

INTRO TO BIOTERRORISM FOR PRIMARY CARE PHYSICIANS

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SLIDE 1: This presentation is designed to provide clinicians with the essential and practical information needed to properly recognize, diagnose and manage illnesses associated with bioterrorism.

SLIDE 2: Bioterrorism is the use or threatened use of a micro-organism or the product of a micro-organism in order to generate fear, morbidity or mortality in a population.

SLIDE 3: Although there are potentially hundreds of organisms that could be used as a biological weapon, the Centers for Disease Control (CDC) has given the highest priority to six diseases that have been designated as Category 'A', or the most likely to be encountered in a biological attack. Agents within this category have several features in common including a high morbidity or mortality and relative ease to produce, store and disperse [1]. This presentation will focus on these six Category A diseases, which are: Anthrax, smallpox, plague, tularemia, botulism and the viral hemorrhagic fevers.

SLIDE 4: Release of biological weapons could be accomplished in a number of ways. However, it is expected that an aerosol release is the most likely to be encountered in a large-scale bioterrorism event if the goal is to sicken or kill large numbers of people. This is because aerosols are odorless, colorless and relatively easy to disperse over vast areas, and for most of the diseases, it is the route that is most contagious and causes the most severe disease. Many of these agents could theoretically be used to target food or water supplies, however, the psychological impact would likely be more of a concern than the clinical effect because of water purification processes, dilution of infectious material into large volumes, stability of the organisms and the logistical difficulties of dispersion [2].

SLIDE 5: As the first responders in this new kind of terrorism, clinicians played a critical role during the 2001 outbreak of anthrax related to contaminated letters [3]. Because of this new threat, all physicians who might see patients at the first presentation of a bioterrorism-related illness should be able to recognize, manage and report such diseases. There are a couple of concepts that are important for the recognition of any bioterrorism event, regardless of the etiology. First, a reasonably high level of suspicion is needed to keep bioterrorism-related illnesses in the differential diagnosis because most of the likely agents are rare, or non-existent as naturally-occurring diseases. The old adage of "When you hear hoofbeats, think horses instead of zebras" needs to be amended to say "...think horses but don't forget to consider the zebras". Secondly, physicians should be conscious of any unusual epidemiologic trends that might suggest a biological weapons release or a naturally emerging infectious disease. For example, an unusual clustering of cases in a geographic area or among persons with common exposures

should raise suspicion. Other red flags include a large number of otherwise healthy people presenting with severe disease, the identification of diseases that are typically not found in the geographic area, and a sudden increase in sick or dead animals, as many of the potential bioterrorism-associated diseases are zoonoses [4].

SLIDE 6: Clinicians should be able to recognize the typical disease syndromes that would be encountered for the Category A diseases [2] and know how to make a preliminary diagnosis. Physicians should also know how to appropriately treat and provide post-exposure prophylaxis for each of the major pathogens. Finally, it is vital that all physicians know how to immediately report any suspected bioterrorism-related illnesses to the appropriate authorities. Generally this will be the local and/or state health departments, as well as the hospital epidemiologist, infectious disease consultants and infection control specialists. The local health departments will be able to provide information on specific samples needed for appropriate diagnostic tests [5].

SLIDE 7: Anthrax is an ancient zoonotic disease primarily affecting herbivorous animals that is caused by the bacterium *Bacillus anthracis*. Of all of the potential pathogens we would expect to encounter as a bioterrorism agent, we have the most knowledge about the use of *B. anthracis* for this purpose.

SLIDE 8: Our understanding of anthrax and its use as a biological weapon stems primarily from information gained through the epidemiologic investigations of inhalational anthrax outbreaks from three sources: the 18 cases in the United States from 1900-1976, associated with various occupational exposures [6,7]; the inadvertent aerosolization of anthrax spores being produced for weapons purposes at a plant in Sverdlovsk, Russia in 1979 [6,8]; and most recently, we have substantial information providing new insights into the presentation and pathogenesis of anthrax infection caused by anthrax spores in powder form that was delivered in letters during the U.S. outbreak of 2001 [9], the first known successful deliberate use of anthrax as an agent of terror.

In the 2001 letter-associated outbreak there were 18 confirmed cases of anthrax in multiple Eastern states. Of the 18, 11 were inhalational and 7 were cutaneous. There were also 4 presumptive cases of cutaneous anthrax. All 5 of the fatal cases were of the inhalational form [10]. Although details of the Sverlovsk outbreak are limited because of the secrecy surrounding the episode, there were at least 77 human cases and 66 deaths, all of which were downwind of the plant [8]. Many were found to be infected with more than one strain, raising fears that multiple strains with differing antibiotic susceptibilities, including genetically altered resistance patterns, could be encountered from a single intentional release [11].

SLIDE 9: There are 3 recognized forms of human anthrax, determined by where spores germinate. Inhalational anthrax, which is the most likely form to be seen in a bioterrorism event, is rare in naturally-occurring disease, comprising less than 5% of all cases. However, the mortality is high, ranging from 45% in the 2001 cases [9] to 89% in 20th century U.S. cases. It is not clear why there is such a discrepancy in mortality between current and past cases, but possible factors include improved antibiotic and

critical care facilities, strain characteristics or inoculum dose. Cutaneous disease is the most common form of natural disease comprising 95% of all cases. Mortality is less than 1% in treated cases [12], but up to 20% in cases that are left untreated [6]. Gastrointestinal disease is rare, comprising less than 5% of all cases worldwide and has never been reported in the United States. Mortality numbers are not well known, however, it is estimated to be at least 50% [13].

The key to anthrax infection is that there must be contact with spores, either through natural or intentional circumstances. Traditional risk factors for naturally-occurring inhalational and cutaneous disease are similar, namely contact with the hides and skins of infected animals. Gastrointestinal infection has occurred by ingestion of meat from infected animals. It has been known for decades that anthrax spores could be aerosolized, thus providing a risk for large scale human exposure. However, the investigations from the 2001 outbreak identified a new risk factor for anthrax that had not been considered in the past: powdered material containing anthrax spores being sent through the mail. Exposure to the spores by handling the contaminated letters led to several cases of cutaneous disease. Also, because the powder was milled into fine particles of 5 microns or less, spores were presumably aerosolized upon mechanical processing of the letters in postal facilities and upon handling of the opened letters, leading to at least 9 of the inhalational anthrax cases. Exposures for the remaining two inhalational cases were hypothesized to be cross-contamination of the mail, but there was insufficient evidence to confirm this [14,15].

