USAMRIID's MEDICAL MANAGEMENT OF BIOLOGICAL CASUALTIES HANDBOOK

Sixth Edition
April 2005

U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FORT DETRICK
FREDERICK, MARYLAND

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PREFACE TO THE SIXTH EDITION

The Medical Management of Biological Casualties Handbook, which has become affectionately known as the "Blue Book," has been enormously successful - far beyond our expectations. Since the first edition in 1993, the awareness of biological weapons in the U.S. has increased dramatically. Over 180,000 copies have been distributed to military and civilian health-care providers around the world, primarily through USAMRIID's on-site and road Medical Management of Biological Casualties course and its four annual satellite broadcasts on this subject.

This sixth edition has been revised and updated. New chapters on plague and viral hemorrhagic fevers have been added as well as two new appendices on the use of investigational new drugs (IND) and antibiotic and vaccine considerations in special populations. References have been expanded and updated for those interested in more in-depth reading on this subject.

Our goal is to make this a reference for the health-care provider on the front lines, whether on the battlefield or in a clinic, who needs basic summary and treatment information quickly. We believe we have been successful in this regard. We want your feedback so that we might make future editions more useful and readable. Thank you for your interest in this important subject.

-The Editors

ACKNOWLEDGMENTS

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DISCLAIMER

The purpose of this handbook is to provide concise supplemental reading material to assist health-care 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 FM 8-284), the information contained in this handbook is **not** official Department of the Army policy or doctrine, and should not be construed as such.

As you review this handbook, you will find specific therapies and prophylactic regimens for the diseases mentioned. The majority of these are based upon standard treatment guidelines; however, some of the regimens noted may vary from information found in standard reference materials. The reason for this is that the clinical presentation of certain diseases caused by a weaponized biological agent may vary from the endemic form of the disease. For ethical reasons, human challenge studies can only be performed with a limited number of these agents. Therefore, treatment and prophylaxis regimens may be derived from in vitro data, animal models, and limited human data. Occasionally you will find various investigational new drug (IND) products mentioned. They are often used in the laboratory to protect health-care workers. These products are not available commercially, and can only be given under a specific protocol with informed consent. For guidelines on the use of IND products, see appendix **L.** IND products are mentioned for the scientific completeness of the handbook, and are not necessarily to be construed as recommendations for therapy.

EXECUTIVE ORDER 13139:
IMPROVING HEALTH PROTECTION OF MILITARY PERSONNEL PARTICIPATING IN PARTICULAR MILITARY OPERATIONS

On 30 September 1999, the President of the U.S. issued Executive Order 13139, which outlines the conditions under which IND and off-label pharmaceuticals can be administered to U.S. service members. This handbook discusses numerous pharmaceutical products, some of which INDs. In certain other 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).

This executive order does not intend to alter the traditional physician-patient relationship or individual physician prescribing practices. Health-care providers remain free to exercise clinical judgement 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 Food and Drug Administration only for their intended use;

•Provides the circumstances and controls under which IND products may be used.

In order 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.

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INTRODUCTION

Medical defense against the use of biological pathogens and toxins as weapons of warfare or terrorism is an area of study previously unfamiliar to many health-care providers. The U.S. military has maintained an ongoing research agenda against biological weapon threats since World War II, but the terrorist attacks on the U.S. mainland in September 2001 and the anthrax mail attacks in October 2001 provided a wake-up call for lawmakers, the public at large, and medical providers of all backgrounds that the threat of biological attacks was real and required planning, training, and resources for response. Consequently, there has been an explosion of interest among health-care practitioners to understand better how to manage the medical consequences of exposure to biological weapons that can lead to mass casualties.

Numerous measures to improve preparedness for and response to biological warfare (BW) or terrorism are ongoing at local, state, and federal levels. Training efforts have increased in both military and civilian sectors. A week-long Medical Management of Chemical and Biological Casualties Course taught at both USAMRIID and USAMRICD trains over 560 military medical professionals each year on biological and chemical medical defense. The highly successful USAMRIID international satellite courses on the Medical Management of Biological Casualties have reached over 110,000 medical personnel since 1997.

Through this handbook and courses noted above, medical professionals learn that effective medical countermeasures are available against many of the bacteria, viruses, and toxins that might be used as biological weapons against our military forces or civilian communities. The importance of this education cannot be overemphasized and it is hoped that health-care professionals will develop a solid understanding of the biological threats we face and the effective medical defenses against these threats.

The global BW threat is serious, and the potential for devastating casualties is high for certain biological agents. There are more than 10 countries around the world suspected to have offensive biological weapons programs. However, with early recognition, intervention, and appropriate use of medical countermeasures either already developed or under development, many casualties can be prevented or minimized.

Even if providers have not read the text thoroughly, the purpose of this handbook is to serve as a concise pocket-sized manual that can be pulled off the shelf (or from a pocket) in a crisis to guide medical personnel in the prophylaxis and management of biological casualties. It is designed as a quick reference and overview, and is not intended as a definitive text on the medical management of biological casualties. More in-depth discussion of the agents covered here may be found in infectious diseases, tropical medicine, and disaster management textbooks.

HISTORY OF BIOLOGICAL WARFARE AND CURRENT THREAT

The use of biological weapons in warfare has been recorded throughout history. During the 12th –15th centuries BC the Hittites are known to have driven diseased animals and people into enemy territory with the intent of initiating an epidemic. In the 6th century BC, the Assyrians poisoned enemy wells with rye ergot, and Solon used the herb hellebore to poison the water source of the city of Krissa during his siege. In 1346, plague broke out in the Tartar army during its siege of Kaffa (at present day Feodosia in Crimea). The attackers hurled the corpses of plague victims over the city walls; the epidemic that followed forced the defenders to surrender, and some infected people who left Kaffa may have started the Black Death pandemic, which spread throughout Europe and is believed to have resulted in the death of one-third of the population of Europe – as many as 25 million people. Russian troops may have used the same tactic against the Swedes in 1710.

On several occasions throughout history, smallpox was used as a biological weapon. Pizarro is said to have presented South American natives with variola virus-contaminated clothing in the 15th century, and the English did the same when Sir Jeffery Amherst ordered his troops to provide Indians loyal to the French with smallpox-laden blankets in 1763 towards the close of the French and Indian Wars. Native Americans defending Fort Carillon sustained epidemic casualties, which directly contributed to the loss of the fort to the English. General George Washington ordered variolation (an early form of smallpox vaccination) for the Continental Army in 1777 after the loss of the siege of Quebec, in part due to devastation rendered on his forces by smallpox, and because of the potential for purposeful spread of smallpox among the colonials by the British.

Use of biological weapons continued into the 1900s; however, the stakes became higher as the science of microbiology allowed for a new level of sophistication in producing agents. There is evidence that during World War I, German agents inoculated horses and cattle with anthrax and glanders at the Port of Baltimore before the animals were shipped to France. In 1937, Japan started an ambitious biological warfare (BW) program, located 40 miles south of Harbin, Manchuria, code-named "Unit 731." Studies directed by Japanese general and physician Shiro Ishii continued there until it was destroyed in 1945. A post-World War II investigation revealed that the Japanese researched numerous organisms and used prisoners of war as research subjects. About 1,000 human autopsies apparently 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 - some have estimated up to 3,000 human deaths. The Japanese also apparently used biological agents in the field: after reported overflights by Japanese planes suspected of dropping plague-infected fleas, plague epidemics ensued in China and Manchuria. By 1945, the Japanese program had stockpiled 400 kilograms of anthrax to be used in a specially designed fragmentation bomb.

In 1943, the U.S. began its own research and development program in the use of biological agents for offensive purposes. Similar programs existed in Canada, the United Kingdom (UK), and probably several other countries. This work was started, interestingly enough, in response to a perceived German BW threat as opposed to a Japanese one. The U.S. research program was headquartered at Camp Detrick (now Fort Detrick), which was a small National Guard airfield before that time, and produced agents and conducted field testing at other sites until 1969, when President Nixon stopped all offensive biological and toxin weapon research and production by executive order. Between May 1971 and May 1972, all stockpiles of biological agents and munitions from the now defunct U.S. program were destroyed in the presence of monitors representing the U.S. Department of Agriculture, the Department of Health, Education, and Welfare, (now Health and Human Services), and the states of Arkansas, Colorado, and Maryland. Included among the destroyed agents were *Bacillus anthracis*, botulinum toxin, *Francisella tularensis*, *Coxiella burnetii*, Venezuelan equine encephalitis virus, *Brucella suis*, and staphylococcal enterotoxin B. The U.S. began a medical defensive program in 1953 that continues today at USAMRIID.

In 1972, the U.S., UK, and USSR signed the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, commonly called the Biological Weapons Convention. Over 140 countries have since added their ratification. This treaty prohibits the stockpiling of biological agents for offensive military purposes, and also forbids research on agents for other than peaceful purposes. To strengthen efforts to combat the BW threat, signatory states agreed in November 2002 to have experts meet annually through 2006 to discuss and promote common understanding and effective action on biosecurity, national implementation measures, suspicious outbreaks of disease, disease surveillance, and codes of conduct for scientists. However, despite this historic agreement among nations, BW research continued to flourish in many countries hostile to the U.S. Moreover, there have been several cases of suspected or actual use of biological weapons. Among the most notorious of these were the “yellow rain” incidents in Southeast Asia, the use of ricin as an assassination weapon in London in 1978, and the accidental release of 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 were thought to be comprised of trichothecene toxins (in particular, T2 mycotoxin). These attacks are grouped under the label “yellow rain.” There has been a great deal of controversy about whether these clouds were truly BW agents. Some have argued that the clouds were nothing more than feces produced by swarms of bees.

In 1978, a Bulgarian exile named Georgi Markov 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 while he was waiting for a bus. 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 supplied by the former Soviet Union.

In April, 1979, an incident occurred in Sverdlovsk (now Yekaterinburg) in the former Soviet Union which appeared to be an accidental aerosol release of *Bacillus anthracis* spores from a Soviet military microbiology facility: Compound 19. Residents living downwind from this compound developed high fever and had difficulty breathing; a large number 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 United States 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 in fact related to military developments at the microbiology facility. In 1994, Meselson and colleagues published an in-depth analysis of the Sverdlovsk incident. They documented that all of the cases from 1979 occurred within a narrow zone extending 4 kilometers downwind in a southerly direction from Compound 19. There were at least 66 fatalities in the 77 patients identified.

In August, 1991, the U.N. carried out its first inspection of Iraq's BW capabilities in the aftermath of the Gulf War. On August 2, 1991, representatives of the Iraqi government announced to leaders of U.N. Special Commission Team 7 that they had conducted research into the offensive use of *B. anthracis*, botulinum toxins, and *Clostridium perfringens* (presumably one of its toxins). This open admission of biological weapons research verified many of the concerns of the U.S. intelligence community. Iraq had extensive and redundant research facilities at Salman Pak and other sites, many of which were destroyed during the war.

In 1995, further information on Iraq's offensive program was made available to United Nations inspectors. Iraq conducted research and development work on anthrax, botulinum toxins, *Clostridium perfringens*, aflatoxins, wheat cover smut, and ricin. Field trials were conducted with *B. subtilis* (a simulant for anthrax), botulinum toxin, and aflatoxin. Biological 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). The extent of Iraq's biological weapons program between 1998 when UNSCOM left Iraq and the U.S. coalition invasion in March 2003 remains unknown. Current information indicates the discovery of a clandestine network of biological laboratories operated by the Iraqi Intelligence Service (Mukhabarat), a prison laboratory complex possibly used for human experimentation, an Iraqi scientist's private culture collection with a strain of possible BW interest, and new research activities involving *Brucella* and Crimean-Congo hemorrhagic fever virus.

The threat of BW has increased in the last two decades, with a number of countries working on the offensive use of these agents. The extensive program of the former Soviet Union is now primarily under the control of Russia. Former Russian president Boris Yeltsin stated that he would put an end to further offensive biological research; however, the degree to which the program was scaled back is not known. Revelations from Ken Alibek, a senior BW program manager who defected from Russia in 1992, outlined a remarkably robust BW program, which included active research into genetic engineering, binary biologicals and chimeras, and capacity to produce industrial quantities of agents. There is also growing concern that the smallpox virus, lawfully stored in only two laboratories at the Centers for Disease Control and Prevention (CDC) in Atlanta and the Russian State Centre for Research on Virology and Biotechnology, may be in other countries around the globe.

There is intense concern in the west about the possibility of proliferation or enhancement of offensive programs in countries hostile to the western democracies, due to the potential hiring of expatriate Russian scientists. Iraq, Iran, and Syria have been identified as countries "aggressively seeking" nuclear, biological, and chemical weapons. Libya was also included; however, Libya has recently renounced further pursuit of offensive programs.

The 1990s saw a well-placed increasing concern over the possibility of the terrorist use of biological 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 had released botulinum toxin (1993 and 1995) and anthrax (1995) from trucks and rooftops. Fortunately, these efforts were unsuccessful. The Department of Defense 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 in 2000. 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 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 and the Dirksen Senate Office Building in Washington, D.C. in February, 2004.

The National Strategy for Homeland Security and the Homeland Security Act of 2002 were developed in response to the terrorist attacks. The Department of Homeland Security (DHS), with over 180,000 personnel, was established to provide the unifying foundation for a national network of organizations and

institutions involved in efforts to secure the nation. Over $8 billion from the DHS has been awarded since March, 2003 to help first responders and state and local governments to prevent, respond to and recover from potential acts of terrorism and other disasters. 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 U.S. Department of Health and Human Services or the U.S. Department of Agriculture; and new steps were imposed to limit access to various biological threat agents. Smallpox preparedness was implemented, including a civilian vaccination program, vaccine injury compensation program, and aid to the States. Prior to the March 2003 invasion of Iraq, state and local health departments and hospitals nationwide conducted smallpox immunizations of healthcare workers and have since prepared statewide bioterrorism response plans.

The threat of the use of biological weapons against U.S. military forces and civilians is more acute than at any time in U.S. history, due to the widespread availability of agents, widespread knowledge of production methodologies, and potential dissemination devices. Therefore, awareness of and preparedness for this threat will require the education of our government officials, health-care providers, public health officials, and law enforcement personnel and is vital to our national security.

DISTINGUISHING BETWEEN NATURAL AND INTENTIONAL DISEASE OUTBREAKS

Determining who is at risk and making appropriate decisions regarding prophylaxis as well as other response measures after a biological weapon attack, whether in a civilian setting as bioterrorism or on the battlefield as biological warfare (BW), will require the tools of epidemiology. With a covert attack, the most likely first indicator of an event will be an increased number of patients presenting to individual care providers or emergency departments with clinical features caused by the disseminated disease agent. The possibility exists that the recognizing authority for something unusual may be other medical professionals, such as pharmacists or laboratorians, who may receive more than the usual numbers of prescriptions or requests for laboratory tests from a number of different care providers. Because animals may be sentinels of disease in humans and many of the high-threat BW agents discussed in this book are zoonoses, it is also possible that veterinarians might recognize an event in animals before it is recognized in humans.

A sound epidemiologic investigation of a disease outbreak, whether natural or human-engineered, will assist medical personnel in identifying the pathogen and lead to the institution of appropriate medical interventions. Identifying the affected population, possible routes of exposure, signs and symptoms of disease, along with rapid laboratory identification of the causative agents, will greatly increase the ability to institute an appropriate medical and public health response. Good epidemiologic information can guide the appropriate follow-up of those potentially exposed, as well as assist in risk communication and responses to the media.

Many diseases caused by weaponized biological agents present with nonspecific clinical features that may be difficult to diagnose and recognize as a biological attack. Features of the epidemic may be important in differentiating between a natural and a terrorist or warfare attack. Epidemiologic clues that may indicate 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 a biological attack may have none. However, if many of the listed clues are recognized, one's index of suspicion for an intentionally spread outbreak should increase.

Once a biological attack or any outbreak of disease is suspected, the epidemiologic investigation should begin. Although, the conduct of the investigation will not differ significantly whether the outbreak is intentional or not, there are some important differences. Because the use of a biological weapon is a criminal act, it will be very important for the evidence gathered to be able to stand up to scrutiny in court. Therefore, samples must be handled through a chain of custody and there must be good communication and information sharing between public health and law-enforcement authorities. In addition, because the attack is intentional, one must be prepared for the unexpected – there is the possibility of multiple outbreaks at different locations as well as the use of

multiple different agents, including mixed chemical and biological agents or multiple biological agents depending upon the intentions of the perpetrator.

The first step in the investigation is to confirm that a disease outbreak has occurred. Because an outbreak generally means there is a higher rate of an illness than is normally seen in a specific population, then it is helpful to have background surveillance data to determine whether what is being seen constitutes a deviation from the norm. 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. In addition, even a single case of a very unusual illness, such as inhalation anthrax, might constitute an outbreak and should be viewed with 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 [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, especially as the potential exists for hysteria and subjective complaints to be confused with actual disease.

Once the attack rate has been determined, the outbreak can be described by time, place, and person. These data will 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 most likely in a biological attack or terrorism situation, individuals are exposed to the disease agent in a fairly short time frame. The early parts of the epidemic curve may be compressed compared to a natural disease outbreak. In addition, the incubation period could be shorter than what is seen with a natural outbreak if individuals are exposed to higher inoculums of the agent than would occur in the natural setting. The peak may occur in days or even hours. 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 determining effective disease control measures.

Once the disease is recognized, appropriate prophylaxis, treatment, and other measures to decrease disease spread, such as isolation (if needed for a contagious illness) would be instituted. The ultimate test of whether control measures are effective is whether they reduce ongoing illness or spread of disease.

Before any event, public health authorities must implement surveillance systems so they can recognize patterns of nonspecific syndromes that could indicate the early manifestations of a BW 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 variation should be followed up promptly with a directed examination of the facts regarding the change. In the past several years, many public health authorities around the country have initiated such syndrome-based surveillance systems in an attempt to achieve near real-time detection of unusual events.

In summary, it is important to understand that the recognition of and preparation for a biological attack will be similar to that for any infectious disease outbreak, but the surveillance, response, and other demands on resources will likely be of an unparalleled intensity. 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 epidemiologic investigating capability, practical training programs, and preparedness plans are essential to prevent and control disease outbreaks, whether they are naturally occurring or intentional.

Table 1. **Epidemiologic Clues of a BW or Terrorist Attack**

- The presence of a large epidemic with 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 exposures
- A disease that is 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 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 exact genetic type among agents isolated from distinct sources at different times and 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
- Disease outbreaks of the same illness occurring in noncontiguous areas
- A disease outbreak with zoonotic impact
- 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)

TEN STEPS IN THE MANAGEMENT OF BIOLOGICAL CASUALTIES ON THE BATTLEFIELD

Military medical personnel will require a firm understanding of certain key elements of biological defense to manage effectively the consequences of a biological attack amidst the confusion expected on the modern battlefield. Civilian providers who might be called upon to respond to a terrorist attack require a similar understanding. Familiarity with the behavior, pathogenesis, modes of transmission, diagnostic modalities, and available treatment options for each of the potential agents thus becomes imperative. Acquiring such an understanding is relatively straightforward once the identity of the agent is known; many references (FM 8-9, FM 8-33, FM 8-284), including this handbook, exist to assist medical personnel in agent-based therapy. A larger problem presents itself when the identity of a causative agent is unknown. In some cases, an attack may be threatened, but it may remain unclear as to whether such an attack has actually occurred. Similarly, it may be unclear whether casualties are due to the intentional release of a biological agent or a chemical agent, or whether they are due to a naturally occurring infectious disease outbreak or an accidental toxic industrial exposure. We recommend here a ten-step process to guide medical personnel in the evaluation and management of outbreaks of unknown origin and etiology. We feel that such an algorithmic approach (as exemplified by the Advanced Trauma Life Support Course (ATLS) sponsored by the American College of Surgeons) is desirable and will be helpful when dealing with the unknown, especially under the austere conditions and chaos expected on the modern battlefield.

I. Maintain an index of suspicion. In the case of chemical or conventional warfare and terrorism, the sinister nature of an attack might be obvious. Victims would likely succumb in close temporal and geographic proximity to a dispersal or explosive device. Complicating discovery of the sinister nature of a biological attack, however, is the fact that biological agents possess inherent incubation periods. These incubation periods, typically days to even weeks long, permit the wide dispersion of victims (in both time and space). Moreover, they make it likely that the 'first responder' to a biological attack would not be the traditional first responder (fire, police, and paramedical personnel), but rather medics, primary care physicians, emergency room personnel, and public health officials. In such circumstances, the maintenance of a healthy 'index of suspicion' is imperative.

Additionally, with many of the biological warfare (BW) diseases, very early treatment is mandatory if patients are to be successfully treated. Anthrax, botulism, plague, and smallpox are readily prevented if patients are provided proper antibiotics, antisera, and/or vaccination promptly after exposure. Conversely, all of these diseases may prove fatal if therapy or prophylaxis is delayed until classic symptoms develop. Unfortunately, symptoms in the early, or prodromal, phase of illness are non-specific, making diagnosis difficult. Moreover, many potential BW diseases, such as brucellosis, Q-fever, and Venezuelan equine encephalitis (VEE), may present simply as undifferentiated

febrile illnesses. Without a high index of suspicion, it is unlikely that medical personnel, especially at lower echelons of care, removed from sophisticated laboratory and preventive medicine resources, will promptly arrive at a proper diagnosis and institute appropriate therapy.

II. Protect yourself. Before medical personnel approach a potential biological casualty, they must first take steps to protect themselves. These steps may involve a combination of physical, chemical, and immunologic forms of protection. On the battlefield, physical protection typically consists of a protective mask. Designed primarily with chemical vapor hazards in mind, the M-40 series mask certainly provides adequate protection against all aerosolized BW threats. In fact, a HEPA-filter (or even a simple surgical) mask will often afford adequate protection against biological agents, although not against chemical threats. Chemical protection refers, in general, to the pre- and/or post-exposure administration of antibiotics; such strategies are discussed on an agent-specific basis elsewhere in this book. Immunologic protection principally involves active vaccination and, in the present climate, applies mainly to protection against anthrax and smallpox. Again, specific vaccination strategies are discussed throughout this book.

III. Assess the patient. This initial assessment is somewhat analogous to the primary survey of ATLS management. As such, airway adequacy should be assessed and breathing and circulation problems addressed before attention is given to specific management. The initial assessment is conducted before decontamination is accomplished and should thus be brief, but the need for decontamination and for the administration of antidotes for rapid-acting chemical agents (nerve agents and cyanide) should be determined at this time. Historical information of potential interest to the clinician should also be gathered, and might include information about illnesses among other unit members, the presence of unusual munitions, food and water procurement sources, vector exposure, vaccination history, travel history, occupational duties, and MOPP status. Physical exam at this point should concentrate on the pulmonary and neuromuscular systems, as well as unusual dermatologic and vascular findings.

IV. Decontaminate as appropriate. Decontamination plays a very important role in the approach to chemical casualty management. The incubation period of biological agents, however, makes it unlikely that victims of a BW attack will present for medical care until days after an attack. At this point, the need for decontamination is minimal or non-existent. In those rare cases where decontamination is warranted, simple soap and water bathing will usually suffice. Certainly, standard military decontamination solutions (such as hypochlorite), 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 biological agents. Routine use of caustic substances, especially on human skin, however, is rarely warranted after a biological attack. More information on decontamination is included elsewhere in this text.

V. Establish a diagnosis. With decontamination (where warranted) accomplished, a more thorough attempt to establish a diagnosis can be carried out. This attempt, somewhat analogous to the secondary survey used in the ATLS approach, should involve a combination of clinical, epidemiological, and laboratory examinations. The amount of expertise and support available to the

clinician will vary at each echelon of care. At higher echelons, a full range of laboratory capabilities might enable prompt definitive diagnoses. At lower echelons, every attempt should be made to obtain diagnostic specimens from representative patients and forward these through laboratory channels. Nasal swabs (important for culture and polymerase chain reaction (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 should be obtained.

Respiratory	**Casualties**
Rapid-Onset	**Delayed-Onset**
Nerve Agents	**Inhalational Anthrax**
Cyanide	**Pneumonic Plague**
Mustard	**Pneumonic Tularemia**
Lewisite	**Q Fever**
Phosgene	**SEB Inhalation**
SEB Inhalation	**Ricin Inhalation**
	Mustard
	Lewisite
	Phosgene
Neurological	**Casualties**
Rapid-Onset	**Delayed-Onset**
Nerve Agents	**Botulism-Peripheral Symptoms**
Cyanide	**VEE-CNS symptoms**

Table 1. Diagnostic Matrix: Chemical & Biological Casualties

While awaiting laboratory confirmation, a physician must attempt to clinically diagnose the infection. Access at higher echelons to infectious disease, preventive medicine, and other specialists, can assist in this process. At lower echelons, the clinician should, at the very least, be familiar with the concept of syndromic diagnosis. Chemical and BW diseases can be generally divided into those that present “immediately” with little or no incubation or have latent periods (principally the chemical agents) and those with a considerable delay in presentation (principally the biological agents). Moreover, BW diseases are likely to present as one of a limited number of clinical syndromes. Plague, tularemia, and staphylococcal enterotoxin (SEB) disease all may present as pneumonia. Botulism and VEE may present with peripheral and central neuromuscular findings, respectively. This allows the construction of a simple diagnostic matrix as shown in Table 1. Even syndromic diagnosis, however, is complicated by the fact that many BW diseases (VEE, Q-fever, brucellosis) may present simply as undifferentiated febrile illnesses. Moreover, other diseases (anthrax, plague, tularemia, smallpox) have undifferentiated febrile prodromes.

VI. 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 might be indicated under certain circumstances. Table 2 was constructed by eliminating from consideration those diseases for which definitive therapy is not warranted, not available, or not critical. Empiric treatment of respiratory casualties (patients with undifferentiated febrile illnesses who might have prodromal anthrax, plague, or tularemia would all be managed similarly) might then be entertained. 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. Keep in mind that such therapy is, in no way, a substitute for a careful and thorough diagnostic evaluation, when conditions permit such an evaluation.

Respiratory	Casualties
Rapid-Onset	Delayed-Onset
Cyanide	Inhalational Anthrax
	Pneumonic Plague
	Pneumonic Tularemia
Neurological	Casualties
Rapid-Onset	Delayed-Onset
Nerve Agents	Botulism

Table 2. CW & BW Diseases Potentially Requiring Prompt Empiric Therapy

VII. Practice good infection control. Standard precautions provide adequate protection against most infectious diseases, including those potentially employed in a biological attack. Anthrax, tularemia, brucellosis, glanders, Q-fever, VEE, and the toxin-mediated diseases are not generally contagious, and victims can be safely managed using standard precautions. Such precautions should be familiar to all clinicians. Under certain circumstances, however, one of three forms of transmission-based precautions would be warranted. Smallpox victims should, wherever possible, be managed using 'airborne 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 require 'contact precautions.'

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

In a civilian context, such notification would typically be made through local and/or regional health department channels. In the U.S., larger cities often have their own health departments. In most other areas, the county represents the lowest echelon health jurisdiction. In some rural areas, practitioners would access the state health department directly. Once alerted, local and regional health authorities are normally well-versed on the mechanisms for requesting additional support from health officials at higher jurisdictions. Each practitioner should have a point of contact with such agencies and should be familiar with mechanisms for contacting them before a crisis arises. A list of useful points of contact is provided in Table 3.

Point of contact	Number / resource
Local Law Enforcement Authorities *	
Local or County Health Department *	
State Health Department *	
CDC Emergency Response Hotline:	770-488-7100
CDC Bioterrorism Preparedness & Response Program:	404-639-0385
CDC Emergency Preparedness Resources:	http://www.bt.cdc.gov
Strategic National Stockpile:	Access through State Health Dept
FBI (general point of contact):	202-324-3000
FBI (suspicious package info):	http://www.fbi.gov/pressrel/pressrel01/mail3.pdf
USAMRIID General Information:	http://www.usamriid.army.mil
USAMRICD Training Materials:	http://ccc.apgea.army.mil
U.S. Army Medical NBC Defense Information:	http://www.nbc-med.org
Johns Hopkins Center for Civilian Biodefense:	http://www.hopkins-biodefense.org
Infectious Diseases Society of America:	http://www.idsociety.org/bt/toc.htm

Table 3. Points of Contact and Training Resources. *Clinicians and Response Planners are encouraged to post this list in an accessible location. Specific local and state points of contact should be included.

IX. Assist in the epidemiologic investigation and manage the psychological consequences. All health-care providers must have a basic understanding of epidemiological principles. Even under austere conditions, a rudimentary epidemiologic investigation may assist in diagnosis and in the discovery of additional BW victims. Clinicians should, at the very least, query patients about illness onset and symptoms, potential exposures, ill unit members, food/water sources, unusual munitions or spray devices, vector exposures; and develop a line listing of potential cases. Such early discovery might, in turn, permit post-exposure prophylaxis, thereby avoiding excess morbidity and mortality. Public health officials would normally conduct more elaborate 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 all available to assist the clinician in conducting an epidemiologic investigation.

In addition to implementing specific medical countermeasures and initiating an epidemiologic investigation, the clinician must be prepared to address the psychological effects of a known, suspected, or feared exposure. Such an exposure (or threat of exposure) can provoke fear and anxiety in the population, and may result in overwhelming numbers of patients seeking medical evaluation. Many of these will likely have unexplained symptoms and many may demand antidotes and other therapies. Moreover, symptoms due to anxiety and autonomic arousal, as well as the side effects of postexposure antibiotic prophylaxis may suggest prodromal disease due to biological-agent exposure, and pose challenges in differential diagnosis. This ‘behavioral contagion’ is best prevented by good, proactive, risk communication from health and government authorities. Such communication should include a realistic assessment of the risk of exposure, information about the resulting disease, and what to do and who to contact for suspected exposure. Risk communication must be timely, accurate, consistent, and well coordinated.

Effective risk communication is predicated upon the pre-existence of thorough risk communication plans and tactical approaches. Similarly, plans must be made to rapidly deploy resources for the initial evaluation and

administration of postexposure prophylaxis (ideally decentralized to unit level on the battlefield or to residential areas in a civilian context). Finally, plans must be made to proactively develop patient and contact tracing and vaccine screening tools, to access stockpiled vaccines and medications, and to identify and prepare local facilities and healthcare teams for the care of mass casualties.

X. Maintain Proficiency and Spread the Word. Fortunately, the threat of BW has remained a theoretical one for most medical personnel. Inability to practice casualty management, however, can lead to a rapid loss of skills and knowledge. It is imperative that the medic maintains proficiency in dealing with this low-probability, but high-consequence problem. This can be done, in part, by availing oneself of several resources. The OTSG (Office of the Surgeon General) (www.nbc-med.org) and USAMRIID (www.usamriid.army.mil) web sites provide a wealth of information, including the full text of this handbook. Numerous satellite television broadcast sponsored by USAMRIID, as well as other video course resources, provide in-depth discussion and training in medical biodefense. CD-ROM training aids are also available, and a field manual (Army FM 8-284) summarizes BW disease management recommendations. Multiple web sites provide a wealth of training materials and information on-line. Finally, medical personnel, once aware of the threat and trained to deal with it, must ensure that other personnel in their units receive training as well. It is only through ongoing training that personnel will be ready to deal with the threat posed by biological weapons. By familiarizing yourself with the contents of this handbook, you have taken a large step towards such readiness.

BACTERIAL AGENTS

Bacteria are unicellular organisms. They vary in shape and size from spherical cells - cocci - with a diameter of 0.5-1.0 μm (micrometer), to long rod-shaped organisms - bacilli - which may be from 1-5 μm. Chains of bacilli may exceed 50 μm in length. The shape of the bacterial cell is determined by the rigid cell wall. The interior of the cell contains the nuclear material (DNA), cytoplasm, and cell membrane, that are necessary for the life of the bacterium. Many bacteria also have glycoproteins on their outer surfaces which aid in bacterial attachment to cell-surface receptors. Under special circumstances, some types of bacteria can transform into spores. The spore of the bacterial cell is more resistant to cold, heat, drying, chemicals, and radiation than the vegetative bacterium itself. Spores are a dormant form of the bacterium and, like the seeds of plants, they can germinate when conditions are favorable.

The term rickettsia generally applies to very small, gram-negative coccobacillary organisms of the genera *Rickettsia* and *Coxiella*. Rickettsiae are unique from classical bacteria in their inability to grow (with rare exceptions) in the absence of a living host cell, but many are susceptible to treatment with antibiotics.

Bacteria generally cause disease in human beings and animals by one of two mechanisms: by invading host tissues, and by producing poisons (toxins). Many pathogenic bacteria utilize both mechanisms. The diseases they produce often respond to specific therapy with antibiotics. It is important to distinguish between the disease-causing organism and the name of the disease it causes (in parentheses below). This manual covers several of the bacteria or rickettsiae considered to be potential BW threat agents: *Bacillus anthracis* (anthrax), *Brucella* spp. (brucellosis), *Burkholderia mallei* (glanders), *Burholderia pseudomallei* (melioidosis), *Yersinia pestis* (plague), *Francisella tularensis* (tularemia), and *Coxiella burnetii* (Q fever).

ANTHRAX

SUMMARY

Signs and Symptoms of Inhalational Anthrax (IA): Incubation period is generally 1-6 days, although longer periods have been noted. Fever, malaise, fatigue, dry cough, and mild chest discomfort progresses to severe respiratory distress with dyspnea, diaphoresis, stridor, cyanosis, and shock. Death typically occurs within 24-36 hr 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 the blood and by blood culture late in the course of illness.

Treatment: Although effectiveness may be limited after symptoms are present, high dose intravenous antibiotic treatment with ciprofloxacin or doxycycline combined with one or two additional antibiotics should be considered. Intensive supportive therapy may be necessary.

Prophylaxis: Oral ciprofloxacin or doxycycline for known or imminent exposure.
An FDA-licensed vaccine is available. Vaccine schedule is 0.5 ml subcutaneously at 0, 2, 4 weeks, then 6, 12, and 18 months (primary series), followed by annual boosters.

Isolation and Decontamination: Standard precautions for healthcare workers. Avoid invasive procedures or autopsy; but if performed, all instruments and 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. All human populations are susceptible. The spores are very stable and may remain viable for many years in soil and water. They resist sunlight for varying periods.

HISTORY AND SIGNIFICANCE

Anthrax spores were weaponized by the U.S. in the 1950s and 1960s before the old U.S. offensive program was terminated. Other countries, including the Soviet Union and Iraq, have weaponized this agent or were suspected of doing so. In the fall of 2001, anthrax spores were delivered in the U.S. mail, resulting in 22 cases of confirmed or suspected anthrax disease. 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. This agent can be produced in either a wet or dried form, stabilized for weaponization by an adversary, and delivered as an aerosol cloud either from a line source such as an aircraft flying upwind of friendly positions, or as a point source from a spray device. Coverage of a large ground area could also be theoretically facilitated by multiple spray bomblets disseminated from a missile warhead at a predetermined height above the ground.

CLINICAL FEATURES

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

Cutaneous anthrax. The cutaneous form (also referred to as "malignant pustule") is the most common naturally occurring form of disease. 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 more recently occurred in as many as 11 people exposed to anthrax spores in the U.S. mail. After a 1 to 12 day (mean 7 days) incubation period, a painless or pruritic papule forms at the site of exposure, enlarging into a round ulcer by the next day. 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 10-20 percent of cases. Treated, mortality is less than 1 percent.

Gastrointestinal (GI) anthrax is rare in humans, and is contracted by eating insufficiently cooked meat from infected animals. Infection is thought to occur as a result of the ingestion of viable vegetative organisms in contrast to spores. The two forms of GI anthrax, oropharyngeal and intestinal, have incubation periods of 1-6 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 (often over a palatine tonsil and unilateral, but variable in location). Other signs and symptoms include dysphagia, regional lymphadenopathy (non-purulent), and severe neck swelling (often unilateral). Edema can lead to airway compromise, and disease can progress to sepsis, with case mortality rates 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 mortality is greater than 50 percent. Some evidence exists for a mild, self-limited gastroenteritis syndrome associated with intestinal anthrax, but this is poorly described.

Inhalational (IA) anthrax. Endemic inhalational anthrax, known as Woolsorters' disease, is also an extremely rare infection contracted by inhaling the spores. It has historically occurred in an industrial 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 to be as a result of intentional exposure to anthrax until proved otherwise. After an incubation period of 1 to 6 days*, presumably dependent upon the dose and strain of inhaled organisms, 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, and despite normal lung physical exams, often have (albeit sometimes subtle at this stage) evidence of mediastinal widening (hemorrhagic mediastinitis) or pleural effusions on chest X-ray or CT scan. These initial symptoms generally last 2-5 days and can be followed by a short period of improvement (hours to 2-3 days), culminating in the abrupt development of severe respiratory distress with dyspnea, diaphoresis, stridor, and cyanosis. Septicemia, shock, and death usually follow within 24-36 hr 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 percent of cases and GI hemorrhage in 80 percent of cases. For the attacks of 2001, mortality was only 45 percent, while before this time mortality rates for IA were > 85 percent. This improvement in outcomes is likely a reflection of advancements in intensive care medicine and the aggressive treatment of recent victims.

*During an outbreak of IA in the Soviet Union in 1979, persons are reported to have become ill up to 6 weeks after an aerosol release occurred. Studies performed in nonhuman primates confirm incubation periods which can be up to 100 days.

DIAGNOSIS

All forms of anthrax disease 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 or cutaneous tularemia may appear similar, these lesions are characteristically painful. Known exposure history or risk 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, use moistened swabs (sterile saline) to swab under an eschar or in the base of an ulcer. 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 immunostaining. Blood culture should be collected in all patients suspected of having anthrax.

Gastrointestinal anthrax. History of exposure to or ingestion of the meat of sick animals should be obtained. Clinical suspicion should be elevated for multiple cases of similar disease. **Oropharyngeal** disease can mimic diphtheria, and vaccination and travel history should be obtained. Gram stain and culture of the oral lesion may often be positive for *B. anthracis* if collected before initiation of antibiotics. **Intestinal** anthrax may mimic acute gastroenteritis, acute abdomen with peritonitis (thus focal and rebound tenderness), or dysentery. Abdominal radiographic studies 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 is variably positive in intestinal anthrax. Peritoneal fluid should be sent for Gram stain, culture, immunostaining, and PCR. Blood should be collected for culture, serology (paired frozen serum 3-4 weeks apart, -70°C) and PCR (lavender tube, refrigerated) in patients with either form of GI disease. Ascitic fluid can be sent for culture, PCR, and immunostaining.

Inhalational anthrax. Early IA is a non-specific syndrome which may be difficult to distinguish clinically from other illnesses. Notably absent in inhalational anthrax are upper respiratory symptoms (rhinorrhea, coryza, congestion) as one would see 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 elevated only slightly at presentation (mean 9,800/microliter in 2001 cases) with a neutrophil predominance. Hemoconcentration may be evidenced by elevated serum sodium and hematocrit. Mildly elevated serum AST and ALT may be present as well as hypoalbuminemia. *Bacillus anthracis* will be detectable even in the early phase of disease by routine blood culture and may even be seen on 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, CSF may show evidence of hemorrhagic

meningitis with numerous gram-positive bacilli. Pleural effusions may be large and bloody; Gram stain may show organisms. If cultures are sterile, blood and other fluids may be sent for PCR; CSF, pleural fluid, and tissue may be sent for immunostaining; and acute and convalescent serum may be collected for serology. All patients suspected of having IA should have a chest radiograph (CXR) to look for mediastinal adenitis (seen as a widened mediastinum or mediastinal "fullness") and pleural effusions. If the radiograph is normal, then chest CT should be performed. In the attacks of 2001, CXR and/or chest CT were abnormal in all cases.

MEDICAL MANAGEMENT

Inhalational. Early initiation of appropriate antibiotics is paramount for patient survival of IA. Initial therapy for adults with IA due to a strain with unknown antibiotic susceptibilities should include ciprofloxacin (400 mg intravenous q 12 hr for adults, and 10-15 mg/kg IV q12 hr (up to 1 g/day) for children) OR doxycycline (200 mg intravenous load, followed by 100 mg intravenous q12 hr for adults and children > 8 yr and >45 kg, and 2.2mg/kg q12 hr for children < 8 yr (up to 200 mg/day))* PLUS one or two additional antibiotics effective against anthrax. Some additional antibiotics to which naturally occurring strains of *B. anthracis* are susceptible include imipenem, meropenem, daptomycin, quinupristin-dalfopristin, linezolid, vancomycin, rifampin, macrolides (e.g., erythromycin, azithromycin, and clarithromycin), clindamycin, chloramphenocol, and aminoglycosides (e.g., gentamicin). While optimal combination antibiotic therapy for IA is not known, many infectious disease physicians have suggested a combination of a quinolone, clindamycin, and rifampin for susceptible *B. anthracis* strains. Penicillin (or other beta-lactam antibiotics) should NEVER be used as monotherapy for severe anthrax disease as *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 infectious disease physician is imperative. If meningitis is suspected, at least one antibiotic with good CSF penetration (e.g., rifampin or chloramphenicol) should be used, as quinolones and tetracyclines do not enter the CSF well. Generally, ciprofloxacin or doxycycline use is avoided during pregnancy and in children due to safety concerns; however, a consensus group and the American Academy of Pediatrics have suggested that ciprofloxacin or doxycycline should still be used as first line therapy in life-threatening anthrax disease until strain susceptibilities are known. In fact, ciprofloxacin has been approved by the FDA for prophylaxis and treatment of anthrax in children. Recommended treatment duration is at least 60 days, and should be changed to oral therapy as clinical condition improves.

