

The concept of genomic warfare is highly speculative and beyond the scope of this handbook. Undoubtedly, as scientific understanding of this technology increases and becomes more widely available, the threat of the development and use of genomic weapons will increase as will the challenge to develop effective medical countermeasures. Ultimately, the capacity to create deadly new organisms through genetic engineering is restrained by technical knowledge and opportunity. However, a determined person with the appropriate knowledge, skills, or access to personnel with such skills may succeed in malevolent creation of GEMs. As scientists develop more sophisticated laboratory procedures and increase their understanding of molecular biology and the genomics of both the pathogens and of humans, the possibility of bioengineering

virulent, antimicrobial-resistant, and vaccine-resistant bacteria and viruses (or other as yet unknown pathogens) for nefarious uses will increase. Although fielded traditional PCR based diagnostics are largely blind to emerging threats, the adoption of field-forward sequencing will offer a powerful new tool to inform Force Health Protection decisions until clinical diagnostics emerge. Additionally, clinicians should remain vigilant to unusual symptom clusters and continue to diagnose using syndromic categorization while maintaining a healthy index of suspicion.

EMERGING THREAT AGNOSTIC COUNTERMEASURES

Developments in genetic and biological engineering, additive manufacturing, and drone technologies are converging.[1] These developments threaten to greatly heighten the risk of biological attack. Both state and nonstate actors are likely to attempt to harness these capabilities to engineer and manufacture new threats. Drone technology will make it simpler to disseminate aerosolized pathogens with a limited ability to detect or counter these threats. Perhaps most worrisome, there may be new threats produced from the use of these technologies, specifically designed to overcome existing counter measures. Many of the threats described in this book still lack specific counter measures despite decades of research.

As a result, the imperative to develop broad nonspecific measures, so-called threat agnostic counter measures, is imperative. The long development times for new drugs, vaccines, and biologics may soon render conventional approaches to bio-warfare countermeasures obsolete. The goal will shift from preventing or treating specific diseases to protecting the host from a much broader range of threats. Primary emphasis will be on preventing the results of severe disease, such as mass casualties, death, and degradation of medical resources.

Along these lines, there have been important developments that may help to shift this traditional paradigm. The COVID-19 response and the host of countermeasures tested during the pandemic shed light on some of these new possibilities. It is the authors hope that by the next addition of this handbook, some of these emerging technologies will be approved available for use. However, at the time of this writing, most outlined are still in development and not FDA cleared for the proposed indications.

Since the publication of the 9th Edition, there has been significant and rapid advancement of disease-agnostic therapies. These include such innovations as targeted anti-inflammatory agents, often referred to as patient-centered treatments, pathogen extraction devices, and other extracorporeal therapies. A few examples follow. These devices and combination therapies may soon serve as non-disease specific means for rescuing critically ill patients and be useful to the field of biodefense countermeasures. This is likely to be increasingly important and may even supplant the conventional “bug-drug” model of development for biodefense countermeasures.

Advances in Diagnosis

Conventional pathogen diagnostics rely on individual pathogen tests, often done in parallel or in series. As outlined in the remainder of this handbook, there remains significant limitations for the diagnosis of many existing threats. The current, state of the art, namely multiplexed, broad spectrum PCR platforms, while an important and useful advance, remain limited, particularly for point-of-care applications. While significant advances in the field were made during

the COVID-19 pandemic, genetic and multi-omic testing methods may soon begin to replace conventional tests for individual organisms. High-throughput next generation sequencing (NGS) technology paired with genetic libraries of pathogenic organisms may soon yield faster diagnoses from single samples using a single testing run. This may substantially reduce the cost and time to diagnosis.[2]

Advances in Prevention

In a biological attack, regardless of etiology, general preventive measures and disinfection reduce the risk of pathogen exposure and spread. Personal protective equipment is useful in areas of known biological threats or contamination from disinfectants essential to the clean-up of contaminated areas. Various forms of light along the electromagnetic spectrum have been proposed as potential disinfectants. These can be combined with photosensitizing agents designed to potentiate the killing efficiency against viruses, bacteria, fungi and even parasitic agents.[3] Such an approach is less susceptible to development of resistance. Blue light at wavelengths of 405-470nm has been shown to have significant species-agnostic reductions in a wide array of bacterial pathogens.[4] This has already been demonstrated in the decontamination of plasma and other resuscitation fluids.[5] Blue light is also currently being used on pre-operative patients to reduce bacterial load and post-op inflammation and outcomes (clinicaltrials.gov NCT0348224).

Emerging pre-exposure vaccine approaches may boost innate immunity to mitigate a wide range of pathogens. Studies have shown beneficial off-target effects of vaccines including BCG (Bacille Calmette-Guerin) and measles against a broad array of childhood illnesses.[6] This includes pathogen agnostic vaccines which can be employed as early as the neonatal period.[7] A universal mosquito saliva vaccine has also shown promise in preventing a range of vector-borne diseases.[8]

Advances in Treatment

Following exposure to biothreats, disease-agnostic interventions employing a multitude of technologies may mitigate pathogenesis through the subclinical, clinical and decompensated stages of disease. Subclinical disease progression may be mitigated by post-exposure prophylaxis employing alternatives to conventional antimicrobial agents. Host-directed antimicrobial therapies (HDT) are currently in development that offer the potential for pathogen agnostic treatment against a variety of agents. Earlier intervention to boost host responses is likely to improve outcomes. During the clinical stage, onset of fever and viremia/septicemia may benefit from early immunomodulatory therapy in addition to specific treatments aimed at suspected pathogens. That being said, understanding the optimal timing and approach to immunomodulation and related interventions for given threats are critical.

Viral threats pose the greatest challenges for developing countermeasures given viral takeover of the host's cellular machinery, and the need to target host cells. There have been a wide variety of approaches to host-directed antiviral therapies. HDT development aims to reduce virulence and viral-induced dysfunctional inflammation. HDTs augment host defenses by targeting critical factors that viruses use to invade and replicate in cells. The intent is to reduce organ damage, morbidity and even mortality without relying on narrow viral protein targets subject to adaptive changes. HDTs are relatively resistant to viral evolution and adaptation, which is the main contributor to conventional drug resistance.[9] The most significant advances in these therapies have been made in approved products for hepatitis B, C and HIV.

There has also seen a surge in development of alternatives to conventional antibiotics. While bacterial threats are less common, and relatively easier to treat, the potential for engineered threat strains with increasing virulence or resistance threatens existing limited countermeasures. These are, for the most part, limited to antibiotics such as tetracyclines, fluoroquinolones and aminoglycosides, and at least one approved anthrax vaccine. Some of the most promising approaches include use of antimicrobial peptides to include innate defense regulatory peptides (IDRP) and synthetic mimics of antimicrobial peptides (SMAMPs). Other less broad-spectrum alternative approaches include bacteriophage viruses, monoclonal antibodies, bacteriocins and bacterial lysins among others.[10]

As clinical disease and immune dysregulation progress, supportive care along with immunomodulators against known dysfunctional host factors may mitigate immune dysregulation and prevent tissue damage. Therapies aimed at direct immunomodulation of the host response are another promising class of threat-agnostic countermeasures. There was significant experience with development and expanded use of these agents during the COVID-19 pandemic. One of the most prominent examples was the use of corticosteroids such as dexamethasone (often in combination with the antiviral remdesivir) to prevent and treat severe COVID-19.[11] Janus kinase and IL-6 inhibitors, among others, were also tested extensively and found to be effective in reducing morbidity, mortality and/or length of hospital stay among those with severe COVID-19.[12-14]

Regenerative medicine technologies also represent an emerging threat-agnostic opportunity to treat infections and modulate immunity. Regenerative medicine will play an increasing role for insult-agnostic treatment, mitigation, and recovery from organ damage. Mesenchymal stem cells have been shown to play a role in treating and preventing infection.[15] Regenerative medicine includes a wide array of approaches, and is not limited to stem cell therapies. The use of extracellular vesicles (ECVs) derived from stem cells and carrying growth factors offer promise as immunomodulators for the treatment for sepsis.[16] A wide range of other technologies are in development and have recently been applied to COVID-19 and septic shock.[17]

As further immune dysregulation and end-organ damage progress to the decompensated stage, miniaturized, automated patient monitoring and extracorporeal life support systems (ECLS) may improve survival in critically ill patients. Automated patient monitoring systems combine non-invasive sensors with digitized patient care stations.[18] Systems are capable of continuous monitoring in austere environments, using simple cell phone applications and interfaces with clinical monitoring systems.[19] ECLS has enjoyed significant miniaturization, simplifying patient management, particularly in the areas of CO_2 filtration and hemodialysis.[20] As ECLS technologies progress and become less invasive, ECLS may be employed earlier in the continuum of care, obviating the need for invasive ventilation and sedation in some cases.

ECLS coupled with regenerative therapies and pathogen extraction technology may improve outcomes in critically ill patients. Early, minimally invasive pathogen extraction systems may prevent deterioration prior to onset of critical illness. An extracorporeal membrane capable of filtering cytokines and endotoxin is currently in clinical development and has shown promise in early trials.[21] Extracorporeal blood purification using other commercial membrane filters (such as AN96) were tested in randomized trials (clinicaltrials.gov NCT04033224, NCT04152174). An extracorporeal “biospleen” has demonstrated proof-of-concept in animals. The device uses opsonin-coated nano-magnets which bind to bacterial pathogens just as in the spleen, and then removes the magnetized pathogen-opsonin complexes .[22] Another novel extracorporeal device immobilizes allogeneic mesenchymal stem cells (MSC) in an in-line filter cartridge to mitigate the inflammatory cascade. By maintaining MSCs outside the body, the device (SBI-101) creates a natural environment and prevents them from differentiating as they normally would if injected. This creates sustained release of anti-inflammatory and regenerative signaling molecules. The device has undergone phase 2 trials for the treatment of acute kidney injury.[23]

PERSONAL PROTECTION

The DOD has currently fielded chemical protective equipment, which includes the protective mask, the Joint Services Lightweight Integrated Suit Technology (JSLIST) chemical protective overgarment (CPO)—which replaces the battle dress overgarment (BDO)—protective gloves; protective footwear covers, and multipurpose rain/snow/CW overboots (MULO) will effectively protect against a bio-agent attack.

Adopted in 2006, the M50/M51 replaced the M40 and M42 MCU2/P series masks and the M45 in the Land Warrior Program. The Joint Service General Purpose Mask (JSGPM) is a family of above-the-neck, Chemical and Biological (CB) respirators that protects against battlefield concentrations of Chemical/ Biological agents, toxins, toxic industrial materials, and radioactive particulate matter. The family consists of the M50 (ground use), M51 (ground vehicle use), M53 (Special Forces), and M53A1 (domestic and military use). The M53A1 can be used in either Air-Purifying Respirator, Powered Air Purifying Respirator, or Self-Contained Breathing Apparatus mode and is the first mask to be approved for both domestic response (National Institute for Occupational Safety and Health certified) and military missions. This lightweight, protective mask incorporates state-of-the-art technology. It is composed of heavy rubber, has a chlorobutyl/silicone base with a polynomial spline eye lens, includes a hydration port, and has a 50% performance improvement over the M40 for Joint force protection requirements. JSGPM replaced the M40/M42 series of protective masks for the Army and Marines and the MCU-2/P series for the Air Force and Navy. This allows it to harmonize with joint service vehicles, weapons, communication systems, individual clothing and protective equipment, and CBRN personal protective equipment. Proper maintenance and periodic replacement of the crucial filter elements is of utmost importance. The filter MUST be replaced when:

- The elements become immersed in water, crushed, cut, or otherwise damaged.
- Excessive breathing resistance is encountered.
- The “ALL CLEAR” signal is given after exposure to a bio-agent.
- Thirty days have elapsed in the combat theater of operations (also, once opened, the filters must be replaced every 30 days).
- Supply bulletins indicate lot number expiration.
- If ordered by the unit commander.

The filter element should be changed only in an uncontaminated environment. Optical inserts are available if the user requires corrective lenses. A mask's drinking tube can be used in a contaminated environment. The wearer should disinfect the canteen and tube by wiping with a 5% hypochlorite solution before use.

The JSLIST is available in seven sizes, woodland and desert patterns, and can be used for 45 days in an uncontaminated environment. Once opened, it can be laundered up to six times and may be worn for 24 continuous hours in a contaminated environment. The JSLIST is replaced using the MOPP-gear exchange procedure described in the Soldier's Manual of Common Tasks. The discarded suit should be incinerated or buried. Chemical protective gloves and overboots come in various sizes and are made from butyl rubber. They may be decontaminated and reissued. The gloves and overboots must be visually inspected and decontaminated as needed after every 12 hours of exposure in a contaminated environment. While the protective equipment will protect against bio-agents, it is noteworthy that even standard uniform clothing of good quality affords reasonable protection against dermal exposure to surfaces covered.

Those casualties unable to continue wearing protective equipment should be held and transported within patient protective wraps designed to protect the patient against further chem/bio-agent exposure. HCWs transporting such patients may consider adding a filter blower unit to generate overpressure, enhance protection, and provide cooling.

Collective protection can be achieved by using either a hardened or unhardened shelter equipped with an air filtration unit that provides overpressure and can protect personnel in a biologically contaminated environment. An airlock ensures that no contamination will be brought into the shelter. Without a dedicated structure, enhanced protection can be afforded within most buildings by sealing cracks and entry ports and providing air filtration with HEPA filters within existing ventilation systems. However, the availability of these shelters is limited, costly to produce and maintain, and difficult to deploy. Personnel should be decontaminated before entering the collective protection unit.

When considering the potential routes of transmission of a biological threat agent, it is essential to remember the most concerning route of exposure to bio-agents is the inhalational route. This makes wearing appropriate personal protective equipment, like masks, essential. Bio-agents can be dispersed as aerosols from point or line source disseminations. Unlike some chemical threats, aerosols of bio-agents disseminated by line source munitions (e.g., sprayed by low-flying aircraft or speedboats along the coast) do not leave hazardous environmental residue (although anthrax spores may persist and could pose a hazard near the dissemination line). In contrast, aerosols generated by point-source munitions (i.e., stationary aerosol generators, bomblets, etc.) are more apt to produce ground contamination, usually near the dissemination point. Point-source munitions leave a prominent signature that may alert the field commander that a BW attack has occurred. Because point-source munitions leave an agent residue, this evidence can be helpful in detection and identification.

Aerosol delivery systems for bio-agents most commonly generate invisible clouds with particles or droplets of < 10 mm. They can remain suspended for extensive periods. The primary risk in such an attack is pulmonary retention of inhaled particles. To a much lesser extent, some particles may adhere to an individual or their clothing, especially near the face. The effective area covered varies by factors, including wind speed, humidity, and sunlight. Without effective real-time detection and alarm systems or direct observation of an attack, the first clue of a BW attack may be mass casualties fitting a clinical pattern compatible with one of the bio-agents. This may occur hours, days, or weeks after an attack.

Toxins may cause direct pulmonary effects or be absorbed and cause systemic toxicity. They are frequently more potent by inhalation than by any other route. Mucous membranes, including conjunctivae, are also vulnerable to many bio-agents. Physical protection is essential, and using full-face masks equipped with small-particle filters, like chemical protective masks, assumes a high degree of importance.

Regarding force protection, other bio-agent delivery routes are considered less significant than inhalation but are nonetheless severe concerns. Contamination of food and water supplies, deliberately or incidentally after an aerosol attack, represents a hazard for infection or intoxication by ingestion. Determining whether food and water supplies are free from contamination is always important and should be made by appropriate preventive medicine authorities in case of a bio-attack.

Intact skin provides an excellent barrier against almost all bioagents—T-2 mycotoxins are an exception due to their dermal activity. However, mucous membranes, abrasions, or otherwise damaged integument can allow for the passage of some bioagents and should, therefore, be protected in the event of an attack.

While biological threat agents can cause illness and even death to their targets, healthcare workers should be particularly cautious when providing care to those infected with these bio-agents or suspected bio-agents. Healthcare workers should be familiar with the appropriate donning and doffing of personal protective equipment to ensure they do not inadvertently infect themselves by improper wear or removal of their protective garments. For additional information on infection control and patient isolation precautions, refer to Appendix I of this manual.

DECONTAMINATION

Biological contamination is the introduction of infectious agents to a body surface, food, water, or inanimate object. In this context, decontamination involves either disinfection, or sterilization to reduce microorganisms to a safe level on contaminated surfaces, making them suitable for use. *Disinfection* is the selective reduction of undesirable microbes to a level below that posing a transmission hazard. *Sterilization* is the elimination of all organisms.

For the control of infectious diseases, decontamination methods play a critical role. The most effective means of rendering microbes harmless (e.g., toxic chemical sterilization) may pose a hazard to humans, or damage equipment. Bio-agents may also be decontaminated by mechanical, chemical, and physical methods.

1) Mechanical decontamination involves measures to remove, but not necessarily neutralize, an agent. An example is drinking water filtration to remove certain water-borne pathogens (e.g., *Dracunculus medinensis, Naegleria fowleri*), or the use of an air filter to remove aerosolized anthrax spores, or soap and water to wash agent from the skin.
2) Chemical decontamination renders bio-agents harmless using disinfectants that may be a liquid, gas, or aerosol. Factors impacting effectiveness include contact time, solution concentration, composition of the contaminated surface, and characteristics of the agent to be decontaminated. Some disinfectants are harmful to humans, animals, the environment, and/or materiel.
3) Physical processes (heat, ionizing radiation, UV light) are other methods that can be employed for decontaminating objects.

It is important that, given the characteristic incubation periods of bio-agents, significant time elapses between the attack, and the patients' onset of symptoms. During this time, it is quite probable that external decontamination of any residual agent may have already occurred through natural means, bathing, and changing of clothes. Thus, it is only in rare circumstances that patients presenting with illness due to a biological attack will require purposeful external decontamination. Though there are not many examples of decontamination in a biowarfare setting, data suggest that current disinfection and sterilization practices are appropriate for managing patient-care, equipment, and environmental surfaces when potentially contaminated patients are evaluated and/or admitted to a health-care facility after exposure to a BW agent.

Skin Decontamination

Soap and water is the preferred method for skin decontamination. Dermal exposure to a suspected biological aerosol should be immediately and vigorously treated by soap and water washing. This removes nearly all the agent from the skin surface but does not kill BW agents, so the water runoff must be

collected and treated before disposal. It is also not advisable to use hot water for skin decontamination since it will open skin pores allowing BW agents to easily penetrate and absorb into the skin. For better results, use tepid or lukewarm water with soap.

Any material that can absorb a liquid and then be brushed or scraped from the skin without abrading the skin can be used as an effective skin or equipment decontaminant to remove liquid agents. A soft towel, baby wipes, clean sawdust, clay, dirt, baking powder, or fuller's earth, can be put on the agent found on the skin or equipment, allowed to be absorbed, and then carefully wiped away.

Reactive Skin Decontamination Lotion (RSDL) has been approved by the Food and Drug Administration for the decontamination of T-2 mycotoxins (in addition to chemical warfare agents). RSDL can be used for the decontamination of intact skin around wounds but is not approved for the decontamination of wounds or use near the eyes.

The use of 0.5 percent hypochlorite (½ percent, dilute household hypochlorite) solution is not recommended for BW agent skin decontamination because it poses risk of causing skin irritation and opens skin pores. If used, the 0.5 percent chlorine solution (9 parts water to 1 part chlorine solution) should be applied on the contaminated areas of the skin with gentle wiping of those areas so that contamination is not spread. It can then be left on the skin for several minutes and then rinsed with clean water. Its oxidation effects are limited, and its protective ratio is not significantly different than soap and water. Chlorine solution must NOT be used in open wounds and corneal opacities may result from chlorine solution being sprayed into the eyes. The 0.5 percent chlorine solution is used for skin decontaminant as a last resort.

Equipment Decontamination

For decontaminating fabric clothing or equipment, a 5% hypochlorite solution should be used, although many fabrics will be damaged with this concentration of hypochlorite. A 5% hypochlorite (full strength household liquid hypochlorite) solution is effective for decontaminating equipment and will kill BW agents. This solution should never be allowed to touch the skin as its alkalinity will redden, burn, and damage skin. Equipment decontaminated with hypochlorite should be thoroughly rinsed with water or soap and water before use. It is important that hypochlorite not be used on sensitive electronic equipment as it will cause oxidation and rust the equipment. This highly reactive oxidant solution will react with some chemicals. For decontaminating equipment, a contact time of 30 min before normal cleaning is required. This is corrosive to most metals and injurious to most fabrics, so it is recommended to rinse thoroughly and oil metal surfaces after completion.

Peracetic acid, a highly biocidal oxidizer, has also been tested in recent years as a method of removing surface contaminants. This solution will inactivate gram-

positive and gram-negative bacteria, fungi, and yeasts in less than five minutes. It is also effective in the presence of soil or organic matter but will take additional solution. Exposure to peracetic acid solution has resulted in elimination of viruses in 15 minutes and bacterial spores in 30 minutes.

Large amounts of soap and water also work well to decontaminate equipment contaminated with BW agents. However, soap and water removes, but does not kill, BW agents so runoff should be collected and killed with hypochlorite or sporicides.

Hypochlorite Solutions

Ampules of calcium hypochlorite—$Ca(ClO)_2$—are currently fielded in the Decontamination Medical Equipment Set (MES) for mixing hypochlorite solutions. The 0.5% solution can be made by adding one 6-ounce container of calcium hypochlorite to 5 gallons of water. The 5% solution can be made by adding eight 6-ounce ampules of calcium hypochlorite to 5 gallons of water (eight ounces of hypochlorite to every one gallon of water). Commercial off-the-shelf bleach can be used when access to the Decontamination MES is not available.

A 0.5% sodium hypochlorite—NaClO—solution is made of one part *Clorox* and nine parts water (1:9) as standard stock *Clorox* is a 5.25% sodium hypochlorite solution with an average pH about 12 which enables long term shelf storage. The solution is then applied with a cloth or swab. The solution should be made fresh daily with the pH adjusted to bring it into the acidic range. When specifically decontaminating for possible weaponized anthrax, a pH adjusted hypochlorite solution is preferred. At acidic pH values of 6.8 or lower, the hypochlorite solution will be 80 to 200 more times more antimicrobial than at the alkaline pH values at which it is manufactured and stored. A small amount of household vinegar is sufficient to lower the pH values to an acidic range.

Diluted hypochlorite at an alkaline pH is a relatively poor disinfectant, but acidified diluted hypochlorite will kill virtually anything in 10 to 20 min. Prepare the 5% hypochlorite solution as above with seven 6-ounce ampoules to four gallons of water and then add 32 ounces of household vinegar.

Recognizable brand names, such as *Clorox*, are recommended for use in these circumstances as branded products have generally been tested and found to be more consistent in quality. These solutions evaporate quickly at high temperatures. If made in advance, they should be stored in closed containers, preferably the containers should be made of plastic, but NOT metal, as the hypochlorite will cause the metal to corrode. Chlorine solutions should always be placed in distinctly marked containers, as without markings it is very difficult to tell the difference between the 5% chlorine solution and the 0.5% solution.

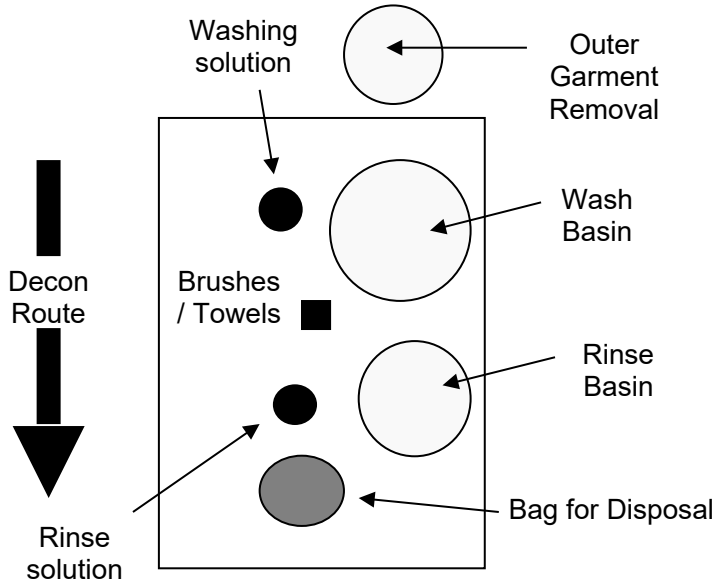
Bio-decontamination Line

A bio-decontamination line can be employed to limit cross-contamination. Small, makeshift lines can be constructed by placing a tarpaulin on the ground and using wash basins (e.g., small children's pools, or large trash bags). Using such a linear method will keep suspected contamination in the basins/bags as personnel move through the line. Movement should be from the "contaminated" end toward the "clean" end, and into the clean basins/bags, thereby leaving contaminated materials behind.

The following steps, correlated with the illustration, constitute one possible set-up for a bio-decontamination line for a small number of contaminated person (CPs).

Figure 2: Operation of a Suggested Bio-decontamination Line

1. Contaminated person (CP) steps into the first basin & removes outer garments & boots.
2. CP steps from the first basin to the wash basin.
3. CP is washed using soap and water.
4. CP steps into the rinse basin & rinses with fresh water.
5. The CP may now step into, or beside, one of the bags & place remaining clothing (undergarments & footwear) or other PPE in a bag for disposal. The disposal bag should be sealed & marked to prevent cross-contamination.
6. Attendant will escort the CP offline.



There are currently no handheld detectors for BW agents that would be appropriate for patient decontamination operations. The external decontamination measures will not address hazards associated with internal sources, such as bodily fluids. For certain highly infectious and communicable (transmissible) disease organisms (for example, Ebola, Marburg, and various hemorrhagic fevers) other associated hazards must be managed appropriately.

If re-aerosolization of agent is a concern, due to the presence of gross contaminant that has been removed from a victim, a damp cloth or towel should be placed directly over the material and a 5% solution of hypochlorite (or equivalent disinfectant) should be liberally applied to saturate it. The saturated fabric/bio-agent should then be properly disposed of IAW established protocol.

Other Decontamination Methods

Bio-agents may be rendered harmless through physical means such as heat and radiation. Agents are rendered completely harmless by sterilization with dry heat for 2 hours at 160°C. If autoclaving with steam at 121°C and 1 atmosphere of overpressure (15 psi), the time may be reduced to 20 minutes, depending on volume. Solar UV radiation has a disinfectant effect, often in combination with drying. This can be used in certain environmental conditions but is hard to standardize for practical/consistent usage for reliable decontamination.

The health hazards of environmental contamination by bio-agents differ from those of persistent or volatile chemical agents. Suspended bio-agents would be eventually inactivated by solar UV light, desiccation, and/or oxidation, with

minimal environmental residues. Possible exceptions include residue near the dissemination line or in the immediate area surrounding point-source munitions. Simulation studies suggest that secondary re-aerosolization would be difficult but may pose a human health hazard.

Environmental decontamination of terrain is costly and difficult. If grossly contaminated terrain, streets, or roads must be passed, the use of dust-binding spray to minimize re-aerosolization may be considered. If it is necessary to decontaminate these surfaces, chlorine-calcium or lye may be used. Otherwise, rely on the natural processes that, especially outdoors, lead to decontamination of agent by drying and solar UV radiation. Rooms in fixed spaces are best decontaminated with aerosolized gases or liquids (e.g., formaldehyde). This is usually combined with surface disinfectants to ensure complete effectiveness.

Innovative decontamination solutions in development, such as enzyme-based decontaminants and advanced chemical neutralizers, provide efficient methods to neutralize hazardous substances. These solutions are designed to be effective against a wide range of BW agents. The use of robotics in hazardous environments has also increased significantly. Robots equipped with detection and decontamination capabilities can operate in areas too dangerous for humans, thereby reducing the risk to first responders (IEEE Xplore, 2018). Mobile decontamination units can be rapidly deployed to the site of an emergency. These units are equipped with tools and materials to carry out decontamination procedures (Public safety Canada, 2021). Automated systems that integrate detection, alarm and response functions streamline the process of managing incidents. These systems can automatically activate containment measures, deploy decontaminants, and alert emergency responders (European Commission, 2021).

For further information, see ATP 4-02.84, *Multi-Service Tactics, Techniques, and Procedures for Treatment of Biological Warfare Agent Casualties*; and ATP 4-02.7, *Multi-Service Tactics, Techniques, and Procedures for Health Service Support in a Chemical, Biological, Radiological, and Nuclear Environment*

FIELD DETECTION

Detection, identification, and characterization of biological agents is a key component in deterring and mitigating biological warfare (BW) and bio-terrorism. This does not come without challenges due to the complex and variable nature of biological agents.[9] Originally, culture-based pathogen detection was used; however, it posed high turnaround times that could stem from days to months; therefore, culture-independent diagnostic tests, to include nucleic acid amplification and next-generation sequencing (NGS), have been developed for rapid pathogen detection.[6] These tests are more suitable for field detection.

The development of real-time detection capability for BW agents and pathogens of military significance has become one of the most challenging, high-priority areas of research within both the DOD and civilian sectors. Equipment fielded to date provide presumptive results only for a limited number of bio-agents. Further efforts are needed to develop unbiased sample analysis in the field.[9] Currently there is real-time detection through target nucleic acids or qPCR instruments. qPCR assays enable the detection of a target agent within a few hours.[9] The real time qPCR is performed in a closed reaction system allowing increased throughput and reducing the chances of carryover contamination. The higher throughput permits a higher assay sensitivity. The field-forward qPCR instruments are more conducive for operations as they are smaller, more lightweight, and rugged instruments.[7] Alternatively, multiplex assays allow analysis of more than one target in a single sample.

Next-generation sequencing (NGS) allows for parallel sequencing with a higher throughput for sequencing millions of reads per run.[8] Advantages of NGS include unbiased, metagenomic analysis of a sample without the need for any prior knowledge, detection of polymicrobial infections, detection of antimicrobial resistance, surveillance, identification of novel pathogens in a single assay, and pathogen identification where other targeted approaches may fail.[9] Traditional NGS was a burden for field operations due to its bulky design and cold storage requirements. Fortunately, technology has advanced to encompass the MinION, a third-generation sequencing device. This pocket-sized device is optimal for the field environment while still providing unbiased identification and characterization of agents. Below are several systems that have been used in the field detection of biological agents:

1. The Dry Filter Unit (DFU) represents a standardized point detection system for bio-agent surveillance and is designed to collect aerosolized bio-particulates from ambient air and then subject them for analysis by several complementary technologies including hand-held assays (HHAs), real-time polymerase chain reaction assays (RT-PCR), and other microbiological confirmatory techniques.[2-5]

2. The Long-Range Biological Standoff Detection System (LRBSDS) is under development and is designed to provide a first-line biological standoff detection capability; that is a detect-to-warn capability.[2] It will employ an infrared laser to detect aerosol clouds at a standoff distance of

up to 30 km. A second-generation system may extend the range to 100 km. This system will be available for fixed-site applications or may be deployable aboard rotary or fixed-winged aircraft. The Short-Range Biological Standoff Detection System (SRBSDS) is in the research and development phase. It will employ UV and laser-induced fluorescence to detect biological aerosol clouds at distances of up to 5 km. The information will be used to provide early warning, enhance contamination avoidance efforts, and as a cue for other detection capabilities. These systems do not identify the bio-agent but may indicate an approaching biological aerosol. The SRBSDS will be designed to differentiate biological aerosols from other non-biological aerosols. Confirmation of a live bio-agent or potent toxin could then be done using the Biological Integrated Detection System (BIDS) or a Biological Weapons Agent-Sampling (BWAS) Kit and a DFU.

3. Hand-held assays (HHA) are simple one-time-use immunochromatographic devices very similar to urine test strips used for home pregnancy tests. These tests provide a yes/no response to the presence of 10 bio-agents within 15 min. HHAs are currently employed in virtually all fielded military biological detection systems and are also present in developmental systems. HHAs are versatile enough to be used in automated readers, as well as read manually. Although reliable, they are designed only for presumptive identification of agents. Samples must subsequently undergo additional testing with complementary technologies before a definitive identification can be made.

4. FilmArray^{R} is a multiplex RT-PCR lab-in-a-box platform capable of providing identification solutions within an hour on raw specimens with minimal sample handling.[12-14] The FilmArray^{R} is configured so that each array/film offers identification panels for different organisms associated with specific subjects and/or syndromes, such as respiratory illnesses, food and water targets, or global fevers. Each panel has a list of approved sample types that are compatible with the kit. Some sample types, like arthropod vectors or soil require additional processing kits.

5. MagPix^{R} is a streamlined imaging-based analyzer that simultaneously measures up to 50 analytes in a single microplate. It has rapid multiplexing allowing for a read time of less than or equal to 60 minutes per 96-well plate. Ultimately, it can provide up to 4,800 results per hour. The MagPix^{R} footprint makes it conducive to deploy, operate, and maintain.

6. MSD^{R}: Mesoscale Defense provides absolute quantitation of protein markers by using multiplex immunoassays based on electrochemiluminescence. It provides a higher sensitivity and broader dynamic range than a traditional ELISA. Currently this device is used only to detect SEB, Ricin and Botulinum.

The above systems provide only presumptive tests for a limited number of bio-agents and are still detect-to-treat systems rather than the desired detect-to-warn systems, but with a higher level of confidence than assays used in the past. There are many other systems under development by the DOD and others that employ innovative detection methods such as oligo arrays, various types of mass spectrometry, quick and efficient sequencing, and single or multiple complementary technologies. These are not standardized systems and have yet to be integrated into the DOD through the formal acquisitions process.

Multiple services and agencies have developed improved tactics, techniques, and procedures to better provide a forward field confirmatory testing capability for both environmental samples and clinical specimens. Units like the 1st Global Medical Field Laboratory (1st GMFL), Navy-FDPMU (Forward Deployed Preventive Medicine Unit), and the Air Force AFBAT (Biological Augmentation Team) have been equipped with RT-PCR instruments to provide genetic analysis of samples that have been collected and tested as presumptively positive. Additionally, these systems have also been installed in the medical laboratories onboard Navy carrier and amphibious ships.

The current Concept of Operations outlines four levels of testing: presumptive, field confirmatory, theater validation, and definitive. Presumptive employs one method of identification and results dictate whether further analysis and reporting needs to be performed. Field confirmatory employs two methods from the same technology and results dictate possible further reporting, further analysis, and certain initiated medical actions. Theater validation employs two methods from two complementary, but different, technologies and results dictate further reporting and analysis, wider initiation of medical actions, and commencement of force health protection measures. Definitive employs more than two different methods from different technologies aimed at fully characterizing the biological threat and guiding future strategic and operational medical decisions and force health protection measures.

Standoff bio-agent detection (detect-to-warn) remains a challenging problem and is currently an area of intense research and development. Tomorrow's detectors promise to be faster, more sensitive, and more reliable than those fielded today. Until such detectors are developed and fielded, we must rely heavily on a layered system of defense to protect against biological attacks including timely and accurate intelligence, analysis of medical surveillance data, proper use of personal and physical protection equipment, use of medical countermeasures (vaccines and chemoprophylactic drugs), post-event deployment of antibiotics and antivirals, and well-developed response protocols.

OPERATIONAL CARE OF THE MILITARY WORKING DOG BY HUMAN HEALTH CARE PROVIDERS

"The capability that military working dogs bring to the fight cannot be replicated by man or machine. By all measures of performance, their yield outperforms any asset we have in our inventory. Our Army would be remiss if we failed to invest more in this incredibly valuable resource."

– GEN David Petraeus (Ret), 2008

Introduction

The intent of this chapter is to provide the basic information necessary for decontamination and treatment of a military working dog (MWD) in the context of a biological warfare agent (BWA) exposure. For basic medical treatment and care of MWDs, readers are directed to the Canine Tactical Combat Casualty Care (K9TCCC) Guidelines[11] and the Joint Trauma System Clinical Practice Guidelines for Military Working Dogs Clinical Management[10].

When handling MWDs, safety is paramount. Potentially dangerous animals and often unpredictable, MWDs are best controlled by the dog handler. Stress, injury, and illness can make a MWD more unpredictable. MWDs should always be muzzled whenever being handled unless a medical issue precludes this. If the standard muzzle issued to handlers is unavailable or compromised due to the contaminated environment, roll gauze looped tightly twice around the muzzle and tied behind the head (below the ears) will suffice[10]. The muzzle should be removed when the animal is no longer being actively handled or if sedated, anesthetized, having difficulty breathing, and in hot temperatures to permit panting.

Decontamination of MWDs

Under natural exposure conditions, MWDs are largely resistant to known BWAs[1-5]. However, their susceptibility in the event of a weaponized BWA exposure is unknown. Even if they remain uninfected, they may still act as a fomite, transferring the agent to the handler, bystanders, and contaminating otherwise clean environments. The best preventive is to decontaminate the MWD if it may have been exposed to a BWA. In a field environment, it is important to decontaminate the handler first and then allow the handler to assist with decontamination of the MWD. Mild sedation in addition to previously described handling requirements (i.e., muzzle) may be required to facilitate decontamination. The mild sedation protocol for MWDs is midazolam 0.3 mg/kg intramuscularly and hydromorphone 0.2 mg/kg intramuscularly[10].

The process for decontaminating an MWD is straightforward but labor-intensive and requires a large volume of water. Before beginning decontamination, elevate the dog onto a working surface to prevent injury or inadvertent contamination while performing decontamination. Start by wetting the MWD. Some breeds of MWD are dual-coated having both a longer, more water-repellant outer coat and a shorter, softer undercoat. Take care to ensure water has soaked through to the skin. Apply a mild soap (e.g., Dove dish soap or dilute chlorhexidine scrub) and use a brush to begin lathering, starting at the head and working backwards. Work along the back and tail, then the chest, and down each leg making sure to get the soap all the way to the skin. Finally, use the brush to lather the abdomen. After the dog is fully lathered, rinse thoroughly with water until no soap is left on the skin or fur. Use a towel(s) to dry off the dog, then move the animal away from the decontamination area as soon as possible. It is critical to cover the MWD's body with a barrier sheet (e.g., towels, blankets, plastic sheeting, etc.) at this point to minimize spray from shaking. Shaking following water immersion is a species-specific response that almost always occurs, and there is a significant potential for contamination of the surrounding environment and personnel with grey water. Do NOT use a dilute bleach solution to decontaminate the dog. It may get into the animal's eyes or cause irritation to the skin, which may facilitate missed BWA (or secondary bacteria) to penetrate the skin and cause infection.

This rinse-wash-rinse protocol remains the currently recommended method for decontamination. However, in the presence of *Bacillus anthracis* spores, this protocol does not result in inactivation of spores[12, 13]. Research efforts are ongoing to establish an effective means of field decontamination that results in spore inactivation for *Bacillus anthracis*.

Treatment of MWDs Exposed to Biological Warfare Agents

As previously noted, MWDs have low susceptibility to many BWAs in the face of natural infection. Their susceptibility in the event of a weaponized BWA remains unknown. In terms of routes of exposure, MWDs are more likely to show GI, inhalational, or ocular symptoms because of their proximity to the ground and frequency with which they both lick and sniff at items[12-13]. If an MWD has been potentially exposed to a BWA, decontamination is highly recommended regardless of whether the dog will become ill or not. In all cases, veterinary consultation following initiation of treatment is recommended to establish a plan for ongoing care and treatment. See Table 1 for dosage recommendations of specified medications.

Botulinum toxins. Botulinum toxin intoxication in an MWD results in the same symmetric, descending flaccid motor paralysis observed in people. Dogs are generally resistant to botulinum toxin, but sporadic case reports do exist[1]. The preferred preventive is inoculation with toxoids appropriate to the exposure, if known. Treatment with a botulinum antitoxin, though variable in efficacy, is the preferred method in a symptomatic MWD.

Plague. Although cats are highly susceptible to infection by *Yersinia pestis*, dogs are highly resistant. Potential symptoms include general lethargy, fever lasting <72 h, lobar pneumonia, and coughing progressing to hemoptysis. Recommended treatments include fluoroquinolones, doxycycline, trimethoprim-sulfonamide, gentamicin, and chloramphenicol. Treatment should be given for 10-21 days, depending on antibiotic selection.[2]

Tularemia. Reported clinical manifestations of tularemia in natural infections in dogs range from subclinical to bacteremia, fever, and respiratory disease. Aminoglycosides (amikacin or gentamicin) are the treatment of choice for tularemia in dogs. Tetracyclines (e.g., doxycycline) and fluoroquinolones are also effective. Treatment for 2-3 weeks is recommended.[3,4]

Anthrax. In the context of natural infection, anthrax infections in dogs are rare. However, weaponized anthrax would likely result in inhalational anthrax, even in MWDs. While the first line of treatment in human infections is ciprofloxacin, individual dogs have widely varying ability to metabolize ciprofloxacin.[15] Tetracyclines (e.g., doxycycline) is the recommended first-line treatment for MWDs. Amoxicillin, chloramphenicol, erythromycin, gentamicin, streptomycin, and sulfonamides may also be used. If administering ciprofloxacin, use it at a higher dose (see Table 1) than considered normal in human patients and for a longer duration than the recommended 45-60d, or until able to switch to either a veterinary product (enrofloxacin) or doxycycline.[5]

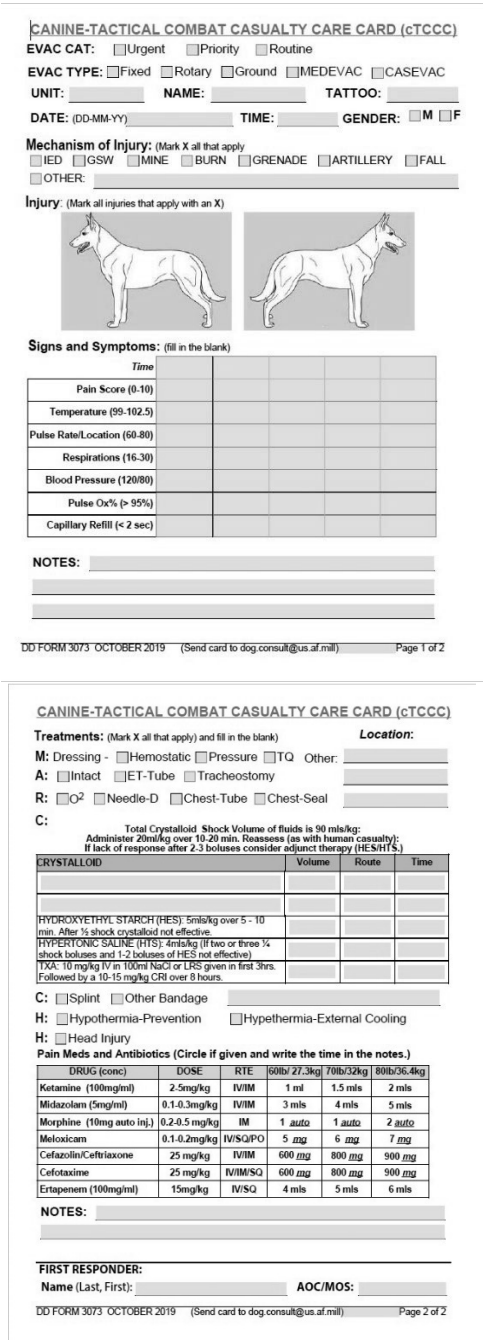
Viral Hemorrhagic Fevers. The two families of viruses classified as Category A agents, arenaviruses and filoviruses, can infect dogs but do not appear to cause any significant disease in the animals.[7,8] Whether dogs represent a potential source for zoonotic infection is unknown.

Smallpox. The natural-occurring variola (smallpox) virus is host-restricted to humans.[16] In the event an exposed MWD develops clinical symptoms following exposure, supportive care is the only recommended treatment. Additionally, secondary bacterial infections are common in poxvirus cases so antibiotic therapy may be indicated in coordination with veterinary staff.

For additional information on the clinical management of MWDs and TC3 guidelines for MWDs, please review the Joint Trauma System Guidelines for MWDs.[10, 11]

Selected Antibiotic Dosages for MWDs[14]

Generic Name	Dose	Frequency	Route	Comments
Amikacin	15-30 mg/kg	24 h	IV	Can be given IV, IM, or SC once daily. Adjust based on kidney function and serum levels when possible.
Amoxicillin	11-15 mg/kg	8-12h	PO	None
Chloramphenicol	40-50 mg/kg	8h	PO, IV, SC, IM	Recommend higher end for BWA
Ciprofloxacin	35 mg/kg	24h	IV or PO	Bioavailability variable in dog
Doxycycline	5-10 mg/kg	12h	IV or PO	Treatment for 14-28 days recommended in most cases
Enrofloxacin	10-20 mg/kg	24 h	IM or PO	Ciprofloxacin is a better option for IV administration if desired, due to potential adverse events with IV administration of enrofloxacin
Erythromycin	10-20 mg/kg	8-12h	PO	Short half-life, low bioavailability, significant GI side effects due to prokinetic effects
Gentamicin	9-12 mg/kg	24h	IV, IM, or SC	Higher dosages (up to 15mg/kg) for septic patients; monitor kidney function and serum levels as in amikacin
Trimethoprim-sulfonamide	30 mg/kg	24h	PO	In severe infections, initial dose may be followed by 15mg/kg PO every 12h



CANINE-TACTICAL COMBAT CASUALTY CARE CARD (cTCCC)

EVAC CAT: ☐Urgent ☐Priority ☐Routine

EVAC TYPE: ☐Fixed ☐Rotary ☐Ground ☐MEDEVAC ☐CASEVAC

UNIT: NAME: TATTOO:

DATE: (DD-MM-YY) TIME: GENDER: ☐M ☐F

Mechanism of Injury: (Mark X all that apply

☐IED ☐GSW ☐MINE ☐BURN ☐GRENADE ☐ARTILLERY ☐FALL

☐OTHER:

Injury: (Mark all injuries that apply with an X)

Signs and Symptoms: (fill in the blank)

Time					
Pain Score (0-10)					
Temperature (99-102.5)					
Pulse Rate/Location (60-80)					
Respirations (16-30)					
Blood Pressure (120/80)					
Pulse Ox% (> 95%)					
Capillary Refill (< 2 sec)					

NOTES:

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CANINE-TACTICAL COMBAT CASUALTY CARE CARD (cTCCC)

Treatments: (Mark X all that apply) and fill in the blank) Location:

M: Dressing - ☐Hemostatic ☐Pressure ☐TQ Other:

A: ☐Intact ☐ET-Tube ☐Tracheostomy

R: ☐O^2 ☐Needle-D ☐Chest-Tube ☐Chest-Seal

C:

Total Crystalloid Shock Volume of fluids is 90 mls/kg:
Administer 20ml/kg over 10-20 min. Reassess (as with human casualty):
If lack of response after 2-3 boluses consider adjunct therapy (HES/HTS.)

CRYSTALLOID	Volume	Route	Time
HYDROXYETHYL STARCH (HES): 5mls/kg over 5 - 10 min. After ⅓ shock crystalloid not effective.			
HYPERTONIC SALINE (HTS): 4mls/kg (If two or three ¼ shock boluses and 1-2 boluses of HES not effective)			
TXA: 10 mg/kg IV in 100ml NaCl or LRS given in first 3hrs. Followed by a 10-15 mg/kg CRI over 8 hours.			

C: ☐Splint ☐Other Bandage

H: ☐Hypothermia-Prevention ☐Hypethermia-External Cooling

H: ☐Head Injury

Pain Meds and Antibiotics (Circle if given and write the time in the notes.)

DRUG (conc)	DOSE	RTE	60lb/ 27.3kg	70lb/32kg	80lb/36.4kg
Ketamine (100mg/ml)	2.5mg/kg	IV/IM	1 ml	1.5 mls	2 mls
Midazolam (5mg/ml)	0.1-0.3mg/kg	IV/IM	3 mls	4 mls	5 mls
Morphine (10mg auto inj.)	0.2-0.5 mg/kg	IM	1 *auto*	1 *auto*	2 *auto*
Meloxicam	0.1-0.2mg/kg	IV/SQ/PO	5 *mg*	6 *mg*	7 *mg*
Cefazolin/Ceftriaxone	25 mg/kg	IV/IM	600 *mg*	800 *mg*	900 *mg*
Cefotaxime	25 mg/kg	IV/IM/SQ	600 *mg*	800 *mg*	900 *mg*
Ertapenem (100mg/ml)	15mg/kg	IV/SQ	4 mls	5 mls	6 mls

NOTES:

FIRST RESPONDER:

Name (Last, First): AOC/MOS:

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Figure 3: Example of the K9 Tactical Combat Casualty Card, DD Form 3073

ARMY BIOCASUALTY OPERATIONAL CONSIDERATIONS

Introduction: The concept of the Army Roles of care has undergone significant evolution since its inception. This structured approach to medical treatment originated during the complex and challenging conditions of World War II, when the necessity for an effective medical care system became paramount. The Army recognized the need for a tiered approach to medical treatment to ensure that wounded soldiers received prompt and appropriate care at each stage of their recovery. Consequently, this led to the establishment of distinct roles of care, ranging from immediate battlefield treatment to comprehensive rehabilitation. Over the subsequent decades, these roles have been refined and expanded, incorporating advancements in medical technology and practices to address the diverse needs of military personnel, particularly in the face of modern threats such as biological warfare within multidomain operation environments.

Background: Medical care is a critical component of the U.S. Army, ensuring that soldiers receive the necessary treatment and support. The process becomes even more crucial in the context of biological warfare. Since the Sars-CoV-2 Pandemic, the U.S. Government and DOD have aggressively worked on our ability to quickly address the threat and return to the American way of life with the publications of The National Biodefense Strategy and Implementation Plan, DOD Biodefense Posture Review, and Army Biological Defense Strategy. An all-hazards clinical approach to biodefense would effectively capture all biothreats in an agnostic manner and would not require the traditional split into endemic and biowarfare agents.[1] In reviewing this section, keep in mind that this is a guide designed as a tool to manage a biological casualty within the Army continuum of care. It is not designed to be a substitute for clinical judgement.

Objective: This chapter will discuss the different roles (Role 1 to Role 4) in U.S. Army medical care, with a focus on the challenges and measures associated with biological warfare agents. The first medical care a Soldier receives (also referred to as unit-level medical care) is Role 1 care which includes immediate response to injuries and illnesses, emphasizing the importance of decontamination operations and basic assessment/management of bioincident. Some additional key components are management of Disease and Nonbattle Injury (DNBI) prevention, combat and operational stress preventive measures, and medical evacuation from supported units [point of injury →battalion aid stations (BAS)/casualty collection points (CCP)] to supporting medical treatment facilities (MTFs). Tactical combat casualty care (immediate far forward care) consists of those lifesaving steps that do not require the knowledge and skills of a physician. The combat medic is the first individual in

[1] (Cardile, 2025, p. 3)

the medical chain that specialty-specific training.[2] At the BAS, the physician and the physician assistant are trained and equipped to provide advanced trauma management to the combat casualty. This element also conducts routine sick call when the operational situation permits.[3]

Upon identification or belief of exposure to a biological agent, the key is to act quickly to treat the patient and contain or prevent the spread of biological agents within the operational environment. Biological agents typically do not require extensive decontamination, in comparison to chemical agents. Simple removal of exposed clothing and a soap/water shower are usually sufficient for decontamination. Clinical laboratory assets include basic hematology, urinalysis, chemistry, serology, and microbiology. Providing Role 1 casualty care for extended periods may be necessary when the tactical situation limits prompt medical care. Recognizing and reacting to a biothreat in a PCC environment is challenging, as units may need to deal with the situation with whatever equipment is available. Effective resource management is essential, including the establishment of a medical supply cache and prioritizing patients based on severity.[4]

The next role of care is the Role 2 MTF (also known as Forward Resuscitative Care) with increased capabilities from the previous Role. Role 2 care involves advanced trauma management and biological agent treatment, including decontamination operations and laboratory capabilities. Care is rendered at the Role 2 MTF by the area support squad, medical treatment platoon of medical companies. The Role 2 MTF has the capability to provide blood products, limited x-ray, clinical laboratory, operational dental support, combat and operational stress control (COSC), preventive medicine, physical therapy, and when augmented, optometry services. The Role 2 MTF provides a greater capability to resuscitate trauma patients than a Role 1. This Role of care provides medical evacuation from supported Role 1 MTFs and provides Role 1 medical treatment on an area support basis for units without organic Role 1 resources. A Role 2 Basic MTF must provide the surgical capability, including damage control surgery and surgical procedures for emergency cases, to deliver life, limb and function saving medical treatment.[5] The surgical capability should be provided within medical timelines. A Role 2 Enhanced MTF must provide all the capabilities of the Role 2 Basic but has additional capabilities because of additional facilities and greater resources, including the capability of stabilizing and preparing casualties for strategic aeromedical evacuation.[6]

Upon identification or belief of a patient exposed to a biological agent, continue to focus on patient care, decontamination, and bio-containment to prevent the spread of biological agents within the MTF. Biological agents typically do not

[2] (Army, 2020)
[3] (Army, 2020)
[4] (Cardile, 2025)
[5] (Army, 2020)
[6] (Army, 2020)

require extensive decontamination, in comparison to chemical agents, simple removal of exposed clothing and a soap/water shower are usually sufficient for decontamination. Clinical laboratory assets include basic hematology, urinalysis, chemistry, serology, and microbiology.

At Role 3, the patient is treated in an MTF staffed and equipped to provide care to all categories of patients, to include resuscitation, initial wound surgery, damage control surgery, and postoperative treatment. This Role of care expands the support provided at Role 2. Patients who are unable to tolerate and survive movement over long distances receive surgical care in a hospital as close to the supported unit as the tactical situation allows. This Role includes provisions for: evacuating patients from supported units, providing care for all categories of patients in an MTF with the proper staff and equipment, and providing support on an area basis to units without organic medical assets.[7] Clinical laboratory assets include all the basic labs at the previous Roles in addition to the following capabilities: aerobic & anaerobic cultures, parasitology, and antibiotic susceptibility. Role 3 can initially evaluate suspected biowarfare patients, collect and properly package samples, establish a chain of custody, and forward samples to a supporting medical laboratory for further analysis (e.g., USAMRIID Special Pathogens Lab).

Role 4 care involves long-term recovery and rehabilitation in fixed medical facilities, including specialized treatment for biological agent exposure. Role 4 medical care is found in continental United States (CONUS)-based Defense Health Agency operated hospitals and other safe havens. The Army Health System (AHS) is more globally integrated and linked to the Military Health System through the Defense Health Agency Role 4 MTFs for the provision of more definitive Role 4 medical care as patients are evacuated to CONUS from Role 3 MTFs in theater. If mobilization requires expansion of military hospital capacities, then the Department of Veteran's Affairs and civilian hospital beds in the National Disaster Medical System are added to meet the increased demands created by the evacuation of patients from the Area of Operations (AO). The support-based hospitals represent the most definitive medical care available within the AHS. Clinical laboratory assets include all the basic labs at the previous roles to include the following additional assets for identification (sensitivity), verification (specification), confirmation and characterization (genome sequencing, molecular epidemiology, and transmissibility).
The goal at this role is recovery of the patient by providing ongoing support, addressing the mental health needs, and long-term monitoring and effective resource management.

Conclusion: This chapter discusses the Roles of U.S. Army medical care (Role 1 to Role 4) in the context of biological warfare, highlighting the importance of quick recognition, early treatment of the patient, decontamination of a biological patient, importance of training, and general capabilities of each MTF. The efficient transition through each role is crucial for providing effective medical

[7] (Army, 2020)

care, especially in the face of biological threats. One of the keys to success in achieving and maintaining resilience to operate within a biological operational environment is knowledge, training, and practicing these skills and guidelines, especially with the challenges of large-scale combat operations within multi-domain operations.

Army Operational Considerations

1. Army, T. U. (2020). *Army Health System Support Planning.* Army. Washington D.C.: Army Technical Publication. Retrieved 02 27, 2025

2. Cardile, A. (2025, February 27). *Clinical Practice Guidelines.* Retrieved from Joint Trauma Surgery : https://jts.health.mil/index.cfm/PI_CPGs/cpgs

AIR FORCE BIOCASUALTY/HIGH CONSEQUENCE INFECTIOUS DISEASE – OPERATIONAL CONSIDERATIONS

The term high consequence infectious diseases (HCID) generally refers to diseases caused by pathogens which exhibit three key features: 1. high morbidity and mortality, 2. communicability (can be transmitted person-to-person), and 3. limited medical countermeasures exist for prevention or treatment. HCID is not limited to naturally occurring diseases but also can include novel or bio-engineered pathogens, as well as those that may be accidentally or deliberately introduced. Transporting persons infected with or exposed to HCID across the continuum of care requires careful planning, dedicated training, and strict adherence to infection prevention and control practices. Directive to be able to transport such patients comes from DOD Instruction 6000.11 (Patient Movement) and delineates United States Transportation Command (USTRANSCOM) as the single manager responsible for global patient movement.[i] Current USTRANSCOM policy is for treatment in place of those infected with or exposed to known or possible HCID.[ii] However, one might desire to transport HCID patients to perform pathogen identification and genetic sequencing, study disease characteristics, and develop targeted therapeutics or preventative measures. Such movement would require an Exception to Policy (ETP) with coordination through referring and receiving geographic combatant commanders, USTRANSCOM Command, and the Assistant Secretary of Defense for Health Affairs.[2] A brief review of DOD experience with HCID air transportation will be provided followed by summation of current resources, capabilities, and considerations.