SLIDE 10: *Bacillus anthracis* is the bacterium that causes anthrax. In its vegetative, metabolically active form it is a large, aerobic encapsulated Gram-positive rod, ranging in size from 3 to 10 microns long and usually 1 micron in width. The organisms are non-motile and non-hemolytic on standard blood agar. Long chains are formed in vitro, while short chains or single bacteria are usually seen in direct examination of clinical specimens, sometimes displaying a “jointed bamboo rod” appearance [6,13]. Gram-positive bacilli in clinical specimens are often considered contaminants by microbiology laboratories, as most other Gram positive bacilli such as *Diphtheroides* and *Corynebacteria* species are commonly isolated non-pathogenic skin flora. If anthrax is suspected, the laboratory should be alerted to ensure full workup of any Gram positive rod. *Bacillus anthracis* is a sporulating organism, thus when exposed to oxygen or a nutrient-depleted milieu, it converts to a hardy inert, but infectious, spore one micron in size that can survive for years, even under harsh conditions. When spores land in a nutrient-rich environment, they germinate into the vegetative bacillus state and begin producing the toxins that lead to disease.

SLIDE 11: This slide demonstrates the “jointed bamboo rod” appearance of Gram positive staining *Bacillus anthracis* in a specimen of CSF from the index case of the 2001 outbreak. Note the short chains and large size [9].

SLIDE 12: This diagram of anthrax pathogenesis nicely illustrates the common processes that occur for all three forms of anthrax. Regardless of exposure route, macrophages engulf the spores where they land. Inhaled spores less than 5 microns can

travel to the alveoli, while larger particles may land in the oropharynx and cause GI disease. Macrophages then carry the spores to regional lymph nodes, except in most cases of cutaneous disease where the infection remains localized in the skin. Within this nutrient-rich environment, the spores germinate into vegetative bacilli. Rapid multiplication results in lysis of macrophages and destruction of local lymph node or subcutaneous architecture. This is the mechanism for the pathognomonic hemorrhagic mediastinal lymphadenitis of inhalational disease. Of note, because the spores do not germinate in the alveoli, a true pneumonia rarely, if ever, develops in inhalational anthrax. During bacterial replication, high levels of the two toxins are produced. Edema toxin causes massive edema at the site of germination in cutaneous disease and large pleural effusions in inhalational. Lethal toxin initiates the cascade of inflammatory mediators that leads to sepsis when systemic disease occurs. High levels of toxin in the blood inevitably lead to death even if the bacteria are quickly killed with antibiotics [6,16].

SLIDE 13: In most cases of inhalational and GI disease, and some untreated cutaneous cases, hi-grade bacteremia with subsequent systemic illness and sepsis occur rapidly. The degree of bacteremia is much higher than most other bacterial infections, and the bacterial burden can be so great that direct Gram stains of peripheral blood buffy coats, such as the one in this photomicrograph, often demonstrate the Gram positive bacilli [17].

SLIDE 14: The clinical features of inhalational anthrax have been fairly well described in the past, and have been further validated by the 2001 outbreak. An asymptomatic incubation period occurs, which can range between 2 and 43 days, generally lasting 4-7 days from exposure to symptom onset. The occasional longer incubation periods are thought to be related to delayed spore germination, which, in animal studies occurred up to 98 days after exposure. Symptoms classically follow a biphasic pattern, starting with a prodrome consisting of a nonspecific flu-like syndrome then progressing to a rapidly fatal fulminant phase. Early symptoms are nearly always constitutional (fevers, malaise, myalgias) and respiratory (dyspnea, nonproductive cough, chest discomfort) and often are gastrointestinal (nausea, vomiting, diarrhea). Notably absent in most cases are nasal symptoms such as rhinorrhea and congestion. This feature may help to differentiate inhalational anthrax in the prodromal stage from upper respiratory viral illnesses. The duration of the prodromal phase is typically several hours to 3 days. It is important to also realize that a flu-like prodrome occurs in many of the other bioterrorism-related diseases. Disease then progresses to a fulminant phase, where patients are critically ill with fever, diaphoresis, respiratory distress and cyanosis. Septic shock, multiorgan failure and disseminated intravascular coagulation ensue. 10-50% may develop hemorrhagic meningitis with headache, meningismus, delirium and coma, which can be the prominent feature upon presentation. Death usually occurs within 36 hours of the fulminant phase onset [6,9].

SLIDE 15: There are no specific laboratory tests to suggest anthrax, but hemoconcentration, manifested as an elevated hematocrit is common. The chest radiograph is always abnormal. The mediastinum is widened in at least 70% cases by the time of presentation, although it may be subtle at first. Pleural effusions and/or infiltrates

probably corresponding to compressive atelectasis are found in greater than 80% [9]. This is a chest x-ray from a naturally occurring case of inhalational anthrax showing the classic widened mediastinum caused by lymphadenopathy, indicated by the shaggy borders, as well as a small left basilar infiltrate and effusion [6].

SLIDE 16: The differential diagnosis for inhalational anthrax is broad. Early disease mimics influenza and other upper respiratory virus infections, however nasal symptoms are typically not present and rapid diagnostic tests, such as nasal swabs for detection of respiratory virus antigens would be negative. In addition, the chest radiograph is always abnormal in inhalational anthrax, even during the early stages, whereas it would be expected to be normal in an uncomplicated case of viral upper respiratory infection [18]. Pneumonia, especially from atypical pathogens, is high on the differential, however sputum production is minimal to absent in inhalational anthrax and there may be no pulmonary infiltrate. The widened mediastinum can be mistaken for primary mediastinitis or a dissecting thoracic aortic aneurysm, but lack of recent surgery and presence of fever should readily differentiate. The meningitis that occurs in many cases presents like other acute bacterial meningitides, however bloody CSF containing Gram positive bacilli should be seen [6].

SLIDE 17: Cutaneous disease usually occurs on the hands, arms, neck or head. The disease occurs whenever spores are able to penetrate the epidermis through visible breaks in the skin or microscopic cuts. The incubation period is typically 3-5 days, but may be up to 12 days, there is no clinical evidence of delayed spore germination in the skin. While mild constitutional symptoms can be seen, progression to severe systemic disease, preceded by lymphangitis and lymphadenopathy, is very rare when treated [6].

SLIDE 18: The hallmark of cutaneous disease is a single or few lesions that are painless throughout all stages, starting with a papule or macule that may be pruritic. Over the next 1-2 days a single or multiple vesicles or a large bulla appears with clear or serosanguineous fluid within. This dries into an ulcer with surrounding gelatinous, often extensive, nonpitting edema. If the edema involves the head or the neck, airway compromise can occur. Several days later this progresses to a black depressed eschar at the base of the ulcer. Purulence or significant erythema and evidence of inflammation should raise the suspicion of secondary bacterial infection such as cellulites [16].

SLIDE 19: This photo is an example of a typical cutaneous anthrax lesion revealing early black eschar formation with surrounding gelatinous edema. Cutaneous anthrax may be misidentified as a brown recluse spider bite and may be similar to ulceroglandular tularemia. There have been rare case reports of possible person-to-person spread of cutaneous disease [19].