*other quinolone antibiotics (levofloxacin, trovofloxacin) or tetracyclines (minocycline, tetracycline) would likely be affective as well, although they have not been specifically approved by the FDA for this purpose.

In the event of a mass-casualty situation intravenous antibiotics may not be available. In this case oral ciprofloxacin OR doxycycline may have to suffice as initial therapy. The doses for ciprofloxacin are 500 mg po bid for adults, and 10-15 mg/kg po bid (up to 1 g/day) for children. The doses for doxycycline are

200 mg po initially then 100 mg po bid thereafter for adults (or children > 8 yr and > 45 kg), and 2.2 mg/kg po bid (up to 200 mg/day) for children < 8 yr.

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 may be available soon as a therapy for IA under an IND from the CDC (see **Appendix L** for instructions on Investigational New Drug (INDs). The role of postexposure anthrax vaccine for patients with IA has not yet been determined.

Cutaneous anthrax Uncomplicated cutaneous anthrax disease should be treated initially with either ciprofloxacin (500 mg po bid for adults or 10-15 mg/kg/day divided bid (up to 1000 mg/day) for children) or doxycycline (100 mg po bid for adults, 5 mg/kg/day divided bid for children less than 8 yr (up to 200 mg/day)). If the strain proves to be penicillin susceptible, then the treatment may be switched to amoxicillin (500 mg po tid for adults or 80 mg/kg po divided tid (up to 1500 mg/day) for children). While *B. anthracis'* genome encodes for beta-lactamases, the organism may still respond to penicillins (such as amoxicillin) if slowly growing as in localized cutaneous disease. In the event that the exposure route is unknown or suspected to be related to a BW event, then antibiotics should be continued for at least 60 days. If the exposure is known to have been due to contact with infected livestock or their products, then 7-10 days of antibiotics may suffice. For patients with significant edema, non-steroidal anti-inflammatory agents (NSAIDS) or corticosteroids may be of benefit. Debridement of lesions is not indicated. If systemic illness accompanies cutaneous anthrax, then intravenous antibiotics should be administered as per the inhalational anthrax recommendations discussed above.

Gastrointestinal anthrax. Documentation of clinical experience in treating oropharyngeal and intestinal anthrax is limited. Supportive care to include fluid, shock, and airway management should be anticipated. Both forms of GI disease should receive the intravenous antibiotic regimen described for inhalational anthrax 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 airway control with 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.

Infection Control. Standard precautions are recommended for patient care in all forms of anthrax disease. There are no data to suggest direct person-to-person spread from any form of anthrax disease. However, for patients with systemic anthrax disease, especially before antibiotic initiation, invasive procedures, autopsy, or embalming of remains could potentially lead to the generation of infectious droplets; thus, such procedures should be avoided when possible. After an invasive procedure or autopsy, the 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. Iodine can be used, but must be used at disinfectant strengths, as antiseptic-strength iodophors are not usually sporicidal. Chlorine, in the form of sodium or calcium hypochlorite, can also be used, but with the caution that the activity of hypochlorites is greatly reduced in the presence of organic material.

The clinical laboratory should be warned before the delivery of anthrax specimens as growth of *B. anthracis* in culture requires BSL-2 precautions.

Animal anthrax experience indicates that incineration of carcasses and contaminated ground is the environmental control method of choice. A prior recommendation was deep burial (at least 6 feet deep) in pits copiously lined with lye (sodium hydroxide); 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 (Anthrax Vaccine Adsorbed (AVA) Bioport, Lansing, MI) is derived from sterile culture fluid supernatant taken from an attenuated (non-encapsulated) strain. Therefore, the vaccine does not contain live or dead organisms. The vaccination series consists of six 0.5-ml subcutaneous doses at 0, 2, and 4 weeks; then 6, 12, and 18 months, followed by yearly boosters. Current Department of Defense policy for missed doses (for those individuals required to remain immune) is to administer the missed dose ASAP and reset the timeline for the series based upon the most recent dose. The Food and Drug Administration (FDA) further clarified in December, 2003 that AVA "is safe and effective for the prevention of anthrax disease - regardless of the route of exposure." AVA is licensed only for preexposure prophylaxis of anthrax in adults. It is available for preexposure use in children, and postexposure prophylaxis (PEP) in adults and children only under INDs through the CDC and DoD. As with all vaccines, the degree of protection depends upon the magnitude of the challenge dose; vaccine-induced protection could presumably be overwhelmed by extremely high spore challenge. Thus, even fully immune personnel should receive antibiotic prophylaxis if exposed to aerosolized anthrax, per the guidelines given below.

Contraindications for use of this vaccine include hypersensitivity reaction to a previous dose of vaccine and age < 18 or > 65. 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 percent of recipients may experience mild discomfort at the inoculation site for up to 72 hr (e.g., tenderness, erythema, edema, pruritus), fewer experience moderate reactions, while less than 1 percent may experience more severe local reactions, potentially limiting use of the arm for 1-2 days. Modest systemic reactions (e.g., myalgia, malaise, low-grade fever) are uncommon, and severe systemic reactions such as anaphylaxis, which precludes additional vaccination, are rare. The vaccine should be stored between 2-6°C (refrigerator temperature, not frozen).

Current DoD policy is to require AVA vaccination for active duty personnel (without specific contraindications) as well as some emergency-essential DoD civilians and contractors to deploy for more than 15 consecutive days or more than 15 cumulative days over 12 months in designated "higher-threat" areas. The vaccination series should be initiated, when feasible, at least 45 days before deployment. DoD has continued to make vaccine available to special mission units, manufacturing and DoD lab workers, and congressionally-mandated anthrax vaccine researchers. Details of the DoD (and service specific guidance) can be found at http://www.anthrax.osd.mil/resource/policies/policies.asp.

Some AVA has been made available to U.S. Department of Health and Human Services (HHS). In 2002 the Advisory Committee on Immunization Practices (ACIP) recommended that HHS prioritize AVA availability to personnel at risk for repeated occupational exposure to anthrax spores, including workers handling environmental specimens (especially powders) and performing confirmatory testing for anthrax in Reference and National labs in the U.S. Laboratory Response Network (LRN), and emergency response personnel who may have to enter anthrax spore-contaminated areas repeatedly.

Antibiotics: No antibiotics are approved for preexposure prophylaxis of anthrax spores. 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 spores of unknown antibiotic susceptibility, prophylaxis with ciprofloxacin (500 mg po bid for adults, and 10-15 mg/kg po bid (up to 1 g/day) for children) OR doxycycline (100 mg po bid for adults or children >8 yr and >45 kg, and 2.2 mg/kg po bid (up to 200 mg/day) for children < 8yr) 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 anthrax immune status and suspected inhaled dose of anthrax. If antibiotic susceptibilities allow, patients who cannot tolerate tetracyclines or quinolone antibiotics can be switched to amoxicillin (500 mg po tid for adults and 80 mg/kg divided tid (≥ 1.5 g/day) in children). The DoD position is that vaccination with AVA is a critical part of postexposure prophylaxis for inhaled anthrax; without vaccine, victims exposed to inhaled anthrax spores are unlikely to develop the immunity necessary to prevent anthrax disease caused by spores that germinate after antibiotics are discontinued. Recently the ACIP agreed that AVA should be made available as an IND to civilians as a part of PEP of inhalational anthrax as well (MMWR, 51(45);1024-26, 15 Nov 2002) Patients who were fully immune* before the attack should continue antibiotics for at least 30 days. If vaccine is available, previously unvaccinated patients should receive at least three doses of AVA at 2-week intervals, and then continue antibiotics for at least 1-2 weeks after receipt of the third dose of AVA. If the vaccine is not available or the patient cannot receive the vaccine for some other reason, antibiotics should be continued for at least 60 days. Upon discontinuation of antibiotics, patients should be closely observed. If clinical signs of anthrax occur, empiric therapy for anthrax is indicated, pending etiologic diagnosis. Optimally, patients should have medical care available upon discontinuation of antibiotics from a fixed medical care facility with intensive care capabilities and infectious disease consultants.

* Vaccinated = completed six doses of AVA and up-to-date on boosters, or minimum of three doses within past 6 months. Those who have already received three doses within 6 months of exposure should continue with their routine vaccine schedule.

BRUCELLOSIS

SUMMARY

Signs and Symptoms: When present, include fever, headache, myalgias, arthralgias, back pain, profuse sweats, chills, weight loss, and generalized malaise. Onset may be acute or insidious. Fever may be intermittent or continuous and recurrences are common. Subclinical infections have been reported. Other manifestations include depression and other mental status changes, localized suppurative organ infection, and osteoarticular complications. Disability may be pronounced, but fatalities are uncommon.

Diagnosis: Diagnosis requires a high index of suspicion, as many infections present as non-specific febrile illnesses or are asymptomatic. Laboratory diagnosis can be made by serum agglutination tests, ELISA, immunofluorescence, and by standard culture. Radiometric and standard blood cultures require a minimum of 10 to 30 days incubation, respectively. Bone marrow cultures may produce a higher yield. Other body fluids may be tested depending on infection distribution (synovial, pleural, CSF). Confirmation may require phage-typing, oxidative metabolism, or genotyping procedures.

Treatment: Antibiotic therapy with doxycycline + rifampin or doxycycline in combination with other medications 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.

Prophylaxis: A human vaccine is not available. Chemoprophylaxis is not recommended after possible exposure to endemic disease. Treatment should be considered for high-risk exposure in the following situations: (1) Inadvertent wound or mucous membrane exposure to infected livestock tissues and body fluids and to livestock vaccines. (2) Exposure to laboratory aerosols or to secondary aerosols generated from contaminated soil particles in calving and lambing areas. (3) Confirmed BW exposure.

Isolation and Decontamination: Brucellosis is spread readily via bodily fluids and aerosols. Standard precautions are appropriate for healthcare workers. If an attack with *Brucella* sp. is suspected, special care should be taken to avoid the generation of secondary aerosols. Person-to-person transmission has been reported via tissue transplantation and sexual contact. Contact surfaces that are free of organic matter can be decontaminated with a 0.5% hypochlorite solution; higher concentrations (>5%) or other disinfectants for gram-negative microorganisms should be used where organic matter cannot be effectively reduced or controlled.

OVERVIEW

Brucellosis is an important disease of livestock in many countries and is caused by infection with one of several species of *Brucellae,* a group of gram-negative cocco-baccillary facultative intracellular pathogens (Table 1).

***Brucella* spp**	**1º Reservoir**	**2º Hosts**	**Geographic Distribution**	**Human Exposure Activity**	**Pathogenicity To Humans**
Abortus	Cattle, Bison, Cervids	Goat, Sheep, Dog, Human	Worldwide	Raw dairy foods, animal husbandry, laboratory	Moderate
Melitensis	Goat, Sheep	Dog, Human	Latin America, Asia, Mediterranean	Raw dairy foods, animal husbandry, laboratory	Highest
Suis	Pig (feral, and domestic)	Dog, Human, Cattle	SE Asia, Scattered and Midwest US, S America	Pork slaughter, processing, feral pig hunting, laboratory	High
Canis	Dog, Coyote		Scattered	Dog breeding and whelping operations	Moderate

Table 1. Select characteristics of brucellosis infection in livestock and humans.

Brucellosis is primarily a disease of the reproductive system of livestock and, depending on the species affected, is associated with infertility, abortion, retained fetal membranes, orchitis, and infection of the male accessory sex glands. Transmission in most livestock is primarily via ingestion of organisms either shed from or contaminated with fetal membranes, aborted fetuses, and uterine discharges, and occasionally from dams to nursing young. *Brucellae* also enter the body through mucous membranes, conjunctivae, wounds, and occasionally through intact skin.

Zoonotic transmission to humans has occurred by contact with infected tissues and discharges (aborted fetuses, fetal membranes and vaginal discharges), blood, urine, and semen. Veterinarians, slaughterhouse workers, ranchers, and other livestock husbandry workers and hunters have been infected in occupational and recreational settings. Transmission to humans also occurs by ingesting 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 laboratory settings. Finally, accidental percutaneous exposure to modified-live livestock vaccines (e.g., veterinarians) has also occurred. *Brucella* spp. most commonly associated with human infection includes *B. abortus, B. melitensis, B. suis*, and rarely *B. canis* (Table 1).

It is estimated that inhalation of only 10 to 100 bacteria is sufficient to cause disease in humans. Subclinical infections are relatively common. Brucellosis has a low mortality 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 BW scenario) would shorten the incubation period, lead to higher clinical attack rates, and result in more prolonged, incapacitating, and disabling disease than in its natural form.

HISTORY AND SIGNIFICANCE

Marston described disease manifestations caused by *B. melitensis* (Mediterranean fever, gastric intermittent fever) among British soldiers on Malta during the Crimean War. Goats were identified as the source. Restrictions on the consumption of unpasteurized dairy goat products soon decreased the incidence of brucellosis among military personnel. *B. abortus (Bang)* was first isolated by Bruce and described by Bang and Stribolt in 1897. Synonyms for human disease vary by region and include undulant fever, Malta fever, rock fever, Gibraltar fever, melitoccie goat fever, Texas fever, Rio Grande fever, Bang fever and Brucella fever. In 1954, *B.suis* became the first agent weaponized by the U.S. at its Pine Bluff Arsenal located in Arkansas. *Brucella* species survive well in aerosols and resist drying. Brucella and all other remaining biological weapons in the US arsenal were destroyed in 1969 when the U.S. offensive biological weapons program was disbanded.

Human brucellosis is now a rare disease in the U.S. with about 100 cases per year reported. Most are reported from CA, FL, TX, and VA, and the majority of these are associated with ingestion of unpasteurized dairy products made outside of the U.S. and privately imported (thus escaping FDA and USDA regulatory food-safety initiatives). 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. Human brucellosis is highly endemic in some Mediterranean basin and Arabian peninsular countries, as well as India, Mexico, and South and Central America. Disease incidence and prevalence vary regionally, with some reporting annual incidences of over 80 cases per 100,000 population. Serologic evidence of *Brucella* spp. exposure on an Arabic peninsular country was near 20% with more than 2% having active disease (WHO). A few regions in Kuwait have reported annual incidences as high as 128 cases per 100,000 population. These highlight a risk to military personnel in the region.

CLINICAL FEATURES

Brucellosis is a systemic disease that can involve any organ system and can present in a variety of clinical manifestations. Untreated, *Brucella* localizes in reticuloendothelial system organs, primarily the liver, spleen, and bone marrow, where granuloma formation ensues. Large granulomas serve as a source for persistent bacteremia.

After an incubation period ranging from 1 week to many months (although most patients are symptomatic within 3-4 weeks), illness can present suddenly, over a few days, or insidiously over weeks to months. Patients usually complain of non-specific symptoms such as fever (90-95% of cases), malaise (80-95%), sweats (40-90%), and myalgias/arthralgias (40-70%). Other common symptoms include fatigue, chills, and backache. Fever is usually intermittent, and can assume an undulant pattern in patients who go untreated for long periods. Neuropsychiatric symptoms including depression, headache, and irritability, are common. GI symptoms (abdominal pain, anorexia, constipation, diarrhea, vomiting) are reported in nearly 70% of adult cases but are less frequent

complaints in children. Cough, dyspnea, chest pain, and testicular pain can occur less frequently. Common physical signs include hepatomegaly (10-70%) and / or splenomegaly (10-30%), arthritis (up to 40%), weight loss, and adenopathy (10-20%).

Osteoarticular complications including bursitis, tenosynovitis, arthritis, osteomyelitis, sacroiliitis, discitis, and paravertebral abscess are reported in 20-60% of all brucellosis cases. Sacroiliitis typically presents acutely with fever and focal lower back pain and occurs in up to 30 percent of cases, predominantly in young men. Arthritis of large, weight-bearing joints of the lower extremities may occur in 20 percent of cases. Arthritis is usually monoarticular, but can be polyarticular up to 30 percent of the time. Spondylitis or vertebral osteomyelitis may affect from up to 30 percent 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.

Gastrointestinal disease can manifest as Ileitis, colitis, or granulomatous or mononuclear infiltrative hepatitis. Hepatitis only progresses to cirrhosis if pre-existing liver disease (hepatitis C or alcoholic liver disease) is present. Pulmonary disease may be present in <1 to 5 percent of cases and may take the form of lung abscess, single or miliary nodules, bronchopneumonia, enlarged hilar lymph nodes, or pleural effusions. While inhalational exposure to *Brucella* has been described in laboratory or abattoir workers, this route of infection has not proven to lead with regularity to any particular form of disease (e.g., pneumonic).

Epididymoorchitis has been described in 2-20 percent of male patients with brucellosis. Patients typically present acutely with scrotal pain and swelling, and continuous 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. However, direct CNS invasion occurs in less than 5 percent of cases of brucellosis. Behavioral disturbances and psychoses appear to occur unrelated to the degree of fever and may be only occasionally associated with the aforementioned syndromes during acute phases. These data coupled with the case histories from a few apparent congenital infections from the UK, suggest the possible existence of a neurotoxicologic process.

Endocarditis occurs in less than 2 percent of cases, but accounts for the majority of brucellosis-related deaths.

Acute brucellosis during the first two trimesters of pregnancy has been reported to lead to spontaneous abortion on up to 40 percent of cases if untreated, while untreated disease may be associated with intrauterine fetal death in only 2 percent of cases with onset in the third trimester.

Disease type and severity may vary with the infecting strain of *Brucella. B. melitensis* is the most pathogenic; human infection is associated with an acute course with 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.

DIAGNOSIS

A high index of suspicion is critical to firmly establish a diagnosis of brucellosis. Animal contact history, consumption of unpasteurized dairy products (including goat), travel to areas where such consumption occurs, and travel to endemic areas should prompt a differential diagnosis consideration of brucellosis. Brucellosis should be suspected when a patient presents with acute or insidious onset of fever, night sweats, undue fatigue, GI symptoms, anorexia, weight loss, headache, arthralgias and splenomegally, and / or hepatomegally.

Brucella species are small, non-motile, non-encapsulated, non-spore forming, slow-growing, coccobacillary gram-negative intracellular aerobes. While traditional culture methods were held for many weeks to show growth, automated blood culture systems will grow *Brucellae* within 7 days in 95% of cases; however, rapid identification systems may mis-identify the organism, often as *Psychrobacter phenylpyruvicus*. If traditional, non-automated blood culture is performed, a biphasic culture method (e.g. Castaneda bottle) may improve the chances of isolation, as may re-culturing onto solid medium every week for 2 months. Clinically, identification to the genus level is adequate to initiate therapy. Speciation is epidemiologically necessary and aids prognostically; however, it requires more specialized analyses.

Blood and bone marrow cultures taken during the acute febrile phase of illness yield the organism in 15-70 percent and 92 percent of cases, respectively. Other fluid cultures are encouraged with accompanying clinical signs (CSF with meningitis, joint fluid with effusion, urine with genitourinary signs). Bone marrow and liver biopsies (granulomatous disease) may also be indicated. Clinical laboratories should always be alerted if a diagnosis of brucellosis is suspected. This permits the use of selective isolation media and the implementation of biosafety level-3 (BSL-3) safety containment.

Diagnostic laboratory criteria include: 1) isolation of *Brucella* sp. from a clinical specimen; 2) at least a fourfold rise in *Brucella* sp. agglutination titer between acute and convalescent sera obtained at least 2 weeks apart and studied at the same laboratory; 3) demonstration by immunofluorescence of *Brucella* sp. 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 at least 160 in one or more serum specimens obtained after onset of symptoms). A confirmed case is a clinically compatible case that is laboratory confirmed.

A serum agglutination test (SAT) for IgM and IgG, and a tube agglutination method for anti-O polysaccharide antibody are available; titers of at least 1:160 by each indicate active disease. ELISA and PCR methods are also available. CSF and joint fluid may also be used for antibody testing with some test kits.

The leukocyte count in brucellosis patients is usually normal but may be low; anemia, neutropenia, and thrombocytopenia may occur in a minority of

cases. AST and ALT may be mildly elevated, and ESR is normal or only mildly elevated in the majority of cases.

Imaging studies may help to identify localized 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 are reasonably sensitive means for detecting sacroiliitis and other axial skeletal infections. Chest radiographs may be unremarkable even with respiratory symptoms. Cranial CT scan may be useful for patients with neurologic signs or deficits. Though cranial CT studies are also often normal, occasional leptomeningitis, cerebral abscess, or other pathology may be identified. Echocardiography may find 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.

MEDICAL MANAGEMENT

The most effective proven treatment for acute brucellosis in adults historically is the combination of doxycycline 100mg po bid for 4-6 weeks plus streptomycin 1 g intramuscular qd for the first 2-3 weeks. If streptomycin is not available, gentamicin probably represents a suitable alternative. For uncomplicated acute brucellosis, combinations of oral antibiotics are usually sufficient, or even preferred, as they are simpler to use in the outpatient setting and have comparable cure rates to doxycycline-aminoglycoside combinations. The most widely recommended combination for adults and children over 8 years old is doxycycline (100 mg PO bid for adults 2.2 mg/kg po bid (up to 200 mg/day) for children) + rifampin (600-900 mg/d PO qd for adults and 15-20 mg/kg (up to 600-900 mg/day) for children) for 4-6 weeks; quinolone (e.g. ofloxacin or ciprofloxacin) + rifampin or trimethoprim-sulfamethoxazole (TMP-SMX) + rifampin may represent suitable alternatives. Relapse rates are 5-10% for most combination oral regimens and higher for monotherapy (up to 30 percent with TMP-SMX alone). During pregnancy and for children < 8 years old the combination of TMP-SMX and rifampin is historically preferred, as doxycycline poses a potential risk to the fetus or young child’s skeletal and dental development. The quinolone-rifampin combination may be a suitable alternative in these patients as well.

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 intramuscular streptomycin for the first 2-3 weeks has been used most frequently. Gentamicin may be a suitable substitute for streptomycin. For skeletal disease 6-8 weeks of antibiotics may be necessary for cure; persisting musculoskeletal complaints may be present in patients with chronic infection and sacroiliitis. Meningoencephalitis and endocarditis should receive at least 90 days of therapy and may require > 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 require surgical excision or drainage.

Patient education is a critical component of medical management and must include emphasis on the importance of antibiotic compliance. Complications are rare in complying patients who are appropriately treated. Periodic follow-up is also critical, and referral to specialists (infectious disease, other) may be indicated. As is the case with all bacterial BW agents, antibiotic resistance can be engineered into the organism, and thus determination of antibiotic susceptibilities in an intentional attack with *Brucella* is paramount.

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

PROPHYLAXIS

The risk of foodborne brucellosis is reduced by avoiding unpasteurized dairy products particularly while traveling in areas where brucellosis occurs in livestock. Travelers should consult with animal health and public health authorities before travel to assess foodborne and endemic brucellosis risks. Most developed countries have largely eradicated brucellosis from domestic cattle herds and sheep and goat flocks by multifaceted control programs. These may include periodic testing and slaughter of positive and contact animals and periodic batch testing of raw milk. Livestock vaccinations are available and are tightly controlled by regional animal health authorities. No licensed human brucellosis vaccine is available.

Chemoprophylaxis is not generally recommended after possible exposure to endemic disease. A 3-6 week course of therapy (with one of the oral regimens discussed above) should be considered after a high-risk exposure such as percutaneous or mucus membrane exposure or aerosolization of infectious material in the laboratory or livestock husbandry setting, or exposure in a BW context. Brucellosis is a reportable human and livestock disease in the U.S. and many other countries.

GLANDERS AND MELIOIDOSIS

SUMMARY

Signs and Symptoms: Incubation period ranges from days to weeks (usually within 14 days) after inhalation. Onset of symptoms may be abrupt or gradual. Inhalational exposure produces fever (usually above 102°F.), rigors, sweats, myalgias, headache, pleuritic chest pain, cervical lymphadenopathy, hepatosplenomegaly, and generalized papular/pustular eruptions. Acute pulmonary disease can progress and result in bacteremia and acute septicemic disease. Both diseases are almost always fatal without treatment.

Diagnosis: Methylene blue or Wright's stain of exudates may reveal scant small bacilli with a safety-pin bipolar appearance. Standard culture medium and procedures can be used to identify both *B. mallei* and *B. pseudomallei* (the causative agents of glanders and melioidosis, respectively). Chest Radiograph may show miliary lesions, small multiple lung abscesses, or infiltrates involving upper lungs with consolidation and cavitation. Abdominal ultrasound may show splenic or hepatic abscesses. Leukocyte counts may be normal or elevated. Serologic tests can help confirm diagnosis, but low titers or negative serology do not exclude the diagnosis.

Treatment: Therapy will vary with the type and severity of the clinical presentation. Patients with localized disease may be managed with oral antibiotics for 60-150 days. Severe illness should be treated initially with a parenteral regimen containing ceftazidime, imipenem, or meropenem (plus trimethoprim-sulfamethoxazole (TMP-SMX) if septicemic) followed by prolonged oral antibiotic therapy and lifelong follow-up. Abscesses respond best to surgical drainage.

Prophylaxis: No vaccines are currently available. Postexposure prophylaxis with TMP-SMX, doxycycline, or ciprofloxacin may be attempted, but is of unproved benefit.

Isolation and Decontamination: Standard precautions for health-care workers. Person-to-person airborne transmission is unlikely, although secondary cases may occur through improper handling of infectious materials. Contact precautions are indicated while caring for patients with skin involvement. Laboratory cultures should be managed under BSL-3 conditions. Environmental decontamination using a 0.5%-1.0% hypochlorite solution should be effective.

OVERVIEW

The causative agents of glanders and melioidosis are *Burkholderia mallei* and *Burkholderia pseudomallei,* respectively. Both are gram-negative bacilli which may have a “safety-pin” appearance on microscopic examination. Both pathogens affect domestic and wild animals, which, like humans, acquire the diseases from inhalation or contaminated injuries.

B. mallei is primarily noted for producing disease in horses, mules, and donkeys. In the past, humans have seldom been infected, despite frequent and often close contact with infected animals. This may be the result of exposure to low concentrations of organisms from infected sites in ill animals and because strains virulent for equids are often less virulent for humans. There are four basic forms of disease in horses and humans. The acute forms are more common in mules and donkeys, with death typically occurring 3 to 4 weeks after illness onset. The chronic form of the disease is more common in horses and humans and causes generalized lymphadenopathy, multiple skin nodules that ulcerate and drain, and induration, enlargement, and nodularity of regional lymphatics on the extremities and in other areas. The lymphatic thickening and induration seen in infected horses is known as ”farcy.” Human cases have occurred primarily in veterinarians, horse and donkey caretakers, and abattoir workers.

B. pseudomallei is widely distributed in water and soil in many tropical and subtropical regions. Melioidosis is endemic in Southeast Asia and northern Australia, where it is most prevalent during the rainy season in people who have direct contact with wet soils and who have predisposing medical conditions (e.g., diabetes mellitus). In northeastern Thailand, *B. pseudomallei* has accounted for 20% of community-acquired septicemia cases and the organism can be isolated from 50% of rice paddies. Melioidosis presents in humans in several distinct forms, ranging from a subclinical illness to an overwhelming septicemia, with up to a 90% mortality rate and death within 24-48 hours after onset. Also, melioidosis can reactivate years after primary infection and result in chronic and life-threatening disease.

These organisms spread naturally to humans by inoculation of contaminated materials into nasal, oral, or conjunctival mucous membranes, through abraded or lacerated skin, or more rarely, by inhalation into the lungs. Person to person spread is rare. Aerosols from cultures have been observed to be highly infectious to laboratory workers. Biosafety level 3 containment practices are required when working with these organisms in the laboratory. Because aerosol spread is efficient, and there is no available vaccine or reliable therapy, *B. mallei* and *B. pseudomallei* have both been viewed as potential BW agents.

HISTORY AND SIGNIFICANCE

Despite the efficiency of spread in a laboratory setting, glanders has only been a sporadic disease in humans, and no epidemics of human disease have been reported. There have been no naturally acquired cases of human glanders

in the U.S. since 1942. Sporadic cases continue to occur in Asia, Africa, the Middle East, and South America. During World War I, glanders was believed to have been spread deliberately by German agents to infect large numbers of Russian horses and mules on the Eastern Front. This had an effect on troop and supply convoys as well as on artillery movement, which were dependent on horses and mules. Human cases in Russia increased with the infections during and after WWI. The Japanese deliberately infected horses, civilians, and prisoners of war with *B. mallei* at the Pinfang (China) Institute during World War II. The U.S. studied this agent as a possible BW weapon in 1943-44 but did not weaponize it. The former Soviet Union is believed to have been interested in *B. mallei* as a potential BW agent after World War II. The low transmission rates of *B. mallei* to humans from infected horses is exemplified by the fact that in China, during World War II, thirty percent of tested horses were positive for glanders, but human cases were rare. *B. mallei* exists in nature only in infected susceptible hosts and is not found in water, soil, or plants.

In contrast, melioidosis is widely distributed in the soil and water in the tropics, and remains endemic in certain parts of the world, even to this day. It is one of the few genuinely tropical diseases and is well established in Southeast Asia and northern Australia. It was first described as a "glanders-like illness" caused by a unique bacillus among debilitated opiate addicts in Rangoon in 1912. As a result of *B. pseudomallei's* potentially long incubation period, French and later U.S. soldiers returning from Viet Nam would sometimes years later develop disease (the "Vietnamese time-bomb"). *B. pseudomallei*, like *B. mallei*, was studied by the U.S. as a potential BW agent, but was never weaponized. It has been reported that the former Soviet Union was experimenting with *B. pseudomallei* as a BW agent.

CLINICAL FEATURES

The manifestations of both glanders and melioidosis are protean; disease can be localized or systemic, acute or chronic, or progress from one form to another over time. Aerosol infection produced by a BW weapon containing either *B. mallei* or *B. pseudomallei* could presumably produce any of these syndromes, although acute respiratory or systemic syndromes are more common after aerosol exposure.

The incubation period varies by route of entry, size of inoculum, virulence of the organism, and host factors. Historic data on naturally acquired human **glanders** suggests that mucus membrane or skin exposure led to symptoms within 1-5 days (range 1-21 days). In the only well-documented cases of human glanders due to respiratory exposure the incubation period varied from 10-14 days. The incubation period of naturally acquired melioidosis is more difficult to determine, as environmental exposure to the agent in endemic regions may be continuous. Many people within endemic regions asymptomatically seroconvert to *B. pseudomallei* early in life. Documented incubation periods for overt melioidosis disease are typically 1-21 days, and extended incubation periods of months to years can occur. Of greater relevance to the weaponization of these agents, animal models of high dose inhalational exposure to either *B. mallei or B. pseudomallei* result in incubation periods that are usually 1-4 days in length.

Acute, septicemic glanders and melioidosis, especially as a result of intentional high-dose aerosol exposure to virulent organisms, can be expected to be clinically indistinguishable; differentiation will depend heavily upon laboratory studies. Such patients would likely present within a few days of exposure with acute onset of fever, chills, malaise, fatigue, and myalgias, with or without cough and pleuritic chest pain. Pneumonia would likely develop and could take multiple forms, including unilateral or bilateral, multifocal, nodular, or lobar consolidation, often progressing to abscess formation and cavitary disease over time. Cough is likely to become productive, and hemoptysis is possible. Failure to provide prompt therapy is likely to lead to fulminant sepsis, shock, and multi-organ system failure. Should the patient survive the initial phase of illness, metastatic septic foci become evident, with hepatic, splenic, and cutaneous abscesses being the most likely, but any organ can potentially be affected.

Differences in the presentation and clinical course of glanders and melioidosis are likely to be more noticeable with mucocutaneous or low inoculum exposures, described below. Natural disease due to both organisms is well-described in the literature.

Glanders. Mucocutaneous exposure usually leads to acute or subacute onset of constitutional signs including fever (may be low-grade or recurring), rigors, sweats, headache, fatigue and myalgias, with localized nodular to erosive infection, mucopurulent discharge, and regional lymphadenopathy. Cutaneous exposure typically leads to local inflammatory nodule formation with subsequent lymphangitis (sometimes with sporotrichoid nodule distribution) and lymphadenitis. Nodules typically break down and ulcerate. With oronasal and ocular entry or involvement, severe headache, photophobia, lacrimation, mucopurulent nasal, and ocular exudates leading to ulceration, may occur. Chronic infection and erosion of the nasal septum and turbinates can lead to severe disfigurement.

Inhalational exposure usually produces the preceding constitutional signs and pulmonary involvement with pleuritic chest pain, cervical adenopathy (particularly with upper respiratory involvement including pharyngitis or purulent rhinitis), and possibly other organ signs such as hepatosplenomegaly. Pulmonary involvement may follow direct inhalation of organisms or arise secondarily via hematogenous spread.

Septicemia may occur at any time during the infectious course, regardless of the mode of entry. When it occurs with initial infection, it is typically rapidly progressive and may include any combination of the previous signs and symptoms as well as tachycardia, jaundice, diarrhea, and granulomatous and necrotizing lesions in virtually any organ. Particularly if systemic invasion occurs from mucosal or cutaneous lesions, a diffuse papular and/or pustular rash may occur that may be mistaken for smallpox. Absent treatment, disseminated infection via hematogenous spread is the rule. Evidence of disseminated infection may include regional or generalized cutaneous pustules, internal organ abscess formation (especially liver, spleen, and lungs) and intramuscular abscess, particularly within skeletal muscles of the legs and arms. Enlargement and induration of regional lymph channels and nodes may also occur. Osteomyelitis, brain abscess, and meningitis have also been reported. Disseminated infections carry a high risk of rapidly progressive septic shock and

subsequent mortality. Chronic symptomatic infection is common (50% of all natural cases) and is eventually always fatal without treatment. Chronic infections with less virulent strains may also be periodically asymptomatic. Mortality rates dropped to 20% for localized disease and 40% overall after sulfadiazine therapy became available; experience with treatment using modern antibiotics is limited.

Melioidosis. Most individuals exposed naturally to *B. pseudomallei* do not develop symptomatic melioidosis. 50-70% of individuals who do develop symptomatic disease have predisposing medical conditions, especially diabetes mellitus (present in up to 50% of melioidosis cases), but also alcoholism, cirrhosis, renal disease, thallassemia, cystic fibrosis, and immunosuppressive drugs (e.g., corticosteroids). Also, melioidosis may remain asymptomatic after initial acquisition, and can remain quiescent for decades; these patients may present with active melioidosis up to 29 years later, often associated with onset of an immune-compromising state.

Mucocutaneous exposure may lead to local nodule / abscess formational and regional lymphadenitis, but this is not as commonly seen as with glanders. In fact, most suspected percutaneous exposures which have led to symptomatic disease initially presented with either pneumonia (presumably via hematogenous spread) and / or sepsis. Rarely, melioidosis will present as a distal, focal abscess with or without obvious site of primary inoculation; most commonly as a primary purulent parotitis in children (more common in Thailand) or as a primary prostatic abscess (more common in northern Australia). For non-septicemic patients with focal disease, and with appropriate surgical and medical therapy, prognosis is good.

Inhalational exposure, either through near drowning or via infectious aerosols, typically results in an acute or subacute pneumonia and septicemia.

Septicemic melioidosis typically presents with fever, rigors, night sweats, myalgia, anorexia, and headache. Additional signs and symptoms can include regional adenopathy, lymphangitis, papular or pustular skin lesions, diarrhea, and hepatosplenomegaly. Most patients (up to 60%) are bacteremic, particularly those with risk factors. With septicemia, flushing, cyanosis, disseminated pustular eruption, regional lymphadenitis and cellulitis may be seen. Pneumonia can be present in 50-80% of cases. Melioidosis pneumonia can present in many forms, but is most commonly seen as a lobar or segmental consolidation with a predilection for the upper lobes, or as multiple, widespread 0.5-1.0 cm nodules. Cavitation is common, sputum is often purulent, and hemoptysis may be present. Even with a primary pneumonic infection, dissemination (if patient survives) is likely to produce cutaneous (10-20% of cases) and internal (especially liver and spleen) abscesses even weeks to months later. Prostatic abscess occurs in 2-15% of cases as well. Without proper treatment most septicemic patients will die within 2-3 days. Poor prognostic indicators for severe melioidosis include positive blood culture within 24 hours of incubation and neutropenia. Overall mortality (treated) for severe melioidosis is up to 50% in Thailand and 19% in Australia. Death is a rare outcome for melioidosis patients who did not have predisposing risk factors. Even after prolonged antimicrobial therapy relapse is common.

DIAGNOSIS

Microbiology. Gram stain of lesion exudates reveals small irregularly staining, gram-negative, bacilli. Methylene blue or Wright's stain may reveal bipolar "safety pin" staining. The organisms can be cultured and identified from abscesses/wounds, secretions, sputum (in pneumonia), and sometimes blood and urine with standard bacteriological medium; adding 1-5% glucose, 5% glycerol, or meat infusion nutrient agar may accelerate growth. Primary isolation requires 48-72 hours in agar at 37.5º C; automated blood culture methods are typically more rapid. *B. pseudomallei* is generally more rapidly growing and less fastidious than *B. mallei*. Selective medium (e.g., Ashdown's media for *B.* pseudomallei) may be necessary for isolation from non-sterile sites (sputum, pharyngeal cultures).

Blood cultures for *B. mallei* are rarely positive upon presentation unless the patient is moribund. In contrast, blood cultures for *B. pseudomallei* septicemia are often positive and urine culture may be positive, especially if prostatitis or renal abscesses are present. Culture of either of these organisms should be performed under BSL-3 precautions due to the high aerosol risk these agents pose to laboratory workers.

Specific, rapid immunoassays may be available in some reference laboratories for *B. pseudomallei* capsular antigens. Polymerase chain reaction (PCR) is sensitive and specific, but available in only a few reference/research laboratories.

Serology. For *B. mallei,* agglutination tests are not positive for at least 7-10 days (sometimes 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 equal to, or exceeds 1:20. For *B. pseudomallei,* a fourfold increase in titer supports the diagnosis of melioidosis. A single IgM titer above 1:160 with a compatible clinical picture suggests active infection; IgG is less useful in endemic regions due to high seroprevalence.

Other laboratory studies. In septicemic glanders, mild leukocytosis with a shift to the left or leukopenia with a relative lymphocytosis may occur. With systemic melioidosis, significant leukocytosis with left shift is common, and leucopenia / neutropenia are poor prognostic indicators; anemia, coagulopathy, and evidence of hepatic or renal dysfunction may be present.

Radiographic studies. Chest radiograph in cases with pneumonia may demonstrate lobar or segmental opacification, or diffuse nodular opacities. Cavitary lesions are common, but effusions and hilar adenopathy are rare. Abdominal ultrasound should be considered on all patients with suspected glanders or melioidosis to exclude the possibility of hepatic and splenic abscesses. Prostatic abscess in melioidosis can be delineated, usually as a heterogeneous multiloculated fluid collection within an enlarged prostate, using transrectal ultrasound, or by computerized tomography or magnetic resonance imaging.

Pathology. Individual chronic lesions may be granulomatous and the pathologic tissue diagnosis may simulate tuberculosis, which can cause confusion in areas where both diseases are endemic **(**such as Thailand**)**.

MEDICAL MANAGEMENT

Supportive Care. Septicemic patients often require aggressive supportive care to include fluid resuscitation, vasopressors, and management of coagulopathy. Large abscesses should be drained when possible; prostatic and parotid abscesses in patients with melioidosis are unlikely to resolve without surgical intervention.

Antimicrobials. The recommended therapy will vary with the type and severity of the clinical presentation. An understanding of appropriate medical management of glanders is confounded by the fact that clinical experience with this disease waned before the modern antibiotic era. Fortunately, *B. mallei* and *B. pseudomallei* have similar antibiotic susceptibility patterns (although, unlike *B. pseudomallei*, natural *B. mallei* strains generally remain susceptible to aminoglycosides and macrolides in vitro) and thus it is generally felt that <u>empiric</u> antibiotic regimens for melioidosis would also work for glanders; any antibiotic regimen must be reevaluated upon receipt of specific antibiotic susceptibilities for the bacterial isolate in question.