The USAMRIID Air Isolation Team (AIT) was the first dedicated DOD team to perform air transport for patients infected with or exposed to HCID. AIT utilized two single patient isolators for missions in a step-wise fashion.[iii] The first was a stretcher/litter-based isolator for the initial patient contact and containment. Then, with direct integration, the patient would be transferred from the smaller litter-based isolator to a larger one known as the Vickers aircraft transport isolator (VATI) for definitive transport and care-in-flight. The AIT would transport patients in the VATI upon standard military transport aircraft back to a biocontainment unit at USAMRIID (Ft. Detrick, Maryland) for monitoring and care if needed. At USAMRIID the patient would also be transferred from the VATI into the biocontainment unit via direct integration so as not to break containment. Due to a myriad of reasons, in 2012 USAMRIID's biocontainment unit and AIT were decommissioned.[iv] The USAF assumed responsibility, under the purview of the Air Mobility Command (AMC), for HCID air transport and began training with a system known as the Patient Isolation Unit (PIU).

Like the AIT mobile assets, the PIU was designed for single-patient movement. However, in response to the DOD's involvement during the 2014 West Africa Ebola outbreak (Operation United Assistance) a new capability that allowed for greater access to patients to administer proper medical care during transport was needed. USTRANSCOM Joint Urgent Operational Need (JUON) TC-0002 was issued to provide aeromedical evacuation for patients with known or suspected exposure to contagious infectious diseases while protecting aircrew, airframe, and all other support personnel, which resulted in the development of the Transport Isolation System (TIS). Ultimately the USAF (and TIS) never transported any persons infected with Ebola. The TIS was subsequently used operationally starting in April 2020 for COVID-19 patient movement. Meanwhile, another USTRANSCOM JUON TC-0003 was issued in March 2020 highlighting the need for high-capacity COVID-19 passenger airlift. In less than 100 days from initial design to product delivery, the Negatively Pressurized Conex (NPC) and the Negatively Pressurized Conex-Lite (NPC-L) were produced, fielded, and are still in inventory today.

The NPC/NPC-L resemble modified shipping containers in appearance. The NPC was designed to transport a maximum of 22 ambulatory patients or up to 8 litter patients whereas the NPC-L can carry 13 ambulatory patients or 2 litter patients. However, maximum capacity for transport is influenced by the pathogen, acuity of illness, and a receiving facility's capability and capacity to receive patients. Both the NPC and NPC-L utilize negative pressure blowers with high-efficiency particulate air (HEPA) filters and are equipped with an anteroom adjacent to the patient room. Medical providers must don appropriate personal protective equipment (PPE) as clinically indicated to provide care within the NPC/NPC-L as the patients are in an open-air environment. Both the NPC and NPC-L have been used operationally during the COVID-19 pandemic and units are prepositioned at several designated locations within and outside of the continental U.S. For mission consideration, the NPC is authorized to be used on a C-17 airframe whereas the NPC-L can be utilized on a C-17 or C-130.

Several caveats exist regarding the use of the NPC/NPC-L as these were produced via JUON for COVID-19. Additional testing is needed to assess the operational and safety impacts to transport and care for patients affected by all HCID. Further, formal sustainment training, programmed funding, and resourcing are needed to train and equip teams for missions using the NPC and NPC-L for HCID patient movement. In addition to use of isolation systems, high level containment transport requires teams trained in validated infection control processes, liquid and solid waste handling procedures, cleaning and disinfection, effective communication, and safe clinical care delivery while in enhanced PPE.[v]

Gaps regarding HCID patient movement and the use of the NPC/NPC-L are currently being addressed. USAF subject matter experts in infectious disease management, infection prevention and control, as well as critical care air transport are part of the Center for Sustainment of Trauma and Readiness Skill

(C-STARS) Omaha, embedded with the University of Nebraska Medical Center (UNMC) and its clinical partner, Nebraska Medicine. C-STARS Omaha is a geographically detached unit from the United States Air Force School of Aerospace Medicine and is uniquely positioned in Nebraska as home to one of 13 Regional Emerging Special Pathogen Treatment Centers and the nation's only National Quarantine Unit. At the time of this writing, members of C-STARS Omaha are actively engaged with AMC and USTRANSCOM developing Concepts of Operations (CONOPS) and establishing an HCID Patient Movement Pilot Unit, focusing on the air transport and medical management of patients infected with or exposed to HCID utilizing the NPC/NPC-L platforms.

The ongoing effort to develop an all-hazards HCID approach on the backbones of NPC/NPC-L will also likely need concurrent development of single patient isolation movement. The NPC/NPC-L require large, fixed wing aircraft for patient movement and indeed have been used for intra-theater and inter-theater missions. The use of smaller, more agile aircraft (including rotary wing) necessitates lower profile isolators which could be used for shorter duration, intra-theater missions. Several single patient transport isolation chambers are commercially available such as the Epi-Shuttle® by EpiGuard, AirBoss ISO-POD®, MilPod Patient Isolation Transport®, Operational Rescue Containment Apparatus (ORCA ™) and Containment and Protection System Utilizing Life Support (CAPSULS) by Isovac Products LLC. Only a few of these products have been FDA-cleared. Like the NPC/NPC-L these single patient isolators utilize negative pressure blowers with HEPA filtration. Providers and transport teams typically do not have to don significant amounts of PPE as engaging the patient is done through sealed glove ports and pass-through ports versus the hands-on open-air approach within the NPC/NPC-L. The current commercially available single patient isolators vary in design regarding rigid or flexible construction, reusable components versus single-use, and placement of glove and pass-through ports. Isolator characteristics to take into consideration for air transportation include ability to undergo airworthiness testing, patient positioning (flat versus variable incline position), patient accessibility, size, and weight.

The USAF is currently developing an all-cause HCID air-transport capability. As stated, USTRANSCOM directive is to treat in place for those exposed to or infected with HCID. An ETP requesting air transport may be considered due to the aforementioned reasons. If granted, movement may rely on non-DOD federal assets such as the Department of State contract with Phoenix Air Group, a civilian air transport company who performed transcontinental transport of U.S.-citizens infected with Ebola virus during the 2014 West Africa Ebola Outbreak.[2] Alternatively, a team comprised of public health technicians, biomedical technicians, aeromedical evacuation specialists, critical care air transport providers, infectious disease physicians, and infection prevention and control experts could be compiled to perform just-in-time training and subsequent deployment. At the time of this writing, several groups within the

USAF are engaged to develop an HCID air transport capability for the DOD. Furthermore, the Office of the Assistant Secretary of Defense for Nuclear, Chemical, and Biological Defense has issued enduring requirements to develop Biocontainment, Isolation, and Quarantine (BIQ) capabilities from the Army, Navy, and Air Force. The USAF was tasked for aid in development of a Portable Patient Transport System (PPTS) which will deliver all-cause HCID air transport capability. The Joint Program Office and USAF held a BIQ demonstration at Travis Air Force Base in November of 2024 to help inform/develop PPTS requirements.

Disclaimer: The NPC/NPC-L are DOD owned and operated assets. The writers and USAF do not endorse any of the registered single patient isolators mentioned in this text; they are used for example purposes only.

BACTERIAL AGENTS

ANTHRAX

Summary

Signs and symptoms of inhalational anthrax (IA): Incubation period is generally 1 to 6 days, although longer periods have been noted. Fever, malaise, fatigue, dry cough, and mild chest discomfort progress to severe respiratory distress with dyspnea, diaphoresis, stridor, cyanosis, and shock. Death typically occurs in 24 to 36 hours after onset of severe symptoms.

Diagnosis: Physical findings are non-specific. A widened mediastinum and pleural effusions may be seen on CXR or CT scan in later stages of illness. The organism is detectable by Gram stain of blood, blood culture, serum levels of anthrax-specific Protective Antigen (PA) and/or Lethal Factor (LF), serology, PCR, and immunohistochemistry.

Treatment: Consult the most recent CDC guidelines for recommendations on all first-line and alternate regimens. For treatment of cutaneous Anthrax without meningitis and without systemic symptoms or extensive facial lesions or edema, doxycycline or minocycline or ciprofloxacin or levofloxacin are recommended. For systemic Anthrax with or without meningitis the optimal empiric regimen includes two bactericidal drugs from different classes plus a protein synthesis inhibitor or an RNA synthesis inhibitor (ciprofloxacin plus meropenem plus minocycline) AND antitoxin (*Raxibacumab* or *Oblitoxaximab*). Intensive supportive therapy will be necessary.

Prophylaxis: An FDA-licensed vaccine (*BioThrax*) is available to the U.S. military for pre-exposure (PrEP) and post-exposure (PEP) prophylaxis. The preferred schedule is 0.5 ml IM at 0, 1, and 6 months (primary series) then 12 and 18 month boosters, followed by annual boosters for PrEP. For known or imminent exposure (PEP) in those who have not received PrEP vaccine, the schedule is 0, 2 and 4 weeks SQ in combination with a PO fluoroquinolone (ciprofloxacin or levofloxacin or moxifloxacin) or doxycycline or clindamycin with duration of antibiotics for 42 days. Current data suggests that the incubation period for inhalational Anthrax may be prolonged due to spore persistence in the lungs. Thus, for potential aerosol exposures, chemoprophylaxis is recommended as an adjunct to immunization for PEP regardless of PrEP status. For an immunocompetent adult who has partially or fully completed PrEP and is exposed via aerosol without appropriate respiratory protection, 30 days of antibiotic PEP is recommended. *Raxibacumab* or *Oblitoxaximab* are FDA-approved for PEP only if other therapies are not available or appropriate.

Isolation and decontamination: Standard precautions for healthcare workers. Avoid invasive procedures or autopsy; but if unavoidable, personal protective equipment (PPE) is mandatory; all instruments and the proximate environment should be thoroughly disinfected with a sporicidal agent (e.g., hypochlorite).

Overview

Bacillus anthracis, the causative agent of anthrax, is a Gram-positive, sporulating rod. The spores are the usual infective form. Naturally occurring anthrax is primarily a zoonotic disease of herbivores, with cattle, sheep, goats, and horses serving as the usual domesticated animal hosts, but other animals may be infected. Humans generally contract the disease when handling contaminated hair, wool, hides, flesh, blood, and excreta of infected animals and from manufactured products such as bone meal. Infection is introduced through scratches or abrasions of the skin, wounds, inhaling spores, eating insufficiently cooked infected meat, or by fly bites. The primary concern for intentional infection by this organism is through inhalation after aerosol dissemination of spores. The spores are very stable and may remain viable for many years in soil and water. They resist sunlight for varying periods.

History & Significance

Anthrax can be produced in either a wet (slurry) or dry (powder) form, stabilized for weaponization, and delivered as an aerosol cloud either from a line source (e.g., boat or aircraft moving upwind of a target), or as a point source (from a spray device). Historically, coverage of large ground areas was also planned by national programs using multiple spray bomblets disseminated from missile warheads at a predetermined height above the ground[1,2] Such anthrax bombs, however, were never deployed on a battlefield. Anthrax was weaponized by the U.S. from the 1940s to the ‘60s until the U.S. offensive BW program was terminated. Other countries, including the Soviet Union and Iraq, have also weaponized it. In 2001, anthrax spores were delivered in the U.S. mail, resulting in 22 cases of confirmed or suspected anthrax disease, of which five people died. [3,4] Anthrax bacteria are easy to cultivate, and spore production is readily induced. Moreover, the spores are highly resistant to sunlight, heat, and disinfectants—properties which create concerns for environmental persistence after an attack.

Clinical Features

Anthrax presents as three distinct clinical syndromes in humans: cutaneous, gastrointestinal, and inhalational disease.[5]

Cutaneous anthrax: The cutaneous form—also referred to as malignant pustule—is the most common naturally occurring form of anthrax. It occurs most frequently on the hands and forearms of persons working with infected livestock or livestock products, but during epizootics it has been transmitted to humans by the bites of flies, and occurred in as many as 11 people exposed to

anthrax spores in the U.S. mail in 2001. After a 1 to 12 day (usually 5 to 7 days) incubation period, a painless or pruritic papule forms at the site of exposure, enlarging into a round ulcer by the next day.[6] Vesicles or bullae containing clear or serosanguinous fluid and bacilli may form on the edge of the ulcer, which can be surrounded by various degrees of non-pitting edema. The ulcer subsequently dries and forms a coal-black scab (eschar), which falls off over the ensuing 1 to 2 weeks. Regional lymphadenopathy with associated systemic symptoms can occur. If untreated, this local infection may disseminate into a fatal systemic infection in 10 to 20% of cases. Treated appropriately, the case fatality rate (CFR) is < 1%.

Gastrointestinal (GI) anthrax: is rare in humans, and is contracted by eating insufficiently cooked meat from infected animals. Infection is thought to occur due to ingestion of viable vegetative organisms rather than spores. Both forms of GI anthrax, oropharyngeal and intestinal, have incubation periods of 1 to 6 days. Disease in ***oropharyngeal*** anthrax is heralded by the onset of fever and severe pharyngitis, followed by oral ulcers which progress from whitish patches to tan or gray pseudomembranes. These lesions vary in location but often form unilaterally over a palatine tonsil. Other signs and symptoms include dysphagia, regional non-purulent lymphadenopathy, and severe neck swelling (often unilateral). Edema can lead to airway compromise, and disease can progress to sepsis, with CFR of 10 to 50%. ***Intestinal*** anthrax begins with fever, nausea, vomiting, and focal abdominal pain. These symptoms can progress to hematemesis, hematochezia or melena, massive serosanguinous or hemorrhagic ascites, and sepsis. Overall CFR is > 50%. Some evidence exists for a mild, self-limited gastroenteritis syndrome associated with intestinal anthrax, but this is poorly described.

Inhalational anthrax (IA): Endemic inhalational anthrax ("woolsorter's disease") is also an extremely rare infection contracted by inhaling *B. anthracis* spores. It has historically occurred in an occupational setting, mainly among workers who handle infected hides, wool, and furs. Because of the rarity of human IA, a single case of this disease should be presumed an intentional exposure to anthrax until proven otherwise. After an incubation period of 1 to 6 days*, a non-specific febrile syndrome begins. Fever, malaise, headache, fatigue, and drenching sweats are often present, sometimes in association with nausea, vomiting, confusion, a nonproductive cough, and mild chest discomfort. Physical findings are typically non-specific in the early phase of the disease. Patients are often tachycardic but may have normal lung physical exams. Chest radiographs or CT scan may show subtle changes including slightly widened mediastinum, (hemorrhagic mediastinitis) or pleural effusions. These initial symptoms generally last 2 to 5 days and can be followed by a short period of apparent improvement (hours to 2 or 3 days), culminating in the abrupt development of severe respiratory distress with dyspnea, diaphoresis, stridor, and cyanosis. Septicemia, shock, and death usually follow within 24 to 36 hours after the onset of respiratory distress unless dramatic life-saving efforts are initiated. Historically, IA has been complicated by hemorrhagic meningitis in up

to 50% of cases and GI hemorrhage in 80%. In the anthrax letter attacks in 2001, victims developed IA following exposure to envelope contents. The CFR among victims was only 45%, despite previously reported CFRs for IA of > 85%. The improved outcome was likely a reflection of rapid and aggressive treatment regimens and advancements in intensive care medicine.

*During the accidental Sverdlovsk outbreak in the Soviet Union in 1979, persons are reported to have become ill up to 6 weeks after an aerosol release. Studies performed in nonhuman primates demonstrate that anthrax spores remain in the lung for up to 100 days.

Diagnosis

All forms of anthrax are diagnosed using a combination of clinical and laboratory findings.

Cutaneous anthrax: The key to diagnosis centers upon the presence of the characteristic painless skin lesion which progresses to a vesicle, ulcer, then eschar, with surrounding edema. While arachnid bites, trauma, burns or cutaneous tularemia may look similar, these are typically painful lesions. Known exposure history or risk factors may also be present. To perform Gram stain and bacterial culture of the lesion, samples should be collected by using two dry Dacron or rayon swabs, ideally with the fluid of an unopened vesicle. If no vesicle is present, apply moistened swabs (sterile saline) under the edge of an eschar or in the base of an ulcer. One swab is sent for Gram stain and culture, the other for PCR testing. Gram stain often demonstrates large Gram-positive bacilli if the patient has not yet received antibiotics. If the Gram stain and culture are negative, collect a 4-mm punch biopsy (or two if both eschar and vesicle are present) of the leading margin of the lesion for general histology and immunohistochemical staining. Blood culture should be performed in all patients suspected of having anthrax.

Gastrointestinal anthrax: History of exposure to, or ingestion of, the meat of sick animals should be elicited. Clinical suspicion should be elevated for multiple cases of similar disease. *Oropharyngeal* disease can mimic diphtheria. Vaccination and travel history should be queried. Gram stain and culture of the oral lesion may be positive for *B. anthracis* if collected before initiation of antibiotics. *Intestinal* anthrax may mimic acute gastroenteritis, acute abdomen with peritonitis (focal with rebound tenderness), or dysentery. Abdominal radiographs are non-specific, sometimes showing diffuse air-fluid levels, bowel thickening, and peritoneal fluid. Surgical findings may include hemorrhagic mesenteric adenitis, serosanguinous to hemorrhagic ascites, bowel ulceration (usually ileum and cecum), edema, and necrosis. Stool culture may identify bacilli with intestinal anthrax. Peritoneal fluid and ascites fluid should be evaluated by culture, Gram stain, immunohistochemistry, and PCR. Blood should be collected for culture, serology (paired frozen sera 3-4 weeks apart, -70ºC) and PCR (lavender tube, refrigerated) in patients with either form of GI disease.

Inhalational anthrax: Early IA is a non-specific syndrome which may be difficult to distinguish clinically from other illnesses. Notably absent in IA are upper respiratory symptoms (rhinorrhea, coryza, congestion) which are typically present in patients with influenza. Pneumonia generally does not occur; therefore, lung exam may be unrevealing, and organisms are not typically seen in the sputum. Patients suspected of having IA should have a complete blood count (CBC), blood culture, and serum electrolytes. White blood cell count is typically mildly elevated at presentation (mean 9,800/μL in the 2001 cases) with a neutrophil predominance. Hemoconcentration may be evidenced by elevated serum sodium and hematocrit. Mildly elevated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) may be present as well as hypoalbuminemia. *B. anthracis* will be detectable even in the early phase of disease by routine blood culture and may even be seen with Gram stain of blood later in the course of the illness; however, even one or two doses of antibiotics will render blood (and other sites) sterile. In patients with neurologic symptoms, cerebrospinal fluid (CSF) may show evidence of hemorrhagic meningitis with numerous Gram-positive bacilli. Pleural effusions may be large and bloody and may also contain numerous Gram-positive bacilli. Blood, CSF, and pleural effusions may be evaluated by Gram stain, immunohistochemistry, and PCR. Acute and convalescent serum may be collected for serology. All patients suspected of having IA should have a CXR to screen for widened mediastinum, enlarged mediastinal lymph nodes, and pleural effusions. In suspected cases CXR and chest CT scan should be performed. In the attacks of 2001, CXR and/or chest CT were abnormal in all cases.

Medical Management

Early initiation of appropriate antibiotics is paramount for patient survival.[7] The below primarily focuses on the treatment for anthrax in adult patients, however, the treatment of pediatric patients has been outlined in detail in other resources.[7,8] In addition, there are other guidelines for management of pregnant or post-partum women.[7,9]

1. **Systemic anthrax:** Per the most recent CDC guidelines systemic is defined as one or more of the following, using cutoffs for adults aged ≥18 years: hyperthermia or hypothermia, tachycardia, tachypnea, hypotension, or neutrophilia or neutropenia. Due to the high mortality of systemic anthrax and potential for natural (up to 10% have resistance to penicillin-based treatments) and genetically engineered antimicrobial drug–resistant strains, empiric use of combination therapy is preferred.[7] Duration of antimicrobial drug treatment should be 2 weeks or longer but duration can be shortened, and IV antimicrobials transitioned to oral medication based on patient improvement and clinical judgement.[7] Per most recent CDC guidelines, if an aerosol exposure might have occurred (e.g., a bioterrorism-related incident or animal skin drum–related event), patients treated for systemic disease who are immunocompetent

do not need further antimicrobial drugs for PEP because they will have developed natural immunity.[7]

2. **Systemic anthrax infection with possible/confirmed meningitis:** To help clinically differentiate whether anthrax meningitis is present, a 4-item screening tool has been developed and revised after a systematic review of systemic anthrax cases since 1880.[7,10-11] The four items assessed are as follows: severe headache, altered mental status, meningeal signs, and other neurological signs (see latest CDC guidelines for full flowchart)[7]. Two or more positive responses support a presumptive diagnosis of anthrax meningitis. These criteria may be helpful in a mass casualty incident in which there are severely limited diagnostics, lumbar puncture for all patients becomes impractical, or supplies run out. Empiric treatment for anthrax in which anthrax meningitis is suspected or cannot be ruled out optimally should include two bactericidal drugs (kills the bacteria) from different classes plus a protein synthesis inhibitor or an RNA synthesis inhibitor (inhibits toxin production) [ciprofloxacin 400mg IV every 8 hours PLUS meropenem 2g IV every 8 hours plus minocycline 200mg IV x1 then 100mg IV every 12 hours] AND antitoxin (raxibacumab 40mg/kg IV x1 or oblitoxaximab 16mg/kg IV x1). There are many possible first-line and alternate treatment regimens, and these are outlined in current CDC guidelines. [7]

3. **Systemic Anthrax without meningitis (inhalational, gastrointestinal, injection, or cutaneous anthrax with systemic involvement, extensive edema, or lesions of the head or neck):** Empiric treatment is the same as systemic anthrax infection with meningitis listed above. Per guidelines, if meningitis is considered unlikely, one bactericidal drug PLUS a protein synthesis inhibitor (meropenem 2g IV every 8 hours PLUS linezolid 600mg IV every 12 hours or doxycycline) or another CDC recommended first-line regimen should be utilized.[7]

   Penicillin (or other beta-lactam antibiotics) should NEVER be used as monotherapy for severe anthrax disease as the *B. anthracis* genome encodes for both constitutive and inducible beta-lactamases and resistance may occur *in vivo* despite apparent *in vitro* susceptibility. Antibiotic choices must be adjusted for strain susceptibility patterns, and consultation with an ID physician is imperative.

   In the event of a mass-casualty situation, IV antibiotics may not be available. In this case, oral ciprofloxacin (or another fluoroquinolone) OR oral doxycycline may have to suffice as initial therapy. The doses for ciprofloxacin are 500 mg PO bid, and for doxycycline are 200 mg PO initially then 100 mg PO bid thereafter for adults.

   Supportive therapy for shock, fluid volume deficit, and adequacy of airway may be needed. In the IA cases from the 2001 attacks, aggressive drainage

of pleural effusions seemed to improve clinical outcome. Corticosteroids may be considered as adjunct therapy in patients with severe edema or meningitis, based upon experience in treating other bacterial diseases. Human anthrax immune globulin can be obtained as a therapy for IA under an IND from the CDC and has been noted to improve mortality in the past.

The two currently FDA approved monoclonal antibodies are available only via the CDC through the SNS: raxibacumab and obiltoxaximab.[12,13] Both bind and neutralize the free protective antigen (PA) component of *Bacillus anthracis* toxin. Both are approved for the treatment of IA in combination with recommended antibiotic regimens (see above).[12,13] Neither cross the blood-brain barrier. Diphenhydramine should be given within 1 hour prior to infusion of both products to reduce the risk of infusion reactions. Dosing is detailed in the package insert. Both may improve mortality if given early during the disease course, but efficacy has only been demonstrated in animal models.

4. **Cutaneous Anthrax without systemic disease/meningitis:** Uncomplicated cutaneous anthrax should be treated initially with doxycycline (100 mg PO bid for adults) OR ciprofloxacin (500 mg PO bid) OR levofloxacin (500mg PO every 24 hours).[7] Alternate regimens are contained in the latest CDC guidance. If exposure is known to have been due to contact with infected livestock or their products, then 7 to 10 d of antibiotics is generally sufficient. If an aerosol exposure might have occurred, patients should transition from a treatment to a PEP regimen. For patients with significant edema, non-steroidal anti-inflammatory drugs (NSAIDS) or corticosteroids may be of benefit. Debridement of lesions is not indicated.
   If systemic illness develops, then IV antibiotics should be administered as discussed above.

5. **Gastrointestinal anthrax:** Documentation of clinical experience in treating oropharyngeal and intestinal anthrax is limited. Supportive care to include fluid for shock and airway management should be anticipated. Both forms of GI disease should receive the IV antibiotic regimen described for systemic disease above. For oropharyngeal anthrax, airway compromise is a significant risk, and consideration should be given for the early administration of corticosteroids to reduce the development of airway edema. If, despite medical therapy, airway compromise develops, early intubation should be considered. Incision and drainage of affected lymph nodes is not generally indicated. No specific guidance exists for drainage of ascites in patients with intestinal anthrax. However, large fluid collections could, at a minimum, compromise respiration and consideration should be given to therapeutic—and potentially diagnostic—paracentesis.

For further information, refer to **Appendix J: Biological Agent Prophylactics & Therapeutics.**

Infection control: Standard precautions are recommended for patient care in all forms of anthrax disease. There is no data to suggest direct person-to-person spread from any form of anthrax. However, for patients with systemic anthrax disease—especially before antibiotics are initiated—invasive procedures, autopsy, or embalming of remains could potentially lead to the generation of infectious droplets; thus, such procedures should be avoided when possible. If unavoidable, all instruments and materials used should be autoclaved or incinerated, and the immediate environment where the procedure took place should be thoroughly disinfected with a sporicidal agent. Chlorine, in the form of sodium or calcium hypochlorite (bleach), can also be used, but with the caution that the activity of hypochlorite is greatly reduced in the presence of organic material. The U.S. Environmental Protection Agency has endorsed the use of bleach to destroy anthrax.

Any clinical laboratory should be warned before the delivery of suspected anthrax specimens, as growth of *B. anthracis* in culture necessitates biosafety level-2 (BSL-2) precautions.

Experience of anthrax in livestock indicates that incineration of carcasses and sterilization of contaminated ground is the environmental control method of choice. Formerly, a recommendation was deep burial ($\geq$ 6 feet deep) in pits copiously lined with sodium hydroxide (lye); however, this practice may still leave a significant proportion of viable spores. This has led a consensus group to recommend "serious consideration" of cremation of human anthrax victim remains.

Prophylaxis

Vaccine: A licensed vaccine, *BioThrax*® or Anthrax Vaccine Adsorbed (AVA) Emergent Biosolutions, Rockville, MD, is derived from sterile culture fluid supernatant taken from an attenuated (non-encapsulated) strain of anthrax. Therefore, it does not contain living or dead organisms. AVA is available for DOD use and is currently licensed for pre-exposure prophylaxis of disease in persons at high risk of exposure and for post-exposure prophylaxis in conjunction with recommended antibacterials in persons 18 through 65 years of age.[14] The preferred schedule is 0.5 ml IM at 0, 1, and 6 months (primary series) then 12 and 18 month boosters, followed by annual boosters for pre-event prophylaxis. For known or imminent exposure (post-exposure prophylaxis), the schedule is 0, 2 and 4 weeks SQ in combination with a PO fluoroquinolone (ciprofloxacin or levofloxacin or moxifloxacin) or doxycycline or clindamycin with duration of antibiotics depending on the treated population (not to exceed 60 days).[14] DOD policy for missed doses (for those individuals required to remain immune) is to administer the missed dose as soon as possible and reset the timeline for the series based upon the most recent dose.[15] As with all vaccines, the degree of protection depends upon the magnitude of the challenge dose of pathogen received; vaccine-induced protection could presumably be overwhelmed by extremely high spore challenge. Thus, even fully vaccinated

personnel should receive antibiotic PEP if exposed to aerosolized anthrax, IAW the guidelines below.[14,16,17,18,19]

Contraindications for use of AVA include hypersensitivity reaction to a previous dose of vaccine and age < 18 or > 65 years. Reasons for temporary deferment of the vaccine include pregnancy, active infection with fever, or a course of immune-suppressing drugs such as steroids. Reactogenicity is mild to moderate. Up to 30% of recipients may experience mild discomfort at the inoculation site for up to 72 h (e.g., tenderness, erythema, edema, pruritus), fewer experience moderate reactions, while < 1% may experience more severe local reactions, potentially limiting use of the arm for 1 to 2 days. Modest systemic reactions (e.g., myalgia, malaise, low-grade fever) are uncommon, and severe systemic reactions such as anaphylaxis, which precludes subsequent vaccination, are rare. The vaccine should be stored between 2 and 6°C (refrigerator temperature, not frozen).

The vaccination series should be initiated, when feasible, ≥ 45 days before deployment. Reference the latest DOD and service-specific guidance for more information.[20] AVA is recommended for persons who handle high concentrations of spores and potentially infected animals and those who work in spore-contaminated areas.

Note that another vaccine, AVA, adjuvanted (Cyfendus®) has been licensed for PEP only and is not readily available to the DOD as it is in the SNS.

Antibiotics: No antibiotic is approved for pre-exposure prophylaxis of anthrax. Thus, official DOD policy is not to initiate prophylactic antibiotics until after an attack is suspected to have occurred. After a suspected exposure to aerosolized anthrax of unknown antibiotic susceptibility, first-line prophylaxis with doxycycline (100 mg PO bid for adults) or ciprofloxacin (500 mg PO bid for adults) or levofloxacin (500mg PO every 24 hours) should be initiated immediately. Should an attack be confirmed as anthrax, antibiotics should be continued for variable lengths of time dependent upon the patient's vaccination status.

AVA is a critical part of PEP for inhaled anthrax; without vaccination, victims inhaling anthrax spores are unlikely to develop the immunity necessary to prevent disease caused by spores that germinate after antibiotics are discontinued. Persons who are exposed to aerosolized *B. anthracis* spores but have not completed the initial priming and booster series for AVA should receive additional AVA doses and PEP-antibiotics as above.[13] ACIP recommends the SC route of administration rather than the IM route for PEP because higher antibody concentrations are achieved by 4 weeks after AVA vaccination. If the PEP-Vx schedule is interrupted, the series does not need to be restarted. Instead, subsequent doses should be administered as soon as possible, and the series should be finished. The number of vaccine doses and duration of PEP-antibiotics will vary depending on the number of previously received doses and patient type:

1. Immunocompetent adults (e.g., healthy, nonpregnant adults aged 18–65 years): PEP-Abx both for the licensed and dose-sparing PEP-Vx regimens can be discontinued 42 days after initiation of vaccine if AVA is administered on schedule for both the licensed and dose-sparing PEP-Vx regimens.
    a. If the AVA series cannot be completed, then antimicrobial therapy should continue for 60 days.
    b. To account for delays in initial vaccination that might occur because of an emergency situation, antimicrobial administration should be initiated as soon as possible and continued for 42 days after the first dose or 2 weeks after the last dose of the vaccine series, whichever comes last.

2. Persons with immunocompromising conditions that might interfere with their ability to develop an adequate immune response or populations for whom data on immune response to AVA are lacking (e.g., children, pregnant women, and adults aged ≥65 years) should continue to receive PEP-Abx for 60 days concurrently with AVA.

3. Those who have received PrEP, if biosafety or respiratory protection measures are breached and exposure to aerosolized *B. anthracis* spores might have occurred (such as in the laboratory): 30-day course of PEP-Abx is recommended, regardless of whether PrEP has been fully or partially completed.[14]

Persons who are exposed to aerosolized *B. anthracis* spores but have not completed the initial priming and booster series for AVA should receive additional AVA doses and PEP-Abx. The number of vaccine doses and duration of PEP-Abx will vary depending upon the number of previously received doses and are summarized in CDC guidance.[13]

Antitoxins: Raxibacumab and obiltoxaximab are approved by the FDA for prophylaxis of IA in adults and children when alternative treatments are not available or are contraindicated.[12,13]

BRUCELLOSIS

Summary

Signs and symptoms are usually non-specific: fever, headache, myalgias, arthralgias, back pain, profuse sweats, chills, weight loss, and malaise. Onset may be acute or insidious. Fever may be intermittent or continuous and recrudescence is common even after antibiotic treatment. Subclinical and chronic infections are common. Osteoarticular complications, notably sacroiliitis, occur with some degree of frequency and are responsible for much of the disability associated with *Brucella spp.* infection. Other manifestations include depression and mental status changes, epididymoorchitis, and localized suppurative infection. Morbidity may be pronounced; fatalities are uncommon.

Diagnosis: requires a high index of suspicion, as most infections present as non-specific febrile illnesses or are asymptomatic. Lab diagnosis can be made by serum agglutination tests, ELISA, immunofluorescence, and by standard culture. Blood cultures often require extended incubation to become positive, even up to 30 days. Bone marrow cultures may produce a higher yield. Other body fluids may be tested depending on the sites affected (e.g., synovial, pleural, CSF).

Treatment: with doxycycline and rifampin (or other antibiotics) for 6 weeks is usually sufficient. More prolonged regimens may be required for patients with complications such as hepatitis, splenitis, meningoencephalitis, endocarditis, or osteomyelitis.

Prophylaxis: No human vaccine is available. Chemoprophylaxis should be considered for high-risk exposures in the following situations: (1) inadvertent wound or mucous membrane exposure to infected livestock tissues or body fluids or to livestock vaccines; (2) exposure to lab aerosols or to secondary aerosols generated from contaminated soil in calving/lambing areas; (3) confirmed bio-warfare/bio-terrorism exposure.

Isolation and decontamination: *Brucella* is spread readily via bodily fluids and certain aerosols, but not by respiratory droplets; standard precautions are thus adequate for the protection of healthcare workers. If an attack with a *Brucella* spp. is suspected, special care should be taken to avoid the generation of secondary aerosols. Contact surfaces that are free of organic matter can be decontaminated with a 0.5% hypochlorite solution; higher concentrations (> 5%), or other disinfectants, should be used where organic matter cannot be effectively reduced or controlled.

Overview

Brucellosis is a zoonotic disease caused by infection with one of ten species of the genus *Brucella,* a group of facultative intracellular gram-negative cocco-baccillary organisms.[1] Four of the ten described species are known to infect humans (Table 1).

Characteristics of Naturally Occurring Brucellosis in Animals & Humans

***Brucella* spp.**	**1º Reservoir**	**2º Hosts**	**Geographic Distribution**	**Human Exposure Activity**	**Pathogenicity To Humans**
B. abortus	Cattle, Bison, Deer	Goat, Sheep, Dog, Human	Worldwide	Raw dairy foods, animal husbandry, laboratory	Moderate
B. melitensis	Goat, Sheep	Dog, Human	Latin America, Asia, Mediterranean	Raw dairy foods, animal husbandry, laboratory	Highest
B. suis	Pig (feral, and domestic)	Dog, Human, Cattle	SE Asia, Scattered & Midwest U.S., S. America	Pork slaughter, processing, feral pig hunting, laboratory	High
B. canis	Dog, Coyote		Scattered	Dog breeding & whelping	Moderate

The global disease burden of *Brucella* spp. is enormous in livestock with conservative estimates of >300 million of the 1.4 billion worldwide cattle population is infected.[2] Brucellosis can be thought of as a venereal disease of livestock and, as such, primarily affects the reproductive system of these animals producing septic abortion, retained fetal membranes, orchitis, and infection of the male accessory sex glands. Transmission occurs primarily via the ingestion of organisms contained in fetal membranes, aborted fetuses, and uterine discharges, and occasionally from dams to nursing young. *Brucella* spp. may also enter the body through mucous membranes, conjunctivae, and wounds.

Brucella spp. infects humans as an incidental host. It's estimated that >500,000 new (human) infections occur annually.[2] Zoonotic transmission to humans has occurred via contact with infected tissues, blood, urine, semen, and gynecologic secretions.[3] Veterinarians, slaughterhouse workers, ranchers, animal husbandry workers, and hunters have consequently been infected in occupational and recreational settings. Transmission to humans also occurs via the ingestion of raw milk and other dairy products from infected animals. Though less common, airborne infections have also occurred in livestock husbandry settings (inhalation of contaminated particles from soil and bedding in birthing areas)

and in lab settings. Finally, accidental percutaneous exposure to modified-live livestock vaccines (e.g., veterinarians) has also occurred.

Infections among abattoir and laboratory workers suggest that the inhalation of as few as 10 organisms is sufficient to cause disease in humans. Subclinical and chronic infections are relatively common. Brucellosis has a low case fatality rate (5% of untreated cases), with rare deaths caused by complications such as endocarditis or meningitis. When disease is naturally occurring, the incubation period may be several days to several months. However, large aerosol doses—as would be expected in a bio-warfare scenario—would shorten the incubation period, lead to higher clinical attack rates, and result in more prolonged, incapacitating, and disabling disease than in the natural form.

History & Significance

Jeffrey Allen Marston first described the disease manifestations associated with *Brucella melitensis* infection among British soldiers on Malta during the Crimean War (1853-56; Florence Nightingale may have been the most famous victim of the so-called Malta fever or Crimean fever). Goats were identified as the source of this outbreak and restrictions on the consumption of unpasteurized dairy goat products soon decreased the incidence among military personnel. *Brucella abortus* was first isolated by David Bruce—hence the genus designation—in 1897. The extraordinary infectivity[4], as well as stability in aerosol form and resistance to desiccation, led bioweaponeers in the UK to focus on *Brucella* spp. during World War II and, in 1954, *B. suis* became the first agent weaponized by the U.S. at its Pine Bluff Arsenal located in Arkansas. Moreover, Ken Alibek, a Soviet defector and former official with the Russian bioweapons program, began his career by perfecting the culture of the *Brucella* spp. It has been alleged that the South African Defense Forces, in the apartheid era, experimented with weaponized *B. melitenisis*. Conversely, their long and variable incubation periods, coupled with the large percentage of asymptomatic infections, mitigate against the use of the *Brucella* spp. as weapons.

Human brucellosis is now rare in the U.S. with about 100 cases reported annually, mostly from CA, FL, TX, and VA, due to pasteurization and the U.S. State-Federal Cattle Brucellosis Eradication Program. Most human cases are associated with the ingestion of unpasteurized dairy products made outside of the U.S. and privately imported (thus escaping FDA and USDA regulatory food-safety measures). Rare infections may still occur in meat processing or livestock handling settings in areas with herds or flocks that are not certified brucellosis-free by regional animal health authorities. In regions where brucellosis is endemic, the disease has far-reaching and negative effects on humans and animals.[1] However, the true incidence of the disease in many areas with endemic brucellosis is likely to be markedly underreported due to poor medical and veterinary infrascture.[1] Human brucellosis is endemic in some African and Mediterranean basin nations, as well as India, Mexico, South and

Central America and many of the republics of the former Soviet Union.[5] Disease incidence and prevalence vary regionally, with some reporting annual incidences of over 80 cases per 100,000 population. Persistent foci of enzootic disease among sheep and goats plague the Middle East today and serologic evidence of *Brucella* spp. exposure among humans on the Arabian Peninsula was near 20% with more than 2% having active disease in one recent WHO study. A few regions in Kuwait have reported annual incidences as high as 128 cases per 100,000 population. These findings highlight a risk to military personnel
in the region.[6]

Clinical Features

Brucellosis is a systemic disease with variable manifestations that can involve virtually any organ system[7,8]; disease type and severity vary with the infecting *Brucella* species. *B. melitensis* is the most pathogenic for humans, in whom infection is associated with an acute course and disabling complications. *B. suis* infection is associated with localized abscess formation and a chronic course. *B. abortus* and *B. canis* infections are associated with frequent relapses and insidious onset.

Untreated, brucellosis will localize in the reticuloendothelial system, primarily the liver, spleen, and bone marrow, where granuloma formation ensues. Large granulomas serve as a source for persistent bacteremia. The incubation period of brucellosis is typically 3 or 4 weeks but can range from as few as 5 days to many months. Illness onset can be abrupt or come insidiously over weeks or months. Although clinical disease varies, the following percentages have been collectively obtained from multiple sources and may represent manifestations from different *Brucella* spp.[7,9,10] Non-specific symptoms such as fever (90-95%), malaise (80-95%), sweats (40-90%), and myalgias/arthralgias (40-70%), are typical. Brucellosis should be considered as a differential diagnosis for fevers of unknown origin especially in malaria-endemic countries.[10] Fever is typically intermittent and can assume an undulant (wave-like) pattern in patients with chronic, untreated infection. Fatigue, chills, and backache are not unusual. Neuropsychiatric symptoms including depression, headache, and irritability, are common.[11] GI symptoms (abdominal pain, anorexia, constipation, diarrhea, vomiting), resembling typhoid fever, are reported in nearly 70% of adult cases. Cough, dyspnea, chest pain, and testicular pain occur less frequently. Common physical findings include hepatomegaly (10-70%) and/or splenomegaly (10-30%), arthritis (up to 40%), weight loss, and adenopathy (10-20%).

Osteoarticular complications of brucellosis, seen in 20-60% of cases, include bursitis, tenosynovitis, arthritis, osteomyelitis, sacroiliitis, discitis, and paravertebral abscess (less common than in spinal tuberculosis[9]). Sacroiliitis typically presents acutely with fever and focal lower back pain and occurs in up to 30% of cases, predominantly in young men. Arthritis of large, weight-bearing joints of the lower extremities may occur in 20%. Arthritis is usually

monoarticular but can be polyarticular up to 30%. Spondylitis or vertebral osteomyelitis may affect up to 30% of all cases of brucellosis. Patients with spondylitis tend to be older and have a more chronic, destructive disease course than those with sacroiliitis or peripheral arthritis; the lumbar vertebrae are most commonly affected.

The liver lesions are common, but vary, ranging from granulomatous or mononuclear infiltrative hepatitis to small necrotizing foci resembling viral hepatitis.[9] As a rule, hepatitis only progresses to cirrhosis if pre-existing liver disease (e.g., hepatitis C or alcoholic liver disease) is present. Besides flu-like GI signs, cases of ileitis, colitis, and peritonitis are not common. Pulmonary disease occurs in 1 to 5% of cases and may take the form of solitary nodules, lung abscesses, miliary lesions, bronchopneumonia, enlarged hilar lymph nodes, or pleural effusions. Rare patients have succumbed to acute respiratory distress syndrome (ARDS) associated with pulmonary brucellosis.[13] While inhalational exposure to *Brucella* has been described in lab and abattoir workers, this route of infection has not proven to lead, with regularity, to any specifically pneumonic form of disease.

Epididymoorchitis, the most common genitourinary complication of brucellosis infection[10], has been described in 2 to 20% of male brucellosis patients and typically presents acutely with scrotal pain and swelling, as well as unremitting fever. Orchitis is unilateral in the majority of cases. Neurologic disease can take the form of meningitis, encephalitis, peripheral neuropathy, brain or epidural abscesses, radiculoneuropathies, or meningovascular syndromes.[9,10,12] However, direct central nervous system invasion occurs in < 5% of brucellosis cases. Behavioral disturbances and psychoses appear to be unrelated to the degree of fever and may be only occasionally associated with the aforementioned neural syndromes during acute phases. Endocarditis, particularly involving native and prosthetic valves, occurs in < 2% of cases, but accounts for the majority of brucellosis-related deaths.[9,10] Acute brucellosis during the first two trimesters of pregnancy has been reported to lead to spontaneous abortion if untreated.

Diagnosis

The gold standard for definitive diagnosis of brucellosis remains bacterial isolation and identification of the bacterium.[9,14] However, in most cases prior to culture and isolation, a high index of suspicion is necessary to even consider brucellosis. A history of contact with susceptible animals, consumption of unpasteurized dairy products (including goat), or travel to endemic areas, should prompt consideration of brucellosis. Patients presenting with fever, night sweats, undue fatigue, GI symptoms, anorexia, weight loss, headache, arthralgias, and hepatosplenomegaly should prompt the consideration of this diagnosis. Additionally, patients with some of the aforementioned complications, such as sacroiliitis or epididymoorchitis merit consideration for brucellosis testing. Brucellosis is a well-established diagnosis in patients with fever of unknown

origin, and a thorough review of risk factors seeking a potential exposure to *Brucella* species should be conducted in such patients.

The leukocyte count in brucellosis is usually normal but may be low; anemia, neutropenia, and thrombocytopenia occur in some cases. AST and ALT may be mildly elevated; the erythrocyte sedimentation rate (ESR) is normal or only mildly elevated in the majority of cases. Microscopically, *Brucella* species are small, non-motile, gram-negative, non-encapsulated, non-spore forming intracellular coccobacilli. Modern automated blood culture systems will grow brucellae within 7 days in 95% of cases, although misidentification is common. Blood and bone marrow cultures taken during the acute febrile phase of illness yield the organism in most cases. CSF, synovial fluid, and urine cultures may prove useful in patients with appropriate clinical signs. Bone marrow and liver biopsies (to detect granulomatous disease) may be useful in select circumstances. Clinical labs should always be alerted if a diagnosis of brucellosis is suspected. This permits the use of selective isolation media and the implementation of BSL-3 containment.

Several laboratory studies may be employed in the definitive diagnosis of brucellosis: culture, serologic testing, immunofluorescence (IF) and molecular diagnostics. Each modality has limitations. Blood cultures are typically negative in patients taking antibiotics. Widely used agglutination tests often give false-positive results. A presumptive diagnosis of brucellosis can be made using a serum agglutination test (SAT) for IgM and IgG, and a tube agglutination method for anti-O polysaccharide antibody is available; titers of $\geq$ 1:160 by each indicate active disease. An ELISA, for detection of Brucella IgM and IgG antibodies in blood samples, is also available, and CSF as well as joint fluid may be used for antibody testing with some test kits. Immunofluorescence staining of biopsy-obtained tissue can be used to demonstrate organisms in select cases.

Polymerase chain reaction (PCR) tests are also available for the detection and rapid diagnosis of *Brucella* species in human blood specimens.[14] Of note, the Rose Bengal Plate Agglutination test (RBT), originally designed for screening in animals, can be used as a rapid screening test for human brucellosis.[15]

Because all modalities have shortcomings, multiple categories of tests may be enlisted to establish the diagnosis. Definitive lab criteria include: 1) isolation of *Brucella* spp. from a clinical specimen; 2) $\geq$ a fourfold rise in *Brucella* spp. agglutination titer between acute and convalescent sera obtained $\geq$ 2 weeks apart and performed at the same lab; 3) demonstration by IF of *Brucella* spp. in a clinical specimen. A probable case is one that is clinically compatible and epidemiologically linked to a confirmed case or that has supportive serology (i.e., *Brucella* agglutination titer of $\geq$ 1:160 in one or more serum specimens obtained after onset of symptoms) or supportive PCR. A confirmed case is a clinically compatible case that is lab-confirmed.

Imaging studies may help to localize infection. Persistent fever after therapy or the prolonged presence of significant musculoskeletal complaints should prompt CT or MR imaging. ^{99m}Technetium and ^{67}gallium scans may reveal sacroiliitis or other axial skeletal infections. CXR in brucellosis patients may be unremarkable even in the presence of respiratory symptoms. ECHO may reveal evidence of endocarditis. Vegetative lesions are most common on the aortic valve (sinus of Valsalva), followed by the mitral valve. Testicular ultrasound may be helpful in distinguishing *Brucella* epididymoorchitis from testicular abscess or tumor.

Clinically, identification to the genus level is adequate to initiate therapy for brucellosis. Species identification is epidemiologically necessary and helps to inform prognosis; however, it requires more specialized analysis.

Medical Management

Historically, the most effective proven treatment for acute brucellosis in adults has been the combination of doxycycline 100 mg PO bid for 4 to 6 weeks plus streptomycin 1 g IM daily for the first 2 to 3 weeks.[15,16,17,18] As streptomycin is no longer widely available, IV gentamicin 3 - 5 mg/kg QD probably represents a suitable alternative. For uncomplicated acute brucellosis, however, combinations of oral antibiotics are usually sufficient and have cure rates approaching those of the doxycycline-aminoglycoside combinations. The most widely recommended combination for adults and children > 8 years old is doxycycline (100 mg PO bid for adults, 2.2 mg/kg PO bid [up to 200 mg/d] for children) + rifampin (600-900 mg/d PO qd for adults,15-20 mg/kg [up to 600-900 mg/d] for children) for 4 to 6 weeks[9,18]; a fluoroquinolone (e.g., ciprofloxacin 500 – 750 mg PO BID) + rifampin or TMP-SMX 1-2 DS PO BID + rifampin may be appropriate alternatives. Relapse rates of 5 to 10% for most combination oral regimens and higher for monotherapy (up to 30% with TMP-SMX alone) complicate therapy.[18] During pregnancy and for children < 8 yrs old, the combination of TMP-SMX and rifampin has been advocated.

Acute, complicated brucellosis (e.g., skeletal disease, endocarditis) often requires long-term triple-drug therapy for effective cure. A combination of oral rifampin and doxycycline (or TMP-SMX in children < 8 years old), plus IV gentamicin for the first 2 to 3 weeks is recommended. For skeletal disease, 6 to 8 weeks of antibiotics may be necessary for cure; persistent musculoskeletal complaints may be present in patients with chronic infection and sacroiliitis. Patients with meningoencephalitis or endocarditis should receive $\geq$ 90 days of therapy and may require treatment for > 6 months. Endocarditis typically responds poorly to antibiotics alone and generally requires surgical excision of the affected valve. Necrotizing orchitis and other suppurative complications of brucellosis may also require surgical management.

Patient education is a critical component of medical management and must include emphasis on the importance of antibiotic compliance. Periodic follow-up

is also critical, and referral to medical specialists may be indicated. As is the case with all bacterial bio-agents, antibiotic resistance can be engineered into the organism, and thus determination of antibiotic susceptibilities in an intentional attack with *Brucella* would be paramount.

Infection control: Standard precautions are adequate in managing brucellosis patients, as the disease is not generally transmissible from person-to-person. Masks, gloves, and eye protection are indicated when performing respiratory procedures and when handling body fluids. BSL-3 containment practices should be used when handling suspected *Brucella* spp. cultures in the laboratory because of potential aerosol exposure.[19]

Prophylaxis

No licensed human brucellosis vaccine is available.[20] Livestock vaccines are available; these live vaccines are potentially hazardous to humans and are thus tightly controlled by regional animal health authorities.

Optimal chemoprophylaxis following known or suspected exposure to *Brucella* spp. remains a matter of dispute. The CDC interim recommendations for high-risk exposures are doxycycline 100 mg PO bid plus rifampin 600 mg PO qd.

Most developed countries have largely eradicated brucellosis from domestic cattle, sheep, and goat herds via multifaceted control programs including periodic testing and slaughter of positive and contact animals as well as periodic batch testing of raw milk. Travelers to developing countries should be aware of prevalent foodborne and endemic brucellosis risks. The risk of foodborne brucellosis is reduced by avoiding unpasteurized dairy products, particularly in areas where brucellosis is known to still occur in livestock.

Brucellosis is a reportable human and livestock disease in the U.S. and in many other countries.

GLANDERS & MELIOIDOSIS

Summary

Symptoms and signs: Incubation periods after inhalation are usually < 14 days but may range from days to weeks for glanders and days to decades for melioidosis. Onset of symptoms may be abrupt or gradual. Respiratory tract disease can produce fever (usually > 102°F), rigors, sweats, myalgias, headache, productive or nonproductive cough, pleuritic chest pain, and cervical lymphadenopathy. Pneumonia can progress rapidly and lead to metastatic infection, causing hepatosplenomegaly and generalized papular/pustular eruptions. Both diseases are usually fatal without treatment.

Diagnosis: *Burkholderia mallei* and *B. pseudomallei* (agents of glanders and melioidosis, respectively) are gram-negative bacilli; methylene blue or Wright's stain of exudates may disclose a "safety-pin" bipolar appearance. CXR may show infiltrates with consolidation and cavitation, multiple small lung abscess, or miliary lesions. Abdominal and pelvic ultrasound, CT or MRI may reveal splenic, hepatic, or prostatic abscesses. Standard cultures and PCR can identify both agents.

Treatment: Initial therapy can consist of IV ceftazidime, meropenem, or imipenem, followed by prolonged oral antibiotic therapy. Surgical drainage is indicated for large abscesses. Life-long follow-up is advised after treatment for melioidosis due to a 10% risk of relapse.

Prophylaxis: No vaccines are currently available. There are no human data or FDA-approved regimens for post-exposure prophylaxis. Notably, TMP-SMX shows promise in animal studies and is recommended after accidental laboratory exposures and should be considered very promptly after a biological attack with either agent. (See also Appendix J.)

Isolation and decontamination: Standard precautions are recommended for health care workers, with contact precautions added for patients with skin lesions. Person-to-person airborne or droplet transmission is unlikely. Cultures must be handled under BSL-3 conditions. Environmental decontamination using a 0.5% to 1.0% hypochlorite solution is effective.

Overview

The etiologic agents of these two diseases are the gram-negative bacilli *Burkholderia mallei* (glanders) and *Burkholderia pseudomallei* (melioidosis).

The natural reservoir of *B. mallei* is limited to horses, mules, donkeys, and some reports of wild cats. Typically, cases are endemic to Eastern Asia, Northern Africa, and the Middle East/Asia particularly areas where equids are still prominently used. Transmission to humans is infrequent, possibly due to low

bacterial loads in lesion discharge and because strains virulent for equids are often less virulent for humans. Cases have occurred among horse and donkey caretakers, abattoir workers, veterinarians, and research laboratory microbiologists. The low transmission rates of *B. mallei* to humans from infected horses is exemplified by the fact that in China, during World War II, 30% of tested horses were positive for glanders, but human cases were rare. Acute presentations are more common in mules and donkeys, with death typically occurring within 3 to 4 weeks. Chronic disease is more common in horses and humans, causing multiple skin nodules that ulcerate and drain, induration and nodular lesions of superficial lymphatic vessels of the extremities, regional lymphadenopathy, and abscesses of internal organs. The cutaneous and lymphatic disease in horses is known as "farcy."[1]

B. pseudomallei is widely distributed in water and soil in tropical and subtropical regions. It spreads to humans by inoculation of abraded or lacerated skin, ingestion of contaminated food or water, or by inhalation. Melioidosis is endemic in Southeast Asia, northern Australia, South America, and now the Southern Gulf Coast of the United States where it is most prevalent during the rainy season among people who have direct contact with wet soils.[17, 18, 20] Most exposed persons do not develop disease; asymptomatic seroconversion is common in endemic regions. Most (50-80%) patients have predisposing conditions including diabetes mellitus, alcoholism, cirrhosis, renal disease, thalassemia, cystic fibrosis, or impaired immunity. Clinical presentations vary from mild disease to overwhelming sepsis with up to a 90% case fatality rate (CFR) and death 24 to 48 hours after onset.[2, 3] Aerosols from cultures are highly infectious to lab workers. BSL-3 containment practices are required when working with cultures of these organisms. Clinical chemistries, hematology, and other laboratory tests may be done under BSL- 2 conditions, but attention should be taken with procedures involving aerosol generation, such as manual CBC differentials. Ordering healthcare providers should over communicate to clinical laboratory personnel a suspicion of any infection of a type like Melioidosis. Also, many biotechnology companies will consider service contracts void if clinical specimens containing BSL-3+/select agents are run through an analyzer. Person-to-person spread is rare.

Because of their virulence, potential transmission by environmental aerosols, lack of available vaccines, and difficult treatment regimens, *B. mallei* and *B. pseudomallei* have been considered potential bio-agents.

History & Significance

B. mallei (glanders) was one of the first bacterial agents to be weaponized in a modern bio-warfare program. During World War I, German agents in Baltimore and other seaports allegedly inoculated horses, mules and donkeys intended for export to Allied forces in Europe. The intent was to disrupt military logistics, as these animals were essential to transportation before the large-scale availability of motorized vehicles. The results of these alleged biological attacks are unknown.

The Japanese allegedly infected horses, civilians, and prisoners of war with *B. mallei* at the Pinfang Institute during World War II. The U.S. also studied this agent as a possible biowarfare weapon in 1943 and 1944 but did not weaponize it. The Soviet Union is believed to have identified *B. mallei* as a potential bio-agent after World War II. Glanders has been eliminated from North America, Europe, and Australia, but sporadic cases still occur among equids in Asia, Africa, the Middle East, and South America. Human cases are rare. A laboratory-acquired case occurred at USAMRIID in 2000.[1,4,5]

B. pseudomallei (melioidosis) is a significant cause of community-acquired pneumonia and sepsis in northern Australia and has accounted for ~20% of community-aquired sepsis in Northern Thailand. Pulmonary melioidosis occurred among U.S. forces during the Vietnam conflict, thought to have been due to inhalation of aerosols of contaminated soil and water generated by helicopter rotor wash in irrigated rice fields.[19] Due to activation of latent infection, French and later U.S. soldiers returning from Vietnam would infrequently develop disease (the "Vietnamese time-bomb") years after exposure. Presently, the estimated global morbidity is ~165,000 cases/year with ~89,000 deaths/year.[22] *B. pseudomallei* was also studied by the U.S. as a potential bio-agent, but never weaponized. It has been reported that the Soviet Union studied and weaponized *B. pseudomallei*.[6] Recently, Melioidosis was transmitted to four U.S. residents from an aerosol exposure to aromatherapy spray (Better Homes and Gardens™) contaminated with *B. pseudomallei*, resulting in two fatalities.[21]

Clinical Features

Incubation periods vary by portal of entry, inoculum, virulence, and host factors. Animal models of high dose inhalational exposure to either *B. mallei or B. pseudomallei* are usually followed by incubation of 1 to 4 days. In the few well-documented human cases of glanders due to respiratory exposure, incubation varied from 10 to 14 days. Incubation following mucus membrane or skin exposure is usually in the range of 1 to 21 days but can be several months. The incubation of naturally acquired melioidosis is more difficult to determine, because exposure in endemic regions may be continuous. Documented incubations of clinically overt melioidosis are typically 1 to 21 days, although periods of several months to years can occur. Uncommonly, patients may present with either disease years after exposure due to activation of latent infection, in the case of melioidosis, usually after the onset of diabetes or other risk factors.[1-3]

The manifestations of both glanders and melioidosis are variable; disease can be acute or chronic, localized or systemic, or progress from one form to another. Inhalation of aerosols produced by bio-weapons containing high inocula of *B. mallei* or *B. pseudomallei* could presumably produce any of these syndromes, although acute respiratory or systemic syndromes would be most likely.