SLIDE 20: Gastrointestinal disease is not well understood because there have been no reported cases in the United States. Gastrointestinal system involvement is not uncommon in the setting of systemic disease, but true GI anthrax is the result of primary germination within the GI tract. There are two recognized subtypes -- one is an oropharyngeal or esophageal type that occurs when inhaled particles larger than 5

microns land in the oropharynx and germinate, or ingested spores are deposited in the mouth or esophagus. This leads to regional lymphadenopathy and edema which cause stridor, sore throat, fever, dysphagia and subsequent sepsis. The more common intestinal form follows ingestion of spores and subsequent germination within the small or large bowel. Early symptoms of nausea, vomiting and malaise, progress to hematochezia, bloody ascites and an acute abdomen [16]. The differential diagnosis for gastrointestinal disease includes gastroenteritis, peritonitis, and any cause of acute abdomen.

SLIDE 21: Because of its mild, nonspecific nature, a high index of suspicion is necessary to make the diagnosis of anthrax in the early stages. This is compounded by the fact that there are no readily available rapid specific tests for confirming anthrax. The presentation of a previously healthy patient with severe flu-like symptoms and a widened mediastinum should prompt immediate treatment and diagnostic measures. The gold standard remains direct culture of clinical specimens onto blood agar with demonstration of typical colony, Gram stain, motility and biochemical features [6]. A culture with the expected characteristics provides a preliminary diagnosis and greater than 90% confirmation that the organism is *Bacillus anthracis*. Direct culture of clinical specimens is widely available and should be performed on blood and other clinical specimens including pleural fluid and CSF, if available. Blood cultures should always be obtained prior to antibiotic initiation as they are positive nearly 100% of the time in inhalational anthrax, but rapid sterilization of blood after a single dose of antibiotics occurs [9]. Sputum is unlikely to be helpful in diagnosing anthrax, but may identify a pathogen if the patient has a bacterial pneumonia instead of anthrax. If anthrax is suspected, the microbiology laboratory should be notified to ensure the workup of any Gram positive bacillus that is isolated. When growth does occur it is usually rapid, within 8-24 hours [6,9]. Preliminarily positive cultures are then sent to a reference laboratory for confirmation via gamma phage lysis and staining for *B. anthracis*-specific capsular or polysaccharide cell wall antigen. More rapid confirmatory tests available only at reference labs including PCR for all clinical specimens have the advantage of being able to confirm anthrax quickly and even when the cultures are negative. As the assays become more commercially available they may have an expanded role in first-line rapid diagnosis. Serologic testing can also be used to retrospectively confirm anthrax infection in cases that are culture-negative [6]. Nasal swabs are a useful epidemiologic tool, but should never be used to rule out exposure to anthrax because of their low sensitivity.

SLIDE 22: The basic components of treatment for severe anthrax disease consist of hospitalization with intensive supportive care and IV antibiotics, which should be started immediately upon suspicion and prior to confirmation of the diagnosis. The 2001 outbreak confirmed that late diagnosis and treatment adversely affects prognosis. Steroids can be considered for treatment of all forms of anthrax when presentation is severe with significant edema, respiratory failure or meningitis [20].

SLIDE 23: As susceptibility data will be delayed, if available at all, antibiotics must be chosen empirically. Based on 2001 data and prior experiences, a recommended regimen for empiric therapy has been proposed, and should be followed until sensitivity patterns allow for adjustment. The CDC recommends the use of intravenous ciprofloxacin at

400mg q12h or doxycycline at 100mg IV q12h, in addition to one or two other antibiotics, choosing from clindamycin, vancomycin, rifampin, penicillin, chloramphenicol or imipenem. Macrolides, cephalosporins and trimethoprim/sulfamethoxazole are usually ineffective and should not be used. The combination of ciprofloxacin, rifampin and clindamycin or vancomycin was successful in the 2001 outbreak. The same empiric therapy for inhalational anthrax should be provided to pregnant women as other adults, with the rationale being that the risks of the antibiotics on the welfare of the fetus are small and are outweighed by the benefits of treating suspected disease [20].

SLIDE 24: Empiric therapy for inhalational disease in children consists of the same drugs but dose adjusted for children at 10-15mg/kg/day of ciprofloxacin q12h with the maximum of 1g a day, and doxycycline at 2.2mg/kg which leads to an adult dosage for children greater than 8 years of age and greater than 45kg. One or two additional antibiotics should be added as in adults. When treating children, the risk to benefit ratio must be weighed, as the two primary drugs have real or theoretical toxicity in that population. The fluoroquinolones rarely cause arthropathy, and doxycycline carries the risk of dental enamel staining. However, in the setting where treatment is necessary for suspected or confirmed anthrax, the risk of the side effects are outweighed by the benefits of providing appropriate antibiotic therapy for this disease with such high morbidity and mortality [20].

SLIDE 25: Empiric therapy for cutaneous disease consists of the same drugs as inhalational and follows the same regimen if there are signs of systemic disease, extensive edema or if lesions are present on the head or the neck. Localized cutaneous infections can be treated with a single oral antibiotic, ciprofloxacin at 500mg bid or doxycycline at 100mg bid being the drugs of choice [20]. Gastrointestinal disease should be treated similarly to inhalational.

SLIDE 26: For all forms of anthrax, the recommended duration of antibiotic therapy is 60 days because of the risk of delayed spore germination. Although delayed germination has not been seen with cutaneous disease, there may be a risk of coexisting inhalation of spores, depending on the mechanism of exposure. The clinical course should be monitored very closely for any evidence of recurrence after cessation of antibiotics. Intravenous antibiotics can be switched to oral antibiotics after clinical improvement is noted and the patient is able to tolerate oral medications. Upon switching to oral therapy the regimen should consist of one or two drugs, including the initially chosen ciprofloxacin or doxycycline, with guidance from susceptibilities. The treatment of children is an exception. Because of the uncertain benefit of treatment for a full 60 days, and the higher risk of drug toxicity from long-term use of fluoroquinolones and tetracyclines, amoxicillin can be considered for use upon switching to oral therapy [20]. There is no role for vaccine in the active treatment of known anthrax disease.

SLIDE 27: Post exposure prophylaxis is the administration of antibiotics with or without vaccine after suspected exposure to anthrax has occurred but before symptoms are present. Once symptoms are present then it is considered active treatment. Post exposure

prophylaxis should be offered to anyone who has had suspected direct exposure to aerosolized anthrax or powders containing anthrax. Prophylaxis should not be offered to contacts of cases unless they were also exposed to the original source. The decision to start post exposure prophylaxis should be based completely on the risk of exposure and not on any laboratory tests, including nasal swabs, as none are sensitive enough to exclude the diagnosis or to reliably detect exposure. Antibiotics should be administered as soon as possible after exposure is suspected. Prompt therapy prior to symptom development is likely to decrease the risk of subsequent disease, and decrease the severity of disease that does develop. The antibiotics should be the same regimen as active treatment for cutaneous disease, with oral ciprofloxacin or doxycycline as the first line for empiric therapy [6]. Ciprofloxacin is preferred over doxycycline in pregnant women because of potential adverse fetal effects, and because there is less certainty of the dramatic positive risk versus benefit ratio for prophylaxis as compared to treatment. If ciprofloxacin cannot be used for pregnant women, then amoxicillin should be the next choice [21]. Because of the unknown magnitude of the risk for delayed spore germination (which was up to 98 days in primates), the known risk of adverse reactions with prolonged antibiotic therapy, and the possible benefit of administering vaccine, there are three options for duration of prophylaxis outlined by the CDC. These include 60 days followed by observation, 100 days and observation, or 100 days and vaccination [22].