Severe disease. Systemic melioidosis should be treated initially with ceftazidime (120 mg/kg/day intravenous in three divided doses), imipenem (60 mg/kg/day intravenous in four divided doses, max 4 g/day), or meropenem (75 mg/kg/day intravenous in three divided doses, max 6g/day). Many experts add trimethoprim / sulfamethoxazole (TMP/SMX) to this regimen (TMP 8 mg/kg/day IV in four divided doses); oral TMP/SMX has been substituted if the intravenous formulation is not available. If ceftazidime or a carbapenem are not available, ampicillin/sulbactam or other intravenous beta-lactam/beta-lactamase inhibitor combinations may represent viable, albeit less-proven alternatives. Intravenous antibiotics should be continued for at least 14 days <u>and</u> until the patient shows clinical improvement. Patients may remain febrile for prolonged periods despite appropriate antimicrobial therapy. Median time to fever resolution is 9 days, but can be significantly longer in patients with large abscesses or empyema that are not drained. Upon completion of intravenous therapy, oral maintenance therapy (with one of the oral treatment regimens listed below) should be continued for at least 4-6 months. Lifelong follow-up is indicated for all patients to identify relapse.

Melioidosis septic shock. Australian researchers have combined intravenous antibiotics with granulocyte colony-stimulating factor (G-CSF) 300 µg intravenous per day for 10 days (or longer if clinical shock persists) in melioidosis patients meeting a diagnosis of septic shock. Mortality in study patients dropped from a historic value of 95% to 10% with G-CSF; however limitations in the study preclude attributing success entirely to G-CSF and further studies are warranted to determine its role in treatment.

Maintenance therapy. Oral antibiotic maintenance therapy of severe melioidosis should continue for at least 20 weeks to reduce the rate of relapse to less than 10%; however, longer courses (6-12 months) may be necessary depending upon response to therapy and severity of initial illness (e.g., longer courses for extrapulmonary suppurative disease). Historically, maintenance therapy in endemic regions has been with a combination of four oral drugs (doxycycline and TMP/SMX for at least 20 weeks, plus chloramphenicol for the first 8 weeks); but side effects with this regimen are common and compliance is poor. Good results have been demonstrated with doxycycline (100 mg po bid) plus TMP/SMX (4 mg/kg/day in two divided doses) for 20 weeks. Amoxicillin/clavulanic acid has been used in some areas and may be the antibiotic of choice during pregnancy or for children less than 8 years old. Combinations including fluoroquinolones show promise, but have not been validated.

Localized disease without toxicity. No consensus exists for antibiotic treatment of mild, localized disease. Monotherapy with oral TMP/SMX (4 mg/kg/day in two divided doses), doxycycline (100 mg b.i.d.), or amoxicillin/clavulanate (60 mg/kg/day in three divided doses) for 60-150 days may be sufficient.

Localized disease with toxicity. One of the two drug maintenance regimens (above) should be used.

Isolation precautions. Standard precautions should be used to prevent person-to-person transmission in proven or suspected cases. Person-to-person airborne transmission is unlikely, although secondary cases may occur through improper handling of infectious materials. Contact precautions are indicated while caring for patients with skin involvement. Environmental decontamination using a 0.5%-1.0% hypochlorite solution is effective.

PROPHYLAXIS

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

Antibiotics: Postexposure chemoprophylaxis may be tried with TMP-SMX or doxycycline based upon success in limited animal studies. These animal studies indicate that ciprofloxacin may have utility as well, but it was associated with higher relapse rates than doxycycline. Optimum duration of prophylaxis is unknown, but at least 10 days and perhaps more should be attempted.

PLAGUE

SUMMARY

Signs and Symptoms: Pneumonic plague begins with sudden onset of symptoms after an incubation period of 1-6 days. Symptoms include high fever, chills, headache, malaise, followed by cough (often with hemoptysis), progressing rapidly to dyspnea, stridor, cyanosis, and death. Gastrointestinal symptoms are often present. Death results from respiratory failure, circulatory collapse, and a bleeding diathesis. Bubonic plague is characterized by swollen painful lymph nodes called buboes (often in the inguinal area), high fever, and malaise. Bubonic plague may progress spontaneously to the septicemic form (septic shock, thrombosis, disseminated intravascular coagulation) or to the pneumonic form. Plague meningitis is also possible.

Diagnosis: Suspect plague if large numbers of previously healthy individuals suddenly develop severe pneumonia, especially if hemoptysis is present with gram-negative coccobacilli in sputum. Presumptive diagnosis can be made by Gram, Wright, Giemsa or Wayson stain of blood, sputum, cerebrospinal fluid, or lymph node aspirates. Definitive diagnosis requires culture of the organism from those sites. Immunodiagnosis is helpful in establishing a presumptive diagnosis.

Treatment: Early administration of antibiotics is critical, as pneumonic plague is invariably fatal if antibiotic therapy is delayed more than 1 day after the onset of symptoms. The treatment of choice is parenteral streptomycin or gentamicin, with doxycycline or ciprofloxacin representing alternatives. Duration of therapy is at least 10-14 days. For plague meningitis add chloramphenicol to the treatment.

Prophylaxis: For asymptomatic persons exposed to a plague aerosol or to a suspected pneumonic plague case, doxycycline 100 mg is given orally twice daily for 7 days or the duration of risk of exposure plus 1 week. Alternative antibiotics include ciprofloxacin, tetracycline, or chloramphenicol. No vaccine is currently available for plague prophylaxis. The previously available licensed, killed vaccine (manufactured by Greer) was effective against bubonic plague, but not against aerosol exposure. No prophylaxis is required for asymptomatic contacts of individuals with bubonic plague.

Isolation and Decontamination: Use Standard precautions for bubonic plague, and respiratory droplet precautions for suspected pneumonic plague. *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 if decontamination is needed. Take measures to prevent local disease cycles if vectors (fleas) and reservoirs (rodents) are present.

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 (e.g., rats, mice, ground squirrels). Humans typically develop disease through contact with infected rodents or, more commonly, their fleas. The biting fleas can transmit the 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. 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 temporary immunity. The organism remains viable in unchlorinated water, moist soil, and grains for several weeks. At near freezing temperatures, it will remain alive from months to years but is killed by 15 minutes of exposure to 55°C. 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 AND SIGNIFICANCE

Throughout recorded history *Yersinia pestis* has been the cause of multiple human pandemics and countless deaths. Plague is now endemic worldwide yet is responsible for only sporadic human disease (200-4500 human cases with 30-200 deaths reported to the WHO annually). The United States worked with *Y. pestis* as a potential BW agent in the 1950s and 1960s before the old offensive BW program was terminated. Other countries are suspected of having weaponized this organism. The former Soviet Union had several separate institutes and thousands of scientists dedicated to researching and weaponizing plague. During World War II, Unit 731, of the Japanese Army, reportedly released plague-infected fleas from aircraft over Chinese cities. This method was cumbersome and unpredictable. The U.S. and Soviet Union developed the more reliable and effective delivery method of aerosolizing the organism. The terrorist potential of plague was brought to light 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 makes it particularly concerning as a biological weapon.

CLINICAL FEATURES

Plague appears in three predominant forms in humans: bubonic, septicemic, and pneumonic. The vast majority of the 1 to 40 human cases reported annually in the United States are from the desert Southwest, where plague is endemic in rural rodent populations. Most naturally occurring human cases in the U.S. are bubonic (85%), with less primary septicemic (13%) or primary pneumonic (1-2%) disease.

Bubonic Plague. The bubonic form may occur after an infected flea bites a human host. The disease begins after a typical incubation period of 2-8 days, with acute and fulminant onset of nonspecific symptoms, including high fever up to 40°C), severe malaise, headache, myalgias, and sometimes nausea and vomiting (25-50%). Up to half of patients will have abdominal pain. Simultaneous with or shortly after the onset of these nonspecific symptoms, the characteristic bubo develops – a swollen, extremely painful, infected lymph node. Buboes are typically 1-10 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. Buboes are most commonly seen in the femoral or inguinal lymph nodes as the legs are the most commonly flea-bitten part of the adult human body. But any lymph nodes can be involved, to include intra-abdominal nodes (presumably through hematogenous extension) which can present as a febrile, acute abdomen. The liver and spleen are often tender and palpable. One quarter of patients will have various types of skin lesions: 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. Secondary septicemia is common, as greater than 80 percent 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 2-6 days of symptom onset in untreated patients. In humans, the mortality of untreated bubonic plague is approximately 60 percent, but this is reduced to less than 5 percent with prompt, effective therapy.

Septicemic Plague. In those that do progress to secondary septicemia, as well as those presenting septicemic but without lymphadenopathy (primary septicemia), the symptoms and signs are similar to other gram-negative septicemias: high fever, chills, malaise, hypotension, tachycardia, tachypnea, nausea, vomiting, and diarrhea. All age groups can be affected, but the elderly seem at increased risk. Plague septicemia can produce thromboses 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; 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 central nervous system and elsewhere. Untreated septicemic plague is virtually 100% fatal, while treated disease has 30-50% mortality.

Pneumonic Plague. Pneumonic plague is an infection of the lungs due to either inhalation of the organisms (primary pneumonic plague), or spread to the lungs from septicemia (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 US in recent decades presented as primary pneumonic plague; 25% of these human cases were in veterinarians or their assistants. Person-to-person spread of pneumonic plague has not occurred in the US since 1925. 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,

eventually productive of bloody sputum. Although bloody sputum is characteristic, it can sometimes be watery or, less commonly, purulent. Gastrointestinal symptoms, including nausea, vomiting, diarrhea, and abdominal pain, may be present. Rarely, a cervical bubo might result from an inhalational exposure. The chest X-ray 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 mortality rate for untreated pneumonic plague is nearly 100 percent. In the U.S. in the past 50 years, four of the seven pneumonic plague patients (57 percent) died. Recent data from the ongoing Madagascar epidemic, which began in 1989, corroborate that figure; the mortality associated with respiratory involvement was 57 percent, while that for bubonic plague was 15 percent.

Pneumonic plague is the only form of plague disease which readily spreads from person to person. From the sparse historical data available on past pneumonic plague epidemics, 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. The majority of secondary cases have been in caregivers at home (80%) or medical professionals (14%) after close contact (within 6ft) with the primary cases.

Plague Meningitis. Meningitis is a rare complication of plague (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.

Nonspecific laboratory findings in all forms of plague disease include a leukocytosis, with a total white blood count up to 20,000 cells per ml or more with increased band forms, and greater than 80 percent polymorphonuclear cells. Platelet counts can be normal or low. One also often finds increased fibrin split products and elevated partial thromboplastin time indicating a low-grade disseminated intravascular coagulation. 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 bubo accompanied by fever, severe malaise and possible rodent exposure in 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 an intentional aerosolization.

Laboratory diagnosis. A presumptive diagnosis can be made microscopically by identification of the coccobacillus in Gram (negative), Wright, Giemsa, Wayson's, or more specific immunofluorescent antibody stained smears from lymph node

needle aspirate, sputum, blood, or cerebrospinal fluid samples. Bubo aspirates can be obtained by inserting a 20 gauge needle on a 10ml syringe containing 1ml of sterile saline; saline is injected and withdrawn until blood tinged. Definitive diagnosis relies on culturing the organism from clinical specimens. The organism grows slowly at normal incubation temperatures (optimal growth at 25-28°C), and may be misidentified by automated systems (often as *Y. pseudotuberculosis)* because of delayed biochemical reactions. It may be cultured on blood agar, MacConkey agar, or infusion broth. It will also grow in automated culture systems. Any patient with suspected plague should have blood cultures performed; as bacteremia can be intermittent, multiple cultures should be obtained, preferably prior to receipt of antibiotics (clinical severity permitting). Confirmatory diagnosis via culture commonly takes 48-72 hours (cultures should be held 5-7 days); thus specific antibiotic therapy for plague must not be withheld pending culture results. Confirmative culture-based diagnosis is conducted via specific bacteriophage lysis of the organism, which is available at reference laboratories.

Most naturally occurring strains of *Y. pestis* produce an F1-antigen in vivo, which can be detected in serum samples by specific immunoassay. 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 previous been exposed to plague or 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, but results are available only retrospectively. Most patients will seroconvert to plague within 1-2 weeks of disease onset, but a minority require 3 or more weeks.

PCR (using specific primers), is not sufficiently developed yet for routine use but it is a very sensitive and specific technique, currently able to identify as few as 10 organisms per ml.

Most clinical assays can be performed in biosafety level 2 (BSL-2) laboratories, whereas 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 mortality approaches 100% if adequate therapy is not initiated within 18-24 hours of onset of symptoms. Initial empiric therapy for systemic disease caused by *Y. pestis* includes at least one of the following antibiotics:

Preferred

- Streptomycin(FDA Approved)*, 1g IM bid (15 mg/kg IM bid for children (up to 2g/day)), or
- Gentamicin 5 mg/kg IM or IV qd, or 2mg/kg loading dose followed by 1.7mg/kg IM or IV q 8 hr (2.5mg/kg IV q 8 hr for children),(adjusted for renal clearance), or

Alternatives

- Doxycycline(FDA Approved), 100 mg IV q12 hr or 200mg IV qd for adults or children ≥ 45kg (2.2 mg/kg IV q 12 hr for children <45 kg), or

- Ciprofloxacin 400 mg IV every 12 hr for adults (for children use 15 mg/g IV q 12 hr (up to 1g/day)), or
- Chloramphenicol, 25 mg/kg IV, then 15mg/kg IV q 6 hr (adjusted for serum levels, and not for children less than 2 years of age)

Intravenous antibiotics can be switched to oral antibiotics as the improvement in the patient's clinical course dictates, to complete at least 10-14 total days of therapy. For treatment of plague meningitis add intravenous chloramphenicol. Patients with uncomplicated bubonic plague often demonstrate resolution of fever and other systemic symptoms within 3-5 days, while more complicated bubonic disease, septicemic, and pneumonic plague often result in extended hospital courses.

It is imperative that antibiotics are adjusted for demonstrated susceptibility patterns for the infecting strain; naturally-occurring strains have been reported which are resistant to streptomycin, tetracyclines, and chloramphenocol, and it is anticipated that weaponized plague could be intentionally rendered antibiotic resistant. Despite typically good in vitro susceptibilities to penicillins and cephalosporins, these antibiotics are generally felt to be ineffective in treating plague; in fact, animal studies suggest that beta-lactam antibiotics may accelerate mortality in bacteremic mice. Macrolide antibiotics are ineffective for plague.

*Streptomycin has historically been the drug of choice for plague and is the only aminoglycoside antibiotic approved by the FDA for treatment of plague; however, since it may not be readily available immediately after a large-scale BW attack, gentamicin and other alternative drugs should be considered first. Requests for streptomycin should be directed to the Roerig Streptomycin Program at Pfizer Pharmaceuticals in New York (800-254-4445).

Supportive therapy includes intravenous crystalloids and hemodynamic monitoring. Although low-grade disseminated intravascular coagulation 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 a risk to others in contact with the patient due to aerosolization of the bubo contents. 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 cases require strict isolation with respiratory droplet precautions for at least 48 hours 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. These might include, but are not limited to, use of flea insecticides, rodent control measures (after or during flea control), and flea barriers for patient care areas.

PROPHYLAXIS

Vaccine. No vaccine is currently available for prophylaxis of plague. A licensed, killed whole-cell vaccine was available in the U.S. from 1946 until November 1998. It offered protection against bubonic plague, but was not effective against aerosolized *Y. pestis*. Presently, an F1-V antigen (fusion protein) vaccine is in development at USAMRIID. It protected mice for a year against an inhalational challenge, and is now being tested in primates.

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

Preexposure prophylaxis: No antibiotics are licensed by the FDA for use before exposure to plague. However, chemoprophylaxis with doxycycline (or ciprofloxacin) may protect against plague based upon in vitro susceptibilities.

Postexposure prophylaxis: Face-to-face contacts (within 2 meters) of patients with pneumonic plague or persons possibly exposed to a plague aerosol (i.e., in a plague BW attack) should be given antibiotic prophylaxis for 7 days or the duration of risk of exposure plus 7 days. If fever or cough occurs in these individuals, a full treatment course with antibiotics should be started.

Preferred empiric prophylaxis:

- Doxycycline 100 mg po bid for adults and children $\geq$45 kg (for children <45 kg use 2.2 mg / kg po bid), or

Alternatives

- Ciprofloxacin 500 mg po bid for adults (20 mg/kg po bid (up to 1 g/day) for children)
- Chloramphenicol, 25mg/kg po qid

Other tetracyclines and fluoroquinolones antibiotics could potentially be substituted for doxycycline and ciprofloxacin, respectively. Trimethoprim-sulfamethoxazole may represent a second-line alternative, should susceptibilities allow. 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 observed for symptoms for a week. If symptoms occur, start treatment antibiotics while awaiting results of diagnostic studies.

Q FEVER

SUMMARY

Signs and Symptoms: A non-specific febrile syndrome may develop 7-41 days (average 2-3 weeks) after exposure. Radiographic evidence of pneumonia is present in up to 50 percent of cases. Duration of acute illness is usually 2 days to 2 weeks. Some patients may develop complications including hepatitis, endocarditis, or granulomatous disease.

Diagnosis: Q fever is often not a clinically distinctive disease. A high index of suspicion is necessary. Confirmatory diagnosis is by serology (indirect fluorescent antibody, complement fixation, ELISA), PCR, or *Coxiella burnetii* positive blood cultures (infection control risk).

Treatment: Q fever may be a self-limited illness; however, the potential for severe complications and relapse warrant that all cases be treated. Acute Q fever should be treated with tetracycline or doxycycline orally for 14-21 days. Chronic Q fever should be treated with combination therapy, either doxycycline plus quinolones for 4 years, or doxycycline plus hydroxychloroquine for 1 ½ to 3 years.

Prophylaxis: Chemoprophylaxis begun too early during the incubation period may delay but not prevent the onset of symptoms. Therefore, tetracycline or doxycycline should be started 8-12 days postexposure and continued for at least 5-7 days. A licensed vaccine is available in Australia and Europe. In the United States, an inactivated whole-cell IND vaccine is available with restrictions to those at high-risk of exposure.

Isolation and Decontamination: Standard precautions are recommended for healthcare workers. Person-to-person transmission is rare. Patients exposed to Q fever by aerosol do not present a risk for secondary contamination or re-aerosolization of the organism. Decontamination is accomplished with soap and water or 0.5 percent hypochlorite solution. The M291 skin decontamination kit will not neutralize the organism.

OVERVIEW

Q fever is a zoonotic disease caused by *Coxiella burnetii*, which is globally distributed. Its natural reservoirs are sheep, cattle, goats, cats, some wild animals (including rodents), and ticks. The organism localizes in the gravid uterus and mammary glands of infected animals and is shed in high numbers at parturition, whether at or before term. Transmission to humans is typically via aerosolization of infectious particles such as from 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, and at necropsy sites. Infection in livestock occasionally results in abortion, stillbirth, and dystocia, but is often asymptomatic. Transmission also occurs by ingesting contaminated raw milk and cheese, through blood product transfusions, vertically (mother to offspring), and by tick vectors. Transmission by infected tick bite is presumed to be important in maintaining livestock reservoirs but is of lesser importance for human disease.

The infectious dose is extremely low; a single organism may lead to infection. Concentrations of the organism in a gram of placental tissue may be as high as 10^9 ID_{50}. Symptomatic or not, infected livestock shed large numbers of organisms in placental tissues and body fluids including milk, urine, and feces. Exposure to infected animals at parturition is an important risk factor for endemic disease. Humans acquire the disease primarily by inhaling aerosols contaminated with the organism. Farmers, abattoir workers, and hunters are at greatest risk for exposure. *Coxiella burnetii* is also a significant hazard in laboratory personnel who are working with the organism. A BW attack with the Q fever organism would likely cause a disease similar to that occurring naturally. Q fever has been a United States notifiable disease since 1999.

HISTORY AND SIGNIFICANCE

Q fever was first described in Australia by Derrick in 1935 after an outbreak of febrile illness among abattoir workers. It was called “Query fever” because the causative agent was initially unknown. Also in 1935, United States researchers isolated a rickettsia-like agent from ticks that were subsequently linked to laboratory-acquired infection, calling it Nine-Mile agent. 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. *Coxiella burnetii* is a rickettsia-like organism that is resistant to heat, desiccation, and many common disinfectants. These features allow it to survive for long periods in the environment. It is highly infectious by the aerosol route and humans are often quite susceptible to disease. A single inhaled organism may produce clinical illness. For all of these reasons, *C. burnetii* could be used as an incapacitating BW agent.

CLINICAL FEATURES

The incubation period of Q fever varies inversely with the inhaled inoculum of *Coxiella,* typically from 7 to 21 days (mean 15, range 2-41). Nearly 60 percent of natural cases result in asymptomatic seroconversion. Of those who develop clinically apparent disease, less than 5 percent will be ill enough to require hospitalization. In symptomatic patients, onset is typically abrupt and heralded by high fever (104-105°F), fatigue, headache, and chills. Sweats, myalgias, dry cough, and nausea are common as well. Fever typically increases to a plateau over 2-4 days then ends abruptly after 1-2 weeks; untreated, fever duration ranges from 5-57 days. Weight loss is not uncommon. While a febrile syndrome with headache is probably the most common clinical presentation, atypical pneumonia or acute hepatitis syndromes are common as well, and tend to follow a geographical distribution; for example, pneumonia predominates in Nova Scotia, while hepatitis predominates in France.

Acute Q fever pneumonia generally presents as a nonspecific febrile (104-105°F) illness, with headache (often severe, retro-orbital), fatigue, chills, myalgias, and sweats, with dry cough developing in 24 to 90 percent of patients 4-5 days after initial onset. Other less common signs and symptoms may include nausea, vomiting, confusion, sore throat, diarrhea, abdominal pain, and chest pain. Physical examination of the chest is usually normal, but may reveal inspiratory rales in some cases. Chest radiograph is abnormal in 90 percent of pneumonia patients, but demonstrates non-specific findings of atypical pneumonia; single or multiple (often bilateral) patchy infiltrates with a predilection for the lower lobes. Rounded or nodular focal opacities, hilar adenopathy, or effusions have less frequently been described. Pleuritic chest pain occurs in about one-fourth of patients with Q fever pneumonia. Mortality rate is <3 percent and most patients recover within several months even without treatment.

Acute Q fever hepatitis, seen in 30-60 percent of reported cases, typically manifests itself only as elevated liver associated enzymes in conjunction with the nonspecific febrile syndrome described already. This mild hepatitis may occur in conjunction with atypical pneumonia or in the absence of a febrile syndrome as well. While hepatomegaly is common, abdominal pain, anorexia, nausea, vomiting, and diarrhea are less so, and jaundice is rare. Fulminant hepatic necrosis leading to death has been described, but is very rare. Most patients recover fully.

Other findings associated with acute Q fever include pericarditis (present in approximately 1 percent), myocarditis (0.5-1 percent of cases, but potentially life-threatening), and meningoencephalitis (0.3-1 percent of cases).

The primary complication of acute Q fever is the development of chronic disease, which develops in less than 5 percent of acute cases and most commonly presents as endocarditis; but it may also present as osteoarticular disease, vascular infection, or granulomatous hepatitis. Most patients who develop chronic Q fever have an underlying condition which predisposes to disease. Endocarditis accounts for 60-70 percent of all chronic Q fever cases; 90 percent of all cases of endocarditis develop in patients with underlying cardiac valvular defects (congenital, rheumatic, degenerative, or infectious).

Approximately 40 percent of patients with cardiac valvular defects who contract acute Q fever will ultimately develop endocarditis. Endocarditis patients usually present with heart failure or valvular dysfunction, often after a remittent febrile illness with malaise, fatigue, weight loss, and sweats. Findings that accompany endocarditis include vegetative lesions on valves (seen on echocardiography in less than 25 percent of patients, predominantly aortic and prosthetic), clubbing of digits, hepatomegaly and splenomegaly (half of patients), arterial emboli (1/3 of patients), and purpura (20 percent of patients). Mortality is less than 10 percent for endocarditis when treated with appropriate antibiotics; however, relapse rates of up to 50 percent occur upon withdrawal of therapy.

Acute Q fever during pregnancy (especially in the first 2 trimesters) is associated with an increased incidence of fetal death, premature delivery, and low birth weights; the majority of these pregnant women will develop chronic Q fever. While antibiotic treatment during pregnancy dramatically reduces the incidence of complications for the fetus, the majority of the mothers still develop chronic Q fever.

DIAGNOSIS

Differential Diagnosis: Q fever usually presents as an undifferentiated febrile illness or a primary atypical pneumonia, making it difficult to distinguish from viral illnesses or pneumonia caused by *Mycoplasma pneumoniae, Legionella pneumophila, Chlamydophila psittaci* and *Chlamydophila pneumoniae* (latter two formerly *Chlamydia* spp.). More rapidly progressive forms of Q fever pneumonia may look like bacterial pneumonias such as tularemia or plague. If significant numbers of soldiers from the same geographic area are presenting over 1 to 2 weeks with a nonspecific febrile illness with associated pulmonary symptoms in about 25 percent of cases, attack with aerosolized *C. Burnetii* should be suspected. Diagnosis may rest on clinical and epidemiologic findings in the event of possible BW attack.

Laboratory Diagnosis: A complete blood count is usually unremarkable excepting leukocytosis and/or thrombocytopenia in up to one third of patients in the acute phase. Thrombocytosis can occur in the convalescent phase. Erythrocyte sedimentation rate (ESR) typically is mildly elevated. Liver function tests in up to 85 percent of patients show a two- threefold elevation in alkaline phosphatase and the transaminases (AST, ALT). 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 (thus liver biopsy may risk hemorrhage), and antinuclear antibodies. Endocarditis patients usually have a significantly elevated erythrocyte sedimentation rate (ESR) often develop anemia, thrombocytopenia, and polyclonal hypergammaglobulinemia.

Blood cultures on standard medium are invariably negative, as *Coxiella* will only grow in living cells or organisms; even so, most laboratories do not attempt cell culture as it poses a significant infection risk to laboratory personnel (BSL-3 precautions) and is often less sensitive than serology. Sputum examination is unremarkable even in patients with productive cough. Mild

lymphocytic pleocytosis is common in the cerebrospinal fluid of patients with meningoencephalitis. Liver biopsy in hepatitis patients or bone biopsy in patients with osteomyelitis may reveal granulomas.

Serological tests are confirmatory and include identification of antibody to *C. burnetii* by indirect fluorescent antibody (widely used and considered dependable), complement fixation (CF) (commonly used but somewhat insensitive), and ELISA (sensitive and easy to perform). Specific IgM antibodies may be detectable as early as the second week after onset of illness. Combined detection of IgM, IgA, and IgG antibodies improves assay specificity and provides accuracy in diagnosis. Two antigenic phases of *C. burnetii* infections exist: phase I (virulent) and phase II (avirulent). Acute Q fever cases usually exhibit a much higher antibody level to phase II (first detected during the second week of illness). Antibodies to phase I antigens of *C. burnetii* generally take longer to appear and indicate continued exposure to the bacteria. High levels of antibody to phase I in later isolates in conjunction with constant or falling levels of antibody to phase II suggest chronic Q fever (Table 1). Antibodies to phase I and II antigens may persist for months or years after initial infection. A fourfold rise in CF antibody titer against phase II antigen is considered positive for acute disease but requires a baseline and repeat sample in 2-4 weeks. CF antibody titer against phase I antigen equal to or greater than 1:200, or phase I IgA equal to or greater than phase II titer is considered positive for chronic disease. The CF test may not be useful if sera have intrinsic anti-complement activity. ELISA is available at USAMRIID in which a single serum specimen can be used to reliably diagnose acute Q fever as early as 1 1/2 - 2 weeks into illness. *Coxiella burnetii* may also be identified in infected tissues by using immunohistochemical staining and DNA detection methods.

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

Table 1. Antibodies generally present during acute and chronic Q Fever infection

Imaging Studies: Chest radiography may reveal atypical pneumonia; pleural effusions are rare. Sonography may reveal granulomatous lesions, particularly of the liver, even in asymptomatic patients. Transesophageal echocardiography is more sensitive in finding the typically small and subendothelial lesions of endocarditis. Chronic Q fever findings include cardiac valve abnormalities (vegetation, regurgitation, abscess) and granulomatous hepatitis.

MEDICAL MANAGEMENT

Standard precautions are recommended for healthcare workers as both nosocomial transmission and infections during autopsy have been documented. Most cases of acute Q fever resolve without antibiotic treatment, but all suspected cases of Q fever should be treated to reduce the risk of complications, some of which are fatal.

Acute Q fever. Doxycycline 100 mg every 12 hours for at least 14 days is the treatment of choice for acute Q fever; alternatively, tetracycline 500 mg every 6 hours could be used. Antibiotics are most effective if begun within 3 days of the onset of symptoms. Fever usually disappears within 3 to 4 days after treatment is begun. Relapse is not uncommon and may be associated with an antibiotic regimen shorter than 2 weeks. Ciprofloxacin and other quinolones are active in vitro and should be considered in patients unable to take tetracycline or doxycycline, but they may require longer courses (14-21 days) to be effective. Quinolones may be a better choice than tetracyclines for patients with meningoencephalitis as they penetrate the cerebrospinal fluid more consistently. Trimethoprim-sulfamethoxazole (TMP-SMX) or macrolides (especially newer macrolides like clarithromycin or azithromycin) may represent alternative therapies for patients for whom doxycycline or quinolones are contraindicated. Ideal therapy for acute Q fever in children less than 8 years old, for whom doxycycline is contraindicated, has not been determined; some clinicians recommend TMP-SMX for this group, while macrolides may be useful alternatives.

Current literature suggests the possibility that hydroxychloroquine or a similar drug chloroquine may play a role in preventing chronic Q fever in selected high-risk patients; for example, some researchers advocate treating acute Q fever in patients with abnormal cardiac valves with at least 12 months of doxycycline plus hydroxychloroquine to prevent progression to endocarditis. Q fever during pregnancy is usually treated with trimethoprim-sulfamethoxazole (TMP-SMX) 160 mg/800mg po bid for the duration of pregnancy; this regimen often does not clear infection, thus a standard 2-3 week course of doxycycline or quinolone may be necessary post-partum.

Chronic Q fever. Successful treatment of Q fever endocarditis is difficult. Combination therapy of doxycycline with quinolones for at least 3-4 years, or doxycycline 100 mg po bid with hydroxychloroquine 200 mg po tid for at least 1 ½ years is recommended. The latter regimen leads to fewer relapses; however, it requires routine eye examination to monitor for hydroxychloroquine-associated ocular toxicity or visual field changes. Valve replacement may be required to cure Q fever endocarditis. Women who have contracted acute Q fever during pregnancy should have specific serum antibody titers determined post-partum; those with evidence of chronic Q fever by serology are often treated with at least 12 months of doxycycline plus hydroxychloroquine. For all forms of chronic Q fever specific serum antibody titers are followed (typically every 3 months); antibiotics should be continued until phase I *C. burnetii* IgG and IgA levels drop to 1:200 or less.

Whether chronic or acute, the importance of following antibiotic therapy protocol must be emphasized, and close follow-up care with an infectious disease specialist is recommended.

PROPHYLAXIS

Vaccine: A licensed Q fever vaccine is available in Australia and Eastern Europe. Administration of vaccine in immune or pre-sensitized individuals may cause severe local induration, sterile abscess formation, and

necrosis at the inoculation site, thus prior exposure must first be determined. This 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 greater than 95 percent protection against aerosol exposure. Protection lasts for at least 5 years. A formalin-inactivated whole cell IND vaccine is available for vaccinating at-risk personnel on an investigational basis in the United States.

Antibiotics: Chemoprophylaxis begun 8-12 days postexposure is effective, with tetracycline 500 mg every 6 hours or doxycycline 100 mg every 12 hours for at least 5–7 days. Chemoprophylaxis given within 1-7 days of exposure is not effective and may only prolong the onset of disease.

Control: As with many zoonoses, work is done with animal health authorities to determine whether the source of an outbreak is naturally occurring or the result of BW. Animal health authorities can also help to control outbreaks that may be propagated by intentionally or unintentionally infected livestock sources, and ensure that diary products are pasteurized and from approved sources.

TULAREMIA

SUMMARY

Signs and Symptoms: Ulceroglandular tularemia presents with a local ulcer and regional lymphadenopathy, fever, chills, headache, and malaise. Typhoidal tularemia presents with fever, headache, malaise, prostration, and often substernal discomfort and a non-productive cough.

Diagnosis: Physical findings are usually non-specific. Chest x-ray may reveal a pneumonic process, mediastinal lymphadenopathy or pleural effusion. Routine culture is possible but difficult. The diagnosis can be established retrospectively by serology.

Treatment: Administration of antibiotics (streptomycin or gentamicin) with early treatment is very effective for naturally acquired disease.

Prophylaxis: A live, attenuated vaccine is available as an investigational new drug. It is administered once by scarification. A 2-week course of doxycycline or ciprofloxacin should be effective as prophylaxis when given after exposure to a susceptible strain.

Isolation and Decontamination: Standard precautions for healthcare workers. Organisms are relatively easy to render harmless by mild heat (55°Celsius for 10 min) and standard disinfectants.

OVERVIEW

Francisella tularensis, the causative agent of tularemia, is a small, aerobic non-motile, gram-negative coccobacillus. Tularemia (also known as rabbit fever and deer fly fever) is a zoonotic disease that humans typically acquire after skin or mucous membrane contact with tissues or body fluids of infected animals, or from bites of infected ticks, deerflies, or mosquitoes. Less commonly, inhaling contaminated aerosols or ingesting contaminated foods or water may produce clinical disease. Respiratory exposure to infectious aerosols would typically cause typhoidal tularemia with pneumonia, but rarely ulceroglandular or oculoglandular forms can be seen as well. The organism is found throughout the temperate northern hemisphere and is the typically the cause of only sporadic human disease (average of 124 cases per year in the U.S. from 1990-2000), but is infrequently the cause of large human epidemics associated with animal epizootics. *F. tularensis* exists in at least two variants, or biovars: Biovar A, which is the predominant cause of human disease in North America; and Biovar B, a less virulent form which predominates in northern Europe and Asia. The organism can remain viable for weeks in water, soil, carcasses, hides, and for years in frozen rabbit meat. It is resistant for months to temperatures of freezing and below. It is easily killed by heat and disinfectants.

HISTORY AND SIGNIFICANCE

Francisella tularensis was identified as a distinct organism in 1911 during an investigation of a plague-like disease in rodents in Tulare County, California. Dr. Edward Francis, USPHS, established the cause of "deer-fly fever" as *Bacterium tularense* and subsequently devoted his life to researching the organism and disease, hence, the organism was later renamed *Francisella tularensis*. *F. tularensis* has been responsible for large epidemics in the past. During WWII and the German siege of Stalingrad, there were perhaps hundreds of thousands of human cases and many were pulmonary, leading to speculation that this may have resulted from the intentional use of tularemia as a biological weapon. However, there was also an ongoing and concurrent epizootic in rodents and thousands of human cases were documented in the area before the siege. This of course suggests a natural cause for the epidemic. In Sweden during the winter of 1966-67, hundreds of cases, most of which were pulmonary, occurred in farmers who processed hay contaminated by infected rodents.

Francisella tularensis was weaponized by the United States in the 1950s and 1960s during the U.S. offensive BW program. In addition, other countries are suspected to have weaponized this agent as well. This organism can be stabilized for weaponization by an adversary and produced in either a wet or dried form for delivery against U.S. forces in fashion similar to the other bacteria discussed in this handbook.

CLINICAL FEATURES

After an incubation period of 3-6 days (range 1-21 days; probably dose dependant), onset is usually acute. Tularemia typically appears in several forms, which can generally be categorized as either typhoidal or ulceroglandular. In humans, as few as 10 to 50 organisms will cause disease if inhaled or injected intradermally, whereas approximately 10^8 organisms are required with oral challenge.

Typhoidal tularemia (5-15 percent of naturally acquired cases) occurs mainly after inhalation of infectious aerosols but can occur after intradermal or gastrointestinal challenge. The disease manifests as a nonspecific syndrome consisting of abrupt onset of fever (38-40°C), headache, malaise, myalgias, and prostration; but unlike most other forms of tularemia disease, it presents without lymphadenopathy. Occasionally patients will present with nausea, vomiting, diarrhea, or abdominal pain. Case fatality rates are approximately 35% in untreated, naturally acquired typhoidal cases. Survivors of untreated tularemia may have symptoms which persist for weeks or, less often, months, with progressive debilitation. Mortality is higher if pneumonia is also present; this is the form of disease most likely to be seen after an aerosol BW attack. Case fatality rates after a BW attack may be greater than the 1-3 percent seen with appropriately treated natural disease.

Ulceroglandular tularemia (75-85 percent of naturally acquired cases cases) is most often acquired through inoculation of the skin or mucous membranes with blood or tissue fluids of infected animals. It is characterized by usually sudden onset of fever (85%), chills (52%), headache (45%), cough (38%), and myalgias (31%), concurrent with the appearance of a painful papule at the site of inoculation. The papule progresses rapidly to pustule then painful ulcer, accompanied by development of painful regional lymphadenopathy. Cutaneous ulcers are generally 0.4-3.0 cm in diameter with heaped-up edges. Lymph nodes are 0.5 to 10 cm in diameter and usually tender. In 5-10 percent of cases there is focal lymphadenopathy without an obvious ulcer present. Enlarged nodes can become fluctuant and spontaneously drain even when the patient has been taking antibiotics, and, if untreated, can persist for months or even years.

In a minority of cases (1-2 percent) the site of primary inoculation is in the eye (oculoglandular disease); this occurs after inoculation of the conjunctivae by contaminated hands, by splattering of infected tissue fluids, or via infectious aerosols. Patients have unilateral, painful, purulent conjunctivitis with preauricular or cervical lymphadenopathy. Chemosis, periorbital edema, and small nodular granulomatous lesions or ulcerations of the conjunctiva are noted in some patients.

Pharyngitis can occur in up to 25 percent of all patients with tularemia. It usually presents as an acute exudative pharyngitis or tonsillitis, sometimes with ulceration and associated painful cervical lymphadenopathy. It may occur as a syndrome of isolated penicillin-unresponsive pharyngitis and mistaken for infectious mononucleosis or other viral pharyngitis.

Pulmonary involvement is present in 47-94 percent of all naturally occurring cases of disease. It may be severe and fulminant or mild and asymptomatic and can be associated with any form of tularemia (seen in 30 percent of ulceroglandular cases), but it is most common in typhoidal tularemia (up to 80 percent of cases). Pneumonitis is asymptomatic in up to 30 percent of cases but more commonly presents with non-productive cough and substernal chest pain and occasionally with pleuritic chest pain, dyspnea, purulent sputum, or hemoptysis. An atypical or interstitial perihilar process is common but fulminant lobar pneumonias, bronchiolitis, cavitary lesions, bronchopleural fistulas, and chronic, granulomatous processes have all been described. Hilar adenopathy is common and pleural effusions have been recorded in 15 percent of cases. Thirty percent of cases of tularemia pneumonia may be accompanied by pharyngitis. Like pneumonic plague, tularemia pneumonia can be primary after the inhalation of organisms or secondary after hematogenous spread from other sites. Untreated, mortality can approach 60 percent.

DIAGNOSIS

Clinical diagnosis. A clue to the diagnosis of tularemia after a BW attack with *F. tularensis* might be a large number of temporally clustered patients presenting with similar nonspecific, febrile, systemic illnesses progressing rapidly to life-threatening pleuropneumonitis. Differential diagnoses include typhoidal syndromes (e.g., typhoid fever, rickettsia, or malaria) or pneumonic processes (e.g., plague, mycoplasma, influenza, Q-fever, staphylococcal enterotoxin B). Even after an aerosol BW attack, a percentage of patients should be expected to present with ulceroglandular disease. Some patients may exhibit a pulse-temperature mismatch (seen as often as 40 percent of the time in naturally acquired disease). The systemic symptoms and signs (fever) of tularemia classically respond quickly to appropriate antibiotics; patients typically improve dramatically within 24-48 hr of initiation of aminoglycosides (e.g. gentamicin), tetracyclines (e.g. doxycycline), or fluoroquinolones (e.g. ciprofloxacin). In contrast patients may remain febrile for weeks while on penicillin or cephalosporins alone.

Radiologic diagnosis. Chest radiographs should be performed if systemic tularemia disease is suspected but findings are often nonspecific. Atypical pneumonia accompanied by hilar adenopathy or pulmonary findings on CXR in the absence of clinical findings of pulmonary disease, could be clues to tularemia in some cases.