Acute glanders and melioidosis after intentional high-inoculum aerosol exposure can be expected to have similar clinical presentations; differentiation will depend upon laboratory studies. Severe pneumonia would likely develop. Patients would likely present within a few days of exposure with the acute onset of fever, chills, malaise, myalgias, and shortness of breath, with or without cough and pleuritic chest pain. Sputum is often purulent, and hemoptysis may occur. Chest X-ray findings vary and may disclose unilateral or bilateral, multifocal, nodular, or lobar consolidation, often progressing to abscess formation and cavitation.

Septicemia may occur at any time, regardless of the portal of entry, and cause fever, rigors, night sweats, myalgia, anorexia, and headache. Bacteremia may cause diffuse seeding of the skin, leading to a regional or generalized papular and/or pustular rash. Disseminated infection may produce abscesses of internal organs (especially liver, spleen, and lungs) and skeletal muscles/bones. These abscesses may result in hepatosplenomegaly and abdominal tenderness. Osteomyelitis, brain abscess, and meningitis have been reported. Disseminated infection carries a high risk of septic shock, end-organ failures, and death. Certain strains of *Burkholderia pseudomallei* have been shown to result in a higher incidence of central nervous system involvement, particularly the ATS2021 strain containing the $bimA_{Bm}$ variant of the *bimA* gene.[23] $bimA_{Bm}$ is a motility factor in *Burkholderia pseudomallei* identical to the same gene in *Burkholderia mallei*.

Rarely, these diseases can present as a focal abscess without an antecedent illness or obvious site of primary inoculation; most commonly in melioidosis as a primary purulent parotitis in children (more common in Thailand) or as a primary prostatic abscess (more common in northern Australia).

Clinical presentations may suggest other bio-agents in the differential diagnosis. A rapidly progressive pneumonia accompanied by sepsis, with respiratory secretions demonstrating gram-negative bacteria with “safety pin” appearance on Wright’s stain suggests pneumonic plague, while a diffuse papular or pustular rash may suggest smallpox.

Natural disease due to both organisms is described in the literature.[1-3] Differences between the clinical presentations of glanders and melioidosis may result from mucocutaneous or low inoculum exposures and are described below.

Glanders: Cutaneous exposure typically leads to local inflammatory nodules with subsequent lymphangitis (sometimes with a sporotrichoid nodular presentation) and regional lymphadenitis. Nodules typically ulcerate and drain. Conjunctivitis can result in photophobia, lacrimation, and purulent discharge. Acute or subacute constitutional symptoms may develop, and can include fever (low-grade or recurring), rigors, sweats, headache, fatigue and myalgias.

Inhalational exposure may produce either upper or lower respiratory tract disease. Rhinitis or pharyngitis may feature constitutional symptoms, headache, purulent exudates, and cervical lymphadenopathy. Chronic infection and erosion of the nasal septum and turbinates can lead to severe disfigurement.

Chronic disease occurs in half of all natural cases and is eventually fatal without treatment. Chronic infections may feature spontaneous clinical remission followed by relapse. CFRs dropped to 20% for localized disease, and to 40% overall, after sulfadiazine therapy became available. Experience during the modern antibiotic era is, however, very limited.

Melioidosis: Mucocutaneous exposure may lead to local nodules/abscesses and regional lymphadenitis. Cutaneous disease may result from local inoculation or from bacteremic seeding of the skin.

Inhalational exposure, either through near drowning or via infectious aerosols, may result in respiratory diseases that can range from a mild bronchitis to a chronic subacute pneumonia, or a severe acute necrotizing pneumonia and septic shock. Sputum is often purulent, and hemoptysis may be present. Radiographic findings commonly feature lobar or segmental consolidation with a predilection for the upper lobes, or multiple, widespread 0.5 to 1.0 cm nodules, or cavitation. Chronic pulmonary disease can follow acute pneumonia, or reactivate years after exposure, with clinical and radiographic findings mimicking those of tuberculosis. Cutaneous and internal (especially hepatic, splenic, and prostatic) abscesses can occur up to weeks or months after exposure or acute disease.

Septicemic melioidosis presents with fever, rigors, night sweats, myalgia, anorexia, and headache. Additional features can include papular or pustular skin lesions, diarrhea, and hepatosplenomegaly. Dissemination is likely to produce cutaneous and internal (especially liver and spleen) abscesses even weeks to months later. Prostatic abscess occurs in 2 to 15% of cases. Poor prognostic indicators include positive blood cultures within 24 hours of incubation and neutropenia. Without proper treatment, most septicemic patients will die within 2 or 3 days. With treatment, CFRs are approximately 40% in Thailand and 14% in Australia.[3] Relapse occurs in approximately 10% of survivors.[3,7,8]

Diagnosis

Microbiology: Gram stain of lesion exudates reveals small irregular staining, gram-negative coccobacilli. Methylene blue or Wright's stain may reveal bipolar "safety pin" staining. The organisms can be cultured from abscesses/wounds, secretions, sputum (in pneumonia), and, possibly, blood and urine with standard media. Primary isolation requires 48 to 72 hours in agar at 37.5º C; automated blood culture methods are typically more rapid. Selective media (e.g., Ashdown's medium for *B. pseudomallei*) may be necessary for isolation from non-sterile sites (sputum, pharynx swabs). *Burkholderia cepacia* selective agar

(often used for care in cystic fibrosis patients) can also be used if unique agars (like Ashdown's agar) are not available.

Blood cultures for *B. mallei* are rarely positive. In contrast, blood cultures for *B. pseudomallei* are often positive and urine culture may be positive, especially if prostatitis or renal abscesses are present. The laboratory should be alerted if these diagnoses are being considered, because of the occupational health hazards posed by these organisms, and because some automated culture systems may misidentify *B. pseudomallei* as *Pseudomonas* spp.[2] Cultures must be performed under BSL-3 precautions due to the high aerosol risk to lab workers. *B. pseudomallei* will grow well on blood, MacConkey, and *B.cepacia* agar, in addition to, Ashdown's media. On Ashdown's, MacConkey, and *B. cepacia* agar, *B. pseudomallei* colonies will have a wrinkled pink appearance after 48+ hours of growth. Suspected *B. pseudomallei* specimens should be kept on all the listed agars for at least 72 hours.

PCR is rapid and specific, but may be less sensitive than cultures, especially for evaluating blood samples. Rapid immunoassays for *B. pseudomallei* capsular antigens are available in some reference laboratories.

Serologic tests: are of limited utility, particularly in endemic areas where background seroprevalence is high.[2,3,10] Indirect hemagglutination assays are the most frequently used serologic tests in endemic regions but are poorly standardized and difficult to perform.[8,9] Currently available tests do not distinguish between the two etiologic agents, and are considered inadequate as a sole method of laboratory confirmation.[2,9,10]

For *B. mallei,* agglutination tests are not positive for $\geq$ 7 to 10 days (or up to 3 weeks), and a high background titer in normal sera (1:320 to 1:640) makes interpretation difficult. Complement fixation (CF) tests are more specific, but less sensitive, and may require 40 days for conversion. CF tests are considered positive if the titer is $\geq$ 1:20. For *B. pseudomallei,* a fourfold increase in titer supports the diagnosis. A single IgM titer > 1:160 with a compatible clinical picture suggests active infection; IgG is less useful in endemic regions due to high seroprevalence.

Other laboratory studies: Clinical chemistries, hematology, and similar clinical laboratory tests not involving cultures may be done under BSL- 2 conditions, but see the above-mentioned precautions to these procedures. Findings may include leukocytosis, anemia, coagulopathy, and abnormal hepatic and renal function tests. In septicemic glanders, mild leukocytosis with a shift to the left or leukopenia with a relative lymphocytosis may occur. In systemic melioidosis, significant leukocytosis with left shift is common, and leucopenia (neutropenia) is a poor prognostic indicator; anemia, coagulopathy, and evidence of hepatic or renal dysfunction may be present.

Radiographic studies: Chest X-Rays may demonstrate lobar or segmental consolidation, diffuse nodular opacities, cavitary lesions, pleural effusions and empyema. Hilar adenopathy is infrequent.[11] Abdominal and pelvic imaging (CT or MRI imaging, or abdominal and pelvic/transrectal ultrasounds) should be considered for all patients with suspected glanders or melioidosis to exclude hepatic, splenic or prostatic abscesses.

Pathology: Melioidosis can cause granulomatous lesions suggesting tuberculosis. This can make diagnosis difficult, especially in areas where both melioidosis and tuberculosis are endemic, such as Thailand.[12]

Medical Management

Supportive Care: Ventilatory support may be necessary for severe pneumonia. Septicemic patients often require aggressive care including fluid resuscitation, vasopressors, and management of coagulopathy. Large abscesses and empyemas should be drained; prostatic and parotid abscesses in patients with melioidosis are unlikely to resolve without surgical intervention. Surgical therapy is not necessary for multiple small hepatic or splenic abscesses, which respond to prolonged antibiotic therapy.

Antimicrobials: Antibiotic regimens for melioidosis are based on clinical trials and medical experience in Thailand and Australia. Although experience with human glanders is limited due to its low incidence during the antibiotic era, the same treatment regimens are recommended for both diseases as these organisms have similar antibiotic susceptibility patterns. (Unlike *B. pseudomallei*, natural *B. mallei* strains generally remain susceptible to aminoglycosides and macrolides *in vitro*.) Revision of empiric therapy is guided by antibiotic susceptibilities of bacterial isolates.

Initial therapy: All cases of both diseases, regardless of clinical severity, should be treated with IV therapy for $\geq$ 10 to 14 days and the patient shows clinical improvement, followed by oral eradication therapy for $\geq$ another 3 months.[2,3] Antibiotic regimens include either ceftazidime (50 mg/kg [up to 2 g]) IV every 6 to 8 hours or meropenem (25 mg/kg [up to 1 g]) IV every 8 hours. Imipenem (25 mg/kg [up to 1 g]) IV every 6 hours is an acceptable alternative but carries a higher risk of central nervous system (CNS) toxicity and is more difficult to dose in renal failure. Meropenem is advised for patients with CNS involvement. A switch to meropenem is indicated if the patient has positive blood cultures after 7 days of therapy, or clinically deteriorates (e.g., develops organ failure or a new focus of infection) at any time during ceftazidime therapy. If ceftazidime or a carbapenem are not available, ampicillin/sulbactam or other intravenous beta-lactam/beta-lactamase inhibitor combinations may be viable, albeit less-proven alternatives. IV therapy may be extended (4 to 8 weeks) for critical illness, severe pulmonary disease, deep-seated abscesses, bone, joint, or CNS involvement.[2,3,8,13] Fever can persist for prolonged periods during appropriate therapy and does not necessarily indicate treatment failure.

Median time to fever resolution is 9 days, but can be significantly longer in patients with large, undrained abscesses.

Maintenance therapy: Upon completion of IV therapy, oral maintenance therapy with TMP/SMX (2 X 160-800 mg [960 mg tablets if > 60 kg]) every 12 hours should be continued for ≥ 3 to 6 months.[2,3,13] Maintenance therapy of severe disease should continue for ≥ 20 weeks to reduce the risk of relapse. Toxicity screening during TMP/SMX maintenance therapy or post-exposure prophylaxis should include complete blood counts, renal function tests and serum electrolytes (weekly during the first 2 to 3 weeks, then biweekly). Folate supplementation (5 mg/day) should be considered for those at risk for folate deficiency.[13] *Augmentin* is advised for resistant isolates (very, very rare) or if the patient is intolerant of TMP/SMX and is used during pregnancy and for children < 8 years old.[3] Life-long follow-up is indicated for melioidosis patients to identify relapse.

Isolation precautions: Person-to-person spread is rare. Standard precautions (i.e., the use of disposable surgical masks; face shields, gloves and gowns, when appropriate, to prevent splashing of mucous membranes and skin) are sufficient to prevent transmission to those caring for patients. Droplet, airborne, or airborne-plus-contact precautions should be used, respectively, if pneumonic plague, pulmonary tuberculosis, or smallpox are serious considerations in the differential diagnosis.[4,16] Environmental decontamination using a 0.5% to 1.0% hypochlorite solution is effective.

For further information, see **Appendix J: Biological Agent Prophylactics & Therapeutics.**

Prophylaxis

Vaccine: There are currently no vaccines available for human use.

Antibiotics: There are no human data or FDA-approved Pre-exposure prophylaxis (PEP) regimens. TMP-SMX has been effective in limited animal studies[14]and should be strongly considered following an intentional exposure. Recommendations for PEP following lab accidents advise TMP/SMX (2 X 160-800 mg (960 mg tablets if > 60 kg]) every 12 hours, with *Augmentin* 20.5 mg/kg every 8 hours as an alternative, especially during pregnancy or for children < 8 years old.[8,13] Toxicity screening and folate supplementation should be considered as discussed for maintenance therapy. Doxycycline 2.5 mg/kg (up to 100 mg) every 12 hours may be considered[8,13], although it has resulted in high relapse rates in animal studies.[13,15] Fluoroquinolones are not recommended, due to poor performance in animal studies of PEP, and high relapse rates during clinical trials for therapy.[8,13] Optimal duration of PEP is unknown, but 3 weeks is recommended by expert consensus.[8,13]

PLAGUE

Summary

Signs and symptoms: Bubonic plague is characterized by enlarged painful lymph nodes ("buboes") proximal to the draining lymph nodes of the flea bite site which is often in the draining inguinal area resulting in high fever and malaise. It may progress spontaneously to the septicemic form (septic shock, thrombosis, disseminated intravascular coagulation) or the pneumonic form (secondary pneumonic plague) with cough, dyspnea, and hemoptysis. Other rare forms include pharyngeal and gastrointestinal plague arising from consumption of contaminated raw meat.

Primary pneumonic plague (following an aerosol bio-agent attack) begins with a sudden onset of symptoms after an incubation period of 1 to 6 days. These include high fever, chills, headache, malaise, followed by cough (often producing purulent, frothy or bloody sputum), chest pain, progressing rapidly to dyspnea, stridor, cyanosis, and death. GI symptoms are often present. Death results from respiratory failure, circulatory collapse, and a bleeding diathesis. Plague meningitis is also possible.

Diagnosis: Suspect plague if large numbers of previously healthy individuals suddenly develop severe pneumonia, especially if hemoptysis is prominent and Gram-negative coccobacilli are present in sputum. Presumptive diagnosis can be made by the rapid diagnostic test based on the F1 antigen, Wright, Giemsa, Wayson, or methylene blue stain of blood, sputum, CSF, or lymph node aspirates. Immuno-diagnosis may be helpful, but definitive diagnosis requires culture of *Yersinia pestis* from one of those sites.

Treatment: Early administration of antibiotics is critical, as pneumonic plague is invariably fatal if treatment is delayed for > 1 day after onset of symptoms. The treatment of choice is parenteral ciprofloxacin, levofloxacin, moxifloxacin or gentamicin, with doxycycline, ofloxacin, gemifloxacin, amikacin, tobramycin, plazomicin, trimethoprim-sulfamethoxazole and chloramphenicol representing acceptable alternatives. Duration of therapy is between 10 and 14 days. For plague meningitis, chloramphenicol, levofloxacin or moxifloxacin are used for treatment.

Prophylaxis: For asymptomatic persons exposed to a plague aerosol or to a suspected pneumonic plague case, ciprofloxacin, levofloxacin, moxifloxacin or doxycycline is given for 7 days, or for the duration of the period of exposure plus 7 days. Alternative antibiotics include ofloxacin, gemifloxacin, tetracycline, omadacycline, minocycline or trimethoprim-sulfamethoxazole. No vaccine is currently available for plague prophylaxis however there are several vaccine candidates currently undergoing clinical trial. (The previously available licensed, killed vaccine was effective against natural bubonic plague, but not

against aerosol exposure). No prophylaxis is required for asymptomatic contacts of individuals with bubonic or septicemic plague without pneumonia.

Isolation and decontamination: Standard precautions are used by medical personnel for bubonic or septicemic plague and respiratory droplet precautions are required for a suspected or known pneumonic plague case. *Y. pestis* can survive in the environment for varying periods, but is susceptible to heat, disinfectants, and exposure to sunlight. Soap and water are effective for decontamination if needed.

Overview

Yersinia pestis is a rod-shaped, non-motile, non-sporulating, Gram-negative bacterium of the family *Enterobacteraceae*. It causes plague, a zoonotic disease of rodents (rats, mice, ground squirrels, etc.). Humans typically develop disease through contact with infected rodents or, more commonly, their fleas.[1,2] The biting fleas transmit bacteria to humans who then typically develop the bubonic form of plague. The bubonic form may progress to the septicemic and/or pneumonic forms. Larger outbreaks of human plague often follow epizootics in which large numbers of host rodents die off, leaving their fleas in search of other sources of a blood meal.[3,4] Pneumonic plague would be the predominant form of disease expected after purposeful aerosol dissemination. All human populations are susceptible. Recovery from the disease is followed by immunity, but the duration of this in humans is currently unknown. (Antibody to F1 can be found in humans more than 10 years following infection).[5] The organism remains viable in unchlorinated water, moist soil, and grains for several weeks. At near freezing temperatures, it will remain alive for months to years, but it is killed by 15 minutes of exposure to 55ºC. It also remains viable for some time (hours to days) in dry sputum, flea feces, and buried bodies, but is killed within several hours of exposure to sunlight.

History & Significance

Historically, *Y. pestis* has been the cause of several human pandemics and countless deaths.[6-10] Recent genetic analysis of cemeteries in the Chüy Valley near Lake Issyk-Kul of modern-day Kyrgyzstan revealed clues to the origin of the medieval Black Death Pandemic of 1346 - 1353 AD.[11] Plague is now endemic worldwide, yet is responsible for only sporadic human disease (200-4,500 human cases including 30-200 deaths reported to the WHO annually).[12,13] Before and during World War II, the Japanese Imperial Army released plague-infected fleas from aircraft over Chinese cities producing outbreaks and deaths.[14] This method was cumbersome and unpredictable. Later, the Soviet Union had several institutes and thousands of scientists dedicated to their ultimately successful project to create and produce an effective *Y. pestis* munition.[15] The U.S. worked with plague as a potential bio-agent in the 1950s and ‘60s, but never successfully weaponized it before its offensive biowarfare program was terminated. Both the U.S. and USSR developed reliable and effective delivery

methods for aerosolizing the organism. The terrorist potential of plague was highlighted in 1995 when Larry Wayne Harris was arrested in Ohio for the illicit procurement of a *Y. pestis* culture through the mail. The contagious nature of pneumonic plague, whether through zoonotic or person-to-person transmission, makes it particularly concerning as a biological weapon.[16,17]

Clinical Features

Human plague can present in one of three predominant forms: bubonic, septicemic, and pneumonic. The vast majority of the 0 to 17 human cases reported annually in the U.S. are from the desert southwest, where plague is endemic in rural rodent populations.[18,19] Most naturally occurring human cases in the U.S. are bubonic (80-85%); primary septicemic cases are less common (15%); and primary pneumonic cases are quite rare (1-2%).[20]

Bubonic plague may occur after an infected flea vector bites a human host. The disease begins after a typical incubation period of 2 to 8 days, with acute and fulminant onset of nonspecific symptoms, including high fever (up to 40˚C), severe malaise, headache, myalgias, and—in 25 to 50%—nausea and vomiting.[1,13,21,22] Up to half of patients will have abdominal pain. Simultaneous with, or shortly following, the onset of these nonspecific symptoms, the characteristic bubo develops—a swollen, extremely painful, infected lymph node.[1,13,21,22] Buboes may be from 1 to 10 cm in diameter with erythema of the overlying skin and variable degrees of surrounding edema. They rarely become fluctuant or suppurate, and lymphangitis is uncommon. They are most commonly seen affecting the femoral or inguinal lymph nodes since the legs are the most commonly flea-bitten part of the adult human body. But any lymph nodes can be involved, including intra-abdominal nodes (presumably through hematogenous extension) which can present as a febrile patient with an acute abdomen. The liver and spleen are often tender and palpable. One quarter of patients will have some type of skin lesion: a pustule, vesicle, eschar or papule (containing leukocytes and bacteria) in the lymphatic drainage of the bubo, and presumably representing the site of the inoculating flea bite. Bacteremia is common, as greater than 80% of blood cultures are positive for the organism in patients with bubonic plague; however, only about a quarter of bubonic plague patients progress to clinical septicemia, typically within 2 to 6 days of symptom onset in untreated patients. The case fatality rate (CFR) of untreated bubonic plague is between 50 to 90%, but this is reduced to 5 to 15% with prompt, effective therapy. In recent decades over 80% of the United States plague cases have been bubonic, with 50% of the cases in people ages of 12 to 45 yr.

Septicemic plague in cases that progress to secondary septicemia, as in primary septicemia, the symptoms and signs are similar to other Gram-negative septicemias: high fever, chills, malaise, hypotension, tachycardia, tachypnea, nausea, vomiting, and diarrhea.[21,22] All age groups can be affected, but the elderly seem to be at increased risk. Plague septicemia can produce thrombi in the acral vessels (presumably assisted by a low-temperature-activated coagulase

protein produced by the organism), possibly leading to necrosis and gangrene, and disseminated intravascular coagulation (DIC); thus, black necrotic appendages may be accompanied by more proximal, purpuric lesions due to endotoxemia in advanced disease. Organisms can spread via the bloodstream to the lungs and, less commonly, to the CNS and elsewhere. Untreated septicemic plague is virtually 100% fatal, while treated disease carries a CFR of 22 to 50%.

Pneumonic plague is an infection of the lungs due to either inhalation of the organisms (primary pneumonic plague) or spread to the lungs from bacteremia (secondary pneumonic plague). Secondary pneumonic plague has been a complication in 12% of bubonic cases in the U.S. over the past 50 years. (28% of human plague cases resulting from exposure to plague-infected domestic cats in the U.S. in recent decades presented as primary pneumonic plague).

Person-to-person spread of pneumonic plague last occurred in the U.S. in 1925 until 2014 when four persons were diagnosed, with three of the cases acquiring the infection from a dog and possibly one person from the infected owner according to the CDC.

After an incubation period varying from 1 to 6 days for primary pneumonic plague (usually 2-4 days, and presumably dose-dependent), onset is acute and often fulminant. The first signs of illness include high fever, chills, headache, malaise, and myalgias, followed within 24 hours by tachypnea and cough, progressing to hemoptysis.[3,21,22] Although bloody sputum is characteristic, it can sometimes be watery or, less commonly, purulent. Nausea, vomiting, diarrhea, and abdominal pain may all be present. Rarely, a cervical bubo might result from inhalational exposure. CXR findings are variable, but most commonly reveal bilateral infiltrates, which may be patchy or consolidated. The pneumonia progresses rapidly, resulting in dyspnea, stridor, and cyanosis. The disease terminates with respiratory failure, and circulatory collapse. The CFR for treated pneumonic plague patients in the U.S. is approximately 50%; if untreated, however, it is nearly 100%. (In the U.S. over the past 21 years, of the 120 cases reported a total of 15 have died for a CFR of 12.5%). Recent data from the ongoing Madagascar epidemic, which began in 1989, corroborate that figure; the CFR associated with respiratory involvement was 57%, while for uncomplicated bubonic plague was 15%.

Pneumonic plague is the only form of the disease which readily spreads from person to person. From the sparse historical data available on past cases, the average secondary infection rate is 1.3 cases per primary case (range: 0 to 6). Transmission has been greatest under crowded, cold, and humid conditions.[23] The majority of secondary cases have been in caregivers at home (80%) or medical professionals (14%) after close proximity ($\leq$ 2 meters) with the primary cases.

Plague meningitis is a rare complication (up to 6% of patients with septicemia, more common in children), most often occurring in bubonic or septicemic plague patients a week or more into illness. Typically, these patients have been receiving sub-therapeutic doses of antibiotics or bacteriostatic antibiotics which do not cross the blood brain barrier well (e.g., tetracyclines). Signs and symptoms are consistent with subacute bacterial meningitis, and CSF demonstrates a leukocytosis with neutrophil predominance and perhaps Gram-negative coccobacilli.

Other syndromes: Plague can also present as a primary pharyngitis and tonsillitis usually with swollen and inflamed anterior cervical lymph nodes.[24] This rare form of plague is acquired from inhalation or ingestion of plague coccobacilli through the consumption of contaminated meat.[20,25] The clinician should be aware of asymptomatic pharyngeal colonization by *Y. pestis* in people with close contact to pneumonic or bubonic cases of plague.[25]

Nonspecific laboratory findings in all forms of human plague include a leukocytosis, with a total WBC up to 20,000 cells per ml or more with increased band forms, and > 80% polymorphonuclear cells. Platelet counts can be normal or low. Increased fibrin split products and elevated partial thromboplastin time, indicating a low-grade DIC, can also be seen. The blood urea nitrogen, creatinine, transaminases, and bilirubin may also be elevated, consistent with multiorgan failure.

Diagnosis

Clinical diagnosis: Diagnosis of plague is based primarily on clinical suspicion. A patient with a painful lymph node accompanied by fever, severe malaise and possible rodent exposure in an endemic area or history of recent travel to an endemic area should raise suspicion of bubonic plague. The sudden appearance of large numbers of previously healthy patients with severe, rapidly progressive pneumonia with hemoptysis strongly suggests pneumonic plague as a result of intentional aerosolization.

Laboratory diagnosis: The rapid diagnostic test at point of care is the F1 capsular antigen of *Y. pestis* (F1RDT) test and is used for suspected cases of plague upon patient presentation to a healthcare facility.[8] A positive F1RDT test supports the clinical suspicion of plague, and a negative result helps to guide physicians to consider other potential diseases.[26-28] For suspected pneumonic plague, sputum samples are tested; whereas aspirated material from infected buboes is used for bubonic plague. For positive F1RDTs, the test needs to be combined with other laboratory evaluations to confirm the diagnosis. However, at this time, F1RDT is not yet approved by the FDA. A presumptive diagnosis can also be made microscopically by identification of the coccobacillus in Wright, Giemsa, Wayson's or methylene blue stains, or more specific immunofluorescence antibody-stained smears from lymph node needle aspirate, sputum, blood, or CSF samples. Although a Gram stain should be used for

classification purposes, it should not be used to seek the "safety pin" appearance characteristic of *Y. pestis*. This characteristic morphology is sometimes not apparent on Gram stain[29] and to an inexperienced microscopist, other members of the *Enterobacteriace* may seem to have it, especially in the early log phase of growth.[30]

Bubo aspirates can be obtained by inserting a 20-gauge needle on a 10 ml syringe containing 1 ml of sterile saline; saline is injected and withdrawn until blood tinged. Definitive diagnosis relies on culturing the organism from clinical specimens (https://www.cdc.gov/plague/healthcare/clinicians.html, accessed on 19 December 2023). The organism grows slowly at normal incubation temperatures (optimally, 25 to 28°C), and may be misidentified by automated systems (often as *Y. pseudotuberculosis)* because of delayed biochemical reactions. It may be cultured on sheep blood agar, MacConkey agar, or brain heart infusion broth.[9] It will also grow in automated culture systems. Any patient with suspected plague should have blood cultures performed (at 28° and 35°C); as bacteremia can be intermittent, multiple cultures should be obtained, preferably before receipt of antibiotics (clinical severity permitting). Confirmatory diagnosis via culture commonly takes 48 to 72 hours (cultures should be held for 5 to 7 days); thus, specific antibiotic therapy for plague must not be withheld pending culture results. Confirmatory culture-based diagnosis is made by specific bacteriophage lysis of the organism,[31] along with PCR to identify *Y. pestis*-specific genes, available at many reference laboratories, especially those participating in the CDC-sponsored Laboratory Response Network (several major civilian and military medical centers). These PCR tests target the F1 antigen gene (*caf1*), *pla* gene, or chromosomal fragments; however, the *pla* gene and chromosomal fragment targets were recently shown to be unreliable for detecting *Y. pestis.*[28] The clinician should be aware of a recent history of the misidentification of *Y. pestis* as *Pseudomonas luteola*, *Acinetobacter lwoffi*, and *Y. pseudotuberculosis* by automated bacterial identification systems. Follow-on antibiotic sensitivity and resistance patterns are essential to identify antibiotic resistance strains, particularly if the outbreak is considered to originate from a biological attack.

Most naturally occurring strains of *Y. pestis* produce an F1-antigen *in vivo*, which can be detected in serum samples by specific immunoassay and would have retrospective significance for an epidemiological investigation. A single anti-F1 titer of >1:10 by agglutination testing is suggestive of plague, while a single titer of >1:128 in a patient who has not previously been exposed to plague, or has not previously received a plague vaccine, is more specific; a fourfold rise in acute vs. convalescent antibody titers in patient serum is probably the most specific serologic method to confirm diagnosis, albeit only retrospectively. Most patients will seroconvert within 1 to 2 weeks of disease onset, but a minority require three or more weeks.

Most clinical assays can be performed in BSL-2 laboratories, but procedures producing aerosols, or yielding significant quantities of organisms, require BSL-3 containment.

Medical Management

Antibiotics: Prompt initiation of appropriate antibiotics is paramount for reducing mortality; this is especially true in primary pneumonic plague, for which CFRs approach 100% if adequate therapy is not initiated within 24 hours of onset of symptoms. Initial empiric therapy for systemic disease caused by *Y. pestis* includes ciprofloxacin,

Levofloxacin, or moxifloxacin as first-line antibiotics recommended for treating pneumonic or septicemic plague.[16,32] Streptomycin was the classic treatment of choice for plague; however, availability is limited. Clinicians are more familiar with the dosing and use of gentamicin so it has become an acceptable alternative antimicrobial for plague. At the same time, gentamicin has poor abscess penetration, so consider alternative or dual therapy for bubonic plague cases.[3]

For treatment recommendations in detail, see **Appendix J: Biological Agent Prophylactics & Therapeutics**.

IV antibiotics can be switched to PO administration as the improvement in the patient's clinical course dictates, to complete $\geq$ 10 to 14 total days of therapy. Patients with uncomplicated bubonic plague often demonstrate resolution of fever and other systemic symptoms in 3 to 5 days, while more complicated cases—including septicemic and pneumonic plague—often result in extended hospital courses.

It is imperative that antibiotics be adjusted to the demonstrated susceptibility patterns of the infecting organism; naturally occurring strains have been reported which are resistant to streptomycin, tetracyclines, and chloramphenicol, and it is anticipated that weaponized plague could be intentionally rendered antibiotic resistant.[8,9,32] Despite typically good *in vitro* susceptibilities to penicillins and cephalosporins, these drugs are generally felt to be ineffective for plague; in fact, animal studies suggest that beta-lactam antibiotics may accelerate mortality in bacteremic mice resulting from endotoxin release upon cell lysis. Macrolide antibiotics are ineffective for plague.

Supportive therapy includes IV crystalloids and hemodynamic monitoring. Although low-grade DIC may occur, clinically significant hemorrhage is uncommon, as is the need to treat with heparin. Endotoxic shock is common, but pressor agents are rarely needed. Finally, buboes rarely require any form of local care, but instead recede with systemic antibiotic therapy. In fact, incision and drainage poses an infection risk to others in contact with the patient due to possible aerosolization of the bubo contents and a risk of secondary infection.

Needle aspiration is recommended for diagnostic purposes and may provide symptomatic relief.

Infection control: Use standard precautions for bubonic and septicemic plague patients. Suspected pneumonic plague requires strict isolation with respiratory droplet precautions for $\geq$ 48 hours after initiation of antibiotic therapy, or until sputum cultures are negative in confirmed cases. Historically, epidemics of pneumonic plague have subsided rapidly with implementation of such relatively simple infection control measures. Pneumonic plague patients being transported should wear a surgical mask when feasible. If competent vectors (fleas) and reservoirs (rodents) are present, measures must be taken to prevent local disease cycles.[8,32] These might include the use of flea insecticides, rodent control measures (after or during flea control), and flea barriers for patient-care areas.[8,9,12]

Prophylaxis

Chemoprophylaxis

Pre-exposure prophylaxis (PrEP) No antibiotic is licensed by the FDA for use before exposure to plague and is not considered necessary for first responders and health care providers who care for pneumonic plague infected patients provided standard and droplet precautions are maintained.[32] However, chemoprophylaxis with doxycycline (or a fluoroquinolone) may protect against plague based upon *in vitro* susceptibilities.

The CDC recommends that if there are shortages of PPE (such as surgical masks), or patient overcrowding, or poor ventilation in treatment areas then pre-exposure might be necessary provided there are sufficient antibiotics available and the risks to the personnel are considered.[17] Although there is no data to support optimal duration, guidelines recommend the pre-exposure antibiotics be discontinued 48 h after the last potential exposure.[33]

Post-exposure prophylaxis (PEP) if personnel are less than 6 feet from the pneumonic plague infected patient with no PPE or if laboratory workers are accidently exposed to infectious materials from infected patients, antimicrobial prophylaxis should be considered for a duration of 7 days.[32] If fever or cough occurs in these individuals, a full treatment course is warranted.

Monotherapy is recommended for scenarios related to an aerosolized biological attack. Empiric PEP should be provided as soon as possible then tailored to the antibiotic resistance pattern of the potentially engineered *Y. pestis* when available.[32]

Chemoprophylaxis is generally not recommended after contact with bubonic or septicemic plague patients; however, individuals making such contacts, especially if sharing the same environment in which the patient received a

natural exposure, should be symptomatically observed for a week. If symptoms are present, start treatment antibiotics while awaiting results of diagnostic studies.

Immunoprophylaxis

Vaccines No vaccine is currently available for prophylaxis of plague. A licensed, killed whole-cell vaccine was formerly manufactured by Greer and available in the U.S. between 1946 and 1998. This vaccine offered protection against bubonic plague but was not effective against aerosolized *Y. pestis*.

The plague bacterium secretes several virulence factors—such as Fraction 1 (F1) and V (virulence) proteins—which as subunit proteins are immunogenic and possess protective properties. As combined recombinant (fusion) proteins, these have been the focus of vaccine development and have shown promise in preclinical studies and in Phase 1 and Phase 2 clinical trials. Efficacy trial requirements are described in the WHO workshop publication[34] and the current state of vaccine development.[35]

An F1-V antigen (fusion protein) vaccine developed at USAMRIID[36] provided 100% protection in monkeys against a high-dose aerosol challenge.[37]

Passive There is no passive immunoprophylaxis (i.e., immune globulin) available for pre- or post-exposure management of plague.

Q-FEVER

Summary

Signs and symptoms: Q-fever may initially present with either acute/primary or persistent focalized (chronic) manifestations; long-term sequelae may be considered a third form. Route and magnitude of exposure largely determine the dominant clinical feature (e.g., pneumonia follows an aerosol exposure). Up to 60% of human infections are clinically inapparent. A non-specific flu-like illness predominates in the remaining 40% with a minority developing immunosuppression, pneumonia or hepatitis. Incubation period is estimated at 1 to 5 weeks (10 to 17 days is most typical) and the duration of symptoms range from a few days to a few months. Persistent focalized (chronic) disease may manifest many months or years after the primary infection; the most frequent and serious presentation being endocarditis, which is usually fatal if not treated.

Diagnosis: Human Q-fever infection is a notifiable disease in the U.S. The combination of frequent subclinical disease, sporadic local occurrence and non-specific signs and symptoms makes Q-fever diagnosis problematic. Q-fever is primarily considered to be a zoonotic disease. Careful history may reveal risk factors (e.g., working around livestock, travelling in endemic areas) during natural infection. The gold standard for acute disease is a fourfold increase in phase II IgG antibody titer by indirect immunofluorescence assay (IFA) of paired acute and convalescent specimens; however, a negative acute titer does not rule out acute Q-fever (seroconversion may be delayed). Other relevant lab approaches include PCR, next generation sequencing (NGS), and *C. burnetii* blood or tissue culture (requires a BSL-3 facility).

Treatment: Acute patients should receive antibiotic treatment, optimally started within the first 3 days of illness. If acute Q-fever is suspected, treatment should not be withheld pending results of lab tests, nor discontinued on account of initially negative serology. Doxycycline (100 mg q12 h) for $\geq$ 14 days is the treatment of choice. For acute patients with pre-existing disease, such as valvulopathy, 12 to 18 months of doxycycline with hydroxychloroquine (200 mg q8 h) may be necessary. Chronic Q-fever should be treated only after lab diagnostic confirmation and may require individualized treatment plans based on disease severity, underlying immune and valvular status, and response to prior treatment. Generally, the same two drugs are administered for 18 months.

Prophylaxis: A licensed vaccine (*Q-Vax*) is available in Australia and Europe. Pre-vaccination screening is essential. Post-exposure prophylaxis in suspected *C. burnetii* exposures has been questioned.

Isolation and decontamination: Standard precautions are recommended for healthcare workers dealing with suspected or confirmed cases. For autopsies, precautions should be taken to prevent aerosolization of body fluids. Patients are not required to wear masks. The spore form of the organism is very hardy and

can survive for years in the environment. Autoclaving and boiling for 10 minutes will kill the organism. Decontamination may be attempted with a 1:100 *Lysol* solution, 1% sodium hypochlorite solution, 5% hydrogen peroxide, or 70% ethanol. The M291 skin decontamination kit will not neutralize the organism.

Overview

Q-fever is a zoonotic disease caused by the obligate intracellular, gram-negative bacterium *Coxiella burnetii*.[1-4] A nationally notifiable disease in the U.S., Q-fever is found world-wide (exception: New Zealand).[5] Its natural reservoirs include sheep, cattle, goats, rabbits, cats, dogs, rodents, birds and ticks. The organism localizes in the gravid uterus and mammary glands of infected animals and is shed in very high numbers at parturition. Infection in livestock occasionally results in abortion, stillbirth, and dystocia, but is most often asymptomatic making the detection and control of infected animals highly important yet extremely challenging.[5] Direct animal contact is not required for transmission to humans. Human infection is typically via aerosolization of infectious particles, especially in premises contaminated with fetal membranes, birth fluids, aborted fetuses, and excreta from infected animals in locations where infected animals and their by-products are processed, as well as at postmortem examination (i.e. necropsy) sites.[5] Transmission to humans may also occur by ingesting contaminated raw milk or cheese, through blood product transfusions or bone marrow transplantations, vertically (i.e., mother to offspring), and by ticks. Person-to-person transmission through sexual contact is rare but considered possible. Hematophagous arthropod vectors (especially ticks) are believed to be important in maintaining disease in livestock and wild animal reservoirs, but not in human disease.[5]

Humans acquire Q-fever primarily by inhaling the aerosolized organism.[1,2] Q-fever pneumonia is considered a noncommunicable disease although there has been anecdotal cases of human-to-human transmission following autopsies through infected aerosols.[5] The infectious dose is extremely low; a single bacterium may lead to infection in 50% of people ($ID_{50} = 1$ organism). Infected livestock, even if asymptomatic, shed large numbers of organisms in placental tissues and body fluids including milk, urine, and feces. The spore-like form of *C. burnetii* can persist in the environment for months making it highly suitable for aerosol delivery (weaponization). Bacterial aerosols can be delivered for at least 30 km by wind resulting in *C. burnetti* infection in patients with no recent contact with animals and far away from the primary contaminated areas.[5] *C. burnetii* is also a significant hazard for the lab personnel.

History & Significance

Q-fever was first described in 1935 in Brisbane, Australia, by Edward Holbrook Derrick after an outbreak of febrile illness among abattoir workers. It was called "Query fever" because the causative agent was initially unknown. No diagnosis

could be made based on the varied patient histories, physical exam findings and investigations. In 1937, Australian researchers Frank Macfarlane Burnet and Mavis Freeman identified a fastidious, intracellular bacterium in guinea pigs that had been injected with body fluids from Derrick's patients. Almost at the same time, in the U.S., a rickettsia-like bacterium was isolated from ticks by Herald Cox. These agents were later determined to be identical. Burnet was first to isolate and describe the organism in 1937, and Cox described vector transmission from ticks in 1938. Owing to the transmission properties described above, the U.S., UK, and USSR researched, weaponized and stockpiled *C. burnetii* during the Cold War.

Due to epidemiological disparities and whether the disease is reportable, the prevalence of Q-fever is highly variable from one country to another. Q-fever did not become a reportable disease in the U.S. until 1999.[5] *C. burnetii* is currently classified by the CDC as a Category B pathogen. During 2007-2010, the largest Q-fever natural outbreak ever reported involved ~4,000 human cases in the Netherlands with chronic Q-fever infections being diagnosed and mortality occurring 8 years after.[6,7,8] Dairy goat farms, located near densely populated areas, were the presumed source of human exposures via the windborne route.

Acute Q-fever cases have been reported in both U.S. and UK military personnel during deployments in support of Operation Iraqi Freedom and Operation Enduring Freedom.[2,9-11,13] Tick bites, sleeping in barns, and environmental exposure due to helicopter-generated (rotor wash) aerosols have been linked to these cases.

Clinical Features

Although the clinical manifestations of Q-fever in humans have not changed, many clinicians and researchers no longer consider Q-fever infection in terms of acute and chronic disease. The clinical presentation and determinants of symptomatology depends on both the virulence of the infecting *C. burnetii* strain and specific risk factors in the infected patient.[5] Additionally, no persistent infection can exist without a focus of infection.[5] Therefore, the location of these infections must be identified in order to determine the treatment strategy that is best suited to the infection (e.g., Q-fever endocarditis, Q-fever vascular infection, Q-fever osteomyelitis, etc.). What most published literature describes as chronic manifestations of Q-fever will be referred to as **"***C. burnetti* persistent focalized infections" in this chapter.

A health care provider will likely be forced to make a presumptive diagnosis that includes Q-fever as a rule out. With varying incubation periods (generally 2 or 3 weeks) highly dependent on the size of the inoculum, and a vague flu-like illness being the most common presentation in acute cases, a clinical diagnosis without additional diagnostic testing is exceptionally difficult. For naturally occurring outbreaks, in which numbers of human cases are typically low (the

2007-2010 Dutch epidemic notwithstanding), most cases may go undiagnosed. Approximately 75% of outbreak victims have been male, with a preponderance in those over 15 years of age. *C. burnetti* persistent focalized infections, are uncommon, but a potentially more serious condition than the acute form. It manifests from a few months to 20 years or so following an acute infection. With the intentional release of large numbers of bacteria, there may be more uniformity in the clinical presentations, as there is expected to be some correlation between the severity and physical manifestation of disease to this route and magnitude of exposure.

Primary (Acute) Q-fever Infection

Asymptomatic and pauci-symptomatic primary infection: Up to 60% of acute infections show no clinical sign of disease.[5,14,18] This may not hold true in an intentional release, as the exposure levels are potentially much higher.

Isolated febrile syndrome or flu-like illness. In natural outbreaks, 40% develop a non-specific, self-limiting flu-like illness, which can include severe headache (mostly retroorbital), joint and/or muscle pain, and fever.[14] Fever is variable, can last more than 15 days in untreated patients[5], but otherwise reaching a peak of 102-105º Fº after 3 days, then returning abruptly to normal after 5 to 14 days in treated individuals.

Pneumonia. Pneumonia (with or without pleural effusion) is an important clinical manifestation in acute cases and may be accompanied by a cough (often productive), fever, dyspnea, and auscultation abormalities.[1,5,18] Severe headaches and other non-specific extrapulmonary flu-like signs are also reported.[5] Resolution of symptoms generally occur within 30 days and the prognosis of *C. burnetti* pneumonia is generally favorable.[5]

Hepatitis. Isolated hepatitis is a frequent presentation especially in countries where Q-fever is endemic.[5,18,19] Elevated liver enzymes (ALP, ALT, and AST reaching 2-3X ULN[17]) with fever, chills, and headache is also a common clinical finding. Painful hepatomegaly has been reported. Jaundice is rare except in severe cases.[5,19] Coinfection with viral hepatitis, particularly in developing countries, can complicate the clinical picture. The prognosis of *C. burnetii* acute hepatitis is good; fatal cases due to hepatic insufficiency are very rare.

Cardiac involvement. Life-threatening pericarditis, myocarditis, and endocarditis are associated with primary infection and generally accounts for approximately 2% of acute cases.[5] Pericardial effusion may suggest pericarditis and/or myocarditis. Still considered a classic manifestation of chronic Q-fever, *C. burnetti* endocarditis should be considered a differential diagnosis during primary infection especially in endemic areas.

Neurologic signs. Rare (1-2% of cases) and can be observed alone or combined with other organ involvement.[5,18] Meningoencephalitis is the most frequent

reported neurologic complication and may manifest alone followed by meningitis (with lymphocytic infiltrates in CSF) and peripheral myelitis.[5]

Rare clinical manifestations. Cutaneous manifestations, including maculopapular or vesicular exanthema, granulomatous panniculitis, and erythema nodosum, are rarely reported.[5,18] Acute lymphadenitis/lymphadenopathy, cholecysitis[12], and bone marrow necrosis are even less frequent. Abortion is virtually inevitable if infection occurs during the first trimester of pregnancy.[1,15]

C. burnetti Persistent Focalized Infections (Chronic Q-fever)

Endocarditis. Q-fever endocarditis is the most frequently reported form and most serious complication of persistent *C. burnetti* infection.[5,18] Patients present with non-specific symptoms such as fever, chills, night sweats, and hepatosplenomegaly. Individuals with pre-existing heart disease (especially mitral and/or aortic insufficiency, mitral or aortic prosthesis, and arterial aneurysms) or immunocompromised are pre-disposed and, if left untreated, usually succumb to disease.[1,2,15-17] The incidence of endocarditis after acute Q-fever in patients with valvulopathy has been estimated to be 39%.[5]

Vascular infections. *C. burnetti* vascular infections are considered the second most common manifestation[5,19] developing after a *C. burnetti* primary infection and when a preexisting lesion, such as aneurysm or vascular graft, is present on a vessel. Abdominal and thoracic aortas are the most frequent sites. Initially, patients present with nonspecific symptoms and diagnosis is made when complications such as aortoduodenal fistulas occur resulting in life threatening conditions such as hemorrhage, graft or aneurysm rupture, or embolic complications.[5,6] Vascular infections, not endocarditis, are the most frequent form of persistent infection reported in the Netherlands following the 2007-2010 epidemic.[5,6]

Osteoarticular infections. Bone and joint *C. burnetti* infections are considered rare, reported in approximately 2% of Q-fever cases in a case study of 1,383 Q-fever infections.[5,18] Although rare, osteomyelitis is one of the most frequent sequelae in children.[20]

Rare manifestations. Primary infection in pregnant women is most often asymptomatic. Various obstetrical complications (e.g., abortion, premature birth, birth defects) or low birth weight have been described particularly when *C. burnetti* infection occurs during the first trimester.[5,20] Isolated persistent lymphadenitis has been reported. *C.burnetti* infection has been associated with an increased risk of lymphoma.[5]

Q-fever chronic fatigue syndrome (CFS). CFS is characterized by persistent fatigue following a primary infection with no signs of persistent infection. Specific organ involvement is not apparent, nor has the pathogenesis been

elucidated. Diagnosis is based on characteristic clinical signs >1 year after acute Q-fever infection with adequate treatment, elevated antibody titers, and absence of clinical and lab evidence of chronic Q-fever (with organ involvement).[1,5]

Diagnosis

The Q-fever differential diagnosis is extensive due to vague clinical symptomatology. A characteristic pattern of cases associated with a geographic area or compressed timeline should raise suspicion. For military personnel, other bio-agents that have overlapping symptoms should also be considered (e.g., anthrax and plague and tularemia pneumonias). Definitive diagnosis requires laboratory testing. Any potential amplification of *C. burnetii* must be performed in a BSL-3 facility.

Serology: Indirect immunofluorescence assay (IFA) is the current reference method for diagnosis of Q-fever.[1,2] Serologic testing should be performed at the time of clinical presentation and 4 to 6 weeks later; patients with negative convalescent samples should not be diagnosed with Q-fever.[2] Seroconversion, or a fourfold rise in titer (which requires a baseline and repeat testing in 2 to 4 weeks), indicates an acute infection. [1,2]

Two antigenic phases of *C. burnetii* infections exist: phase I (virulent) and phase II (avirulent).[1,2] Acute Q-fever cases usually exhibit a much higher antibody level to phase II antigen (first detected during the second week of illness). Specific IgM against phase II antigen may be detectable as early as the second week after onset of illness, with a concomitant increase in phase II IgG. Phase II specific IgG antibodies typically peak at 8 weeks post symptom onset[1,19] and remain elevated for up to 3 months. Elevated IgG of > 1:200 and IgM > 1:25 to phase II also supports an acute infection.[1] Combined detection of IgM, IgA, and IgG improves assay specificity and provides accuracy in diagnosis.

An elevated phase I antibody titer greater than or equal to the phase II antibody titer is the hallmark of chronic (persistent focal) Q-fever infection (Table 1).[1,19] Antibodies to phase I antigens generally take longer to appear and indicate continued exposure to bacteria. Antibodies to phase I and II antigens may persist for months or years after initial infection. In chronic disease, a 1:800 to 1:1024 IgG or > 1:59 IgA against phase I antigen suggest a chronic infection exists.

Antibodies Generally Present during Acute and Chronic Q-fever Infection

	IgA Phase		IgM Phase		IgG Phase	
Infection Stage	I	II	I	II	I	II
Acute			X	X		X
Chronic	X				X	

Blood chemistry/Complete blood count (CBC): CBC is usually unremarkable; leukocytosis being an exception (14 to 21x10^9/L) in about 25% of cases. Thrombocytopenia may also be seen in up to a third of patients in the acute phase, with thrombocytosis developing during the recovery phase. Typically, the estimated sedimentation rate (ESR) is mildly elevated. Abnormal liver enzymes are the most common abnormal blood chemistry finding, showing a 2- or 3-fold elevation in alkaline phosphatase (ALP) and the transaminases in up to 85% of patients.[1,21] Bilirubin is usually normal. Hepatitis patients, and those with chronic Q-fever, frequently have circulating autoantibodies, including anti-smooth muscle, anti-cardiolipin, anti-phospholipid, anti-clotting factor (liver biopsy may risk hemorrhage), and antinuclear antibodies. Endocarditis usually causes a significantly elevated ESR, often with anemia, thrombocytopenia, and polyclonal hypergammaglobulinemia. Mild lymphocytic pleocytosis is common in the cerebrospinal fluid (CSF) of patients with meningoencephalitis.

Imaging studies: Thoracic radiographs are non-specific and may be normal in up to 10% of those with acute Q-fever. While radiography is necessary to diagnose pneumonia, it cannot distinguish Q-fever from other etiologies.[1,19] A transesophageal echocardiogram (TEE) and/or transthoracic echocardiogram (TTE) help identify symptomatic heart disease that could pre-dispose individuals to develop chronic Q-fever especially if valvular defects are suspected.[2] Though TEEs are more sensitive, TTEs should be considered in patients with acute Q-fever, especially those with significant murmurs on physical exam or with a history of valvulopathy. TEE, more sensitive in finding small subendothelial valvular lesions, should be performed in patients with negative or inconclusive TTE findings but still suspected of endocarditis. Negative TTE or TEE should not rule out a diagnosis of chronic Q-fever endocarditis.[1,2]

PCR: Rapid, sensitive and specific detection of *C. burnetii* origin DNA in samples ranging from serum to whole blood (in anticoagulant tubes) to tissue biopsies (to include excised heart valves).[1,2] As there are usually bacteria present in the serum in acute infection, PCR allows for detection well before serum antibodies against Q-fever emerge. Therefore, *C. burnetti* DNA may be detected by real time PCR (RT-PCR) prior to positive serology and has become the most frequently used PCR system for diagnosis.[5] In chronic Q-fever cases, PCR can be performed on CSF, pleural fluid, bone marrow, bone marrow biopsies, and liver biopsies.

Next Generation Sequencing (NGS): Identifies a wide range of microbial DNA at one time and can be useful for Q-fever diagnosis, given the non-specific disease symptoms that often accompany this disease.[19,22]

Culture: Perform in dedicated bio-containment laboratories. Isolating the bacterium from tissue samples is highly specific, but the process lacks sensitivity.[1,2] Standard plate or liquid media will not support the growth of

C. burnetii; the bacterium is an obligate intracellular organism and requires mammalian cells to replicate. Bacterial isolation and amplification may be carried out using HEL cells and Shell Vial centrifugation. The shell vial technique is the most frequently used method.[5] Blood cultures on standard media are invariably negative; *C. burnetii* will only grow in living cells or organisms. In patients with chronic Q-fever endocarditis, routine blood cultures are negative.

Other studies: Sputum examination is unremarkable even in patients with productive cough. Liver or bone biopsies in patients with hepatitis or osteomyelitis, respectively, may reveal non-specific fibrin-ring granulomas with a central lipid vacuole resembling a doughnut; this finding is frequent in hepatic biopsies.[5,18] During persistent infection, microscopic examination of cardiac valves and vascular tissue can be informative.[5] Immunohistochemistry and/or fluorescence *in situ* hybridization (FISH) for bacterial antigen and *C. burnetii* DNA, respectively, can be tested on fresh or formalin-fixed paraffin-embedded tissues.[19] Generally, *C. burnetti* antigen will not be detected by immunohistochemistry but should still be considered with microscopic examination. However, in chronic cases, immunohistochemistry performed on heart valve specimens may detect *C. burnetti* antigen in patients with culture negative endocarditis.[1,5]

Medical Management

There is no single management strategy given the clinical polymorphism of *C. burnetti* infection.[5] An infectious disease (ID) specialist should be consulted in any suspected case, especially one with a history of acute Q-fever. Standard precautions are recommended for providers dealing with suspected or confirmed cases. After treatment of *C. burnetti* infection, the duration of treatment and follow-up are determined according to the results of screening for risk factors (e.g., age, valvulopathy, preexisting vascular aneurysms, comorbidities, etc.) of persistent infection.

Primary (Acute) Q-fever Infection

Adults: The treatment of choice is doxycycline 100 mg PO twice q12 h for $\geq$ 14 days.[1,2,17] Moxifloxacin 400 mg daily for 14 days could be used as an alternative. These are most effective if begun within 3 days of the onset of symptoms. Relapse is not uncommon and may be associated with an antibiotic regimen discontinued within 2 weeks. In cases with known valvulopathy or such discovered on exam, 12 months of prophylactic therapy with hydroxychloroquine (HCQ) and doxycycline should be considered following consultation with an ID specialist.[2] TMP-SMX is used for acute symptomatic Q-fever in pregnant women and children. Follow-up serological testing is recommended in all patients treated for acute Q-fever. Pregnant women diagnosed with acute Q-fever should be treated with TMP-SMX throughout the duration of the pregnancy.

Children: < 8 years with uncomplicated acute Q-fever may be treated with TMP-SMX or a shorter duration (5 days) of doxycycline.[1]

C. burnetti Persistent Focalized Infections (Chronic Q-fever)

Due to variation in the acute and chronic clinical course, disease severity, immune and valvular status, and an individual's response to treatment, successful treatment of chronic Q-fever is difficult. Doxycycline 100 mg PO q 12 hours, with HCQ 200 mg PO tid, for ≥ 18 months is recommended for adults, especially those with endocarditis.[1, 2] A similar approach is recommended with osteoarticular infections with surgical debridement. Routine eye examinations should be performed to monitor for HCQ- and doxycycline-associated ocular toxicity (e.g., photosensitivity and hypersensitivity to sunlight) or visual field changes.[2,23] Alternatively, combination therapy of doxycycline with a fluoroquinolone has been evaluated; but may not be as effective (i.e., more relapses) when compared to the doxycycline/HCQ combination. Due to the *in utero* effects of TMP-SMX and doxycycline, acute Q-fever infection during pregnancy requires special attention. These women should have specific serum antibody titers determined post-partum; then those with evidence of chronic Q-fever are often treated with ≥ 12 months of doxycycline and HCQ.[1,2] For all forms of chronic Q-fever, specific serum antibody titers are followed; but the optimum length of serologic follow-up remains to be determined. The current recommendation in cases of proven Q-fever endocarditis is serologic testing for 5 years (or longer) based on the individual's response to therapy.[2,8,20,21] A four-fold decrease in the phase I IgG and IgA titers and the disappearance of phase II IgM at 1 yr have been suggested as evidence of cure.

Long term sequelae

A chronic fatigue syndrome has been reported as a possible long-term complication of acute Q-fever infection.[1] Treatment is largely symptomatic and may require a combination of physical and pharmacological interventions.

For further information, see **Appendix J: Biological Agent Prophylactics & Therapeutics.**

Prophylaxis

Immunoprophylaxis: A licensed Q-fever vaccine (*Q-Vax*) for humans is available in Australia and Eastern Europe.[1,26] It is not commercially available in the U.S. so most workers in high-risk occupations are not vaccinated. Administration in already immune or pre-sensitized individuals may cause severe local induration, sterile abscess formation, and necrosis at the inoculation site. Determination of prior exposure is accomplished by an intradermal skin test using 0.02 mg of vaccine. Vaccination with a single dose of this killed suspension of *C. burnetii* provides complete protection against naturally occurring Q-fever, and > 95% protection against aerosol exposure. Protection

lasts for ≥ 5 years. There are no U.S. approved veterinary Q-fever vaccines. Historically, an experimental formalin-inactivated whole cell vaccine was offered to at-risk laboratory workers in the U.S. under an Investigational New Drug (IND) protocol with informed consent but is no longer available.