SLIDE 28: The anthrax vaccine adsorbed (AVA) is a vaccine that has been in use in the United States for many years. Because of production problems, there is a limited supply that is controlled by the Department of Defense. It is an inactivated cell-free filtrate of the vaccine strain of *Bacillus anthracis*. The vaccine prevents disease in >95% of animals exposed to lethal aerosols. Although there are no human efficacy data, an older generation vaccine provided > 90% protection in cutaneous disease, and a trend toward protection versus inhalational [23]. The vaccine does have some documented adverse effects, mostly as recorded from a database of over 1.6 million doses given to military personnel as of April, 2000. There were no deaths recorded and <10% resulted in moderate to severe local reactions including erythema and edema. Fewer than 1% had systemic reactions such as fever and malaise [24].

SLIDE 29: There is no known person-to-person transmission of inhalational anthrax, however there have been rare case reports of possible transmission of cutaneous disease [19]. As the risk of transmission is thought to be extremely low, standard precautions are recommended for patient contact, which includes the use of gloves when a draining lesion is present. Biosafety Level 2 (BSL-2) handling is all that is necessary for standard clinical specimens. However, for environmental samples, large volumes, or dealing with powders that could contain anthrax spores, BSL-3 handling should be used [6].

SLIDE 30: Smallpox is the disease caused by variola virus.

SLIDE 31: Smallpox was perhaps the most devastating infectious disease scourge known to mankind, causing more deaths and suffering than any other [25]. As a result of possibly the greatest achievement of modern public health, there have been no cases of smallpox since a laboratory accident in 1978, one year after the global eradication of

naturally-occurring disease [26]. There is precedence, however, to be concerned about smallpox as a biological weapon. It was used in the French and Indian War when blankets known to have infectious materials on them were given to Native Americans, leading to smallpox outbreaks among the tribes. More recently, its production in the former Soviet Union specifically for the use as a biological weapon was confirmed. The potential of its use as a bioweapon, however, is contingent upon the availability of the virus and this is a question of debate. There are officially only two stocks of virus in existence -- one at the CDC in Atlanta, Georgia and the second at a similar biological lab in Russia. However, the security of the stock that has been in Russian hands is in question because of the fallout from the collapse of the former Soviet Union. Because it is estimated that less than 20% of the United States population has substantial immunity, smallpox is an attractive weapon to be used by those wishing to cause a high mortality [27,28]. For these reasons, there is legitimate concern that an extinct disease poses a substantial risk.

Slide 32: Smallpox afflicted only humans, as there are no known animal hosts. In a largely unvaccinated population, smallpox had a mortality of 25-30%. Although the case fatality is lower than other potential bioterrorism agents such as anthrax, smallpox has the potential for secondary spread from person to person. Transmission occurs primarily through close face-to-face contact via droplet nuclei. However, smallpox can also be transmitted via an airborne route in the setting of an infected patient with a severe cough, and from direct aerosol inhalation. One of the most concerning things about smallpox is that there is person to person transmission, and the secondary attack rate would likely be 25-40% in unvaccinated contacts, meaning that at least one of every three or four persons exposed would develop disease. Historically, three to four contacts were infected per index case. However, it is expected that up to 10-20 contacts in a mostly nonimmune population could be infected. There is very high potential for nosocomial spread as evidenced by several cases occurring throughout a hospital where an infected patient with a cough was kept in an isolation room [27,29].

Slide 33: Variola virus is in the *Orthopoxviridae* family of DNA viruses. There are 2 strains including *Variola major*, which was the cause of the majority of fatal disease with a mortality of 25-30% and was prominent in India, Asia and Northern Africa. *Variola minor* is a second strain with a milder disease and a lower mortality, usually less than 1%, which was the predominant form seen in the United States and Europe in the 20th century [30,31]. *Vaccinia* is another *Orthopox* virus, and the virus used for the current smallpox vaccine. Cowpox, used by Jenner in his first vaccinations against smallpox, and monkeypox are other *Orthopox* viruses that rarely cause disease in humans [26].

Slide 34: The pathogenesis of smallpox begins when the virus lands on respiratory or oral mucosa. Macrophages engulf the organism and carry it to the regional lymph nodes where a primary transient viremia develops. The reticuloendothelial organs are invaded and overwhelmed leading to a secondary viremia. White blood cells are subsequently infected and then migrate to capillaries and invade the dermis causing dermal cell infection and an influx of additional leukocytes and mediators that lead to the formation

of deep vesicles. A further inflammatory response occurs systemically which is triggered by the viremia and leads to sepsis, multiorgan failure and often, death [27,32].

SLIDE 35: There are three stages of disease starting with an asymptomatic incubation stage that typically lasts 12-14 days, with a range of 7-17 days. This is followed by a prodromal phase that begins very acutely as a nonspecific, flu-like illness almost always accompanied by fevers and prostration. The prodrome lasts for 3-5 days and ends with the eruption of the characteristic rash. Patients become infectious approximately one day prior to the appearance of the rash, corresponding to the development of oral mucosal lesions. The classical smallpox rash is characteristic and can be distinguished from other rashes based on its distribution, its grouping and the deep tense vesicles that are formed [27].

SLIDE 36: This photograph of an infant shows the classic distribution of the rash. It appears in a centrifugal pattern where the lesions first occur on the head and face and then the distal arms and legs including the palms and soles with relative sparing of the trunk [27].

SLIDE 37: This photograph shows the characteristic grouping where all lesions within a localized area are in the same stage of development. This is unlike chicken pox where crops of lesions in different stages of development are noted in the same location. The vesicles are also deeper and more tense than chickenpox. The severity of the rash correlates with the mortality, where the most severe rashes have the lowest survival rates.

SLIDE 38: The progression of smallpox lesions can be noted on this slide. The earliest stage is maculopapular, leading to deep vesicles, then pustules and finally scabs that then separate leaving a permanent scar. Scab separation marks the end of the period of infectiousness [27].

SLIDE 39: The one disease that is most likely to be misidentified as smallpox in the setting of an outbreak is chicken pox. In addition to the grouping of the lesions, the critical differentiation can be made by the distribution of the rash. Chickenpox is distributed centripetally, starting on the trunk and sparing the palms and soles [33].

SLIDE 40: The diagnosis of smallpox is a clinical one and in the setting of an outbreak, the classic syndrome and rash are all that is necessary for confirmation. Any suspicious rash during the setting of an outbreak must be considered smallpox until proven otherwise. Traditional confirmatory methods have included electron microscopy of vesicle fluid that can rapidly confirm the presence of an *Orthopoxvirus* but does not prove variola is the species. This requires culture on chick membrane or cell culture, which is specific but slow. Newer rapid tests including PCR are available at reference labs [27].