Laboratory diagnosis. Initial laboratory evaluations are generally nonspecific. Peripheral white blood cell counts usually range from 5,000 to 22,000 cells per microliter. Differential blood cell counts are normal with occasional lymphocytosis late in the disease process. Hematocrit, hemoglobin, and platelet levels are usually normal. Mild elevations in lactic dehydrogenase, serum transaminases, and alkaline phosphatase are common. Rhabdomyolysis may be associated with elevations in serum creatine kinase and urinary myoglobin levels. Cerebrospinal fluid is usually normal, although mild abnormalities in protein, glucose, and blood cell counts have been reported.

Tularemia can be diagnosed by recovering the organism in culture from blood, ulcers, conjunctival exudates, sputum, gastric washings, and pharyngeal exudates. Recovery of organisms may even be possible after the institution of appropriate antibiotic therapy. However, unless tularemia is suspected, delays in diagnosis are probable as the organism does not grow well in standard clinical laboratory medium. *F. tularensis* produces small, smooth, opaque colonies after 24 to 48 hr on medium containing cysteine or other sulfhydryl compounds (e.g., glucose cysteine blood agar, thioglycollate broth). Isolation represents a clear hazard to laboratory personnel and culture should only be attempted in BSL-3 containment.

Most diagnoses of tularemia are made serologically using bacterial agglutination or enzyme-linked immunosorbent assay (ELISA). Antibodies to *F. tularensis* appear within the first week of infection but levels adequate to allow confidence in the specificity of the serologic diagnosis (titer > 1:160) do not appear until more than 2 weeks after infection. Because cross-reactions can occur with *Brucella* spp., *Proteus* OX19, and *Yersinia* organisms and because antibodies may persist for years after infection, diagnosis should be made only if a fourfold or greater increase in the tularemia tube agglutination or microagglutination titer is seen during the course of the illness. Titers are usually negative the first week of infection, positive the second week in 50-70 percent of cases, and reach a maximum in 4-8 weeks.

MEDICAL MANAGEMENT

Treatment. Initial empiric therapy for systemic disease caused by *F. tularensis* includes at least one of the following antibiotics:

Preferred

- Streptomycin[*], 1g IM bid (15 mg/kg IM bid for children), or
- Gentamicin 5 mg/kg IM or IV qd (2.5 mg / kg IM or IV q8 hr for children), or

Alternatives

- Doxycycline, 100 mg IV q12 hr for adults or children ≥ 45kg (2.2 mg/kg IV q 12 hr for children <45 kg), or
- Ciprofloxacin 400 mg IV every 12 hr for adults (for children use 15 mg/g IV q 12 hr (up to 1g/day)), or
- Chloramphenicol, 15 mg/kg IV q 6 hr

*Streptomycin has historically been the drug of choice for tularemia and is the only aminoglycoside antibiotic approved by the FDA for treatment of tularemia; however, since it may not be readily available immediately after a large-scale BW attack, gentamicin and other alternative drugs should be considered first. Requests for streptomycin should be directed to the Roerig Streptomycin Program at Pfizer Pharmaceuticals in New York (800-254-4445).

Intravenous antibiotics can be switched to oral antibiotics as the improvement in the patient's course dictates. Length of therapy depends upon the antibiotic used. Chloramphenicol and tetracyclines (doxycycline) have been associated with relapse with courses lasting even 2 weeks and thus should be continued for <u>at least</u> 14-21 days. Streptomycin, gentamicin, and ciprofloxacin should be continued for <u>at least</u> 10-14 days. It is quite possible that any intentional use of tularemia as a weapon will employ a strain of the organism

which is resistant to our preferred antibiotics. Thus testing the strain for antibiotic susceptibilities is of paramount importance. A clinical clue to resistance would be failure of the patient to improve dramatically after 24-48 hr of antibiotics.

Infection Control. Because there is no known human-to-human transmission of tularemia, neither isolation nor quarantine is necessary. Standard precautions are appropriate for care of patients with draining lesions or pneumonia. Strict adherence to the drainage / secretion recommendations of standard precautions is required, especially for draining lesions, and for the disinfection of soiled clothing, bedding, equipment, etc. Heat and disinfectants easily inactivate the organism. Laboratory workers should not attempt to grow the organism in less than BSL-3 conditions.

PROPHYLAXIS

Vaccine: An investigational (IND) live-attenuated vaccine (live vaccine strain - LVS), administered by scarification, has been given to > 5,000 persons without significant adverse reactions. The vaccine prevents typhoidal and ameliorates ulceroglandular forms of laboratory-acquired tularemia. Aerosol challenge tests in laboratory animals and human volunteers have demonstrated significant protection. As with all vaccines, the degree of protection depends upon the magnitude of the challenge dose. Vaccine-induced protection could be overwhelmed by extremely high doses of the tularemia bacteria.

Immunoprophylaxis. There is no passive immunoprophylaxis (i.e., immune globulin) available for pre- or postexposure management of tularemia.

Preexposure prophylaxis: No antibiotics are licensed by the FDA for use before exposure to tularemia. However, chemoprophylaxis with ciprofloxacin or doxycycline) may protect against tularemia based upon in vitro susceptibilities.

Postexposure prophylaxis:

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 mg/kg po bid (up to 1 g/day) for children)

Postexposure prophylaxis should ideally begin within 24 hr of exposure and continue for at least 14 days. These oral antibiotic dosages may also be appropriate for treatment in mass casualty settings in which intravenous antibiotics are not available.

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

VIRAL AGENTS

Viruses are the simplest microorganisms and consist of a nucleocapsid protein coat containing genetic material, either RNA or DNA. In some cases, the viral particle is also surrounded by an outer lipid layer. Viruses are much smaller than bacteria and vary in size from 0.02 μm to 0.2 μm (1 μm = 1/1000 mm). Viruses are intracellular parasites and lack a system for their own metabolism; therefore, they depend upon the synthetic machinery of their host cells. This means that viruses, unlike the bacteria, cannot be cultivated in synthetic nutritive solutions, but require living cells in order to replicate. The host cells can be from humans, animals, plants, or bacteria. Every virus requires its own special type of host cell for replication, because a complicated interaction occurs between the cell and virus. Virus-specific host cells can be cultivated in synthetic nutrient solutions and then infected with the virus in question. Another common way of producing viruses is to replicate them on chorioallantoic membranes (from fertilized eggs). Virus production is expensive, demanding, and time-consuming. A virus typically brings about changes in the host cell that eventually lead to cell death. This handbook covers three types of viruses which could potentially be employed as BW agents: smallpox, alphaviruses (e.g., VEE), and hemorrhagic fever viruses.

SMALLPOX

SUMMARY

Signs and Symptoms: Clinical manifestations begin acutely with malaise, fever, rigors, vomiting, headache, and backache. Two to 3 days later, lesions appear which quickly progress from macules to papules, and eventually to pustular vesicles. They are more abundant on the extremities and face, and develop synchronously.

Diagnosis: Neither electron nor light microscopy are capable of discriminating variola from vaccinia, monkeypox, or cowpox. Culture and polymerase chain reaction (PCR) diagnostic techniques are more accurate in discriminating variola and other *Orthopoxviruses*.

Treatment: At present, there is no effective chemotherapy, and treatment of a clinical case remains supportive.

Prophylaxis: Immediate vaccination or revaccination should be undertaken for all personnel exposed.

Isolation and Decontamination: Patients should be considered infectious from onset of rash until all scabs separate and should be isolated using droplet and airborne precautions during this period. In the civilian setting, strict quarantine of asymptomatic contacts for 17 days after exposure may prove to be impractical and impossible to enforce. A reasonable alternative would be to require contacts 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 and remain in isolation until all scabs separate.

OVERVIEW

Smallpox is caused by the Orthopox virus, variola, which is known to exist in at least two strains, *Variola major* and the milder form, *Variola minor*. Despite the global eradication of smallpox and continued availability of a vaccine, the potential weaponization of variola continues to pose a military threat. This threat may be attributed to the aerosol infectivity of the virus, the relative ease of large-scale 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 of infection constitutes a nosocomial hazard from the time of onset of a smallpox patient's exanthem until scabs have separated. Quarantine should be applied to secondary contacts for 17 days postexposure. Vaccinia vaccination and vaccinia immune globulin each possess some efficacy in postexposure prophylaxis.

HISTORY AND SIGNIFICANCE

Endemic smallpox was declared eradicated in 1980 by the World Health Organization (WHO). Although two WHO-approved repositories of variola virus remain at the Centers for Disease Control and Prevention (CDC) in Atlanta and at Russian State Centre for Research on Virology and Biotechnology (Koltsovo, Novosibirsk Region) Russian Federation, the extent of clandestine stockpiles in other parts of the world remains unknown. The WHO Advisory Committee on Variola Virus Research recommended that all stocks of smallpox be destroyed by 30 June 2002. However, destruction has been delayed annually since that time by the WHO Health Assembly due to concerns over the need for further study of the virus given its potential as a biological warfare agent.

The United States stopped routinely vaccinating its military population in 1989, but began vaccination again in 2003 for troops deployed to Southwest Asia and the Republic of Korea. Routine civilian vaccination in the United States was discontinued in 1972. Thus much of the population is now susceptible to *Variola major*. Variola may have been used by the British Army against Native Americans by giving them contaminated blankets from the beds of smallpox victims during the eighteenth century. Japan considered the use of smallpox as a BW weapon in World War II and it has been considered as a possible threat agent against U.S. forces for many years. In addition, the former Soviet Union is reported to have produced and stockpiled massive quantities of the virus for use as a biological weapon. It is not known whether any of these stockpiles may still exist in Russia.

CLINICAL FEATURES

The incubation period of naturally-acquired smallpox averages 12 days, although it could range from 7-19 days after exposure. Clinical manifestations begin acutely with malaise, high fever (to 104°F), rigors, vomiting, headache, backache, and prostration; 15% of patients develop delirium. Approximately 10% of light-skinned patients exhibit an erythematous rash during this phase. Two to three days later, an enanthem consisting of small, painful ulcerations of the tongue

and oropharynx appears concomitantly or within 24 hours of a discrete rash about the face, hands, and forearms.

After 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 2-3-mm papules over 2 to 3 days, then to 2-5-mm vesicles within 1 to 2 more days. Four to 7 days after rash onset, the vesicles become 4-6mm 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. From 8 to 14 days after onset, the pustules form scabs that leave depressed depigmented scars upon healing. Death, if it occurs, is usually during the second week of clinical disease. The precise cause of death is not entirely understood, but is often attributed to toxemia, with high levels of circulating immune complexes. Although variola concentrations in the throat, conjunctiva, and urine diminish with time, the virus can be readily recovered from scabs throughout convalescence. Therefore, patients should be isolated and considered infectious until all scabs separate.

During 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 1% mortality in unvaccinated victims. However, the prototypical disease caused by *Variola major* resulted in mortality of 3% and 30% in the vaccinated and unvaccinated, respectively. Mortality rates 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. Higher mortality was 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 common in severely ill patients.

Other clinical forms associated with *Variola major* - flat-type and hemorrhagic-type smallpox - were notable for severe mortality. Flat-type smallpox occurred in about 6% of all cases and was most common in children. Hemorrhagic smallpox occurred in about 2-3% of all cases, was more common in pregnant women and immunocompromised individuals, 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 normal, with discrete hemorrhagic areas forming at lesion sites. Mortality was approximately 95% in both flat and hemorrhagic forms.

Partially immune patients, especially those vaccinated more than 3 years before smallpox exposure, could develop less severe forms of disease. Modified smallpox is a clinical form of disease characterized by fewer lesions which are more superficial, associated with a less pronounced fever and a more rapid resolution of disease, 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.

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

Animal studies suggest that unnaturally large inhaled inoculae of poxviruses may result in a significantly shortened incubation period (even 3-5 days) and fulminant pulmonary disease with or without appearance of rash before death; the implications of these findings for human disease resulting from intentional smallpox aerosolization is unknown at this time.

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* in unvaccinated household contacts was 58.4% and in vaccinated household contacts 3.8%.

A relative of variola, monkeypox, occurs naturally in equatorial Africa. In 2003, an outbreak of 81 primary human cases occurred in the U.S. due to exposure to exotic pets, some of which had been imported from Africa. Descriptions of human monkeypox in Africa revealed a disease that could be clinically indistinguishable from smallpox with the exception of a generally lower case fatality rate and notable enlargement of cervical and inguinal lymphadenopathy appearing 1-2 days before the rash in 90% of cases. The U.S. cases in 2003 tended to be less severe, with often localized lesions only, no mortality, and no secondary transmission to other humans.

DIAGNOSIS

Smallpox must be distinguished from other vesicular exanthems, such as chickenpox, erythema multiforme with bullae, or allergic contact dermatitis. 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 without 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. Providers who collect or process specimens should be vaccinated and should exercise contact and airborne precautions. Specimens should be collected only under the direction of public health officials. Typical variola specimens might include scrapings of skin lesions, lesion fluid, crusts, blood, or pharyngeal swabs.

A method of presumptive diagnosis is demonstration of characteristic poxvirus virions on electron microscopy of vesicular scrapings. Under light microscopy, aggregations of variola virus particles, called Guarnieri bodies, can be found. 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 is capable of discriminating variola from vaccinia, monkeypox, or cowpox.

Definitive diagnosis of variola has classically required isolation of the virus and characterization of its growth on chicken egg chorioallantoic membrane or in cell culture. Culture of variola is presently available only at Laboratory Response Network (LRN) national laboratories (CDC and USAMRIID) under BSL-4 conditions. Several nucleic acid techniques have been developed for specific poxvirus identification, with PCR becoming more widely available.

Neutralizing antibodies to variola form in the first week of illness and may be present for many years. Hemaglutination-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.

Associated laboratory 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 DIC (disseminated intravascular coagulation) developed.

MEDICAL MANAGEMENT

Medical personnel must be able to recognize a vesicular exanthem and consider the etiology as potentially variola, and then quickly initiate appropriate countermeasures. Any confirmed case of smallpox should be considered an international emergency with immediate reporting to public health authorities. People who have been exposed to known cases of small pox should be monitored for a minimum of 17 days from exposure regardless of their vaccination status; such individuals should be immediately isolated using droplet and airborne precautions at the onset of fever. In a civilian setting, strict quarantine of asymptomatic contacts may prove to be impractical and impossible 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 and remain in isolation until all scabs separate. Patients should be considered infectious until all scabs separate. 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 PPE regardless of vaccination status. Weaponized smallpox strains encountered in the future may be genetically altered to render the current vaccine ineffective, a possibility demonstrated unequivocally in similar poxvirus animal models.

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 alarming in two hospital outbreaks.

Indirect transmission by contaminated bedding or by 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.

Vaccination with a verified clinical "take" (vesicle with 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 existed a significant prospect of smallpox exposure.

Antivirals for use against smallpox are under investigation. Cidofovir has had significant *in vitro* and *in vivo* activity in animal studies. Whether it would offer benefit superior to immediate postexposure vaccination in humans has not been determined. While cidofovir is a licensed drug, its use for treating smallpox is "off-label" and thus it should be administered as an investigation new drug (IND). See Appendix L for guidelines on the administration of IND drugs. Topical antivirals such as trifluridine or idoxuridine may be useful for treating smallpox ocular disease.

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

PROPHYLAXIS

Vaccine: Smallpox vaccine (vaccinia virus) is most often administered by intradermal inoculation with a bifurcated needle, a process that became known as scarification because of the permanent scar that resulted. The current smallpox vaccine is the Wyeth Dryvax™, which is a licensed product derived from calf lymph; future smallpox vaccines will be grown on human cell cultures. Primary vaccinees receive three punctures with the needle, repeat vaccinees receive 15. Vaccination after exposure to weaponized smallpox or a case of smallpox may prevent or ameliorate disease if given as soon as possible and preferably within 7 days after exposure. A vesicle typically appears at the vaccination site 5-7 days after inoculation, with associated erythema and induration. The lesion forms a scab and gradually heals over the next 1-2 weeks; the evolution of the lesion may be more rapid, with less severe symptoms, in those with previous immunity.

Side effects include low-grade fever and axillary lymphadenopathy. The attendant erythema and induration of the vaccination vesicle is frequently misdiagnosed as bacterial superinfection. More severe vaccine reactions (more common in primary vaccinees) include inadvertent inoculation 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 1/10000 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.

Vaccination is *contraindicated* in the following conditions: immunosuppression, HIV infection, history or evidence of eczema, other active severe skin disorders, during pregnancy, or current household, sexual, or other close physical contact with person(s) possessing one of these conditions. 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 state that, with the exception of significant impairment of systemic immunity, there are no absolute contraindications to *postexposure* 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.

Passive Immunoprophylaxis: Vaccinia immune globulin (VIG) is indicated for treating some complications to the smallpox (vaccinia) vaccine (generalized vaccinia with systemic illness, ocular vaccinia without keratitis, eczema vaccinatum, and progressive vaccinia), and should be available when administering vaccine. It is available as an IND through both DoD and the CDC in both intramuscular and intravenous formulations. A formulation of VIG-IV has been licensed, but is currently in very limited supply. The dose for prophylaxis or treatment is 100 mg/kg for the intravenous formulation (first line). 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 IM (third line). Due to the large volume of the intramuscular formulation (42 ml in a 70-Kg person), the dose would be given in multiple sites over 24-36 hours. Because of decreased potency of existing lots of VIG-IM, DoD does not currently have an active IND application for it. Limited data suggest that VIG may also be of value in postexposure 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 after exposure, administration of both products (vaccine and VIG), if available, is reasonable.

VENEZUELAN EQUINE ENCEPHALITIS (VEE)

SUMMARY

Signs and Symptoms: Incubation period 1-6 days. VEE presents as an acute systemic febrile illness with encephalitis developing in a small percentage (4% children; < 1% adults). Symptoms include generalized malaise, spiking fevers, rigors, severe headache, photophobia, and myalgias for 24-72 hr. Nausea, vomiting, cough, sore throat, and diarrhea may follow. Full recovery from malaise and fatigue takes 1-2 weeks. The incidence of CNS disease and associated morbidity and mortality could be much higher after a BW attack.

Diagnosis: Clinical and epidemiological diagnosis. Physical findings are nonspecific. The white blood cell count may show a striking leukopenia and lymphopenia. Virus isolation may be made from serum, and in some cases throat or nasal swab specimens. Both neutralizing and IgG antibody in paired sera or VEE-specific IgM present in a single serum sample indicate recent infection.

Therapy: Treatment is supportive only. Treat uncomplicated VEE infections with 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: A live, attenuated vaccine is available as an investigational new drug (IND). A second, formalin-inactivated, killed vaccine is available for boosting antibody titers in those initially receiving the first vaccine. There is no postexposure immunoprophylaxis. In experimental animals, alpha-interferon and the interferon-inducer poly-ICLC have proven highly effective as post-exposure prophylaxis.

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

OVERVIEW

The Venezuelan equine encephalitis (VEE) virus complex is a group of eight mosquito-borne alphaviruses that are endemic in northern South America and Trinidad and cause rare cases of human encephalitis in Central America, Mexico, and Florida. These viruses can cause severe diseases in humans and equidae (horses, mules, burros, and donkeys). Natural infections are acquired by the bites of a wide variety of mosquitoes. Equidae serve as amplifying hosts and sources of mosquito infection.

Western and eastern equine encephalitis viruses are similar to the VEE complex, are often difficult to distinguish clinically, and share similar aspects of transmission and epidemiology. The human infective dose for VEE is thought to be approximately 10-100 organisms, which is one of the principal reasons that VEE is considered a militarily effective BW agent. Neither the population density of infected mosquitoes nor the aerosol concentration of viral particles has to be great to allow significant transmission of VEE in a BW attack. There is no evidence of direct human-to-human or horse-to-human transmission. Natural aerosol transmission is not known to occur. VEE particles 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 the VEE virus complex.

HISTORY AND SIGNIFICANCE

Initially isolated from moribund horses in 1936, VEE was shown to be capable of causing disease in humans as well in 1952 by researchers in Colombia. Between 1969 and 1971, an epizootic of a "highly pathogenic strain" of VEE emerged in Guatemala, moved through Mexico, and entered Texas in June 1971. This strain was virulent in both equidae and humans. In Mexico, there were 8,000-10,000 equine deaths, "tens of thousands" of equine cases, and 17,000 human cases (no human deaths). Over 10,000 horses in Texas died. Once the Texas border was breached, a national emergency was declared and resources were mobilized to vaccinate horses in 20 states. Ninety five percent of all horses and donkeys were vaccinated; over 3.2 million animals. In addition equine quarantines were established and control of mosquito populations was obtained with the use of broad-scale insecticides along the Rio Grande Valley and the Gulf Coast. A second VEE outbreak in 1995 in Venezuela and Columbia involved over 75,000 human cases and as many as 300 deaths.

VEE is better characterized than EEE or WEE, primarily because it was tested as a BW agent during the U.S. offensive program in the 1950s and 1960s. Other countries have also been or are suspected to have weaponized this agent. In compliance with President Nixon's National Security Decision No. 35 of November 1969 directing the destruction of the BW microbial stockpile, all existing stocks of VEE in the U.S. were destroyed under supervision.

These viruses could theoretically be produced in large amounts in either a wet or dried form by relatively unsophisticated and inexpensive systems. This

form of the VEE virus complex could be intentionally disseminated as an aerosol and would be highly infectious. It could also be spread by the purposeful dissemination of infected mosquitoes, which can probably transmit the virus throughout their lives. The VEE complex is relatively stable during the storage and manipulation procedures necessary for weaponization.

In natural human epidemics, severe and often fatal encephalitis outbreaks in equidae (30-90% mortality) always precede disease in humans. However, a biological warfare attack with virus intentionally disseminated as an aerosol would most likely cause human disease as a primary event or simultaneously with equidae. During natural epidemics, illness or death in wild or free ranging equidae may not be recognized before the onset of human disease, thus a natural epidemic could be confused with a BW event, and data on onset of disease should be considered with caution. A more reliable method for determining the likelihood of a BW event would be the presence of VEE outside of its natural geographic range. A biological warfare attack in a region populated by equidae and appropriate mosquito vectors could initiate an epizootic / epidemic.

CLINICAL FEATURES

Susceptibility in humans is high (90-100%), and nearly 100 percent of those infected develop overt illnesses. The overall case fatality rate for VEE is less than 1 percent, although it is somewhat higher in the very young or aged. Recovery from an infection results in excellent short-term and long-term immunity to the infective strain, but may not protect against other strains of the virus.

VEE is primarily an acute, incapacitating, febrile illness with encephalitis developing in only a small percentage of the infected population. Most VEE infections are mild (in contrast to clinically apparent EEE and WEE infection, in which encephalitis is common). After an incubation period as short as 28 hr but typically 2-6 days, onset of prostrating illness is usually sudden. This acute phase of illness is often manifested by generalized malaise, chills, spiking high fevers ($38^{o}C$-$40.5^{o}C$), rigors, severe headache, photophobia, and myalgias prominent in the legs and lumbosacral area. Nausea and vomiting are also common. Physical signs may include tachycardia, conjunctival injection, erythematous pharynx, and muscle tenderness. These severe symptoms generally subside within 2-4 days, to be followed by asthenia (malaise and fatigue) lasting for 1-2 weeks before full recovery. A biphasic illness, with recurrence of the acute symptoms 4-8 days after initial onset of disease, has been described infrequently. Generally, about 10 percent of patients in natural epidemics will be ill enough to require hospitalization.

During natural epidemics, approximately 4 percent of infected children (<15 years old) and less than 1 percent of adults will develop signs of severe CNS infection, with as high as 35 percent fatality for children and 10 percent for adults. Adults rarely develop neurologic complications during natural infections. Mild CNS findings would include lethargy, somnolence, photophobia or mild confusion, with or without nuchal rigidity. Seizures, ataxia, paralysis, or coma follow more severe CNS involvement. Experimental aerosol challenges in animals suggest that the incidence of CNS disease and associated morbidity and

mortality could be much higher after a BW attack, as the VEE virus may travel along the olfactory nerve and spread directly to the CNS. School aged children may be more susceptible to a fulminant form of disease characterized by depletion of lymphoid tissues, encephalitis, interstitial pneumonitis, and hepatitis, which follows a lethal course over 48-72 hr. VEE infection during pregnancy may cause encephalitis in the fetus, placental damage, spontaneous abortion, or severe congenital neuroanatomical anomalies.

DIAGNOSIS

Diagnosis of VEE is suspected on clinical and epidemiological grounds, but confirmed by virus isolation, serology, ECL, or PCR. A variety of serological tests are applicable, including IgM, ELISA, indirect FA, hemagglutination inhibition, complement-fixation, and IgG. For persons without prior known exposure to VEE complex viruses, a presumptive diagnosis may be made by identifying IgM antibody in a single serum sample taken 5-7 days after onset of illness. PCR procedures are available for confirmation, but are generally available only as a rear laboratory capability.

Samples suitable for performing diagnostic tests include blood culture (only in appropriate BSL-3 containment), acute and convalescent sera, and cerebrospinal fluid. Viremia during the acute phase of the illness (but not during encephalitis) is generally high enough to allow detection by antigen-capture ELISA or ECL. 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 or suckling mice (a Gold Standard identification assay for VEE). VEE should be isolated only in a BSL-3 laboratory.

The white blood cell count is often normal at the onset of symptoms and then usually shows a striking leucopenia, lymphopenia, and sometimes a mild thrombocytopenia by the second to third day of illness. Each of these abnormalities will usually resolve over the ensuing 1-2 weeks. Temporary, mild elevations of lactic dehydrogenase, AST, and alkaline phosphatase may also be present. In patients with encephalitis, the cerebrospinal fluid pressure may be increased and contain up to 1,000 white blood cells / mm^3 (predominantly mononuclear cells) and a mildly elevated protein concentration.

An outbreak of VEE may be difficult to distinguish from influenza on clinical grounds. Clues to the diagnosis might include the appearance of a small proportion of neurological cases, lack of person-to-person spread, or disease in equines. A BW aerosol attack could lead to an epidemic of febrile meningoencephalitis featuring seizures and coma. In a BW context, the differential diagnosis would include other causes of aseptic meningitis and meningoencephalitis.

MEDICAL MANAGEMENT

No specific viral therapy exists; hence treatment is supportive only. Patients with uncomplicated VEE 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 treated in a screened room or in quarters treated with a residual insecticide for at least 5 days after onset, or until afebrile, as human cases may be infectious for mosquitoes for at least 72 hr. Patient isolation and quarantine are otherwise not required; sufficient contagion control is provided by the implementing Standard Precautions augmented with the need for vector control while the patient is febrile. Patient-to-patient transmission by means of respiratory droplet infection has not been proven. The virus can be destroyed by heat (80°C for 30 min) and standard disinfectants.

PROPHYLAXIS

Vaccine: There are two IND human unlicensed VEE vaccines. The first investigational vaccine (designated TC-83) was developed in the 1960s and is a live, attenuated cell-culture-propagated vaccine produced by the Salk Institute. This vaccine is not effective against all of the serotypes in the VEE complex. It has been used to protect several thousand persons against laboratory infections and is presently licensed for use in equidae (and was used in the 1970-71 Texas epizootic in horses), but is an IND vaccine for humans. The vaccine is given as a single 0.5-ml subcutaneous dose. Fever, malaise, and headache occur in approximately 20 percent of vaccinees, and may be moderate to severe in 10 percent of those vaccinees to warrant bed rest for 1-2 days. Another 18 percent of vaccinees fail to develop detectable neutralizing antibodies, but it is unknown whether they are susceptible to clinical infection if challenged. Temporary contraindications for use include a concurrent viral infection or pregnancy. Individuals with diabetes or a close family history of diabetes should not receive this vaccine.

A second investigational (IND) vaccine (designated C-84) has been tested but not licensed in humans and is prepared by formalin-inactivation of the TC-83 strain. This vaccine is not used for primary vaccination, but is used to boost nonresponders to TC-83. Administer 0.5 ml subcutaneously at 2-4-week intervals for up to three inoculations or until an antibody response is measured. Periodic boosters are required. The C-84 vaccine alone does not protect rodents against experimental aerosol challenge. Therefore, C-83 is used only as a booster immunogen for the TC-84 vaccine.

As with all vaccines the degree of protection depends upon the magnitude of the challenge dose; vaccine-induced protection could be overwhelmed by extremely high doses of the pathogen. Research is underway to produce a recombinant VEE vaccine.

Immunoprophylaxis: At present, there is no preexposure or postexposure immunoprophylaxis available. Animal studies of VEE-neutralizing immune serum have given mixed results.

Chemoprophylaxis: In experimental animals, alpha-interferon and the interferon-inducer poly-ICLC have proven highly effective for postexposure chemoprophylaxis of VEE. There are no clinical data on which to assess efficacy of these drugs in humans.

VIRAL HEMORRHAGIC FEVER

SUMMARY

Signs and Symptoms: Viral hemorrhagic fevers (VHF) are illnesses characterized by fever and bleeding diathesis. Manifestations of VHF often include flushing of the face and chest, petechiae, frank bleeding, edema, hypotension, and shock. Malaise, myalgias, headache, vomiting, and diarrhea occur frequently.

Diagnosis: Definitive diagnosis is usually made at a reference laboratory with advanced biocontainment capability. An early clinical diagnosis is crucial. Any patient with a compatible clinical syndrome should suggest the possibility of a viral hemorrhagic fever.

Treatment: Intensive supportive care may be required. Antiviral therapy with intravenous ribavirin may be useful in *Bunyaviridae* and *Arenaviridae* infections (specifically Lassa fever, Rift Valley fever, Crimean-Congo hemorrhagic fever, and hemorrhagic fever with renal syndrome due to Old World Hantavirus infection) and should be used only under an investigational new drug (IND) protocol. Convalescent plasma may be effective in Argentine or Bolivian hemorrhagic fevers (available only as IND).

Prophylaxis: The only licensed VHF vaccine is the 17D yellow fever vaccine. Experimental vaccines for other VHF are not readily available. Prophylactic ribavirin may be effective for some *Bunyaviridae* and *Arenaviridae* infections (available only as IND).

Isolation and Decontamination: All VHF patients should be cared for using strict contact precautions, including hand hygiene double gloves, gowns, shoe and leg coverings, and faceshield or goggles. Airborne precautions should be instituted to the maximum extent possible. At a minimum, a fit-tested, HEPA filter-equipped respirator (such as an N-95 mask), a battery-powered, air-purifying respirator, or a positive pressure supplied air respirator should be worn by personnel sharing an enclosed space with or coming within six feet of a VHF patient. Multiple patients should be cohorted to a separate building or a ward with an isolated air-handling system. Ideally, VHF patients should be isolated in a negative pressure isolation room with 6-12 air exchanges per hour. Environmental decontamination is accomplished with hypochlorite or phenolic disinfectants.

OVERVIEW

The VHFs are a diverse group of illnesses caused by RNA viruses from four viral families. They are unified by their potential to present as a severe febrile illness accompanied by shock and a hemorrhagic diathesis. The *Arenaviridae* include the etiologic agents of Lassa fever and Argentine, Bolivian, and Venezuelan hemorrhagic fevers. The *Bunyaviridae* include the members of the *Hantavirus* genus that cause hemorrhagic fever with renal syndrome (HFRS), the Congo-Crimean hemorrhagic fever virus from the *Nairovirus* genus, and the Rift Valley fever virus from the *Phlebovirus* genus. The *Filoviridae* include Ebola and Marburg viruses. Finally, the *Flaviviridae* include dengue, yellow fever, and two viruses in the tick-borne encephalitis group that cause VHF, Omsk hemorrhagic fever virus and Kyasanur Forest disease virus . These viruses are spread in a variety of ways; some may be transmitted to humans through a respiratory portal of entry. Although evidence for weaponization does not exist for many of these viruses, they are included in this handbook because of their *potential* for aerosol dissemination, weaponization, or likelihood for confusion with similar agents that might be weaponized.

HISTORY AND SIGNIFICANCE

Because these viruses are so diverse and occur in different endemic geographic locations, a comprehensive discussion is beyond the scope of this handbook. However, each viral infection possesses a number of different features that may provide insight into their possible importance as biological threat agents.

Arenaviridae: Lassa virus causes Lassa fever in West Africa, where endemic transmission is related to infected *Mastomys* rodents. Over 5,000 deaths in West Africa are attributed to Lassa each year, with between 200,000 – 300,000 annual infections. Argentine hemorrhagic fever (AHF) is caused by Junin virus and was first described in 1955 among field workers who harvested corn. From 300 to 600 cases per year occur in areas of the Argentine pampas. Bolivian, Brazilian, and Venezuelan hemorrhagic fevers are caused by the related Machupo, Guanarito, and Sabia viruses, respectively. Nosocomial transmission is probably possible with all Arenavirus infections but is frequently a problem with Lassa fever. Lassa infection of health-care workers has been attributed to parenteral exposures, contact with body fluids, and aerosols generated by patients. These viruses are transmitted from their rodent reservoirs to humans through inhalation of dusts contaminated with rodent excreta.

Bunyaviridae: Congo-Crimean hemorrhagic fever (CCHF) is a tick-borne disease with a widespread distribution from sub-Saharan Africa through southeastern Europe, Central Asia and the Indian sub-continent. It may also be spread by contact with the body fluids or slaughtered meat of infected animals and in health-care settings. Rift Valley fever (RVF) is a mosquito-borne disease that occurs in Central Africa. The hantaviruses are rodent-borne viruses with a wide geographic distribution. Hantaan and closely related Old World hantaviruses cause hemorrhagic fever with renal syndrome (HFRS). Hantaan

virus infection is also known as Korean hemorrhagic fever or epidemic hemorrhagic fever. This is the most common human disease due to hantaviruses. It was described before WW II in Manchuria along the Amur River, among United Nations troops during the Korean conflict, and subsequently in Japan, China, and in the Russian Far East. Severe disease also occurs in some Balkan states, including Bosnia, Serbia, and Greece. Nephropathia epidemica is a milder disease that occurs in Scandinavia and other parts of Europe and is caused by a virus carried by bank voles (small rodents of the genus *Microtus* and related genera). New World hantaviruses cause hantavirus pulmonary syndrome (HPS) in the Americas. However, HPS generally does not present as a hemorrhagic fever. The hantaviruses are transmitted to humans via inhalation of dusts contaminated with rodent excreta.

Filoviridae: Ebola hemorrhagic fever was first recognized in the western equatorial province of the Sudan and a nearby region of Zaire in 1976. A second outbreak occurred in Sudan in 1979. In 1995 a single index case resulted in a large outbreak (316 cases) in Kikwit, Zaire. Subsequent epidemics have occurred in Gabon, Ivory Coast, Uganda, Republic of Congo, and Sudan. The Zaire and Sudan species of Ebola virus cause severe disease with high mortality. Reasearchers have been unable to identify a natural reservoir, and it is not known why this disease appears intermittently. A related virus (Ebola Reston) was isolated from monkeys imported into the United States from the Philippine Islands in 1989. Infected monkeys developed hemorrhagic fever. While subclinical infections occurred among exposed animal handlers, Ebola Reston has not been identified as a human pathogen. Marburg epidemics have occurred on six occasions: five times in Africa, and once in Europe. The first recognized outbreak occurred in Marburg, Germany and in Yugoslavia, among people exposed to African green monkeys. It resulted in 31 cases with seven deaths. Filoviruses may be spread from human to human by direct contact with infected blood, secretions, organs, or semen. Ebola Reston apparently spread from monkey to monkey and from monkeys to humans by the respiratory route.

Flaviviridae: Yellow fever and dengue are two mosquito-borne diseases that have great importance in the history of military campaigns and military medicine. Tick-borne flaviruses include the agents of Kyasanur Forest disease in India, and Omsk hemorrhagic fever in Siberia.

All of the VHF agents (except for dengue virus) are laboratory infectious hazards by aerosol. The aerosol infectivity for many VHF agents has been studied and quantified in experimental animal models. VHF agents cause severe disease, and many have an extremely high rate of fatality. For these reasons, the VHF agents are considered a significant threat for BW use.

CLINICAL FEATUREs

Diseases classified as VHF can be diverse in clinical presentation. In their most severe form, these diseases manifest as the VHF syndrome, with capillary leak, bleeding diathesis and hemodynamic compromise leading to shock. Early symptoms of VHF are nondescript in most cases, consisting of fever and constitutional symptoms such as malaise, myalgias and headache. This

constellation of findings is difficult to discern from any number of viral, bacterial or parasitic diseases.

Diversity of clinical features among the VHF infections probably stems from varying mechanisms of pathogenesis. For example, an immunopathogenic mechanism has been identified for dengue hemorrhagic fever, which usually occurs among patients previously infected with a heterologous dengue serotype. A prominent theory explaining this phenomenon is called "antibody-dependent enhancement." Disseminated intravascular coagulation (DIC) is thought to underlie the hemorrhagic features of Rift Valley, Marburg and Ebola fevers; but in most VHFs, the etiology of the coagulopathy is multifactorial (e.g., hepatic damage, consumptive coagulopathy, and primary marrow dysfunction).

Why some infected persons develop full-blown VHF while others do not remains an unresolved issue. Virulence of the infecting agent clearly plays a large role. The VHF syndrome occurs in a majority of patients manifesting disease from filoviruses, CCHF and the South American hemorrhagic fever viruses, while it occurs in a small minority of patients with dengue, RVF and Lassa fever. The reasons for variation among patients infected with the same virus are still unknown but probably stem from a complex system of virus-host interactions.

Differentiating the various VHF's prior to laboratory diagnosis may be difficult. Epidemiologic features may be helpful, especially the proportion of cases with mild or moderate disease compared to the proportion with severe disease. Astute clinicians who are familiar with the clinical presentations of the various VHF diseases may be able to pick out unique features that implicate one disease over the others. Clinical manifestations of the various VHF's are discussed below. Table 1 provides a summary of disease characteristics.

Arenaviridae: The clinical features of the South American hemorrhagic fevers (SAHF's) are quite similar, but they differ significantly from those of Lassa fever. The onset of the SAHF's is insidious, resulting in high unremitting fever and constitutional symptoms. A petechial or vesicular enanthem involving the palate and tonsillar pillars is quite common, as is conjunctival injection and flushing of the upper torso and face. Patients frequently have associated neurologic disease, with initial hyporeflexia followed by gait abnormalities and cerebellar dysfunction. Seizures portend a grave prognosis. Mortality from the SAHF's is high, ranging from 15% to over 30%.

By contrast, most natural infections with Lassa virus are mild or non-apparent. Less than 10% of infections result in severe disease, but mortality in these patients can be as high as 25%. Hemorrhagic phenomena are relatively uncommon, but patients frequently have retrosternal chest pain, sore throat and proteinuria. Syndromes with features of encephalitis and/or meningitis are sometimes present, as are convalescent cerebellar syndromes. Serum AST levels of 115 U/L or greater are indicative of a poor prognosis – this is often considered a criterion for treatment. Eighth nerve deafness is a common feature of Lassa fever. It occurs in the second or third week of illness and may be permanent. Transient alopecia is not uncommon during convalescence.

Bunyaviridae: CCHF is generally a severe, hemorrhagic disease. Onset is abrupt, and GI and meningeal symptoms occur frequently. Petechiae

and ecchymoses are common, as is mucosal bleeding. Hepatitis and jaundice probably results from direct viral cytotoxicity. Thrombocytopenia can be profound. Mortality ranges from 20% to 50%.

RVF is usually a self-limited, nondescript febrile illness. About 10% of patients develop retinitis with cotton-wool exudates in or around the macula. This may result in permanent vision loss. Only 1% develop hemorrhagic manifestations or severe hepatic disease, usually occurring as a second febrile phase after defervescence from an initial febrile phase of 3 – 7 days. A small minority of patients develops encephalitis after the initial febrile illness.

The severity of HFRS depends largely on the infecting virus. Puumala virus causes a relatively mild form of disease called *nephropathica endemica* that is associated with rare mortality. The most severe form of HFRS is caused by Hantaan virus and is known by various names including Korean hemorrhagic fever. Disease onset is usually abrupt and is associated with fever, malaise, myalgia, headache and lassitude. Some characteristic features are flushing of the face and neck, conjunctival and pharyngeal injection, cutaneous and mucosal petechiae (occuring by day 4-5), and profound low back pain. Mild DIC, thrombocytopenia and capillary leak syndrome may ensue leading to hypovolemic shock. Renal dysfunction is pathognomonic, frequently progressing to oliguric renal failure. A subsequent diuretic phase often accompanies convalescence, and fluid management may be a significant challenge. Death occurs in 5% to 15% of Hantaan infections.

Filoviridae: Ebola and Marburg infections present similiarly. Onset is abrupt with fever, constitutional symptoms, nausea, vomiting, diarrhea, abdominal pain, lymphadenopathy, pharyngitis, conjuctival injection, jaundice and pancreatitis. Deleirum, obtundation and coma are common. Hemorrhagic features develop as the disease progresses. A large number of patients develop a maculopapular rash around day 5, but this may be difficult to appreciate in dark-skinned persons. Death occurs at the beginning of the second week of illness. Mortality ranges from 25% (Marburg) to over 80% (Ebola Zaire).