Chemoprophylaxis: Doxycycline 100 mg PO q12 hours, or tetracycline 500 mg PO q6 hours, for ≥ 5 to 7 days begun 8 to 12 days post-exposure has been considered effective. For pregnant women, although there are no official guidelines, TMP-SMX (160 mg/800 mg PO bid) may be considered for the duration of the pregnancy. Based on a 1956 challenge trial, however, it is believed that commencing prophylaxis within 7 days of exposure is not effective and may prolong the onset of clinical disease.[27] (Such prophylaxis prevented symptomatic illness but not infection in this study.) Whether chemoprophylaxis after an episode of Q-fever decreases the incidence of endocarditis in high-risk patients is not known.

In 2013, based on the weakness of the available data, the CDC's Q-fever Working Group (WG) failed to endorse the use of chemoprophylaxis for lab workers after a known or potential exposure. The use of post-exposure prophylaxis (PEP) after a bio-terrorism release of *C. burnetii,* provided that the timing of exposure is known, has received some support from authorities.[28] At the same time, even this was questioned by the CDC WG and the benefit of any kind of PEP against *C. burnetii* was repudiated.[1]

Isolation, Decontamination & Control

Standard precautions alone are recommended for HCWs dealing with suspected or confirmed cases. Patients are not required to wear masks. For autopsies, or when handling surgical or tissue biopsies, precautions should be taken to prevent aerosolization of body fluids. The spore form of the organism is very hardy and can survive for years in the environment. It can probably survive direct UV light, dilute bleach and typical disinfectants. Autoclaving and boiling for 10 min will kill the organism in samples no longer needed.

Decontamination may be attempted with a 1:100 *Lysol* solution, 1% sodium hypochlorite solution, 5% hydrogen peroxide, or 70% ethanol. The M291 skin decontamination kit will not neutralize the organism.

Surveillance and reporting of Q fever are essential components of public health education and disease prevention efforts. Investigations must be coordinated with animal health authorities to determine whether the source is naturally occurring or the result of an intentional release.[1] Animal health authorities can also help to control outbreaks that may be propagated by intentionally or unintentionally infected livestock sources and ensure that dairy products are pasteurized and from approved sources.

TULAREMIA

Summary

Signs and symptoms: Historically, Tularemia manifests in two primary forms: typhoidal and ulceroglandular. Typhoidal tularemia presents with fever, chills, headache, malaise, and often non-productive cough and chest discomfort without a clear entry point. Ulceroglandular tularemia shares these systemic symptoms but features a visible entry site, typically an ulcer with regional lymph node swelling. Both forms stem from the bacterium Francisella tularensis. Infection routes include insect bites, animal contact, or ingestion of contaminated food or water. Other less common clinical forms exist, varying in severity and organ involvement.

Diagnosis: The large differential diagnosis involving both typhoidal and pneumonic syndromes present challenges to diagnosing tularemia. Chest X-rays may reveal pneumonia, hilar lymphadenopathy, or pleural effusion. Definitive diagnosis requires careful culture of blood, sputum, ulcers, or pharyngeal samples. Direct fluorescence antibody (DFA) and PCR tests provide presumptive results. Serological tests confirm the diagnosis retrospectively. The diverse symptoms, mimicking other conditions, demand high suspicion and appropriate laboratory testing for accurate identification.

Treatment: Early treatment with parenteral antibiotics (gentamicin) is very effective for naturally acquired disease.

Prophylaxis: Following exposure to a susceptible strain, a 2-week course of doxycycline or ciprofloxacin can be administered orally as post-exposure prophylaxis.

Isolation and decontamination: Standard precautions are recommended for healthcare workers. Organisms are relatively easy to render harmless by heat and standard disinfectants.

Overview

Francisella tularensis, is a small, aerobic non-motile, gram-negative coccobacillus that causes tularemia, also known as “rabbit fever” and “deer fly fever”. This zoonotic disease can infect humans through various routes, including insect bites from infected arthropods (e.g., ticks, deerflies, or—only in Eurasia—mosquitoes), contact with infected animals (e.g., rabbits), contaminated water or food, and inhalation of bacteria. Tularemia presents in different forms, with ulceroglandular form (“glandular” refers to regional lymphadenopathy) being the most common, characterized by skin ulcers and swollen lymph nodes. Other forms include glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal tularemia in which a clinically obvious portal of entry is absent. As part of typhoidal disease, pneumonia may

occur after apparent inhalation of contaminated aerosols, or after apparent ingestion of contaminated foods or water.[1, 2, 3, 5]

F. tularensis is found throughout the temperate northern hemisphere, causing sporadic human infection (~200 cases/yr in the U.S.).[14,15] It has two main biovars: the more virulent Biovar A, prevalent in North America, and the less virulent Biovar B, common in northern Europe and Asia. The bacterium can survive for extended periods in water, mud, and frozen animal carcasses, but is easily destroyed by heat and disinfectants.[3,7]

History & Significance

F. tularensis was identified in 1911 during an investigation of a plague-like disease in ground squirrels in Tulare County, California. Edward Francis, a US Public Health Service physician, established it as the cause of the "deer fly fever" and devoted his life to its research, leading to its renaming as *Francisella tularensis*.[8] During WWII's German siege of Stalingrad, there were possibly hundreds of thousands of human cases, many pneumonic, sparking speculation about the Soviet Union's use of tularemia as a biological weapon; however, an ongoing rodent epizootic and harsh local conditions suggest a natural cause for the epidemic.[9]

F. tularensis was weaponized by both the U.S. and the USSR during the early Cold War (late 1940s and '50s).[3] The Soviets weaponized a virulent American strain given to them by U.S. scientists in 1949. Ironically, the tularemia vaccine later developed in the U.S. (LVS) was based on a strain obtained from the Soviets in the 1950s.[4]

Clinical Features

After an incubation period of 3 to 6 days (range 1-21 days; a shorter incubation period often correlates with a higher infectious dose), tularemia typically presents with acute onset. The disease manifests in several forms: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal. These can be broadly categorized as either typhoidal or ulceroglandular. The infectious dose varies by route: as few as 10 organisms cause disease via intradermal injection, 10 – 50 organisms cause illness via inhalation, while ~10^8 organisms are required with oral ingestion.[5]

Typhoidal tularemia: (~25% of naturally acquired cases) occurs mainly after inhalation of infectious aerosols but can occur after any route of exposure. The disease manifests as a nonspecific syndrome with abrupt onset of fever (38-40°C), chills, headache, cough, myalgias, and malaise, without an obvious portal of entry or peripheral lymphadenopathy. Some patients develop gastrointestinal symptoms. Case fatality rates (CFRs) may be 30–60% in untreated cases, but as low as 1–3% with optimal treatment. Survivors of untreated tularemia may have persistent symptoms for weeks to months. Fatality is higher if pneumonia

manifests, which would likely occur after an aerosolized bio-warfare attack. Severe typhoidal disease may be complicated by meningitis, pericarditis, endocarditis, or septicemia; renal and hepatic damage.[3, 5]

Ulceroglandular tularemia: (~75% of naturally acquired cases) is typically acquired through inoculation of the skin or mucous membranes with infected animal fluids or insect bites. It presents with systemic symptoms of typhoidal disease and a painful papule at the inoculation site, which progresses rapidly to pustule and a painful ulcer. Painful regional lymphadenopathy develops, with enlarged nodes potentially becoming fluctuant and draining spontaneously, even during antibiotic treatment. Untreated, lymphadenopathy can persist for months or years.[1]

In rare cases, oculoglandular disease occurs when the eye is the primary inoculation site, usually from contaminated hands, infected tissue fluids,or aerosols. Symptoms include unilateral painful purulent conjunctivitis with preauricular or cervical lymphadenopathy. Some patients develop chemosis, periorbital edema, and small nodular granulomatous lesions or conjunctival ulcerations.[3, 5]

Pharyngitis occurs in up to 25% of tularemia cases (i.e., *oropharyngeal disease*), often following ingestion of contaminated food or water. It typically presents as an acute exudative pharyngitis or tonsillitis, sometimes with ulceration, and painful cervical lymphadenopathy.[1] This syndrome may resemble penicillin-unresponsive pharyngitis and be mistaken for infectious mononucleosis or other viral pharyngitis.[5]

Pulmonary involvement: is seen on CXR in ~45% of naturally occurring tularemia cases, ranging from asymptomatic to severe. Symptoms may include non-productive cough, pleuritic chest pain, and dyspnea; purulent sputum or hemoptysis are rare. 30% of patients with CXR findings may be asymptomatic. Pulmonary involvement is most common in typhoidal tularemia (83% of cases), indicating direct inhalation, but also occurs in 31% of ulceroglandular cases, suggesting hematogenous spread.[1] Untreated tularemic pneumonia may have CFRs approaching 60%.

Diagnosis

Clinical approach: A bio-warfare attack with *F. tularensis* might present as a cluster of patients with similar nonspecific febrile illnesses rapidly progressing to life-threatening pleuropneumonitis.[3] Some patients may exhibit a temperature/pulse mismatch (Faget sign; seen in up to 40% of naturally acquired disease). Fever typically responds within 24 to 48 hours with appropriate antibiotics, though patients may remain febrile for weeks if treated with ineffective drugs.

A CXR is essential for suspected systemic tularemia, even without pulmonary symptoms. Patterns may include pulmonary infiltrates (unilobar or multilobar/diffuse), pleural effusion, hilar adenopathy, or, less commonly, oval density or cavitation.[5, 10]

The differential diagnosis of tularemic pneumonia is broad, including typhoidal syndromes (e.g., typhoid fever, rickettsia, or malaria) and various pneumonic processes (e.g., pneumonic plague, influenza, Q-fever, SEB intoxication, *Mycoplasma pneumoniae, Chlamydophila pneumoniae*, psittacosis, legionellosis, and others). Inhalational anthrax, pulmonary TB and other viral and fungal lung infections should also be considered.[3,5] Even after an aerosol bio-warfare attack, some patients may present with ulceroglandular disease.

Laboratory diagnosis: Initial lab findings are generally nonspecific. WBC counts typically range from 5,000 to 22,000 cells per microliter with possible lymphocytosis. Hematocrit, hemoglobin, and platelet levels are usually normal. Mild elevations in lactose dehydrogenase, serum transaminases, and alkaline phosphatase are common. Rhabdomyolysis may cause elevated serum creatine kinase and urinary myoglobin.[1]

Definitive diagnosis of tularemia requires isolating *F. tularensis from clinical specimens such as* blood, ulcers, conjunctival exudates, pharyngeal exudates, sputum, gastric washings, and CSF. Recovery is possible even after antibiotic initiation. Delays in diagnosis are likely if tularemia is not suspected, as *F. tularensis* grows poorly on standard media, requiring cysteine-enriched media or other sulfhydryl compounds for 48 to 72 hours to produce small, smooth, opaque colonies. Physicians should proactively alert the laboratory of suspected tularemia for biosafety precautions. Due to laboratory hazards, isolation should only be attempted in BSL-3 facilities.[3, 11] State health authorities, USAMRIID, or the CDC should be contacted for specimen handling.

Rapid presumptive diagnosis can be made using PCR or direct immunofluorescence immunoassay (DFA).[3]

Serological confirmation of tularemia involves demonstrating a 4-fold increase in antibody titer between acute and convalescent specimens collected at least two weeks apart.[3] While antibodies appear within the first week of infection, diagnostically significant titers (titer > 1:160) typically develop after two weeks. Serological tests include bacterial agglutination and ELISA, but their delayed response limits immediate diagnostic utility. Cross-reactions can occur with *Brucella* spp., *Proteus* OX19, and *Yersinia* species. Additionally, antibodies may persist for years post-infection; therefore, diagnosis should rely on a 4-fold or greater titer increase during illness progression. Titers are usually negative in the first week of infection, positive in 50-70% of cases by the second week, and reach peak at 4-8 weeks.

Medical Management

Treatment: For detailed treatment information, see **Appendix J: Biological Agent Prophylactics & Therapeutics.**

Chloramphenicol (at a higher dose, along with streptomycin) is historically reserved to treat meningitis. However, it is no longer considered a first-line treatment for tularemia including meningitis cases.[5,16] For potential bioweapon-related cases, rapid antibiotic susceptibility testing is crucial. Lack of significant improvement within 24-48 hours of appropriate antibiotic therapy may indicate resistance. In mass casualty settings, oral doxycycline or ciprofloxacin are preferred for both adults and children, with ciprofloxacin given for 10 days and doxycycline for 14-21 days.[3]

Infection control: No known human-to-human transmission of tularemia, therefore, neither isolation nor quarantine is necessary. Standard precautions are adequate for patient care, including cases with pneumonia or draining lesions.[11] The organism is easily inactivated by heat and disinfectants.[7]

Prophylaxis

Vaccine: Currently, no licensed tularemia vaccine is available in the United States or European Union. Historically, a live attenuated vaccine strain (LVS) was used experimentally and offered to over 5,000 at-risk laboratory workers in the U.S. from the 1960s until the early 2020s under an Investigational New Drug (IND) protocol with informed consent.[13]

Immunoprophylaxis: There is no passive immunoprophylaxis (i.e., specific parenteral immune globulin) available for pre- or post-exposure management of tularemia.

Pre-exposure chemoprophylaxis: No antibiotics are licensed by the FDA for use before exposure to tularemia. Based on *in vitro* susceptibilities, however, cipro or doxy may offer protection.

Post-exposure chemoprophylaxis: Initial empiric PEP against *F. tularensis* includes one of the following antibiotics.[3]

Preferred:

- Doxycycline 100 mg PO bid for adults and children ≥ 45 kg (for children < 45 kg use 2.2 mg/kg PO bid), or
- Ciprofloxacin 500 mg PO bid for adults (15-20 mg/kg PO bid (up to 1 g/d) for children)

PEP should ideally begin within 24 hours of exposure and continue for 7-14 days. These oral antibiotic dosages may also be appropriate for treatment in mass casualty settings in which the optimal drugs, IV antibiotics, are not available in quantity.

Chemoprophylaxis is generally not recommended after potential natural (tick bite, rabbit, or other animal) exposures.

VIRAL PATHOGENS

SMALLPOX (*Variola*) & MPOX

Summary

Signs and symptoms: Begins with malaise, fever, rigors, vomiting, headache, and backache. Two to three days later, skin lesions appear, quickly progress (more or less simultaneously) from macules to papules, and eventually to pustular vesicles. They are centrifugal (more abundant on the extremities and face than the trunk).

Diagnosis: Initially, must be clinical. Neither electron nor light microscopy is capable of discriminating *Variola* (smallpox) from vaccinia, mpox (formerly known as monkeypox), or cowpox. Vaccinia and cowpox disease in humans are typically localized and self-limiting except in the immune compromised or those with some other underlying conditions (i.e., eczema). Variola and mpox viruses typically cause widespread systemic disease. Clinical management of suspected mpox is the same as for smallpox. PCR is accurate in discriminating *Variola* from other orthopoxviruses.

Treatment: Tecovirimat and brincidofovir are FDA-approved anti-viral treatments for smallpox. These antivirals are not FDA licensed for treatment of mpox; however, CDC recommends their use under Expanded Access (Emergency)-IND (Investigational New Drug).

Post-Exposure Prophylaxis: Immediate vaccination or revaccination should be instituted for all personnel exposed to smallpox virus. For both ACAM2000 and JYNNEOS, their use is most effective during the first 4 days after exposure.

Isolation and decontamination: Patients should be considered infectious from the onset of a rash until all scabs have separated and the rash fully healed. Patients should be isolated under both contact and airborne precautions. Strict quarantine of asymptomatic contacts for 17-21 days (historically, 17 days has been recommended for smallpox and 21 days are currently recommended for mpox) after exposure to mpox may be advisable but could prove difficult to enforce. A reasonable alternative would be to require contacts to check for new symptoms and temperatures daily. Any fever above 38°C (101°F) during the monitoring period after exposure to a confirmed smallpox or mpox case would suggest secondary infection. The febrile contact should then be isolated immediately, ideally at home, until the diagnosis is either confirmed or ruled out. Isolation should continue until all scabs have separated and a layer of epithelial cells has formed where the scab dehisced.

Overview

Smallpox was caused by an *Orthopoxvirus* called *Variola*. Two strains existed, *Variola major*—with a fatality rate of 10 to 30%—and the milder *Variola minor*, which killed < 1% of its victims.[1] Following aerosol exposure, droplet nuclei containing virus were inhaled into the lower respiratory tract, travelled to regional lymph nodes, and there replicated causing primary viremia and systemic disease. Despite global eradication of smallpox and continued availability of a vaccine, the potential weaponization of *Variola* may continue to pose a military or terrorist threat. Of special concern is the aerosol infectivity of the virus, the relative ease of large-scale virus production, and an increasingly *Orthopoxvirus*-naive populace. Although the fully developed cutaneous eruption of smallpox is unique, earlier stages of the rash could be mistaken for chicken pox (varicella). Secondary spread would constitute a nosocomial hazard from the time of onset of a patient's exanthem until the lesions have healed.[2] Quarantine is recommended for secondary contacts with a person with smallpox for 17 days post-exposure. Vaccination and vaccinia immune globulin each possess some efficacy in post-exposure prophylaxis for smallpox.[3] Tecovirimat and brincidofovir are both FDA-approved treatment for smallpox.[4] Cidofovir may also be of benefit but is not currently licensed and would have to be used under an Emergency Investigational New Drug (EIND).[5]

History & Significance

Smallpox, an ancient disease, was responsible for an estimated 300–500 million deaths worldwide during the 20th century. Earlier, smallpox-laden objects are believed to have been used by the British Army as a crude bio-weapon against Native Americans and, later, the rebelling American colonials feared its use by the British during the American Revolution.[6] The United States studied smallpox virus as a possible bio-weapon during the 1950s and ‘60s and the Soviet Union produced and stockpiled massive weaponized quantities.

Endemic smallpox was declared eradicated in 1980 by the World Health Organization (WHO) after an immense vaccination effort.[1] Although two WHO-approved repositories of *Variola* virus remain at the Centers for Disease Control and Prevention (CDC) in Atlanta and at the Russian State Centre for Research on Virology and Biotechnology (Koltsovo, Novosibirsk Region) Russian Federation, the extent of clandestine stockpiles and misplaced samples in other parts of the world remains unknown.[7] The WHO Advisory Committee on *Variola* virus research has recommended repeatedly that all stocks of smallpox be destroyed. However, destruction has been deferred periodically since 1986 by the WHO Health Assembly due to concerns over the need for further study of the virus given its potential as a bio-agent.[3]

The U.S. military ended routine smallpox vaccination in 1989 but began again in 2003 for troops deployed to Southwest Asia and the Republic of Korea.

Routine civilian vaccination in the U.S. was discontinued in 1972. Thus, most of the American, and indeed the world, population is now susceptible to infection with *Variola* or any other orthopox virus.

The full-length sequences of several *Variola* strains have been published. Rapid advances in synthetic biology now make it at least theoretically possible to reconstruct *Variola* solely from fragments produced utilizing a DNA synthesizer. The construction of a *Mycoplasma* organism as well as a polio virus (the former with a genome three times larger than *Variola*) has demonstrated the feasibility of such an accomplishment. Thus, the old strategy of closely supervising existing stocks of *Variola* no longer ensures that a determined and sophisticated adversary could not produce and use a smallpox bio-weapon.[8,9]

Clinical Features

The incubation period of naturally acquired smallpox averages 12 days, although it can range from 7 to 19 days after exposure. Following the primary viremia, virus disseminates to other lymphoid tissues, spleen, liver, bone marrow, and lung causing a secondary viremia. Clinical manifestations begin with malaise, high fever (to 104^{o} F), rigors, vomiting, headache, backache, and prostration; 15% of patients develop delirium. Two to three days later, an enanthem consisting of small, painful ulcerations of the tongue and oropharynx appears simultaneously with (or within 24 hours of) a discrete rash about the face, hands, and forearms.[7, 10, 11]

After development of eruptions on the lower extremities, the rash spreads centrally to the trunk over the next week. The exanthem typically begins as small, erythematous macules which progress to 2 or 3 mm papules over 2 to 3 days, then to 2 to 5 mm vesicles within another 1 or 2 days. Four to seven days after rash onset, the vesicles become 4 to 6 mm umbilicated pustules, often accompanied by a second, smaller fever spike. Lesions are more abundant on the extremities and face, and this centrifugal distribution is an important diagnostic feature. In distinct contrast to varicella, lesions on various segments of the body remain generally synchronous in their stages of development. Between 8 and 14 days after onset, the pustules form scabs that leave depressed depigmented scars after healing. Death, if it occurs, is usually during the second week of clinical disease. The precise cause of death is not entirely understood, but was historically attributed to toxemia, with high levels of circulating immune complexes. Although *Variola* virus concentrations in the throat, conjunctiva, and urine diminish with time, it can be readily recovered from scabs throughout convalescence; therefore, patients should be isolated and considered infectious until all scabs have separated and lesions have healed.[7, 11]

In the 20th century, two distinct types of smallpox were recognized. *Variola minor* was distinguished by milder systemic toxicity and more diminutive pox lesions and caused a 1% case fatality rate (CFR) in unvaccinated victims.

However, the prototypical disease caused by *Variola major* resulted in a CFR of about 3% and 30% in the vaccinated and unvaccinated, respectively. CFRs were higher in certain populations (e.g., Pacific islanders and Native Americans), at extremes of age, during pregnancy (average 65% for ordinary smallpox), and in people with immunodeficiencies. Greater fatalities were associated with higher concentrations of lesions, with confluence of lesions portending the worst prognosis. Smallpox during pregnancy resulted in an increased incidence of spontaneous abortions. Acute complications of smallpox included viral keratitis or secondary ocular infection (1%), encephalitis (<1%), and arthritis (up to 2% of children). Bronchopneumonia was also seen in severely ill patients.[1, 2]

Two other clinical forms of *Variola major*—termed flat-type and hemorrhagic-type smallpox—were notable for severe mortality. Flat-type smallpox occurred in 2 to 5% of all cases and was most common in children. Hemorrhagic smallpox occurred in 2 to 3% of all cases, was more common in pregnant women and the immunocompromised, and presented with both early and late forms. Early hemorrhagic disease had a shorter incubation period, often large areas of ecchymosis, and fulminant progression to death, sometimes before lesions had even formed. In the late form, the disease progression was typical, with discrete hemorrhagic areas forming at lesion sites. CFRs were approximately 95% in both flat and hemorrhagic forms in unvaccinated individuals.[2, 12]

Partially immune patients, especially those vaccinated several years before smallpox exposure, could develop less severe forms of disease. This modified smallpox is a clinical form characterized by fewer lesions which are more superficial, associated with a less pronounced fever and a more rapid resolution, often with lesion crusting within 10 days of onset. Some previously immune individuals or infants with maternal antibodies could develop a short-lived febrile syndrome without rash upon exposure to smallpox virus.[12]

Long-term sequelae in smallpox survivors include blindness from corneal scarring (1-4%), growth abnormalities in children, and disfiguring or even physically debilitating dermal scarring.[1]

Animal studies suggest that unnaturally large, inhaled inoculum of poxvirus may result in a significantly shortened incubation period (even as little as 3 to 5 days) and fulminant pulmonary disease with or without appearance of rash before death; the implications of these findings for human disease as a result of intentional smallpox aerosolization are unknown.[14]

Historically, smallpox tended to spread slowly through communities. Smallpox could become endemic in densely populated regions even in a population with up to 80% vaccination rates. Increased person-to-person spread of disease was associated with: 1) exposure to cases with confluent rash or severe enanthem; 2) exposure to cases with severe bronchiolitis and cough; 3) low humidity environment; 4) crowding (as in winter or rainy seasons). The average secondary attack rate of *Variola major* was 58.4% in unvaccinated household contacts and 3.8% in vaccinated household contacts.[1]

Mpox

Mpox virus (MPXV), a relative of *Variola*, occurs naturally in equatorial Africa.[15] Mpox was first discovered in laboratory monkeys in 1958 when two outbreaks of a pox-like disease occurred in colonies of monkeys kept for research in an animal care facility in Copenhagen, Denmark.[15a] Though primarily a zoonotic disease, mpox made its jump into humans in 1970 when the first case occurred in a child in the Democratic Republic of Congo (DRC, formerly Zaïre).[15b] The child died from measles several days after recovery from mpox. The WHO recognizes two distinct mpox clades: clade I (with subclades Ia and Ib) and clade II (with subclades IIa and IIb). Clade I is endemic in the Congo basin and Central Africa regions and has a CFR of 3.5-10%; whereas clade II is endemic in West Africa and has a significantly lower mortality rate of 1% or less. A notable difference in the clinical presentation of smallpox and mpox is that of cervical and inguinal lymphadenopathy appearing 1 to 2 days before the rash in 90% of cases of mpox.[16, 16a] In 2003, an outbreak of 78 confirmed or suspected human cases occurred in the U.S. due to exposure to exotic pets, some of which had been imported from Africa.[15] These cases tended to be less severe, often only with localized lesions, no deaths, and no secondary transmission to other humans. (The west African strain involved, however, was apparently atypically less virulent than prototypical monkeypox disease in Africa.)[17]

A global outbreak of mpox clade II occurred in May 2022, and as of Nov 8, 2024, this outbreak infected more than 102,000 people with more than 220 deaths. Since 2024, Clade I has surged causing 9,000 cases globally. On 14 August 2024, the WHO Director General declared that the increase in mpox cases in the DRC and its expansion to neighboring countries constitutes a Public Health Emergency of International Concern (PHEIC).[17a] The causative agent of this outbreak is MPXV clade Ib which is predominantly affecting non-endemic areas in the DRC and neighboring countries. MPXV clade Ia continues to primarily affect endemic areas within the DRC. Whereas the ongoing outbreak of clade II is mainly in Nigeria and other endemic countries in West and Central Africa with clade IIb being associated with the global mpox epidemic.[17a] Although subclades designated “b” can occur in the general population, they occur more commonly among men-who-have-sex-with-men (MSM).

Confirmatory identification of mpox, to include clade differentiation is available at select sites to include the CDC and the U.S. Army Medical Research Institute of Infectious Diseases in Fort Detrick, Maryland. The CDC recommends Jynneos, a two-dose vaccine for those that are at high risk of transmission to include healthcare workers at risk of occupational exposure, travel to countries with mpox outbreaks, and known or suspected exposure. [32]

Diagnosis

Smallpox must be distinguished from other vesicular exanthems, such as chickenpox, erythema multiforme with bullae, allergic contact dermatitis and other orthopoxvirus infections. In a confirmed outbreak, smallpox would likely be a clinical diagnosis. Particularly problematic to the necessary infection control measures would be the failure to recognize relatively mild cases of smallpox in persons with partial immunity, or extremely severe cases in patients who bypass classical disease. Therefore, isolation of suspected cases, quarantine of potential exposures, and initiation of medical countermeasures should be promptly followed by an accurate laboratory diagnosis. Contact and airborne precautions should be implemented and providers who attend at bedside or collect or process specimens should be vaccinated. Specimens should be collected only upon the direction of public health officials, who will provide further guidance. Typical *Variola* specimens might include scrapings of skin lesions, lesion fluid, crusts, blood, or pharyngeal swabs. The CDC has prepared a useful poster and diagnostic algorithm[18] to assist in decision making.

A method of presumptive diagnosis is the demonstration of characteristic poxvirus virions with electron microscopy of vesicular scrapings. Under light microscopy, aggregations of *Variola* virus particles, called Guarnieri bodies, can be seen. Another rapid but relatively insensitive test for Guarnieri bodies in vesicular scrapings is Gispen's modified silver stain, in which cytoplasmic inclusions appear black. However, none of the above laboratory tests are capable of discriminating *Variola* from vaccinia, mpox, or cowpox.[3]

Identification of *Variola* has classically required isolation of the virus and characterization of its growth on chicken egg chorioallantoic membranes. Real-time PCR assays are now available and provide a rapid and specific diagnosis. Specific smallpox PCR diagnosis is presently available only at facilities participating in the Laboratory Response Network (LRN). A real-time PCR assay that detects all orthopoxviruses (including vaccinia) may be available from the 1st Global Field Medical Laboratory (GFML), Aberdeen Proving Ground, MD, for a presumptive diagnosis.[19, 20]

Neutralizing antibodies to *Variola* form in the first week of illness and may persist for many years. Hemagglutination-inhibition antibodies are detectible by the 16th day of infection and complement fixation antibodies by the 18th, but both begin to decrease after 1 year.[7]

Associated lab findings, including the complete blood counts (CBC) of patients with ordinary smallpox, often exhibited a neutropenia and lymphocytosis during the eruptive stage. Neutrophils could become elevated during the late pustular stage when secondary bacterial infections would occur. Mild thrombocytopenia was common. In hemorrhagic smallpox, thrombocytopenia was progressive and severe as disseminated intravascular coagulation developed.[1]

Medical Management

Medical personnel must be able to recognize a vesicular exanthem and consider the etiology as potentially *Variola*, and then quickly initiate appropriate isolation precautions and countermeasures. Any confirmed case of human smallpox should be considered an international emergency mandating immediate notification of public health authorities. Those exposed to known cases of smallpox should be monitored for a minimum of 17 days from the time of exposure regardless of their vaccination status; such individuals should be immediately isolated using contact and airborne precautions from the onset of fever. In a civilian setting, strict quarantine of asymptomatic contacts may prove to be impractical to enforce. A reasonable alternative would be to require contacts to remain at home and to check their temperatures daily. Any fever above 38°C (101°F) during the 17 days after exposure to a confirmed case would suggest the development of smallpox. The contact should then be isolated immediately, preferably at home, until smallpox is either confirmed or ruled out. Patients should be considered infectious until all scabs have separated and must remain in isolation until that time. Immediate vaccination or revaccination should also be undertaken for all personnel exposed to either weaponized *Variola* virus or a clinical case of smallpox. Caregivers should be vaccinated and continue to wear appropriate personal protective equipment regardless of vaccination status. Weaponized smallpox strains encountered in the future may be genetically altered to render the current vaccine ineffective, a possibility validated in experimental animal models using similar poxviruses.[1, 7]

The potential for airborne spread to other than close contacts is controversial. In general, close person-to-person contact is required for transmission to reliably occur. Nevertheless, *Variola*'s potential for airborne spread in conditions of low relative humidity was demonstrated during two hospital outbreaks. Indirect transmission by contaminated bedding or by other fomites was infrequent. Some close contacts harbored virus in their throats without developing disease and hence might have served as a means of secondary transmission.[7]

Vaccination with a verified clinical “take” (vesicle with subsequent scar formation) within the past 3 years is considered to render a person immune to smallpox. However, given the difficulties and uncertainties under wartime conditions of verifying the adequacy of troops' prior vaccination, routine revaccination of all potentially exposed personnel would seem prudent if there exists a significant likelihood of smallpox exposure.[1, 21]

The antivirals, tecovirimat (TPOXX) (2018) and brincidofovir (TEMBEXA) (2021), are FDA-approved as treatments for smallpox and are maintained in the Strategic National Stockpile by the Assistant Secretary for Preparedness and Response (ASPR).[4] Neither antiviral is FDA approved for the treatment of mpox; however, the CDC recommends these antivirals for the treatment of mpox under Expanded Access (Emergency) IND. Cidofovir is not FDA-

approved for the treatment of orthopoxvirus infection, smallpox or mpox, but is commercially available for other treatments.[5] (see Appendix J).

Supportive care is imperative for successful management of smallpox patients; measures include maintenance of hydration and nutrition, pain control, and management of secondary infections.[7]

Prophylaxis

Vaccines: There are two vaccines currently approved by the FDA for use against smallpox and mpox: *ACAM 2000*® and *JYNNEOS*®.[24, 25a, 25b]

ACAM2000 is produced in cell culture and is a live smallpox vaccine made from vaccinia virus. It is administered by percutaneous inoculation with 15 pricks (jabs) of a bifurcated needle, a process known as scarification because of the small, permanent scar that results. Vaccination with *ACAM2000*® after exposure to smallpox may prevent or ameliorate disease if given as soon as possible and preferably within 4 days of exposure. A vesicle typically appears at the vaccination site 5 to 7 days after inoculation, with associated erythema and induration. The lesion forms a scab and gradually heals over the next 1 or 2 weeks; the evolution of the lesion may be more rapid, with less severe symptoms, in those with previous immunity.[1, 7]

ACAM2000® vaccination side effects include low-grade fever and axillary lymphadenopathy. The attendant erythema and induration of the vaccination vesicle is frequently misdiagnosed as bacterial superinfection or cellulitis. More severe vaccine reactions (more common in primary vaccinees) include inadvertent ***autoinoculation*** of the virus to other sites such as the face, eyelid, or other persons (~ 6/10,000 vaccinees), and ***generalized vaccinia***, which is a systemic spread of the virus to produce mucocutaneous lesions away from the primary vaccination site (~3/10,000 vaccinees). Approximately 1/10,000 primary vaccinees will experience a transient, acute ***myopericarditis***. Rare, but often fatal, adverse reactions include ***eczema vaccinatum*** (generalized cutaneous spread of vaccinia in patients with eczema), ***progressive vaccinia*** (systemic spread of vaccinia in immunocompromised individuals), and ***post-vaccinia encephalitis***.[7, 25-27]

Vaccination with *ACAM2000*® is *contraindicated* in the following conditions unless a smallpox outbreak is documented: immunosuppression, HIV infection, history or evidence of eczema, other active severe skin disorders, pregnancy, or current household, sexual, or other close physical contact with person(s) possessing one of these conditions.[28] In addition, vaccination should not be performed in breastfeeding mothers, in individuals with serious cardiovascular disease or with three risk factors for cardiovascular disease, or individuals who are using topical steroid eye medications or who have had recent eye surgery. Despite these caveats, most authorities, including current CDC guidelines, state that, with the exception of significant impairment of systemic immunity, there

are no *absolute* contraindications to post-exposure vaccination of a person who experiences *bona fide* exposure to *Variola*. However, concomitant vaccine immune globulin administration is recommended for pregnant and eczematous persons in such circumstances.[3, 7]TPOXX may be used to treat severe adverse reaction from vaccinia virus vaccination under IND protocols.

JYNNEOS® (Smallpox and Monkeypox Vaccine, Live, Non-replicating) was approved by the FDA in September 2019 for the prevention of smallpox and mpox in adults 18 years of age and older. *JYNNEOS*® is a live vaccine produced from a modified form of the vaccina virus called Modified Vaccina Ankara, an attenuated, non-replicating orthopoxvirus and is administered as subcutaneous injection of two doses given 4 weeks apart. The most common side effects include pain, redness, swelling, itching, and firmness at the injection site, muscle pain, headache and fatigue. The vaccine is part of the Strategic National Stockpile.[29, 30] No cardiac disease has been associated with JYNNEOS administration. JYNNEOS can be administered safely to patients with atopic dermatitis and certain other immune related diseases that would contraindicate a replicating smallpox vaccine.

Passive Immunoprophylaxis: *Vaccinia Immune Globulin* (VIG) is indicated for some complications of the smallpox vaccine (*generalized vaccinia* with systemic illness, *ocular vaccinia* without keratitis, *eczema vaccinatum*, and *progressive vaccinia*), and should be available whenever administering vaccine. A formulation of VIG-IV has been licensed (2005) but is currently in very limited supply.[31] The dose for prophylaxis or treatment is 100 mg/kg for the IV formulation (first line treatment). If VIG-IV is not available, cidofovir may be of use for treating vaccinia adverse events (second line). The intramuscular VIG formulation (VIG-IM) is dosed 0.6 ml/kg (third line). Due to the large volume of the IM formulation (42 ml in a 70 kg person), the dose would be given in multiple sites over 24 to 36 hours. Limited data suggest that VIG may also be of value in post-exposure prophylaxis of smallpox when given within the first week after exposure, and concurrently with vaccination. Vaccination alone is recommended for those without contraindications to the vaccine. If greater than 1 week has elapsed since exposure, administration of both products (vaccine and VIG), if available, is reasonable.[4, 6]

EQUINE ENCEPHALITIDES (VEE, EEE, & WEE)

Summary

Signs and symptoms: Incubation periods are 27.5 hours to 4 days (Venezuelan equine encephalitis – VEE), 4 to 10 days (eastern equine encephalitis – EEE), and 5 to 10 days (western equine encephalitis – WEE) in natural disease. These encephalitides all present as acute systemic febrile illnesses, however, the prevalence of encephalitis varies depending on the virus and are likely underdiagnosed due to insufficient diagnostics and surveillance efforts (~4% of children, < 1% of adults for VEE; 4 to 5% for EEE; up to 33% in infants and 13% in adults for WEE). Symptoms include generalized malaise, spiking fevers, rigors, severe headache, photophobia, and myalgias. Nausea, vomiting, cough, sore throat, and diarrhea may follow. Full recovery from malaise and fatigue takes 1 or 2 weeks. If encephalitis ensues, anticipate vomiting, stiff neck, drowsiness, paresis, impaired respiratory regulation, seizures, or coma. The incidence of neurologic disease could increase after an aerosol exposure.

Diagnosis: is primarily clinical, based on epidemiology. Physical findings are nonspecific and easily misdiagnosed as other agents, such as dengue virus. Leukopenia with a striking lymphopenia is seen in VEE and leukocytosis with a neutrophilia in EEE and WEE. If within the first 7 days, virus may be isolated/detected from serum for VEE. Virus isolation is not typically successful for EEE and WEE. Serologic or Cerebrospinal Fluid (CSF) testing remains the primary method for diagnosing EEE and WEE. Positivity in a virus-specific IgM assay should be confirmed by a neutralizing antibody test at a state or national laboratory.

Treatment: is **s**upportive. Uncomplicated infections benefit from analgesics to relieve headache and myalgia. Patients who develop encephalitis may require anticonvulsants and intensive supportive care to maintain fluid and electrolyte balance, ensure adequate ventilation, and avoid complicating secondary bacterial infections.

Prophylaxis: Currently, there are no FDA licensed vaccines or post-exposure prophylaxis (PEP) for VEE, EEE, or WEE.

Isolation and decontamination: Patient isolation and quarantine are not required. Standard precautions (augmented with vector control) while the patient is febrile is recommended for VEE. There is no evidence of direct human-to-human or horse-to-human transmission. The virus can be destroyed by heat (80^{o} C for 30 min) and standard disinfectants.

Overview

Alphaviruses are enveloped, single-stranded, positive-sense ribonucleic acid (RNA) viruses that belong to the *Togaviridae* family. There are 31 species in the *Alphavirus* genus, which can be antigenically or genetically classified into eight complexes.[1-3] The alphaviruses have been categorized into Old World or New World viruses historically based on their geographical distribution and predominant disease characteristic, arthralgia or encephalitis. The Old World alphaviruses, traditionally found in Africa and Asia, primarily cause a rash and arthritis. Examples include chikungunya virus, O'nyong-nyong virus, and Ross River virus. The New World alphaviruses, found in the Americas, include Venezuelan equine encephalitis virus (VEEV), eastern equine encephalitis virus (EEEV), and western equine encephalitis virus (WEEV), can cause encephalitis in equines (horses, donkeys, mules, burros) and humans.[1]

The VEEV complex consists of eight closely related subtypes that differ in regard to ecology, epidemiology, and virulence for humans and equines.[4] Subtypes IAB and IC are known as the epizootic strains and are responsible for large-scale epidemics in North, Central, and South America. Subtypes ID and IE are the enzootic strains, which may cause disease in humans, but lack virulence for equines.[5,6]

The EEEV complex is divided into two distinct lineages which vary in geographic, epidemiologic, phylogenetic, and pathogenic characteristics.[7,8] Virus species in North America, referred to as EEEV, are enzootic along the eastern seaboard, Great Lakes, and Gulf Coast of North America and the Caribbean. EEEV is responsible for most human cases, with significant mortality rates in humans (30-50%) and equines (80-90%). The South American species, Madariaga virus (MADV), enzootic in Central America and South America, appears to be much less virulent, but lineage III viruses can cause fever and encephalitis.[2,7]

The WEEV complex includes several virus species that differ in their ecology and virulence; however, only the WEEV species causes encephalitic disease in humans.[1]

Alphaviruses cycle between invertebrate insect vectors and vertebrate reservoir hosts. For many alphaviruses, the insect vectors are mosquitoes and the vertebrate hosts are birds or small mammals.[1] In most cases, humans and equines are incidental hosts and become infected during outbreaks in the late summer and early fall, especially after periods of heavy rainfall. For VEEV, infection in horses results in development of a high viremia enabling amplification and spread of virus to new mosquitoes during a blood meal (PMID: 16549790). Unlike VEEV, in which there are often massive epizootics in horses and spillover epidemics in humans, EEEV and WEEV usually result in either individual cases or limited outbreaks in both horses and humans.[1,5,9]

Alphaviruses are also highly infectious by aerosol. In fact, VEEV, EEEV, and WEEV possess many of the required characteristics for strategic or tactical weapon development, including ease of large-scale production, virus stability, potential for aerosolization, and virulence.[5] VEEV is of particular concern because it produces overt disease in nearly all human infections and can produce a self-sustaining natural outbreak since equines are amplifying hosts. However, there is no evidence of direct human-to-human or horse-to-human transmission. Natural aerosol transmission is not known to occur. Alphaviruses are not considered stable in the environment and are thus not as persistent as the bacteria responsible for Q fever, tularemia, or anthrax. Heat and standard disinfectants can easily kill VEEV, EEEV, and WEEV.

VEEV is more characterized than EEEV or WEEV, primarily because it was tested as a bio-warfare agent during the U.S. offensive biological weapons program in the 1950s and '60s, as well as by the USSR in the same period and later. In compliance with President Nixon's November 1969 directive mandating the destruction of all existing stocks of U.S. biological and chemical weapons, all VEEV weapon stocks under U.S. control were destroyed under supervision.[10]

History and Epidemiology

Although the first clear epidemic of equine encephalitis occurred in 1831[11], it was not until 100 years later that three distinct, but antigenically related, virus complexes were recovered from horses with severe equine encephalitis: WEEV was isolated in the San Joaquin Valley in California in 1930[12], EEEV was isolated in Virginia and New Jersey in 1933[13-15], and VEEV in Venezuela in 1936.[1,16,17]

Since its initial isolation, VEEV has caused several major epizootics/epidemics, primarily in Central and South America, involving hundreds of thousands of human cases and even more in equines.[2,6] Equines, especially horses, are very susceptible to epizootic VEEV, leading to high morbidity and mortality. Importantly, horses are also amplifying hosts for epizootic VEEV, meaning the resulting viremia permits mosquito transmission and therefore fuels epizootics. Epidemics are the consequence of spillover during epizootics: humans become infected by mosquitoes that previously fed on infected horses.[5,6] Infected humans can shed high levels of VEEV in their nasal secretions; however, direct human-to-human transmission has never been documented.[18] Additionally, human viremia following endemic VEEV infection is sufficient to infect potential vectors, although extensive human disease has never been documented in the absence of equine amplification or enzootic vectors.[19] The most recent significant outbreak occurred in Venezuela and Columbia in 1995, resulting in over 100,000 human cases, 3000 of which experienced neurologic complications and 300 deaths.[18] The total number of equine cases was not reported but was probably similar in magnitude to human numbers.[20] Epizootic VEEV has not been isolated in the U.S. since 1971. That being said, since its initial isolation and prior to more stringent personal protective measures, at least 150

symptomatic laboratory-acquired infections have been reported, most of which were known or thought to be aerosol infection.[21]

EEEV is endemic along the eastern seaboard, Great Lakes, and Gulf Coast regions of the U.S., and typically results in a low number of human cases annually. On average, eleven human cases with neuro-invasive disease are attributed to EEE each year. At the same time, in 2019, there were 38 human cases, including twelve deaths, and 184 equine cases, most fatal. Interestingly, an increase in detection of EEEV in other species—eagles, chickens, alpaca, wolves—were also observed. Outbreaks in humans occur in the late summer or early fall, are usually associated with heavy rainfall and warmer water temperatures, and are frequently preceded by cases of equine encephalitis. Humans, horses, and other mammals are considered dead-end hosts.[1]

Historically, WEEV has caused epizootics and epidemics in the western U.S.; however, few cases have been reported in recent years. Several states in the U.S. had human and/or equine outbreaks during the 1930s, '40s, and '50s, with equine epizootics being more severe than the human epidemics.[22,23] There were an average of 34 human cases of neuroinvasive disease attributed to WEEV per year in the U.S. from 1955 to 1984, but those numbers have declined rapidly and none have been reported in the past 25 years.[1,24,25] Major outbreaks also occurred in South America during the 1970s and 1980s, but only isolated cases have been reported in Argentina in 1996 and Uruguay in 2009[26,27] until November 2023 when a large WEEV outbreak occurred in Argentina and Uruguay resulting in 112 confirmed (another 105 suspected) human cases and 127 confirmed (2421 suspected) equine cases[28,29]. Like EEEV, WEEV infection in humans and equines are considered dead-end[1].

Natural human epidemics are almost always preceded by epizootics, characterized by severe and often fatal (30-90%) encephalitic outbreaks in equids. An intentional aerosol dissemination would most likely cause human disease as a primary event or simultaneously with equids. Occasionally during natural epidemics, illness or death in wild or free-ranging equines may not be recognized before the onset of human disease; therefore, a natural epidemic could be confused with a nefarious release, and data on the onset of disease should be considered with caution. A more reliable method for determining the likelihood of an intentional event would be the presence of any of these alphaviruses outside of their natural geographic range. For VEEV, an intentional aerosol release in a region populated by equines and appropriate mosquito vectors could initiate an epizootic/epidemic outbreak.

Clinical Features

VEEV, EEEV, and WEEV cause similar nonspecific prodromal syndromes in humans; unfortunately, the consequences vary by virus complex. VEEV epidemics are explosive, often resulting in thousands of cases, but VEEV causes the least amount of neurological disease of the encephalitic alphaviruses. Human

susceptibility to VEEV is high (90-100%), requiring as few as 10 viral particles by aerosol.[30] Nearly 100% of those infected develop overt illness with the vast majority presenting as undifferentiated flu-like illness[26] and < 1% of adults and < 4% of children developing encephalitis.[31] The overall case fatality rate (CFR) for VEE is < 1%. It is somewhat higher in those that develop encephalitis and may be as high as 35% in children and 10% in adults who develop VEE.[32] Recovery from an infection results in excellent short-term and long-term immunity to the infective subtype, but may not protect against other subtypes of the virus.

VEEV primarily results in an acute, incapacitating, febrile illness with most infections being self-limiting (in contrast to clinically apparent EEEV and WEEV infection, in which encephalitis occurs with increased frequency). After an incubation period as short as 27.5 hours but typically 2 to 4 days, onset of prostration is usually sudden. This acute phase of illness is often manifested by generalized malaise, chills, spiking high fevers (38° C - 40.5° C), rigors, severe headache, photophobia, and myalgias. Nausea, vomiting, and diarrhea are also common. Physical signs may include tachycardia, conjunctival injection, erythematous pharynx, and muscle tenderness. Severe symptoms generally subside within 2 to 4 days, followed by asthenia (malaise and fatigue) lasting another 1 or 2 weeks before full recovery. A biphasic illness, with recurrence of the acute symptoms 4 to 8 days after initial onset of disease, has been described infrequently.[33] Mild Central Nervous System (CNS) findings include lethargy, somnolence, photophobia or mild confusion, with or without nuchal rigidity. Seizures, ataxia, paralysis, or coma follow more severe CNS involvement. Generally, about 10% of patients in natural epidemics will be ill enough to require hospitalization.[32] Experimental aerosol challenges in animals suggest that the incidence of CNS disease and associated morbidity and mortality could be much higher after an aerosol exposure, as the virus may travel along the olfactory nerve and spread directly to the CNS and result in acute neurological signs.[5,34-39] School-age children may be more susceptible to a fulminant form of the disease characterized by depletion of lymphoid tissues, encephalitis, interstitial pneumonitis, and hepatitis, which follows a lethal course over 48 to 72 hours.[40] For those who survive encephalitic involvement, neurological recovery is usually complete, although one report documented motor disorders and an increased incidence of seizures in children following VEE outbreaks.[41] VEEV infection during pregnancy may cause encephalitis in the fetus, placental damage, spontaneous abortion, or severe congenital neuroanatomical anomalies.[18,42]

EEEV outbreaks are usually more limited due to a low incidence of human infection (< 3% of the population at risk). Additionally, the neurological attack rate in one outbreak was estimated at 1 in every 23 cases of human infection.[43,44] Of those who develop clinical symptoms, only about 4 or 5% will go on to develop encephalitis (full-blown EEE). EEE is the most severe of the alphavirus encephalitides, with CFRs ranging from 30 to 70% and severe neurologic sequelae in those that survive.[43,45] The initial clinical presentation of EEEV

infection is indistinguishable from that of VEEV or WEEV, with patients presenting with "flu-like" symptoms. The incubation period is slightly longer, ranging from 4 to 10 days. Adults can exhibit a febrile prodrome for up to 11 days before the onset of neurological disease[46]; however, onset of illness in children is more rapid. The nonspecific prodrome is followed by severe headache, high fevers, lethargy, and seizures, often with rapid progression to coma and death.[45,47,48] In a retrospective study of 15 cases of EEE in children, fever, headache, and seizures were the most common clinical findings, with 87% of the patients becoming stuporous or comatose during the first 3 days of hospitalization. Radiographic lesions were noted in the basal ganglia, thalami, and cerebral cortex. Importantly, this study found an association between a short prodrome (i.e., the time between initial nonspecific symptoms and the first major neurologic symptom) and an increased risk of death or severe disease. The eight patients which had a poor outcome all had a prodrome of 2 days or less, and all four deaths occurred in this group.[49]

Similarly, the initial clinical presentation of WEEV infection is indistinguishable from that of VEEV or EEEV. Infection with WEEV results in encephalitis (full-blown WEE) less often than EEEV infection. CFRs in natural epidemics range from 8 to 15%[4,22], while the CFR associated with lab accidents involving aerosol exposure has been closer to 40%.[50] The incubation period is 5 to 10 days for natural infection. In lab monkeys exposed by aerosol, the incubation period is 4 to 5 days.[51] A large percentage of patients with vector-borne infections are either asymptomatic or present with a nonspecific febrile illness or aseptic meningitis. The ratio of encephalitis cases per infection has been estimated to vary from 1:1,150 in adults, 1:58 in children to 1:1 in infants.[52] The severity of the syndrome and the incidence of inapparent infection almost certainly depend on the strain and dose of virus and the route of infection. Patients with the most severe infections usually die within 7 days of clinical illness, while other patients begin a gradual convalescence after the first week of encephalitic symptoms. Most adults ≤60 years of age recover completely but may take months to years to recuperate from fatigability, recurrent headaches, emotional lability, and impaired concentration.[53] Some patients have neurologic sequalae such as motor weakness, cognitive deficits, or a seizure disorder. Like VEEV and EEEV, children carry a higher incidence of neurological sequelae, ranging from < 1% in those older than 1 year, to > 50% in newborns. Congenital infection in the last trimester of pregnancy has been described, with resultant encephalitis in the infants.[54]

Diagnosis

A diagnosis of VEE, EEE, or WEE is suspected on clinical and epidemiological grounds, but confirmed by virus isolation for VEEV, or by serology or PCR for VEEV, EEEV, and WEEV. A variety of serological tests are applicable, including IgM, ELISA, indirect fluorescent antibody, hemagglutination inhibition, complement-fixation, and IgG. For persons without prior known exposure to alphaviruses, a presumptive diagnosis may be made by identifying

IgM antibody in a single serum sample taken 5 to7 days after onset of illness. PCR procedures are available for confirmation but are generally available only as a rear echelon laboratory capability.

Samples suitable for performing diagnostic tests include blood culture for VEEV, or acute and convalescent sera, and CSF for VEEV, EEEV, or WEEV. Viremia during the acute phase of the illness—but not during encephalitis—is generally high enough to allow detection by antigen-capture ELISA for VEEV. Virus isolation is time consuming but may be performed from serum and throat or nasal swab specimens collected in the first 3 days of illness by inoculation of cell cultures of suckling mice. VEEV, EEEV, and WEEV should be isolated only in a BSL-3 laboratory.

In the cases of VEEV infection, the WBC is often normal at the onset of symptoms and then usually shows a leukopenia with a striking lymphopenia, and sometimes a mild thrombocytopenia by the second to third day of illness. Each of these abnormalities will usually resolve over the ensuing 1 or 2 weeks. In EEE, there may be an initial leukopenia, which then becomes a leukocytosis characterized by a neutrophilia. Temporary, mild elevations of LDH, AST, and ALP may also be present. In patients with encephalitis, CSF pressure may be increased and contain up to 1,000 WBCs/mm^3 (mostly mononuclear cells, unless very early in infection) and a mildly elevated protein concentration.

On purely clinical grounds, an alphavirus outbreak may be difficult to distinguish from one caused by influenza. Clues might include the appearance of a small proportion of neurological cases, lack of person-to-person spread, or concurrent encephalitis in equines. An intentional aerosol attack could lead to an epidemic of febrile meningoencephalitis featuring seizures and coma. In the case of nefarious aerosol release of virus, the differential diagnosis would include other causes of aseptic meningitis and meningoencephalitis.

Medical Management

No specific antiviral therapy exists; hence treatment is supportive only. Patients with uncomplicated alphaviral infection may be treated with analgesics to relieve headache and myalgia. Nausea and emesis can lead to dehydration and necessitate IV fluids in some cases. Patients who develop encephalitis may require anticonvulsants and intensive supportive care to maintain fluid and electrolyte balance, ensure adequate ventilation, and avoid complicating secondary bacterial infections. In the presence of mosquito vectors, patients should be housed in a screened room or in quarters treated with a residual insecticide for $\geq$ 5 days after onset, or until afebrile, as human cases of VEE may be infectious for mosquitoes for $\geq$ 72 hours. Patient isolation and quarantine are otherwise not required; sufficient contagion control is provided by implementing standard precautions augmented with vector control while the patient is febrile. Patient-to-patient transmission by means of respiratory droplet

infection has not been shown to occur. The virus can be destroyed by heat (80° C for 30 min) and standard disinfectants.

Prophylaxis

Vaccine: Currently there are no licensed vaccines available for VEEV, EEEV, or WEEV in the United States or European Union. Historically, a live attenuated strain of VEEV, TC-83, was available to at-risk laboratory workers under the Special Immunization Program under an Investigational New Drug (IND) protocol until the early 2020s. Formalin-inactivated IND vaccines for EEEV and WEEV were also available to laboratory personnel prior to cessation of the program. Research is underway to produce improved, second-generation VEE, EEE, and WEE vaccines.[55-57]

Immunoprophylaxis: At present, there are no licensed pre- or post-exposure immunoprophylactics for the equine encephalidities. In animal models, protection from subcutaneous and aerosol exposure to VEEV has been demonstrated by passive transfer of neutralizing monoclonal antibodies administered 24 hours pre- or up to 48 hours post-infection.[58-61]

Chemoprophylaxis: Like vaccines and immunoprophylactics, there are no licensed chemoprophylactics for VEEV, EEEV, or WEEV. In experimental animals, innate immune modulators, such as α-interferon, CpG and poly-ICLC, have proven highly effective for post-exposure chemoprophylaxis.[62-64] The nucleoside analog, Molnupiravir, the prodrug to beta-d-N(4)-Hydroxycytidine has also shown promise in mice[65] and nonhuman primates against VEEV.

VIRAL HEMORRHAGIC FEVERS (VHFs)

Summary

Signs and symptoms: Viral hemorrhagic fevers (VHFs) are illnesses characterized by fever, potential bleeding diathesis and systemic manifestations, including facial and chest flushing, petechiae, edema, hypotension, frank bleeding, and shock. Malaise, myalgias, headache, vomiting, and diarrhea (sometimes high volume) occur frequently.

Diagnosis: Diagnosis is usually made at a reference laboratory with bio-containment (BSL-4) capability. Early recognition and diagnosis are crucial for appropriate management and to minimize potential nosocomial spread. Any patient with compatible signs and symptoms should prompt consideration of a VHF.

Treatment: Intensive supportive care may be required. There are two approved monoclonal antibody treatments for *Zaire ebolavirus*: Ebanga™ and Inmazeb®. Antiviral therapy (IV ribavirin) under an investigational new drug (IND) protocol may be useful in *Bunyaviridae* and *Arenaviridae* infections (specifically Lassa fever, Crimean-Congo hemorrhagic fever, and hemorrhagic fever with renal syndrome due to Old World hantavirus infection). Convalescent plasma may be effective in Argentine or Bolivian hemorrhagic fevers as an IND.

Prophylaxis: There are two licensed VHF vaccines in the U.S. for viral hemorrhagic fevers: the 17D yellow fever vaccine and rVSV vaccine (Ervebo®) for Ebola Zaire. Experimental vaccines for other VHFs are not readily available. Prophylactic ribavirin may be effective for some *Bunyaviridae* and *Arenaviridae* infections (only as an IND).

Isolation and decontamination: Strict contact precautions (hand hygiene, double gloves, gowns, shoe/leg coverings & face shield or goggles) and droplet precautions (private room or cohorting, surgical mask) are mandatory. Airborne precautions (negative-pressure isolation room with 6 to 12 air exchanges/hour) should also be instituted as feasible and especially for aerosol-inducing procedures, along with a HEPA filter-equipped respirator (N-95 mask), but a positive pressure-supplied air respirator (PAPR) should be considered for personnel sharing an enclosed space with, or within 6 feet of, the patient. Multiple confirmed patients may be cohorted in a separate ward or building with dedicated air-handling when feasible. Environmental decontamination is accomplished with hypochlorite or phenolic disinfectants.