SLIDE 41: The management of confirmed or suspected cases consists primarily of supportive care for those infected, and isolation. There is no specific antiviral treatment for those already showing symptoms. Supportive care is critical including careful

attention to electrolyte and volume status, and ventilatory and hemodynamic support. Antibiotics are only required in the uncommon setting of secondary bacterial infections, such as *Staphylococcus aureus* cellulitis. Isolation of the patient is a vital component of the management of smallpox. Vaccination does not provide benefit to those truly infected who are already symptomatic, but can be considered in the treatment regimen in case the diagnosis of smallpox is wrong in a patient who was at risk of exposure [27].

SLIDE 42: Post exposure prophylaxis should be provided to those who have suspected exposure prior to symptom onset. This would include persons exposed to an original aerosol or contacts of cases, defined as those in the same household or who have had direct face-to-face contact with the patient after fever onset [27]. Vaccine is partially protective if given within 3-4 days of exposure and may reduce the incidence of disease by 2-3-fold and mortality by 50% [34]. Administration of *Vaccinia* immune globulin (VIG) in conjunction with vaccination may provide up to 70% greater protection versus incidence and death versus vaccination alone if given within the first few days after exposure. The passive immunity lasts for approximately two weeks, and presumably provides protection until active immunity from the vaccine develops [32,35]. The antiviral agent cidofovir can prevent disease in animals exposed to other pox viruses and may be effective as a post exposure prophylactic option for smallpox if given within two days of exposure [27,36].

SLIDE 43: The smallpox vaccine used in the United States, called Dryvax, consists of live attenuated *Vaccinia* virus. As of early 2002, there are an estimated 15 million dosages available in the United States, although it is likely that the current supply can be stretched by dilution. The stock is controlled by the CDC and is still viable despite being more than 20 years old [37]. In an outbreak setting, vaccination can reduce the secondary attack rate by ten-fold [38]. It has the highest efficacy in those who are vaccinated multiple times. Duration of efficacy of a single immunization is unknown but is likely to provide substantial protection for at least 3-5 and possibly up to 10 years, and to have at least a 3-fold decrease in mortality for 20 years. Revaccination can grant 30+ years of immunity that may persist life long [27,39,40].

SLIDE 44: The vaccine does have serious complications with up to 3 in 100,000 vaccinees reporting significant adverse reactions and nearly 1 in 1,000,000 deaths. Likelihood of adverse effects are 3-4-fold higher in infants and in primary vaccinees [41].

SLIDE 45: Most of the adverse effects of the vaccine are attributable to *Vaccinia* viremia. Encephalitis is the most feared adverse effect, occurring in 1 out of every 300,000 primary vaccinees. Mortality from this complication, for which there is no treatment, is 25%, and those who do survive often have permanent neurological sequelae. *Vaccinia gangrenosum* and *Vaccinia necrosum* are also highly feared serious adverse effects with mortality nearing 100% in those who are untreated. Eczema vaccinatum, which occurs in vaccinees or their contacts who have a history of eczema, is manifested by vaccinia lesions that appear in areas of skin involved by eczema. Mortality can be up to 40% in children less than 2 years old. All complications except encephalitis can be treated successfully with VIG [32,41].

SLIDE 46: This photograph shows an infant with *Vaccinia necrosum* at the inoculation site who died of this complication.

SLIDE 47: There are several critical infection control issues to be considered in the setting of a smallpox outbreak. They involve the proper handling of infected patients and case contacts. First, all suspected cases must be isolated following standard, contact and airborne precautions requiring the placement in a negative pressure room with HEPA filtration, and the use of gowns, gloves and N95 masks. If the clinical situation allows, home isolation is an option, especially during a large outbreak. When possible, only recently vaccinated caregivers should be assigned to suspected cases [27,42].

SLIDE 48: Contacts of suspected smallpox cases must first be correctly identified based on the fact that the period of infectiousness effectively begins with the eruption of the rash, and lasts until all lesions have scabbed over. Because the rash is preceded by a fever, a temperature greater than 38.0°C is an adequate trigger to isolate a case contact. Persons at risk include those exposed to a suspected case after fever onset via direct contact with secretions or face-to-face contact within 3 meters. Also, because of the high risk of nosocomial spread, all patients and staff in a hospital containing a suspected case should be considered contacts. All contacts should be immediately vaccinated (with concomitant VIG for those with relative contraindications) and observed 17 days for the development of fever. Isolation is not necessary before a fever is detected. Quarantining of patients or contacts may be necessary from a public health standpoint [27,42].

SLIDE 49: Plague is the zoonotic disease caused by the bacterium *Yersinia pestis*.

SLIDE 50: Plague is an ancient disease that is thought to be responsible for over 200 million deaths, many of which occurred during three pandemics, including the infamous Black Death of 14th century Europe. Populations were decimated by up to 50% during these pandemics [43]. *Yersinia pestis* is one of the agents that the Japanese attempted to use as a biological weapon in World War II at the Unit 731 camp in Manchuria, and it is thought to have been produced in large quantities by the former Soviet Union bioweapons program [44].

SLIDE 51: Plague remains endemic in many areas of the world, including the southwestern United States, where up to 10 cases per year are reported [45]. Globally, there are approximately 1700 cases each year [43]. Plague is primarily a disease of rodents, but can be transmitted to humans through the bite of infected fleas, the most common route, or through direct contact with infected animals. Additionally, plague can be transmitted via inhalation of infectious droplets from persons with pneumonic plague or from infected animals, particularly cats [46,47]. Experiments with primates have confirmed that an infectious aerosol of *Y. pestis* can be created, and this would likely be the form encountered in a bioterrorist event [44].

SLIDE 52: There are 3 predominant forms of human plague: pneumonic, bubonic and septicemic. Pneumonic plague can be either primary, which is the development of

pneumonia from direct inhalation of organisms, or secondary through the hematogenous spread of organisms from any primary site to the lungs. Two percent of United States plague cases are primary pneumonic, while 12% of bubonic and septicemic cases spread to secondary pneumonic. Presentation and clinical course are similar for both with an overall case fatality of 50-70%, which nears 100% when treatment is delayed by 18-24 hours or after onset of symptoms. In the United States, 84% of cases are bubonic, caused by flea bites or handling infected animals, and characterized by buboes, or tender and markedly swollen regional lymph nodes. Mortality is less than 5% in treated cases, but can reach 40-60% in cases that go untreated or where treatment is significantly delayed. Bubonic plague would not likely be the initial form of disease in a bioterrorism-related outbreak, but could occur after development of an epidemic and subsequent widespread rodent infection. Septicemic plague accounts for 13% of cases in the United States and consists of a severe systemic illness without preceding lymphadenopathy or pneumonia. Any route of exposure can lead to septicemic plague, and this form of disease might be seen after aerosol exposure in rare individuals who did not develop a substantial pneumonia. Mortality is 30-50% despite treatment and greater than 90% when treatment is delayed [43,45].