Flaviviridae: Yellow fever is classically described as a severe biphasic illness, but it is apparent that a large number of infections are mild or subclinical. The initial phase of illness lasts about one week and consists of fever, constitutional symptoms, GI symptoms and other undifferentiated symptoms. Objective findings are unimpressive except for the frequent appearance of relative bradycardia (Faget's sign) and leukopenia. Facial flushing and conjuctival injection may also be present. After a period of clinical improvement and defervescence (hours to days) some patients develop a second febrile phase. This so called period of intoxication is characterized by high fever, severe constitutional symptoms, obtundation, skin and mucous membrane hemorrhages, severe hepatitis and profound jaundice. Liver associated enzyme elevation occurs in a pattern consistent with hepatocellular damage, and bilirubin may be quite high. Proteinuria is almost universal and is an excellent diagnostic clue. As severe disease progresses, renal failure consistent with hepatorenal syndrome may ensue. Death occurs in 50% of patients with the hemorrhagic form of yellow fever.

The two members of the TBE complex causing hemorrhagic disease (KFD, OHF) have similar clinical syndromes and are often biphasic. The first phase is a febrile viral syndrome of varying severity, associated with conjuctival suffusion, facial flushing, lymphadenopathy and splenomegaly. In its most

severe form, this syndrome may be accompanied by diffuse mucosal hemorrhaging and petechiae. Hemorrhagic pulmonary edema is a relatively common and unique feature. A second phase of illness may occur 1-3 weeks after remission. This second phase involves mainly neurologic disease. Mortality ranges from < 3% (OHF) up to 10% (KFD). Survivors may experience complications of iritis, keratitis or neuropsychiatric abnormalities.

Table 2: Comparison of VHF agents and diseases

	Virus	Disease	Endemic area	Mortality	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 and transaminases, jaundice, renal failure	17-D live attenuated vaccine very effective in prevention, no post-exposure 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 (not avail. in US) may offer some cross-protection
Filoviruses	Ebola virus	Ebola hemorrhagic fever	Africa, Philippines (Ebola Reston)	50-90% for Sudan/Zaire	Common	Severe illness, maculopapular rash, profuse bleeding and DIC	Anecdotal success with immune serum transfusion
	Marburg virus	Marburg hemorrhagic fever	Africa	23-70%	Yes		
Bunyaviruses	CCHF	Crimean-Congo hemorrhagic fever	Africa, SE Europe, Central Asia, India	30%	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 , Significant threat to livestock – epidemics of abortion and death of young	Effective livestock vaccines in Africa 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)	5% 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 convalescence common	Ribavirin effective in clinical trial with non-randomized controls
	Junin	Argentine hemorrhagic fever	Argentinean pampas	30%	Rare	Prominent GI complaints, late neurologic syndrome	Immune plasma, Ribavirin effective Candid 1 vaccine

Machupo	Bolivian hemorrhagic	Bolivia	25-35%	Rare	Similar to AHF	protective but not avail. in U.S. Immune plasma effective, ribavirin probably effective, Candid 1 vaccine protects monkeys

DIAGNOSIS

VHF should be considered in any patient presenting with a severe, acute febrile illness and evidence of vascular involvement (postural hypotension, petechiae, easy bleeding, flushing of face and chest, non-dependent edema). Symptoms and signs suggesting additional organ system involvement are common (headache, photophobia, pharyngitis, cough, nausea or vomiting, diarrhea, constipation, abdominal pain, hyperesthesia, dizziness, confusion, tremor) but usually do not dominate the picture, with the exceptions listed above under "Clinical Features." A positive tourniquet test has been particularly useful in dengue hemorrhagic fever, but should be sought in other hemorrhagic fevers as well.

A detailed travel history and a high index of suspicion are essential in making the diagnosis of VHF. Patients with arenavirus or hantavirus infections often recall proximity to rodents or their droppings; but as the viruses are spread to humans by aerosolized excreta or environmental contamination, actual contact with the rodent reservoir is not necessary. Large mosquito populations are common during RVF, yellow fever, or dengue transmission, but a history of mosquito bite is too common to be of diagnostic importance. Tick bites or nosocomial exposure are of some significance in suspecting CCHF. Large numbers of military personnel presenting with VHF manifestations in the same geographic area over a short time period should trigger immediate alarm. A natural outbreak is possible in an endemic setting, but a large number of cases should also suggest a BW attack.

The clinical laboratory can be very helpful 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 their presence is characteristic of AHF, Bolivian hemorrhagic fever, and HFRS. High AST elevation correlates with severity of Lassa fever, and jaundice is a poor prognostic sign in yellow fever.

In most geographic areas, the major differential diagnosis is malaria. Bear in mind that parasitemia in patients partially immune to malaria does not prove that symptoms are due to malaria. Other diseases in the differential diagnosis may include typhoid fever, nontyphoidal salmonellosis, leptospirosis, rickettsial infections, shigellosis, relapsing fever, fulminant hepatitis, and meningococcemia. Non-infectious illnesses that could mimic VHF include acute leukemia, lupus erythematosus, idiopathic or thrombotic thrombocytopenic purpura, hemolytic uremic syndrome and the multiple causes of DIC.

Definitive diagnosis in an individual case rests on specific virologic 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. Diagnosis by virus replication and identification requires 3 to 10 days or longer. Polymerase chain reaction (PCR) assays are being developed at USAMRIID and the CDC, and they may be helpful in making a presumptive diagnosis. With the exception of dengue, specialized microbiological containment is required for safe handling of these viruses. Appropriate precautions should be observed in collection, handling, shipping, and processing of diagnostic samples. Both the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia) and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID, Frederick, Maryland) have diagnostic laboratories functioning at the highest (BL-4 or P-4) containment level.

MEDICAL MANAGEMENT

General principles of supportive care apply to the hemodynamic, hematologic, pulmonary, and neurologic manifestations of VHF, regardless of the specific etiologic agent. Intensive care is required for the most severely ill patients. Health-care providers employing vigorous fluid resuscitation of hypotensive patients must be mindful of the propensity of some VHFs (e.g., HFRS) 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.

Management of the hemorrhagic component of VHFs mirrors that for any patient with a systemic coagulopathy. Red blood cells, platelets and clotting factors should be replaced, guided by clinical indicatation and coagulation studies. Intramuscular injections, aspirin, and other anticoagulant drugs should be avoided. Steroids are not indicated.

The antiviral drug ribavirin is available for therapy of Lassa fever, HFRS, CCHF, and RVF under an IND protocol. A controlled clinical trial has clearly indicated that parenteral ribavirin reduces morbidity in HFRS. Several trials have suggeste that ribavirin 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. Both the CDC and DOD (USAMRIID) have IND protocols for the treatment of viral hemorrhagic fevers with intravenous ribavirin. Since the supply of intravenous ribavirin is small, 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 United States. Since it is not approved for use in VHF, it should be used under an IND protocol if possible. Dosing recommendations for IV and oral ribavirin are included in Table 2. Side effects of ribavirin include modest reversible hemolytic anemia and bone marrow suppression. Ribavirin is teratogenic in laboratory animals, but no human data exist. Risks to the fetus must be weighed against the potential life-saving benefit in pregnant women with grave illness due to one of these VHFs.

Safety in infants and children has not been established for intravenous ribavirin, but inhaled ribavirin has been used extensively in the treatment of respiratory syncitial 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, Omsk hemorrhagic fever and Kyanasur Forest disease).

Argentine hemorrhagic fever responds to therapy with two or more units of convalescent plasma containing adequate amounts of neutralizing antibody and given within 8 days of onset. Bolivian hemorrhagic fever appears to respond to passive immune therapy as well. Convalescent serum or immune globulin for South American HF's is not readily available in the United States. This therapy is investigational and should be done only in consultation with experts.

TABLE 2: Recommended ribavirin dosing for treatment of viral hemorrhagic fevers* (from Borio et al. JAMA 2002;287:2391-2405)

		Intravenous	Oral
Adults	**Loading dose**	**30 mg/kg IV (max 2 grams) once**	**2000 mg PO once**
	Maintenance dose	**16 mg/kg IV (max 1 gram) q6 hours for 4 days** **8 mg/kg IV (max 500 mg) q8 hours for 6 days**	**Wt > 75 kg: 600 mg PO bid for 10 days** **Wt < 75 kg: 400 mg PO in AM, 600 mg PO in PM for 10 days**
Children	**Loading dose**	**Same as for adult**	**30 mg/kg PO once**
	Maintenance dose	**Same as for adult**	**7.5 mg/kg PO bid for 10 days**

***for confirmed or suspected arenavirus or bunyavirus or VHF of unknown etiology**

PROPHYLAXIS

The 17D live attenuated yellow fever vaccine is the only licensed vaccine available for any of the hemorrhagic fever viruses. The Candid 1 vaccine for Argentine hemorrhagic fever is a live, attenuated, investigational vaccine developed at USAMRIID. It was highly efficacious in a randomized, controlled trial in Argentinean agricultural workers, and it appears to protect against Bolivian hemorrhagic fever in non-human primates. Unfortunately, Candid 1 is no longer manufactured and is not available. Both inactivated and live-attenuated RVF vaccines are currently under investigation. There are presently no vaccines for the other VHF agents available for human use in the United States. Several local vaccines for OHF, KFD, HFRS and CCHF are used in endemic areas, but they have not been rigorously studied.

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

Close personal contacts or 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 during the established incubation period. After a presumed BW attack with an unknown VHF virus, any fever of 101°F or greater should prompt patient evaluation and consideration for immediate treatment with intravenous ribavirin. 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).

ISOLATION AND CONTAINMENT

These viruses pose special challenges for hospital infection control. With the exception of dengue and hantaviruses, VHF patients harbor significant quantities of potentially infectious virus in blood, body fluids or secretions. Special caution must be exercised in handling hypodermic needles and other sharps which could result in parenteral exposure. Strict adherence to VHF-specific barrier precautions will prevent nosocomial transmission in most cases.

Lassa, CCHF, Ebola, and Marburg viruses may be particularly prone to nosocomial spread. In several instances, secondary infections among contacts and medical personnel without direct body fluid exposure have been well documented. These instances suggest a rare phenomenon of aerosol transmission of infection. Therefore, when a VHF is suspected, additional infection control measures are indicated. The patient should be hospitalized in a private room with an adjoining anteroom to be used for putting on and removing protective barriers, storage of supplies, and decontamination of laboratory specimen containers. A negative pressure isolation room with 6–12 air exchanges per hour is ideal for all VHF patients and is strongly advised for patients with significant cough, hemorrhage, or diarrhea. All persons entering the room should wear double gloves, impermeable gowns with leg and shoe coverings (contact isolation), eye protection and HEPA (N-95) masks or positive-pressure air-purifying respirators (PAPRs).

In the absence of a large, fixed medical treatment facility, 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 in a ward with an air-handling system separate from the rest of the building. Access should be restricted to necessary personnel. Personnel should wear contact and respiratory protection while in this patient care area. Personnel should undergo an external decontamination procedure at the point of leaving the patient care area. A building, room or designated area that is separated from the patient care area should be established for donning and removing 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 incinerated or autoclaved.

Laboratory specimens should be double-bagged, and the exterior of the outer bag should be decontaminated before transport to the laboratory. Excreta and other contaminated materials should be autoclaved, or decontaminated by the liberal application of appropriate disinfectants. Clinical laboratory personnel are at significant risk for exposure and should employ a biosafety cabinet (if available) with barrier and respiratory precautions when handling specimens.

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.

No carrier state has been observed for any VHF, but excretion of virus in urine or semen may occur for some time during convalescence. Survivors should avoid sexual contact for at least 3 months. Should the patient die, there should be minimal handling of the remains, which should ideally be sealed in leak-proof material for prompt burial or cremation.

BIOLOGICAL TOXINS

Toxins are harmful substances produced by living organisms (animals, plants, microbes). The following features distinguish them from chemical agents, such as VX, cyanide, or mustard. Toxins are not man-made, are non-volatile (no vapor hazard), are usually not dermally active (mycotoxins are the exception), and may be much more toxic (based on weight) than chemical agents. A toxin's lack of volatility is a very important property and makes them unlikely to produce either secondary or person-to-person exposures, or a persistent environmental hazard.

A toxin's utility as an aerosol weapon is determined by its toxicity, stability, and ease of production. The bacterial toxins, such as botulinum toxins, are the most toxic substances by weight known (Appendix I). Less toxic compounds, such as the mycotoxins, are thousands of times less toxic than botulinum, and have limited aerosol potential. The relationship between aerosol toxicity and the quantity of toxin required for an effective open-air exposure is shown in Appendix J, which demonstrates that for some agents such as the mycotoxins and 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, and are easily denatured by environmental factors (heat, dessication, or ultraviolet light), thus posing little downwind threat. Finally, some toxins, such as saxitoxin, might be both stable and highly toxic, but are so difficult to extract that they can only feasibly be produced in minute quantities.

As with all biological weapons, potential to cause incapacitation as well as lethality must be considered. Depending on 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 and will almost certainly create panic and disruption of the affected population. Several toxins, such as staphylococcal enteroxin B (SEB), pose a significant incapacitating threat by causing illness at doses much lower than those required for lethality.

The four toxins most likely to be used as biological agents are botulinum toxins, ricin, SEB, and T-2 mycotoxins.

BOTULINUM

SUMMARY

Signs and Symptoms: Symptoms usually begin with cranial nerve palsies, including ptosis, blurred vision, diplopia, dry mouth and throat, dysphagia, and dysphonia. These findings are followed by symmetrical descending flaccid paralysis, with generalized weakness and progression to respiratory failure. Symptoms begin as early as 12-36 hours after inhalation, but may take several days to develop after exposure to low doses of toxin.

Diagnosis: Diagnosis is primarily clinical. Biological agent attack should be suspected if multiple casualties simultaneously present with progressive descending flaccid paralysis. Laboratory confirmation can be obtained by bioassay (mouse neutralization) of the patient's serum. Other helpful assays include enzyme-linked immunosorbent assay (ELISA) or electrochemiluminescence (ECL) for antigen in environmental samples, polymerase chain reaction (PCR) for bacterial DNA in environmental samples, or nerve conduction studies and electromyography.

Treatment: Early administration of trivalent licensed antitoxin or heptavalent antitoxin (IND product) may prevent or decrease progression to respiratory failure and hasten recovery. Intubation and ventilatory assistance is needed for respiratory failure. Tracheostomy may be required for long-term airway maintenance.

Prophylaxis: Pentavalent toxoid vaccine (which protects from types A, B, C, D, and E (although potency concerns for B - E); but not F or G) is available as an IND product for those at high risk of exposure. Because the original toxoid components were produced in 1970, recent evidence suggests that immunologic protection for serotypes B through E may not be adequately obtained with this currently available pentavalent toxoid vaccine.

Isolation and Decontamination: Standard precautions for healthcare workers. Botulinum toxin is not dermally active and secondary aerosols are not a hazard from patients. Decontaminate with soap and water. Botulinum toxin is inactivated by sunlight within 1-3 hours. Heat (80°C for 30 min, 100°C for several minutes) and chlorine (>99.7% inactivation by 3 mg/L FAC in 20 min) also destroy the toxin.

OVERVIEW

The botulinum toxins are a group of seven related neurotoxins produced by the spore-forming bacillus *Clostridium botulinum* and two other *Clostriidum* species. These toxins, types A through G, are the most potent neurotoxins known; paradoxically, they have been used therapeutically to treat spastic conditions (strabismus, blepharospasm, torticollis, tetanus) and cosmetically to treat wrinkles. The spores are ubiquitous; they germinate into vegetative bacteria that produce toxins during anaerobic incubation. Industrial-scale fermentation can produce large quantities of toxin for use as a BW agent. There are three epidemiologic forms of naturally occurring botulism - foodborne, infantile, and wound. Botulinum toxin can be delivered by aerosol or used to contaminate food or water supplies. When inhaled, these toxins produce a clinical picture very similar to foodborne intoxication, although the time to onset of paralytic symptoms after inhalation may actually be longer than for foodborne cases, and may vary by type and dose of toxin. The clinical syndrome produced by these toxins is known as "botulism."

HISTORY AND SIGNIFICANCE

Botulinum toxins have caused numerous cases of botulism when ingested in improperly prepared or canned foods. Many deaths have occurred from such incidents. It is feasible to deliver botulinum toxins as an aerosolized biological weapon, and several countries and terrorist groups have weaponized them. Botulinum toxins were weaponized by the U.S. in its now defunct offensive BW program. Evidence obtained by the United Nations in 1995 revealed that Iraq had filled and deployed over 100 munitions with nearly 10,000 liters of botulinum toxin. The Aum Shinrikyo cult in Japan weaponized and attempted to disseminate botulinum toxin on multiple occasions in Tokyo before their 1995 sarin attack in the Tokyo subway.

TOXIN CHARACTERISTICS

The botulinum toxins are the most toxic compounds, per weight of agent, known to humanity, requiring only one nanogram (10^{-9} g) per kg of body weight to kill 50 percent of the animals studied. Botulinum toxin type A is 15,000 times more toxic by weight than VX and 100,000 times more toxic than sarin (GB), two of the well-known organophosphate nerve agents.

Botulinum toxins are proteins with molecular masses of approximately 150,000 daltons. Each of the seven distinct, but related neurotoxins, A through G, is produced by a different strain of *Clostridium botulinum.* All seven types act by similar mechanisms of inhibition of 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. Although intelligence suggests attack by aerosol dispersal is the most likely scenario for the use of botulinum toxins, the agent could be used to sabotage food supplies.

Enemy Special Forces or terrorists might use this method in certain scenarios to produce foodborne botulism in specific targets.

These large proteins are easily denatured by environmental conditions. The toxins are detoxified in air within 12 hours. Sunlight inactivates the toxins within 1-3 hours. Heat destroys the toxins in 30 minutes at $80^{O}C$ and in several minutes at $100^{O}C$. 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 procedure; and 84% inactivated by 20 minutes at 0.4% mg/L FAC, similar to municipal water treatment procedures.

MECHANISM OF TOXICITY

Botulinum toxin consists of two polypeptide subunits (A and B chains). The B subunit binds to receptors on the axons of motor neurons. The toxin is taken into the axon, where the A chain exerts its cytotoxic effect; it inactivates the axon, preventing release of acetylcholine and blocking neuromuscular transmission (pre-synaptic inhibition). Recovery follows only after the neuron develops a new axon, which can take months. The presynaptic inhibition affects both cholinergic autonomic (muscarinic) and motor (nicotinic) receptors. This interruption of neurotransmission causes cranial nerve and skeletal muscle paralysis seen in clinical botulism.

Unlike the situation with nerve agent intoxication, where there is too much acetylcholine 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 H).

CLINICAL FEATURES

The onset of symptoms of inhalation botulism usually occurs from 12 to 36 hours after exposure, but can vary according to the amount of toxin absorbed, and could be reduced following a BW attack. Recent primate studies indicate that the signs and symptoms may not appear for several days when a low dose of the toxin is inhaled versus a shorter time period following ingestion of toxin or inhalation of higher doses.

Cranial nerve palsies are prominent early, with eye symptoms such as blurred vision due to mydriasis, diplopia, ptosis, and photophobia, in addition to other cranial nerve signs such as dysarthria, dysphonia, and dysphagia. 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 foodborne botulism.

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. Dilated pupils (mydriasis) are seen in approximately 50 percent of cases.

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

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 strongly suggests botulinum intoxication. Foodborne outbreaks tend to occur in small clusters and have never occurred in soldiers on military rations such as MREs (Meals, Ready to Eat). Higher numbers of cases in a theater of operations should raise at least the consideration of a BW` attack with aerosolized botulinum toxin.

Individual cases might be confused clinically with other neuromuscular disorders such as Guillain-Barre syndrome, myasthenia gravis, or tick paralysis. The edrophonium or Tensilon® test may be transiently positive in botulism, so it may not distinguish botulinum intoxication from myasthenia. The cerebrospinal fluid 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. 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. Atropine overdose is distinguished from botulism by its central nervous system excitation (hallucinations and delirium) even though the mucous membranes are dry and mydriasis is present. The clinical differences between botulinum intoxication and nerve agent poisoning are depicted in Appendix H.

Laboratory testing is generally not critical to the diagnosis of botulism. Mouse neutralization (bioassay) remains the most sensitive test, and serum

samples should be drawn and sent to a laboratory capable performing of this test. PCR might detect *C. botulinum* genes in an environmental sample. Detecting toxin in clinical or environmental samples is sometimes possible by ELISA or ECL. Clinical samples can include serum, gastric aspirates, stool, and respiratory secretions. 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.

MEDICAL MANAGEMENT

Supportive care, including prompt respiratory support, can be lifesaving. Respiratory failure due to paralysis of respiratory muscles is the most serious effect and, generally, the cause of death. Reported cases of botulism before 1950 had a mortality rate of 60%. With tracheotomy or endotracheal intubation and ventilatory assistance, fatalities are less than 5 percent today, although initial unrecognized cases may have a higher mortality. Preventing nosocomial infections is a primary concern, along with hydration, nasogastric suctioning for ileus, bowel and bladder care, and preventing decubitus ulcers and deep venous thromboses. Intensive and prolonged nursing care may be required for recovery, which may take up to 3 months for initial signs of improvement, and up to a year for complete resolution of symptoms.

Antitoxin: Early administration of botulinum antitoxin is critical, as the antitoxin can only neutralize the circulating toxin in patients with symptoms that continue to progress. When symptom progression ceases, no circulating toxin remains, and the antitoxin has no effect. Antitoxin may be particularly effective in foodborne cases, where presumably toxin continues to be absorbed through the gut wall. Animal experiments show that after aerosol exposure, botulinum antitoxin is very effective if given before the onset of clinical signs. If the antitoxin is delayed until after the onset of symptoms, it does not protect against respiratory failure.

Several different antitoxin preparations are available in the U.S. A licensed trivalent (types A, B, E) equine antitoxin is available from the Centers for Disease Control and Prevention (the CDC) for cases of foodborne botulism. This product has all the disadvantages of a horse serum product, including the risks of anaphylaxis and serum sickness. A bivalent human intravenous antiserum (types A and B) was licensed in October 2003 by the FDA and is available from the California Department of Health Services for treating infant botulism. Two "despeciated" equine heptavalent antitoxin preparations against all seven serotypes have been prepared by cleaving the Fc fragments from horse IgG molecules, leaving $F(ab)_2$ fragments. The original product was developed by USAMRIID, and is currently available under IND status. It has been effective in animal studies. However, 4% of horse antigens remain, so there is still a risk of hypersensitivity reactions. A newer heptavalent IND preparation by a commercial manufacturer is available through USAMRIID or the CDC.

Use of the equine antitoxin requires compliance with the IND protocol. Administration of the antitoxin may first require skin testing with escalating dose challenges to assess the degree of an individual's sensitivity to horse serum before full dose administration of the vaccine. Skin testing is performed by

injecting 0.1 ml of a 1:10 dilution (in sterile physiological saline) of antitoxin intradermally in the patient's forearm with a 26 or 27 gauge needle. The injection site is monitored and the patient is observed allergic reaction for 20 minutes. The skin test is positive if any of these allergic reactions occur: hyperemic areola at the site of the injection > 0.5 cm; fever or chills; hypotension with decrease of blood pressure > 20 mm Hg for systolic and diastolic pressures; skin rash; respiratory difficulty; nausea or vomiting; generalized itching. Equine-derived botulinum $F(ab')_2$ antitoxin is NOT administered if the skin test is positive. If no allergic symptoms are observed, the antitoxin is administered as a single dose intravenously in a normal saline solution, 10 ml over 20 minutes.

With a positive skin test, desensitization can be attempted by administering 0.01 - 0.1 ml of antitoxin subcutaneously, doubling the previous dose every 20 minutes until 1.0 - 2.0 ml can be sustained without any marked reaction. Preferably, desensitization should be performed by an experienced allergist. Medical personnel administering the antitoxin should be prepared to treat anaphylaxis with epinephrine, intubation equipment, and intravenous access.

PROPHYLAXIS

Vaccine: A pentavalent toxoid of *C. botulinum* toxin types A, B, C, D, and E is available as an IND for preexposure prophylaxis. It will likely remain under IND status as efficacy testing in humans is not feasible. This product has been administered to several thousand volunteers and occupationally at-risk workers, and historically induced serum antitoxin levels that correspond to protective levels in experimental animals. At-risk laboratory workers remain the primary recipients. The currently recommended primary series of 0, 2, and 12 weeks, followed by a 1 year booster induces protective antibody levels in > 90 percent of vaccinees after 1 year. Adequate antibody levels are transiently induced after three injections, but decline before the 1-year booster. Previously, additional need for boosters was determined by antibody testing. Since 2001, the potency of the vaccine appears to be declining. In fact, in 2004 the vaccine failed potency tests for B, C, D, and E serotypes. In the future, changes may be made to the protocol, to add a dose at 6 months and to add annual booster doses. Laboratory workers should be aware that the vaccine cannot be used as the sole protection against a possible laboratory exposure to A-E serotypes.

Contraindications to the vaccine include sensitivities to alum, formaldehyde, and thimerosal, or hypersensitivity to a previous dose. Reactogenicity is mild, with 2 to 4 percent of vaccinees in a passive surveillance system reporting erythema, edema, or induration at the local site of injection which peaks at 24 to 48 hours. The frequency of such local reactions increases with subsequent inoculations; after the second and third doses, 7 to 10 percent will have local reactions, with higher incidence (up to 20 percent or so) after boosters. Severe local reactions are rare, consisting of more extensive edema or induration. Systemic reactions are reported in up to 3 percent, consisting of fever, malaise, headache, and myalgia. Incapacitating reactions (local or systemic) are uncommon. More recent data based on active surveillance revealed 23 percent reported local reactions and 7.4 percent reported systemic reactions. The vaccine should be stored at 2-8°C (not frozen).

The vaccine is typically recommended for selected individuals or groups who work with the botulinum toxins in the laboratory. Because of the challenges of administering an IND product in an operational environment, and due to the concerns related to vaccine potency, only those individuals who have an extremely high risk of exposure to botulinum toxins in the field should be considered for receipt of the vaccine. There is no indication at present for using botulinum antitoxin as a prophylactic modality except under extremely specialized circumstances.

Posteposure prophylaxis, using the heptavalent antitoxin, has been demonstrated effective in animal studies; however, human data are not available, so it is not recommended for this indication. The antitoxin should be considered for this purpose only in extraordinary circumstances.

RICIN

SUMMARY

Signs and Symptoms: Fever, chest tightness, cough, dyspnea, nausea, and arthralgias occur 4 to 8 hours after inhalational exposure. Airway necrosis and pulmonary capillary leak resulting in pulmonary edema may occur within 18-24 hours, followed by severe respiratory distress and death from hypoxemia in 36-72 hours.

Diagnosis: Acute lung injury in large numbers of geographically clustered patients suggests exposure to aerosolized ricin. The rapid time course to severe symptoms and death would be unusual for infectious agents. Serum and respiratory secretions should be submitted for antigen detection (ELISA). Acute and convalescent sera provide retrospective diagnosis. Nonspecific laboratory and radiographic findings include leukocytosis and bilateral interstitial infiltrates.

Treatment: Management is supportive and should include treatment for pulmonary edema. Gastric lavage and cathartics are indicated for ingestion, but charcoal is of little value for large molecules such as ricin.

Prophylaxis: There is currently no vaccine or prophylactic antitoxin available for human use, although vaccination appears promising in animal models. Using a mask is currently the best protection against inhalation.

Isolation and Decontamination: Standard precautions for healthcare workers. Ricin is non-volatile, and secondary aerosols are not expected to be a danger to healthcare providers. Decontaminate with soap and water. Hypochlorite solutions (0.1% sodium hypochlorite) inactivate ricin.

OVERVIEW

Ricin is a potent protein cytotoxin derived from the beans of the castor plant (*Ricinus communis*). Castor beans are ubiquitous worldwide, and the toxin is fairly easy to extract; therefore, ricin is widely available. When inhaled as a small particle aerosol, this toxin may produce pathologic changes within 8 hours and severe respiratory symptoms followed by acute hypoxic respiratory failure in 36-72 hours. When ingested, ricin causes severe gastrointestinal symptoms followed by vascular collapse and death. This toxin may also cause disseminated intravascular coagulation, microcirculatory failure, and multiple organ failure if given intravenously in laboratory animals.

HISTORY AND SIGNIFICANCE

Ricin's significance as a potential BW toxin relates in part to its wide availability. Worldwide, one million tons of castor beans are processed annually in the production of castor oil; the waste mash from this process is 3-5 percent ricin by weight. The toxin is also quite stable and extremely toxic by several routes of exposure, including the respiratory route. Ricin was apparently used in the assassination of Bulgarian exile Georgi Markov in London in 1978. Markov was attacked with a specially engineered weapon disguised as an umbrella, which implanted a ricin-containing pellet into his body. This technique was used in at least six other assassination attempts in the late 1970s and early 1980s. In 1994 and 1995, four men from a tax-protest group known as the "Minnesota Patriots Council," were convicted of possessing ricin and conspiring to use it (by mixing it with the solvent dimethylsulfoxide) to murder law enforcement officials. In 1995, a Kansas City oncologist, Deborah Green, attempted to murder her husband by contaminating his food with ricin. In 1997, a Wisconsin resident, Thomas Leahy, was arrested and charged with possession with intent to use ricin as a weapon. In October 2003, ricin powder was discovered in a South Carolina postal facility and in February 2004 in the mail room of a United States senator. There were no injuries and these events remain under investigation as of July 2004. Ricin has a high terrorist potential due to its ready availability, relative ease of extraction, and notoriety in the press.

TOXIN CHARACTERISTICS

Ricin is actually made up of two hemagglutinins and two toxins. The toxins, RCL III and RCL IV, are dimers with molecular masses of about 66,000 daltons. The toxins are made up of two polypeptide chains, an A chain and a B chain, which are joined by a disulfide bond. Large quantities of ricin can be produced relatively easily and inexpensively by low-level technology. 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 target, or used to contaminate food or water. Ricin is stable under ambient conditions, but is detoxified by heat (80°C for 10 minutes or 50°C for about an hour at pH 7.8) and chlorine (>99.4 percent inactivation by 100 mg/L FAC in 20 minutes). Low chlorine concentrations, such as 10 mg/L FAC, as well as iodine at up to 16

mg/L, have no effect on ricin. Ricin's toxicity is marginal when comparing its LD_{50} to other toxins, such as botulinum and SEB (incapacitating dose). An enemy would need to produce it in large quantities to cover a significant area on the battlefield, limiting its large-scale use.

MECHANISM OF TOXICITY

Ricin's cytotoxicity is due to inhibition of protein synthesis. The B chain binds to cell-surface receptors and the toxin-receptor complex is taken into the cell; the A chain has endonuclease activity and extremely low concentrations will inhibit DNA replication and protein synthesis. 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. There is a latent period of 8 hours after inhalation exposure before histologic lesions are observed in animal models. In rodents, ricin is more toxic by the aerosol route than by other routes.

CLINICAL FEATURES

The clinical picture depends on the route of exposure. After aerosol exposure, signs and symptoms depend on the dose inhaled. Accidental sublethal aerosol exposures, which occurred in humans in the 1940s, were characterized by onset of fever, chest tightness, cough, dyspnea, nausea, and arthralgias within 4 to 8 hours. The onset of profuse sweating some hours later was commonly the sign of termination of most of the symptoms. 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, are sufficient to cause death from acute respiratory distress syndrome (ARDS) and respiratory failure. Time to death in experimental animals is dose dependent, occurring 36-72 hours after inhalation exposure. 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, intravascular injection can cause minimal pulmonary perivascular edema due to vascular endothelial injury. Ingestion causes necrosis of the gastrointestinal epithelium, local hemorrhage, and hepatic, splenic, and renal necrosis. Intramuscular injection causes severe local necrosis of muscle and regional lymph nodes with moderate visceral organ involvement.

DIAGNOSIS

An attack with aerosolized ricin should be primarily diagnosed by the clinical features in the appropriate epidemiological setting. 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 pathogens could present with similar signs and symptoms. Other biological threats, such as SEB, Q fever, tularemia, plague, and some chemical warfare

agents like phosgene, need to be included in the differential diagnosis. Ricin-induced pulmonary edema would be expected to occur much later (1-3 days postexposure) compared to that induced by SEB (about 12 hours postexposure) or phosgene (about 6 hours postexposure). Ricin intoxication is expected to progress despite treatment with antibiotics, as opposed to an infectious process. Ricin intoxication does not cause mediastinitis as seen with inhalational anthrax. Ricin patients do not plateau clinically as occurs with SEB intoxication. Additional supportive clinical or diagnostic features after aerosol exposure to ricin include the following: bilateral infiltrates on chest radiographs, arterial hypoxemia, neutrophilic leukocytosis, and a bronchial aspirate rich in protein compared to plasma which is characteristic of high-permeability pulmonary edema.

Specific ELISA and ECL tests of serum and respiratory secretions, or immunohistochemical stains of tissue may be used where available to confirm the diagnosis. Ricin is an extremely immunogenic toxin, and paired acute and convalescent sera should be obtained from survivors to measure antibody response. PCR can detect castor bean DNA in most ricin preparations.

MEDICAL MANAGEMENT

Management of ricin-intoxicated patients depends on the route of exposure. Patients with pulmonary intoxication are managed by appropriate respiratory support (oxygen, intubation, ventilation, PEEP, and hemodynamic monitoring) and treatment for pulmonary edema, as indicated. Gastrointestinal intoxication is best managed by vigorous gastric lavage, followed by use of cathartics such as magnesium citrate. Superactivated charcoal is of little value for large molecules such as ricin. Volume replacement of gastrointestinal fluid losses is important. In percutaneous exposures, treatment is primarily supportive.

PROPHYLAXIS

The M 40 protective mask is effective in preventing aerosol exposure. Although a vaccine is not currently available, candidate vaccines are under development which are immunogenic and confer protection against lethal aerosol exposures in animals. Preexposure prophylaxis with such a vaccine is the most promising defense against a BW attack with ricin.

STAPHYLOCOCCAL ENTEROTOXIN B

SUMMARY

Signs and Symptoms: A latent period of 3-12 hours after aerosol exposure is followed by sudden onset of fever, chills, headache, myalgia, and nonproductive cough. Some patients may develop shortness of breath and retrosternal chest pain. Patients tend to plateau rapidly to a fairly stable clinical state. Fever may last 2 to 5 days, and cough may persist for up to 4 weeks. Patients may also present with nausea, vomiting, and diarrhea. Gastrointestinal symptoms are thought to be more profound if toxin is swallowed or ingested. High levels of exposure result in toxic shock and death.

Diagnosis: Diagnosis is clinical. Patients present with a febrile respiratory syndrome without CXR abnormalities. Large numbers of patients presenting in a short period of time with typical symptoms and signs of SEB aerosol exposure suggest an intentional attack with this toxin.

Treatment: Treatment is limited to supportive care. Artificial ventilation may be needed for very severe cases, and attention to fluid management is important.

Prophylaxis: Use of protective mask. There is currently no human vaccine available to prevent SEB intoxication.

Isolation and Decontamination: Standard precautions for healthcare workers. SEB is not dermally active and secondary aerosols are not a hazard from patients. It can be decontaminated with soap and water and any contaminated food should be destroyed.

OVERVIEW

Staphylococcus aureus produces a number of exotoxins, one of which is SEB. Such toxins are referred to as exotoxins as they are excreted from the organism, and as they normally exert their effects on the intestines, they are called enterotoxins. SEB is one of the pyrogenic toxins that commonly causes food poisoning in humans after the toxin is produced in improperly handled foodstuffs and is subsequently ingested. SEB has a very broad spectrum of biological activity. This toxin causes a markedly different clinical syndrome when inhaled than it characteristically produces when ingested. Significant morbidity is produced in individuals who are exposed to SEB by either portal of entry to the body.

HISTORY AND SIGNIFICANCE

SEB is the second most common source of outbreaks of food poisoning. Often these outbreaks occur in a setting such as a church picnic or other community event, due to common-source exposure in which contaminated food is consumed. Although an aerosolized SEB toxin weapon would not likely produce significant mortality, it could render 80 percent or more of exposed personnel clinically ill and unable to perform their mission for 1-2 weeks. The demand on the medical and logistical systems could be overwhelming. For these reasons, SEB was one of the seven biological agents stockpiled by the U.S. during the time of its offensive bioweapons program, which was terminated in 1969.

TOXIN CHARACTERISTICS

Staphylococcal enterotoxins are proteins of 23-29 kilodaltons molecular mass (SEB is 28,494 daltons). They are extracellular products of coagulase-positive staphylococci. Up to 50% of clinical isolates of *S. aureus* produce exotoxins. They are produced in culture medium and also in foods when there is overgrowth of the organisms. Related toxins include toxic-shock syndrome toxin-1 (TSST-1) and exfoliative toxins. SEB is one of at least seven antigenically distinct enterotoxins that have been identified. These toxins are moderately stable; SEB is inactivated after a few minutes at 100°C. SEB causes symptoms when inhaled at very low doses in humans: a dose of several logs lower (at least 100 times less) than the lethal dose by the inhaled route would be sufficient to incapacitate 50 percent of those exposed. This toxin could also be used to sabotage food or small-volume water supplies.

MECHANISM OF TOXICITY

Staphylococcal enterotoxins belong to a class of potent immune stimulants known as bacterial superantigens. Superantigens bind to monocytes at major histocompatibility complex type II receptors rather than the usual antigen-binding receptors. This leads 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.

CLINICAL FEATURES

Symptoms of SEB intoxication begin after a latent period of 3-12 hours after inhalation, or 4-10 hours after ingestion. Initial symptoms after either route may include nonspecific flu-like symptoms such as fever, chills, headache, and myalgias. Subsequent symptoms depend upon the route of exposure. Oral exposure results in predominantly gastrointestinal symptoms: nausea, vomiting, and diarrhea. Inhalation exposures produce predominantly respiratory symptoms: nonproductive cough, retrosternal chest pain, and dyspnea. Gastrointestinal symptoms may accompany respiratory exposure due to inadvertent swallowing of the toxin after normal mucocilliary clearance, or simply as a systemic manifestation of intoxication. Gastrointestinal symptoms have been seen in ocular exposures in which ingestion was not thought to have occurred. Ocular exposure results in conjunctivitis with associated periorbital edema.

Respiratory pathology is due to the activation of pro-inflammatory cytokine cascades in the lungs, leading to pulmonary capillary leak and pulmonary edema. Severe cases may result in acute pulmonary edema and respiratory failure.

Fever may last up to 5 days and range from 103 to 106°F, with variable degrees of chills and prostration. The cough may persist up to 4 weeks, and patients may not be able to return to duty for 2 weeks.

Physical examination in patients with SEB intoxication is often unremarkable. Conjunctival injection may be present, and postural hypotension may develop due to fluid losses. Chest examination is unremarkable except in the unusual case where pulmonary edema develops. The chest X-ray is usually normal, but in severe cases increased interstitial markings, atelectasis, and occasionally pulmonary edema or ARDS may develop.

DIAGNOSIS

Diagnosis of SEB intoxication is based on clinical and epidemiologic features. Because the symptoms of SEB intoxication may be similar to several respiratory pathogens such as influenza, adenovirus, and mycoplasma, the diagnosis may initially be unclear. All of these might present with fever, nonproductive cough, myalgia, and headache. SEB attack would cause cases to present in large numbers over a very short period of time, probably within a single 24 hours. Influenza or community-acquired pneumonia should involve

patients presenting over a more prolonged time interval. Naturally occurring staphylococcal food poisoning does not present with pulmonary symptoms. SEB intoxication tends to plateau rapidly to a fairly stable clinical state, whereas inhalational anthrax, tularemia pneumonia, or pneumonic plague would all continue to progress if left untreated. Tularemia and plague, as well as Q fever, are often associated with infiltrates on chest radiographs. Other diseases, including hantavirus pulmonary syndrome, *Chlamydia* pneumonia, and various chemical warfare agents (mustard, phosgene via inhalation) are in the initial differential diagnosis.

Laboratory confirmation of SEB intoxication includes antigen detection (ELISA, ECL) on environmental and clinical samples, and gene amplification (PCR – to detect staphylococcal genes) on environmental samples. SEB may not be detectable in the serum by the time symptoms occur; regardless, a serum specimen should be drawn as early as possible after exposure. Data from rabbit studies clearly show that the presence of SEB in the serum is transient; however, accumulation in the urine **was** detected for several hours post exposure **in these animals (unpublished, USAMRIID)**. Therefore, urine samples should also be obtained and tested for SEB. Respiratory secretions and nasal swabs may demonstrate the toxin early (within 24 hours of exposure). Because most patients develop a significant antibody response to the toxin, acute and convalescent sera should be drawn for retrospective diagnosis. Nonspecific findings include a neutrophilic leukocytosis, an elevated erythrocyte sedimentation rate, and chest x-ray abnormalities consistent with pulmonary edema.