Overview

The term VHF is a syndrome caused by four viral families of lipid-enveloped, single-stranded RNA viruses: *Arenaviridae, Bunyaviridae, Filoviridae,* and *Flaviviridae*. They all have the potential to present as severe febrile illnesses accompanied by shock and potential hemorrhagic diathesis. The *Arenaviridae* include the etiologic agents of Lassa fever and Argentine (Junin), Bolivian (Machupo), Brazilian (Sabia) and Venezuelan (Guanarito) hemorrhagic fevers. The *Bunyaviridae* include members of the *Hantavirus* genus causing hemorrhagic fever with renal syndrome (HFRS) or respiratory failure; the Crimean-Congo hemorrhagic fever virus from the *Nairovirus* genus; and the Rift Valley fever virus from the *Phlebovirus* genus. The *Filoviridae* include Ebola and Marburg viruses. The *Flaviviridae* include dengue, yellow fever, and two tick-borne viruses that cause VHF—Omsk hemorrhagic fever (OHF) virus and Kyasanur Forest disease (KFD) virus. These viruses are transmitted by multiple means: through blood/body fluid exposure, from animals to humans by a vector, inhalation, or ingestion of excretions/secretions of rodents. Rarely, some may be transmitted person-to-person through a respiratory portal of entry. The Soviet Union weaponized both Ebola and Marburg viruses[1]; other VHF viruses are included here because of their *potential* for aerosol dissemination, weaponization, or likelihood for confusion with similar agents that might be weaponized.

History & Significance

The VHF viruses are diverse and occur in different endemic geographic locations; hence, a comprehensive discussion is beyond the scope of this handbook. However, each viral infection possesses different features that provide potential importance as bio-agents. All of the VHF agents (except dengue) are lab aerosol and percutaneous infection hazards (even dengue has been nosocomially transmitted by blood splash). The aerosol infectivity for many has been studied in experimental animal models. VHF agents cause severe disease, and many have high fatality rates. For these reasons, they are considered significant potential biowarfare and bio-terrorism threats.[2]

Arenaviridae: Lassa virus causes Lassa fever in West Africa, where transmission occurs from exposure to rodent reservoirs of the *Mastomys* genus,[6] which are common there and often found nesting in homes, and are occasionally consumed.[7] Over 5,000 deaths in West Africa are attributed to Lassa each year, from 100,000 and 300,000 annual infections.[6,7] Argentine hemorrhagic fever (AHF), caused by Junin virus, was first described in 1955 among corn harvestors[2]. Historically, 300 to 600 cases occurred annually in the Argentine pampas, but have declined lately. Bolivian (BHF), Brazilian, and Venezuelan hemorrhagic fevers are caused by related Machupo, Sabia, and Guanarito viruses, respectively. Arenaviruses transmit from rodents to humans through inhalation of dust contaminated with or potentially direct contact with rodent excreta. Nosocomial transmission is likely possible with all arenavirus

infections but is a significant problem with Lassa fever.[3,6,7] Lassa infection of healthcare workers has been attributed to parenteral exposures, contact with body fluids, and patient-generated aerosols.[7]

Bunyaviridae: Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease with a widespread distribution from Africa through southeastern Europe, Central Asia and the Indian sub-continent. It may also be spread by contact with body fluids or slaughtered meat of infected animals and in health-care settings.[7] The 2009 death of a U.S. soldier infected with CCHF in Afghanistan, who died in Germany, was a reminder of the endemic disease risk around the world. Nosocomial spread in a Spanish hospital, where it was not known to be endemic demonstrates the ongoing risk of this pathogen.[36] Rift Valley fever (RVF) is a mosquito-borne disease that occurs in Central/East Africa in addition to transmission by handling infected tissues (animal slaughter), and by aerosol (particularly lab workers).[9] In 2000, a large outbreak occurred in Yemen and Saudi Arabia.[9] RVF virus is on the DHHS and USDA Select Agent lists indicating both human and animal agricultural significance.[10] The hantaviruses are rodent-borne with a wide geographic distribution. Hantaan and other Old World hantaviruses cause hemorrhagic fever with renal syndrome (HFRS). Hantaan causes Korean hemorrhagic fever or epidemic hemorrhagic fever, the most common human disease due to hantaviruses. It was described before WW II in Manchuria, among UN troops during the Korean conflict, and subsequently in Japan, China, and in the Russian Far East.[2,11] Severe HFRS from other hantaviruses (Dobrava virus) occurs in the Balkans. Puumala virus carried by bank voles causes *Nephropathia epidemica*, a milder disease occurring in Scandinavia and elsewhere in Europe.[12] New World hantaviruses (i.e., Sin Nombre, Andes) cause hantavirus pulmonary syndrome (HPS) in the Americas, which leads to respiratory and cardiovascular failure rather than hemorrhagic fever. Similar to arenaviruses, hantaviruses generally transmit to humans via inhalation of dust contaminated with rodent excreta.[11]

Filoviridae: Five species of Ebola virus (Tai Forest, Reston, Sudan, Ebola (aka – Zaire), and Bundibugyo) have been identified. Ebola-Zaire and Ebola-Sudan cause severe disease with high case fatality rates (CFRs).[13] Ebola hemorrhagic fever (EHF) was first recognized in Sudan (Ebola-Sudan) and a nearby region of Zaire (Ebola-Zaire) in 1976. In 1995, a single index case resulted in a large outbreak (316 cases) in Kikwit, Zaire.[14] Subsequent epidemics of Ebola-Zaire and Ebola-Sudan have occurred in Gabon, Ivory Coast, Uganda, Democratic Republic of Congo (former Zaire), and Sudan. In February 2014, the largest EHF outbreak to date began in Guinea and spread to neighboring Liberia and Sierra Leone causing a reported 28,610 infections and 11,308 deaths. A subsequent outbreak from 2018-2020 in eastern Democratic Republic of the Congo caused 3,470 cases and 2,287 deaths.[37] Ebola-Reston was isolated from monkeys imported into the U.S. from the Philippines in 1989 that developed hemorrhagic fever. Since then, there have been subsequent outbreaks in primate facilities in the U.S. and EU. Several animal handlers sero-converted after exposure, but did not manifest clinical disease; therefore, Ebola-Reston

currently appears not to be a human pathogen.[2] In 2008, pigs were identified in the Philippines to be co-infected with Ebola-Reston and a porcine-specific virus. Again, some pig handlers sero-converted without clinical disease.[16] Any role of pigs in the natural ecology of this disease remains unclear. In 1994, a scientist contracted Ebola-Taï Forest virus after working with post-mortem infected chimpanzee tissues, but recovered.[17] In 2007, a VHF outbreak in the Bundibugyo District in western Uganda (149 cases, 25% CFR) was confirmed as the fifth Ebola species.[18] Data implicate bats as the reservoir, although the link to humans, periodic resurfacing, and ecology of these diseases remain unclear.[2]

Only a single species of Marburg virus (Lake Victoria) is known, including two closely related viruses, Marburg and Ravn. The first recognized outbreak with 37 cases and 7 deaths occurred in Marburg, Germany, and Yugoslavia in 1967, among people exposed to African green monkeys imported from Uganda.[2,19] Marburg epidemics continue to be sporadic and mostly in Africa. In 2005, the largest outbreak occurred in Angola, with 356 deaths and most fatalities in children.[19] The Egyptian fruit bat (*Rousettus aegyptiacus*), found throughout Africa, is thought to be the reservoir.[20]

Human-to-human transmission may occur by direct contact with infected blood, secretions, organs, or semen.[2] Lab monkeys have been infected via airborne transmission experimentally, although the significance for human outbreaks remains unknown.[21]

Flaviviridae: Yellow fever and dengue are two mosquito-borne viruses with great historical military importance. Tick-borne flaviruses include the agents of Kyasanur Forest disease in India, and Omsk hemorrhagic fever in Siberia.[2]

Clinical Features

Hemorrhagic fever viruses can cause illnesses with diverse clinical presentations. Early symptoms consist of fever and constitutional symptoms, including malaise, myalgias, and headache, which are difficult to distinguish from other diseases, especially in tropical regions where many occur.[2,22] The most severe cases may develop full-blown VHF syndrome, with petechiae/purpura, capillary leak, bleeding diathesis, and hemodynamic compromise with shock and end organ failure.

Diversity of clinical features among the VHFs probably stems from varying mechanisms of pathogenesis. For example, an immunopathogenic mechanism likely occurs with dengue hemorrhagic fever, related to prior infection with a heterologous dengue serotype (dubbed "antibody-dependent enhancement."). Disseminated intravascular coagulation (DIC) may underlie the hemorrhagic features of Rift Valley, Marburg, and Ebola fevers, but the etiology of the coagulopathy is multifactorial (e.g., hepatic damage, consumptive coagulopathy, and primary marrow dysfunction) and the exact pathogenesis is still being pursued.[2]

Why some patients develop full-blown VHF versus others remains unresolved, but probably stems from complex virus-host interactions. Virulence of the specific infecting agent plays a large role, with a majority of patients manifesting disease from filoviruses, CCHF, and the South American hemorrhagic fever (SAHF) viruses, while occurring in a minority of dengue, RVF, and Lassa fever patients. Extremes of age and higher viral loads are associated with more severe disease.

Differentiating between VHFs before laboratory diagnosis is challenging. Epidemiological context may help, especially discerning the proportion of cases with mild/moderate/severe disease or known travel to endemic areas. Clinical manifestations of the VHFs are discussed below. Table 1 provides a further summary.

Arenaviridae: The clinical features of the SAHFs are similar, but differ significantly from Lassa fever. Onset of SAHFs is insidious, with high unremitting fever and constitutional symptoms. A petechial or vesicular enanthem involving the palate and tonsillar pillars is common, as is conjunctival injection and upper torso/facial flushing. Patients frequently have associated neurologic disease, with initial hyporeflexia followed by gait abnormalities and cerebellar dysfunction. Seizures portend a grave prognosis. Fatality rates are high, from 10% to 30%.[3,23]

In contrast, approximately 80% of natural infections with Lassa virus are mild or non-apparent.[7] The other 20% develop severe disease, with 15-20% CFR in hospitalized patients. Overall CFR for Lassa virus infection is ~1%, but wide disparities in reporting makes this approximate. Patients frequently have retrosternal chest pain, a sore throat and proteinuria. Encephalitis and/or meningitis may occur, as well as convalescent cerebellar syndromes. Serum AST levels in the hundreds/thousands (U/L) portend a poor prognosis. Deafness (transient or permanent) is a common sequela and occurs in up to one-third of afflicted patients annually.[7,23]

Bunyaviridae: CCHF is generally a severe, hemorrhagic disease. Onset is abrupt and GI and meningeal symptoms occur frequently. Petechiae, ecchymoses, and mucosal bleeding are common. Hepatitis and jaundice probably result from direct viral cytotoxicity. Thrombocytopenia can be profound. CFRs range from 5% to 30% but as high as 62% has been reported.[24,25]

RVF is usually a self-limiting, nondescript febrile illness. The most common sequela of an RVF infection is retinitis, and up to 10% of these patients have some residual vision loss. Only 1% develop hemorrhagic manifestations or severe hepatic disease, usually as a second febrile phase after the initial 3-7 day febrile phase. A small minority of patients develop encephalitis after the initial febrile illness.[9,25]

The severity of HFRS depends on the infecting hantavirus. Puumala virus, common in northern Europe and Russia, causes a milder disease (*nephropathica endemica*) with rare fatalities. Hantaan causes the most severe form (5%-15% CFR) and disease progression occurs in four phases: an initial **febrile phase**, with abrupt onset of fever, malaise, myalgia, headache, and lassitude. Some characteristic features are flushing of the face and neck, conjunctival and pharyngeal injection, cutaneous and mucosal petechiae (occurring by day 4 or 5), and profound lower back pain. In the second, **hypotensive phase,** mild DIC, thrombocytopenia, and capillary leak syndrome may ensue with hypovolemic shock. In the **oliguric phase**, renal dysfunction is pathognomonic, frequently progressing to oliguric renal failure. The final **diuretic phase** often accompanies convalescence, which requires judicious fluid management.[11,25]

Filoviridae: Ebola and Marburg infections present similarly. Onset is abrupt with fever, constitutional symptoms, nausea, vomiting, diarrhea, abdominal pain, lymphadenopathy, pharyngitis, conjunctival injection, and pancreatitis. Many patients develop a maculopapular rash around day 5, which may be difficult to appreciate in dark-skinned persons. Increased liver enzymes, BUN, creatinine, clotting times, and d-dimers occur, but decreased fibrinogen are typical laboratory findings. Delirium, obtundation, and coma are common. Hemorrhagic features develop as the disease progresses, and usually manifest as oozing from intravenous or venipuncture sites, subconjunctival hemorrhage, petechiae, gingival bleeding, rather than frank hemorrhage. Death usually occurs during the second week of illness. Fatality rates from 25% (Bundibugyo) to over 80% (Marburg/Ebola Zaire) have been observed, although they can be significantly impacted by intensive care.[2,21,38]

Flaviviridae: Yellow fever is classically described as a severe biphasic illness, but many infections are mild or subclinical. The initial phase lasts ~1 week with fever, constitutional symptoms, GI symptoms and other undifferentiated features. Objective findings are unimpressive except for potential occurrence of relative bradycardia (Faget's sign) and leukopenia. Facial flushing and conjunctival injection may also occur. After a period of clinical improvement and defervescence (hours to days) some patients develop a second febrile phase. This "period of intoxication" is characterized by high fever, severe constitutional symptoms, obtundation, skin and mucous membrane hemorrhages, severe hepatitis, and profound jaundice. Liver enzyme elevation occurs consistent with hepatocellular damage, with significant bilirubin elevation. Proteinuria is almost universal and an excellent diagnostic clue. As severe disease progresses, renal failure consistent with hepatorenal syndrome may ensue. Death may occur in over 50% of patients with the hemorrhagic form.[2,26,27]

The two members of the tick-borne encephalitis complex causing hemorrhagic disease (Kyasanur Forest and Omsk) have similar clinical syndromes and are often biphasic. The first phase is a febrile syndrome of varying severity, associated with conjunctival suffusion, facial flushing, lymphadenopathy,

and splenomegaly. In severe cases, it may be accompanied by diffuse mucosal hemorrhaging and petechiae. Hemorrhagic pulmonary edema is relatively common and distinct. A second illness phase may occur 1-3 weeks after remission, involving mainly neurologic disease. Fatality ranges < 3% (Omsk) up to 10% (Kyasanur Forest). Survivors may experience neurologic complications after initial recovery.[2,28,29]

Dengue virus has not typically been considered a potential biological weapon threat, as it has not been shown in the laboratory to infect by aerosol. However, transmission by blood splashes in hospitals has occurred.

For a comparison of VHF agents and diseases see Appendix D.

Diagnosis

A VHF should be considered in anyone presenting with a severe, acute febrile illness and evidence of vascular instability (postural hypotension, petechiae, easy bleeding, facial/chest flushing, non-dependent edema), especially with appropriate epidemiologic risk. Signs/symptoms suggesting additional organ system involvement are common (headache, photophobia, pharyngitis, cough, nausea/vomiting, diarrhea, constipation, abdominal pain, hyperesthesia, dizziness, confusion, tremor) with variations noted under Clinical Features and Table 1. A positive tourniquet test has been useful in dengue hemorrhagic fever and may be of use in other hemorrhagic fevers.[2,22]

A detailed travel/exposure history and a high index of suspicion are essential in making the diagnosis. Patients with arenavirus or hantavirus infections often recall proximity to rodents or their droppings, but direct contact with the infected rodents is not necessary. Large mosquito populations are common during RVF, yellow fever, or dengue transmission, but mosquito bites are too common to be of diagnostic significance. Tick bites or nosocomial exposure may be significant in suspecting CCHF. A large outbreak among military personnel with VHF manifestations is possible in an endemic setting, but should also be considered a red flag for a potential bio-agent attack.[2,22]

The clinical laboratory can provide helpful information in presumptive diagnosis of VHF. Thrombocytopenia (exception: Lassa) and leukopenia (exceptions: Lassa, Hantaan, and CCHF) are the rule. Proteinuria and/or hematuria are common, and characteristic of AHF, BHF, and HFRS. AST elevation is nonspecific for, but correlates with, severity of Lassa fever; jaundice is a poor prognostic sign in yellow fever. Higher viral loads, renal failure, a high AST/ALT ratio (7-12 times higher AST), and low calcium (<6 mg/dl) appear to be poor prognostic factors for filoviral disease.[2,22,30]

In most geographic areas, malaria is the major differential diagnosis consideration; however, parasitemia alone in patients partially immune to malaria does not prove that clinical features are due to malaria. Other diseases in the differential diagnosis include typhoid, non-typhoidal salmonellosis,

leptospirosis, rickettsiae, shigellosis, relapsing fever, fulminant hepatitis, and meningococcemia. Non-infectious illnesses could mimic VHF, including acute leukemia, lupus erythematosus, idiopathic or thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, and multiple causes of DIC.[2]

Definitive diagnosis rests on specific virology diagnosis. Most patients have readily detectable viremia at presentation (exception: hantavirus infections). Rapid enzyme immunoassays can detect viral antigens in acute sera from patients with AHF, Lassa fever, RVF, CCHF, and yellow fever. Lassa- and Hantaan-specific IgM often are detectable during the acute illness. Lack of antibody production in response to filoviral infection is a poor prognostic sign. Diagnosis by virus replication and identification requires 3 to 10 days or longer. PCR assays for Ebola are widely available now, and a saliva test has been approved to supplement PCR testing. Assays for other VHFs have been developed at USAMRIID and the CDC, and they may be helpful in making a presumptive diagnosis when available. With the exception of dengue, specialized microbiological containment with appropriate precautions should be observed in collection, handling, shipping, and processing of diagnostic samples. Both the CDC (Atlanta, Georgia) and USAMRIID (Frederick, Maryland) have diagnostic laboratories at the BSL-4 or P-4 containment level, although other organizations around the U.S. now also have BSL-4 laboratories.[2,30]

Medical Management

General principles of supportive care apply to managing the hemodynamic, hematologic, pulmonary, and neurologic aspects of VHFs, regardless of the specific etiologic agent. Intensive care is required for the most severely ill patients. Healthcare providers should utilize vigorous fluid resuscitation of hypotensive patients judiciously, due to the propensity of some VHFs (e.g., HFRS, dengue) for pulmonary capillary leak. Vasoactive or inotropic agents are frequently required. The benefits of intravascular devices and invasive hemodynamic monitoring must be carefully weighed against the significant risk of hemorrhage. Restlessness, confusion, myalgia, and hyperesthesia should be managed by conservative measures, including the judicious use of sedatives and analgesics. Mechanical ventilation, renal dialysis, and anti-seizure therapy may be required. Secondary infections may occur as with any patient managed with invasive procedures and devices.[2] Management during recent outbreaks has frequently included treatment with antimalarials and broad spectrum antibiotics.

Management of the hemorrhagic component of VHFs is controversial but the approach mirrors that for any patient with a systemic coagulopathy. Aggressive treatment of mild bleeding in the absence of a definitive diagnosis of VHF is contraindicated. In cases of severe bleeding, red blood cells, platelets, and clotting factors should be replaced, guided by clinical indication and coagulation parameters. IM injections, aspirin, and other anticoagulant drugs should be avoided. Steroids are not indicated.[2]

The antiviral drug ribavirin is available for therapy of Lassa fever, HFRS, and CCHF under an IND protocol. A controlled clinical trial has clearly indicated that parenteral ribavirin reduces morbidity in HFRS. Several trials have suggested that it lowers both the morbidity and mortality of Lassa fever. In the HFRS field trials, treatment was effective if begun within the first 4 days of fever and continued for a 10-day course. The DOD (Force Health Protection Division, USAMMDA) has IND protocols for the treatment of VHFs with IV ribavirin. Because the supply of IV ribavirin is limited, oral ribavirin may be required in a mass-casualty situation. Oral ribavirin is licensed for the treatment of hepatitis C infection and is commercially available in the U.S. Because it is not approved for use in VHFs, it should only be used under an IND protocol or EUA. Dosing recommendations for IV and PO ribavirin are in Table 2. Side effects include modest reversible hemolytic anemia and bone marrow suppression. Ribavirin is teratogenic in lab animals, but no human data exists. Potential risks to the fetus must be weighed against the potential life-saving benefit in pregnant women with severe illness. Safety in infants and children has not been established for IV ribavirin, but inhaled ribavirin has been used extensively in the treatment of respiratory syncytial virus infection in infants. Ribavirin has poor *in vitro* and *in vivo* activity against the filoviruses (Ebola and Marburg) and the flaviviruses (dengue, yellow fever, OHF and KFD).[2]

AHF responds to therapy with two or more units of convalescent plasma containing adequate amounts of neutralizing antibody and given within 8 days of onset. BHF appears to respond to passive immune therapy as well. Convalescent serum or immune globulin for SAHFs is not readily available in the U.S. This therapy is investigational and should be given only in consultation with experts.[2]

A randomized controlled trial of four treatments for Ebola Zaire was conducted during the 2018-2020 DRC outbreak. Case fatality rates for two of the drugs (REGN-EB3, Mab114) demonstrated a decline by nearly half from the average of 65%, with further declines in patients who presented early in their disease course, less ill, or with lower viremia to fatality rates of 10-12%.[33] Both drugs are now licensed for treatment of Ebola Zaire under the trade names Inmazeb® and Ebanga™, respectively. Dosing recommendations are shown in **Appendix J: Biological Agent Prophylactics & Therapeutics** under Hemorrhagic Fever Viruses.

Prophylaxis

The 17D live attenuated yellow fever vaccine is licensed for prevention of yellow fever in travelers. A vaccine, Ervebo®, based on a recombinant vesiculostomatitis vector is licensed against Ebola Zaire. New vaccines are being tested against Marburg virus, with one incorporating a chimpanzee adenovirus vector used for healthcare workers in a 2024 outbreak in Rwanda. The *Candid 1 vaccine* for AHF is a live, attenuated, investigational vaccine developed at USAMRIID. It was highly efficacious in a randomized, controlled

trial in Argentine agricultural workers and it appears to protect against BHF in monkeys. Unfortunately, *Candid 1* is no longer manufactured and is not available in the U.S. Both inactivated and live-attenuated RVF vaccines are currently under investigation. At this time, there are no vaccines for the other VHF agents available for human use in the U.S. Several local vaccines for OHF, KFD, HFRS, and CCHF are used in their respective endemic areas, but these have not been rigorously studied.[2]

Persons with percutaneous or mucocutaneous exposure to blood, body fluids, secretions, or excretions from a patient with suspected VHF should immediately wash the exposed skin surfaces with soap and water and irrigate mucous membranes with copious amounts of water or saline solution.

Close personal contact or anyone, including medical personnel, exposed to blood or secretions from VHF patients (particularly Lassa fever, CCHF, and filoviral diseases) should be monitored for symptoms (fever and other signs) for the established incubation period. After a presumed bio-agent attack with an unknown VHF virus, any fever of 101ºF or greater should prompt evaluation and consideration for immediate treatment with IV ribavirin until the particular agent is determined. However, the utility of post-exposure, pre-symptomatic ribavirin prophylaxis is questionable. The DOD IND protocol for ribavirin therapy of CCHF and Lassa fever may allow for prophylactic treatment of exposed personnel, in consultation with protocol investigators. Most patients will tolerate this regimen well, but should be under surveillance for breakthrough disease (especially after drug cessation) or adverse drug effects (principally anemia).[2,22] Individuals with potential exposures to Ebola should be evaluated for consideration of either post-exposure vaccine (under IND) versus potential use of one of the licensed monoclonal antibody preparations, based on risk assessment.

Isolation and Decontamination

Some of these viruses pose special challenges for hospital infection control. Except for dengue and hantaviruses, VHF patients harbor significant levels of potentially infectious virus in blood, body fluids, or secretions. Special caution must be exercised in handling hypodermic needles and other sharps that could result in parenteral exposure. Strict adherence to standard and contact precautions will prevent nosocomial transmission in most cases.[2, 32] Lassa, CCHF, Ebola and Marburg viruses are particularly prone to nosocomial spread due to periods of high viremia corresponding with significant gastrointestinal fluid loss and bleeding propensity. Secondary infections among contacts and medical personnel without direct body fluid exposure have been documented. These have prompted concern of an atypical aerosol transmission of infection. In the 2024 Rwanda Marburg outbreak, most cases occurred in healthcare workers. Therefore, when a VHF is suspected, additional infection control measures are indicated, including isolation in a private room with an adjoining anteroom to be used for donning and doffing protective equipment,

storage of supplies, and decontamination of lab specimen containers. A negative-pressure room, with 6 to 12 air exchanges per hour, is ideal for any VHF patient and is strongly advised for those with significant cough, hemorrhage, or diarrhea. All persons entering the room should use standard and contact precautions (i.e., double gloves, impermeable gowns with leg/shoe coverings, eye protection, no uncovered skin) as well as HEPA (e.g., N-95) masks or powered, air-purifying respirators (PAPRs). Note, however, that person-to-person aerosol transmission of these viruses, if it occurs, is a rare phenomenon.[4,5,22] The CDC provides general guidance[32] and provides more stringent guidance for unstable patients and definite VHF infection versus stable and merely suspected cases.[41,42]

In the absence of a large, fixed MTF, or in the event of an overwhelming number of casualties, isolation rooms may not be available for all casualties. At a minimum, VHF patients should be cohorted in a separate building, or ward, with an air-handling system separate from the rest of the facility, when feasible. Access should be restricted to those required to care for the patients. Personnel should undergo external decon procedures upon exiting the contaminated patient-care area. A building, room or other dedicated area that is separated from the patient-care area should be established for donning and doffing protective gear. All waste (including linens) leaving the patient-care area should be decontaminated with bleach or quaternary ammonium compounds and double-bagged in clearly labeled biohazard waste bags. Ideally, this waste will be autoclaved or incinerated.[4,5,22]

Clinical specimens should be double-bagged, and the exterior of the outer bag decontaminated before transport to the lab. Excreta and other contaminated materials should be autoclaved or decontaminated by liberal application of appropriate disinfectants. Clinical lab personnel are at significant risk for exposure and should employ a biosafety cabinet (if available) with barrier and respiratory precautions when handling specimens. Point-of-care tests are ideal with appropriate precautions, when feasible; otherwise, clinical specimens should be handled in a designated, isolated space within the lab. Access to this space should be limited and thorough decontamination of the space and equipment should be routine.[4,22]

No carrier state has been observed for any VHF, but excretion of virus in urine or semen may occur for some time during convalescence. Rare cases of infection relapse (eye, meninges) in survivors have occurred post-infection, likely related to survival in protected spaces, such as the eye, brain, and testes.[34,35] Survivors should avoid sexual contact or use condoms for $\geq$ 3 months after recovery and preferably until such time as their semen tests negative. In fatal cases, there should be minimal handling of the remains, which should ideally be sealed in leak-proof material for prompt burial or cremation.[4,22]

BIOLOGICAL TOXINS

Introduction

Toxins are harmful substances produced by living organisms (animals, plants, or microbes). They are distinguished from chemical agents—such as VX, cyanide, or mustard—by the facts that they are extracted from natural sources and are more complex chemical structures or proteins. Toxins are similar to chemical agents, in that they may have rapid onset of symptoms after exposure (within hours) and have similar or higher toxicity by weight (*potency*). Most toxins are non-volatile and thus are unlikely to produce either secondary or person-to-person exposures, or to create a persistent environmental hazard.

Potency, stability, and ease of production determine a toxin's utility as an aerosol weapon. Bacterial toxins, such as botulinum neurotoxins, are some of the most toxic substances (by weight) known (Appendix G). The relationship between aerosol toxicity and the quantity of toxin required for an effective open-air exposure is shown in Appendix H: for some agents, such as ricin, very large (ton) quantities would be needed for an effective open-air attack in a dispersed tactical environment. Stability limits the open-air potential of some toxins, for example, botulinum and tetanus toxins are large-molecular-weight proteins that are easily denatured by environmental factors (such as adsorption to soils or degradation by UV light), thus limiting the downwind threat. Some toxins (e.g., certain botulinum serotypes) may be effective terrorist weapons when delivered by contamination of the food supply; endemic accidental exposures occur with regular frequency with some foodborne toxins (botulinum, SEB), and can be difficult to distinguish from a subtle attack. Finally, some toxins, might be both stable and highly toxic, but are present in low concentrations or are difficult to extract from natural sources and thus can only be produced in quantities insufficient for bioterrorism or biowarfare.

As with all bio-agents, the potential to cause incapacitation as well as lethality characterizes the threat. Depending upon the goals of an adversary, incapacitating agents may be more effective than lethal agents. Large numbers of ill patients might overwhelm the medical and evacuation infrastructure, may require specific medical treatment not normally available in hospitals on a large scale (e.g., mechanical ventilation, antitoxins), and will assuredly create panic and disruption in the affected community. Several toxins, such as Staphylococcal Enterotoxin B (SEB), pose a significant incapacitating threat by causing illness at doses much lower than those required for lethality.

A number of toxins have been weaponized by major state bio-weapons programs in the past. During the Cold War, the former U.S., UK, and USSR bio-warfare programs weaponized both botulinum toxins and SEB. In Iraq, in the 1980s, Saddam Hussein, expended great effort to weaponize botulinum and aflatoxin. The four toxins considered most likely to be used as bio-agents today are botulinum toxins, ricin, SEB, and T-2 mycotoxins; these are therefore the

ones discussed here. As natural products, new toxins are being consistently discovered and researched; some of these may pose future hazards through natural outbreaks from changing climate patterns or weaponization by terrorists or nation states.

BOTULINUM

Summary

Signs and symptoms: Symptoms are dose-dependent and typically emerge within 12 to 36 hours of exposure but may take days with low toxin doses. They often begin with minor visual changes or abdominal discomfort (in foodborne cases) and progress to neurologic manifestations, including ptosis (drooping eyelids), blurred vision, diplopia (double vision), dry mouth and throat, dysphagia (difficulty swallowing), and dysphonia (voice impairment). These symptoms may advance to symmetrical descending flaccid paralysis, generalized weakness, and ultimately progression to respiratory failure.

Diagnosis: Initial treatment and management of suspected botulism rely on clinical findings. A bio-agent attack should be suspected if multiple casualties simultaneously present with progressive descending flaccid paralysis. Laboratory diagnosis of botulism involves detecting botulinum toxin in serum, stool, or food samples using mouse bioassay, ELISA, or molecular assays. Isolation of *Clostridium botulinum* from stool or wound cultures can also confirm the diagnosis. As laboratory confirmation may take days, treatment should not be delayed in cases of high clinical suspicion. Electrodiagnostic studies like nerve conduction and electromyography are inconsistently reliable and often operationally challenging, limiting their diagnostic utility.

Treatment: Treatment includes supportive care, such as mechanical ventilation when needed, and prompt administration of equine derived botulinum antitoxin. *Heptavalent Botulism AntiToxin* (*BAT*) is a combination of antibodies to botulinum toxin types A-G, licensed for the treatment of symptomatic botulism in adults and children and may prevent or decrease progression to respiratory failure and hasten recovery after exposure to all serotypes of botulinum neurotoxin (BoNT). Botulism Immune Globulin (BabyBIG) is a preparation of human-derived antibodies specifically used to treat infantile botulism.

Prophylaxis: The pentavalent toxoid vaccine, previously used for protection against types A-E, was discontinued in 2011 due to potency loss.
While no replacement vaccine is available, several candidate vaccines are under investigation.

Isolation and decontamination: Standard precautions are recommended for healthcare workers. BoNT is not dermally active and secondary aerosols are not a hazard from patients. Decontaminate with soap and water. BoNTs are inactivated by sunlight in 1 to 3 hours. Heat (80^{O}C for 30 min, 100^{O}C for several min) and chlorine (>99.7% inactivation by 3 mg/L free available chlorine [FAC] in 20 minutes) also destroy BoNTs.

Overview

The botulinum neurotoxins (BoNTs) are a group of seven related proteins produced by the spore-forming bacillus *Clostridium botulinum* (Types A through G) and three other *Clostridium* species (*C. butyricum* [Type E], *C. baratii* [Type F], and *C. argentinense* [Type G]).[1] Botulinum toxin type H, discovered in 2017, is still under investigation to fully understand its structure and potential implications for human disease.[2] These toxins are the most potent neurotoxins known; paradoxically, they have been used therapeutically to treat spastic conditions (strabismus, blepharospasm, torticollis, tetanus) and cosmetically to efface wrinkles. The spores are ubiquitous; they germinate into vegetative bacteria that produce toxins during anaerobic incubation. Industrial-scale fermentation can potentially produce large quantities of toxin for use as a bio-agent. There are three epidemiologic forms of naturally occurring botulism—foodborne, intestinal (infant or adult intestinal), and wound botulism. BoNT could be delivered via aerosol or used to contaminate food or water supplies. The clinical syndrome (regardless of route of intoxication) produced by all these toxins is known as botulism.

History & Significance

BoNTs have caused numerous cases of botulism when ingested in improperly prepared or canned foods.[3] Many deaths have occurred from such incidents. Historical outbreaks of infant botulism have often been linked to environmental exposure or contaminated sources such as honey.[4] Historical outbreaks of wound botulism, primarily associated with traumatic injuries and more recently with intravenous drug use or black-tar heroin, highlight the role of Clostridium botulinum spores contaminating wounds and thriving in anaerobic conditions.[5] It is theoretically possible, although difficult, to deliver BoNTs as an aerosolized biological weapon.[6] Several countries and terrorist groups have weaponized BoNTs in the past.[1] BoNTs were weaponized by Imperial Japan (1930s), the U.S. (1940s-50s) in its now defunct offensive biowarfare program, and by the USSR. Evidence obtained by the UN in 1995 revealed that Iraq had filled and deployed over 100 munitions with nearly 10,000 liters of botulinum toxin.

Toxin Characteristics

BoNTs are proteins with a molecular mass of about 150,000 daltons.[2,6] Each of the seven toxin serotypes act to inhibit presynaptic acetylcholine release. The toxins produce similar effects when inhaled or ingested, although the time course may vary depending on the route of exposure and the dose received.

These large proteins are readily denatured by environmental conditions. They are detoxified in open air within 12 hours. Sunlight inactivates the toxins in 1 to 3 hours. Heat destroys the toxins in 30 minutes at 80^{O}C and in several minutes at 100° C. In water, the toxins are >99.7% inactivated by 20 minutes of exposure

to 3 mg/L free available chlorine (FAC) similar to the military disinfection procedures; and 84% inactivated by 20 minutes at 0.4% mg/L FAC, similar to municipal water treatment procedures.

Mechanism of Toxicity

BoNT consists of two polypeptide subunits (A and B chains). The B chain binds to receptors on the axons of motor neurons.[2] The whole toxin is transported into the axon, where the chains separate and the A chain exerts its cytotoxic effect by preventing release of acetylcholine (ACh) and blocking neuromuscular transmission (pre-synaptic inhibition). Recovery follows only after the neuron develops new axonal sprouts, a process which can take months. The presynaptic inhibition affects both autonomic (muscarinic) and motor (nicotinic) cholinergic receptors. This interruption of neurotransmission may affect cranial nerves and nerves innervating skeletal muscle (resulting in paralysis) and the autonomic nervous system (nonreactive and dilated pupils, constipation, dry mouth, orthostatic hypotension).

Unlike the situation with nerve agent intoxication, where there is too much ACh due to inhibition of acetylcholinesterase, the problem in botulism is lack of the neurotransmitter in the synapse. Thus, pharmacologic measures such as atropine are *not* indicated in botulism and could exacerbate symptoms (see **Appendix J: Biological Agent Prophylactics & Therapeutics**).

Clinical Features

The onset of symptoms of inhalational botulism usually occurs between 12 and 36 hours after exposure, but this is highly dose dependent.[7] Signs and symptoms may not appear for several days when a low dose of the toxin is inhaled compared to a mere matter of hours after ingestion or inhalation of higher doses. The early symptoms of botulism, such as mild visual disturbances or abdominal discomfort, can be easily confused with common illnesses, making botulism difficult to discern. Infant botulism may present with weak cry and inability to feed.[4]

The toxin effects lead to a descending paralysis signaled by cranial nerve palsies early in the disease state, with eye symptoms such as blurred vision due to mydriasis (dilated pupils), diplopia, ptosis, and photophobia, in addition to other cranial nerve signs such as dysarthria, dysphonia, and dysphagia.[8] Flaccid skeletal muscle paralysis follows, in a symmetrical, descending, and progressive manner. Collapse and obstruction of the upper airway may occur due to weakness of the oropharyngeal musculature. As the descending motor weakness involves the diaphragm and accessory muscles of respiration, respiratory failure may occur abruptly. Progression from onset of symptoms to respiratory failure has occurred in as little as 24 hours in cases of severe food-borne botulism.[3]

The autonomic effects of botulism are manifested by typical anticholinergic signs and symptoms: dry mouth, ileus, constipation, and urinary retention. Nausea and vomiting may occur as nonspecific sequelae of an ileus. Mydriasis is seen in approximately 50% of cases.[7]

Sensory symptoms usually do not occur. BoNT does not cross the blood/brain barrier and does not cause CNS disease. However, the psychological sequelae of botulism may be severe and require specific intervention.[8]

Physical examination usually reveals an afebrile, alert, and oriented patient, although the paralysis may limit the patient's ability to respond. Postural hypotension may be present. Mucous membranes may be dry and crusted and the patient may complain of dry mouth or sore throat. There may be difficulty with speaking and swallowing. Gag reflex may be absent. Pupils may be dilated and even fixed. Ptosis and extraocular muscle palsies may also be present. Variable degrees of skeletal muscle weakness may be observed depending on the degree of progression in an individual patient. Deep tendon reflexes may be diminished or absent. With severe respiratory muscle paralysis, the patient may become cyanotic or exhibit narcosis from CO_2 retention.

Diagnosis

The occurrence of an epidemic of afebrile patients with progressive symmetrical descending flaccid paralysis would strongly suggest botulinum intoxication. Food-borne outbreaks have most often occurred in small clusters. Higher numbers of confirmed cases in a theater of operations should at least raise the consideration of a bio-agent attack with BoNTs.

Individual cases might be confused clinically with other neuromuscular disorders such as Guillain-Barre syndrome, myasthenia gravis, or tick paralysis[7]. The edrophonium or *Tensilon*® test may be transiently positive in botulism, so it may not distinguish botulinum intoxication from myasthenia. The CSF in botulism is normal and the paralysis is generally symmetrical, which distinguishes it from enteroviral myelitis. Mental status changes generally seen in viral encephalitis should not occur with botulinum intoxication.

It may become necessary to distinguish nerve agent and/or atropine poisoning from botulinum intoxication.[8] Nerve agent poisoning produces copious respiratory secretions, miotic pupils, convulsions, and muscle twitching, whereas normal secretions, mydriasis, difficulty swallowing, and progressive muscle paralysis is more likely in botulinum intoxication. The clinical differences between botulinum intoxication and nerve agent poisoning are depicted in Appendix F.

Laboratory testing is generally not critical to the diagnosis of botulism. Botulism is foremost a clinical diagnosis, and lab results can be delayed and inconclusive.

Routine diagnostic tests, such as complete blood counts, cerebrospinal fluid (CSF) analysis, and radiologic studies, are typically normal in patients with botulism. Laboratory diagnosis of botulism involves detecting botulinum toxin in serum, stool, or food samples using mouse bioassay, ELISA, or molecular assays. Isolation of *Clostridium botulinum* from stool or wound cultures can also confirm the diagnosis.[7] Mouse neutralization (bioassay) remains the gold standard test; therefore, serum samples should be drawn and sent to a laboratory capable of performing this assay. PCR (polymerase chain reaction) is increasingly used as a diagnostic method for botulism. It can detect the genetic material of Clostridium botulinum and its toxin genes in clinical samples such as stool, wound, or food, providing a faster and more specific alternative to the mouse bioassay. That being said, PCR may not be as widely available or universally accepted as the detection of botulinum toxin itself. Detecting toxin in clinical or environmental samples is possible on various immunoassay platforms. Survivors do not usually develop an antibody response due to the very small amount of toxin necessary to produce clinical symptoms. Exposure does not confer immunity.

Electrodiagnostic studies have been described in the evaluation of botulism including nerve conduction studies, electromyography, and repetitive nerve stimulation. Each are inconsistently reliable and often operationally challenging, limiting their diagnostic utility.

Medical Management

Supportive care, including respiratory support, can be lifesaving. Respiratory failure due to paralysis of respiratory muscles is the most serious complication and primary cause of death.[6,7] Prior to 1950, the case fatality rate (CFR) was 60%, but with appropriate interventions (e.g., ventilation, botulinum immunoglobulin), CFRs are now under 5%. However, unrecognized cases may have higher fatality, and care must focus on preventing infections, managing hydration and bowel function, and preventing complications like decubitus ulcers and thromboses. Recovery may take up to a year, with intensive care required for several months.[9,10]

Antitoxins: Early administration of botulinum antitoxin is critical, as it neutralizes the circulating toxin in patients with symptoms that would continue to progress without it.[11,12] The antitoxin has no effect on toxin already bound to the nerve terminals, so antitoxin should *never* be withheld from the patient, even when treatment has been delayed.

Two different antitoxin preparations are available in the U.S. A bivalent human IV antiserum (types A and B, *BabyBIG*) was licensed in 2003 by the FDA and is available from the California Department of Health Services for treating infant botulism.[13] This purified immunoglobulin is derived from pooled adult plasma from persons who were vaccinated with pentavalent botulinum toxoid (see

below) and selected for their high titers of neutralizing antibody against botulinum neurotoxins type A and B.

HBAT (Heptavalent Botulinum Antitoxin) is an equine-derived antitoxin that targets all seven botulinum toxin serotypes (A-G).[11,12] It was developed by USAMRIID and later manufactured by Cangene Corporation, with FDA approval granted in 2013. HBAT is used for the treatment of non-infant botulism in adults and children. It is administered intravenously, with a slow infusion to monitor for allergic reactions, starting at 0.5 mL/min for the first 30 minutes, increasing to 2 mL/min if no reactions occur. The product is distributed by the CDC in the U.S. for emergency use.

Botulinum Antitoxin, Heptavalent, Equine, Types A, B, C, D, E, F, and G (*HE-BAT*) is also still available to the military under IND protocols. Use requires compliance with the experimental protocol, including skin testing to assess sensitivity to horse serum. Skin testing involves injecting 0.1 mL of a 1:10 dilution of antitoxin intradermally, monitoring for allergic reactions for 20 minutes. A positive skin test—indicated by symptoms like hyperemia, hypotension, or respiratory difficulty—prevents antitoxin administration. If no reaction occurs, the antitoxin is given intravenously over 20 minutes; desensitization can be attempted with escalating doses for those with a positive skin test. Ideally, desensitization would be performed by an experienced allergist. Medical personnel administering *HE-BAT* should ensure ready IV access and be prepared to treat anaphylaxis with epinephrine and intubation, if necessary.

Prophylaxis

Vaccine: The pentavalent toxoid (PBT) of *C. botulinum* toxin types A, B, C, D, and E which was previously administered as an IND for pre-exposure prophylaxis was discontinued on 30 November 2011 due to declining efficacy and an increasing rate of adverse events. Several vaccine candidates are under investigation including DNA-based, viral vector-based, and recombinant protein-based options, with DNA vaccines utilizing plasmids or viral vectors encoding BoNT heavy chain receptor binding domains.[14]

Antitoxin: There is no official indication at present for using a botulinum antitoxin as a prophylactic modality, except under extremely specialized circumstances. Post-exposure prophylaxis, using a heptavalent antitoxin, has been demonstrated effective in animal studies; however, as human data are not available, it is generally not recommended. This usage of heptavalent antitoxin may be considered after a known high-risk exposure to BoNT has occurred (e.g., a high-risk laboratory mishap) for all exposed, as an extraordinary measure.

RICIN

Summary

Signs and symptoms: Fever, chest tightness, cough, dyspnea, nausea, abdominal pain, anuria, headache and arthralgias may occur within 8 hours after inhalational exposure. Airway necrosis and pulmonary edema may occur within 18 to 24 hours, followed by Acute Respiratory Distress Syndrome (ARDS) and death from hypoxemia in 36 to 72 hours**.**

Diagnosis: Acute lung injury in multiple geographically clustered patients may suggest exposure to aerosolized ricin. Nonspecific lab and x-ray findings include leukocytosis and bilateral interstitial infiltrates. The rapid progression would be unusual for infectious agents. Serum and respiratory secretions should be submitted for antigen detection by immunoassay. Analysis of convalescent sera (detection of circulating anti-ricin antibodies) allows retrospective diagnosis of survivors after about two weeks.

Treatment: Supportive care and includes management of pulmonary edema. Gastric lavage and/or activated charcoal may be indicated for ricin ingestion to reduce toxin absorption (very narrow therapeutic window). Fluid and electrolyte management is indicated for any route of exposure; positive–pressure ventilation may be needed after aerosol exposure. Vasopressors should be used as needed for hypotension.

Prophylaxis: There is currently no licensed vaccine or prophylactic anti-toxin available for human use. A mutant recombinant RTA chain vaccine, *RiVax*, has been shown safe and immunogenic in humans in two phase 1 trials. A second recombinant RTA chain vaccine, *RVEc*, has shown promise in animal models and two phase 1 human trials.

Isolation and decontamination: Ricin patients are not contagious; standard patient precautions are appropriate. Ricin is non-volatile and secondary aerosols are not expected to be a hazard. Decontaminate with soap and water. Hypochlorite solution (0.1% sodium hypochlorite) and Reactive Skin Decontamination Lotion (RSDL) can inactivate ricin.

Overview

Ricin is a protein cytotoxin derived from the beans of the castor plant (*Ricinus communis*). Castor beans are ubiquitous in tropical and subtropical regions worldwide, and the toxin is fairly easy to extract. About two million metric tons of castor seeds are processed annually in the production of castor oil; the waste mash from this process is 3 to 5% ricin by weight. It is quite stable and extremely toxic by several routes of exposure. When inhaled as a small-particle aerosol, it may produce pathologic changes within hours and severe respiratory symptoms followed by acute hypoxic respiratory failure in 36 to 72 hours.[1]

The severity of intoxication by aerosolization is dependent on the particle size: the smaller the particle size, the further the toxin can travel into the lungs, causing damage to alveoli resulting in reduced blood oxygenation.[2] When ingested, ricin causes severe GI symptoms followed by vascular collapse, but rarely results in death.[3] IM injection causes induration and necrosis locally and, if sufficient toxin is absorbed systemically, may cause fever, nausea, vomiting, tachycardia, hypotension, leukocytosis, lymphoid necrosis, renal failure, hematemesis, liver failure, and death.[3,4] Ricin also causes disseminated intravascular coagulation, microcirculatory failure, and multiple organ failure when given IV in lab animals.

History & Significance

Ricin's significance as a potential bio-agent relates, in part, to its wide availability. During WWI, ricin dust clouds were considered as one method of dissemination while "W bombs" ("Compound W" was a code name for weaponized ricin) were produced, but never used, during WWII.[5,6] Ricin was suspected in the assassination of Bulgarian exile Georgi Markov in London in 1978.[4,5] In 1994 and '95, four men from a tax-protest group known as the "Minnesota Patriots Council," were convicted of possessing ricin and conspiring to use it to murder law enforcement officials. In 1995, a Kansas City oncologist, Deborah Green, attempted to murder her husband by ricin food contamination. In 1997, a Wisconsin resident, Thomas Leahy, was arrested and charged with possession with intent to use ricin as a weapon. In 2003, an envelope with a threatening note and a sealed container of ricin was found in a South Carolina postal facility, though no confirmed cases of ricin-associated illness were identified.[7] In April 2013, letters containing ricin were sent to Mississippi Senator Roger Wicker and the White House. James Everett Dutschke of Tupelo, Mississippi, was arrested and charged with the attempted use of a biological weapon. In June 2013 actress Shannon Richardson was arrested for allegedly sending letters containing ricin to New York City mayor Michael Bloomberg and President Barack Obama.[6] In addition to its ready availability and ease of extraction, these incidents have added to ricin's media notoriety and may have increased its appeal to would-be bioterrorists.

Toxin Characteristics

Ricin is a type II ribosome inactivating protein (RIP) made up of two polypeptide chains, an A chain and a B chain, which are joined by a disulfide bond.[8] Large quantities of low-purity ricin can be extracted from castor beans relatively easily and inexpensively, though producing high-purity ricin extract is significantly more technologically challenging. Ricin can be prepared in liquid or crystalline form, or it can be lyophilized to make a dry powder. It can be disseminated as an aerosol, injected into a victim, or used to contaminate food or water. Ricin is stable under typical ambient conditions, but is detoxified by heat exceeding 80^{O} C.[6] The active ingredient of Reactive Skin Decontamination Lotion (RSDL) has shown *in vitro* effectiveness in neutralizing ricin.[9] Ricin's

toxicity (LD_{50}) is marginal compared to other toxins, such as botulinum and staphylococcal enterotoxin B (SEB) (incapacitating dose). Estimates suggest that eight metric tons of ricin could achieve only a 50% casualty rate over an area of 100 km, significantly limiting ricin's battlefield utility.[2,10] Abrin, a ricin-like type II RIP found in the rosary pea plant (*Abrus precatorius*), can cause illness clinically similar to ricin, but is less of a concern for weaponization as the plant is less widely cultivated.[11]

Mechanism of Toxicity

Ricin's cytotoxicity is primarily due to inhibition of protein synthesis. The B chain binds to cell-surface receptors containing β-1,4-linked galactose residues and the toxin-receptor complex is endocytosed.[12] The A chain enzymatically inactivates ribosomes by depurinating ribosomal RNA (rRNA), inhibiting protein synthesis and activating cellular signaling pathways leading to apoptosis, inflammation, and tissue injury.[6,12] In rodents, the histopathology of aerosol exposure is characterized by necrosis of upper and lower respiratory epithelium, causing tracheitis, bronchitis, bronchiolitis, and interstitial pneumonia with perivascular and alveolar edema.[13] There is a several hour latent period after inhalational exposure before histologic lesions are observed in animal models. Ricin appears most toxic by inhalation compared to other routes.

Clinical Features

The clinical picture depends on the route of exposure. Although lethal human aerosol exposures have not been described, the severe pathophysiologic changes seen in the animal respiratory tract, including necrosis and severe alveolar flooding, were sufficient to cause death from ARDS and respiratory failure. Time to death in experimental animals is dose dependent, occurring 36 to 72 hours after inhalation.[14] Exposed humans can be expected to develop severe lung inflammation with progressive cough, dyspnea, cyanosis, and pulmonary edema.

By other routes of exposure, ricin is not a direct lung irritant; however, IV injection can cause minimal pulmonary perivascular edema due to vascular endothelial injury. Ingestion causes necrosis of the GI epithelium, local hemorrhage, and hepatic, splenic, and renal necrosis. Only 13 deaths have been recorded since the late 1880s out of 875 reported cases of accidental ingestion.[15] Ingestion of ricin is rarely lethal due to the degradation of the toxin by the low pH of the stomach acid. IM injection causes severe local necrosis of muscle and regional lymph nodes with moderate visceral organ involvement. Of six reported cases of intentional injection of ricin, five resulted in death.[15]

Diagnosis

An attack with aerosolized ricin would be primarily diagnosed by observation of the clinical features in the appropriate epidemiological context. Acute lung injury affecting a large number of geographically clustered cases should raise suspicion of an attack with a pulmonary irritant such as ricin, although other pulmonary agents could present with similar signs and symptoms. Other biological threats, such as SEB, Q fever, tularemia, plague, and chemical warfare agents like phosgene, need to be included in the differential diagnosis. Ricin-induced pulmonary edema would be expected to occur much later (1 to 3 days post-exposure) compared to that induced by SEB (about 12 hours post-exposure) or phosgene (about 6 hours post-exposure). Ricin intoxication will progress despite treatment with antibiotics, in contrast to an infectious process. Ricin intoxication does not cause mediastinitis as with inhalational anthrax. Ricin patients do not plateau clinically as with SEB intoxication. Additional supportive clinical or diagnostic features after aerosol exposure to ricin include the following: bilateral infiltrates on CXR, arterial hypoxemia, neutrophilic leukocytosis, and a bronchial aspirate rich in protein compared to plasma, which is characteristic of high-permeability pulmonary edema.

Specific immunoassays of serum and respiratory secretions, skin and/or nasal swabs, or immunohistochemical stains of tissue may be used where available to confirm the diagnosis. Due to the rapid cellular uptake and distribution of ricin, early detection is critical to patient care and survival.[16] Ricin has a biphasic half-life, an alpha and beta phase, limiting detection to 24 hours post-intoxication.[17] Biochemical methods and platforms are used for ricin detection using labeled, antibody-bound magnetic beads (Luminex MAGPIX), capture and detection antibodies (Handheld Assay Detection Devices and MSD PR2 model 1900), or by liquid chromatography/mass spectrometry (LC/MS) .[4]

PCR can be used to detect castor bean DNA in crude ricin preparations, which can be helpful in confirming ricin in suspicious white powder cases.

Ricin is an extremely immunogenic toxin, and paired acute and convalescent sera should be obtained from survivors to measure antibody response for retrospective confirmation.

Medical Management

There is no currently available direct antidote or medical countermeasure to ricin intoxication; good supportive care is the best available option. Management of ricin-intoxicated patients varies according to the exposure route. Patients with pulmonary intoxication are managed by the appropriate level of respiratory support (oxygen, intubation, ventilation, positive end-expiratory pressure [PEEP], and hemodynamic monitoring) and treatment for pulmonary edema, as indicated.

In ricin ingestion cases, gastric lavage may be useful to remove toxin within one hour of ingestion; activated charcoal should also be administered if not contraindicated due to airway protection or vomiting.[12,18,19] Volume and electrolyte replacement of GI fluid losses is important. In animal models, anti-ricin antibodies may mitigate the damage caused by ricin if administered quickly, but are not currently clinically available for human use.[20,21] Future directions for ricin therapeutics research includes monoclonal and polyclonal antibodies, vaccines, and small-molecule inhibitors of ricin enzymatic function and uptake into cells.[22]

Prophylaxis

The M-50 protective mask is effective in preventing aerosol exposure. Although a vaccine is not currently available, candidate vaccines are under development. USAMRIID developed a ricin toxin A (RTA) chain vaccine, *RVEc*™, that was well tolerated in human Phase I human trials, and immunogenic conferring protection against lethal aerosol exposures in animals.[22,23] The second vaccine candidate is another recombinant RTA chain, *RiVax* which has shown promise in animal models and appeared safe in Phase I human trials.[22] Pre-exposure prophylaxis with such vaccines is currently the most promising anticipated defense against a bio-warfare attack with ricin.

STAPHYLOCOCCAL ENTEROTOXIN B (SEB)

Summary

Signs and symptoms: SEB intoxication via ingestion begins within 1 to 12 hours and manifests as nausea, vomiting, abdominal cramps, and/or diarrhea that resolve within 24 to 48 hours. Inhalational intoxication manifests as sudden onset (after an asymptomatic latent period of 1.5 to 18 hours) of high fever, chills, headache, malaise, myalgia, and nonproductive cough. Some may develop shortness of breath and retrosternal chest pain. Symptoms tend to plateau quickly at a stable clinical state. Fever generally lasts 2 to 5 days, after which the other symptoms resolve. Pulmonary edema or ARDS may occur in severe cases, and delivery of high doses may result in toxic shock and death. Inhalational intoxication patients may also present with nausea, vomiting, and diarrhea, as well as upper respiratory tract symptoms (sore throat/hyperemic pharynx, rhinorrhea and/or sinus congestion), or conjunctival injection. GI symptoms are likely to be more profound if toxin is swallowed. Conjunctivitis and localized periocular swelling may occur after direct ocular exposure.

Diagnosis: is clinical, informed by epidemiological features. After aerosol exposure, patients present with a febrile illness and respiratory symptoms, but CXR is usually normal. Large numbers of patients presenting in a short time with typical symptoms and signs of SEB aerosol exposure suggest an intentional attack with this toxin. Foodborne intoxication would be suggested by several individuals presenting with GI symptoms within hours after ingestion of a common source food.

Treatment: Supportive. Artificial ventilation may be needed for very severe cases, and attention to fluid management is essential.

Prophylaxis: Protective mask. There is currently no human vaccine available.

Isolation and decontamination: Standard precautions are recommended for healthcare workers. Secondary aerosols are not a hazard. Ocular exposure to SEB (i.e., direct eye contact from contaminated hands) has resulted in SEB intoxication (conjunctivitis, local swelling). Dermal exposure to concentrated SEB solutions may cause dermatitis. Soap and water are recommended for decontamination of skin. SEB contaminated food should be destroyed. Direct sunlight likely accelerates decay of SEB, but the specific persistence (duration in hours/days) on surfaces is unknown.