SLIDE 53: *Yersinia pestis* is one of the three pathogenic *Yersinia* species within the family Enterobacteriaceae. The other two, less virulent species, are *Y. enterocolitica* and *Y. pseudotuberculosis* [48]. It is a nonmotile, intracellular, aerobic Gram-negative coccobacillus that has a characteristic bipolar appearance on Wright, Giemsa and Wayson's stains. It is among the most virulent human pathogens, with an antiphagocytic capsule, lipopolysaccharide endotoxin and other virulence factors [46,49].

SLIDE 54: This photo demonstrates the characteristic bipolar, or "safety pin", appearance of *Yersinia pestis* on Wright, Giemsa and Wayson stains.

SLIDE 55: Primary pneumonic plague ensues after live organisms are inhaled into the alveoli where they cause a severe lobular pneumonia that often progresses rapidly to dense lobar consolidation with necrosis and subsequent high-grade bacteremia. The bacteremia can lead to seeding of multiple organs and a typical Gram negative sepsis syndrome mediated by the lipopolysaccharide endotoxin [46].

SLIDE 56: The incubation period is typically 1-4 days, but can be as long as 6 days. Disease starts suddenly with non-specific symptoms resembling an acute flu-like illness, including fevers, chills, myalgias, malaise and headache. There are often prominent gastrointestinal symptoms including nausea, vomiting, diarrhea and abdominal pain, before more specific symptoms of pneumonia appear. Patients typically progress from feeling well to having severe pneumonia with severe dyspnea, cough, chest pain, and stridor within 24 hours. Hemoptysis is a common finding reflecting the degree of necrosis occurring in the lung, and may be a helpful clue in the differential diagnosis. Sepsis, manifested by hypotension, multi-organ failure and DIC, ensues in inadequately treated patients. Purpuric lesions and gangrene of the digits are complications of the sepsis and DIC that result from any form of the disease [44,46]. The differential diagnosis of pneumonic plague includes any severe pneumonia, and should be considered

in any case of severe Gram negative pneumonia without nosocomial exposure, especially if there is no response to typical antibiotic therapy.

SLIDE 57: This is an example of a chest x-ray showing an extensive left lower lobe consolidation from pneumonic plague.

SLIDE 58: Bubonic plague also begins with flu-like symptoms 2-8 days after exposure, but is accompanied by painful, enlarged, and sometimes draining lymph nodes called “buboes” proximal to the inoculation site, generally in the groin or axilla. Lymph node destruction and hi-grade bacteremia with systemic disease and sepsis occur in severe cases. Septicemic plague presents only as the severe systemic disease after nonspecific flu-like symptoms and without preceding lymphadenopathy or pneumonia, making initial diagnosis extremely difficult [44].

SLIDE 59: Timely diagnosis is difficult as there are no tests available that are rapid, specific and confirmatory, making a high index of suspicion necessary. A preliminary diagnosis can be made when bipolar staining bacilli are visualized in samples prepared with Wayson, Giemsa or Wright stain. Culture of blood, sputum, bubo fluid and CSF onto blood and MacConkey agar can confirm the presence of *Y. pestis* if the appropriate biochemical tests are available. Serologic tests identifying the presence of the F1 capsular antigen are helpful retrospectively. Rapid confirmatory tests such as PCR and fluorescent antibody assays are generally only available at reference laboratories [44,49].

SLIDE 60: Treatment of *Yersinia pestis* consists primarily of antibiotic therapy that must be initiated rapidly upon first suspicion and prior to confirmation. Historically, monotherapy with an appropriate antibiotic results in rapid improvement. Aminoglycosides are first line therapy, particularly streptomycin, which is approved by the Food and Drug Administration (FDA) for this purpose at 1g IM bid for adults. Gentamicin is also recommended and is easier to administer, because it can be given intravenously and can be dosed once daily at 5mg/kg. Tetracyclines can be used, such as doxycycline 100mg IV bid for adults, which is also the first choice if oral therapy is required, for example in the setting of mass casualties. Other alternatives include ciprofloxacin 400mg IV q12 hours for adults, which is effective versus *Y. pestis* in vitro, although there are no human data. Other fluoroquinolones may be effective as well, but have not been studied. Chloramphenicol is the first choice for plague meningitis as it penetrates the blood-brain barrier. The dose for chloramphenicol is 25mg/kg given intravenously q 6h maintaining levels between 5mcg/ml and 20mcg/ml. Children under 2 years old should not be treated with chloramphenicol if at all possible to avoid the "grey baby" syndrome [44,49].

SLIDE 61: Antibiotics that are generally ineffective and should not be used for *Yersinia pestis* include beta-lactams such as penicillins and cephalosporins, rifampin, aztreonam and macrolides. Natural antibiotic resistance to the drugs of choice is rare, but it should be anticipated that genetically-engineered antibiotic resistance may be encountered in a bioterrorism scenario. Intravenous antibiotics can be switched to oral therapy if available for the drug being used after clinical improvement occurs. Duration of therapy should be

for 10-14 days, or at least 3 days after becoming afebrile with clinical improvement [44,46].

SLIDE 62: Post-exposure prophylaxis with oral doxycycline or ciprofloxacin should be administered for 7 days to anyone who may have had inhalational exposure to *Yersinia pestis* within the prior 6 days, either as an aerosol or as droplets from a patient with pneumonic plague. Significant exposure is defined as a household or hospital contact, or being within 2 meters of the infected patient. Contacts who refuse prophylaxis should be observed closely for 7 days and started on full treatment regimens for the development of any cough or fever [44,46].

SLIDE 63: A vaccine produced from a killed virulent strain was used in the United States in the past, but has not been commercially available since 1999. It was effective against bubonic plague only and did have some adverse effects [44].

SLIDE 64: It is thought that pneumonic plague is transferred person-to-person via respiratory droplets. Thus, any suspected pneumonic plague patients should be placed in respiratory droplet isolation*, where a surgical mask is required. These precautions should be maintained until the patient has received at least 48 hours of appropriate antibiotics and is clinically improving. Patients with draining buboes should be in contact isolation. Routine processing of clinical specimens can be performed in a Biosafety Level 2 (BSL-2) setting, but BSL-3 is necessary for high-risk procedures such as grinding and shaking [44].

* *Editor's Note*

Transcript reflects updated/corrected information.

SLIDE 65: Tularemia is the disease caused by *Francisella tularensis*.

SLIDE 66: Tularemia is a relatively modern disease, first described in the early 20th Century in Tulare County, California. Although less deadly than many other high priority biological weapon candidates, it causes severe incapacitation. Both the United States and former Soviet Union weaponized *F. tularensis* in their prior bioweapons program, and it was tested by the Japanese at the infamous Unit 731 in Manchuria during World War II [50,51].