MEDICAL MANAGEMENT

Currently, therapy is limited to supportive care. Close attention to oxygenation and hydration is important, and in severe cases with pulmonary edema, ventilation with positive end-expiratory pressure, vasopressors and diuretics may be necessary. Acetaminophen for fever, and cough suppressants may make the patient more comfortable. The value of steroids is unknown. Most patients can be expected to do quite well after the initial acute phase of their illness, but will be unfit for duty for 1 to 2 weeks. Severe cases risk death from pulmonary edema and respiratory failure.

PROPHYLAXIS

Although there is currently no human vaccine against SEB intoxication, several vaccine candidates are in development. Preliminary animal studies have been encouraging. A vaccine candidate is nearing transition to advanced development for safety and immunogenicity testing in humans. Experimentally, passive immunotherapy can reduce mortality in animals, but only when given within 4-8 hours after inhaling SEB. Because of the rapidity of SEB binding with MHC receptors (<5 minutes in vitro) active vaccination is considered the most practical defense. Interestingly, most people have detectable baseline antibody titers to SEB; however, immunity acquired through natural exposure to SEB does not completely protect from an aerosol challenge (although it may reduce the emetic effect).

T-2 MYCOTOXINS

SUMMARY

Signs and symptoms: Exposure causes skin pain, pruritis, 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. Toxin also produces similar effects after ingestion or eye contact. Severe intoxication results in prostration, weakness, ataxia, collapse, shock, and death.

Diagnosis: The toxin should be suspected if an aerosol attack occurs in the form of "yellow rain" with droplets of variously pigmented oily fluids contaminating clothes and the environment. Confirmation requires testing of blood, tissue and environmental samples.

Treatment: There is no specific antidote. Treatment is supportive. Soap and water washing, even 4-6 hours after exposure, can significantly reduce dermal toxicity; washing within 1 hour may prevent toxicity entirely. 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, contact with contaminated skin and clothing can produce secondary dermal exposures. Contact precautions are warranted until decontamination is accomplished. After decontamination, standard precautions are recommended for healthcare workers. Environmental decontamination requires the use of a hypochlorite solution under alkaline conditions such as 1% sodium hypochlorite and 0.1M NaOH with 1 hour contact time.

OVERVIEW

The trichothecene (T-2) mycotoxins are a group of over 40 compounds produced by fungi of the genus *Fusarium*, a common grain mold. They are small-molecular-weight compounds, and are extremely stable in the environment. They are the only threat-agent toxin that is dermally active, causing blisters within a relatively short time after exposure (minutes to hours). Dermal, ocular, respiratory, and gastrointestinal exposures can be expected after an aerosol attack with mycotoxins.

HISTORY AND SIGNIFICANCE

The potential for T-2 use as a biological toxin was demonstrated to the Russian military shortly after World War II when flour contaminated with *Fusarium* was unknowingly baked into bread that was ingested by civilians. Some developed a protracted lethal illness called alimentary toxic aleukia (ATA) characterized by initial symptoms of abdominal pain, diarrhea, vomiting, prostration, and within days fever, chills, myalgias and bone marrow depression with granulocytopenia and secondary sepsis. Survival beyond this point allowed for the development of painful pharyngeal / laryngeal ulcerations and diffuse bleeding into the skin (petechiae and ecchymoses), melena, hematochezia, hematuria, hematemesis, epistaxis, and vaginal bleeding. Pancytopenia, and gastrointestinal ulceration and erosion were secondary to the ability of these toxins to profoundly arrest bone marrow and mucosal protein synthesis and cell-cycle progression through DNA replication.

Mycotoxins allegedly were released from aircraft in the "yellow rain" incidents in Laos (1975-81), Kampuchea (1979-81), and Afghanistan (1979-81). It has been estimated that there were more than 6,300 deaths in Laos, 1,000 in Kampuchea, and 3,042 in Afghanistan. The victims were usually unarmed civilians or guerrilla forces. These groups were not protected with masks or chemical protective clothing and had little or no capability of destroying the attacking enemy aircraft. These attacks occurred in remote jungle areas, which made confirmation of attacks and recovery of agent extremely difficult. Some investigators have claimed that the “yellow clouds” were, in fact, bee feces produced by swarms of migrating insects. This theory fails to account for the deaths, however. Much controversy has centered upon the veracity of eyewitness and victim accounts, but there is evidence to make these allegations of BW agent use in these areas possible.

TOXIN CHARACTERISTICS

The trichothecene mycotoxins are low-molecular-mass (250-500 daltons) nonvolatile compounds produced by filamentous fungi (molds) of the genera *Fusarium*, *Myrotecium, Trichoderma, Stachybotrys* and others. The structures of approximately 150 trichothecene derivatives have been described in the literature. These substances are relatively insoluble in water but are highly soluble in ethanol, methanol and propylene glycol. The trichothecenes are extremely stable to heat

and ultraviolet light inactivation. They retain their bioactivity even when autoclaved; heating to 1500° F for 30 minutes is required for inactivation. Hypochlorite solution alone does not effectively inactivate the toxins. Rather, adding 0.1M NAOH to a 1% hypochlorite solution, with 1 hour contact time is required. Soap and water effectively remove this oily toxin from exposed skin or other surfaces.

MECHANISM OF TOXICITY

The mycotoxins appear to have multiple mechanisms of action, many of which are poorly understood. Their most notable effect stems from their ability to rapidly inhibit protein and nucleic acid synthesis. Thus, they are markedly cytotoxic to rapidly dividing cells such as in the bone marrow, GI tract (mucosal epithelium), skin, and germ cells. Because this cytotoxic effect imitates the hematopoietic and lymphoid effects of radiation sickness, the mycotoxins are referred to as "radiomimetic agents." The mycotoxins also alter cell membrane structure and function, inhibit mitochondrial respiration, and inactivate certain enzymes.

CLINICAL FEATURES

In a BW attack with trichothecenes, the toxin(s) can adhere to and penetrate the skin, be inhaled, or be ingested. In the alleged yellow rain incidents, symptoms of exposure from all three routes coexisted. Contaminated clothing may serve as a reservoir for further toxin exposure. Early symptoms beginning within minutes of exposure include burning skin pain, redness, tenderness, blistering, and progression to skin necrosis with leathery blackening and sloughing of large areas of skin. Upper respiratory exposure may result in nasal itching, pain, sneezing, epistaxis, and rhinorrhea. Pulmonary and tracheobronchial toxicity produces dyspnea, wheezing, and cough. Mouth and throat exposure causes pain and blood-tinged saliva and sputum. Anorexia, nausea, vomiting, and watery or bloody diarrhea with crampy abdominal pain occur with gastrointestinal toxicity. 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 fatal cases. Death may occur in minutes, hours, or days. The most common symptoms are vomiting, diarrhea, skin involvement with burning pain, redness and pruritis, rash or blisters, bleeding, and dyspnea. A late effect of systemic absorption is pancytopenia, predisposing to bleeding and sepsis.

DIAGNOSIS

Clinical and epidemiological findings provide clues to the diagnosis. High attack rates, dead animals of multiple species, and physical evidence such as yellow, red, green, or other pigmented oily liquids suggest mycotoxins. Rapid onset of symptoms in minutes to hours supports a diagnosis of a chemical or toxin attack. Mustard and other vesicant agents must be considered but they have an odor, are visible, and can be rapidly detected by a field chemical test (M8 paper, M256 kit). Symptoms from mustard toxicity are also delayed for several hours. Inhalation of

SEB or ricin aerosols can cause fever, cough, dyspnea, and wheezing but does not involve the skin.

Specific diagnosis of T-2 mycotoxins in the form of a rapid diagnostic test is not presently available in the field. Serum and urine should be collected and sent to a reference laboratory for antigen detection. The mycotoxins and metabolites are eliminated in the urine and feces; 50-75 percent is eliminated within 24 hours; however, metabolites can be detected as late as 28 days after exposure. Pathologic specimens include blood, urine, lung, liver, and stomach contents. Environmental and clinical samples can be tested using a gas liquid chromatography-mass spectrometry technique. 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 4-6 hours after exposure. The M291 skin decontamination kit can also be used to remove skin-adherent T-2. Standard burn care is indicated for cutaneous involvement. Standard therapy for poison ingestion, including the use of superactivated charcoal to absorb swallowed T-2, should be administered to victims of an unprotected aerosol attack. Respiratory support may be necessary. The eyes should be irrigated with normal saline or water to remove toxin.

PROPHYLAXIS

Physical protection of the skin, mucous membranes, and airway (use of chemical protective mask and clothing) are the only proven effective methods of protection during an attack. Immunological (vaccines) and chemoprotective pretreatments are being studied in animal models, but are not available for field use. Topical skin protectant may limit dermal exposure. Soap and water washing, even 1 hour after dermal exposure to T-2, effectively prevents dermal toxicity.

Emerging Threats and Future Biological Weapons

OVERVIEW

The appearance of a new or reemerging infectious disease has global implications. During the past 20 years, over 30 new lethal pathogens have been identified. A classic example of this type of emerging threat is pandemic influenza. In 1918 as WWI came to an end, the Spanish flu struck with devastating consequences. In less than 1 year, this virus was able to circumnavigate the globe and kill an estimated 40 million people. More recently, the emergence of severe acute respiratory syndrome (SARS) in Southeast Asia was due to a coronavirus which jumped the species barrier from animals to humans and rapidly spread to 29 countries in less than 90 days. Novel infectious agents such as SARS or influenza appear to be occurring with increasing frequency and with a greater potential for serious consequences. Many factors contribute to the emergence of new diseases including environmental changes, global travel and trade, social upheaval, and genetic changes in infectious agents, host, or vector populations. Once a new disease is introduced into a suitable human population, it often spreads rapidly and with devastating impact on the medical and public health infrastructure. If the disease is severe, it may lead to social disruption, and cause severe economic impact. Emerging disease outbreaks may be difficult to distinguish from the intentional introduction of infectious diseases for nefarious purposes; consideration must be given to this possibility before any novel infectious disease outbreak is deemed to be of natural origin.

As scientists develop more sophisticated laboratory procedures and increase their understanding of molecular biology and the human genetic code, the possibility of bioengineering more virulent, antibiotic-resistant and vaccine-resistant pathogens for nefarious uses becomes increasingly likely. It is already theoretically possible to synthesize and weaponize certain biological response modifiers (BMRs) as well as to engineer genomic weapons capable of inserting novel DNA into host cells. The potential to cause widespread disease and death with any of the aforementioned is incalculable and should be of great concern to all. Fortunately, scientists and policy makers have begun to address the issue and a robust research agenda to develop medical countermeasures is underway.

EMERGING AND REEMERGING INFECTIONS

Because these diseases are so diverse and are endemic to different geographic locations, their complete description is beyond the scope of this handbook. However, some recently discovered infections may become important future threats as agents of BW or terrorism. An overview of the more important pathogens follows. It must be kept in mind, however, that the most worrisome emerging infectious disease may well be the one not yet recognized. Recent experience with HIV, Ebola, SARS, monkeypox, West Nile virus, and hundreds of other “new” diseases teach us that we will continue to be surprised. Clinicians and public health officials today must remain vigilant for outbreaks of novel or unexplained diseases.

Avian Influenza: Avian influenza or highly pathogenic avian influenza (HPAI) has periodically caused human infections primarily through close contact with avian species, most often through occupational contact at chicken or duck farms in South East Asia. For example, in May 2004, there was a large outbreak of avian influenza involving the H5N1 strain and human cases have been reported in two countries from this region. Thus far, no human-to-human transmission has been reported, but the potential for genetic reassortment between avian and human or animal strains of influenza exists. A recent report in the journal *Science*, linked the influenza virus responsible for the 1918 epidemic to a possible avian origin. If true, avian influenza may pose a much greater danger to human populations than previously reported. The disease presents in humans like other types of influenza virus. It usually begins with fever, chills, headaches and myalgias, and often involves the upper & 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, (+) RT-PCR for H5N1 and (+) neutralization assay for H5N1 influenza strain. In vitro studies suggest the neuraminidase inhibitor class of drugs may have clinical efficacy for treating and preventing avian Influenza infection.

Human Influenza: The threat for pandemic spread of human influenza viruses is substantial. The pathogenicity of human influenza viruses is directly related to their ability to rapidly alter their eight viral RNA segments; the new antigenic variation results in the formation of new hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins, which may go unrecognized by an immune system primed against heterologous strains.

Two distinct phenomena contribute to a renewed susceptibility to influenza infection among persons who have had influenza illness in the past. 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 such 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 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-year cycles for the last several centuries. During the last century, influenza pandemics occurred three times: 1918 (“Spanish influenza”, a H1N1 virus), 1957 (Asian influenza, a H2N2 subtype strain), and in 1968 (Hong Kong influenza, a H3N2 variant). The 1957-58 pandemic caused 66,000 excess deaths, and the 1968 pandemic caused 34,000 excess deaths in the U.S. 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 communities was between 25-40%, and case mortality rate

averaged 2.5%. A re-emergent 1918-like influenza virus would have tremendous societal effects, even in the event that antiviral medications are effective against more lethal influenza virus.

Illness – Signs and Symptoms: The onset of illness usually begins 18 to 72 hours after initial exposure, depending upon the dose of transmitted virus. Severity of illness correlates temporally with viral shedding. Death and desquamation of respiratory epithelial cells leads to inflammation and edema in the respiratory tract, with transient hyper-reactivity of the airway and consequent pulmonary dysfunction. While the virus is deposited in the respiratory tract, the signs and symptoms of influenza are systemic. The severity of flu symptoms can range from mild upper respiratory symptoms at one extreme, to fatal pneumonia on the other.

Typical signs and symptoms in adults include an abrupt onset of fever (usually >100ºF) that peaks within 24 hours and persists for from 1 to 5 days, nonproductive cough, chills, headache, myalgia, sore throat, anorexia, and malaise. Malaise may be severe and last for several days. This factor may be useful in differentiating influenza infection from other common respiratory illnesses. Other symptoms may include substernal chest pain, photophobia, and gastrointestinal symptoms including nausea, abdominal pain, and diarrhea, (although these symptoms are rarely prominent). Hospitalization or death related to influenza arises primarily from its complications, especially from secondary bacterial pneumonia.

The influenza virus may be isolated from respiratory tract specimens within 24 hours of the onset of illness. In general, viral shedding peaks at 3 to 5 days after exposure and is usually complete by 5 to 10 days. Young children tend to shed higher viral titers for more prolonged periods. Rapid tests (antigen detection, PCR) for influenza may be done at community hospitals under BSL 2 conditions. The CDC recommends that viral cultures for suspected avian influenza (H5N1) not be attempted except by reference laboratories under BSL 3 conditions; viral cultures from patients suspected of having influenza H5N1 should not be attempted in most clinical laboratories. Requests for testing within CONUS should come through public health departments, which should contact the CDC Emergency Operations Center at 770-488-7100 before sending specimens. OCONUS labs should follow theater policy.

Vaccine: Influenza vaccine strains are selected by extrapolating information gained from circulating viruses seen in the waning stages of the previous season's influenza activity cycle. Typically, two Influenza A strains, and an Influenza B strain are selected for the trivalent influenza vaccine. According to the Advisory Committee on Immunization Practices (ACIP), when an influenza vaccine accurately reflects the strains of circulating viruses, clinical efficacy in preventing infection or its complications may be quite high. The ACIP recommends that persons aged 50 years and older and those between 6 and 23 months old (and close contacts of children 0 – 23 months of age) be routinely vaccinated, as should anyone aged 6 months or older who is at increased risk for complications of influenza. In addition, vaccinating hospital and outpatient care employees can also minimize transmission to these individuals by infected caregivers. Influenza vaccine is recommended for women in the second or third trimesters of pregnancy during the flu season. Influenza vaccine is also

recommended for those at risk for coming into contact with avian influenza; although the avian strains are not covered by the vaccine, it may prevent co-infection with circulating human strains, and reduce the risk of genetic reassortment and emergence of novel strains.

Antivirals: The antiviral agents approved for use in the treatment of uncomplicated illness due to influenza include amantadine, rimantadine, Tamiflu™ (oseltamivir phosphate) and Relenza® (zanamivir).

Through their action on the viral M2 protein, amantadine and rimantadine interfere with the replication of the type A virus. When administered within the first 48 hours of illness, either of these two agents improves time to recovery by 1 to 2 days. Amantadine is indicated for treating influenza in both children and adults; insufficient data have been accumulated to approve a pediatric indication for rimantadine. In addition to their therapeutic indications, both antiviral agents are also indicated to prevent the development of illness after exposure to type A viruses.

The most significant safety issues associated with either amantadine or rimantadine involve the development of central nervous system side effects, such as nervousness, anxiety, lightheadedness, or impaired concentration. The frequency of these side effects is significantly greater in persons taking amantadine than in those taking rimantadine. Because transmissible viral resistance to amantadine or rimantadine can occur, it is appropriate to discontinue treatment after 3 to 5 days or within 24 to 48 hours after symptoms resolve.

Tamiflu™ (oseltamivir phosphate) is approved by the FDA for treating uncomplicated acute illness due to influenza infection in children (1 year and older), adolescents, and adults who have been symptomatic for no more than 2 days. It is also approved by the FDA for the prophylaxis of influenza in adults and children 13 years of age or older. Relenza® (zanamivir) is approved by the FDA for treating uncomplicated acute illness due to influenza virus in children (7 years and older), adolescents, and adults who have been symptomatic for no more than 2 days. Both Tamiflu™ and Relenza® are active against type A and type B viral strains. Relenza must be inhaled, while Tamiflu may be administered orally.

SARS and SARS-CoV: SARS-associated coronavirus (SARS-CoV) emerged as the cause of severe acute respiratory syndrome (SARS) during 2003. That year, SARS was responsible for approximately 900 deaths and over 8,000 infections in people from at least 29 countries worldwide. Before a case definition had been clearly established, Chinese authorities reported to the World Health Organization (WHO) over 300 cases of an atypical pneumonia with five related deaths; all originated from Guangdong Province in China during February 2003. The infection quickly spread by travel to Hong Kong, and from there to Vietnam, Canada, and other locations. Only eight laboratory-confirmed cases occurred in the U.S. but there is concern the U.S. population is vulnerable to a widespread outbreak of SARS such as occurred in China, Hong Kong, Toronto, and Taiwan in 2003.

Case Definition and Clinical Presentation: A SARS case definition evolved from this initial report to the WHO by Chinese health authorities in February 2003. A case was initially defined by clinical criteria that included potential exposure to an existing case and fever with pneumonia or respiratory distress syndrome as a suspected or probable case. In April 2003, this was modified to include isolation of the SARS-CoV virus from culture which would then constitute a confirmed case of SARS.

Key clinical features of SARS-CoV infection include an incubation period of 2-10 days, early systemic symptoms followed within 2-7 days of dry cough and / or shortness of breath often without respiratory tract symptoms, development of radiographically confirmed pneumonia by day 7-10 of illness, and the development of lymphopenia in most cases.

Case Detection: Most SARS-CoV patients have a clear history of exposure to either a SARS patient or to a setting in which SARS-CoV is known to exist. Laboratory tests may be helpful but do not reliably detect infection early during the illness. SARS-CoV should be suspected in patients requiring hospitalization for radiographically confirmed pneumonia or acute respiratory distress syndrome of unknown etiology and:

One of the following risk factors during the 10 days before the onset of illness

- Travel to China, Hong Kong, or Taiwan, or close contact with an ill person having a history of such travel
- Employment in an occupation associated with a risk for SARS-CoV exposure, or
- Part of a cluster of cases of atypical pneumonia without an alternative diagnosis

Infection Control: A "respiratory hygiene / cough etiquette" strategy should be adopted in all SARS-affected health-care facilities. All patients admitted to the hospital with suspected pneumonia should be:

- placed in droplet isolation until it is determined that isolation is no longer indicated (standard precautions are appropriate for most community-acquired pneumonias; droplet precautions for non-avian influenza.),
- Screened for risk factors of possible exposure to SARS-CoV,
- Evaluated with a chest radiograph, pulse oximetry, complete blood count, and additional workup as indicated.

If the patient has a risk factor for SARS, droplet precautions should be implemented pending an etiologic diagnosis. When there is a high index of suspicion for SARS-CoV disease, the patient should be placed on SARS isolation precautions immediately (including contact and airborne precautions) and all contacts of the ill patient should be identified, evaluated, and monitored.

Treatment: While the use of ribavirin, high-dose corticosteroids, and interferons has been attempted, it is unclear what effect they have had on clinical outcome. At this time, no definitive therapy has been established. Empiric antibiotic treatment for community acquired pneumonia by the current American

Thoracic Society/Infectious Diseases Society of America guidelines is recommended pending etiologic diagnosis.

Laboratory Testing: Diagnostic tests for SARS-CoV include antibody testing using an enzyme immunoassay (EIA) and reverse transcription polymerase chain reaction (RT-PCR) tests for respiratory, blood and stool specimens. In the absence of known SARS-CoV transmission, testing is only recommended in consultation with public health authorities. Requests for testing within CONUS should be come through public health departments, which should contact the CDC Emergency Operations Center at 770-488-7100 before sending specimens. OCONUS labs should follow theater policy. It is important to test for influenza, respiratory syncitial virus (RSV), pneumococcus, *Chlamydia*, *Mycoplasma*, and *Legionella*; the identification of one of these agents excludes SARS by case definition.

RT-PCR Diagnostics: Clinical samples can be obtained during the first week of illness with an NP swab plus OP swab and a serum or plasma specimen. After the first week of illness, an NP swab plus OP swab and a stool specimen should be obtained.

Serologic Diagnostics: Serum specimens for SARS-CoV antibody testing should be collected when the diagnosis is first suspected and at later times if indicated. An antibody response can occasionally be detected during the first week of illness, is likely to be detected by the end of the second week of illness, and at times may not be detected until >28 days after onset of symptoms.

Collecting Respiratory Specimens for RT-PCR Testing: Respiratory specimens from any of several different sources may be collected for viral and/or bacterial diagnostics: 1) nasopharyngeal wash / aspirates, 2) nasopharyngeal swabs, 3) oropharyngeal swabs, 4) bronchoalveolar lavage, 5) tracheal aspirate, 6) pleural fluid tap, 7) sputum, and 8) postmortem tissue. The preferred specimens of choice are nasopharyngeal washes / aspirates.

Nipah and Hendra Viruses: They are closely related but distinct paramyxoviruses that comprise a new genus within the family *Paramyxoviridae*. The Nipah virus was discovered in Malaysia in 1999 during an outbreak of a zoonotic infection involving mostly pigs and some human cases. Hendra was identified in a similar outbreak involving a single infected horse and three human cases in Southern Australia in 1994. It is believed certain species of fruit bats are the natural hosts for these viruses and remain asymptomatic. Horses (Hendra) or pigs (Nipah) act as amplifying hosts. The mode of transmission from animal to humans appears to require direct contact with tissues or body fluids, or with aerosols generated during butchering or culling. Personal protective equipment including gowns, gloves, respiratory, and eye protection is advised for agricultural workers culling infected animal herds. Thus far, human to human transmission of these viruses has not been reported.

In symptomatic cases, the onset of disease begins with flu-like symptoms and rapidly progresses to encephalitis with disorientation, delirium, and coma. Fifty percent of those with clinically apparent infections have died from their disease. There is currently no approved treatment for these infections and therefore therapy relies heavily on supportive care. The antiviral drug,

ribavirin, has been used in past infections but its effectiveness remains unproven in clinically controlled studies. Although no person-person transmission is known to have occurred, barrier nursing and droplet precautions are recommended as respiratory secretions and other bodily fluids are known to harbor the virus. The clinical laboratory should be notified before sending specimens, as these may pose a laboratory hazard. Specimens for viral isolation and identification should be forwarded to a reference laboratory. Requests for testing within COMNUS should come through public health departments, which should contact the CDC Emergency Operations Center at 770-488-7100 before sending specimens. OCONUS labs should follow theater policy.

Bioengineered Threats

The rapid advance of biotechnology has tremendous potential to alter the present and future threat of biological weapons. Already, complete or partial genomic sequence data for many of the most lethal human pathogens (such as anthrax and plague bacilli and the smallpox virus) is published and widely available through the internet. In addition to this enormous explosion in our knowledge of human pathogens is a parallel understanding of the complexities of the human immune response to foreign agents and toxins. Such knowledge has led to a deeper understanding of the development of basic immunity to a variety of different human infectious diseases. With this increase in scientific knowledge has come the power to manipulate the immune system at its most fundamental level. As we prepare for future threats, we must not ignore the potential quantum leap biotechnology offers our potential enemies in developing new BW threats. In fact, there is mounting evidence that novel biological agents have already been produced by former adversaries. Examples of such novel threat agents and the potential effects they might have on human subjects have been detailed in the scientific and popular literature. Examples of novel biologic threats that could be produced through the use of genetic engineering technology include:

1) microorganisms resistant to antibiotics, standard vaccines and/or therapeutics.
2) innocuous microorganisms genetically altered to produce a toxin, poisonous substance, or endogenous bioregulator.
3) microorganisms possessing enhanced aerosol and environmental stability characteristics.
4) immunologically altered microorganisms which are able to defeat standard threat identification, and/or diagnostic methods.
5) combinations of the above with improved delivery systems.

Examples of potential hybrid microorganisms that fit the criteria listed above have already been described by several investigators and many of these organisms are routinely used in university laboratories around the country.

Biological Response Modifiers

Biological response modifiers (BRMs) direct the myriad complex interactions of the immune system. BRMs include erythropoietins, interferons, interleukins, colony-stimulating factors, stem cell growth factors, monoclonal antibodies, and tumor necrosis factor inhibitors, and vaccines.

A growing understanding of the structure and function of various BRMs is driving the discovery and creation of many novel compounds including synthetic analgesics, antioxidants, antiviral, and antibacterial substances. For example, BRMs are being used to treat debilitating rheumatoid arthritis by targeting cytokines that contribute to the disease process. By neutralizing or eliminating these targeted cytokines, BRMs may reduce symptoms and decrease inflammation. Recently marketed BRM-based medications include Etanercept (brand name: Enbrel) and infliximab (brand name: Remicade), which have been used to target the tumor necrosis factor alpha (TNFa) cytokine, as well as Anakinra (brand name: Kineret), which targets interleuken –1 (IL-1).

BRMs may also be used as anti-carcinogens, to:

- stop, control, or suppress processes that permit cancer growth.
- make cancer cells more recognizable, and therefore more susceptible, to destruction by the immune system.
- boost the killing power of immune system cells, such as T cells, NK cells, and macrophages.
- alter cancer cells' growth patterns to promote behavior like that of healthy cells
- Block or reverse the process that changes a normal cell or a precancerous cell into a cancerous cell.
- enhance the body’s ability to repair or replace normal cells damaged or destroyed by other forms of cancer treatment, such as chemotherapy or radiation.
- prevent cancer cells from spreading to other parts of the body.

More of these promising new drugs are currently in development. It can be readily theorized that research to develop various BRMs can be subverted to a malicious end. That is, instead of using BRMs to suppress cancer growth or disease susceptibility, the potential exists that such compounds could be developed to have the opposite effect, causing illness and death to those exposed to a BRM-based agent.

GENOMIC WEAPONS

Genetic vectors have been used to transfer human and foreign genes into human cells for therapeutic purposes. As in the case of bioregulators discussed above, this exciting technology that offers the promise of treating or curing a myriad of human aliments also offers the potential to cause great harm. Viral vectors such as adenovirus and vaccinia, as well as naked or plasmid DNA have been engineered for the sole expressed purpose of delivering foreign genes into new cells. For the moment, adenovirus and adeno-associated vectors seem to be the favorite viral vectors for experiments involving the introduction of foreign

genes into human cell types. These vectors have high transduction efficiency, ability to invade non-dividing cells and the ability to infect a wide variety of cell-types. Most successful gene therapy experiments have involved the use of this viral vector as the source for the introduction of new genetic based therapies.

At this point the concept of genomic warfare is highly speculative and beyond the scope of this handbook. Little has been published in the open source literature on this topic, however, the article by Black, John L., “Genome projects and gene therapy: gateways to next generation biological weapons,” 2003. Military Medicine, 168, 11:864-71, offers an insightful discussion of this topic. 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.

DETECTION

Accurate and timely intelligence is required to develop an effective defense against the use of biological weapons. Once an agent has been dispersed, detecting the biological agent before its arrival over the target (and in time for personnel to don protective equipment), is referred to as "detect to warn." However, the concept of "detect to warn" is an ideal standard that to date has not been fully achieved. Interim systems for detecting dispersed biological agents are just now being fielded in limited numbers. Until highly accurate reliable detectors become widely available, the first indication that a biological attack has occurred will most likely be ill patients. Therefore, the timely monitoring of medical surveillance data resulting in "detection to treat" is critical for detecting a BW attack in time to potentially affect the outcome of those who may have been exposed but who are not yet ill.

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 the DoD and civilian sectors. Sensors fielded to date provide presumptive results only for a limited number of BW agents. While several systems have been deployed, and several more are in the technology demonstration stage of development, the following systems are currently available:

1. The biological integrated detection system (BIDS) is a HMMWV (high mobility multi-purpose wheeled vehicle)-mounted system that concentrates aerosol particles from environmental air, then subjects the particle sample to antibody-based detection schemes for selected agents. It is presently capable of detecting eight BW agents within 45 minutes.

2. The interim biological agent detection system (IBADS) is a semi-automatic version of the BIDS designed for shipboard use. It is capable of detecting the same eight BW agents as the BIDS but within 25 minutes.

3. Portal shield is an independent aerosol collector capable of detecting up to eight BW agents within 25 minutes using antibody-based detection. It is designed for fixed installations and can be networked and interfaced with chemical warfare sensors.

4. The joint biological point detection system (JBPDS) is designed to detect 10 BW agents. Like the portal shield it can operate as part of a network. It is designed to a have a process time of less than 18 minutes, decreasing to less than 10 minutes in future versions. JBPDS is intended to be used on multiple platforms and by all military services.

5. The dry filter unit (DFU) represents a standardized point detection system for biological 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. Samples may be processed at nearby labs or delivered

to established high-volume laboratories set up specifically for such purposes. More information is available at http://www.dcfp.navy.mil/cbrd/ca/dfu.htm. There is also a biological weapons agent-sampling (BWAS) kit designed for manual sampling and testing with the HHA.

6. 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. It will employ an infrared laser to detect aerosol clouds at a standoff distance of up to 30 kilometers. 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 ultraviolet and laser-induced fluorescence to detect biological aerosol clouds at distances of up to 5 kilometers. 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 agent but may indicate an approaching aerosol. The SRBSDS will be designed to differentiate biological aerosols from other non-biological aerosols. Confirmation of a live BW agent or toxin could then be done using the BIDS or a BWAS Kit and a DFU.

7. Hand-held assays are simple one-time-use immunochromatography devices that are very similar to the urine test strips used for home pregnancy tests. These tests provide a yes-no response to the presence of 10 biological agents within 15 minutes. A skilled user may derive a semi-quantitative measure of an agent's presence by the degree of color change. HHAs are currently employed in virtually all fielded military biological detection systems (BIDS, portal shield, DFUs, JBPDS), and are also present in developmental systems. HHAs are quite versatile. They may be used in automated readers or can be 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.

8. The joint biological agent identification and diagnostic system (JBAIDS), is similar to the ruggedized advanced pathogen identification device (RAPID). Both employ RT-PCR technology to identify BW agents. They are designed as portable, reusable systems capable of confirmatory identification of BW agents and pathogens. The systems rely on technically advanced processes and critical reagents provided through each respective program. The JBAIDS program has a "spiral upgrade" structure to allow for advances in technology, which will lead to decreases in the weight and cube of the system, lessen the technical expertise required to use the system, and lessen the time required to obtain results. The associated critical reagents program will lead to an increase in the number of agents that can be detected.

9. The Zebra (Z) chip project represents an attempt to develop a comprehensive surveillance network to detect biothreats and emerging diseases. It consists of four elements. These include a Zebra diagnostic platform, which in its present manifestation includes a gene chip (Z-chip). This chip consists of an array of DNA probes designed to detect various gene sequences. When DNA from an unknown infectious agent is placed on the chip, the DNA will adhere to areas

where it locates matching sequences. The resulting DNA pattern will reveal the identity of the infectious organism.

The above systems represent a vast improvement over capabilities available only a few years ago. However, they mostly provide only presumptive tests for a limited number of agents and are still “detect-to-treat” systems rather than the desired “detect-to-warn” systems. There are many other systems under development by the DoD and others. Some employ innovative detection methods such as gene chips and various types of mass spectrometry. Others employ either single or multiple complementary technologies. Some are simply improved aerosol collectors with no inherent BW agent identifying technology. Other government agencies are working on systems similar to portal shield that will use both antibody- and genetic-based detection schemes to yield confirmatory results for both domestic and military use.

Someday, we hope to have a reliable “detect-to-warn” capability. In the meantime the services have developed improved tactics, techniques, and procedures to better provide a forward confirmatory testing capability for both environmental samples and clinical specimens. Units like the 520th TAML (Theater Area Medical Laboratory), FDPMU (Forward Deployed Preventive Medicine Unit), and the Air Force FFBAT (Biological Augmentation Team) have been equipped with RT-PCR instruments such as the light-cycler® and RAPID® to provide for 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.

These labs as well as CONUS labs test for multiple biomarkers using other technologies such as immunochemical methods. A single positive test provides for a presumptive identification of an agent as false positives are possible with nearly all laboratory tests. Confirming the presence of an agent requires that at least two tests analyzed by different technologies be performed on the sample because the probability of two tests generating false positive results simultaneously is quite low. Three or more positive tests provide definitive confirmation, as does the ‘gold standard’ of culturing the organism.

Standoff BW 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 most 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 collective physical protection equipment, use of medical countermeasures (vaccines and other chemoprophylactic measures), post-event deployment of antibiotics and antivirals, and well developed response protocols.

PERSONAL PROTECTION

The currently fielded chemical protective equipment which includes the protective mask; the Joint Services lightweight integrated suit technology (JSLIST), which replaces the battle dress overgarment (BDO); protective gloves; and multi-purpose overboots (MULO) will protect against a biological agent attack. At the time of this writing improved gloves are under development. A Joint Service general purpose mask is also under development.

The standard issue mask, the M40, is available in three sizes, and when worn correctly, will protect the face, eyes, and respiratory tract. The M40 employs a single, standard screw-on C2A1 filter element which involves two separate but complementary mechanisms: 1) impaction and adsorption of agent molecules onto ASC Whetlerite carbon filtration media, and 2) static electrical attraction of particles which are able to pass carbon filtration media on first pass. Proper maintenance and periodic replacement of the crucial filter elements is of utmost importance. The filter MUST be replaced under the following circumstances:

1. The elements become immersed in water, crushed, cut, or otherwise damaged.
2. Excessive breathing resistance is encountered.
3. The "ALL CLEAR" signal is given after exposure to a biological agent.
4. Thirty days have elapsed in the combat theater of operations (the filters must be replaced every 30 days once opened).
5. Supply bulletins indicate lot number expiration.
6. When so ordered by the unit commander.

The filter element must only be changed in a non-contaminated environment. Two styles of optical inserts for the protective mask are available for personnel requiring visual correction. The wire frame style is considered to be the safer of the two and is more easily fitted into the mask. A prong-type optical insert is also available. A drinking tube on the mask allows the wearer to drink while in a contaminated environment. Note that the wearer should disinfect the canteen and tube by wiping with a 5 percent hypochlorite solution before use.

The JSLIST is available 7 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 by 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 both 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 biological agents, it is noteworthy that even standard uniform clothing of good quality affords a reasonable protection against dermal exposure of surfaces covered.

Those casualties unable to continue wearing protective equipment should be held and / or transported within casualty wraps designed to protect the patient against further chemical-biological agent exposure. Adding a filter blower unit to provide overpressure enhances protection and provides cooling.

Collective protection by using either a hardened or unhardened shelter equipped with an air filtration unit providing overpressure can protect personnel in the biologically contaminated environment. An airlock ensures that no contamination will be brought into the shelter. In the absence of a dedicated structure, enhanced protection can be afforded within most buildings by sealing cracks and entry ports, and providing air filtration with high efficiency particulate air (HEPA) filters within existing ventilation systems. The key problem is that these shelters can be very limited in military situations, very costly to produce and maintain, and difficult to deploy. Personnel must be decontaminated before entering the collective protection unit.

The inhalational route is the most important route of exposure to biological agents. BW agents are dispersed as aerosols from point or line source disseminations. Unlike some chemical threats, aerosols of agents disseminated by line source munitions (e.g., sprayed by low-flying aircraft or speedboats along the coast) do not leave hazardous environmental residua (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 generator, bomblets, etc.) are more apt to produce ground contamination, but only in the immediate vicinity of dissemination. Point-source munitions leave an obvious signature that may alert the field commander that a BW attack has occurred. Because point-source munitions always leave an agent residue, this evidence can be exploited for detection and identification purposes.

Aerosol delivery systems for BW agents most commonly generate invisible clouds with particles or droplets of $< 10\ \mu m$. They can remain suspended for extensive periods. The major risk in such an attack is pulmonary retention of inhaled particles. To a much lesser extent, some particles may adhere to an individual or his clothing, especially near the face. The effective area covered varies with many factors, including wind speed, humidity, and sunlight. In the absence of an effective real-time detection and alarm systems or direct observation of an attack, the first clue may be mass casualties fitting a clinical pattern compatible with one of the biological agents. This may occur hours or days after the attack.

Toxins may cause direct pulmonary toxicity or be absorbed and cause systemic toxicity. Toxins are frequently as potent as or more potent by inhalation than by any other route. A unique clinical picture may sometimes be seen which is not observed by other routes (e.g., pulmonary edema after SEB exposure). Mucous membranes, including conjunctivae, are also vulnerable to many BW agents. Physical protection is then quite important and the use of full-face masks equipped with small-particle filters, like the chemical protective masks, assumes a high degree of importance.

Other routes for delivering biological agents are thought to be less important than inhalation, but are nonetheless potentially significant. Contamination of food and water supplies, either purposefully or incidentally after an aerosol BW attack, represents a hazard for infection or intoxication by ingestion. Assurance that food and water supplies are free from contamination should be provided by appropriate preventive medicine authorities in the event of an attack.

Intact skin provides an excellent barrier for most biological agents, T-2 mycotoxins are an exception because of their dermal activity. However, mucous membranes and abraded, or otherwise damaged integument can allow for passage of some BW agents and should be protected in the event of an attack.

DECONTAMINATION

Contamination is the introduction of an infectious agent on a body surface, food or water, or other inanimate objects. Decontamination involves either disinfection or sterilization to reduce microorganisms to an acceptable level on contaminated articles, thus rendering them suitable for use. Disinfection is the selective reduction of undesirable microbes to a level below that required for transmission. Sterilization is the killing of all organisms.

Decontamination methods have always played an important role in the control of infectious diseases; however, we are often unable to use the most effective means of rendering microbes harmless (e.g., toxic chemical sterilization), as these methods may injure people, damage tissue, and damage materials that are to be decontaminated. BW agents may 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*), or in a BW context, 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 BW agents harmless by the use of disinfectants that are usually in the form of 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 materiel.

3) Physical processes (heat, radiation) are other methods that can be employed for decontaminating objects.

It is important to note that, given the inherent incubation periods of biological agents, significant time may have elapsed between the attack and when patients present with illness due to the attack. During this time it is quite probable that external decontamination of any residual agent may have already occurred. Thus, it is only in rare circumstances that patients presenting with illness due to BW attack will require external decontamination.

Dermal exposure to a suspected BW aerosol should be immediately treated by soap and water. Careful washing with soap and water removes nearly all of the agent from the skin surface. Hypochlorite solution or other disinfectants are reserved for gross contamination (i.e., after the spill of solid or liquid agent from a munition directly onto the skin). In the absence of chemical or gross biological contamination, these disinfectants will confer no additional benefit, may be caustic, and may predispose to colonization and resistant superinfection by reducing the normal skin flora. Grossly contaminated skin surfaces should be washed with a 0.5% sodium hypochlorite solution, if available, with a contact time of 10 to 15 minutes. If reaerosolization of agent is a concern due to the presence of large amounts of gross contaminant. a damp cloth or towel should be placed

directly over the area and a 5% solution of hypochlorite (or equivalent disinfectant) should be liberally applied to saturate the gross contaminant. The saturated fabric/biological agent should then be properly disposed of per established protocol.