Overview

Staphylococcus aureus produces many distinct exotoxins, one of which is staphylococcal enterotoxin B (SEB).[1-5] Such toxins are referred to as *exotoxins* since they are excreted from the organism. These toxins (a common cause of food poisoning due to improperly handled food) are also known as *enterotoxins* as they exert their effects mainly on the intestines if ingested.[6,7] SEB has been identified as a potential weapon of bio-terrorism as it is one of the more potent staphylococcal enterotoxins, and may result in significant morbidity after inhalation of low (nanogram) doses.[8] Inhalational SEB intoxication is manifested as a nonspecific febrile illness (sudden onset of high fevers, chills, myalgia, malaise, and cough) that may be associated with significant respiratory symptoms and result in incapacitation of most military personnel for 1 to 2 weeks.[8-10]

History & Significance

Staphylococcal enterotoxins are a common cause of food poisoning outbreaks.[11] These accidental intoxications often occur in a group setting or community event, and are due to improperly handled food and temperature holding, combined with ingestion of a common contaminated food source. Although an aerosolized SEB weapon would not likely produce significant fatalities, it could render most exposed personnel clinically ill and unable to perform their mission for 1 or 2 weeks.[9] The resulting demand on medical and logistical systems could be overwhelming. For these reasons, SEB was one of the seven bio-agents weaponized and stockpiled by the U.S. during its offensive bio-weapons program (1943-1969). SEB toxin could also be used to sabotage food or small-volume water supplies.

Toxin Characteristics

Staphylococcal enterotoxins are proteins ranging between 22 and 30 kilo-daltons molecular mass.[6] Up to 50% of clinical isolates of *S. aureus* produce exotoxins. They are produced in culture media and in foods when there is overgrowth of the bacterium. Related toxins include toxic-shock syndrome toxin-1 (TSST-1) and exfoliative toxins. SEB is one of several identified classes of antigenically distinct enterotoxins.[1-3,6,12] These toxins are moderately stable. They are resistant to inactivation by proteolytic enzymes in the GI tract, such as pepsin.[5] Staphylococcal enterotoxins are heat stable (may be heat resistant under various conditions of pH, salt concentration, media, and toxin purity).[5,10,13,14] SEB causes symptoms when inhaled at even very low (nanogram) doses in humans: a dose of several logs lower ($\geq$ 100 times less) than the lethal inhalational dose would be sufficient to incapacitate 50% of those exposed.[8,10] GI symptoms from SEB ingestion may occur with doses as low as 50 µg.[7] Dermal exposure to SEB (dose as low as 1 µg/cm^2) may cause dermatitis.[9,15,16] Persons exposed to SEB should decontaminate skin using soap and water for $\geq$ 15 minutes (irrigate eyes

for 15 minutes with water for ocular exposures). While sunlight may result in decay of SEB, the specific persistence (duration in hours or days) is unknown.[17]

Mechanism of Toxicity

Staphylococcal enterotoxins belong to a class of potent immune stimulants known as bacterial superantigens. Superantigens bind to major histocompatibility complex type II receptors on antigen-presenting cells, leading to the direct stimulation of large populations of T-helper cells while bypassing the usual antigen processing and presentation. This induces a brisk cascade of pro-inflammatory cytokines (such as tumor necrosis factor, interferon, interleukin-1 and interleukin-2), with recruitment of other immune effector cells, and relatively deficient activation of counter-regulatory negative feedback loops. This results in an intense inflammatory response that injures host tissues. Released cytokines are thought to mediate many of the toxic effects of SEB.[6,12,18-22]

Clinical Features

Symptoms of SEB intoxication usually begin after a latent period of 3 to 12 hours (range 1.5 to 18 hours) after inhalation, or 1 to 6 hours (range: 1 to 12 hours) after ingestion.[7,9] Symptoms depend upon the route of exposure. Ingestion results in predominantly GI symptoms: nausea, vomiting, abdominal cramps, and, less commonly, diarrhea.[6,7,11] Inhalation results in a non-specific febrile illness, characterized by the sudden onset of high fever (range: 103° to 105° F), chills, headache, malaise, myalgia, and cough; some patients may develop retrosternal chest pain and dyspnea.[9] Pulmonary edema or ARDS may occur in severe cases (attributed to activation of pro-inflammatory cytokine cascades in the lungs that leads to pulmonary capillary leak and pulmonary edema).[23] GI symptoms may also accompany respiratory exposure due to inadvertent swallowing of the toxin after normal mucocilliary clearance, or simply as a systemic manifestation. Upper respiratory symptoms (sore throat, rhinorrhea, sinus congestion, profuse postnasal drip) and conjunctival injection may develop in some patients.[9,16] Ocular exposure may result in localized purulent conjunctivitis, periorbital edema.[9,16] Dermal exposure to concentrated SEB solutions (including dermal patch tests containing SEB) may cause dermatitis (erythema, induration, and fine scaling of the skin).[9,15,16]

Symptoms from ingestion of SEB generally resolve in 24 to 48 hours. Fever, chills, prostration, and other symptoms due to inhalation generally last from 2 to 5 days, but a cough may persist for up to 4 weeks; patients may not be able to return to duty for 2 weeks.[9,18,24] Symptoms from ocular exposure generally resolve in 3 to 5 days.[9]

Physical examination of patients with SEB intoxication is often unremarkable. In inhalational intoxication, conjunctival injection or hyperemia of the pharynx may be present, and postural hypotension may develop due to fluid losses. Chest

examination is unremarkable unless pulmonary edema develops. CXR is usually normal, but severe cases may exhibit increased interstitial markings, atelectasis, and occasionally pulmonary edema or acute respiratory distress syndrome (ARDS). Leukocytosis is common, with WBC counts often ≥10,000 cells/mm^3 (range: 8,000 to 29,000 cells/mm^3).[9,18]

Diagnosis

Diagnosis of SEB intoxication is based on clinical and epidemiologic features. Because the symptoms of inhalational SEB intoxication resemble respiratory pathogens including influenza, adenovirus, and mycoplasma, the diagnosis may initially be unclear. All these illnesses might present with fever, nonproductive cough, myalgia, and headache. The presence of leukocytosis and upper respiratory tract findings in SEB intoxication may further contribute to misdiagnosis as an infectious process.[9] A SEB attack would result in an onset of illness in most cases within a single 24 hour period. Influenza or community-acquired pneumonia should involve patients presenting over a more prolonged interval. Symptoms of SEB intoxication tends to plateau rapidly to a stable clinical state, whereas inhalational anthrax, tularemia pneumonia, or pneumonic plague would all continue to progress if left untreated. Tularemia, plague, and Q fever are often associated with infiltrates on CXR, unlike SEB intoxication. The initial differential diagnosis may also include hantavirus pulmonary syndrome or inhalation of various chemical agents (mustard, phosgene) or other bio-toxins. Naturally occurring staphylococcal food poisoning does not present with pulmonary symptoms.

Lab confirmation of SEB intoxication includes immunological antigen detection assays (immunochromatographic lateral flow assays [hand-held devices], enzyme-linked immunosorbent assays [ELISA], electrochemiluminescence [ECL] assays, and time-resolved fluorescence [TRF] assays) on environmental and clinical samples, and gene amplification (PCR, to detect staphylococcal genes) on environmental samples.[25-37] SEB has also been detected using reverse passive latex agglutination assays, radioimmunoassays, immunoblotting, mass spectrometry, macroarray systems, and biosensor-based techniques (i.e., surface plasmon resonance detection).[38-46] While it has been detected in the serum of four ICU patients, successful detection in the serum is uncommon.[47] Studies in mice have detected low levels (range: 45 to 100 ng/ml) in the serum within 2 hours after intranasal challenge and up to 36 hours post-challenge.[32] The toxin was cleared rapidly from the serum, and was detected in the urine for several hours post-exposure.[32] Therefore, serum and urine specimens to assess for SEB should be obtained as early as possible after inhalational SEB exposure. Respiratory secretions and nasal swabs (within 24 hours of exposure) to assess for SEB may also be obtained.[18]

Medical Management

Currently, therapy is limited to supportive care. Individuals with inhalational exposure to SEB should be closely monitored for signs of respiratory compromise or hypotension. Oxygen supplementation should be provided, if clinically indicated. Mechanical ventilation or vasopressors may be required in severe cases.[18] Fluid support may be required in SEB intoxication with severe GI symptoms (nausea, vomiting, diarrhea) or shock. Acetaminophen (for fever and myalgias), cough suppressants, and antiemetics should be employed, as needed. Most patients with SEB inhalational intoxication improve within 5 days after the onset of illness, but will likely be unfit for duty for 1 to 2 weeks. FDA-approved immunosuppressants (including the steroid dexamethasone) have shown efficacy in animal trials in reducing mortality from SEB; however, their use in treating human exposure cases would be off-label.[48]

Prophylaxis

Because of the rapidity of SEB binding with MHC Class II receptors (<5 min *in vitro*), active vaccination is considered the most practical defense. There is currently no approved human vaccine to prevent SEB intoxication. In animal studies, vaccine candidates have demonstrated protection against SEB challenges.[49-53] A recombinant attenuated mutant SEB vaccine candidate demonstrated safety and immunogenicity in a Phase I human trial.[54] Experimentally, passive immunotherapy can reduce fatalities in animals, but only if given within 4 to 8 hours after inhalation or immediately post-challenge to within 4 hours after intra-peritoneal challenge.[55-60] Interestingly, many healthy persons may have detectable antibody titers to SEB and other staphylococcal superantigens through natural exposure. While these antibodies might provide some protection during *S. aureus* septicemia, it is not known if these naturally acquired antibodies would provide any protective effect against aerosol SEB exposure.[61,62]

T-2 MYCOTOXINS

Summary

Signs and symptoms: Exposure causes skin pain, pruritus, redness, vesiculation, necrosis, and sloughing of the epidermis. Effects on the airway include nose and throat pain, nasal discharge, itching and sneezing, cough, dyspnea, wheezing, chest pain, and hemoptysis. Similar effects occur after ingestion or eye contact. Severe intoxication results in weakness, ataxia, collapse, prostration, shock, and death.

Diagnosis: Suspect mycotoxin if an aerosol attack occurs in the form of "yellow rain" with droplets of variously pigmented oily fluids contaminating clothes and the environment, especially if field tests for vesicant chemical agent are negative. No rapid diagnostic test for mycotoxins is available for clinical field use. Confirmation requires lab-based testing of blood, tissue, or environmental samples.

Treatment: No specific antidote; treatment is supportive. Soap and water washing, even 4-6 h after exposure, can significantly reduce dermal toxicity; washing within 1 h may prevent toxicity entirely. Reactive Skin Decontamination Lotion (RSDL) skin decontamination kit should be used if available. Superactivated charcoal should be given orally if the toxin is swallowed.

Prophylaxis: The only defense is to prevent exposure by wearing a protective mask and clothing (or topical skin protectant) during an attack. No specific immunotherapy or chemotherapy is available for use in the field.

Isolation and decontamination: Outer clothing should be removed and exposed skin decontaminated with soap and water. Eye exposure should be treated with copious saline irrigation. Secondary aerosols are not a hazard; however, direct contact with contaminated skin or clothing can produce secondary dermal exposures. Contact precautions are warranted until decontamination is completed. After decontamination, standard precautions are recommended for healthcare workers. A 3-5% solution of sodium hypochlorite should be used for environmental decontamination.

Overview

Mycotoxins are metabolites of fungi produced through secondary biochemical pathways. *Trichothecene* compounds are one of several classes of mycotoxins, which also include the aflatoxins, rubratoxins, ochratoxins, and fumonisins. Trichothecenes are a large family of chemically related metabolites produced by a variety of molds and are important for their effect on crops. *T-2 mycotoxins* are trichothecenes also produced by several mold species; they are low-molecular-weight compounds that are resistant to heat and UV light thus rendering them extremely stable in the environment. Unlike other biological

toxins—and unlike the propagating bio-agents—T-2 mycotoxins are potent dermal irritants.[1] Delivered in a sufficient dose, they can cause severe skin, and potentially systemic, reactions. In an intoxicated human or animal, they are rapidly metabolized to HT-2, T2-triol, and T-2 tetraol within hours of exposure. Possible dermal, ocular, respiratory, and GI exposures, and their characteristic signs and symptoms, should be anticipated after an aerosol attack with mycotoxins.[2-4]

History & Significance

The potential for T-2 mycotoxin use as a weapon was demonstrated during and after World War II in Orenburg, Russia when >10% of the civilian population was affected after ingesting bread made with wheat flour unintentionally contaminated with the common mold *Fusarium*.[5] Some developed a protracted, ultimately fatal, illness christened as alimentary toxic aleukia (ATA) and characterized initially by abdominal pain, diarrhea, vomiting, prostration, and within days, fever, chills, myalgias and bone marrow depression with granulocytopenia and secondary sepsis. Survival beyond this point was accompanied by the development of painful pharyngo-laryngeal ulcerations and diffuse bleeding into the skin (petechiae, then ecchymoses), melena, hematochezia, hematuria, hematemesis, epistaxis, and vaginal bleeding. Pancytopenia and GI ulceration/erosion developed secondary to the profound depression of bone marrow and mucosal protein synthesis and to cell-cycle progression through DNA replication.

Owing to their environmental stability and dissemination potential, it was realized that the T-2 mycotoxins could be weaponized. Controversy still prevails over the “yellow rain” incidents where mycotoxins allegedly were released from aircraft by the Soviet Union and its allies during the conflicts in Laos (1975-81), Cambodia (1979-81), and Afghanistan (1979-81).[6] It was estimated that more than 6,300, 1,000, and 3,042 deaths resulted in those three countries, respectively.[7] The victims included both unarmed civilians and guerrilla forces. These groups were not protected with gas masks or chemical protective clothing. The attacks supposedly occurred in remote jungle areas, which made definitive confirmation of reports and recovery of agent extremely difficult. Some authorities have asserted that the “yellow clouds” were, in fact, bee feces produced by swarms of the migrating insects.[8] This theory failed to account for the reported deaths and injuries. Much of the debate centered upon the veracity of eyewitness and victim accounts, but there is evidence to consider serious allegations of biological (or chemical) weapon use.[9-12] A recent history of the Soviet biological weapons program indicates that there is evidence of Soviet-era offensive mycotoxin weapons research and development.[13]

According to UNSCOM, Saddam Hussein is known to have produced, weaponized, and stockpiled the mycotoxin known as aflatoxin in Iraq, by the end of the 1980s.[14,15]

Trichothecene mycotoxin accidental exposures have typically involved ingestion of contaminated foodstuffs. Fatal pulmonary hemorrhages in infants occurring in the U.S. state of Ohio 1993-1996 raised suspicion that the cause may have been due to such exposure in homes secondary to mold overgrowth.[16] Cases of sudden infant death syndrome (SIDS) have been attributed to *Stachybotrys* mycotoxin exposure in homes secondary to mold overgrowth resulting from flooding.[17]

Toxin Characteristics

The trichothecene mycotoxins are low-molecular-mass (250-500 daltons) non-volatile compounds produced by filamentous fungi (molds). The structures of approximately 150 trichothecene derivatives have been described and are produced by more than 350 species, most notably of the genera *Fusarium*, *Myrotecium, Trichoderma,* and *Stachybotrys*.[18] These substances are relatively insoluble in water but are highly soluble in organic solvents such as acetone, ethanol, methanol and propylene glycol. Trichothecenes can vaporize when heated in organic solvents. Extraction of these mycotoxins from fungal cultures yields a yellow-brown liquid that evaporates into a yellow greasy crystalline product (some speculate this to be the substance found in "yellow rain"). T-2 mycotoxin is unusual among the bio-agents in that systemic toxicity can result from any of the major routes of exposure—transdermal, oral, or inhalational.

The trichothecenes are extremely stable and resistant to heat and UV light inactivation. They retain their bioactivity even when autoclaved; heating to 900° F for 10 minutes or 500°F for 30 minutes is required for inactivation. A 3-5% solution of sodium hypochlorite is effective for inactivating T-2 mycotoxins and the efficacy can be further enhanced with the addition of small amounts of alkali.[19] The U.S. Army's decontaminating agents DS-2 and Supertropical bleach inactivate T-2 toxin within 30 to 60 minutes. In lab animals, washing contaminated skin with soap and water within 4 to 6 hours removed 80-98% of the toxin, which prevented dermal lesions and death.

Mechanism of Toxicity

Trichothecenes are potent inhibitors of protein synthesis and have a pronounced effect on actively proliferating cells, such as those found in the skin, GI tract, and bone marrow. Because this cytotoxic effect mimics the hematopoietic and lymphoid effects of radiation sickness, the mycotoxins are referred to as "radiomimetic agents." T-2 mycotoxins interfere with peptidyl transferase activity and inhibit either the initiation or elongation process of translation. The mycotoxins also alter cell membrane structure and function, inhibit mitochondrial respiration, and inactivate certain enzymes. Recent molecular studies suggest that T-2 mycotoxins also induce apoptosis (programmed cell death) through a reactive oxygen species–mediated mitochondrial pathway.[20] It is estimated that T-2 mycotoxin is about 400 times more potent in producing skin injury than mustard.[21]

Clinical Features

Clinical signs, symptoms, and severity will vary depending on the route of exposure, duration of exposure (acute, subacute, chronic), toxin concentration, and total dose. In a bio-warfare attack, the toxin or toxins could adhere to and penetrate the skin, be inhaled, or be ingested. In the alleged yellow rain incidents, symptoms of exposure from all three routes seemed to coexist. Contaminated clothing may serve as a reservoir for further (secondary) toxin exposure. Early symptoms beginning within minutes of exposure include burning skin pain, redness, tenderness, blistering, and progression to skin necrosis with eventual leathery blackening and sloughing of large areas of skin. Upper respiratory exposure may result in nasal itching, pain, sneezing, epistaxis, and rhinorrhea. Pulmonary and trachea-bronchial toxicity would produce dyspnea, wheezing, and cough. Mouth and throat exposure could cause pain and blood-tinged saliva and sputum. Anorexia, nausea, vomiting, and watery or bloody diarrhea with cramps and abdominal pain will likely occur with ingestion. Eye pain, tearing, redness, foreign body sensation, and blurred vision may follow ocular exposure. Skin symptoms occur in minutes to hours and eye symptoms in minutes. Systemic toxicity can occur via any route of exposure, and results in weakness, prostration, dizziness, ataxia, and loss of coordination. Tachycardia, hypothermia, and hypotension follow in severe cases. Death may occur in minutes, hours, or days. The most common symptoms are vomiting, diarrhea, skin involvement with burning pain, redness and pruritus, rash or blisters, bleeding, and dyspnea. A late effect of systemic absorption is pancytopenia, predisposing to bleeding and sepsis.

No human mortality or morbidity data have been reported for T-2 mycotoxin use as a bioweapon. Information regarding fatalities from the few instances of accidental ingestion of contaminated food is quite varied, with 10 to 60% case fatality rates reported in Russia's Orenburg district in the 1940s.

Diagnosis

Clinical and epidemiological findings provide clues to the diagnosis. High attack rates, dead animals of multiple species, along with physical evidence such as yellow, red, green, or other pigmented oily liquids, suggest mycotoxin exposure. Rapid onset of symptoms in minutes to hours supports a diagnosis of a chemical or toxin attack. In addition, the coexistence of cutaneous, ocular, respiratory, and GI symptoms may support the suspicion of mycotoxin exposure. Mustard and other vesicant agents must be considered, especially if there is a distinctive odor and visible residue; rapid detection is by a field chemical test (M8 paper, M256 kit). Symptoms of mustard toxicity are also delayed for several hours after exposure. Inhalation of SEB or ricin aerosols can cause fever, cough, dyspnea, and wheezing, but does not affect the skin.

There are several commercial immunoassay kits on the market that detect trichothecene mycotoxins in grain and feed, but no data exist to differentiate the

expected environmental background levels of these substances from potential toxic and/or intentional contamination. Unfortunately, no rapid diagnostic test is currently available for field clinical use. Serum and urine should be collected and sent to a reference laboratory for antigen detection. The mycotoxins and their metabolites are eliminated in urine and feces; 50-75% is eliminated within 24 hours; however, metabolites can be detected as late as 28 days after exposure. Pathologic specimens yielding diagnosis may include blood, urine, lung, liver, and stomach contents. Environmental and clinical samples can be tested using gas chromatography (GC), a high-performance liquid chromatography (HPLC)-mass spectrometry (MS) combination technique, or various enzyme-linked immunosorbent assay (ELISA) techniques. GC-MS and HPLC-MS are the preferred and most sensitive methods for detecting mycotoxins. This system can detect as little as 0.1-1.0 parts per billion of T-2, which is sensitive enough to measure T-2 levels in the plasma of toxin victims.

Medical Management

No specific antidote or therapeutic regimen is currently available. All therapy is supportive. If a soldier is unprotected during an attack, the outer uniform should be removed as soon as possible. The skin should be thoroughly washed with soap and water. This may reduce dermal toxicity, even if delayed for 4 to 6 hours after exposure.[22] (Contaminated clothing as well as wash waste from the decontamination process should be exposed to bleach [5% sodium hypochlorite] for 6 h or more to neutralize any residual mycotoxin.) The RSDL skin decontamination kit can also be used to remove skin-adherent T-2.

Treatment for cutaneous involvement will resemble standard burn care. The eyes should be irrigated copiously with normal saline or water to remove toxin if eye pain or tearing is apparent. Standard therapy for poison ingestion, including the use of superactivated charcoal to absorb swallowed T-2 toxin, should be administered to victims of an unprotected aerosol attack. Some have advocated activated charcoal use even after inhalational exposure as the toxin that is adherent to the oral mucosa may thus be bound.[23] Respiratory support may be necessary. Serial lymphocyte count may identify patients who will become immunocompromised. For systemic intoxication, some survival benefit was seen with administration of dexamethasone, diphenhydramine, naloxone, methylthiazolidine-4-carboxylate, metoclopramide, magnesium sulfate, and sodium bicarbonate in animal studies. No similar studies have been conducted in humans. Likewise, the utility of administering colony-stimulating factors to patients presenting with bone marrow suppression in this context is purely theoretical.

Prophylaxis

Physical barrier protection of the skin, mucous membranes, and airway (use of HAZMAT suits or chemical protective mask and clothing, such as MOPP gear) are the only practical effective methods of protection during an attack. The Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), has been shown to block dermal irritation in animal studies and can be applied at closure points of chemical over-garments as well as to any skin-exposed areas.[24] It is FDA-approved for use against dermally active toxins. RSDL acts by a combination of physical removal and nucleophilic breakdown, which renders the original toxic substance (chemical or biological) non-toxic.[25]

Candidate immunologic products (vaccines and monoclonal antibodies), and chemo-protective pre-treatments, are being studied in animal models, but are not available for field use.

Appendix A: Glossary of Medical Terms & Acronyms

This glossary is a list of medical terms and bio-defense acronyms used in this book. Some entries were adapted from: Stedman's Electronic Medical Dictionary *(Williams & Wilkins, Baltimore, MD, 2006) and Mandell et al,* Principles and Practice of Infectious Diseases *(7th Edition, Churchill Livingstone, 2009). Any omissions were accidental.*

Acetylcholine (ACH, Ach) – The neurotransmitter substance at cholinergic synapses, which causes cardiac inhibition, vasodilation, gastrointestinal peristalsis, and other parasympathetic effects. It is liberated from preganglionic and postganglionic endings of parasympathetic fibers and from preganglionic fibers of the sympathetic as a result of nerve injuries, whereupon it acts as a transmitter on the effector organ; it is hydrolyzed into choline and acetic acid by acetylcholinesterase before a second impulse may be transmitted.
ACIP – Advisory Committee on Immunization Practices; Overseen by the CDC.
Active vaccination –The act of artificially stimulating the body to develop antibodies against infectious disease by the administration of vaccines or toxoids.
Adenopathy – Swelling or morbid enlargement of the lymph nodes.
AF – Air Force.
AFMAN – U.S. Air Force Manual.
AHF – Argentine hemorrhagic fever, a VHF.
AIDS – Acquired Immunodeficiency Syndrome.
AIGIV – Anthrax Immune Globulin, Intravenous.
Airborne precautions – *See* Transmission-based precautions.
Aleukia – Absence or extremely decreased number of leukocytes in the circulating blood.
ALP – Alkaline phosphatase.
ALT – Alanine aminotransferase, a liver enzyme.
AM – Morning (Latin, *ante meridiem*).
A.M.P.L.E. – Pneumonic for a bio-agent medical history: Allergies/Arthropods, Medications/MOPP status, Past medical history (travel, vaccine, occupational), Last meal, Expose (decon).
Analgesic – 1. A compound capable of producing analgesia, i.e., one that relieves pain by altering perception of nociceptive stimuli without producing anesthesia or loss of consciousness. 2. Characterized by reduced response to painful stimuli.
Anaphylaxis – The term is commonly used to denote the immediate, transient kind of immunologic (allergic) reaction characterized by contraction of smooth muscle and dilation of capillaries due to release of pharmacologically active substances (histamine, bradykinin, serotonin, and slow-reacting substance), classically initiated by the combination of antigen (allergen) with mast cell-fixed, cytophilic antibody (chiefly IgE).
Anderson's Fallacy – **Belief that only hummingbirds have rapid heart rates.**
Anticonvulsant – An agent that prevents or arrests seizures.
Antigen – Any substance that, as a result of coming in contact with appropriate cells, induces a state of sensitivity or immune responsiveness and that reacts in a demonstrable way with antibodies or immune cells of the sensitized subject in vivo or in vitro.
Antitoxin – An antibody formed in response to and capable of neutralizing a biological poison; a serum prepared from animals vaccinated against a specific toxin.
AR – Army Regulation.
ARDS – Acute Respiratory Distress Syndrome.
Arthralgia – Severe pain in a joint, especially one not inflammatory in character.
ASAP – As soon as possible.
ASD(HA) – Assistant Secretary of Defense for Health Affairs.
AST – Aspartate aminotransferase, a liver enzyme.
Asthenia – Weakness or debility.
Ataxia – An inability to coordinate muscle activity during voluntary movement, so that smooth movements occur. Most often due to disorders of the cerebellum or the posterior columns of the spinal cord; may involve the limbs, head, or trunk.
Atelectasis – Decrease or loss of air in all or part of the lung, with resulting loss of lung volume itself.
ATLS – Advanced Trauma Life Support.
OASD(NCB) – Office of the Assistant Secretary of Defense for Nuclear, Chemical, and Biological Defense Programs.

Atropine – An anticholinergic, with diverse effects (tachycardia, mydriasis, cycloplegia, constipation, urinary retention) attributable to reversible competitive blockade of acetylcholine at muscarinic type cholinergic receptors; used in the treatment of poisoning with organophosphate insecticides or nerve gases.
Augmentin - A formulation of ampicillin and clavulanic acid.
AVA – Anthrax Vaccine Adsorbed.
BDO – Battle dress overgarment.
BHF – Bolivian Hemorrhagic Fever, a VHF.
BID or bid – Twice each day.
BIDS – Biological Integrated Detection System.
Bilirubin – A yellow bile pigment formed from hemoglobin during normal and abnormal destruction of erythrocytes. Excess bilirubin is associated with jaundice.
Bio-agent – Biological agent (*q.v.*); biological threat agent.
Biocontainment – In laboratory biosafety, is the physical containment of highly pathogenic organisms or agents (bacteria, viruses, and toxins), usually by isolation in environmentally and biologically secure cabinets or rooms, to prevent accidental infection of workers or release into the surrounding community during scientific research. Often confused with "isolation" and "quarantine".
Biological agent – A bacterium, virus, protozoan, parasite, fungus, or toxin that can be used purposefully as a weapon in bio-terrorism or biological warfare; biological threat agent, bio-weapon, or bio-agent.
Bio-toxin – See toxin.
Bio-surveillance – The gathering, analysis and interpretation of data related to disease activity and threats to human and animal health to achieve early warning, detection and situational awareness (DoD definition).
Blood agar – A mixture of blood and nutrient agar used for the cultivation of many medically important microorganisms.
BMR – Biological response modifier.
BoNT – Botulinum neurotoxin.
BRM – Biological response modifier.
Bronchiolitis – Inflammation of the bronchioles often associated with bronchopneumonia.
Bronchitis – Inflammation of the mucous membrane of the bronchi.
Brucella – A genus of encapsulated, nonmotile bacteria (family Brucellaceae) containing short, rod-shaped to coccoid, gram-negative cells. These organisms are parasitic, invading all animal tissues and causing infection of the genital organs, the mammary gland, and the respiratory and intestinal tracts, and are pathogenic for humans and various species of domestic animals. They do not produce gas from carbohydrates.
BSAT – Biological Select Agent or Toxin (see Appendix C).
BSL – Bio-safety level.
BSV – Bio-surveillance (*q.v.*).
BTRP – Biological Threat Reduction Program.
Bubo – Inflammatory swelling of one or more lymph nodes, usually in the groin; the confluent mass of nodes usually suppurates and drains pus.
Bulla, gen. and pl. bullae – A large blister greater than 1 cm in diameter appearing as a circumscribed area of separation of the epidermis from the subepidermal structure (subepidermal *bulla*) or as a circumscribed area of separation of epidermal cells (intraepidermal *bulla*) caused by the presence of serum, or occasionally by an injected substance.
BW – Biological warfare; bio-warfare (less commonly, biological weapons).
BWAS – Biological Weapons Agent Sampling [kit].
BWC – Biological Weapons Convention (1972, 1975).
C – Celsius or centigrade.
CA – California.
CADS – Chemical Agent Decon Set.
Carbuncle – Deep-seated pyogenic infection of the skin and subcutaneous tissues, usually arising in several contiguous hair follicles, with formation of connecting sinuses; often preceded or accompanied by fever, malaise, and prostration.
Case Fatality Rate (CFR) – The proportion or percentage of deaths within a designated population of people with a particular disease, over the course of the disease. (*Cf.* mortality rate.)
CBC – Complete blood count.

CBDP – The DoD's Chemical and Biological Defense Program.
CBEP – Cooperative Biological Engagement Program.
CBRN(E) – Chemical, biological, radiological, and nuclear (and explosives).
CCHF – Crimean-Congo hemorrhagic fever; a viral hemorrhagic fever.
CDC – U.S. Centers for Disease Control and Prevention, Atlanta, Georgia.
cDNA – complementary DNA; DNA synthesized from a messenger RNA (mRNA) template in a reaction catalyzed by the enzyme reverse transcriptase.
Cerebrospinal – Relating to the brain and the spinal cord.
CF – Complement fixation.
Cf – Latin *confer*, meaning "compare" or "consult".
CFR – Case Fatality Rate (*q.v.*); also Code of Federal Regulations.
CFS – Chronic Fatigue Syndrome.
Chemoprophylaxis – Prevention of disease by the use of chemicals or drugs.
Cholinergic – Relating to nerve cells or fibers that employ acetylcholine as their neurotransmitter.
CJCS – Chairman of the Joint Chiefs of Staff.
Cipro – Ciprofloxicin, a flouroquinolone antibiotic.
Cm(s) – Centimeter(s).
CMV – Cytomegalovirus.
CNS – Central nervous system.
Coagulopathy – A disease affecting the coagulability of the blood.
Coccobacillus – A short, thick bacterial rod of the shape of an oval or slightly elongated coccus.
Conjunctiva, pl. conjunctivae – The mucous membrane investing the anterior surface of the eyeball and the posterior surface of the lids.
Contact precautions – *See* Transmission-based precautions.
CONUS – Continental United States.
CPO – Chemical protective overgarment.
CPT – Current Procedural Terminology; maintained by the American Medical Association.
CSF – Cerebrospinal fluid.
CSTARS – Center for Sustainment of Trauma and Readiness Skills (Associated with USAFSAM).
CT – Computed tomography.
CTR - DTRA's Cooperative Threat Reduction program.
Cutaneous – Relating to the skin.
CW – Chemical warfare.
CXR – Chest X-ray; chest radiograph.
Cyanosis – A dark bluish or purplish coloration of the skin and mucous membrane due to deficient oxygenation of the blood, evident when reduced hemoglobin in the blood exceeds 5 g per 100 ml.
D or d – Day(s).
DARPA – Defense Advanced Research Projects Agency.
Decon – Decontamination.
DEOC – The CDC Director's Emergency Operations Center.
DFA – Direct fluorescence antibody or direct immunofluorescence immunoassay (*see* Fluorescent antibody).
DFU – Dry filter unit.
DHHS – United States Department of Health and Human Services; Oversees FDA, CDC, etc.
DHS – United States Department of Homeland Security.
Diathesis –The constitutional or inborn state disposing to a disease, group of diseases, or metabolic or structural anomaly.
DIC – Disseminated intravascular coagulation.
Diplopia –The condition in which a single object is perceived as two objects. SYN: double vision.
Disinfection – Application of a **disinfectant** (antimicrobial chemical agent) to non-living objects to destroy surface microorganisms; does not necessarily constitute **sterilization** (*q.v.*), especially as resistant bacterial spores may survive.
Distal – Situated away from the center of the body, or from the point of origin; specifically applied to the extremity or distant part of a limb or organ.
DNA – Deoxyribonucleic acid.
DoD –United States Department of Defense.
DODI – DoD Instruction.
DODD – DoD Directive.
Doxy – The antibiotic doxycycline.

Droplet precautions – *See* Transmission-based precautions.
DTRA – The DoD's Defense Threat Reduction Agency.
DURC – Dual use research of concern.
DVD – Digital versatile disc (or digital videodisk)
Dysarthria – A disturbance of speech and language due to emotional stress, to brain injury, or to paralysis, incoordination, or spasticity of the muscles used for speaking.
Dysphagia, dysphagy – Difficulty in swallowing.
Dysphonia – Altered voice production.
Dyspnea – Shortness of breath, a subjective difficulty or distress in breathing, usually associated with disease of the heart or lungs; occurs normally during intense physical exertion or at high altitude.
Dystocia – Slow or difficult labor or delivery.
Ecchymosis – A purplish patch caused by extravasation of blood into the skin, differing from petechiae only in size (larger than 3 mm diameter).
ECG – Electocardiogram; electrocardiography.
Echo – Echocardiogram.
ECL – Electrochemiluminescence**.**
Eczema – Generic term for inflammatory conditions of the skin, particularly with vesiculation in the acute stage, typically erythematous, edematous, papular, and crusting; followed often by lichenification and scaling and occasionally by duskiness of the erythema and, infrequently, hyperpigmentation; often accompanied by sensations of itching and burning.
ED_{50} – Median effective dose; the dose that produces the desired effect; when followed by a subscript (generally "ED_{50}"), it denotes the dose having such an effect on a certain percentage (e.g., 50%) of the test animals.
Edema – An accumulation of an excessive amount of watery fluid in cells, tissues, or serous cavities.
EDP – Especially dangerous pathogen(s).
EEE or EEEV – Eastern Equine Encephalitis [virus].
EIND – Emergency IND (*q.v.*); See Appendix K.
Electrochemiluminescence – A method used to identify microorganisms. Similar in operation to ELISA, FA and sandwich antibody assays. A capture antibody bound to a magnetic bead captures the target microorganism. Another antibody labeled with a ruthenium tris-bipyridyl compound ($Ru(bpy)_3^{2+}$) is introduced. A magnet is used to pull the beads to an electrode which is used to excite the ruthenium compound which then emits light. The light is detected revealing the presences of the target organism. The method is easily automated and is generally faster than either ELISA or FA.
ELISA – Enzyme-linked immunosorbent assay (*q.v.*).
EM – Electron microscopy; electron microscope.
Enanthem, enanthema – A mucous membrane eruption, especially one occurring in connection with one of the exanthemas.
Encephalitis (*pl.* **encephalitides**) – Inflammation of the brain.
Endotoxemia – Presence in the blood of endotoxins.
Endotracheal intubation – Passage of a tube through the nose or mouth into the trachea for maintenance of the airway during anesthesia or for maintenance of an imperiled airway.
Enterotoxin – A cytotoxin specific for the cells of the intestinal mucosa.
Enzyme-Linked Immunosorbent Assay (ELISA) – A method used to detect a microbial antigen or an antibody to a microbial antigen. It works by chemically linking an enzyme to an antibody that recognizes and adheres to the desired antigen or antibody. Any unbound antibody-enzyme complex is removed. A chemical that is converted by the enzyme into a fluorescent compound is applied and allowed to react. The fluorescence is then detected to reveal the presence or absence of the antigen or antibody.
EO – Executive Order.
Epidemic – The rapid spread of infectious, or other, disease to a large number of people in a given population within a short period of time; a threshold number of cases within a specific time frame is often pre-designated by experts to trigger notification.
Epidemic curve – A pattern, often presented as a histogram, depicting an outbreak of disease; useful in identifying the transmission method or source, and in predicting the future rate of infection.
Epistaxis – Profuse bleeding from the nose.
Epizootic – 1. Denoting a temporal pattern of disease occurrence in an animal population in which the disease occurs with a frequency clearly in excess of the expected frequency in that population during a given time interval. 2. An outbreak (epidemic) of disease in an animal population; often with the implication that it may also affect human populations.
Erythema – Redness of the skin due to capillary dilatation.

Erythema multiforme – An acute eruption of macules, papules, or subdermal vesicles presenting a multiform appearance, the characteristic lesion being the target or iris lesion over the dorsal aspect of the hands and forearms; its origin may be allergic, seasonal, or from drug sensitivity, and the eruption, although usually self-limited (e.g., multiforme minor), may be recurrent or may run a severe course, sometimes with fatal termination (e.g., multiforme major or Stevens-Johnson syndrome).
Erythrocyte – A mature red blood cell.
Erythropoiesis – The formation of red blood cells.
ESR – Erythrocyte sedimentation rate ("sed rate").
EU – European Union.
EUA – Emergency Use Authorization. (See Appendix K.)
Exanthema – A skin eruption occurring as a symptom of an acute viral or coccal disease, as in scarlet fever or measles.
Extracellular – Outside the cells.
Extraocular – Adjacent to but outside the eyeball.
F – Fahrenheit.
FA – Fluorescent antibody (*q.v.*).
FAC – Free available chlorine.
Fasciculation – Involuntary contractions, or twitchings, of groups (fasciculi) of muscle fibers, a coarser form of muscular contraction than fibrillation.
FBI – U.S. Federal Bureau of Investigation.
FDA – U.S. Food and Drug Administration; Part of DHHS.
FD&C Act – Federal Food, Drug and Cosmetic Act (1938).
Febrile – Denoting or relating to fever.
FEMA – Federal Emergency Management Agency.
FHP – Force Health Protection.
FL – Florida.
FM – Field Manual.
Fomite – Objects, such as clothing, towels, and utensils that possibly harbor a disease agent and are capable of transmitting it.
Formalin – A 37% aqueous solution of formaldehyde.
Fluorescent antibody – A microbiological method to detect microorganisms, usually bacteria. An antibody with an attached fluorescent molecule is applied to a slide containing the bacteria and washed to remove unbound antibodies. Under UV light the bacteria to which antibodies are bound will fluoresce, revealing their presence. An antibody may be applied *primarily* (DFA: direct fluorescence antibody, or direct immunofluorescence assay) or *secondarily*, using two antibodies (IFA: indirect fluorescence antibody, or indirect immunofluorescence assay).
Fulminant hepatitis – Severe, rapidly progressive loss of hepatic function due to viral infection or other cause of inflammatory destruction of liver tissue with associated coagulopathy and encephalopathy.
G-CSF – Granulocyte-colony stimulating factor.
GEM – Genetically engineered microorganisms.
Generalized vaccinia – Secondary lesions of the skin after vaccination, which may occur in subjects with previously healthy skin but are more common in the case of traumatized skin, especially in the case of eczema (eczema vaccinatum). In the latter instance, generalized vaccinia may result from mere contact with a vaccinated person. Secondary vaccinial lesions may also occur after transfer of virus from the vaccination to another site by means of the fingers (autoinnoculation).
GI – Gastrointestinal.
Glanders – A chronic debilitating disease of horses and other equids, as well as some members of the cat family, caused by *Pseudomonas mallei*; it is transmissible to humans. It attacks the mucous membranes of the nostrils of the horse, producing an increased and vitiated secretion and discharge of mucus, and enlargement and induration of the glands of the lower jaw.
GLC – Gas liquid chromatography.
G or g – Gram(s).
Granulocytopenia – Less than the normal number of granular leukocytes in the blood.
Guarnieri bodies – Intracytoplasmic acidophilic inclusion bodies observed in epithelial cells in variola (smallpox) and vaccinia infections, and which include aggregations of Paschen body's or virus particles.
H or h – Hour(s).
HA – Hemagglutination assay.
HBAT – *Heptavalent Botulinum Antitoxin*.

HCQ – Hydroxychloroquine.
HCW – Health care worker.
HE-BAT – *Botulism Antitoxin, Heptavalent, Equine* (A, B, C, D, E, F and G).
HEL – Human erythroleukemia.
Hemagglutination – The agglutination of red blood cells; may be immune as a result of specific antibody either for red blood cell antigens per se or other antigens that coat the red blood cells, or may be nonimmune, as in hemagglutination caused by viruses or other microbes.
Hemagglutinin – A substance, antibody or other, that causes hemagglutination.
Hematemesis – Vomiting of blood.
Hematuria – Any condition in which the urine contains blood or red blood cells.
Hemopoietic – Pertaining to or related to the formation of blood cells.
Hemodynamic – Relating to the physical aspects of the blood circulation.
Hemolysis – Alteration, dissolution, or destruction of red blood cells in such a manner that hemoglobin is liberated into the medium in which the cells are suspended, e.g., by specific complement-fixing antibodies, toxins, various chemical agents, tonicity, alteration of temperature.
Hemolytic Uremic Syndrome – Hemolytic anemia and thrombocytopenia occurring with acute renal failure.
Hemoptysis – The spitting of blood derived from the lungs or bronchial tubes as a result of pulmonary or bronchial hemorrhage.
HEPA – High-Efficiency Particulate Air [filter].
Hepatic – Relating to the liver.
Heterologous – 1. Pertaining to cytologic or histologic elements occurring where they are not normally found. 2. Derived from an animal of a different species, as the serum of a horse is heterologous for a rabbit.
HFRS – Hemorrhagic fever with renal syndrome. A viral hemorrhagic fever syndrome caused by viruses of the genus *Hantavirus*, Bunyaviridae family, with renal impairment as the primary organ manifestation.
HHA – Hand held assay.
HHS – See DHHS.
Histogram – A graphical representation of the distribution of data. (See epidemic curve).
HPLC-MS – High-performance liquid chromatography-mass spectrometry.
HPS – Hantavirus pulmonary syndrome.
HQ – Headquarters.
HSPD – Homeland Security Presidential Directive.
HUS – Hemolytic-uremic syndrome.
Hyperemia – The presence of an increased amount of blood in a part or organ.
Hyperesthesia – Abnormal acuteness of sensitivity to touch, pain, or other sensory stimuli.
Hypotension – Subnormal arterial blood pressure.
Hypovolemia – A decreased amount of blood in the body.
Hypoxemia – Subnormal oxygenation of arterial blood, short of anoxia.
IA – Inhalational anthrax.
IATA – International Air Transport Association.
IAW – In accordance with.
ICB – intracellular bacterial [pathogen].
ICD – International Classification of Diseases; published by the WHO.
ICLC – Interstitial Cajal-like cells.
ICU – Intensive care unit.
ID – Infectious disease.
IDE – Investigational Device Exemption; similar to an IND.
Idiopathic – Denoting a disease of unknown cause.
IE – Information Exchanges.
IF – Immunofluorescence.
IFA – Indirect immunofluorescence assay, or indirect immunofluorescence antibody (*see* Fluorescent antibody).
Ig – Immunoglobulin.
IHR – International Health Regulations.
IM – Intramuscular; intramuscularly.
IMDG – International Maritime Organization Dangerous Goods [code].

Immunoassay – Detection and assay of substances by serological (immunological) methods; in most applications the substance in question serves as antigen, both in antibody production and in measurement of antibody by the test substance.
Incubation period – the period between exposure to a pathogen (bacterium, virus, fungus) and the first symptoms or signs of infection (*cf* **latent period**).
IND – Investigational New Drug; FDA's terminology for an experimental drug or vaccine, not approved for general use.
Induration – 1. The process of becoming extremely firm or hard, or having such physical features. 2. A focus or region of indurated tissue.
Inguinal – Relating to the groin.
Inoculation – Introduction into the body of the causative organism of a disease.
IRB – Institutional Review Board.
Isolation – Voluntary or compulsory separation and confinement of an individual known or suspected to be infected with a contagious disease agent (whether ill or not) to prevent further infections. In a system devised, and periodically revised, by the CDC, various levels comprise application of one or more "precaution" (e.g., contact, droplet, airborne). (*Cf* **biocontainment**, **quarantine**).
ESSENCE – Electronic Surveillance System for the Early Notification of Community-based Epidemics.
IV – Intravenous; intravenously.
In vitro – In an artificial environment, referring to a process or reaction occurring therein, as in a test tube or culture media.
In vivo – In the living body, referring to a process or reaction occurring therein.
JAMA – *Journal of the American Medical Association*.
JBPDS – Joint Biological Point Detection System.
JBAIDS – Joint Biological Agent Identification and Diagnostic System.
JPEO-CBRND – The DoD's Joint Program Executive Office for Chemical, Biological, Radiological, and Nuclear Defense.
JSGPM – Joint Service General Protective Mask (U.S. Army XM-50).
JSLIST – Joint Services Lightweight Integrated Suit Technology.
KFD – Kyasanur Forest disease [virus]; a tick-borne encephalitis.
Kg – Kilogram(s).
KGB – The USSR's "Committee for State Security" [*Komitet gosudarstvennoy bezopasnosti*].
Latent period – The period between exposure to a toxin and the first symptoms or signs of intoxication (*cf* **incubation period**).
LD_{50} – In toxicology, the LD'_{50} of a particular substance is a measure of how much constitutes a lethal dose. In toxicological studies of substances, one test is to administer varying doses of the substance to populations of test animals; that dose administered which kills half the test population is referred to as the LD_{50}.
LDH – lactate dehydrogenase, a liver enzyme.
LED – light-emitting diode.
Leukopenia – The antithesis of leukocytosis; any situation in which the total number of leukocytes in the circulating blood is less than normal, the lower limit of which is generally regarded as 4000-5000 per cubic mm.
LOC – Lines of Communication.
LRN – Laboratory Response Network (See Appendix M-4).
Lumbosacral – Relating to the lumbar vertebrae and the sacrum.
Lumen, pl. lumina – The space in the interior of a tubular structure, such as an artery or the intestine.
LVS – Live vaccine strain (an IND tularemia vaccine).
Lymphadenopathy – Any disease process affecting a lymph node or lymph nodes.
Lymphopenia – A reduction, relative or absolute, in the number of lymphocytes in the circulating blood.
Macula, pl. maculae - 1. A small spot, perceptibly different in color from the surrounding tissue. 2. A small, discolored patch or spot on the skin, neither elevated above nor depressed below the skin's surface.
MADV – Madariaga Virus.
MCBC – The week-long Medical Management of Chemical and Biological Casualties course, jointly taught by USAMRICD and USAMRIID on a quarterly basis. (See also MMBC.)
MCM – Medical countermeasure.

MD – Maryland.
Mediastinitis – Inflammation of the cellular tissue of the mediastinum.
Mediastinum – The median partition of the thoracic cavity, covered by the mediastinal pleura and containing all the thoracic viscera and structures except the lungs.
Megakaryocyte – A large cell with a polyploid nucleus that is usually multilobed; megakaryocytes are normally present in bone marrow, not in the circulating blood, and give rise to blood platelets.
Melena – Passage of dark-colored, tarry stools, due to the presence of blood altered by the intestinal juices.
Meningism – A condition in which the symptoms simulate a meningitis, but in which no actual inflammation of these membranes is present.
Meningococcemia – Presence of meningococci (*N. meningitidis*) in the circulating blood.
Meninges – Any membrane; specifically, one of the membranous coverings of the brain and spinal cord.
MERS-CoV – Middle East respiratory syndrome coronavirus.
Microcyst – A tiny cyst, frequently of such dimensions that a magnifying lens or microscope is required for observation.
Microscopy – Investigation of minute objects by means of a microscope.
Min – Minute(s).
Mm – millimeter(s).
MMBC – USAMRIID's Medical Management of Biological Casualties course, a sub-component of the MCBC (*q.v.*).
MOPP – Mission Oriented Protective Posture; U.S. Army terminology for NBC personal protective gear (mask, hood, suit, boots).
Mo(s) – Month(s).
Mortality rate – A measure of the number of deaths (in general, or due to a specific cause) in some populations, scaled to the size of that population, per unit time. (*Cf.* **Case fatality rate**).
Mg – Milligram(s).
Moribund – Dying; at the point of death.
MRI – Magnetic resonance imaging.
MTF – Medical treatment facility.
Mucocutaneous – Relating to mucous membrane and skin; denoting the line of junction of the two at the nasal, oral, vaginal, and anal orifices.
MULO - Multipurpose rain/snow/CW overboots.
MVA – Modified vaccinia virus Ankara.
MWD – Military Working Dog.
Myalgia – Muscular pain.
Mydriasis – Dilation of the pupil.
NA – Neuraminidase.
NAAK – Nerve Agent Antidote Kit; consists of prefilled autoinjectors for the rapid administration of atropine and pralidoxime.
NATO – North Atlantic Treaty Organization.
NBC – Nuclear, Biological and Chemical.
Narcosis – General and nonspecific reversible depression of neuronal excitability, produced by a number of physical and chemical agents, usually resulting in stupor rather than in anesthesia.
NDBR – National Drug Biological Research [Company]; used with vaccine lot numbers.
Necrosis – Pathologic death of one or more cells, or of a portion of tissue or organ, resulting from irreversible damage.
NEDSS – National Electronic Disease Surveillance System.
Nephropathia epidemica – A generally benign form of epidemic hemorrhagic fever reported in Scandinavia.
Neutrophilia – An increase of neutrophilic leukocytes in blood or tissues; also frequently used synonymously with leukocytosis, inasmuch as the latter is generally the result of an increased number of neutrophilic granulocytes in the circulating blood (or in the tissues, or both).
NIBC – The National Integrated Biodefense Campus at Fort Detrick, Maryland.
NICBR – The National Interagency Confederation for Biological Research.
Nosocomial – Denoting a new disorder (not the patient's original condition) associated with being treated in a hospital, such as a hospital-acquired infection.
NPC/NPC-L – Negatively Pressurized Conex/Negatively Pressurized Conex-Lite. USAF biocontainment mobile assets for fixed wing aircraft.

NSAID – Non-steroidal anti-inflammatory drug.
NSB – National Strategy for Biosurveillance.
OCONUS – Outside the Continental United States.
ODP – Office of Domestic Preparedness; Overseen by both the U.S. Department of Justice and DHS.
OHF – Omsk hemorrhagic fever [virus]; a tick-borne encephalitis.
Oliguria – Scant urine production.
Oropharynx – The portion of the pharynx that lies posterior to the mouth; it is continuous above with the nasopharynx via the pharyngeal isthmus and below with the laryngopharynx.
Orphan drug – A drug effective in a rare or exotic medical condition, but which remains commercially undeveloped owing to its limited profitability; granting "orphan status," for the creation of financial incentives, is a matter of public policy in many countries; the concept applies to many vaccines as well.
Osteomyelitis – Inflammation of the bone marrow and adjacent bone.
Outbreak – An occurrence of disease greater than expected for a particular time and place; outbreaks may be epidemics (*q.v.*), affecting a region in a country or a group of countries, or a pandemic, affecting populations globally.
PA – Physician assistant.
Pancytopenia – Pronounced reduction in the number of erythrocytes, all types of white blood cells, and the blood platelets in the circulating blood.
Pandemic – Denoting a disease affecting or attacking the population of an extensive region, country, continent; extensively epidemic.
PAPR – Powered air-purifying respirator.
Papule – A small, circumscribed, solid elevation up to 1 cm in diameter on the skin.
Parasitemia – The presence of parasites in the circulating blood; used especially with reference to malarial and other protozoan forms, and microfilariae.
Passive immunity – Providing temporary protection from disease through the administration of exogenously produced antibody (i.e., transplacental transmission of antibodies to the fetus or the injection of immune globulin for specific preventive purposes).
PBT – Pentavalent botulinum toxoid.
PCR – Polymerase chain reaction (*q.v.*).
PEP – Post-exposure prophylaxis.
Percutaneous – Denoting the passage of substances through unbroken skin, for example, by needle puncture, including introduction of wires and catheters.
Perivascular – Surrounding a blood or lymph vessel.
Petechia, pl. petechiae – Minute hemorrhagic spots, of pinpoint to pinhead size, in the skin, which are not blanched by pressure.
Pharyngeal – Relating to the pharynx.
Pharyngitis – Inflammation of the mucous membrane and underlying parts of the pharynx.
Phosgene – Carbonyl chloride; a colorless liquid below 8.2°C, but an extremely poisonous gas at ordinary temperatures; it is an insidious gas, as it is not immediately irritating, even when fatal concentrations are inhaled.
Photophobia – Light-induced pain, especially of the eyes; for example, in uveitis, the light-induced movement of the iris may be painful. SYN: photodynia, photalgia.
Pleurisy – Inflammation of the pleura.
PM – Afternoon or evening (Latin, *post meridiem*).
PO – By mouth; orally.
Polymerase chain reaction (PCR) – An *in vitro* molecular biology method for enzymatically synthesizing and amplifying defined sequences of DNA. Can be used for improving DNA-based diagnostic systems for identifying unknown bio-agents.
Polymorphonuclear – Having nuclei of varied forms; denoting a variety of leukocyte.
Polyuria – Excessive excretion of urine.
POW – Prisoner of war.
PPE – Personal protective equipment.
Presynaptic – Pertaining to the area on the proximal side of a synaptic cleft.
Prophylaxis, pl. prophylaxes – Prevention of disease or of a process that can lead to disease.
Prostration – A marked loss of strength, as in exhaustion.
Proteinuria – Presence of urinary protein in concentrations greater than 0.3 g in a 24-h urine collection or in concentrations greater than 1 g/l in a random urine collection on two or more occasions $\geq$ 6 h apart; specimens must be clean, voided midstream, or obtained by catheterization.