SLIDE 67: In its natural form tularemia is a rare sporadic disease found primarily in moderate climates. It is endemic in the United States, Japan, Russia and Europe where it occasionally causes epidemics. There are approximately 125 cases reported annually in the U.S. since 1990, with the majority being reported from Midwestern states. Tularemia is a zoonotic infection of small mammals, particularly rabbits, that can be transmitted to humans via direct skin contact with infected animals, arthropod bites or aerosolization. Exposure by aerosol is the likely method that would be encountered in a bioterrorism event and has been reported after presumed aerosolization of infected animals by a lawn mower, illustrating the ease by which this route can cause disease. Overall mortality in

the U.S. is less than 2%, but this rises to 30% to 60% for the typhoidal and pneumonic forms of disease when treatment is delayed [50,52-54].

SLIDE 68: Human tularemia occurs in 6 recognized forms, determined primarily by route of infection. Pneumonic tularemia that results from inhalation of aerosolized organisms is rare but is associated with the most severe disease. It should be noted that all forms of tularemia may develop secondary pulmonary features, but the designation of pneumonic tularemia should be confined to primary disease that is acquired via inhalation. The most common form of tularemia is ulceroglandular, accounting for 45-85% of U.S. cases. This results from inoculation of organisms into skin via arthropod bites or animal contact with subsequent local ulcer formation and lymphadenopathy in the proximal draining lymph nodes. Occasionally, lymphadenopathy occurs without an ulcer leading to the designation of glandular disease. Oculoglandular disease occurs when *F. tularensis* is inoculated into the eye, and the oropharyngeal form most often occurs via ingestion of contaminated meat. The typhoidal form is marked by a lack of preceding skin ulcer, lymphadenopathy or pneumonia, similar in concept to the septicemic form of plague. It is thought that typhoidal disease primarily results from inhalation of organisms. A bioterrorism attack involving an aerosol of *F. tularensis* would be expected to cause primarily pneumonic disease, but all forms could be seen, particularly typhoidal [50,53].

SLIDE 69: The organism, *Francisella tularensis*, is a small, intracellular, aerobic pleomorphic Gram negative coccobacillus that is nonmotile and does not form spores. It has a thin envelope that allows it to live for weeks in cool, moist conditions. It stains very faintly on Gram stain, making it difficult to visualize in clinical specimens. Growth on culture is slow, generally taking 2-3 days to first appear, and it requires cysteine-enriched medium. There are 2 major strains, or biovars. Type A is the predominant strain in the U.S. and causes the most severe disease. Type B is found primarily in Europe and Asia, and mortality is rare [50,52,53,55].

SLIDE 70: The pathogenesis of *Francisella tularensis* is similar for all forms of tularemia. There is initial infection at the inoculation site. When the lung is the portal of entry, an acute bronchiolitis and pneumonitis leads to an aggressive immune response causing extensive suppuration and consolidation and eventual fibrosis. The pleura is also frequently involved manifested as pleural thickening and effusions. Organisms migrate to regional lymph nodes and can spread hematogenously to other organs where a similar suppurative immune response occurs if treatment is delayed. Severe systemic disease marked by sepsis, DIC, multiorgan failure and death can occur when the disease spreads hematogenously, which is most commonly seen with typhoidal and pneumonic forms [50,56]

SLIDE 71: All forms of tularemia are preceded by a common non-specific influenza-like illness characterized by the sudden onset of high fevers, chills, profuse sweats and myalgias often localizing to the lower back. Symptoms generally arise 2-5 days after exposure, but can be seen sooner in high dose aerosol exposure. The incubation period can uncommonly spread out to 3 weeks. Pleuropulmonary involvement including cough,

chest pain and dyspnea occur in up to 40% of cases not exposed through inhalation. Interestingly, pulse/temperature dissociation, or relative bradycardia, is a relatively common, albeit nonspecific phenomenon seen in 40% of patients in one series, that may be helpful in differentiating tularemia from other diseases [50,51,57].

SLIDE 72: After the initial flu-like prodrome, primary pneumonic tularemia can have variable manifestations from mild disease to fulminant pneumonia with sepsis. Patients usually present with fever, minimally productive cough, pleuritic pain and dyspnea. Hemoptysis is occasionally present and leukocytosis is common. Chest radiographs typically show lobar and often bilateral patchy infiltrates with pleural effusions and sometimes hilar adenopathy [50,53]. Secondary pneumonic tularemia has similar findings in addition to the features coinciding with the primary disease process. The differential diagnosis for pneumonic tularemia includes other atypical pneumonias including pneumonic plague. Ulceroglandular tularemia is characterized by a solitary painful ulcer at the inoculation site that starts as a maculopapular lesion 2 days after the prodromal illness appears and progresses to a pustule then a slow-healing ulcer with raised edges [51-53]. Painful lymphadenopathy with overlying erythema usually appears proximal to the inoculation site, but is not always present.

SLIDE 73: This photo shows the typical skin ulcer of ulceroglandular tularemia.

SLIDE 74: A high index of suspicion is required to diagnose tularemia as there are no readily available rapid and specific confirmatory tests. Gram stains are rarely helpful, culture lacks specificity and takes several days to grow and serological testing is retrospective only. Blood cultures are rarely positive, but sputum and pharyngeal washings have higher yields for *F. tularensis*. Fluorescent antibody assays (DFA), PCR and immunohistochemical assays can give a presumptive diagnosis within hours but are usually only available at reference labs [50,53,58].

SLIDE 75: The treatment of choice is streptomycin at 1 gram IM bid in adults, which has proven clinical efficacy with nearly 100% cure rates and is FDA-approved for this indication. The second choice is gentamicin with near 90% cure rates and greater convenience because it can be given intravenously and once daily. Failures with aminoglycosides are usually related to insufficient duration of therapy, severe comorbid illnesses, or delay in treatment initiation. Alternatives such as doxycycline and tetracycline are effective, however, they have a higher risk of relapse and must be continued for a longer course of therapy [50,53,59].

SLIDE 76: Chloramphenicol is another effective option that should be considered if meningitis is suspected, however its high relapse rate precludes monotherapy. Ciprofloxacin has been shown to have very good success anecdotally in several cases, with no reported failures and only one relapse. An advantage of the tetracyclines and fluoroquinolones is that they can be directly converted to oral therapy upon clinical improvement. Beta-lactams and macrolides are clinically ineffective and should be avoided, despite good in vitro activity for ceftriaxone. Ten days is a sufficient duration of therapy for aminoglycosides, whereas ciprofloxacin should be continued for 14 days.

Doxycycline, tetracycline and chloramphenicol treatment durations should be 14-21 days [50,59-61].

SLIDE 77: For persons who quickly become aware of a suspected exposure to aerosolized *F. tularensis*, such as in a laboratory accident or an announced bioterrorism attack, oral doxycycline or ciprofloxacin should be given for prophylaxis for 2 weeks. In a more likely scenario where a bioterrorism release is not discovered until after the first cases present, then it is recommended that all potentially exposed persons be monitored for development of a fever without initiation of antibiotics as the incubation period is generally short. An active treatment regimen of antibiotics should be started immediately if a temperature over 38° C occurs during a 14 day period after presumed exposure. Persons in contact with tularemia patients do not require prophylaxis unless they were at risk for the original exposure themselves, as there is no known person-to-person transmission [50].