Ampules of calcium hypochlorite (HTH) are currently fielded in the Chemical Agent Decon Set for mixing hypochlorite solutions. The 0.5 percent solution can be made by adding one 6-ounce container of calcium hypochlorite to 5 gallons of water. The 5 percent solution can be made by adding eight 6-ounce ampules of calcium hypochlorite to 5 gallons of water. These solutions evaporate quickly at high temperatures so if they are made in advance they should be stored in closed containers. Also the chlorine solutions should be placed in distinctly marked containers because it is very difficult to tell the difference between the 5 percent chlorine solution and the 0.5 percent solution.

A 0.5 percent sodium hypochlorite solution is made of one part Clorox and nine parts water (1:9) as standard stock Clorox is a 5.25% sodium hypochlorite solution. The solution is then applied with a cloth or swab. The solution should be made fresh daily with the pH in the alkaline range.

Generally, soap and water wash is the preferred method for BW agent decontamination of patients. Chlorine solution must NOT be used in (1) open body-cavity wounds (as it may lead to the formation of adhesions), or (2) brain and spinal cord injuries. However, this solution (0.5% strength) may be instilled into non-cavity wounds and then removed by suction to an appropriate disposal container. Within about 5 minutes, this contaminated solution will be neutralized and non-hazardous. Copious irrigation with saline or other surgical solutions should be subsequently performed. Corneal opacities may result from chlorine solution being sprayed into the eyes.

For decontaminating fabric clothing or equipment, a 5 percent hypochlorite solution should be used, although many fabrics will be damaged with this concentration of hypochlorite. For decontaminating equipment, a contact time of 30 minutes before normal cleaning is required. This is corrosive to most metals and injurious to most fabrics, so rinse thoroughly and oil metal surfaces after completion.

BW agents may be rendered harmless through such physical means 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 degrees centigrade and 1 atmosphere of overpressure (15 pounds per square inch), the time may be reduced to 20 minutes, depending on volume. Solar ultraviolet radiation has a disinfectant effect, often in combination with drying. This is effective in certain environmental conditions but is hard to standardize for practical usage for decontamination purposes.

The health hazards of environmental contamination by biological agents differ from those posed by persistent or volatile chemical agents. Aerosolized particles in the 1-5 μm size range will remain suspended by brownian motion and can disseminate widely. Suspended BW agents would be eventually inactivated by solar ultraviolet light, desiccation, and oxidation. Little, if any environmental residues would remain. Possible exceptions include residua near the

dissemination line or in the immediate area surrounding point-source munitions. BW agents deposited on the soil would be subject to degradation by environmental stressors and competing soil microflora. Simulant studies at Dugway Proving Ground suggest that secondary reaerosolization 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 reaerosolization 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 the decontamination of agent by drying and solar ultraviolet 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 decontamination.

For further information on decontamination see FM 3-5, NBC Decontamination, FM 4-02.7 Health Service Support in a NBC Environment, Army FM 8-284 Treatment of Biological Warfare Agent Casualties.

Electronic copies of all DoD publications are available at the Washington Headquarters Services, ESC Directorate Directives and Records Division, http://www.dtic.mil/whs/directives/index.html

Appendix A: Glossary of Medical Terms

Adapted from Stedman's Electronic Medical Dictionary,
Williams & Wilkins, Baltimore, MD, 1996 and
Principles and Practice of Infectious Diseases,
Mandell et al, Third Edition.

Acetylcholine (ACH, Ach) - The neurotransmitter substance at cholinergic synapses, that 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.

Active immunization -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.

AHF – Argentine hemorrhagic fever, a viral hemorrhagic fever

Aleukia - Absence or extremely decreased number of leukocytes in the circulating blood.

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).

Anticonvulsant - An agent which prevents or arrests seizures.

Antitoxin - An antibody formed in response to and capable of neutralizing a biological poison; an animal serum containing antitoxins.

Arthralgia - Severe pain in a joint, especially one not inflammatory in character.

AST - Abbreviation for 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 - Absence of gas from a part or the whole of the lungs, due to failure of expansion or resorption of gas from the alveoli.

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.

Bilirubin - A red bile pigment formed from hemoglobin during normal and abnormal destruction of erythrocytes. Excess bilirubin is associated with jaundice.

Blood agar - A mixture of blood and nutrient agar used for the cultivation of many medically important microorganisms.

Bronchiolitis - Inflammation of the bronchioles, often associated with bronchopneumonia.

Bronchitis - Inflammation of the mucous membrane of the bronchial tubes.

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 man and various species of domestic animals. They do not produce gas from carbohydrates.

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 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.

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.

CCHF – Congo-Crimean hemorrhagic fever, a viral hemorrhagic fever

Cerebrospinal - Relating to the brain and the spinal cord.

Chemoprophylaxis - Prevention of disease by the use of chemicals or drugs.

Cholinergic - Relating to nerve cells or fibers that employ acetylcholine as their neurotransmitter.

CNS - Abbreviation for 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.

CSF - Abbreviation for cerebrospinal fluid.

Cutaneous - Relating to the skin.

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.

Diathesis -The constitutional or inborn state disposing to a disease, group of diseases, or metabolic or structural anomaly.

Diplopia -The condition in which a single object is perceived as two objects.

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.

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.

Ecchymosis - A purplish patch caused by extravasation of blood into the skin, differing from petechiae only in size (larger than 3 mm diameter).

ECL - See below for Electrochemiluminescence**.**

Electrochemiluminescence - A method used to identify microorganisms. It is a relatively new technique for this purpose and is 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.

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} - 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.

ELISA – See below for Enzyme Linked Immunosorbent Assay.

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 - A method used in microbiology to detect microorganisms such as bacteria or viruses. It works by chemically linking an enzyme to an antibody that recognizes and adheres to the desired microorganism. Any unbound antibody-enzyme complex is removed and chemical which 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 microorganism.

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.

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.

FA - See below for Fluorescent antibody.

Fasciculation - Involuntary contractions, or twitchings, of groups (fasciculi) of muscle fibers, a coarser form of muscular contraction than fibrillation.

Febrile - Denoting or relating to fever.

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 method used in microbiology 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 antibody. Under UV light the bacteria to which antibodies are bound will fluoresce revealing their presence.

Fulminant hepatitis - Severe, rapidly progressive loss of hepatic function due to viral infection or other cause of inflammatory destruction of liver tissue.

Generalized vaccinia - Secondary lesions of the skin following 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 following transfer of virus from the vaccination to another site by means of the fingers (autoinnoculation).

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.

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.

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 which 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.

Hemopoietic - Pertaining to or related to the formation of blood cells.

Hematuria - Any condition in which the urine contains blood or red 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.

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.

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.

Idiopathic - Denoting a disease of unknown cause.

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.

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.

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.

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}

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 cu mm.

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.

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.

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.

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.

Moribund - Dying; at the point of death.

Mucocutaneous - Relating to mucous membrane and skin; denoting the line of junction of the two at the nasal, oral, vaginal, and anal orifices.

Myalgia - Muscular pain.

Mydriasis - Dilation of the pupil.

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.

Necrosis - Pathologic death of one or more cells, or of a portion of tissue or organ, resulting from irreversible damage.

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).

Nosocomial - Denoting a new disorder (not the patient's original condition) associated with being treated in a hospital, such as a hospital-acquired infection.

Oliguria - Scanty 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.

Osteomyelitis - Inflammation of the bone marrow and adjacent bone.

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.

Papule - A small, circumscribed, solid elevation 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).

PCR - see below for polymerase chain reaction.

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, since it is not immediately irritating, even when fatal concentrations are inhaled.

Photophobia - Morbid dread and avoidance of light. Photosensitivity, or pain in the eyes with exposure to light, can be a cause.

Pleurisy - Inflammation of the pleura.

Polymerase chain reaction - An in vitro method for enzymatically synthesizing and amplifying defined sequences of DNA in molecular biology. Can be used for improving DNA-based diagnostic procedures for identifying unknown BW agents.

Polymorphonuclear - Having nuclei of varied forms; denoting a variety of leukocyte.

Polyuria - Excessive excretion of urine.

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-hour urine collection or in concentrations greater than 1 g/l in a random urine collection on two or more occasions at least 6 hours 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.

Retinitis - Inflammation of the retina.

Retrosternal - Posterior to the sternum.

Rhinorrhea - A discharge from the nasal mucous membrane.

RVF –Rift Valley Fever, a viral hemorrhagic fever

Sarin - A nerve poison which is a very potent irreversible cholinesterase inhibitor and a more toxic nerve gas than tabun or soman.

Scarification -The making of a number of superficial incisions in the skin. It is the technique used to administer tularemia and smallpox vaccines.

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 following as a consequence of a disease.

Shigellosis - Bacillary dysentery caused by bacteria of the genus *Shigella*, often occurring in epidemic patterns.

Soman - An extremely potent cholinesterase inhibitor, similar to sarin and tabun.

Sterile abscess - An abscess whose contents are not caused by pyogenic bacteria.

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.

Superinfection - A new infection in addition to one already present.

Tachycardia - Rapid beating of the heart, conventionally applied to rates over 100 per minute.

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.

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.

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.

Vaccine - A suspension of attenuated live or killed microorganisms (bacteria, viruses, or rickettsiae), or fractions thereof, administered to induce immunity and thereby prevent infectious disease.

Vaccinia - An infection, primarily local and limited to the site of inoculation, induced in man by inoculation with the vaccinia (coxpox) virus in order to confer resistance to smallpox (variola). On about the third day after vaccination, 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 day, 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 days. Syn: chickenpox

Variola - Syn: smallpox.

Variolation - The historical practice of inducing immunity against smallpox by “scratching” the skin with the purulency from smallpox skin pustules. The first inoculation for smallpox is said to have been done in China about 1022 B.C.

Viremia - The presence of virus in the bloodstream.

Virion - The complete virus particle that is structurally intact and infectious.

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: Patient Isolation Precautions

Standard Precautions

- Wash hands after patient contact.
- Wear gloves when touching blood, 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, 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 care when handling sharps and use a mouthpiece or other ventilation device as an alternative to mouth-to-mouth resuscitation when practical.
Standard precautions are employed in the care of ALL patients

Airborne Precautions

Standard Precautions plus:

- Place the patient in a private room that has monitored negative air pressure, a minimum of six air changes/hour, and appropriate filtration of air before it is discharged from the room.
- Wear respiratory protection when entering the room.
- Limit movement and transport of the patient. Place a mask on the patient if they need to be moved.

Conventional Diseases requiring Airborne Precautions: Measles, Varicella, Pulmonary Tuberculosis.

Biothreat Diseases requiring Airborne Precautions: Smallpox.

Droplet Precautions

Standard Precaution plus:

- Place the patient in a private room or cohort them with someone with the same infection. If not feasible, maintain at least 3 feet between patients.
- Wear a mask when working within 3 feet of the patient.
- Limit movement and transport of the patient. Place a mask on the patient if they need to be moved.

Conventional Diseases requiring Droplet Precautions: Invasive *Haemophilus influenzae* and meningococcal disease, drug-resistant pneumococcal disease, diphtheria, pertussis, mycoplasma, GABHS, influenza, mumps, rubella, parvovirus.

Biothreat Diseases requiring Droplet precautions: Pneumonic Plague.

Contact Precautions

Standard Precautions plus:

- Place the patient in a private room or cohort them with someone with the same infection if possible.
- Wear gloves when entering the room. Change gloves after contact with infective material.
- Wear a gown when entering the room if contact with patient is anticipated or if the patient has diarrhea, a colostomy or wound drainage not covered by a dressing.
- Limit the movement or transport of the patient from the room.
- 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 of patients with the same pathogen. If not feasible, adequate disinfection between patients is necessary.

Conventional Diseases requiring Contact Precautions: MRSA, VRE, *Clostridium difficile*, RSV, parainfluenza, enteroviruses, enteric infections in the incontinent host, skin infections (SSSS, HSV, impetigo, lice, scabies), hemorrhagic conjunctivitis.

Biothreat Diseases requiring Contact Precautions: Viral Hemorrhagic Fevers.

For more information, see: Garner JS. Guidelines for Infection Control Practices in Hospitals. Infect Control Hosp Epidemiol 1996;17:53-80.

Appendix C: BW Agent Characteristics

Disease	Transmit Human to Human	Infective Dose (Aerosol)	Incubation Period	Duration of Illness	Lethality (approx. case fatality rates)	Persistence of Organism	Vaccine Efficacy (aerosol exposure)
Anthrax	No	8,000-50,000 spores	1-6 days	3-5 days (usually fatal if untreated)	High	Very stable - spores remain viable for > 40 years in soil	2 dose efficacy against up to 1,000 LD_{50} in monkeys
Brucellosis	No	10 -100 organisms	5-60 days (usually 1-2 months)	Weeks to months	<5% untreated	Very stable	No vaccine
Cholera	Rare	10-500 organisms	4 hours - 5 days (usually 2-3 days)	$\geq$ 1 week	Low with treatment, high without	Unstable in aerosols & fresh water; stable in salt water	No data on aerosol
Glanders	Low	Unknown, Potentially low	10-14 days via aerosol	Death in 7-10 days in septicemic form	> 50%	Very stable	No vaccine
Melioidosis	Low	Unknown, Potentially low	1-21 days (up to years)	Death in 2-3 days with septicemic form (untreated)	19 – 50% for severe disease	Very stable; survives indefinitely in warm moist soil or stagnant water	No vaccine
Plague	Moderate, Pneumonic	500 - 15000 organisms	1-7 days (usually 2-3 days)	1-6 days (usually fatal)	High unless treated within 12-24 hours	For up to 1 year in soil; 270 days in live tissue	3 doses not protective against 118 LD_{50} in monkeys
Tularemia	No	10-50 organisms	1-21 days (average 3-6)	$\geq$ 2 weeks	Moderate if untreated	For months in moist soil or other media	80% protection against 1-10 LD_{50}
Q Fever	Rare	1-10 organisms	7-41 days	2-14 days	Very low	For months on wood and sand	94% protection against 3,500 LD_{50} in guinea pigs
Smallpox	High	Assumed low (10-100) organisms)	7-17 days (average 12)	4 weeks	High to moderate	Very stable	Vaccine protects against large doses in primates
Venezuelan Equine Encephalitis	Low	10-100 organisms	2-6 days	Days to weeks	Low	Relatively unstable	TC 83 protects against 30-500 LD_{50} in hamsters

Disease	Transmit Human to Human	Infective Dose (Aerosol)	Incubation Period	Duration of Illness	Lethality (approx. case fatality rates)	Persistence of Organism	Vaccine Efficacy (aerosol exposure)
Viral Hemorrhagic Fevers	Moderate	1-10 organisms	4-21 days	Death between 7-16 days	High to moderate depends on agent	Relatively unstable - depends on agent	No vaccine
Botulism	No	0.001 μg/kg is LD_{50} for type A (parenteral), 0.003 μg/kg (aerosol)	12 hours -5 days	Death in 24-72 hours; lasts months if not lethal	High without respiratory support	For weeks in nonmoving water and food	3 dose efficacy 100% against 25-250 LD_{50} in primates
Staph Enterotoxin B	No	0.03 μg / person (80kg) incapacity-ation	3-12 hours after inhalation	Hours	< 1%	Resistant to freezing	No vaccine
Ricin	No	3-5 μg/kg is LD_{50} in mice	18-24 hours	Days - death within 10-12 days for ingestion	High	Stable	No vaccine
T-2 Mycotoxins	No	Moderate	2-4 hours	Days to months	Moderate	For years at room temperature	No vaccine

Appendix D: BW Agents
Vaccines, Prophylaxis, and Therapeutics

ANTHRAX

VACCINE/TOXOID	DEVELOPMENT
Bioport BioThrax™ Anthrax Vaccine (AVA) Preexposure [A]: licensed for adults 18-65yr old, 0.5 mL SC @ 0, 2, 4 wk, 6, 12, 18 mo then annual boosters Postexposure[IND]: DoD Contingency Use Protocol for volunteer anthrax vaccination SC@ 0, 2, 4 wk in combination with approved and labeled antibiotics Pediatric Annex [IND] for postexposure use.	Recombinant protective antigen (rPA) vaccine
CHEMOPROPHYLAXIS	
Ciprofloxacin[A]: 500 mg PO bid (adults), 15mg/kg (up to 500mg/dose) PO bid (peds) [A], **or** Doxycycline[A]: 100 mg PO bid (adults), 2.2mg/kg (up to 100mg/dose) PO bid (peds < 45kg) [A] or (if strain susceptible): Penicillin G procaine: 1,200,000U q 12 hr (adults)[A], 25,000U/kg (maximum 1,200,000 unit) q 12 hr (peds)[A], **or** Penicillin V Potassium: 500 mg q 6 hr (adults), **or** Amoxicillin: 500mg PO q 8 hr (adults and children>40kg), 15mg/kg q 8 hr (children<40kg)**,** **Plus**, AVA (postexposure)[IND] 1. Fully immunized (completed 6 shot primary series and up-to-date on annual boosters, or 3 doses within past 6 mo): continue antibiotics for at least 30 days. 2 Unimmunized: 3 doses of AVA 0.5cc SQ at 0, 2, 4 weeks[IND]. Continue antibiotics for at least 7-14 days after 3rd dose. 3 No AVA used: continue antibiotics for at least 60 days	
CHEMOTHERAPY	
Inhalational, Gastrointestinal, or Systemic Cutaneous Disease: Ciprofloxacin : 400 mg IV 1 12 h initially then by mouth (adult) [A] 15 mg/kg/dose (up to 400mg/dose) q 12 h (peds)[A], or Doxycycline: 200 mg IV, then 100 mg IV q 12 h (adults) [A] 2.2mg/kg (100mg/dose max) q 12 h (peds < 45kg) [A], or (if strain susceptible), Penicillin G Procaine: 4 million units IV q 4 h (adults) [A] 50,000U/kg (up to 4M U) IV q 6h (peds) [A] **PLUS**, One or two additional antibiotics with activity against anthrax. (e.g. clindamycin plus rifampin may be a good empiric choice, pending susceptibilities). Potential additional antibiotics include one or more of the following: clindamycin, rifampin, gentamicin, macrolides, vancomycin, imipenem, and chloramphenicol. Convert from IV to oral therapy when the patient is stable, to complete at least 60 days of antibiotics. **Meningitis:** Add Rifampin 20mg/kg IV qd or Vancomycin 1g IVq12h	Anthrax Immune Globulin (AIG)
COMMENTS	
In 2002 the American Committee on immunization Practices (ACIP) recommended making anthrax vaccine available in a 3-dose regimen (0, 2, 4 weeks) in combination with antimicrobial postexposure prophylaxis under an IND application for unvaccinated persons at risk for inhalational anthrax. Penicillins should be used for anthrax treatment or prophylaxis only if the strain is demonstrated to be PCN-susceptible. According to CDC recommendations, amoxicillin prophylaxis is appropriate only after 14-21 days of fluoroquinolone or doxycycline and only for populations with contraindications to the other drugs (children, pregnancy) Oral dosing (versus the preferred IV) may be necessary for treatment of systemic disease in a mass casualty situation. **Cutaneous Anthrax**: Antibiotics for cutaneous disease (without systemic complaints) resulting from a BW attack involving BW aerosols are the same as for postexposure prophylaxis. Cutaneous anthrax acquired from natural exposure could be treated with 7-10 days of antibiotics.	AIG is serum from human AVA recipients with high anti-PA titers.

(A) Approved for this use by the FDA
(IND) Available as an investigational new drug for this indication (i.e. NOT an FDA-approved use)

Brucellosis

VACCINE/TOXOID
None
CHEMOPROPHYLAXIS
Can try one of the treatment regimens for 3-6 weeks, for example: Doxycycline : 200mg po qd (adults)[(A)], **plus** Rifampin: 600mg PO qd
CHEMOTHERAPY
Inhalational, Gastrointestinal, or Systemic Cutaneous Disease **Significant infection:** Doxycycline: 100mg PO bid for 4-6 wks (adults)[(A)], 2.2 mg/kg PO bid (peds), **plus** Streptomycin 1g IM qd for first 3 wks (adults)[(A)], **or** Doxycycline[(A)] **+** Gentamicin (if streptomycin not available) **Less severe disease:** Doxycycline 100mg PO bid for 4-6 wks (adults)[(A)], **plus** Rifampin 600-900 mg/day PO qd for 4-6 wks (adults)[(A)], 15-20mg/kg (up to 600-900mg) qd or divided bid (peds) Others used with success: TMP/SMX 8-12mg/kg/d divided qid, **plus** Rifampin (may be preferred therapy during pregnancy or in children <8yrs), Or Ofloxacin + Rifampin **Long-term (up to 6 mo) therapy for meningoencephalitis, endocarditis:** Rifampin **+** a tetracycline **+** an aminoglycoside (first 3 weeks)
COMMENTS
Ideal chemoprophylaxis is unknown. Chemoprophylaxis not recommended after natural exposure. Avoid monotherapy (high relapse). Relapse common for treatments less than 4-6 weeks.

Glanders & Meliodosis

VACCINE/TOXOID
None
CHEMOPROPHYLAXIS
PO TMP/SMX or Doxycyline for at least 10 days may be tried. Ciprofloxacin is a possible alternative, but has been associated with increased relapse rates in animal treatment models.
CHEMOTHERAPY
Severe Disease: ceftazidime (40mg/kg IV q 8hrs), **or** imipenem (15mg/kg IV q 6hr max 4 g/day), **or** meropenem (25mg/kg IV q 8hr, max 6g/day), **plus**, TMP/SMX (TMP 8 mg/kg/day IV in four divided doses) Continue IV therapy for at least 14 days and until patient clinically improved, then switch to oral maintenance therapy (see "mild disease" below) for 4-6 months. **Melioidosis with septic shock:** Consider addition of G-CSF 30ug/day IV for 10 days. **Mild Disease:** Historic: PO doxycycline and TMP/SMX for at least 20 weeks, **plus** PO chloramphenicol for the first 8 weeks. Alternative: doxycycline (100 mg po bid) **plus** TMP/SMX (4 mg/kg/day in two divided doses) for 20 weeks.
COMMENTS
Little is known about optimum therapy for glanders, as this disease has been rare in the modern antibiotic era. For this reason, most experts feel initial therapy of glanders should be based on proven therapy for the similar disease, melioidosis. One potential difference in the two organisms is that natural strains of *B. mallei* respond to aminoglycosides and macrolides, while *B. pseudomallei* does not; thus, these classes of antibiotics may be beneficial in treatment of glanders, but not melioidosis. **Severe Disease:** If ceftazidime or a carbapenem are not available, ampicillin/sulbactam or other intravenous beta-lactam/beta-lactamase inhibitor combinations may represent viable, albeit less-proven alternatives. **Mild Disease:** Amoxicillin/clavulanate may be an alternative to Doxycycline plus TMP/SMX, especially in pregnancy or for children <8yr old.

(A) Approved for this use by the FDA
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Plague

VACCINE/TOXOID	DEVELOPMENT
	Recombinant F1-V Antigen Vaccines, DoD & UK
CHEMOPROPHYLAXIS	
Ciprofloxacin: 500 mg PO bid x 7 d (adults), 20mg/kg (up to 500mg) PO bid (peds), **or** Doxycycline: 100 mg PO q 12 h x 7 d (adults), 2.2 mg/kg (up to 100mg) PO bid (peds), **or** Tetracycline: 500 mg PO qid x 7 d (adults)	
CHEMOTHERAPY	
Streptomycin: 1g q 12hr IM (adults) [(A)], 15mg/kg/d div q 12hr IM (up to 2 g/day)(peds) [(A)], **or** Gentamicin: 5 mg/kg IM or IV qd or 2 mg/kg loading dose followed by 1.7 mg/kg IM or IV (adults), 2.5 mg/kg IM or IV q8h (peds). **Alternatives:** Doxycycline: 200 mg IV once then 100 mg IV bid until clinically improved, then 100 mg PO bid for total of 10-14 d (adults) [(A)], **or** Ciprofloxacin: 400mg IV q 12 h until clinically improved then 750 mg PO bid for total 10-14 d, **or** Chloramphenicol: 25 mg/kg IV, then 15 mg/kg qid x 14 d. A minimum of 10 days of therapy is recommended (treat for at least 3-4 days after clinical recovery). Oral dosing (versus the preferred IV) may be necessary in a mass casualty situation. **Meningitis:** add Chloramphenicol 25mg/kg IV, then 15mg/kg IV qid.	FDA-approved therapeutics
COMMENTS	
Greer inactivated vaccine (FDA licensed) is no longer available. Streptomycin is not widely available in the US and therefore is of limited utility. Although not licensed for use in treating plague, gentamicin is the consensus 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 (25mg/kg PO qid), oral formulations are available only outside the US. Alternate therapy or prophylaxis for susceptible strains: trimethoprim-sulfamethoxazole Other fluoroquinolones or tetracyclines may represent viable alternatives to ciprofloxacin or doxycycline, respectively.	

Q Fever

VACCINE/TOXOID
Inactivated Whole Cell Vaccine (Pre-exposure only): Licensed (Australian) Qvax™ ; IND DoD vaccine in U.S. for at-risk laboratory personnel.
CHEMOPROPHYLAXIS
Doxycycline: 100 mg PO bid x 5 d (adults), 2.2mg/kg PO bid (peds), **or** Tetracycline: 500 mg PO qid x 5d (adults) Start postexposure prophylaxis 8-12 d post-exposure.
CHEMOTHERAPY
Acute Q-fever: Doxycycline: 100 mg IV or PO q 12 h x at least 14 d (adults)[(A)], 2.2 mg/kg PO q 12 h (peds), **or** Tetracycline: 500 mg PO q 6 hr x at least 14 d **Alternatives:** Quinolones (eg ciprofloxacin), **or** TMP-SMX, **or** Macrolides (eg clarithromycin or azithromycin) for 14-21 days**.** Patients with underlying cardiac valvular defects: Doxycycline plus Hydroxychloroquine 200mg PO tid for 12 months **Chronic Q Fever:** Doxycycline plus quinolones for 4 years, **or** Doxycycline plus hydroxychloroquine for 1.5-3 years.
COMMENTS
DoD Q-Fever vaccine manufactured in 1970. Significant side effects if administered inappropriately; sterile abscesses if prior exposure/skin testing required prior to vaccination. Time to develop immunity – 5 weeks. Initiation of postexposure prophylaxis within 7 days of exposure merely delays incubation period of disease. Tetracyclines are preferred antibiotic for treatment of acute Q fever except in: 1. <u>Meningoencephalitis</u>: fluoroquinolones may penetrate CSF better than tetracyclines 2. <u>Children < 8yrs (doxycycline relatively contraindicated)</u>: TMP/SMX or macrolides (especially clarithromycin or azithromycin). 3. <u>Pregnancy</u>: TMP/SMX 160mg/800mg PO bid for duration of pregnancy. If evidence of continued disease at parturition, use tetracycline or quinolone for 2-3 weeks.

(A) Approved for this use by the FDA
(IND) Available as an investigational new drug for this indication (i.e. NOT an FDA-approved use)

Tularemia

VACCINE/TOXOID

Live attenuated vaccine (Preexposure) (IND)

DoD Laboratory Use Protocol for vaccine. Single 0.1ml dose via scarification in at-risk researchers.

CHEMOPROPHYLAXIS

Ciprofloxacin: 500 mg PO q 12 h for 14 d, 20mg/kg (up to 500mg) PO bid (peds), **or**

Doxycycline: 100 mg PO bid x 14 d (adults), 2.2mg/kg (up to 100mg) PO bid (peds<45kg), **or**

Tetracycline: 500 mg PO qid x 14 d (adults)

CHEMOTHERAPY

Streptomycin: 1g IM q12 h days x at least 10 days (adults)(A), 15mg/kg (up to 2g/day) IM q12h (peds)(A) , **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 x at least 10 days (adults), 2.5mg/kg IM or IV q 8 h (peds), **or**

Alternatives:
Ciprofloxacin 400 mg IV q 12 h for at least 10d (adults), 15mg/kg (up to 400mg) IV q 12 h (peds), **or**

Doxycycline: 200 mg IV, then 100 mg IV q 12 h x 14-21 d (adults)(A), 2.2mg/kg (up to 100mg) IV q 12 h (peds<45kg), **or**

Chloramphenicol: 25mg/kg IV q 6 h x 14-21 d, **or**

Tetracycline: 500 mg PO qid x 14-21 d (adults)(A)

COMMENTS

Vaccine manufactured in 1964.

Streptomycin is not widely available in the US and therefore is of limited utility. 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 days; doxycycline and chloramphenicol are associated with high relapse rates with course shorter than 14-21 days. 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 less than 2 yrs. While Chloramphenicol is potentially an alternative for post-exposure prophylaxis (25mg/kg PO qid), oral formulations are available only outside the US.

Botulinum Toxins

<table>
<tr><th>VACCINE/TOXOID</th><th>DEVELOPMENT</th></tr>
<tr><td>Pentavalent Toxoid Vaccine (IND) (Preexposure use only)

IND for pre-exposure prophylaxis for high risk individuals only.</td><td>DoD rBONT Heptavalent Vaccine</td></tr>
<tr><td colspan="2">CHEMOPROPHYLAXIS</td></tr>
<tr><td>DoD equine antitoxins(IND)
In general, botulinum antitoxin is not used prophylactic ally. 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.</td><td></td></tr>
<tr><td colspan="2">CHEMOTHERAPY</td></tr>
<tr><td>CDC trivalent equine antitoxin for serotypes A, B and E. A and B are licensed and E is a CDC IND Product.

BabyBig™, California Health Department, types A and B Human lyophilized IgG(A)

HE-BAT, DoD heptavalent equine botulism antitoxin, types A-G (IND)

HFabBAT, DoD de-speciated heptavalent equine botulism antitoxin, types A-G (IND)</td><td>Monoclonal antibodies</td></tr>
<tr><td colspan="2">COMMENTS</td></tr>
<tr><td>Pentavalent Toxoid Vaccine failed potency testing for Serotypes B, C, D and E. Must initiate series 13 weeks before potential exposure for optimum protection.

Skin test for hypersensitivity before equine antitoxin administration.</td><td></td></tr>
</table>

(A) Approved for this use by the FDA
(IND) Available as an investigational new drug for this indication (i.e. NOT an FDA-approved use)

Ricin Toxin

VACCINE/TOXOID	DEVELOPMENT
	Formalin treated toxoid vaccine; De-glycosylated A-chain vaccine
CHEMOPROPHYLAXIS	
CHEMOTHERAPY	
COMMENTS	
Inhalation: supportive therapy G-I: gastric lavage, cathartics.	Availability of ricin vaccine contingent upon transition of candidate to advanced development and upon availability of funds.

Staphylococcus Enterotoxins

VACCINE/TOXOID	DEVELOPMENT
	DoD recombinant SEB Vaccine
CHEMOPROPHYLAXIS	
CHEMOTHERAPY	
COMMENTS	
Supportive care including assisted ventilation for inhalation exposure.	Currently insufficient funding for JVAP development to IND product.

Encephalitis Viruses

VACCINE/TOXOID	DEVELOPMENT
JE inactivated vaccine [(A)] VEE Live Attenuated Vaccine[(IND)] (DoD Laboratory Use Protocol for Preexposure) TC-83 strain, for initial immunizations VEE Inactivated Vaccine[(IND)] (DoD Laboratory Use Protocol for Preexposure) C-84 strain, for booster immunizations EEE Inactivated Vaccine[(IND)] (DoD Laboratory Use Protocol for Preexposure) WEE Inactivated Vaccine[(IND)] (DoD Laboratory Use Protocol for Preexposure)	VEE (V3526) Vaccine.
CHEMOPROPHYLAXIS	
None	
CHEMOTHERAPY	
No specific therapy. Supportive care only.	
COMMENTS	
VEE TC-83 vaccine manufactured in 1965. Live, attenuated vaccine, with significant side effects. 25%-35% or recipients require 2-3 days bed rest. Time to develop immunity – 8 weeks. VEE TC-83 reactogenic in 20%. No seroconversion in 20%. Only effective against subtypes 1A, 1B, and 1C. VEE C-84 vaccine used for non-responders to VEE TC-83. Must be given prior to EEE or WEE (if administered subsequent, antibody response decreases from 81% to 67%). EEE vaccine manufactured in 1989. Antibody response is poor, requires 3-dose primary (one month) and 1-2 boosters (one month apart). Primary series yields antibody response in 77%; 5%-10% of non-responders after boosts. Time to immunity – 3 months. WEE vaccine manufactured in 1991. Antibody response is poor, requires 3-dose primary (one month) and 3-4 boosters (one month apart). Primary series antibody response in 29%, 66% after four boosts. Time to develop immunity – six months. EEE and WEE inactivated vaccines are poorly immunogenic. Multiple immunizations are required.	

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Hemorrhagic Fever Viruses

VACCINE	EXPERIMENTAL / IN DEVELOPMENT
Yellow Fever live attenuated 17D vaccine [A] AHF live attenuated Candid-1 vaccine (effective in clinical trials but no license or current IND) (x-protection for BHF) RVF inactivated TSI-GSD-200 vaccine[IND] (DoD IND for high-risk laboratory workers)	-Ebola vaccines (adenovirus vectored & DNA*) -Live attenuated RVF vaccines (Clone 13 & MP-12) -Hantavirus DNA vaccine
CHEMOPROPHYLAXIS	
Lassa fever and CCHF: Ribavirin 500mg PO q 6 hr for 7 days (Not FDA approved for this use)	
CHEMOTHERAPY	
<u>Ribavirin (CCHF/Lassa*)[IND]</u>: Loading Dose: 33mg/kg (max dose: 2.64g), followed by Day 1-4: 16mg/kg (max dose 1.28g) q6hrs, Day 5-10: 8mg/kg (max dose 0.64g) q8hrs *(HFRS)[IND] : 8mg/kg dose is given only for 3 days (7 days of Rx total) <u>Ribavirin (VHF of unknown etiology)</u>: 30 mg/kg (up to 2g) IV initial dose; then 16 mg/kg (up to 1g) IV q 6 h x 4 d; then 8 mg/kg (up to 500mg) IV q 8 h x 6 d (adults)[not FDA approved or IND] <u>Mass Casualty Situation (Arenavirus, Bunyavirus, or VHF of unknown etiology. Not FDA-approved or IND)</u>: Ribavirin 2000mg PO; then 600mg PO bid (if > 75kg), or 400mg PO in AM & 600mg PO in PM (if ≤ 75kg) for 10 days (adults). 30mg/kg, then 15mg/kg divided bid for 10 days (peds)	Passive antibody for AHF, BHF (clear benefit in trials, no licensed or IND product available).
COMMENTS	
Aggressive supportive care & management of hypotension and coagulopathy very important. Human antibody used with apparent beneficial effect in uncontrolled human trials of AHF. Human experience w/postexposure ribaririn use for VHF exposure is limited to a few cases exposed to CCHF and Lassa. Any use for this purpose should be ideally under IND. Consensus statement in 2002 JAMA suggests using Ribavirin to treat clinically apparent VHF infection of unknown etiology using doses from CCHF/Lassa/KHF IND.	*Ebola DNA vaccine currently in human Phase I trials at NIH

Smallpox

VACCINE/TOXOID	DEVELOPMENT
Wyeth Dryvax™ (1:1) (Preexposure)[A] Aventis Pasteur Smallpox Vaccine (APSV) (Preexposure)[IND] Cell Culture derived Vaccines (all NYCBOH strain): - Dynport Vaccine (Preexposure)[IND] - Acambis/Acambis-Baxter Vaccines (ACAM1000 and ACAM2000) (Preexposure)[IND]	Attenuated Vaccinia Vaccines: Acambis Modified Vaccinia Ankara (MVA) VaxGen LC16m8 strain
CHEMOPROPHYLAXIS	
Wyeth Dryvax™ (1:1) (Postexposure)[IND] Use of Smallpox Vaccine in Response to Bioterrorism: Wyeth Dryvax™ (1:5 dilution)[IND] CDC IND. If Dryvax™ (1:5) used up, not available, or need both vaccines, then use: APSV (1:5 dilution)[IND]	DoD IND for APSV (1:5) Contingency Use
CHEMOTHERAPY	
Cidofovir for treatment of smallpox[IND]: - Probenecid 2g PO 3 h prior to cidofovir infusion. - infuse 1L NS 1 h prior to cidofovir infusion - Cidofovir 5mg/kg IV over 1 hr - repeat probenecid 1g PO 2 h and again 8 h after cidofovir infusion completed. For Select Vaccine Adverse reactions (Eczema vaccinatum, vaccinia necrosum, ocular vaccinia w/o keratitis, severe generalized vaccinia): 1. VIG IV (Vaccinia Immune Globulin – intravenous formulation). 100mg/kg IV infusion. 2. Cidofovir 5mg/kg IV infusion (as above). 3. VIG-IM (Vaccinia Immune Globulin – intramuscular formulation). 0.6ml/kg IM.	Oral formulations of cidofovir derivatives Monoclonal Vaccinia Immune Globulins
COMMENTS	
Dryvax™ - Wyeth calf lymph vaccinia vaccine 100 dose vials undiluted: 1 dose by scarification. Greater than 97% take after one dose within 14 days of administration. Dryvax™ is effective (either preventing <u>or</u> attenuating resulting disease) up to at least 4 days post exposure. Dryvax™ (1:1) FDA license approved 25 Oct 2002. APSV also known as Salk Institute (TSI) vaccine, a frozen, liquid formulation using the NYCBOH vaccine strain via calf-lymph production also used in the Dryvax™ Pre and post exposure vaccination recommended if > 3 years since last vaccine. Recommendations for use of smallpox vaccine in response to bioterrorism are periodically undated by the Centers for Disease Control & Prevention. Most recent recommendations can be found at http:www.cdc.gov.	

(A) Approved for this use by the FDA
(IND) Available as an investigational new drug for this indication (i.e. NOT an FDA-approved use)

Appendix E: Medical Sample Collection for Biological Threat Agents

This guide helps determine which clinical samples to collect from individuals exposed to aerosolized biological threat agents or environmental samples from suspect sites. Proper collection of specimens from patients is dependent on the time-frame following exposure. Sample collection is described for “Early post-exposure”, “Clinical”, and “Convalescent/ Terminal/ Postmortem” time-frames. These time-frames are not rigid and will vary according to the concentration of the agent used, the agent strain, and predisposing health factors of the patient.

- Early post-exposure: when it is known that an individual has been exposed to a bioagent aerosol; aggressively attempt to obtain samples as indicated

- Clinical: samples from those individuals presenting with clinical symptoms

- Convalescent/Terminal/Postmortem: samples taken during convalescence, the terminal stages of infection or toxicosis or postmortem during autopsy

Shipping Samples: Most specimens sent rapidly (less than 24 h) to analytical labs require only blue or wet ice or refrigeration at 2 to 8˚C. However, if the time span increases beyond 24 h, contact the USAMRIID “Hot-Line” (1-888-USA-RIID) for other shipping requirements such as shipment on dry-ice or in liquid nitrogen.

Blood samples: Several choices are offered based on availability of the blood collection tubes. Do not send blood in all the tubes listed, but merely choose one. 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 also preferred over citrated blood for bacterial cultures.

Pathology samples: routinely include liver, lung, spleen, and regional or mesenteric lymph nodes. Additional samples requested are as follows: brain tissue for encephalomyelitis cases (mortality is rare) and the adrenal gland for Ebola (nice to have but not absolutely required).