Pruritus – Syn: itching.
Ptosis, pl. ptoses – In reference to the eyes, drooping of the eyelids.
Pulmonary edema – Edema of the lungs.
Pyrogenic – Causing fever.
Q or q – Latin, *quaque*, meaning "each" or "every".
QD or qD – Each day.
QID or qid – Four times each day.
Quarantine – The compulsory separation and confinement, with restriction of movement, of healthy individuals or groups who have potentially been exposed to a contagious disease agent to prevent further infections should infection occur. (*Cf* **biocontainment**, **isolation**).
Q.v. – Latin, *quod vide*, "which see".
Reactogenicity – The property of a vaccine being able to produce common, "expected" adverse reactions, especially excessive immunological responses and associated signs and symptoms—fever, sore arm or redness at injection site, etc.
Retinitis – Inflammation of the retina.
Retrosternal – Posterior to the sternum.
Rhinorrhea – A discharge from the nasal mucous membrane.
RNA – Ribonucleic acid.
RODS – Real-time Outbreak and Disease Surveillance.
RT – Reverse transcriptase.
RT-PCR – Reverse transcription-polymerase chain reaction (*q.v.*).
RTA – Ricin Toxin A [chain].
RTB – Ricin Toxin B [chain].
RVF – Rift Valley fever, a VHF.
SA – Select Agent (*q.v.*).
SAP – CDC's Select Agent (*q.v.*) Program.
SAHF – South American Hemorrhagic Fevers (i.e., AHF and BHF).
Sarin – A nerve poison which is a very potent irreversible cholinesterase inhibitor and a more toxic nerve gas than tabun or soman.
SARS – Severe Acute Respiratory Syndrome [virus].
Scarification – The making of a number of superficial incisions in the skin. It is the technique used to administer tularemia and smallpox vaccines.
Scud – NATO reporting name (SS-1 Scud) for a series of tactical ballistic missiles developed by the USSR and exported widely to other countries, including Iraq.
SEB – Staphylococcal Enterotoxin B.
Select Agent – A bio-agent that, since 1997, has been declared by the DHHS, or by the USDA, to have the "potential to pose a severe threat to public health and safety". (See also BSAT and Appendix C).
Septic shock – 1. Shock associated with sepsis, usually associated with abdominal and pelvic infection complicating trauma or operations; 2. Shock associated with septicemia caused by gram-negative bacteria.
Sequela, pl. sequelae – A condition after a consequence of a disease.
Shigellosis – Bacillary dysentery caused by bacteria of the genus *Shigella*, often occurring in epidemic patterns.
SNS – Strategic National Stockpile; Repository of drugs, vaccines, etc., overseen jointly by CDC and DHS.
Soman – An extremely potent cholinesterase inhibitor, similar to sarin and tabun.
SOP – Standard [or standing] operating procedure.
SQ – Subcutaneous; subcutaneously.
SRBSDS – Short Range Biological Standoff Detection System.
ST-246 – The oral antiviral tecovirimat (*Arestvyr*®), an IND.
Standard precautions – A set of uniform or comprehensive measures designed to prevent the inadvertent transmission of communicable diseases between patient and HCW. They are employed during *every* patient encounter, regardless of whether or not the patient is thought to harbor an infectious disease. (See Appendix I.)
Sterile abscess – An abscess whose contents are not caused by pyogenic bacteria.
Sterilization – Process that eliminates (removes) or kills all forms of life, including transmissible agents (bacteria [including spores], viruses, fungi) present on a surface, contained in a fluid, in

medication, or in a substance such as biological culture media; achieved by applying heat, chemicals, irradiation, high pressure, and/or filtration.
Stridor – A high-pitched, noisy respiration, like the blowing of the wind; a sign of respiratory obstruction, especially in the trachea or larynx.
Superantigen – An antigen that interacts with the T-cell receptor in a domain outside of the antigen recognition site. This type of interaction induces the activation of larger numbers of T cells compared to antigens that are presented in the antigen-recognition site leading to the release of numerous cytokines.
Superinfection – A new infection in addition to one already present.
Tachycardia – Rapid beating of the heart, conventionally applied to rates over 100 per minute.
TB – Tuberculosis.
TBE – Tick-borne encephalitis [viruses].
TEE – Transesophageal echocardiogram.
Teratogenicity – The property or capability of producing fetal malformation.
Thrombocytopenia – A condition in which there is an abnormally small number of platelets in the circulating blood.
TID or tid – Thrice each day.
TMM – The U.S. Army's *Textbook of Military Medicine* series.
TMP-SMX – The combination antibiotic trimethoprim-sulfamethoxazole.
TMT – The DoD's Transformational Medical Technologies Initiative.
Toxin or bio-toxin – A poisonous substance produced within living cells or organisms; typically they are peptides, proteins or smaller molecules.
Toxoid – A modified bacterial toxin that has been rendered nontoxic (commonly with formaldehyde) but retains the ability to stimulate the formation of antitoxins (antibodies) and thus producing an active immunity. Examples include botulinum, tetanus, and diphtheria toxoids.
Tracheitis – Inflammation of the lining membrane of the trachea.
Transmission-based precautions – Measures implemented in addition to Standard Precautions (*q.v.*), in select circumstances, to prevent the transmission of specific disease agents known or suspected to be present in a patient; may include (1) *Contact Precautions* to preclude disease transmission via blood, body fluids, or fomites; (2) *Droplet Precautions* when transmission via macroscopic respiratory droplets is a risk, or (3) *Airborne Precautions* when microscopic (~ 3-6 micron) "droplet nuclei" provide a possible vehicle of disease transmission. (See Appendix I.)
TTE – Transthoracic echocardiogram.
TTX – Table Top Exercise.
TX – Texas.
UK – United Kingdom.
UN – United Nations.
UNSCOM – United Nations Special Commission; an inspection regime created by the UN to ensure Iraq's compliance with its policies concerning production and use of WMD after the Persian Gulf War.
Urticaria – An eruption of itching wheals, usually of systemic origin; it may be due to a state of hypersensitivity to foods or drugs, foci of infection, physical agents (heat, cold, light, friction), or psychic stimuli.
USAFSAM – United States Air Force School of Aerospace Medicine.
USAMMDA – U.S. Army Medical Materiel Development Agency, Fort Detrick, Maryland.
USAMRICD – U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland.
USAMRIID – U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.
USC – United States Code.
USDA – United States Department of Agriculture; oversees regulation of BSATs (*q.v.*) affecting agriculture. (See Appendix C).
USSR - Union of Soviet Socialist Republics.
UV – Ultraviolet [light].
VA – Virginia.
Vaccine – A suspension of attenuated live or killed microorganisms (bacteria, viruses, or rickettsiae), or fractions thereof (for example, specific protein subunits or naked DNA), administered to induce immunity and thereby prevent infectious disease.
Vaccinia – An infection, primarily local and limited to a site of inoculation, induced in humans with the vaccinia virus (a relative of cowpox) to confer resistance to smallpox (variola). On about the 3rd d, papules form at the site of inoculation which become transformed into umbilicated vesicles and later

pustules; they then dry up, and the scab falls off on about the 21st d, leaving a pitted scar; in some cases there are more or less marked constitutional disturbances.
Varicella – An acute contagious disease, usually occurring in children, caused by the varicella-zoster virus, a member of the family *Herpesviridae*, and marked by a sparse eruption of papules, which become vesicles and then pustules, like that of smallpox although less severe and varying in stages, usually with mild constitutional symptoms; incubation period is about 14 to 17 d. Syn: chickenpox.
Variola – Smallpox or smallpox virus.
Variolation – The historical practice of inducing immunity against smallpox by inoculating the skin with matter from skin pustules of a smallpox victim. Said to have first been done in Ancient China.
VEE/VEEV – Venezuelan Equine Encephalitis [virus].
VHC – Refers to DoD's regional Vaccine Health Centers.
VHF – Viral Hemorrhagic Fever.
VIGIV – *Vaccinia Immune Globulin, Intravenous.*
Viremia – The presence of virus in the bloodstream.
Virion – The complete virus particle that is structurally intact and infectious.
WBC – White blood cell.
WEE/WEEV – Western Equine Encephalitis [virus].
WHO – The UN's World Health Organization.
Wk(s) – Week(s).
WMD – Weapon(s) of Mass Destruction; see also NBC.
Wt – Weight.
Yr(s) – Year(s).
Zoonosis – An infection or infestation shared in nature by humans and other animals that are the normal or usual host; a disease of humans acquired from an animal source.

Appendix B: CDC Bio-agent Categories A, B & C

Categories of Bio-terrorism Agents/Diseases Adapted from: http://www.bt.cdc.gov/agent/agentlist-category.asp		
Category	Definition	Examples
A	High-priority agents include organisms that pose a risk to national security because they are… • Highly transmissible person-to-person. • Highly fatal & have the potential for major public health impact. • Might cause public panic & social disruption. • Require special action/expertise for public health preparedness.	• Anthrax (*Bacillus anthracis*) • Botulism (*Clostridium botulinum* toxin) • Plague (*Yersinia pestis*) • Smallpox (Variola major type) • Tularemia (*Francisella tularensis*) • Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo])
B	Second highest priority agents because they have… • Moderate ease of dissemination/transmission. • Moderate morbidity rates & low fatality rates. • Require specific diagnostic capability & enhanced disease surveillance by the CDC.	• Brucellosis (*Brucella* species) • Epsilon toxin of *Clostridium perfringens* • Food safety threats (e.g., *Salmonella* species, *Escherichia coli* O157:H7, *Shigella*) • Glanders (*Burkholderia mallei*) • Melioidosis (*Burkholderia pseudomallei*) • Psittacosis (*Chlamydia psittaci*) • Q fever (*Coxiella burnetii*) • Ricin toxin from *Ricinus communis* (castor beans) • Staphylococcal enterotoxin B • Typhus fever (*Rickettsia prowazekii*) • Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis]) • Water safety threats (e.g., *Vibrio cholerae*, *Cryptosporidium parvum*)
C	Third highest priority agents include emerging pathogens that could be engineered for mass dissemination because they are… • Available. • Easy to produce and disseminate. • High potential for morbidity & fatality rates or major health impact.	Emerging infectious diseases such as… • Nipah virus • Hantavirus

Appendix C: Biological Select Agents and Toxins (BSATs)

Under U.S. law, "Biological Select Agents and Toxins" (BSATs)—or simply Select Agents for short—are bio-agents which, since 1997,[1] have been declared by the U.S. Department of Health and Human Services (HHS) or by the U.S. Department of Agriculture (USDA) to have the "potential to pose a severe threat to public health and safety". These bio-agents are divided into three broad categories: (1) HHS select agents and toxins (affecting humans); (2) USDA select agents and toxins (affecting agriculture); and (3) Overlap select agents and toxins (affecting both).

The U.S. Centers for Disease Control and Prevention (CDC) administers the Select Agent Program (SAP), which regulates the laboratories which may possess, use, or transfer select agents within the United States. The SAP was established to satisfy requirements of the USA PATRIOT Act of 2001 and the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, which were enacted in the wake of the September 11, 2001 attacks and the subsequent 2001 anthrax attacks.

The active use of BSATs in biomedical research prompts concerns about dual use. The Federal government has created the National Science Advisory Board for Biosecurity, a critical component of a set of federal initiatives to promote biosecurity in life science research. This advisory board is composed of government, education and industry experts who provide policy recommendations on ways to minimize the possibility that knowledge and technologies emanating from vitally important biological research will be misused to threaten public health or national security. For most recent updates visit https://www.selectagents.gov/sat/list.htm. For nucleic acids that are capable of producing infectious forms of select agent viruses, please reference the following guidance: https://www.selectagents.gov/compliance/guidance/nucleic/index.htm.

List of Select Agents

Tier 1 BSATs are indicated by an asterisk (*).[2]

I. HHS select agents and toxins

Pathogens

Bacteria

- Botulinum neurotoxin-producing species of *Clostridium**
- *Bacillus cereus* Biovar *anthracis**
- *Coxiella burnetii*
- *Francisella tularensis**
- *Rickettsia prowazekii*
- *Rickettsia rickettsii*
- *Yersinia pestis**

Viruses

- Coronavirus:
  - SARS-associated coronavirus (SARS-CoV)
  - SARS-CoV/SARS-CoV-2 chimeric viruses[3]
- Encephalitis viruses:
  - Eastern equine encephalitis virus
  - Tick-borne encephalitis-complex (flavi) viruses
    - Far Eastern subtype
    - Siberian subtype
- Influenza viruses:
  - Highly Pathogenic Avian Influenza H5N1 virus
  - Reconstructed 1918 influenza virus[4]
- Orthopoxviruses:
  - Monkeypox virus
  - Variola major virus* (smallpox virus)
  - Variola minor virus* (Alastrim)
- Viral hemorrhagic fevers (VHFs):
  - Arenaviruses
    - Chapare virus
    - Guanarito virus (Venezuelan hemorrhagic fever)
    - Junin virus (Argentine hemorrhagic fever)
    - Lassa fever virus
    - Lujo virus
    - Machupo (Bolivian hemorrhagic fever)
    - Sabiá virus (Brazilian hemorrhagic fever)
  - Bunyaviruses
    - Crimean-Congo hemorrhagic fever virus
  - Filoviruses
    - Orthoebolaviruses*
      - Bundibugyo virus (species *orthoebolavirus bundibugyoense*)
      - Ebola virus (species *orthoebolavirus zairense*)
      - Sudan virus (species *orthoebolavirus sudanense*)
      - Taï Forest virus (species *orthoebolavirus taiense*)
    - Orthomarburgviruses*
      - Marburg virus
      - Ravn virus
  - Flaviviruses:
    - Kyasanur Forest disease virus
    - Omsk hemorrhagic fever virus

Toxins

- Abrin
- Botulinum neurotoxins*
- *Clostridium perfringens* epsilon toxin
- Conotoxins
- Ricin
- Saxitoxin
- Shiga-like ribosome inactivating proteins
- Shiga toxin
- Staphylococcal enterotoxins
- Tetrodotoxin
- Type A trichothecenes:
    - Diacetoxyscirpenol
    - T-2 toxin

II. Overlap select agents and toxins

Bacteria

- *Bacillus anthracis**
- *Bacillus anthracis* Pasteur strain
- *Brucella abortus*
- *Brucella melitensis*
- *Brucella suis*
- *Burkholderia mallei**
- *Burkholderia pseudomallei**

Viruses

- Hendra virus
- Nipah virus
- Rift Valley fever virus
- Venezuelan equine encephalitis virus[5]

III. USDA select agents and toxins

For animals

Bacteria

- *Mycoplasma mycoides*
- *Mycoplasma capricolum*

Viruses

- African horse sickness virus
- African swine fever virus
- Avian influenza virus (highly pathogenic)
- Classical swine fever virus
- Foot-and-mouth disease virus*
- Goat pox virus
- Lumpy skin disease virus
- Newcastle disease virus
- Peste des petits ruminants virus
- Rinderpest virus*

- Sheep pox virus
- Swine vesicular disease virus

For plants

Bacteria

- *Ralstonia solanacearum*
- *Rathayibacter toxicus*
- *Xanthomonas oryzae*

Fungi or fungus-like pathogens

- *Peronosclerospora philippinensis* (*Peronosclerospora sacchari*)
- *Coniothyrium glycines* (formerly *Pyrenochaeta glycines and Phoma glycinicola*)
- *Sclerophthora rayssiae*
- *Synchvitrium endobioticum*

1. *Additional Requirements for Facilities Transferring or Receiving Select Agents*, Title 42 CFR Part 72 and Appendix A; 15 April 1997 (DHHS).
2. Select agent regulations were revised in October 2012 to designate thirteen "Tier 1" agents with a documented risk of causing a high consequence event higher than other BSATs. Criteria for Tier 1 status were (1) Ability to produce a mass casualty event or devastating effects to the economy; (2) Communicability; (3) Low infectious dose; and (4) History of or current interest in weaponization based on threat reporting. In the same revision Chapare virus, Lujo virus, and SARS-associated coronavirus (SARS-CoV) were added to the list of select agents. Department of Health and Human Services (2012), "Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review", *Federal Register* / Vol. 77, No. 194 / Friday, October 5, 2012 / Rules and Regulations, pg 61084. Government Printing Office [www.gpo.gov] [FR Doc No: 2012-24389].
3. Resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors.
4. This refers to reconstructed, replication-competent forms of the 1918 flu pandemic virus containing any portion of the coding regions of all eight gene segments.
5. Modified Venezuelan Equine Encephalitis Virus TC-83(A3G) strain is a select agent.
6. Criteria for removal from the BSAT list were (1) Low potential for causing mortality; (2) Endemicity in the U.S. (animal agents); and (3) Difficulty in producing quantities necessary for high consequence event.

Appendix D: Comparison of VHF Agents and Diseases

	Virus	Disease	Endemic area	Fatality	Nosocomial transmission	Characteristic features	Countermeasures
Flavivirus	Yellow fever virus	Yellow fever	Africa, South America	Overall 3-12%. 20-50% if severe second phase develops	No	Often biphasic, severe second phase with bleeding, very high bilirubin, transaminases, jaundice, renal failure	17-D live attenuated vaccine very effective preventive, no postexposure countermeasure available
	KFD virus	Kyasanur Forest disease	Southern India	3-5%	No	Flu-like syndrome with addition of cough, GI symptoms, hemorrhage, bradycardia	Formalin-inactivated vaccine available in India
	OHF virus	Omsk hemorrhagic fever	Siberia	0.2-3%	No	Frequent sequelae of hearing loss, neuropsych complaints, alopecia	TBE vaccines may offer some cross-protection
Filoviruses	Ebola virus	Ebola virus disease	Africa, Philippines (Ebola Reston)	40-90% for Sudan/Zaire	Common	Severe illness, maculopapular rash, profuse bleeding and DIC	Ervebo® vaccine (Zaire only) Monoclonal antibodies[33] (Zaire only)
	Marburg virus	Marburg hemorrhagic fever	Africa	23-70%	Yes		

	Virus	Disease	Endemic area	Fatality	Nosocomial transmission	Characteristic features	Countermeasures
Bunyaviruses	CCHF	Crimean-Congo hemorrhagic fever	Africa, SE Europe, Central Asia, India	30% (highly variable)	Yes	Often prominent petechial/ ecchymotic rash	Anecdotal success with ribavirin
	RVF	Rift valley fever	Africa	<0.5%	No	Hemorrhagic disease rare, classically associated with retinitis and encephalitis,	Effective livestock vaccines in Africa
	RVF (continued)	Rift Valley Fever				Significant threat to livestock – epidemics of abortion and death of young	Human killed vaccine – DOD IND, live attenuated vaccine in clinical trials
	Hantavirus (Hantaan, Dobrava, Seoul, Puumala)	Hemorrhagic fever with renal syndrome (HFRS)	Europe, Asia, South America (rare)	Variable. 5%-15% for Asian HFRS	No	Prominent renal disease, marked polyuric phase during recovery, usually elevated WBC	Effective locally produced vaccines in Asia (not avail. in U.S.). Experimental vaccine at USAMRIID. Ribavirin effective in randomized, controlled clinical trial
Arenaviruses	Lassa virus	Lassa fever	West Africa	1-2%	Yes	Frequent inapparent/mild infection, hearing loss in	Ribavirin effective in clinical trial with non-randomized controls

	Virus	Disease	Endemic area	Fatality	Nosocomial transmission	Characteristic features	Countermeasures
						convalescence common	
	Junin	Argentine hemorrhagic fever	Argentinean pampas	10-30%	Rare	Prominent GI complaints, late neurologic syndrome	Immune plasma, Ribavirin effective, Candid 1 vaccine protective but not avail. in US
	Machupo	Bolivian hemorrhagic	Bolivia	25-35%	Rare	Similar to AHF	Immune plasma effective, ribavirin probably effective, Candid 1 vaccine protects monkeys

Appendix E: Summary of Bio-agent Characteristics

Disease	Degree of person-to-person transmission	Infective Dose (Aerosol)/ LD_{50}*[8]	Incubation Period[2]	Duration of Illness	Case fatality rate (CFR)	Persistence of organism outside host	Vaccine efficacy (aerosol exposure)
Anthrax	None	8,000-50,000 spores	1-6 d	3-5 d (usually fatal if untreated)	High	Very stable; spores remain viable for > 40 yrs in soil	2 dose efficacy against up to 1,000 LD_{50} in monkeys
Brucellosis	None	10 -100 organisms	5-60 d (usually 3-4 wks)	Wks to mos	<5% untreated	Very stable	No vaccine
Glanders	Low	Unknown, Potentially low	10-14 d via aerosol	Death in 7-10 d in septicemic form	> 50%	Very stable	No vaccine
Melioidosis	Low	Unknown, Potentially low	1-21 d (up to yrs)	Death in 2-3 d with septicemic form (untreated)	19 – 50% for severe disease	Very stable; survives indefinitely in warm moist soil or stagnant water	No vaccine
Plague	Moderate (for pneumonic form)	500 - 15000 organisms	1-7 d (usually 2-3 d)	1-6 d (usually fatal)	High unless treated within 12-24 h	For up to 1 yr in soil; 270 d in live tissue	No vaccine
Q Fever	Rare	1-10 organisms	7-41 d	2-14 d	Very low	For mos on wood & sand	94% protection against 3,500 LD_{50} in guinea pigs
Tularemia	None	10-50 organisms	1-21 d (average 3-6 d)	3-6 wks	Moderate if untreated	For mos in moist soil or other media	No vaccine
Smallpox	High	Assumed low	7-17 d (average 12 d)	4 wks	High to moderate	Very stable	Protects against large doses in primates

[8] In this Table, "Infective Dose" refers to bacteria and viruses, while "LD50" refers to toxins.
[2] In this Table, "Incubation Period" implies "Latent Period" where toxins are indicated.

Venezuelan Equine Encephalitis	Rare	10-100 organisms	27.5 h to 4 d	Days to wks	Low	Relatively unstable	No vaccine
Viral Hemorrhagic Fevers	Moderate	1-10 organisms	4-21 d	Death between 7-16 d	High to moderate (depends on agent)	Relatively unstable – (depends on agent)	Vaccines for YF and Ebola Zaire
Botulism	None	0.001 μg/kg is LD_{50} for type A (parenteral), 0.003 μg/kg (aerosol)	12 h to 5 d	Death in 24-72 h; lasts mos if not lethal	High without respiratory support	For wks in non-moving water & food if shaded from UV light	No Vaccine
Staph Enterotoxin B	None	0.03 μg / person (80kg) incapacitation	1.5-18 h after inhalation	Hours to days	< 1%	Unknown; Resistant to freezing	No vaccine
Ricin	None	3-5 μg/kg is LD_{50} in mice	4-24 h	Days - death within 2-3 days	High	Stable	No vaccine
T-2 Mycotoxins	None	Moderate	2-4 h	Days to mos	Moderate	For yrs at room temp	No vaccine

Appendix F: Differential Diagnosis of Chemical Nerve Agent, Botulinum Toxin & SEB Intoxication following Inhalation Exposure

	Chemical Nerve Agent	Botulinum Toxin	SEB
Time to Symptoms	Minutes	Hours (12-48)	Hours (1.5-18)
Nervous	Convulsions, Muscle twitching	Progressive, descending skeletal muscle flaccid paralysis	Headache, muscle aches
Cardiovascular	Slow heart rate	Normal rate	Normal or rapid heart rate
Respiratory	Difficulty breathing, airway constriction	Normal, then progressive paralysis	Nonproductive cough; Severe cases: chest pain/difficulty breathing
Gastrointestinal	Increased motility, pain, diarrhea	Decreased motility	Nausea, vomiting and/or diarrhea
Ocular	Small pupils	Droopy eyelids, large pupils, disconjugate gaze	May see “red eyes” (conjunctival injection)
Salivary	Profuse, watery saliva	Normal; difficulty swallowing	May be slightly increased quantities of saliva
Death	Minutes	2-3 d	Unlikely
Response to Atropine/2PAM-CL	Yes	No	Atropine may reduce gastrointestinal symptoms through anticholinergic effects

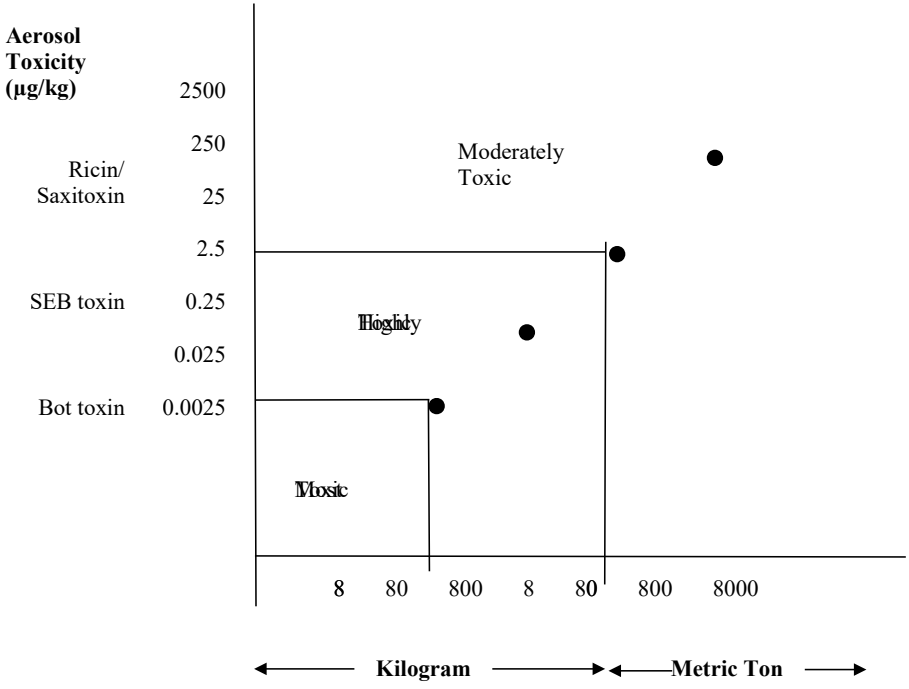
Appendix G: Comparative Lethality of Selected Toxins & Chemical Agents in Laboratory Mice*

Agent	LD_{50} (µg/kg)	Molecular Weight	Source
Botulinum neurotoxin A	0.001	150,000	Bacterium
Shiga toxin	0.002	55,000	Bacterium
Tetanus toxin	0.002	150,000	Bacterium
Abrin	0.04	65,000	Plant (Rosary Pea)
Diphtheria toxin	0.10	62,000	Bacterium
Maitotoxin	0.10	3,400	Marine Dinoflagellate
Palytoxin	0.15	2,700	Marine Soft Coral
Ciguatoxin	0.40	1,000	Marine Dinoflagellate
Textilotoxin	0.60	80,000	Elapid Snake
C. perfringens toxins	0.1 – 5.0	35-40,000	Bacterium
Batrachotoxin	2.0	539	Arrow-Poison Frog
Ricin (Aerosol)	3.0	64,000	Plant (Castor Bean)
alpha-Conotoxin	5.0	1,500	Cone Snail
Taipoxin	5.0	46,000	Elapid Snake
Tetrodotoxin	8.0	319	Puffer Fish
alpha-Tityustoxin	9.0	8,000	Scorpion
Saxitoxin	10.0 (Inhal 2.0)	299	Marine Dinoflagellate
VX	15.0	267	Chemical Agent
SEB (rhesus/aerosol)	27.0 (ED_{50}~pg)	28,494	Bacterium

Anatoxin-a(S)	50.0	500	Blue-Green Algae
Microcystin	50.0	994	Blue-Green Algae
Soman (GD)	64.0	182	Chemical Agent
Sarin (GB)	100.0	140	Chemical Agent
Aconitine	100.0	647	Plant (Monkshood)
T-2 Toxin	1,210.0	466	Fungal Myotoxin

* Unless otherwise stated, LD_{50} data is determined by intravenous route, and marine toxins are determined by intraperitoneal route.

Appendix H: Aerosol Toxicity in LD50 vs. Quantity of Toxin



Aerosol toxicity in LD_{50} (see also Appendix G) vs. quantity of toxin required to provide a theoretically effective open-air exposure, under ideal meteorological conditions, to an area 100 km^2. Ricin, saxitoxin and botulinum toxins kill at the concentrations depicted. (Devised by William Patrick III and Richard Spertzel, 1992: Based on Cader K.L., "BWL Tech Study #3: Mathematical models for dosage and casualty resulting from single point and line source release of aerosol near ground level", DTIC #AD3 10-361, Dec 1957.)

Appendix I: Patient Isolation Precautions

Precautions are steps taken by healthcare professionals to prevent the spread of infectious pathogens. The Centers for Disease Control and Prevention (CDC) have outlined two types of precautions: standard precautions and transmission-based precautions. Standard Precautions provide measures designed to prevent the inadvertent transmission of communicable diseases among patients and between patients and providers. Standard Precautions should be employed during EVERY healthcare encounter, regardless of whether there is a confirmed infectious diagnosis. Standard precautions assume that every person is potentially infected or colonized with an organism that could be transmitted in the healthcare setting.[1] In select circumstances, additional transmission-based precautions are used to augment standard precautions when additional controls are necessary to interrupt the route(s) of transmission that may not be accounted for with standard precautions alone.[1] There are three subcategories of Transmission-Based Precautions: (1) *Contact Precautions* are used to mitigate the transmission of infectious agents through direct or indirect contact with a patient or the patient's environment. (2) *Droplet Precautions* prevents the transmission of pathogens spread from close respiratory or mucous membrane contact with respiratory secretions that do not travel more than three feet.[2] (3) *Airborne Precautions* are used to prevent the transmission of infectious pathogens that remain suspended in the air over a longer distance (>3 feet).

Standard Precautions:

- Wash hands with soap and water or use an alcohol-based sanitizer before and after patient contact, between procedures on the same patient, and between patients.
- Wash hands when hands are visibly soiled.
- Wear gloves when touching blood, other body fluids, secretions, excretions, and contaminated items.
- Wear a mask and eye protection, or a face shield during procedures likely to generate splashes or sprays of blood, other body fluids, secretions, or excretions.
- Handle used patient-care equipment and linen in a manner that prevents the transfer of microorganisms to people or equipment.
- Use safe injection practices and dispose of sharp instruments safely.
- Use respiratory hygiene/cough etiquette.
- Use a mouthpiece or other ventilation device as an alternative to mouth-to-mouth resuscitation when practical.

Transmission-based Precautions:

Contact Precautions

Standard Precautions plus:

- Place the patient in a private room or cohort them with someone with the same infection if possible; if cohorting is employed, maintain ≥ 3 feet of spatial separation between patients.
- Wear a gown and gloves when entering the room if contact with patient or other surfaces patient has touched is anticipated, especially if patient has diarrhea, a colostomy, or wound drainage not covered by a dressing.
- Don personal protective equipment (PPE) upon room entry and discard before exiting the patient room to contain the pathogen(s). Change gloves after contact with infective material.
- Limit the movement or transport of the patient from the room and, if needed, lightly cover open wounds for patient transport.
- Ensure that patient-care items, bedside equipment, and frequently touched surfaces receive daily cleaning.
- Dedicate use of noncritical patient-care equipment (such as stethoscopes) to a single patient, or cohort patients with the same pathogen. Use single-use/disposable equipment if possible. If not feasible, thorough disinfection between patients is necessary.

1. Droplet Precautions

Standard Precautions plus:

- Place the patient in a private room or cohort them with someone with the same infection. If not feasible, maintain ≥ 3 feet between patients.
- Wear a surgical mask when working within 3 feet of the patient.
- Don gown and gloves for all interactions.
- Limit movement and transport of the patient. Place a mask on the patient if they must be moved out of their room.

2. Airborne Precautions

Standard Precautions plus:

- Place the patient in a private, airborne infection isolation room (AIIR) that has monitored negative air pressure, a minimum of 12 air changes per hour for new construction or renovation and 6 air exchanges per hour for existing facilities, and appropriate HEPA filtration of exhausted air.
- Wear appropriate, fit-tested, respiratory protection when entering the room. NIOSH certified N95 masks or higher-level respirators are effective against particles as small as 1-5 micrometers.

- Limit movement and transport of the patient. Place a surgical mask on the patient if they need to be moved (Caution- DO NOT place N95 masks on patients who have respiratory difficulty).

Patient Isolation

1. Occupational Safety and Health Administration. (n.d.). *Worker protections against occupational exposure to infectious diseases.* https://www.osha.gov/bloodborne-pathogens/worker-protections
2. Siegel, J., Rhinehart, E., Jackson, M., Chiarello, L., *2007 Guideline for isolation precautions:Preventing transmission ofiInfectious agents in healthcare settings*. (Updated 2024). *https://www.cdc.gov/infection-control/media/pdfs/Guideline-Isolation-H.pdf*

Appendix J: Biological Agent Prophylactics & Therapeutics

NB: (IND = Available as an investigational new drug for this indication (i.e. NOT an FDA-approved use).

BACTERIAL PATHOGENS:

Anthrax

VACCINE
BioThrax® Anthrax Vaccine (AVA) (Emergent BioSolutions). Preexposure (PrEP): licensed for adults 18-65-yr old, 0.5 mL IM @ 0, 1-, and 6-months (primary series) then 12-, and 18-month boosters, followed by annual boosters. Postexposure (PEP): licensed for adults 18-65-yr old, SQ at 0, 2-, 4-weeks in combination with approved & labeled antibiotics. Pediatric Annex (IND) for postexposure use IM at 0, 2-, 4-weeks in combination with approved & labeled antibiotics.
CHEMOPROPHYLAXIS
After suspected exposure to aerosolized *B. anthracis* of unknown antibiotic susceptibility, prophylaxis with doxycycline (100 mg PO q 12 h for adults) OR ciprofloxacin (500 mg PO q 12 h for adults) OR levofloxacin (500mg PO every 24 hours) should be initiated immediately. Other alternate regimens per CDC guidance at https://www.cdc.gov/mmwr/volumes/72/rr/rr7206a1.htm. Per ACIP guidance, the number of vaccine doses and duration of PEP-antibiotics will vary depending on the number of previously received doses and patient type: 1. **Immunocompetent adults** (e.g., healthy, nonpregnant adults aged 18–65 years): PEP- antibiotics both for the licensed and dose-sparing PEP regimens can be discontinued 42 days after initiation of vaccine if AVA is administered on schedule for both the licensed and dose-sparing regimens. If the AVA series cannot be completed, then antimicrobial therapy should continue for 60 days. 2. **Persons with immunocompromising conditions** that might interfere with their ability to develop an adequate immune response or populations for whom data on immune response to AVA are lacking (e.g., children, pregnant women, and adults aged ≥65 years) should continue to receive PEP- antibiotics for 60 days concurrently with AVA. 3. **Those who have received PrEP**, if biosafety or respiratory protection measures are breached and exposure to aerosolized *B. anthracis* spores might have occurred (such as in the laboratory): 30-day course of PEP- antibiotics is recommended, regardless of whether PrEP has been fully or partially completed. Persons who are exposed to aerosolized *B. anthracis* spores but have not completed the initial priming and booster series for AVA should receive additional AVA doses and PEP-antibiotics. The number of vaccine doses and duration of PEP-antibiotics will vary depending upon the number of previously received doses and are summarized in CDC guidance at https://www.cdc.gov/mmwr/volumes/68/rr/rr6804a1.htm.

CHEMOTHERAPY

Systemic and or inhalation* with or without Meningitis

Top 3 CDC recommended treatment regimens** in order of priority (additional alternate regimens are available at https://www.cdc.gov/mmwr/volumes/72/rr/rr7206a1.htm:

Regimen 1: Two bactericidal drugs from different antimicrobial drug classes plus a Protein Synthesis Inhibitor (PSI) or Rifampin (RNAI). For example: ciprofloxacin 400mg IV every 8 hours PLUS meropenem 2g IV every 8 hours plus minocycline 200mg IV x1 then 100mg IV every 12 hours].

Regimen 2: One bactericidal drug plus a PSI. For example: meropenem 2g IV every 8 hours + doxycycline 200mg IV x1 then 100mg IV every 12 hours.

Regimen 3: One bactericidal drug plus a second bactericidal drug from a different antimicrobial drug class. For example: meropenem 2g IV every 8 hours + ciprofloxacin 400mg IV every 8 hours.

*Per most recent CDC guidelines, if an aerosol exposure might have occurred (e.g., a bioterrorism-related incident or animal skin drum–related event), patients treated for systemic disease who are immunocompetent do not need further antimicrobial drugs for PEP because they will have developed natural immunity.
**Each regimen should include antitoxin (raxibacumab 40mg/kg IV x1 or oblitoxaximab 16mg/kg IV x1) as an adjunct.

Alternate treatment regimens are outlined in current CDC guidelines at https://www.cdc.gov/mmwr/volumes/72/rr/rr7206a1.htm.

Management of pediatric and pregnant/postpartum women are available at: https://pediatrics.aappublications.org/content/133/5/e1411 and https://wwwnc.cdc.gov/eid/article/20/2/13-0611_article.

COMMENTS

Penicillins should be used for anthrax treatment or prophylaxis only if the strain is demonstrated to be PCN-susceptible. Oral dosing (versus the preferred IV) could be necessary for treatment of systemic disease in a mass casualty situation.

Cutaneous anthrax: Antibiotics for cutaneous disease (without systemic complaints) resulting from a bio-agent aerosol attack are the same as for post-exposure prophylaxis. Cutaneous anthrax acquired from natural exposure could be

Brucellosis

VACCINE

None currently available.

CHEMOPROPHYLAXIS

A human vaccine is not available. Chemoprophylaxis is not recommended after possible exposure to endemic disease. Prophylaxis should only be considered for high-risk exposure in the following situations: (1) inadvertent wound or mucous membrane exposure to infected livestock tissues & body fluids & to livestock vaccines, (2) exposure to laboratory aerosols or to secondary aerosols generated from contaminated soil particles in calving & lambing areas, (3) confirmed bio-warfare/bio-terrorism exposure. **Despite extensive studies, optimal antibiotic therapy for brucellosis remains in dispute.**

CHEMOTHERAPY

Doxycycline & rifampin (or other antibiotics) for 6 weeks is sufficient in most cases. More prolonged regimens may be required for patients with complications such as hepatitis, splenitis, meningoencephalitis, endocarditis, or osteomyelitis.

Inhalational, Gastrointestinal, or Systemic Disease

Complicated Infection - Long-term (up to 6 mo) therapy for meningoencephalitis, endocarditis:
Rifampin + a tetracycline + an aminoglycoside (first 3 wks).

Uncomplicated infection:
Doxycycline: 100 mg PO q 12 h for 4-6 wks (adults), **plus** Streptomycin 1 g IM q 24 h for first 2-3 wks (adults); **OR** Doxycycline **plus** Gentamicin 3-5 mg/kg per d for 7 d (if streptomycin not available).

WHO guidelines for adults & children older than 8 yrs recommend rifampin (600-900 mg) & doxycycline q 24 h 24 h for 6 wks minimum. Treatment in children younger than 8 yrs requires rifampin & TMP-SMX.

Less severe disease:
Doxycycline 100 mg PO q 12 h for 6 wks (adults) **OR** Ciprofloxacin 500-750 mg PO q12h **OR** TMP-SMX 1-2 DS tabs PO q 12 h, **PLUS** Rifampin 600-900 mg/d PO q 24 h 24 h for 4-6 wks (adults).

COMMENTS

The CDC interim PEP recommendations for high-risk exposures to *Brucella* spp. are: doxycycline 100 mg PO q 12 h, plus rifampin 600 mg PO q 24 h.

Avoid monotherapy (high relapse). Relapse common for treatments less than 4-6 wks.

Glanders & Melioidosis

VACCINE

None currently available.

CHEMOPROPHYLAXIS

No FDA approved prophylaxis exists.

The antibiotic susceptibility pattern for *B. mallei* is similar to that of *B. pseudomallei*, with *B. mallei* exhibiting resistance to a number of antibiotics.

PO TMP/SMX (2 X 160-800 mg [960 mg tablets]) if > 60 kg q 12 h plus folate 5 mg/d for 21 d should be given ASAP after exposure.

Amoxicillin/clavulanic acid (*Augmentin*) 20.5 mg/kg/dose every 8 h is an alternative, especially during pregnancy or for children < 8 yr old. Doxycycline 2.5 mg/kg (up to 100 mg) q 12 h may be considered but carries risk of relapse. Fluoroquinolones should not be used for PEP, based upon animal studies & high relapse rates in human clinical trials for therapy.

CHEMOTHERAPY

No FDA approved therapy exists.

Ceftazidime (50 mg/kg [up to 2 g]) IV q 6 to 8 h, meropenem (25 mg/kg [up to 1 g]) IV q 8 h, or imipenem (25 mg/kg [up to 1 g]) IV q 6 h. Meropenem is advised for patients with neurologic involvement or renal insufficiency. A switch to meropenem is indicated if the patient has positive blood cultures after 7 d of therapy, or clinically deteriorates (e.g., develops organ failure or a new focus of infection) at any time during ceftazidime therapy.

Continue IV therapy for ≥ 14 d & until patient clinically improved. IV therapy may be extended (4 to 8 wks) for critical illness, severe pulmonary disease, deep-seated abscesses, bone, joint, or CNS involvement. Continue with PO maintenance therapy with TMP/SMX (2 X 160-800 mg [960 mg tablets]) if > 60 kg q 12 h for 3 to 6 mos.

COMMENTS

Both *B. mallei* & *B. pseudomallei* are sensitive to carbapenems, & most strains are also susceptible to ceftazidime & piperacillin. *B. pseudomallei* exhibits resistance to diverse antibiotics, including 1st- & 2nd-generation cephalosporins, penicillins, macrolides & aminoglycosides.

If ceftazidime or a carbapenem are not available, ampicillin/sulbactam (*Augmentin*) or other IV beta-lactam/beta-lactamase inhibitor combinations may represent viable, albeit less-proven alternatives. *Augmentin* may be an alternative to TMP/SMX, especially in pregnancy or for children < 8 yrs old. See main text for recommendations for toxicity screening & folate supplementation during prolonged courses of TMP/SMX.

Plague

VACCINE/TOXOID

None currently available.

CHEMOPROPHYLAXIS

Ciprofloxacin 500–750 mg every 12 h PO (adults); 15 mg/kg every 12 h PO (maximum 750 mg/dose (peds), **OR**

Levofloxacin 500–750 mg every 24 h PO (adults); Body weight < 50 kg: 8 mg/kg every 12 h PO (maximum 250 mg/dose), **OR**
Body weight ≥ 50 kg: 500–750 mg every 24 h PO (peds).

Moxifloxacin 400 mg every 24 h PO (adults), **OR**

Doxycycline 100 mg every 12 h PO (adults); < 45 kg: 2.2 mg/kg every 12 h PO.
Body weight ≥ 45 kg: 100 mg every 12 h PO (peds).

CHEMOTHERAPY

Fluoroquinolones, doxycycline, & gentamicin are used for plague & are approved by the FDA for this purpose.
Ciprofloxacin 400 mg every 8 h IV or 750 mg every 12 h PO (adults), **OR**

Levofloxacin 750 mg every 24 h IV or PO adult & pediatric patients > 50kg; 500mg administered by slow IV infusion over 60 min q 24 h for 10 to 14 d. Pediatric patients < 50kg & > 6 mos of age: 8 mg/kg (not to exceed 250 mg per dose) by slow IV infusion every 12 h for 10 to 14 d, **OR**

Moxifloxacin 400 mg every 24 h IV or PO, **OR**

Gentamicin 5 mg/kg IM or IV qd, or 2 mg/kg loading dose followed by 1.7 mg/kg IM or IV q 8 h (2.5 mg/kg IV q 8 h for children), adjusted for renal clearance, **OR**

Streptomycin 1g q 12 h IM (adults) 15mg/kg/d divided q 12 h IM (up to 2 g/d)(peds).

Alternatives: Doxycycline 200 mg IV once then 100 mg IV q 12 h until clinically improved, then 100 mg PO q 12 h for total of 10-14 d (adults), **OR** ofloxacin 400mg every 12 h PO, **OR** Gemifloxacin 320 mg every 24 h PO, **OR** amikacin 15-20 mg every 24 h IV or IM, **OR** tobramycin 5-7 mg/kg every 24 h IV or IM, **OR** trimethoprim-sulfamethoxazole 5 mg/kg (trimethoprim component) every 8 h IV or PO.

A minimum of 10 d of therapy is recommended (treat for ≥ 3-4 d after clinical recovery). Oral dosing (versus the preferred IV) could be necessary in a mass casualty situation.

Meningitis: add chloramphenicol 25 mg/kg IV, then 15 mg/kg IV q 6 h **PLUS** levofloxacin 750 mg every 24 h IV or PO **OR** moxifloxacin 400 mg every 24 h IV or PO.

COMMENTS

Streptomycin is not widely available in the U.S. & is of limited use. Although not licensed for use in treating plague, gentamicin is the common choice for parenteral therapy by many authorities. Reduce dosage in renal failure.

Chloramphenicol is contraindicated in children less than 2 yrs. While chloramphenicol is potentially an alternative for post-exposure prophylaxis (25 mg/kg PO q 6 hrs), oral formulations are available only outside the U.S.

Alternate therapy or prophylaxis for susceptible strains: ofloxacin, gemifloxacin, omadacycline, TMP-SMX

Q Fever

VACCINE/TOXOID

None currently available in the U.S.
Inactivated Whole Cell Vaccine (Pre-exposure only): Licensed (Australia) *Qvax*™

CHEMOPROPHYLAXIS

Doxycycline 100 mg PO q 12 h x 5-7 d (adults), 2.2 mg/kg PO q 12 h (peds), **OR** tetracycline 500 mg PO q 6 h x 5-7 d (adults); start post-exposure prophylaxis 8-12 d postexposure.

CHEMOTHERAPY

Doxycycline is the first line treatment for all adults & for children with severe illness. Treatment should be initiated whenever Q fever is suspected & started again if the patient relapses.

Acute Q-fever: Doxycycline 100 mg IV or PO q 12 h x ≥ 14 d (adults), 2.2 mg/kg PO q 12 h (peds < 45 kg), **OR** Tetracycline 500 mg PO q 6 h x ≥ 14 d.

Alternatives: Quinolones (e.g., ciprofloxacin, moxifloxacin), **OR** TMP-SMX, **OR** Macrolides (e.g., clarithromycin or azithromycin) for 14-21 d. Patients with underlying cardiac valve defects: Doxycycline plus hydroxychloroquine 200 mg PO q 8 h for 12 mos.

Chronic Q Fever: Doxycycline plus quinolones for 4 yrs, **OR** doxycycline plus hydroxychloroquine for 1.5-3 yrs.

COMMENTS

Initiation of post-exposure prophylaxis within 7 d of exposure merely delays incubation period of disease.

Tetracyclines are preferred antibiotic for treatment of acute Q fever except in:
1. Meningoencephalitis: fluoroquinolones may penetrate CSF better than tetracyclines.
2. Children < 8 yrs (doxycycline relatively contraindicated): TMP/SMX or macrolides (especially clarithromycin or azithromycin).
3. Pregnancy: TMP/SMX 160 mg/800 mg PO q 12 h for duration of pregnancy. If evidence of continued disease at parturition, use tetracycline or quinolone for 2-3 wks. Doxycycline is contraindicated during pregnancy.

Tularemia

VACCINE

None currently available.

CHEMOPROPHYLAXIS

Ciprofloxacin 500 mg PO q 12 h for 14 d, 15-20 mg/kg (up to 500 mg) PO q 12 h (peds), **OR**

Doxycycline 100 mg PO q 12 h x 14 d (adults), 2.2 mg/kg (up to 100 mg) PO q 12 h (peds < 45 kg), **OR**

Tetracycline 500 mg PO q 6 h x 14 d (adults)

CHEMOTHERAPY

Streptomycin 1 g IM q 12 h for ≥ 10 d (adults), 15 mg/kg (up to 2 g/d) IM q 12 h (peds), **OR**

Gentamicin 5 mg/kg IM or IV q 24 h, or 2 mg/kg loading dose followed by 1.7 mg/kg IM or IV q 8 h x ≥ 10 d (adults), 2.5 mg/kg IM or IV q 8 h (peds), **or**

Alternatives:
Ciprofloxacin 400 mg IV q 12 h for ≥ 10 d (adults); 15-20 mg/kg (up to 1 g/d) IV q 12 h (peds), **OR**

Doxycycline 200 mg IV, then 100 mg IV q 12 h x 14-21 d (adults), 2.2 mg/kg (up to 100 mg) IV q 12 h (peds < 45 kg), **OR**

Chloramphenicol 15-25 mg/kg IV q 6 h x 14-21 d, **OR**

Tetracycline 500 mg PO q 6 h x 14-21 d (adults)

COMMENTS

Streptomycin is not widely available in the U.S. & is of limited use. Gentamicin, although not approved for treatment of tularemia, likely represents a suitable alternative. Adjust gentamicin dose for renal failure.

Treatment with streptomycin, gentamicin, or ciprofloxacin should be continued for 10 d; doxycycline & chloramphenicol are associated with high relapse rates with courses shorter than 14-21 d. IM or IV doxycycline, ciprofloxacin, or chloramphenicol can be switched to oral antibiotic to complete course when patient clinically improved.

Chloramphenicol is contraindicated in children < 2 yrs. While chloramphenicol is potentially an alternative for post-exposure prophylaxis (25 mg/kg PO q 6 h), oral formulations are available only outside the U.S.

VIRAL PATHOGENS

Smallpox & MPOX

VACCINE

-*ACAM2000*® (Pre-exposure)
- *JYNNEOS*® (Pre-exposure)

CHEMOPROPHYLAXIS

- Acambis Vaccine (*ACAM2000*) (Post-exposure)
- JYNNEOS (Post-Exposure EUA)

CHEMOTHERAPY

Tecovirimat (TPOXX®) 600 mg/d PO with full glass of water & food for 14 d. May be extended if necessary, **OR**

Brincidofovir ≥ 48 kg: 200 mg susp or tab weekly x2, **OR**

IV Cidofovir protocol for treatment of smallpox or other orthopox infection:(IND)

1. Probenecid 2g PO 3 h before cidofovir infusion. Infuse 1L NS 1 h before cidofovir infusion.
2. If tolerated, infuse 2nd liter normal saline 1-3 h with/after cidofovir.
3. Cidofovir 5 mg/kg IV over 1 h.
4. Repeat probenecid 1g PO 2 h & 8 h after cidofovir infusion complete.

For select **Vaccine Adverse Reactions** (Eczema vaccinatum, vaccinia necrosum, ocular vaccinia w/o keratitis, severe generalized vaccinia): 1st choice - VIGIV (Vaccinia Immune Globulin, Intravenous) (Cangene Corporation) 6000U/kg IV infusion. 9000 U/kg for the patient that does not respond to the 6000 U/kg dose. See CDC guidelines at www.https://www.cdc.gov/smallpox/clinicians/vaccine-medical-management6.html

Note: VIG is NOT recommended for mild instances of accidental implantation, implantation-associated ocular keratitis, mild or limited generalized vaccinia, erythema multiforme, or encephalitis postvaccination),

OR Cidofovir 5 mg/kg IV infusion (as above),(IND)
OR Tecovirimat (TPOXX®) 600 mg/d PO with full glass of water & food for 14 d. May be extended if necessary.(IND)

COMMENTS

Pre- & post-exposure vaccination recommended if > 3 yrs since last vaccine.

Recommendations for use of smallpox vaccine in response to bio-terrorism are periodically updated by the CDC & the most recent recommendations can be found at http://www.cdc.gov.

In Nov 2021, ACIP unanimously voted in favor of JYNNEOS as an alternative to ACAM2000 for primary vaccination and booster doses for orthopoxvirus infection among persons at risk for such exposures. MMWR Morb Mortal Wkly Rep. 2022;71(22):734.Epub 2022 Jun 3.

Encephalitis Viruses

VACCINE

JE inactivated vaccine *JE-VAX*® (Sanofi-Pasteur) and JE inactivated vaccine *JE-VC* (Ixiaro), does not contain thimerosal.

CHEMOPROPHYLAXIS

None currently available.

CHEMOTHERAPY

No specific therapy. Treatment consists of corticosteroids, anticonvulsants, & supportive care measures.

COMMENTS

Previous IND vaccines under the DoD Special Immunizations Program are no longer available.

Hemorrhagic Fever Viruses

VACCINE

Yellow fever live-attenuated 17D vaccine, given as a single shot, with a booster dose every 10 yrs.

Ervebo (rVSV) vaccine for Ebola Zaire; single dose; booster doses to be determined.

Candid 1 AHF vaccine [(IND)] (Cross-protection for BHF).

Tick-Borne Encephalitis vaccine approved in Europe.

Hantavirus vaccine approved in the Republic of Korea, reported to be 75% effective after 3 doses.

CHEMOPROPHYLAXIS

Lassa fever & Crimean-Congo Hemorrhagic Fever (CCHF): Ribavirin optimal dose & duration unknown, not FDA approved for this use.

CHEMOTHERAPY

Ebola Zaire:

- Ebanga™ (Ansuvimab-zykl): Single IV infusion of 50mg/kg over 60minutes.
- Inmazeb® (Atoltivimab/maftivimab/odesivimab): Single IV infusion 50mg/kg of each component over 2-4 h based on weight.

Ribavirin for confirmed or probable/suspected arenavirus (Lassa fever), nairovirus (CCHF), hantavirus (Hemorrhagic Fever with Renal Syndrome [HFRS]), or VHF of unknown etiology: IND IV Ribavirin Protocols under Force Health Protection Division/USAMMDA for 1) HFRS & 2) CCHF or Lassa fever. IV ribavirin is not licensed by FDA & must be used either under a FHP Division protocol or under FDA's expanded access.

Decision to initiate ribavirin treatment will be based on epidemiological, clinical & clinical lab results as diagnostic lab results may not be available. Always rule out malaria before starting treatment.

Treatment of HFRS with IND ribavirin:
Adults:

- Loading dose: 30 mg/kg IV (max 2 g) once, **OR** 2000mg PO once; followed by:
  - Day 1-4: 16 mg/kg IV (max 1 g) q 6 h (16 doses total),
  - Day 5-7: 8 mg/kg IV (max 0.5 g) q 8 h (9 doses total), **OR**
  - Maintenance Oral: Wt > 75 kg 600 mg PO q 12 h for 10 d; Wt < 75 kg 400 mg PO in AM, 600 mg PO in PM for 10 d.

Pediatrics:

- Loading dose (peds): IV same as for adult, **OR** Oral 30 mg/kg PO one time; followed by:
- Maintenance dose (peds): IV same as for adult. Oral 15mg/kg every 5 h for 4 d; 7.5 mg/kg every 8 h for 6 d*.

Treatment of CCHF or Lassa fever with IND ribavirin:
Adults:

- Loading dose: 30 mg/kg (max dose: 2 g), **OR** 2000mg PO once; followed by:
  - Day 1-4: 16 mg/kg IV (max dose: 1 g) q 6 h (16 doses),
  - Day 5-10: 8mg/kg IV (max dose: 0.5 g) q 8 h (18 doses), **OR**
    - Administered in 50-100 mL Normal Saline over 30-40 min with an infusion pump.
  - Maintenance Oral: Wt > 75 kg 600 mg PO q 12 h for 10 d; Wt <75 kg 400 mg PO in AM, 600 mg PO in PM for 10 d.

Pediatrics: • Loading dose (peds): IV same as for adult, **OR** Oral 30 mg/kg PO one time; followed by: • Maintenance dose (peds): IV same as for adult. Oral 15mg/kg q 5 h for 4 d; 7.5 mg/kg q 8 h for 6 d*. *Recommended dosing from WHO.
COMMENTS
Aggressive supportive care & management of hypotension & coagulopathy are imperative.

TOXINS:

Botulinum Neurotoxin

VACCINE
None currently available.
CHEMOPROPHYLAXIS
DoD Botulinum Antitoxin, Heptavalent, Equine, Types A, B, C, D, E, F, and G (HE-BAT) (IND) In general, botulinum antitoxin is not used prophylactically. Under special circumstances, if the evidence of exposure is clear in a group of individuals, some of whom have well defined neurological findings consistent with botulism, treatment can be contemplated in those without neurological signs.
CHEMOTHERAPY
Heptavalent (A-G) equine botulinum antitoxin (H-BAT) (Cangene Corporation) available through the CDC. FDA-approved for use in the Strategic National Stockpile. BabyBIG™, California Health Department, types A & B Human lyophilized IgG, for treatment of infant botulism.
COMMENTS
Could need to perform skin test for hypersensitivity before equine antitoxin administration. Antitoxin levels observed 2-4 wks after dose 3 of the primary series (wk 13).

Ricin Toxin

VACCINE
None currently available.
CHEMOPROPHYLAXIS
None currently available.
CHEMOTHERAPY
None currently available.
COMMENTS
Inhalation: supportive therapy; Ingestion: gastric lavage, activated charcoal, supportive therapy.

Staphylococcus Enterotoxins

VACCINE
Modified recombinant SEB protein vaccine (STEBVax) completed Phase I trial. No licensed FDA vaccine available.
CHEMOPROPHYLAXIS
None currently available.
CHEMOTHERAPY
None currently available.
COMMENTS
Inhalation: supportive therapy. Ingestion: gastric lavage, cathartics.

Appendix K: Investigational Medical Products (INDs, etc.) & Emergency Use Authorizations (EUAs)

Overview

DoD Components are expected to use medical products (i.e., drugs, biologics, or devices) approved, licensed, or cleared by the U.S. Food and Drug Administration (FDA) for general commercial marketing, when available, to provide required medical countermeasures. Under current DoD policy, personnel will be provided the best available, operationally relevant medical countermeasures for chemical, biological, radiological, and nuclear (CBRN) threats per DoD Instruction (DoDI) 6200.02.

Drugs are chemical substances intended for use in the medical diagnosis, cure, treatment, or prevention of disease. *Biologics* are blood and blood products, vaccines, allergenics, cell and tissue-based products, and gene therapy products. A *medical device* is an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including a component part, or accessory intended for use in the diagnosis, prevention or treatment of disease.

Unapproved medical products, or approved medical products used "off-label" may be used as medical countermeasures under an Emergency Use Authorization (EUA), an Investigtional New Drug (IND) application, or investigational device exemption (IDE) issued by the FDA. This is appropriate when used under a force health protection program and only if compliant with the regulatory requirements below and with the approval of the Assistant Secretary of Defense for Health Affairs (ASD(HA)).

A medical product may be administered for a use not described in the labeling based on standard medical practice in the United States. "Standard medical practice" refers to the authority of an individual health care practitioner to prescribe any legally marketed medical product to a patient for any condition or disease within a legitimate health care practitioner-patient relationship. These instances fall outside of a DoD force health protection program. FDA regulatory requirements for INDs and EUAs apply to medical care provided to military and civilian DoD healthcare beneficiaries, DoD-affiliated personnel, and others receiving treatment at DoD medical treatment facilities located both CONUS and OCONUS.

EXECUTIVE ORDER 13139:
IMPROVING HEALTH PROTECTION OF MILITARY PERSONNEL PARTICIPATING IN PARTICULAR MILITARY OPERATIONS

On 30 September 1999, the President issued Executive Order 13139, outlining conditions where Investigational New Drug (IND) and off-label pharmaceuticals can be administered to U.S. service members. This handbook discusses numerous pharmaceutical products, some of which are available only under INDs.
In certain cases, licensed pharmaceuticals are discussed for use in a manner (or for a condition) other than that for which they were originally licensed (i.e., an “off-label" indication).

The executive order did not intend to alter the traditional physician-patient relationship or individual physician prescribing practices. Healthcare providers remain free to exercise clinical judgment and prescribe licensed pharmaceutical products as they deem appropriate for the optimal care of their patients. This policy does, however, potentially influence recommendations that might be made by U.S. Government agencies and that might be applied to large numbers of service members outside of the individual physician-patient relationship. The following text presents a brief overview of EO 13139 for the benefit of the individual provider.

EO13139 – Provides the Secretary of Defense guidance regarding the provision of IND products or products unapproved for their intended use as antidotes to chemical, biological, or radiological weapons; stipulates that the U.S. Government will administer products approved by the U.S. Food and Drug Administration only for their intended use; and provides the circumstances and controls under which IND products may be used.

To administer an IND product:

- Informed consent must be obtained from individual service members
- The President may waive informed consent (at the request of the Secretary of Defense and only the Secretary of Defense) if:
    - Informed consent is not feasible
    - Informed consent is contrary to the best interests of the service member
    - Obtaining informed consent is not in the best interests of national security

Investigational New Drugs (IND)

INDs are FDA applications to use unapproved drugs or biological products subject to FDA regulations under 21 CFR 312 and include:

- Drugs not approved, or biological products not licensed, by the FDA which
    - do not yet have permission from the FDA to be legally marketed and sold in the United States (“unapproved product”), or
    - are entirely new drugs, vaccines, or therapeutics not licensed by the FDA for any human use.

- Drugs unapproved for the applied use ("off-label"). These are FDA-approved drugs or licensed biological products administered for a use not described in the FDA-approved labeling of the drug or biological product ("unapproved use of an approved product")**.**

INDs can be made available under a number of mechanisms:

- As part of a clinical research study (see 21 CFR 312 for details)
- As part of an Expanded Access program (see 21 CFR 312 subpart I and the following):
    - http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM351261.pdf and
    - http://www.fda.gov/ForConsumers/ByAudience/ForPatientAdvocates/AccesstoInvestigationalDrugs/ucm176098.htm
- Under an Emergency Use Authorization (for details see below and refer to the following):
    - http://www.fda.gov/regulatoryinformation/guidances/ucm125127.htm
    - http://www.fda.gov/EmergencyPreparedness/Counterterrorism/ucm182568.htm

Investigational Device Exemptions (IDE)

An investigational device, including *in vitro* diagnostic tests, is a device which has not been approved or cleared for marketing by the FDA. Investigational devices are regulated under 21 CFR 812. There may be circumstances under which a health care provider may wish to use an unapproved device to save the life of a patient or to help a patient suffering from a serious disease or condition for which no alternative therapy exists. Patients/physicians faced with these circumstances may have access to investigational devices under one of five main mechanisms: emergency use, emergency research compassionate use, treatment use, continued access.

These mechanisms can be utilized during a certain timeframe in the IDE process if the criteria are met. FDA approval is required except in the case of emergency use. Details for each mechanism are defined under 21 CFR 812.36, and are described at http://www.fda.gov/medicaldevices/deviceregulationandguidance/howtomarketyourdevice/investigationaldeviceexemptionide/ucm051345.htm

Emergency Use Authorization (EUA)

An EUA is a special authority under U.S. federal law. The FDA issues an EUA to allow use of an "unapproved medical product" or an "unapproved use of an approved medical product" during a declared emergency by the Secretary of Health and Human Services (DHHS) involving a heightened risk of attack on

the public or military forces. An EUA is generally intended for situations affecting, or potentially affecting, many individuals (> 10,000).

Recent examples of using medical products under an EUA come from the medical response to the 2009 H1N1 pandemic influenza. The declaration of emergency issued by the DHHS Secretary justified the authorization of the emergency use of certain approved neuraminidase inhibitors for unapproved uses (i.e., oseltamivir and zanamivir) and use of an unapproved antiviral drug, peramivir.

Another example was the authorization of the emergency use of *in vitro* diagnostics for detection of 2009 H1N1 influenza virus. This EUA impacted DOD due to using these diagnostics on our deployed Joint Biological Agent Identification Diagnostic System (JBAIDS) platforms in theater. More recently, EUAs were granted for diagnostic testing for influenza H7N9 (2013) and the Middle Eastern Respiratory Syndrome Coronavirus (MERSCoV) (2013).

Recent changes included in the Pandemic and All Hazards Preparedness Reauthorization Act (PAHPRA) of 2013 allow, under specific conditions and regulatory requirements, medical countermeasures that are not FDA-approved or cleared to be pre-positioned for use in a declared emergency. This prepositioning can be supported by a pre-EUA submission to the FDA. This submission describes the design and manufacture of the product and provides all available safety and efficacy data for FDA review and is periodically updated to reflect new data. Acceptance of such a submission by FDA expedites response time in case of a declared emergency.

Refer to the FDA's online materials for further guidance on "Emergency Use Authorization of Medical Products":
http://www.fda.gov/regulatoryinformation/guidances/ucm125127.htm and
http://www.fda.gov/EmergencyPreparedness/Counterterrorism/ucm182568.htm

There are currently a large number of EUAs issued for treatments, preventives and diagnostics resulting from the response to SARS COV-2, and more recently MPox. These include vaccines, drugs, antibody therapeutics, diagnostics, and devices such as ventilators. A full list of current EUAs can be found here: https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization

Regulatory requirements for using INDs, IDEs, and products under an EUA

Investigational medical products are subject to FDA regulations 21 CFR 312, as amended (for drugs and biologics) and 21 CFR 809 and 812 (for devices), and for all military users, DoDI 6200.02 series. Use of products under an EUA for a force health protection program are subject to DoDI 6200.02, section 564 of the Federal Food, Drug, and Cosmetic Act [21 U.S.C.], sections 1107 and 1107a of title 10, U.S.C. and applicable FDA requirements. DoDI 6200.02 establishes

DoD policy, assigns responsibilities, and prescribes procedures concerning the application of FDA rules to DoD force health protection programs involving FDA unapproved medical products required to be used under an EUA, IND, or IDE application.

Current IND Medical Countermeasures
Current medical countermeasures administered as INDs by Force Health Protection Division (FHP), USAMMDA include vaccines, drugs, and immunoglobulins to prevent and/or treat diseases caused by biothreat agents and infectious diseases of military interest. Examples of drugs or biologics currently available under INDs include:

- **IV ribavirin** (Virazole®) is FDA-approved for the treatment of severe lower respiratory tract infections due to respiratory syncytial virus in hospitalized infants and young children. FHP can provide IV ribavirin under IND protocols to treat some forms of viral hemorrhagic fever (CCHF, Lassa fever) or hemorrhagic fever with renal syndrome (Hantavirus). Early treatment is critical. The protocol can be activated so that treatment can begin prior to obtaining a positive diagnostic laboratory test result.
- **Tecovirimat** (*TPOXX*®) is an FDA-approved oral antiviral drug for the treatment of smallpox and MPox. Tecorvirimat was previously available under an IND protocol for treatment of generalized vaccinia reaction and orthopox infections, but as of 2018 was FDA approved.
- **IV cidofovir (Vistide®) or brincidofovir (TEMBEXA®)** are licensed for treating cytomegalovirus retinitis in HIV patients. Brincidofovir is approved for treatment of Smallpox. While cidofovir is not approved for orthopox indications, it is commercially available and was previously available under a treatment IND for generalized vaccina and orthopox infections.
- **IV Pentostam®** (sodium stibogluconate) is approved to treat visceral leishmaniasis only through the CDC within CONUS. IV Pentostam is available to DOD personnel through IND protocol for visceral leishmaniasis.
- Antivenoms for snake bites.

To activate one of the IND indications, call the FHP 24/7 emergency line at 301-401-2768. For a full listing of all products currently available under FHP IND protocols or EUA applications, go to the USAMMDA Force Health Protection website. https://usammda.health.mil/index.cfm/fhp/overview

Receipt & Administration of INDs for Military Healthcare Providers
If an IND drug or biological product protocol exists already, contact USAMMDA FHP at 301-619-1104, 24/7 at 301-401-2768. E-mail to usarmy.detrick.medcom-usammda.list.fhp@mail.mil. If the use of the IND is indicated, USAMMDA will coordinate with the treatment site to ship the medical product.

There are several available options, depending on the specific product, to determine who will administer the IND product and where:

- Designate an investigator for the IND at the requesting site. The proposed investigator must meet eligibility criteria (completed training, signed FDA form 1572, CV, license and copy of protocol, etc.) and be approved by the Sponsor. This can be arranged through USAMMDA FHP Division.
- DOD may have pre-trained providers established at major MEDCENs to assist with treatment or potentially travel to the patient to administer the IND product.

If no satisfactory FDA-approved medical product is available as a medical countermeasure against a particular threat at the time of need under a force health protection program, contact USAMMDA FHP. USAMMDA FHP will coordinate with the appropriate entities to use an unapproved product under an IND application, or to initiate the request for an EUA to treat large populations. (DoDI 6200.02 series applies).

Process for obtaining VIG-IV, cidofovir and tecovirimat
VIGIV is licensed by FDA to treat complications of vaccinia vaccination, but not approved to treat mpox. CDC holds an expanded access IND protocol allowing use of stockpiled VIGIV for the treatment of orthopoxviruses (including mpox) in an outbreak. Information is available at https://www.cdc.gov/mpox/hcp/clinical-care/index.html

Military Health Care Providers VIG-IV stocks are prepositioned for DOD in CONUS and OCONUS. Contact your DOD Regional Vaccine Healthcare Centers (VHC) office during normal business hours or the DOD VHC Network's Vaccine Clinical Call Center 24/7 at 1-866-210-6469 to obtain VIG-IV. Military providers requesting cidofovir must consult with an infectious disease or allergy-immunology specialist. Consultations will be arranged via the DOD Vaccine Healthcare Centers (VHC) Network's Vaccine Clinical Call Center (866-210-6469, available 24/7). The infectious disease or allergy-immunology specialist physician, in consultation with the VHC, will contact the CDC Director's Emergency Operations Center (DEOC) at 770-488-7100 and consult with on-call staff in the Division of Bioterrorism and Response (BDPR). The CDC is the release authority for cidofovir under a CONUS IND protocol.

Civilian Health Care Providers Civilian health care providers should first contact their State Health Department when seeking consultation for civilian patients experiencing a severe or unexpected adverse event following smallpox vaccination or when requesting cidofovir. If further consultation is required, or cidofovir recommended, the physician and State Health Department can request consultation through the CDC Director's Emergency Operations Center as above.

Process for obtaining botulinum antitoxin
In 2013, FDA approved a new heptavalent botulinum antitoxin (HBAT, Cangene Corporation) to treat botulism and include in the Strategic National Stockpile (SNS). HBAT is the only botulinum antitoxin currently available in the U.S. for naturally occurring non-infant botulism and is available only from the CDC. Providers who suspect botulism in a patient should call their state health department's emergency 24-hour telephone number or the CDC DEOC (770-488-7100) to report, obtain clinical consultation and if indicated, request release of HBAT. To obtain BabyBIG® for suspected infant botulism, the patient's physician must contact the Infant Botulism Treatment and Prevention Program (IBTPP) on-call physician at (510) 231-7600 to review treatment indications. https://www.cdc.gov/botulism/php/national-botulism-surveillance/index.html

Other Biodefense Related Programs

DoD's Chemical and Biological Defense Program (CBDP)

Enacted by Congress in 1993, Public Law 103-160 created the DOD Chemical and Biological Defense Program (CBDP). The CBDP has implemented steps to assess and mitigate risks associated with emerging bio-agent and infectious disease threats, including analysis of non-traditional agents.

Defense Threat Reduction Agency (DTRA)
The Defense Threat Reduction Agency (DTRA) is the intellectual, technical and operational leader for the U.S. Department of Defense (DOD) and U.S. Strategic Command in the effort to combat biological, chemical and nuclear threats. DTRA efforts include basic and animal research on novel countermeasure product candidates and supporting scientific disciplines. While many disease threats have limited commercial markets, successful candidates will have "dual-use" potential for commonly occurring diseases to ensure the DOD has a steady supply of the needed countermeasures.

Biological Threat Reduction Program (BTRP)

The Cooperative Threat Reduction (CTR) program was established in 1991 to help states of the former Soviet Union safeguard and dismantle stockpiles of nuclear, chemical and biological weapons, related materials, and delivery systems. DTRA executes the CTR program in coordination with partner governments and other U.S. Government agencies. In addition to nuclear proliferation, the CTR works to safeguard dangerous bio-agents through the cooperative Biological Threat Reduction program (BTRP) and International Cooperative Biological Engagement Program (CBEP). BTRP and CBEP aim to eliminate, secure, detect, and surveil for especially dangerous pathogens. The BTRP helps build capacity in partner countries by improving detection, diagnostics, monitoring, and reporting of endemic and epidemic diseases. Both work to counter threats posed by select agents, related materials, expertise, other

emerging infectious disease risks to prevent these agents from reaching state or non-state actors.

Defense Advanced Research Projects Agency (DARPA)
Historically, bioweapons may be mass-produced as quickly as a year after discovery, while developing countermeasures takes many more years. Using current methods and technologies, researchers require decades of study to understand new threat agents. The gap between threat weaponization and developing prevention and/or treatment options leaves U.S. forces vulnerable. DARPA also manages the Advanced Manufacturing of Pharmaceuticals (AMP) program. AMP aims to create rapid, flexible, cost-effective production technology capable of producing millions of doses of countermeasures rapidly (within 3 months). DARPA's Rapid Threat Assessment (RTA) program provides information to speed medical countermeasure production.

Joint Program Executive Office Chemical, Biological, Radiological, Nuclear Defense (JPEO-CBRND)
The Joint Program Executive Office for Chemical, Biological, Radiological and Nuclear Defense (JPEO-CBRND) falls under the Office of the Secretary of Defense. JPEO-CBRND is the advanced clinical development acquisitions activity for CBRN countermeasures. As such, they manage products in the clinical stages of development to include Phase 1-3 clinical trials, commercial manufacturing scale-up, and fielding. JPEO's goals include diagnosing CBRN threats before the onset of symptoms, provide new, improved medical countermeasures to enable a single treatment for diverse threats, rapid medical countermeasure responses, and genomic sequencing.

Relevant Intra-agency Agreements
Public Law (PL) 115-92

On December 12, 2017, the President signed Public Law No. 115-92 (P.L. 115-92), an Act to amend the Federal Food, Drug, and Cosmetic Act (FD&C Act) to authorize additional emergency uses for medical products to reduce deaths and severity of injuries caused by chemical, biological, radiological, or nuclear (CBRN) agents or agents that may cause, or are otherwise associated with, an imminently life-threatening and specific risk to the U.S. military forces and for other purposes. P.L. 115-92 requires enhanced collaborations and communication between the U.S. Department of Defense (DoD) and the U.S. Food and Drug Administration (FDA) on DoD's medical product priorities (MPPs) for military emergencies.

P.L. 115-92 amended the FD&C Act to provide, among other things, specific policy for increased DoD-FDA collaboration on the development and availability of MPPs. In summary, P.L. 115-92:

- Expands FDA's emergency use authorization (EUA) authority under §564 of the FD&C Act to allow FDA to issue EUAs for emergency use of unapproved medical products or unapproved uses of approved medical products to address additional types of threats (beyond CBRN agents) related to attack with an "agent or agents that may cause, or are otherwise associated with, an imminently life-threatening and specific risk to the United States military forces";
- Allows the Secretary of Defense to request, and authorizes FDA to take specific actions to expedite the development of medical products, and the review of investigational submissions, applications for approval/licensure, and submissions/notifications for clearance for such medical products reasonably able to diagnose, prevent, treat, or mitigate a specific and life-threatening risk to the U.S. military; and requires semi-annual review between DoD and FDA on DoD's MPP portfolio and requires quarterly DoD-CBER meetings for CBER-regulated MPPs.

DHA Agreement #: DHA-2023-S-2440 – Memorandum of Agreement (MOA) between the Defense Health Agency and the United States Food and Drug Administration for data sharing and information collaboration.
The purpose of this agreement is to enhance information sharing, create more efficient inter-agency activities to promote product risk identification, validation and analysis, and collaborate to develop methods to evaluate safety, efficacy, and use of drugs, biologics, medical devices, and other regulated products. The MOA covers broad topics in product development to include:

- Pandemic response and preparedness including:
  - COVID-19 therapeutics approved and under emergency use authorization (EUA)
  - Patient characteristics for those treated under EUA
  - Linking SARS-COV2 variant data to patient health records to evaluate the safety and efficacy of vaccines and therapeutics
  - Estimate incidence of hospitalization after mAb treatments across SARS-COV2 variant types
- Biosimilar products – evaluate use patterns and health outcomes for biosimilars
- Pregnancy and lactation – maternal, TNF inhibitor exposure and pregnancy outcomes
- Therapeutics efficacy and safety – thromboembolism risk
- Method development and evaluation
- Drug utilization – provider characteristics, settings (e.g., Theater), medication errors, adverse events for drugs with Risk Evaluation and Mitigation Strategies (REMS)

Appendix L: Use of Drugs/Vaccines in Vulnerable Populations

Pediatric patients

Large-scale attacks on civilian targets, as well as collateral damage inflicted during armed conflict will undoubtedly involve pediatric victims who may be more susceptible than adults to the effects of certain biological, chemical, and radiological agents for a number of anatomic, physiologic, immunologic, developmental, and programmatic reasons:

1. Smaller mass means that children may be thrown farther in a blast, and a relatively large, unprotected abdomen and greater organ-to-body-mass ratio further increases the risk of serious injury following such an event.
2. A thinner and less-keratinized epidermis makes dermally-active chemical (but not generally biological) agents a greater risk to children than adults. It also makes children more susceptible to transcutaneous heat and fluid loss which may accompany the fever, chills, and diaphoresis associated with many infectious diseases.
3. A larger surface area per unit volume exacerbates these problems.
4. A small relative blood volume also makes children more susceptible to the volume losses associated with enteric infections such as cholera, and GI intoxications might be seen with exposure to the staphylococcal enterotoxins.
5. Children's high minute ventilation compared with that of adults increases the threat of agents delivered via the inhalational route. Their propensity for engaging in "high-energy" activity exacerbates this risk.
6. The fact that children live "closer to the ground" further compounds this effect when heavier-than-air substances are involved. Their propensity for putting fomites in their mouths risks additional exposure to these substances.
7. An immature blood-brain barrier may heighten the likelihood that bloodborne pathogens may gain access to the CNS. Similarly, it may increase the risk of CNS toxicity from nerve agents.
8. Developmental considerations, such as an inability to distinguish fantasy from reality, a lack of communication skills, and an inability to follow the directions of public safety personnel, make it less likely that a child would readily flee an area of danger, thereby increasing exposure to these various adverse effects.
9. Children have a unique susceptibility to certain potential bio-agents. While adults generally suffer only a brief, self-limited incapacitating illness following infection with Venezuelan equine encephalitis (VEE) virus, young children are more likely to experience seizures, permanent neurologic sequelae, and death. In the case of smallpox, waning herd

immunity may disproportionately affect children. Vaccine-induced immunity to smallpox probably diminishes significantly after 3 to 10 yrs. Although most adults are considered susceptible to smallpox, given that routine civilian immunization in the U.S. ceased in the early 1970s, older adults may have some residual protection from death, if not from the development of disease. Today's children are among the first to grow up in a world without any individual or herd immunity to smallpox.

10. Children may experience unique disease manifestations not seen in adults; suppurative parotitis is a common characteristic occurring among children with melioidosis but is not generally seen in adults with *Burkholderia pseudomallei* infection. Lassa, in young infants, can present as a unique 'swollen baby' syndrome, with a mortality rate as high as 75%. Children with plague may be more likely to develop meningitis as well as digital necrosis owing, perhaps, to their smaller vasculature.

11. Many of the drugs useful in treating such casualties are unfamiliar to pediatricians or have relative contraindications in childhood. The fluoroquinolones and tetracyclines are commonly cited as prophylactic and therapeutic agents of choice against anthrax, plague, tularemia, brucellosis, and Q fever. While both classes are often avoided in children, potential morbidity and mortality from these diseases far outweighs the minor risks associated with short-term use of these agents. Of note, ciprofloxacin received, as its first licensed pediatric indication, FDA approval for use in the prophylaxis of anthrax following inhalational exposure during a terrorist attack. Doxycycline and levofloxacin are now licensed specifically in children for the same indication and levofloxacin is also licensed for post-exposure prophylaxis of children against plague. Antibiotic dosing guidelines relevant to pediatric patients, pregnant women, and breastfeeding mothers are provided in Table K-1 below.

12. Immunizations potentially useful in preventing bio-agent–induced diseases often lack approval for use in pediatric patients. The currently available anthrax vaccine is licensed only for those between 18 and 65 yrs of age. The plague vaccine, currently out of production and probably ineffective against inhalational exposures, was approved only for individuals aged 18 to 61 yrs. The ACAM 2000 smallpox vaccine, a live product employing vaccinia virus, can cause fetal vaccinia and demise when given to pregnant women (see below). Jynneos® is a newer, live but non-replicating, vaccine licensed for the prevention of both smallpox and monkeypox in adults 18 years and older but might be utilized in younger children under an Emergency Use Authorization (EUA).

13. Some useful pharmaceutical agents are not available in pediatric dosing regimens. The military utilizes the "Antidote Treatment Nerve Agent Autoinjector" (ATNAA), consisting of atropine and pralidoxime combined in a single syringe, for the rapid treatment of nerve agent casualties. Many emergency departments and some ambulances stock a civilian version known as Duodote®, but these are approved only for

adults and children weighing >41 kg. (although separate atropine autoinjectors specifically formulated for children have been approved by the FDA).

14. Although physical protective measures and devices (e.g., "gas masks") are likely to be of little utility in a civilian bio-terrorism setting, such commercially available devices are often unavailable in pediatric sizes. Additionally, Israeli experience during the first Gulf War suggests that frightened parents may improperly use such masks on their children, resulting in inadvertent suffocation.
15. In the event of a large-scale bioterrorist attack, there may be an insufficient number of pediatric hospital beds. In any large disaster, excess bed capacity might potentially be provided at civilian and Veterans Affairs hospitals under the auspices of the National Disaster Medical System, but that system makes no specific provision for pediatric beds.

Nursing mothers

Many pharmaceuticals are excreted in breast milk (see Table 1) and may thus be ingested by nursing infants. Such medications, if contraindicated in infants, should be avoided by breastfeeding mothers whenever possible. It is generally recommended that fluoroquinolones, tetracyclines, and chloramphenicol be avoided by nursing mothers. As these drugs may represent the treatment of choice for many bio-agents, practitioners must weigh the risks of administering these drugs against the potential adverse consequences of using a less effective medication. In some cases, temporary cessation of nursing while taking the offending drug may be necessary. Antibiotics generally considered safe in nursing mothers include the aminoglycosides, penicillins, cephalosporins, and macrolides.

Pregnant patients

Many medications approved for safe use in adults may pose a risk to the developing fetus. In 2015, the FDA retired its letter-based (A,B,C,D,X) drug safety categorization scheme. Clinicians should consult specific FDA guidance provided for any drug they contemplate using in a pregnant patient.

Tetracyclines and fluoroquinolones often constitute empiric therapies of choice for many bio-agent diseases yet remain relatively contraindicated during pregnancy. Animal studies indicate that tetracyclines can retard skeletal development in the fetus; embryotoxicity has also been described in animals treated early in pregnancy. There are few adequate studies of fluoroquinolones in pregnant women; existing published data, albeit sparse, do not demonstrate a substantial teratogenic risk associated with fluoroquinolone use during pregnancy. In cases for which either fluoroquinolones or tetracyclines are recommended for initial empiric prophylaxis (e.g., inhalational anthrax, plague, or tularemia), tolerated fluoroquinolone may represent the lower risk option. After antibiotic susceptibility data are available, antibiotics should be switched to lower risk alternatives if possible.

Live vaccines (e.g., measles-mumps-rubella) are also generally contraindicated during pregnancy, although vaccine risks must be weighed against the risk of disease (to both the vaccinated mother and her fetus). For example, the administration of the ACAM2000 smallpox vaccine (vaccinia) to pregnant women presents a very tangible risk to the fetus (in the form of fetal vaccinia infection), although that risk is likely to be less than the risk of maternal smallpox.

The immunocompromised patient

Immunocompromised individuals may be more susceptible to diseases caused by bio-agents or may develop more severe disease than immunocompetent patients. Nonetheless, consensus groups generally recommend using the same antimicrobial regimens recommended for their immunocompetent counterparts. One important difference in the management of immunocompromised patients concerns the receipt of live vaccines, such as the currently licensed ACAM2000 smallpox vaccine. Generally, it is best to manage these individuals on a case-by-case basis and in concert with immunologists and/or infectious disease specialists.

Table L-1. Antimicrobials in Special Populations

Class of Drug	Drug name	Breast milk	Standard Pediatric oral dosing regimens[1, 2]	Standard Pediatric parenteral dosing regimens[1, 2]
	Gentamicin	(+) small		3 - 7.5 mg/kg/d in 3 doses (IV or IM)
	Amikacin	(+) small		15 - 22.5 mg/kg/d in 3 doses (max 1.5 g/d) (IV or IM)
	Streptomycin	(+) small		30 mg/kg/d in 2 doses (max 2 g/d)(IM only)
Aminoglycosides	Tobramycin	(+) small		3 - 7.5 mg/kg/d in 3 doses (IV or IM)
	Imipenem	(?)		60 mg/kg/d in 4 doses (max 4 g/d) (IV or IM)
Carbapenems	Meropenem	(?)		60-120 mg/kg/d in 3 doses (max 6 g/d) (IV)
	Ceftriaxone	(+) trace		80 - 100 mg/kg in 1 or 2 doses (max 4 g/d) (IV or IM)
	Ceftazidime	(+) trace		125-150 mg/kg/d in 3 doses (max 6 g/d) (IV or IM)
	Cephalexin	(+) trace	25-50 mg/kg/d in 3-4 doses	
	Cefuroxime	(+) trace	20-30 mg/kg/d in 2 doses (max 2 g/d)	100-150 mg/kg/d in 3 doses (max 6 g/d) (IV or IM)
Cephalosporins	Cefepime	(+) trace		150 mg in 3 doses (max 4 g/d) (IV or IM)
Chloramphenicol	Chloramphenicol	(+)	50-100 mg/kg/d in 4 doses (formulation not avail in U.S.)	50-100 mg/kg/d in 4 doses (max 4 g/d) (IV)
	Ciprofloxacin	(+)	30 mg/kg/d in 2 doses (max 1.5 g)	20-30 mg/kg/d in 2 doses (max 1 g/d)(IV)
Fluoroquinolones	Levofloxacin	(+)	16 mg/kg/d in 2 doses	16 mg/kg/d in 2 doses (IV)
Glycopeptides	Vancomycin	(+)		40-60 mg/kg/d in 4 doses (max 4 g/d) (IV)
Lincosamides	Clindamycin	(+)	10-20 mg/kg/d in 3-4 doses (max 1.8 gm/d)	25-40 mg/kg/d in 3-4 doses (max 2.7 g/d) (IV or IM)

Class of Drug	Drug name	Breast milk	Standard Pediatric oral dosing regimens[1, 2]	Standard Pediatric parenteral dosing regimens[1, 2]
Lipopeptides	Daptomycin	(?)		4 mg/kg once daily (IV)
Macrolides	Azithromycin	(+)	5-12 mg/kg/d once daily (max 600 mg/d)	
	Clarithromycin	(?)	15 mg/kg/d in 2 doses (max 1 g/d)	
	Erythromycin	(+)	30-50 mg/kg/d in 2-4 doses (max 2 g/d)	15-50 mg/kg/d in 4 doses (max 4 g/d) (IV)
Monobactams	Aztreonam	(+) trace		90-120 mg/kg/d in 3-4 doses (max 8 g) (IV or IM)
Oxalodinones	Linezolid	(+)	20-30 mg/kg/d in 3 doses (max 800/mg/d)	20-30 mg/kg/d in 3 doses (max 1200/mg/d)(IV)
Penicillins	Amoxicillin	(+) trace	25-90 mg/kg/d in 3 doses (max 1.5 g/d)	
	Ampicillin	(+) trace	50-100 mg/kg/d in 4 doses (max 4 g/d)	200-400 mg/kg/d in 4 doses (max 12 g/d) (IV or IM)
	Penicillin G	(+) trace		25,000-400,000 U/kg/d in 4-6 doses (max 24 mil U/d) (IV or IM)
	Nafcillin	(+) trace		100-150 mg/kg/d in 4 doses (max 12 g) (IV or IM)
Rifampin		(+)	10-20 mg/kg/d in 1-2 doses (max 600 mg/d)	10-20 mg/kg/d in 1-2 doses (max 600 mg/d)
Streptogramins	Dalfopristin-Quinupristin	(+)		22.5 mg/kg/d in 3 doses (IV)
Sulfonamides	Trimethoprim/ Sulfamethoxazole	(+) trace	8-12 mg/kg/d TMP in 4 doses (max 320 mg/d TMP)	8-12 mg/kg/d TMP in 4 doses (IV)
Tetracyclines	Doxycycline	(+)	2-4 mg/kg/d in 1-2 doses (max 200 mg/d)	2-4 mg/kg/d in 1-2 doses (max 200 mg/d)(IV)
	Tetracycline	(+)	20-50 mg/kg/d in 4 doses (max 2 g)	10-25 mg/kg/d in 2-4 doses (max 2 g) (IV)
Brincidofovir		(+)	>10 kg: 6 mg/kg once weekly for 2 doses; 10-48 kg: 4 mg/kg once weekly for 2 doses; >48 kg: 200 mg once weekly for 2 doses	

Class of Drug	Drug name	Breast milk	Standard Pediatric oral dosing regimens[1, 2]	Standard Pediatric parenteral dosing regimens[1, 2]
Cidofovir		(?)		5 mg/kg once with probenecid & hydration
Oseltamivir		(+)	1-12 yrs old: ≤15 kg: 30 mg twice daily; 15-23 kg: 45 mg 2X/d; 23-40 kg: 60 mg 2X/d; >40 kg: adult dose	
Ribavirin		(?)	30 mg/kg once, then 15 mg/kg/d in 2 doses (VHFs)	Same as for adults, dosed by weight (IV)
Tecovirimat		(+)	13-25 kg: 200 mg twice daily; 25-40 kg: 400 mg twice daily; 40-120 kg: 600 mg twice daily; >120 kg: 600 mg every 8 hours	3-35 kg: 6 mg/kg every 12 hours by IV infusion over 6 hours; 35-120 kg: 200 mg every 12 hours by IV infusion over 6 hours; >120 kg: 300 mg every 12 hours by IV infusion over 6 hours
NB: (1) The above doses are for children outside of the neonatal period. Neonatal doses may be different. (2) Pediatric antibiotic doses included in this table represent generic doses for severe disease. They may not accurately reflect expert consensus for treatment for anthrax, plague, or tularemia. For those diseases, refer to the specific chapter for recommendations.				

Appendix M-1: Indicated Clinical Specimens for Bio-agent Laboratory Diagnosis

Disease	Face or Nasal Swab [B]	Blood Culture [E]	Smear [F]	Acute & Convalescent Sera	Stool	Urine	Other
Anthrax	+	+	Pleural fluid & CSF; mediastinal lymph node; spleen	+	+/-	-	Cutaneous lesion aspirates or 4mm punch biopsy, toxin detection
Brucellosis	+	+	-	+	-	-	Bone marrow and blood are the most effective for culture
Glanders & Melioidosis	+	+	Sputum and abscess aspirates	+	-	+/-	Abscess culture
Plague	+	+	Sputum	+	-	-	Bubo aspirate, CSF, sputum, lesion scraping, lymph node aspirate. Never dissect bubo.
Tularemia	+	+	+	+	-	-	

Q-fever	+	[D]	Lesions	+	-	-	Lung, spleen, lymph nodes, bone marrow biopsies
Venezuelan Equine Encephalitis [A]	+	[C]	-	+	-	-	CSF
Viral Hemorrhagic Fevers [A]	+	[C]	-	+	-	-	Liver
Botulism: *C. botulinum* toxins (A-G)	+	-	Wound tissues	+	+/-	-	Serum or other fluids for toxin detection/ mouse bioassay
Staphylococcus Enterotoxin B	+	-	-	+	+	+	Lung, kidney
Ricin Toxin	+	-	-	+	+	+	Spleen, lung, kidney
T-2 Mycotoxins	+	-	-	-	+	+	Serum, stool, or urine for metabolites

Notes:

[A] All specimens collected for viral examination should be placed into universal or viral transport media [1, 2]

[B] Swabs should all be Nylon, Rayon, or Dacron heads with plastic stems [3]

[C] Virus isolation from blood or throat swabs in appropriate containment.

[D] *C. burnetii* can persist for days in blood and resists desiccation. EDTA anti-coagulated blood preferred. Culturing should not be done except in biosafety level-3 containment.

[E] All blood for culture should be collected from $\geq$ 2 different sites (e.g. left arm and right arm) to control for possible skin contamination

[F] All collected sputum specimens should be graded for acceptance to rule out possible presence of normal mouth flora

Appendix M-2: Medical & Environmental Bio-agent Sample Collection, Packaging & Shipment

This appendix provides guidance in determining clinical and environmental samples to collect from exposure to biological threat agents and the timing of collection. It also covers general testing methodology, packaging and shipment, and the collection of environmental samples from suspect sites. Practical and regulatory parameters for packaging and shipping collected specimens are outlined.

Proper collection of clinical specimens from patients in the context of possible bio-agent exposure/infection is dependent upon the time that has elapsed since the apparent exposure. Time-frames for sample collection can be succinctly categorized as "Early post-exposure," "Clinical," and "Convalescent/Terminal/Postmortem."

- **Early post-exposure** – period immediately after exposure to a bio-agent (aerosol or otherwise); aggressively attempt to obtain samples as indicated.
- **Clinical** – period when individuals are presenting with clinical symptoms.
- **Convalescent/Terminal/Post-mortem** – period of convalescence, terminal stages of infection, toxicosis, or post-mortem (e.g., during autopsy).

These timeframes are not rigid and will vary according to the concentration of the agent used, the agent strain, predisposing health factors of the patient and other considerations. Tables L-2-1, L-2-2 and L-2-3 present recommend timing of sample collections for bacteria/rickettsia, toxins, and viruses, respectively.

Shipping Clinical Samples
To maintain integrity, specimens sent within 24 hours to analytical labs require blue or wet ice or refrigeration at 2-8˚C. If the time span increases beyond 24 hours or if other procedural questions do arise, contact the USAMRIID "Hot-Line" (1-888-USA-RIID) for pertinent questions.

Blood samples. Blood sample collection is dependent on the type of testing being performed. Several choices are offered based on availability of the blood collection tubes and testing requested. Do not send blood in all the tubes listed in the attached tables, rather verify with your testing location on validated testing methods and their specimen requirements. This is intended as a guide for standard practices. Tiger-top tubes that have been centrifuged are preferred over red-top clot tubes with serum removed from the clot, but the latter will suffice. Blood culture bottles are usually preferred over citrated blood for bacterial cultures, but make sure that specimens are collected from two different sites (such as left and right arm) to mitigate blood contamination with skin flora.

Pathology specimens. Post-mortem, routinely includes liver, lung, spleen, and regional or mesenteric lymph nodes. Additional samples requested are as follows: brain tissue for encephalomyelitis cases (although fatality is rare), adrenal gland for Ebola/Marburg cases (not absolutely required) and bone marrow. Culture of bone marrow for brucellosis has higher sensitivity than blood culture.[1]

Fixative considerations. If the transit time is short and/or refrigerated, specimens can be sent in sterile normal saline or a sterile container. A 10% buffered formalin is the standard pathology fixative, however, it will prevent any cell culture because infections are frequently not or only intermittently bacteremic. Formalin is an excellent tissue penetrator, but it can interfere with PCR and RT-PCR.[2, 3] Alcohols also produce excellent tissue histology, although pathologists are not used to testing samples immersed in alcohol. Alcohols have low tissue penetration, so tissue samples should be sliced thin (3-4 mm) or minced for fixation. The volume of any fixative (formalin, alcohol etc.) should be several times the volume of tissue and submerse sample entirely.

The gold standard for storage of PCR samples is at -70˚C or in liquid nitrogen; obviously liquid nitrogen may not always be readily available outside of fixed facilities. There are also specialized products available: Ambion's RNAlater® is a tissue preservative for RNA at room temperature.[4] Biomatrica® has a full range of products for room temperature storage of samples for molecular testing.[5] Specialized products may not be necessary, however, especially in a field-expedient situation. DNA and RNA viruses have been shown to be detectable by PCR/real time-PCR even after six months of room temperature storage in alcohol. This was demonstrated in 100% ethanol but would probably work in other alcohols.[6]

Regulatory requirements. The world has changed since the WHO Smallpox Eradication Program routinely shipped and carried thousands of live smallpox samples without creating any concern or incidents, which was normal for all diagnostic and research samples. It was said in those days that samples were carried VIP ("Virus in Pocket"). Since the 2001 anthrax letter mailings, several new sets of laws and regulations from multiple authorities that control shipment of biological samples have been imposed. Although written for a study of insect vector samples, Coleman *et al.*[7] provides an excellent summary. It is exceedingly difficult to obtain reliable shipping advice for biological pathogens, particularly the Biological Select Agents and Toxins (BSATs, or SAs). The regulations are often complex, the certifications needed are difficult to obtain, and the procedures can be baffling. The effect of this complexity could impede research, put patients at medical risk, and/or place medical personnel at legal risk. Laboratory and shipper hesitation could result in a compromise of specimen integrity, such as thawing at border check points, hindrance at State boundaries, etc. Post 9/11 bio-defense legislation has resulted in more extensive regulations of SA research and/or surveillance work that affects how SAs are collected, stored, secured, and shipped.[8] All of these factors must be integrated into the sampling and specimen transportation process and awareness for planning purposes is of significant importance.

With these impediments in mind, there appear to be three basic approaches available to people left with the responsibility to do practical work: (1) Send the samples as "general diagnostic samples" without testing, or with only preliminary testing (or presumptive clinical diagnosis of a patient). It must be noted that these samples will most likely still fall within the category of hazardous material/dangerous goods: infectious substances/toxins. (2) Fix or otherwise kill the samples rendering them suitable only for molecular analysis, serology, or staining methods, but not any kind of assay requiring a live organism. (3) Ship samples which have been identified as SAs under the required safeguards and permits in accordance with prescribed public statutes and DOD directives. Utilization of couriers on military aircraft or the medical evacuation chain may facilitate the process, though it doesn't obviate regulatory requirements. Coordination with the Laboratory Response Network (LRN) and/or the Defense Laboratory Network (DLN) can also assist this process.[9]

There are several numerous regulations that govern the packaging, shipment, and receipt of SAs and/or infectious substance practices: 1) 49 CFR Parts 100-185, 2) International Air Transport Association: Dangerous Goods Regulations (IATA), 3) DoD Regulations 4500.9-R, 4) Air Force Manual 24-204, 5) International Maritime Organization Dangerous Goods Code 36-12 (IMDG), 5) 42 CFR Part 73, 6) 7 CFR Part 331, 7) 9 CFR Part 121, 8) and Army Regulation 50-1. Within all of these regulations, the materials of interest to this reading audience are broken down into the following categories of decreasing generality:(1) hazardous materials/dangerous goods, (2) infectious substances/toxins, or (3) BSATs. Thus, a BSAT is considered both an infectious substance/toxin and a hazardous material/ dangerous good. 49 CFR Parts 100-185 outline the procedures and policies for packaging and receiving dangerous goods, particularly dangerous infectious substances/toxins. IATA outlines the guidelines adopted by the commercial airline industry for transport of hazardous materials/dangerous goods, particularly infectious substances/toxins, but, most importantly, lists those air carriers that will and will not transport and what their individual requirements are. DOD R4500.9-R dictates to DOD personnel the procedures for moving hazardous material/ dangerous goods, including infectious substances/toxins, in accordance with U.S. Federal law and DOD policies and delineates the responsible parties with roles and responsibilities. AFMAN 24-204 applies both U.S. Federal law and DoD R4500.9-R to movement of hazardous materials/dangerous goods via military aircraft and delineates the responsible parties with roles and responsibilities. IMDG Code 36-12 describes the guidelines for movement of hazardous materials/dangerous goods via surface movement at sea. 42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121 are the regulatory statutes that describe and control all aspects of BSATs from the perspective of U.S. Federal law, the Centers for Disease Control and Prevention (CDC), and the U.S. Department of Agriculture (USDA) Animal Plant Health Inspection Service (APHIS). Finally, AR 50-1 integrates U.S. Federal law with DOD/Army policy to build a framework of directives for U.S. Army personnel dealing with BSATs, particularly safety and security.[10-14]

While the various regulations provide clear guidance on BSAT procedures used within or into the U.S., very little guidance exists regarding the packaging and shipment of BSATs in specimens during military deployments or other OCONUS contingency operations. Current practice during military deployments is to implement procedures that best meet the intent of relevant U.S. BSAT laws and regulations. However, this may be mitigated by existing partner nation laws and/or regulations if they exist. An important consideration during military deployments is whether a diagnostic specimen is considered a hazardous material/infectious substance and/or a BSAT (i.e., Class 6.1 or 6.2 Dangerous Good: 49 CFR Parts 101-185), as determination triggers a variety of specific actions/responses. In general, diagnostic specimens are considered to contain BSATs if they fall under the definitions set by 42 CFR 73.3. Moreover, strong guidelines for procedures to identify specimens as highly suspicious and reportable have been set from a collaboration between the CDC, Association of Public Health Laboratories, and American Society for Microbiology, known as Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases.[15] In a field environment during combat/contingency operations, the DoD identified four levels of identification for bio-agents: presumptive, field confirmatory, theater validation, and definitive.[16] *Presumptive* employs one method of identification and results dictate whether further analysis and reporting needs to be performed. *Field confirmatory* employs two methods from the same technology and results dictate possible further reporting, further analysis, and certain initiated medical actions. *Theater validation* employs two methods from two complementary, but different, technologies and results dictate further reporting and analysis, wider initiation of medical actions, and commencement of force health protection measures. *Definitive* employs more than two different methods from different technologies aimed at fully characterizing the biological threat and guiding future strategic and operational medical decisions and force health protection measures.

Specimen preparation procedures frequently inactivate any biological pathogens that are present; even though a confirmatory assay identified a particular pathogen, that sample would not be considered BSAT as no viable pathogen is present. Lab personnel should exercise caution when making a determination that a positive specimen is not a BSAT as it is extremely difficult in a field setting to determine whether a viable pathogen is present. Additionally, any portion of the diagnostic sample that did not undergo nucleic acid extraction or other sterilizing procedures may, inevitably, still contain viable BSATs. Nucleic acid from positive-stranded RNA viruses can be used to produce infectious virus and is considered a BSAT according to 42 CFR 73.3. Military personnel conducting diagnostic testing for BSATs should understand the rules, regulations, and statutes pertaining to BSATs and how they could pertain to combat/contingency operations. Individual unit criteria and SOPs for determining whether specimens contain a BSAT must be well articulated, and clearly define procedures for securing, transporting, and destroying these specimens in accordance with Army, DOD, and U.S. laws and regulations. Personnel conducting diagnostic testing must understand that specimens that do not meet established criteria of a BSAT may still pose a considerable safety and force health protection threat from any

exposure. Personnel regularly interacting with potential infectious substances should also be fully aware of classification schemes for these substances (for purposes of packaging and transportation) and safety precautions.

Environmental Samples

Environmental specimens should be collected as soon as possible after recognition of a bio-agent release to determine the nature of a bio-aerosol or other delivery system. The sooner the environmental specimen is taken (in conjunction with early post-exposure clinical samples) the less difficult it will be to identify the agent and become aware of all the important factors surrounding the exposure.

Specimens taken well after an attack may still allow identification of the agent used. While this information would likely be too late to inform useful prophylactic measures, it may be used, when combined with other information, for intelligence purposes, the gathering of forensic evidence, the future development of countermeasures, and the prosecution of war crimes or other criminal proceedings. Although not strictly a medical responsibility, such sample collection issues are the same as for during, or shortly after the attack, and medical personnel may be the only personnel with the requisite specimen collection expertise on site.

If time and conditions permit, medical post-exposure planning and risk assessments should be performed. As in any hazardous materials situation, a clean line and exit/entry strategy should be designed for post exposure mitigation. Depending on the situation, personnel protective equipment (PPE) should be donned. The standard M40 gas mask and Mission Oriented Protective Posture (MOPP) level 4 is effective protection against bio-agent exposure. If it is possible to have a clean line, then a three-person team is recommended, with one clean and two dirty. The former would help decontaminate the latter. Specimens may be used in a criminal prosecution. Specimen collection should be documented both written and with pictures to include who, what, where, when, why, and how. Take into consideration that documentation materials may need to be decontaminated later, thus will have to be rugged and resistant to such treatment. The types of samples taken can be extremely variable. Some of the possible samples are:

- Aerosol collections in buffer solutions
- Soil
- Swabs
- Dry powders
- Container of unknown substance
- Vegetation
- Food / water
- Body fluids or tissues

Aerosol collection during an attack would be ideal, assuming you have the appropriate collection device; otherwise, anything that appears to be contaminated can be either sampled with swabs if available, or with absorbent paper or cloth. The item itself could be collected if not too large. After an attack, samples from dead animals or human remains can be taken (refer to Appendix M-3, *Laboratory Assays for Bio-agent Identification*, for appropriate specimens). All samples should ideally be double bagged in *Ziploc*® bags (the outside of the inner bag decontaminated with dilute bleach before placing in the second bag) labeled with time and place of collection along with any other pertinent data.

Table M-2-1 Bacteria and Rickettsia: Timing of Sample Collection

Early Post-Exposure	Clinical	Convalescent/ Terminal/ Postmortem
Anthrax Bacillus anthracis 0 – 24 h Nasal & throat swabs, induced respiratory secretions for culture1, FA & PCR.	24 to 72 h Serum (TT, RT) for toxin assays; Blood (E, C, H) for PCR; Blood (BC, C) for culture.2	3 to 10 d Serum (TT, RT) for toxin assays; Blood (BC, C) for culture; Pathology samples.
Plague Yersinia pestis 0 – 24 h Nasal swabs, sputum, induced respiratory secretions for culture, FA & PCR.	24 – 72 h Blood (BC, C) & bloody sputum for culture & FA (C); F-1 Antigen assays (TT, RT), PCR (E, C, H).	>6 d Serum (TT, RT) for IgM later for IgG; Pathology samples.
Tularemia Francisella tularensis 0 – 24 h Nasal swabs, sputum, induced respiratory secretions for culture, FA & PCR.	24 – 72 h Blood (BC, C) for culture; Blood (E, C, H) for PCR; Sputum for FA & PCR.	>6 d Serum (TT, RT) for IgM & later IgG, agglutination titers; Pathology samples.

BC: Blood culture bottle	E: EDTA (3-ml)	TT: Tiger-top (5 – 10 ml)
C: Citrated blood (3-ml)	H: Heparin (3-ml)	RT: Red top if no TT

1: Sputum specimens for culture should always be evaluated/scored for contamination with saliva. Negative results from sputum specimens not graded to be clinically relevant could still be positive but may not be perceived as such due to salivary contamination.17

2: Blood for culture should be collected in the appropriate blood culture media and should be collected from > 2 different sites to control for contamination. No more than 3 sets of blood culture specimens should be taken in a 24 h period.

Early Post-Exposure	Clinical	Convalescent/ Terminal/ Postmortem
Glanders *Burkholderia mallei* 0 – 24 h Nasal swabs, sputum, induced respiratory secretions for culture & PCR.	24 – 72 h Blood (BC, C) for culture; Blood (E, C, H) for PCR; Sputum & drainage from skin lesions for PCR & culture.	>6 d Blood (BC, C) & tissues for culture; Serum (TT, RT) for immunoassays; Pathology samples.
Brucellosis *Brucella abortus*, *suis, & melitensis* 0 – 24 h Nasal swabs, sputum, induced respiratory secretions for culture & PCR.	24 – 72 h Blood (BC, C) for culture; Blood (E, C, H) for PCR.	>6 d Blood (BC, C) & tissues for culture; Serum (TT, RT) for immunoassays; Pathology samples.
Q-Fever Coxiella burnetii 0 – 24 h Nasal swabs, sputum, induced respiratory secretions for culture & PCR.	2 to 5 d Blood (BC, C) for culture in eggs or mouse inoculation; Blood (E, C, H) for PCR.	>6 d Blood (BC, C) for culture in eggs or mouse inoculation; Pathology samples.

BC: Blood culture bottle
C: Citrated blood (3-ml)

E: EDTA (3-ml)
H: Heparin (3-ml)

TT: Tiger-top (5 - 10 ml)
RT: Red top if no TT

Table M-2-2 Toxins: Timing of Sample Collection

Early Post-Exposure	Clinical	Convalescent/ Terminal/ Postmortem
Botulism Botulinum toxin from *Clostridium botulinum* 0 – 24 h Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) & toxin assays; Serum (TT, RT) for toxin assays.	24 to 72 h Nasal swabs, respiratory secretions for PCR (contaminating bacterial DNA) & toxin assays.	>6 d Usually no IgM or IgG; Pathology samples (liver & spleen for toxin detection).
Ricin Intoxication Ricin toxin from castor beans 0 – 24 h Nasal swabs, induced respiratory secretions for PCR (contaminating castor bean DNA) & toxin assays; Serum (TT) for toxin assays.	36 to 48 h Serum (TT, RT) for toxin assay; Tissues for immunohisto-logical stain in pathology samples.	>6 d Serum (TT, RT) for IgM & IgG in survivors.
Staph enterotoxicosis Staphylococcus Enterotoxin B 0 – 3 h Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) & toxin assays; Serum (TT, RT) for toxin assays.	2 - 6 h Urine for immunoassays; Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) & toxin assays; Serum (TT, RT) for toxin assays.	>6 d Serum for IgM & IgG; Note: Only paired antibody samples will be of value for IgG assays…most adults have antibodies to staph enterotoxins.
T-2 toxicosis 0 – 24 h postexposure Nasal & throat swabs, induced respiratory secretions for immunoassays, HPLC/ mass spectrometry (HPLC/MS).	1 to 5 d Serum (TT, RT), tissue for toxin detection	>6 d post-exposure Urine for detection of toxin metabolites

BC: Blood culture bottle
C: Citrated blood (3-ml)

E: EDTA (3-ml)
H: Heparin (3-ml)

TT: Tiger-top (5 - 10 ml)
RT: Red top if no TT

Table M-2-3 Viruses: Timing of Sample Collection

Early Post-Exposure	Clinical	Convalescent/ Terminal/ Postmortem
Equine Encephalomyelitis VEE, EEE and WEE viruses 0 – 24 h Nasal swabs & induced respiratory secretions for RT-PCR & viral culture (in viral transport media).	24 to 72 h Serum & throat swabs for culture (TT, RT), RT-PCR (E, C, H, TT, RT) & Antigen ELISA (TT, RT), CSF, Throat swabs up to 5 d	>6 d Serum (TT, RT) for IgM; Pathology samples plus brain.
Ebola/Marburg 0 – 24 h Nasal swabs & induced respiratory secretions for RT-PCR & viral culture (in viral transport media).	2 to 5 d Serum (TT, RT) for viral culture.	>6 d Serum (TT, RT) for viral culture; Pathology samples plus adrenal gland.
Pox (Smallpox, monkeypox) Orthopoxvirus 0 – 24 h Nasal swabs & induced respiratory secretions for PCR & viral culture (in viral transport media).	2 to 5 d Serum (TT, RT) for viral culture.	>6 d Serum (TT, RT) for viral culture; Drainage from skin lesions/ scrapings for microscopy, EM, viral culture, PCR; Pathology samples.

BC: Blood culture bottle
C: Citrated blood (3-ml)

E: EDTA (3-ml)
H: Heparin (3-ml)

TT: Tiger-top (5 - 10 ml)
RT: Red top if no TT

Appendix M-3: Laboratory Assays for Bio-agent Identification

Disease	Agent	Gold Standard	Antigen Detection	IgG	IgM	PCR
Anthrax	*Bacillus anthracis*	Culture[1]	X	X	X	X
Brucellosis	*Brucella* spp.	Culture[1]	X	X	X	X
Glanders	*Burkholderia mallei*	Culture[1]		X	X	X
Melioidosis	*Burkholderia pseudomallei*	Culture[1]	X	X	X	X
Plague	*Yersinia pestis*	Culture or 4-fold increase in Ab titer to F1 antigen[1]	X	X	X	X
Tularemia	*Francisella tularensis*	Culture (Chocolate Agar or BYCE) or 4 fold increase in Ab titer to *F. tularensis* antigen[1]	X	X	X	X
Q Fever	*Coxiella burnetii*	Acute: 4-fold increase in Ab titer to *C. burnetii*. Chronic: IFA to phase I antigen >1:800[1]	X	X	X	X
Smallpox	Orthopoxviruses	Reference laboratory testing only; generally PCR assays[1]	X	X		X
Venezuelan Equine Encephalitis	VEE virus	Paired Sera Serology or Virus Specific IgM in sera or CSF[2]	X	X	X	X

Viral Hemorrhagic Fevers	Filoviruses	Serology/PCR[3]	X	X	X	X
	Hantaviruses	Serology/PCR[3]	X	X	X	X
Botulism	Bot Toxins (A-G)/ *Clostridium botulinum*	Toxin Present in Sera (Serology Test) or Isolation of *C. botulinum* from sample[1]	X			*
Saxitoxin	Saxitoxin	HPLC-MS[4]				X
Staph Enterotoxin B	SEB Toxin	ELISA[4]	X	X		*
Ricin	Ricin Toxin	ELISA[4]	X	X	X	X
T-2 Mycotoxins	T-2 Mycotoxins	LC-MS or HPLC-MS[4]	X			
Tetrodototoxin	Tetrodotoxins	HPLC-MS[4]	X			

* Toxin gene detected – only works if cellular debris including genes present as contaminant. Purified toxin does not contain detectable genes.
See Glossary (App. A) for acronyms/initialisms. Not all the indicated assays are available in field laboratories.

Appendix M-4: Laboratory Response Network (LRN)

https://www.cdc.gov/laboratory-response-network/php/about/index.html

History

In 1995, a growing global concern about the potential use of biological and chemical agents in acts of terrorism prompted the United States government to act. Presidential Decision Directive 39 was issued, outlining a U.S. policy on counterterrorism, directing how Federal agencies work together to carry out counter-terrorism policy and respond to any attacks on U.S. soil. This directive specifically recognized the need for a coordinated national effort to address these emerging threats. In response to a subsequent congressional mandate, the Laboratory Response Network (LRN) was established by the Department of Health and Human Services (HHS) and Centers for Disease Control and Prevention (CDC). The CDC, in partnership with the Federal Bureau of Investigation (FBI) and the Association of Public Health Laboratories (APHL), created the LRN for the purpose of strengthening the nation's capacity to provide immediate and sustained laboratory testing and communication in the event of public health emergencies, particularly in response to acts of bioterrorism.

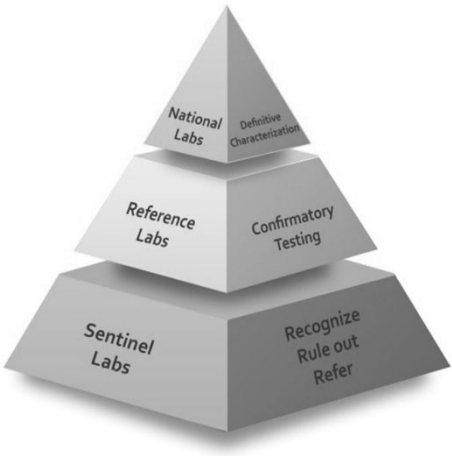
Growth and Evolution of the LRN

From the time of its establishment in 1999 to the present, the LRN has been successful in integrating the public and private health laboratory communities, in addition to expanding its original focus of medical laboratories to military laboratories, veterinary laboratories, agricultural laboratories, food and water testing laboratories, and international laboratories. The original designation of laboratories as Levels A, B, C, and D was revised to Sentinel, Reference, and National laboratories. They now function as an integrated network, with the major goal being to ensure that the nation's public health and private sector laboratories, along with other select laboratories, are prepared and equipped to respond to a biological or chemical act of terrorism in an appropriate and integrated manner. Its objective was to ensure an effective laboratory response to bioterrorism by helping to improve the nation's public health laboratory infrastructure, which had limited ability to respond to bioterrorism. Today, the LRN is an integrated network of state and local public health, federal, military, and international laboratories that can respond to bioterrorism, chemical terrorism and other public health emergencies. The LRN is a unique asset in the nation's growing preparedness for biological and chemical terrorism. The LRN is divided into the Laboratory Response Network for Biological Threats (LRN-B) which detects biological threats and emerging infectious diseases and the Laboratory Response Network for Chemical Threats (LRN-C) which detects and responds to chemical terrorism and public health emergencies.

LRN Mission

"The LRN is a national security asset that, with its partners, will develop, maintain and strengthen an integrated domestic and international network of laboratories to respond quickly to biological, chemical, and radiological threats and other high priority public health emergency needs through training, rapid testing, timely notification and secure messaging of laboratory results.

National Laboratories
LRN-B National laboratories, include those operated by CDC, U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), and the National Bioforensics Analysis Center (NBFAC). They are responsible for specialized characterization of organisms, bioforensics, select agent activity, and handling highly infectious biological agents.



Reference Laboratories
LRN-B member laboratories are responsible for investigation and/or referral of specimens. There are approximately 120 domestic state and local public health, military, veterinary, agriculture, and food laboratories. These laboratories receive reagents, protocols, and specialized training to perform testing for multiple agents in high risk environmental or clinical samples. Reference laboratories also provide training and guidance to thousands of sentinel laboratories across the U.S.

Sentinel Laboratories
LRN-B Sentinel laboratories play a key role in the early detection of biological agents. Sentinel laboratories provide routine diagnostic services, rule-out, and referral steps in the identification process. These laboratories can evaluate whether samples should be sent to an LRN reference laboratory for further testing. The LRN works with the American Society for Microbiology and state public health laboratory directors to ensure that sentinel laboratories, such as private and commercial laboratories, are part of the LRN. There are an estimated 25,000 private and commercial laboratories in the United States. Most of these laboratories are hospital-based, clinical institutions, and commercial diagnostic laboratories.

NOTE: If believe that you have been exposed to a biological or chemical agent, or if you believe an intentional biological threat will occur or is occurring, please contact your local health department and/or your local police or other law enforcement agency.

Local public health laboratories (LRN), private laboratories, and commercial laboratories with questions about the LRN should contact their state public health laboratory director.

CDC Office of Laboratory Systems and Response
Division of Laboratory Systems
Laboratory Readiness and Informatics Branch
Mail Stop H24-2
1600 Clifton Road NE,
Atlanta, GA 30333

CDC National Center for Environmental Health
Division of Laboratory Sciences
Emergency Response Branch
Mail Stop S102-2
4770 Buford Highway NE,
Atlanta, GA 30341

Association of Public Health Laboratories
8515 Georgia Ave, Suite 700
Silver Springs, MD 20910
Website: www.aphl.org
Email: info@aphl.org

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