Bacteria and Rickettsia

Early post-exposure	Clinical	Convalescent/ Terminal/Postmortem
Anthrax *Bacillus anthracis* 0 – 24 h Nasal and throat swabs, induced respiratory secretions for culture, FA, and PCR	24 to 72 h Serum (TT, RT) for toxin assays Blood (E, C, H) for PCR. Blood (BC, C) for culture	3 to 10 days 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, and PCR	24 – 72 h Blood (BC, C) and bloody sputum for culture and FA (C), F-1 Antigen assays (TT, RT), PCR (E, C, H)	>6 days 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 and PCR	24 – 72 h Blood (BC, C) for culture Blood (E, C, H) for PCR Sputum for FA & PCR	>6 days Serum (TT, RT) for IgM and later IgG, agglutination titers. 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

Bacteria and Rickettsia

Early post-exposure	Clinical	Convalescent/ Terminal/Postmortem
Glanders *Burkholderia mallei* <u>0 – 24 h</u> Nasal swabs, sputum, induced respiratory secretions for culture and PCR.	<u>24 – 72 h</u> Blood (BC, C) for culture Blood (E, C, H) for PCR Sputum & drainage from skin lesions for PCR & culture.	<u>>6 days</u> Blood (BC, C) and tissues for culture. Serum (TT, RT) for immunoassays. Pathology samples.
Brucellosis *Brucella abortus*, *suis,* & *melitensis* <u>0 – 24 h</u> Nasal swabs, sputum, induced respiratory secretions for culture and PCR.	<u>24 – 72 h</u> Blood (BC, C) for culture. Blood (E, C, H) for PCR.	<u>>6 days</u> Blood (BC, C) and tissues for culture. Serum (TT, RT) for immunoassays. Pathology samples
Q-Fever *Coxiella burnetii* <u>0 – 24 h</u> Nasal swabs, sputum, induced respiratory secretions for culture and PCR.	<u>2 to 5 days</u> Blood (BC, C) for culture in eggs or mouse inoculation Blood (E, C, H) for PCR.	<u>>6 days</u> 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

Toxins

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) and toxin assays. Serum (TT, RT) for toxin assays	24 to 72 h Nasal swabs, respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays.	>6 days Usually no IgM or IgG Pathology samples (liver and 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) and 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 days Serum (TT, RT) for IgM and IgG in survivors
Staph enterotoxicosis *Staphylococcus* Enterotoxin B 0 – 3 h Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays. Serum (TT, RT) for toxin assays	2 - 6 h Urine for immunoassays Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays. Serum (TT, RT) for toxin assays	>6 days Serum for IgM and IgG Note: Only paired antibody samples will be of value for IgG assays…must 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 days Serum (TT, RT), tissue for toxin detection	>6 days postexposure 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

Viruses

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 and viral culture (in viral transport media)	24 to 72 h Serum & Throat swabs for culture (TT, RT), RT-PCR (E, C, H, TT, RT) and Antigen ELISA (TT, RT), CSF, Throat swabs up to 5 days	>6 days Serum (TT, RT) for IgM Pathology samples plus brain
Ebola 0 – 24 h Nasal swabs & induced respiratory secretions for RT-PCR and viral culture (in viral transport media)	2 to 5 days Serum (TT, RT) for viral culture	>6 days 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 and viral culture (in viral transport media)	2 to 5 days Serum (TT, RT) for viral culture	>6 days 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

Environmental samples can be collected to determine the nature of a bioaerosol either during, shortly after, or well after an attack. The first two, along with early post exposure clinical samples, can help identify the agent in time to initiate prophylactic treatment.

Samples taken well after an attack may allow identification of the agent used. While the information will most likely be too late for useful prophylactic treatment, this information along with other information may be used in the prosecution of war crimes or other criminal proceedings. This is not strictly a medical responsibility. However, the sample collection concerns are the same as for during or shortly after a bioaerosol attack and medical personnel may be the only personnel with the requisite training.

If time and conditions permit planning and risk assessments should be performed. As in any hazmat situation, a clean line and exit and entry strategy should be designed. Depending on the situation, personnel protective equipment should be donned. The standard M40 gas mask is effective protection against bioaerosols. 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. The samples may be used in a criminal prosecution, what, where, when, how, etc. of the sample collection should be documented both in writing and with pictures. Consider using waterproof disposable cameras, and waterproof notepads. Since these items may need to be decontaminated. 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

What is collected will depend on the situation. 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. Well after the attack, samples from dead animals or human remains can be taken (refer to Appendix F 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.

Appendix F: Specimens for Laboratory Diagnosis

Disease	Face or Nasal Swab[1]	Blood Culture	Smear	Acute & Convalescent Sera	Stool	Urine	Other
Anthrax	+	+	Pleural & CS fluids mediastinal lymph node spleen	+	+/- (GI dz)	-	Cut. lesion aspirates or 4mm punch biopsy
Brucellosis	+	+	-	+	-	-	Bone marrow and spinal fluid cultures; tissues, exudates
Cholera	-	-	-	+	+	-	
Glanders & Melioidosis	+	+	Sputum and abscess aspirates	+	-	+/-	Abscess culture
Plague	+	+	Sputum	+	-	-	Bubo aspirate, CSF, sputum, lesion scraping, lymph node aspirate
Tularemia	+	+	+[2]	+	-	-	
Q-fever	+	+[4]	Lesions	+	-	-	Lung, spleen, lymph nodes, bone marrow biopsies
Venezuelan Equine Encephalitis	+	[3]	-	+	-	-	CSF
Viral Hemorrhagic Fevers	+	[3]	-	+	-	-	Liver
Botulism	+	-	-	-	-	-	Serum or other fluids for mouse bioassay
Staph Enterotoxin B	+	-	-	+	+	+	Lung, kidney
Ricin Toxin	+	-	-	+	+	+	Spleen, lung, kidney
T-2 Mycotoxins	+	-	-	-	+	+	Serum, stool, or urine for metabolites
Clostridial Toxins	+	-	Wound tissues	+	+	-	

[1]Within 18-24 hours of exposure
[2]Fluorescent antibody test on infected lymph node smears. Gram stain has little value.
[3]Virus isolation from blood or throat swabs in appropriate containment.
[4]*C. burnetii* can persist for days in blood and resists desiccation. EDTA anticoagulated blood preferred. Culturing should not be done except in BL3 containment.

Appendix G: BW Agent Lab Identification

Disease	Agent	Gold Standard	Antigen Detection	Immunoassays IgG	IgM	PCR	Animal
Aflatoxin	Aflatoxins	Mass spectrometry	X				
Anthrax	*Bacillus anthracis*	FA/Std. Microbiology	X	X	X	X	X
Brucellosis	*Brucella sp.*	FA/Std. Microbiology	X	X	X	X	X
Cholera	*Vibrio cholerae*	Std. Microbiology/serology	X(toxin)	X	X	X	
Glanders	*B. mallei*	Std. Microbiology		X	X	X	
	B. pseudomallei	Std. Microbiology		X	X	X	
Plague	*Yersinia pestis*	FA/Std. Microbiology	X	X	X	X	X
Tularemia	*F. tularensis*	FA/Std. Microbiology	X	X	X	X	X
Q Fever	*C. burnetii*	FA/eggs or cell Cx/serology	X	X	X	X	X
Smallpox	Orthopox Viruses	Virus isolation/ FA/neutralization	X	X		X	X
Venezuelan Equine Encephalitis	Arboviruses (incl. alphaviruses	Virus isolation/FA, neutralization	X	X	X	X	X
Viral Hemorrhagic Fevers	Filoviruses	Virus isolation/neutralization	X	X	X	X	X
	Hantaviruses	Virus isolation/ FA/neutralization	X	X	X	X	X
Botulism	Bot Toxins (A-G)/*C. botulinum*	Mouse neutralization/ standard microbiology	X			*	X
Saxitoxin	Saxitoxin	Bioassay		(neutralizing antibodies)		X	
Shigellosis	*Shigella sp.*	Std. Microbiology	X			X	
Staph Enterotoxin B	SEB Toxin	ELISA	X	X		*	X
Ricin	Ricin Toxin	ELISA	X	X	X	X	X
T-2 Mycotoxins	T-2 Mycotoxins	Mass spectrometry	X				
Tetrodototoxin	Tetrodotoxins	Bioassay	X	(neutralizing antibodies)			X
	C. perfringens/ toxins	Std. Micro./ELISA (alpha & enterotoxin)	X	X		X	

* Toxin gene detected – only works if cellular debris including genes present as contaminant. Purified toxin does not contain detectable genes
ELISA - enzyme-linked immunosorbent assays
FA - indirect or direct immunofluorescence assays
Std. Micro./serology - standard microbiological techniques available, including electron microscopy
Not all assays are available in field laboratories.

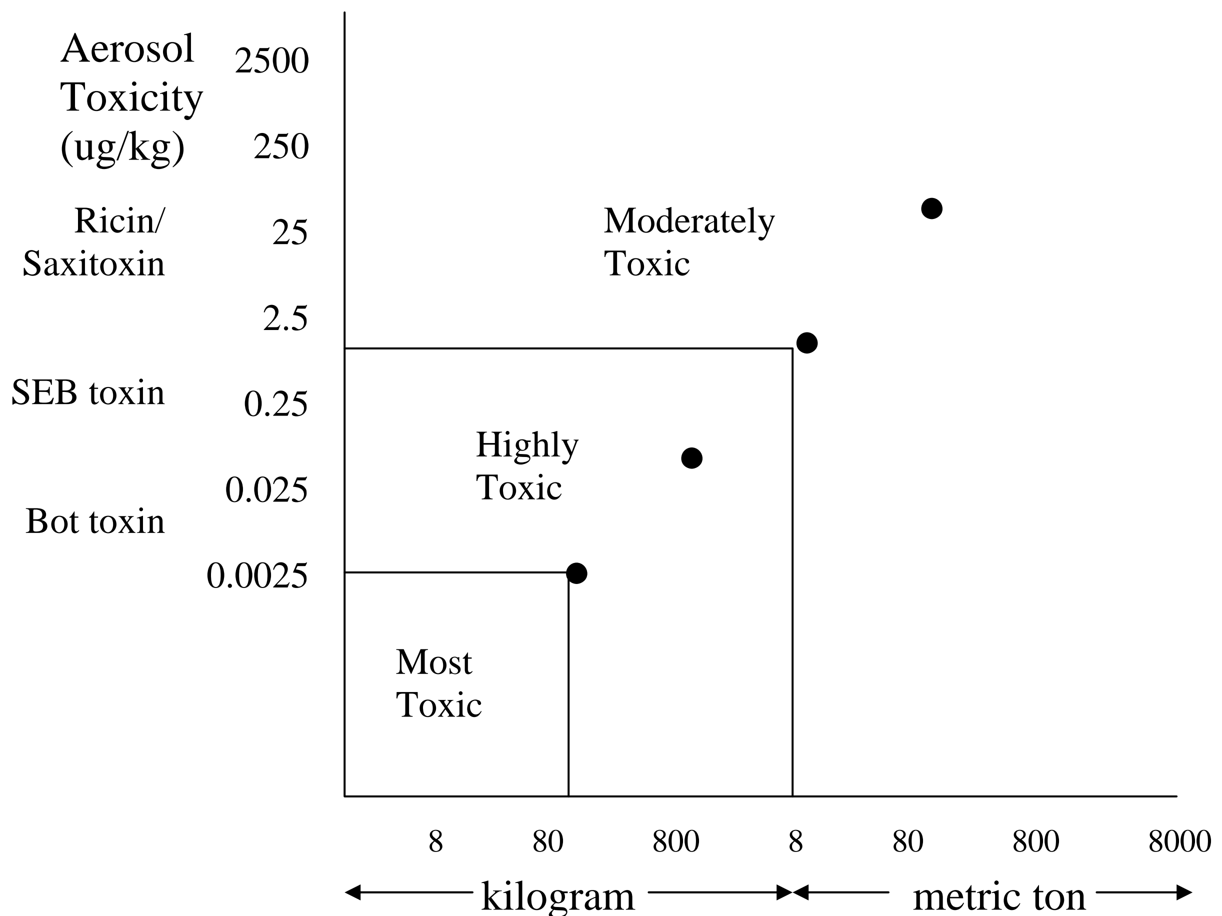
Appendix H: Differential Diagnosis of Chemical Nerve Agent, Botulinum Toxin and SEB Intoxication following Inhalation Exposure

	Chemical Nerve Agent	**Botulinum Toxin**	**SEB**
Time to Symptoms	Minutes	Hours (12-48)	Hours (1-6)
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	Difficult breathing, airway constriction	Normal, then progressive paralysis	Nonproductive cough; Severe cases; chest pain/difficult 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 infection)
Salivary	Profuse, watery saliva	Normal; difficulty swallowing	May be slightly increased quantities of saliva
Death	Minutes	2-3 days	Unlikely
Response to Atropine/2PAM-CL	Yes	No	Atropine may reduce gastrointestinal symptoms

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

Agent	LD_{50} (μg/kg)	Molecular Weight	Source
Botulinum toxin	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	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

Appendix J: Aerosol Toxicity in LD_{50} vs. Quantity of Toxin



Aerosol toxicity in LD_{50} (see Appendix C) 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. (Patrick and 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 K: References

Master Web Resource list

Mr. Greg Banner (gregory.banner@health.ri.gov) Emergency Manager for the Rhode Island Department of Health maintains a master list of web sites useful for medical emergency planning. The list is updated regularly and includes numerous CBRNE related sites. It is available as a WORD document and may be downloaded at: http://www.health.ri.gov/biot/web_sites.doc

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Investigational New Drugs

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http://www.fda.gov/cder/regulatory/applications/ind_page_1.htm#Introduction

Appendix L: Investigational New Drugs (IND)

An **investigational drug** is a drug that is under study in humans, but does not yet have permission from the U.S. Food and Drug Administration (**FDA**) to be legally marketed and sold in the United States. These FDA regulatory requirements also apply to medical care provided to military and civilian DoD healthcare beneficiaries OCONUS. Investigational drugs are either: **1)** entirely new drugs, vaccines, or therapeutics which have never been licensed by the FDA for ANY human use; or, **2)** drugs, vaccines, blood products, or therapeutics which are currently licensed for specific indications, but not for the purpose for which you intend to use them.

Examples

- The USAMRIID tularemia LVS vaccine has not been licensed by the FDA, and thus its use is investigational.
- Anthrax Vaccine Adsorbed (AVA) is licensed for PREexposure prevention of anthrax in adults. It would be considered investigational when used for POSTexposure prophylaxis of anthrax, or for use in children.

A drug that has been approved by the FDA for ANY indication may be prescribed by an <u>individual physician</u> for "off-label" use on a <u>case-by-case basis</u>. For example, cidofovir is licensed for treating cytomegalovirus retinitis in HIV patients, but not for the treating generalized *Vaccinia*. A physician could decide to prescribe cidofovir for an individual case of generalized *Vaccinia*. In that situation, the physician assumes the legal risk as would occur with any medical intervention. But because this is not an FDA-licensed indication for the drug, it cannot legally be official policy (e.g., of the hospital, the DoD, etc.) to treat all cases of generalized *Vaccinia* with cidofovir.

<u>The Drug Licensure Process:</u> if a drug, vaccine, or other therapeutic appears promising from rigorous testing in animals for treating or preventing a specific human disease, a **sponsor** may apply for FDA approval to study the drug in people. This is called an **Investigational New Drug (IND) Application**. Once the IND is approved, clinical trials may begin. **Clinical trials** are research studies designed to determine the safety and effectiveness of the drug in people. Once clinical trials are completed, the sponsor submits the study results in a **New Drug Application (NDA)** or **Biologics License Application (BLA)** to the FDA. This application is carefully reviewed and, if the drug is found to be reasonably safe and effective, it is approved. This whole process can take years to a decade or longer. One of the major historical hurdles for licensing products to treat or prevent diseases caused by the "classic" BW agents is demonstrating effectiveness of the measure in the natural setting. Because these diseases occur in humans rarely, it is impossible to test the effectiveness and it is unethical to challenge humans with potentially deadly disease just to test a new drug. Fortunately, the FDA recently enacted the animal rule, which will allow a new drug to be licensed for BW diseases if it meets strict criteria for effectiveness in acceptable animal models.

<u>The IND Process</u>: the IND process involves an agreement between the sponsor and the FDA to use the product in question in rigidly defined conditions and with very close administrative supervision defined by a research protocol that has been approved by a human ethical and scientific review board and the FDA. The sponsor for all DoD IND protocols is the U.S. Army Surgeon General, whose representative is the U.S. Army Medical Materiel Development Activity (USAMMDA). The Centers for Disease Control and Prevention (CDC) sponsors BW-related INDs for U.S. civilians. IND products must be administered only by specifically trained **investigators**, who must have received **Good Clinical Practices (GCP)** training, are familiar with the investigational protocol, and have signed an **FDA form 1572** (a legally binding "contract" with the FDA to follow the IND protocol "to the letter"). All recipients of the product (**subjects**) must meet specific eligibility criteria and acknowledge having received informed consent with their signature before receiving the product. Therefore, participation in the protocol is voluntary and cannot be required or coerced. This informed consent requirement can only be released by a presidential waiver, under very special and limited circumstances. A mandatory requirement for the investigational use of a product is documentation of the administration of the product, with strict accountability of product shipment, storage conditions, and for any doses that were given. All use of an IND product requires monitoring for adverse events, or **AE**, after product use.

Instructions for Receipt and Administration of Investigational Drugs for Military Healthcare Providers

1. **Call USAMRIID.** Military healthcare providers should call USAMRIID to discuss the case with the medical officer on call, who is familiar with the protocols for administration of the IND products (1-888-USA-RIID during duty hours or DSN: 343-2257 or commercial 301-619-2257 during non-duty hours to reach the 24-hour security desk at USAMRIID). If the use of the investigational product is indicated, USAMRIID will coordinate with USAMMDA for shipping the product.

2. **Determine who will administer the product and where**. There are several options.
   a. Designate an investigator for the IND at the requesting site. The proposed investigator must meet eligibility criteria (GCP training, signed FDA form 1572 and copy of protocol, etc…) and be approved by the sponsor. This can be arranged through USAMMDA.
   b. DoD has pre-trained, designated investigators who are already established at several of the major MEDCENs who could potentially travel to the patient to administer the IND product. Alternatively, the patient could be evacuated to the nearest medical center with a pre-trained, designated investigator who will administer the product.
   c. The U.S. Army has previously designated certain qualified individuals to serve on a Special Medical Augmentation Team (SMART-IND) to administer IND products. For large numbers of casualties, or the need for a time-critical IND administration,

USAMMDA might consider sending a SMART-IND team to run the protocol and administer the IND product.

Instructions for Receipt and Administration of Investigational Drugs for Civilian Healthcare Providers

1. Civilian healthcare providers should first contact their **state health departments** for guidance.

2. If further consultation is required
   a. For smallpox or smallpox vaccine AE related products, call **CDC Clinical Information Line (CIL)** at 1-877-554-4625.
   b. For botulinum antitoxin, state health departments should call 770-488-7100. The call will be taken by the **CDC Emergency Operations Center**, which will page the Foodborne and Diarrheal Diseases Branch medical officer on call.
   c. For other questions, consider calling the **CDC Drug and Immunobiologics Service** (404-639-3670).

Appendix M: Use of Drugs / Vaccines in Special or Vulnerable Populations in the Context of Bioterrorism.

(The pediatric patient, nursing mothers, pregnant patient and the immunocompromised)

Pediatric patients

Management of pediatric patients exposed to BW agents may be problematic for several reasons. Some antimicrobials and vaccines are not licensed for use in children. Additionally, most investigational new drug (IND) applications do not include children in their subject groups. For example, the Anthrax Vaccine (AVA) is licensed only for pre-exposure use in people aged 18-65. While AVA may be effective in preventing anthrax in children as well, it has not been studied in pediatric populations. Smallpox vaccine can be used only in patients 6 months of age or older.

Some vaccines, even though licensed for use in children, are more problematic in children than in adults. Smallpox vaccine is much more likely to lead to postvaccinial encephalitis, an often-fatal condition, when given to young children. Yellow fever vaccine is more likely to cause severe encephalitis in young infants than it is in adults.

Some antimicrobials are relatively contraindicated in children due to real or perceived risks which do not appear to be present in adult populations. Tetracyclines and fluoroquinolones are the two classes of antibiotics that generate the most concern since they are the drugs of choice for treating or preventing many BW diseases.

Tetracyclines. This class of antibiotics is generally contraindicated in children less than 8 years old because the antibiotic and its pigmented breakdown products can cause permanent dental staining and, more rarely, enamel hypoplasia during odontogenesis. The degree of staining is proportional to the total dose received and is thus dependent upon both dose and duration of therapy. Thus, doxycycline, which is given only twice per day, represents a lower risk than other tetracyclines. Tetracyclines may also cause reversible delay in bone growth rate during the course of therapy. Despite these relative contraindications, the American Academy of Pediatrics (AAP) recommends tetracyclines for treating certain severe illnesses that respond poorly to other antibiotics (e.g. Rocky Mountain spotted fever and other rickettsial diseases), specifically including treatment or prevention of anthrax disease.

Fluoroquinolones. This class of antibiotics is generally contraindicated in patients less than 18 years old because it is associated with cartilage damage in juvenile animal models. While sporadic cases of arthropathy in humans have been reported, they have primarily been associated with adults and children receiving pefloxacin, a fluoroquinolone commonly used in France. Ciprofloxacin, which has been used extensively in children, has not thus far been associated with arthropathy and seems to be well tolerated. For this reason, the AAP

recognizes that fluoroquinolones may be used in children in special circumstances, specifically including treating or preventing anthrax. In fact, ciprofloxacin is specifically licensed by the FDA for postexposure prophylaxis against anthrax IN CHILDREN.

General guidance. In pediatric cases of suspected BW exposure or disease in which the empiric treatment of choice is a drug with limited pediatric experience, one may be left with few viable alternatives than to treat with such a drug. For example, if a 5-year-old child is suspected to have been exposed to an aerosol of *Bacillus anthracis* of unknown antibiotic susceptibility, the best initial choice of antibiotic may be ciprofloxacin or doxycycline (In fact, for this reason, the FDA and AAP recommend either of these drugs for empiric postexposure prophylaxis of inhalational anthrax). If the organism is later determined to be susceptible to penicillins, then one could switch to amoxicillin to complete the course of antibiotics. If the organism is not susceptible to penicillin but is susceptible to doxycycline and ciprofloxacin, then ciprofloxacin may represent a better choice for continued prophylaxis, as arthropathy from fluoroquinolones thus far has proved rare in children, whereas the necessarily prolonged course of doxycycline (perhaps 60 days) could lead to significant dental staining. If the same child was exposed to *Yersinia pestis* susceptible to both ciprofloxacin and doxycycline, doxycycline might be an equally good choice as ciprofloxacin, as the short (7 day) course of postexposure prophylaxis is unlikely to result in dental staining. Clinicians must use judgment in these cases, taking into account the organism's antibiotic susceptibilities, the available prophylaxis or treatment options, and the risk versus benefit to the individual patient. Antimicrobial doses are often different in children, and prescribed according to patient weight. Some representative antibiotics and their pediatric doses are included in Table 1.

Nursing mothers

Some medications are excreted in breast milk (see Table 1), and thus may be ingested by nursing infants. Such medications, if contraindicated in infants and orally absorbed, should also be avoided by breastfeeding mothers if possible. It is generally recommended that fluoroquinolones, tetracyclines, and chloramphenicol be avoided in nursing mothers. Obviously, these drugs may represent the treatment of choice for many BW agents; thus, practitioners must again weigh the risks of administering these drugs with the potential adverse consequences of using a less effective medication. In some cases, temporary cessation of nursing while on the offending drug may be necessary. Antibiotics generally considered safe during nursing are aminoglycosides, penicillins, cephalosporins, and macrolides.

Pregnant patients

Some medications that are useful and safe for treating diseases in women may nonetheless pose specific risks during pregnancy. FDA has developed the following pregnancy risk categories. **A**: studies in pregnant women show no risk; **B**: animal studies show no risk but human studies are not adequate or animal toxicity has been shown but human studies show no risk; **C**: animal studies show toxicity, human studies are inadequate but benefit of use may exceed risk; **D**: evidence of human risk but benefits may outweigh risks; **X**: fetal abnormalities in

humans, risk outweighs benefit. Pregnancy risk categories for representative therapeutics are included in Table 1.

Again, tetracyclines and fluoroquinolones must be addressed, as they are empiric treatments of choice for many BW diseases yet relatively contraindicated in 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 ciprofloxacin use during pregnancy. In cases for which either ciprofloxacin or doxycycline are recommended for initial empiric prophylaxis (e.g., inhalational anthrax, plague, or tularemia), ciprofloxacin if tolerated may represent the lower risk option; then, after antibiotic susceptibility data are gained, antibiotics should be switched to lower risk alternatives if possible.

While most vaccinations are to be avoided during pregnancy, killed vaccines are generally considered to be of low risk. While live vaccines (e.g., measles-mumps-rubella) are contraindicated during pregnancy, a notable exception is the administration of the smallpox vaccine (vaccinia) to pregnant women after a known or highly suspected exposure to the smallpox virus during an outbreak.

The immunocompromised patient

While immunocompromised individuals may be more susceptible to BW disease or may develop more severe disease than immunocompetent patients, consensus groups generally recommend using the same antimicrobial regimens recommended for their immunocompetent counterparts. The most obvious difference in management of these patients concerns receipt of live vaccines, such as the currently licensed smallpox vaccine, or the LVS tularemia 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 1. Antimicrobials in Special Populations

Class of Drug	Pregnancy category	Drug name	breast milk	Pediatric Oral Dose	Pediatric parenteral dose
Aminoglycosides	C	Gentamicin	(+) small		3 - 7.5 mg/kg/day in 3 doses (IV or IM)
	D	Amikacin	(+) small		15 - 22.5 mg/kg/day in 3 doses (max 1.5g/day) (IV or IM)
	D	Streptomycin	(+) small		30 mg/kg/day in 2 doses (max 2g/day)(IM only)
	D	Tobramycin	(+) small		3 - 7.5 mg/kg/day in 3 doses (IV or IM)
Carbapenems	C	Imipenem	(?)		60 mg/kg/day in 4 doses (max 4g/day) (IV or IM)
	B	Meropenem	(?)		60-120 mg/kg/day in 3 doses (max 6g/day) (IV)
Cephalosporins	B	Cefriaxone	(+) trace		80 - 100 mg/kg in 1 or 2 doses (max 4g/day) (IV or IM)
	B	Ceftazidime	(+) trace		125-150 mg/kg/day in 3 doses (max 6g/day) (IV or IM)
	B	Cephalexin	(+) trace	25-50 mg/kg/day in 3-4 doses	
	B	Cefuroxime	(+) trace	20-30 mg/kg/day in 2 doses (max 2g/day)	100-150 mg/kg/day in 3 doses (max 6g/day) (IV or IM)
	B	Cefepime	(+) trace		150mg in 3 doses (max 4g/day) (IV or IM)
Chloramphenicol	C		(+)	50-100 mg/kg/day in 4 doses(formulation not avail in US)	50-100 mg/kg/day in 4 doses (max 4g/day) (IV)
Fluoroquinolones	C	Ciprofloxacin	(+)	30 mg/kg/day in 2 doses (max 1.5g)	20-30 mg/kg/day in 2 doses (max 800mg/day)(IV)
Glycopeptides	C	Vancomycin	(+)		40-60 mg/kg/day in 4 doses (max 4g/day) (IV)
Lincosamides	B	Clindamycin	(+)	10-20 mg/kg/day in 3-4 doses (max 1.8g/day)	25-40 mg/kg/day in 3-4 doses (max 2.7g/day) (IV or IM)
Lipopeptides	B	Daptomycin	(?)		4 mg/kg once daily (IV)
Macrolides	B	Azithrothromycin	(+)	5-12 mg/kg/day once daily (max 600mg/day)	
	C	Clarithromycin	(?)	15 mg/kg/day in 2 doses (max 1g/day)	
	B	Erythromycin	(+)	30-50 mg/kg/day in 2-4 doses (max 2g/day)	15-50 mg/kg/day in 4 doses (max 4g/day) (IV)
Monobactams	B	Aztreonam	(+)trace		90-120 mg/kg/day in 3-4 doses (max 8g) (IV or IM)
Oxalodinones	C	Linezolid	(+)	20-30 mg/kg/day in 3 doses (max 800/mg/day)	20-30 mg/kg/day in 3 doses (max 1200/mg/day)(IV)
Penicillins	B	Amoxicillin	(+) trace	25-9 0mg/kg/day in 3 doses (max 1.5g/day)	
	B	Ampicillin	(+) trace	50-100 mg/kg/day in 4 doses (max 4g/day)	200-400 mg/kg/day in 4 doses (max 12g/day) (IV or IM)
	B	Penicillin G	(+) trace		25000-400000U/kg/day in 4-6 doses (mag 24milU/day) (IV or IM)
	B	Nafcillin	(+) trace		100-150 mg/kg/day in 4 doses (max12g) (IV or IM)
Rifampin	C		(+)	10-20 mg/kg/day in 1-2 doses (max 600mg/day)	10-20 mg/kg/day in 1-2 doses (max 600mg/day)
Streptogramins	B	Dalfopristin-Quinupristin	(+)		22.5 mg/kg/day in 3 doses (IV)
Sulfonamides	C	Trimethoprim/Sulfamethoxazole	(+) trace	8-12 mg/kg/dayTMP in 4 doses (max 320 mg/day TMP)	8-12 mg/kg/dayTMP in 4 doses (IV)
Tetracyclines	D	Doxycycline	(+)	2-4 mg/kg/day in 1-2 doses (max 200mg/day)	2-4 mg/kg/day in 1-2 doses (max 200mg/day)(IV)
	D	Tetracycline	(+)	20-50 mg/kg/day in 4 doses (max 2g)	10-25 mg/kg/day in 2-4 doses (max 2g) (IV)
Cidofovir	C		(?)		5mg/kg once with probenecid and hydration
Oseltamivir	C		(+)	1-12 years old: $\leq$15 kg: 30 mg twice daily; 15-23 kg: 45 mg 2X/day; 23-40kg: 60mg 2X/day; >40kg: adult dose	
Ribavirin	X		(?)	30 mg/kg once, then 15 mg/kg/day in 2 doses (VHFs)	Same as for adults, dosed by weight (IV)

Note: (1) The above dose are for children outside of the neonatal period. Neonatal doses may be different
Note: (2) Pediatric antibiotic doses included in this table represent generic doses for severe disease. They may not accurately reflect expert consensus for treatment of some specific BW diseases (anthrax, plague, tularemia). For those diseases, refer to the specific chapter for recommendations.

Appendix N: Emergency Response Contacts FBI & Public Health

Federal Bureau of Investigation (FBI) Field Offices (listed by city)

A

FBI Albany
200 McCarty Avenue
Albany, New York 12209
albany.fbi.gov
(518) 465-7551

FBI Albuquerque
Suite 300
415 Silver Avenue, Southwest
Albuquerque, New Mexico 87102
albuquerque.fbi.gov
(505) 224-2000

FBI Anchorage
101 East Sixth Avenue
Anchorage, Alaska 99501-2524
anchorage.fbi.gov
(907) 258-5322

FBI Atlanta
Suite 400
2635 Century Parkway, Northeast
Atlanta, Georgia 30345-3112
atlanta.fbi.gov
(404) 679-9000

B

FBI Baltimore
7142 Ambassador Road
Baltimore, Maryland 21244-2754
baltimore.fbi.gov
(410) 265-8080

FBI Birmingham
Room 1400
2121 8th. Avenue N.
Birmingham, Alabama 35203-2396
birmingham.fbi.gov
(205) 326-6166

FBI Boston
Suite 600
One Center Plaza
Boston, Massachusetts 02108
boston.fbi.gov
(617) 742-5533

FBI Buffalo
One FBI Plaza
Buffalo, New York 14202-2698
buffalo.fbi.gov
(716) 856-7800

C

FBI Charlotte
Suite 900, Wachovia Building
400 South Tyron Street
Charlotte, North Carolina 28285-0001
charlotte.fbi.gov
(704) 377-9200

FBI Chicago
Room 905
E.M. Dirksen Federal Office Building
219 South Dearborn Street
Chicago, Illinois 60604-1702
chicago.fbi.gov
(312) 431-1333

FBI Cincinnati
Room 9000
550 Main Street
Cincinnati, Ohio 45202-8501
cincinnati.fbi.gov
(513) 421-4310

FBI Cleveland
Federal Office Building
1501 Lakeside Avenue
Cleveland, Ohio 44114
cleveland.fbi.gov
(216) 522-1400

FBI Columbia
151 Westpark Blvd
Columbia, South Carolina 29210-3857
columbia.fbi.gov
(803) 551-4200

D

FBI Dallas
One Justice Way
Dallas, Texas 75220
dallas.fbi.gov
(972) 559-5000

FBI Denver
Federal Office Building, Room 1823
1961 Stout Street, 18th. Floor
Denver, Colorado 80294-1823
denver.fbi.gov
(303) 629-7171

FBI Detroit
26th. Floor, P. V. McNamara FOB
477 Michigan Avenue
Detroit, Michigan 48226
detroit.fbi.gov
(313) 965-2323

E

FBI El Paso
660 S. Mesa Hills Drive
El Paso, Texas 79912-5533
elpaso.fbi.gov
(915) 832-5000

H

FBI Honolulu
Room 4-230, Kalanianaole FOB
300 Ala Moana Boulevard
Honolulu, Hawaii 96850-0053
honolulu.fbi.gov
(808) 566-4300

FBI Houston
2500 East TC Jester
Houston, Texas 77008-1300
houston.fbi.gov (713) 693-5000

I

FBI Indianapolis
Room 679, FOB
575 North Pennsylvania Street
Indianapolis, Indiana 46204-1585
indianapolis.fbi.gov
(317) 639-3301

J

FBI Jackson
Room 1553, FOB
100 West Capitol Street
Jackson, Mississippi 39269-1601
jackson.fbi.gov
(601) 948-5000

FBI Jacksonville
Suite 200
7820 Arlington Expressway
Jacksonville, Florida 32211-7499
jacksonville.fbi.gov
(904) 721-1211

K

FBI Kansas City
1300 Summit
Kansas City, Missouri 64105-1362
kansascity.fbi.gov
(816) 512-8200

FBI Knoxville
Suite 600, John J. Duncan FOB
710 Locust Street
Knoxville, Tennessee 37902-2537
knoxville.fbi.gov
(865) 544-0751

L

FBI Las Vegas
John Lawrence Bailey Building
700 East Charleston Boulevard
Las Vegas, Nevada 89104-1545
lasvegas.fbi.gov
(702) 385-1281

FBI Little Rock
Suite 200
Two Financial Centre
10825 Financial Centre Parkway
Little Rock, Arkansas 72211-3552
littlerock.fbi.gov
(501) 221-9100

FBI Los Angeles
Suite 1700, FOB
11000 Wilshire Boulevard
Los Angeles, California 90024-3672
losangeles.fbi.gov
(310) 477-6565

FBI Louisville
Room 500
600 Martin Luther King Jr. Place
Louisville, Kentucky 40202-2231
louisville.fbi.gov
(502) 583-3941

M

FBI Memphis
Suite 3000, Eagle Crest Bldg.
225 North Humphreys Blvd.
Memphis, Tennessee 38120-2107
memphis.fbi.gov
(901) 747-4300

FBI North Miami Beach
6320 Northwest Second Avenue
North Miami Beach, Florida 33169-6508
miami.fbi.gov
(305) 944-9101

FBI Milwaukee
Suite 600
330 East Kilbourn Avenue
Milwaukee, Wisconsin 53202-6627
milwaukee.fbi.gov
(414) 276-4684

FBI Minneapolis
Suite 1100
111 Washington Avenue, South
Minneapolis, Minnesota 55401-2176
minneapolis.fbi.gov
(612) 376-3200

FBI Mobile
One St. Louis Centre
200 N. Royal Street
Mobile, Alabama 36602
mobile.fbi.gov
(334) 438-3674

N

FBI Newark
1 Gateway Center, 22nd. Floor
Newark, New Jersey 07102-9889
newark.fbi.gov
(973) 792-3000

FBI New Haven
600 State Street
New Haven, Connecticut 06511-6505
newhaven.fbi.gov
(203) 777-6311

FBI New Orleans
2901 Leon C. Simon Dr.
New Orleans, Louisiana 70126
neworleans.fbi.gov
(504) 816-3000

FBI New York
26 Federal Plaza, 23rd. Floor
New York, New York 10278-0004
newyork.fbi.gov
(212)384-1000

FBI Norfolk
150 Corporate Boulevard
Norfolk, Virginia 23502-4999
norfolk.fbi.gov
(757) 455-0100

O

FBI Oklahoma City
3301 West Memorial Drive
Oklahoma City, Oklahoma 73134
oklahomacity.fbi.gov
(405) 290-7770

FBI Omaha
10755 Burt Street
Omaha, Nebraska 68114-2000
omaha.fbi.gov
(402) 493-8688

P

FBI Philadelphia
8th. Floor
William J. Green Jr. FOB
600 Arch Street
Philadelphia, Pennsylvania 19106
philadelphia.fbi.gov
(215) 418-4000

FBI Phoenix
Suite 400
201 East Indianola Avenue
Phoenix, Arizona 85012-2080
phoenix.fbi.gov
(602) 279-5511

FBI Pittsburgh
3311 East Carson St.
Pittsburgh, PA 15203
pittsburgh.fbi.gov
(412) 432-4000

FBI Portland
Suite 400, Crown Plaza Building
1500 Southwest 1st Avenue
Portland, Oregon 97201-5828
portland.fbi.gov
(503) 224-4181

R

FBI Richmond
1970 E. Parham Road
Richmond, Virginia 23228
richmond.fbi.gov
(804) 261-1044

S

FBI Sacramento
4500 Orange Grove Avenue
Sacramento, California 95841-4205
sacramento.fbi.gov
(916) 481-9110

FBI St. Louis
2222 Market Street
St. Louis, Missouri 63103-2516
stlouis.fbi.gov
(314) 231-4324

FBI Salt Lake City
Suite 1200, 257 Towers Bldg.
257 East, 200 South
Salt Lake City, Utah 84111-2048
saltlakecity.fbi.gov
(801) 579-1400

FBI San Antonio
Suite 200
U.S. Post Office Courthouse Bldg.
615 East Houston Street
San Antonio, Texas 78205-9998
sanantonio.fbi.gov
(210) 225-6741

FBI San Diego
Federal Office Building
9797 Aero Drive
San Diego, California 92123-1800
sandiego.fbi.gov
(858) 565-1255

FBI San Francisco
450 Golden Gate Avenue, 13th. Floor
San Francisco, California 94102-9523
sanfrancisco.fbi.gov
(415) 553-7400

FBI San Juan
Room 526, U.S. Federal Bldg.
150 Carlos Chardon Avenue
Hato Rey
San Juan, Puerto Rico 00918-1716
sanjuan.fbi.gov
(787) 754-6000

FBI Seattle
1110 Third Avenue
Seattle, Washington 98101-2904
seattle.fbi.gov
(206) 622-0460

FBI Springfield
Suite 400
400 West Monroe Street
Springfield, Illinois 62704-1800
springfield.fbi.gov
(217) 522-9675

T

FBI Tampa
Room 610, FOB
500 Zack Street
Tampa, Florida 33602-3917
tampa.fbi.gov
(813) 273-4566

W

FBI Washington
Washington Metropolitan Field Office
601 4th Street, N.W.
Washington, D.C. 20535-0002
washingtondc.fbi.gov
(202) 278-2000

State and Territorial Public Health Directors (listed by state)

Alabama
Alabama Department of Public Health
State Health Officer
Phone No. (334) 206-5200
Fax No. (334) 206-2008

Alaska
Division of Public Health
Alaska Department of Health and Social Services
Director
Phone No. (907) 465-3090
Fax No. (907) 586-1877

American Samoa
Department of Health
American Samoa Government
Director
Phone No. (684) 633-4606
Fax No. (684) 633-5379

Arizona
Arizona Department of Health Services
Director
Phone No. (602) 542-1025
Fax No. (602) 542-1062

Arkansas
Arkansas Department of Health
Director
Phone No. (501) 661-2417
Fax No. (501) 671-1450

California
California Department of Health Services
State Health Officer
Phone No. (916) 657-1493
Fax No. (916) 657-3089

Colorado
Colorado Department of Public Health & Environment
Executive Director
Phone No. (303) 692-2011
Fax No. (303) 691-7702

Connecticut
Connecticut Department of Public Health
Commissioner
Phone No. (860) 509-7101
Fax No. (860) 509-7111

Delaware
Division of Public Health
Delaware Department of Health and Social Services
Director
Phone No. (302) 739-4700
Fax No. (302) 739-6659

District of Columbia
DC Department of Health
Acting Director
Phone No. (202) 645-5556
Fax No. (202) 645-0526

Florida
Florida Department of Health
Secretary and State Health Officer
Phone No. (850) 487-2945
Fax No. (850) 487-3729

Georgia
Division of Public Health
Georgia Department of Human Resources
Director
Phone No. (404) 657-2700
Fax No. (404) 657-2715

Guam
Department of Public Health & Social Services
Government of Guam
Director of Health
Phone No. (67l) 735-7102
Fax No. (671) 734-5910

Hawaii
Hawaii Department of Health
Director
Phone No. (808) 586-4410
Fax No. (808) 586-4444

Idaho
Division of Health
Idaho Department of Health and Welfare
Administrator
Phone No. (208) 334-5945
Fax No. (208) 334-6581

Illinois
Illinois Department of Public Health
Director of Public Health
Phone No. (217) 782-4977
Fax No. (217) 782-3987

Indiana
Indiana State Department of Health
State Health Commissioner
Phone No. (317) 233-7400
Fax No. (317) 233-7387

Iowa
Iowa Department of Public Health
Director of Public Health
Phone No. (515) 281-5605
Fax No. (515) 281-4958

Kansas
Kansas Department of Health and Environment
Director of Health
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