SLIDE 78: A live, attenuated vaccine is available that is primarily used by researchers exposed to *F. tularensis*. The precise efficacy is unknown, but it is thought to reduce the incidence of typhoidal disease, and the severity of ulceroglandular. Because of the short incubation period of tularemia, and the time required to develop an immune response from immunization, use of the vaccine is not recommended as post-exposure prophylaxis [50,52].

SLIDE 79: There has been no documented person-to-person transmission of tularemia, even in the pre-antibiotic era. Thus, standard precautions are sufficient for patient handling in hospital settings. While clinical specimens can be handled with routine procedures, pure culture of *Francisella tularensis* is a danger to laboratory workers, with several reported cases of inhalationally acquired typhoidal or pneumonic infection. Thus, cultures with colonial growth require handling in BSL-2 conditions in a safety cabinet. Therefore, when tularemia is suspected the microbiology lab should be alerted prior to receiving the specimens to be cultured [50].

SLIDE 80: Botulism is the disease caused by intoxication with the botulinum neurotoxin produced by *Clostridium botulinum*.

SLIDE 81: Botulinum toxin is the most lethal substance known, with less than 1 microgram sufficient to cause fatal human disease. This toxin already has a history of use or attempted use as a biological weapon. The Japanese experimented with it in Manchuria during World War II, and it was produced by the former U.S. and Soviet Union bioweapons programs. After the Persian Gulf War, it was confirmed that Iraq had produced enough concentrated botulinum toxin to kill the entire global population and that dozens of missiles and bombs had been armed with it. In the early 1990's a Japanese cult unsuccessfully attempted to disperse botulinum toxin in Tokyo. Because of its potency, its history of use as a biological weapon, and its potential for aerosolization or food contamination, it is one of the most likely agents expected to be encountered in a bioterrorist attack. Contamination of municipal water supplies is unlikely because standard purification processes inactivate the toxin [62,63].

SLIDE 82: Naturally-occurring botulism is a rare disease with an annual incidence of approximately 100 cases in the U.S., one fourth of which are associated with foodborne outbreaks. Botulism is the result of exposure to and uptake into the body of botulinum toxin, produced by the bacterium *Clostridium botulinum*, which is found in soil throughout the world. The disease is not an infection, but an intoxication, and cannot be transmitted from person-to-person. There are three main mechanism of botulinum toxin entry into the body. The classic route of entry, leading to foodborne botulism outbreaks, is from the ingestion of preformed botulinum toxin in contaminated, often improperly prepared canned foods that contained *C. botulinum* spores. Intoxication also rarely occurs when *C. botulinum* spores germinate in a wound, typically from injection drug use, with subsequent toxin production. The majority of reported cases in the U.S. are infant botulism, which occurs sporadically as a consequence of *C. botulinum* colonization of the intestinal tract with subsequent absorption of toxin without actual infection. Rarely, this intestinal form can occur in adults. A fourth method of delivery is inhalational. Animal studies and rarely reported cases of laboratory accidents confirm that this is an efficient mechanism of delivery that quickly leads to intoxication. This is the most likely route of infection to be encountered in a large scale bioterrorism event, however foodborne botulism is a likely candidate for smaller scale events with a primary goal of spreading fear. Overall mortality for U.S. cases is less than 10%, and is rare when diagnosis is promptly made and mechanical ventilation is available. For these reasons, index cases of foodborne outbreaks have a higher mortality, and subsequent cases are more likely to receive timely antitoxin [63-66].

SLIDE 83: *Clostridium botulinum* is a large Gram positive spore-forming strictly anaerobic bacterium. It rarely infects humans, but it produces one of seven closely related neurotoxins, depending on the strain. These toxins are designated Type A through G. Types A, E and B are the most frequently encountered in the U.S., thus outbreaks with other types should raise greater suspicion for a possible bioterrorism-related event. All toxin types have the same general mechanism of action causing the same syndrome, but differ slightly in their structure and proteolytic activity [63,64]

SLIDE 84: This slide nicely illustrates the pathogenesis of botulism. The toxin is taken up by skeletal muscle motor neurons where it irreversibly inhibits the release of acetylcholine, resulting in post-synaptic muscle paralysis. The paralysis persists until axonal branches regenerate [63].

SLIDE 85: Regardless of the route of intoxication the same neurologic syndrome develops. After an incubation period of 12-72 hours, the classic syndrome appears. This is a flaccid paralysis that starts with acute symmetric cranial nerve palsies manifested by visual changes, ptosis and dysphasia, followed by descending complete skeletal muscle paralysis, including the diaphragm, leading to respiratory failure. Autonomic nervous system disturbances can include urinary retention and orthostasis. The symmetric, descending nature of the syndrome with a lack of fever and a normal mental status help to differentiate botulism from other neurological diseases [63,64].

SLIDE 86: The limited differential diagnosis for botulism includes myasthenia gravis, which would have a sustained response to anticholinesterases; Guillaine-Barre syndrome, which would involve an ascending paralysis with paresthesias and areflexia; stroke, which would likely be asymmetric and have associated abnormalities on brain imaging; tick paralysis, which would develop ascending paralysis and paresthesias with the presence of a tick; and poliomyelitis, which would be asymmetric and follow a preceding viral illness. In addition to the neurologic syndrome, there are often other features present depending on the route of intoxication. Foodborne illness is usually associated with nausea, diarrhea and dry mouth; infant botulism is often accompanied by constipation; and wound botulism would result from visible wounds [63,64].

SLIDE 87: A high index of suspicion is necessary for early presumptive diagnosis as there are no readily available rapid confirmatory tests. Diagnosis is primarily made on clinical presentation. Laboratory confirmation can be achieved in most cases by detection of toxin in serum or stool via a mouse bioassay that is available at reference laboratories. Anaerobic culture of stool is sometimes positive for *C. botulinum*. These tests require days to complete. The rapid detection of toxin by ELISA* may be available at some reference labs [63,64,66].

** Editor's Note*

Transcript reflects updated/corrected information.

SLIDE 88: Because botulinum toxin inhibits acetylcholine release irreversibly, the paralysis can last for weeks to months before axonal branches regenerate. Therapy is primarily supportive, with all severe cases requiring prolonged ventilatory and nutritional support. Complications related to ventilation are the primary cause of death, thus prevention of secondary infections, such as aspiration pneumonia, is critical. If antibiotics become necessary, aminoglycosides and clindamycin should be avoided because of their neuromuscular blockade activity. Passive immunization with antitoxin is the other mainstay of management, which has been shown to decrease mortality. Antitoxin contains antibodies that bind and inactivate circulating toxin, effectively halting the progression of paralysis, but it does not reverse paralysis that has already occurred. Thus, the antitoxin must be administered as quickly as possible upon suspicion of disease, without waiting for confirmation. There are two types of antitoxin available. The first is a licensed trivalent horse serum derived antitoxin containing antibodies to the main toxin Types A, B and E. It is available from the CDC through local health departments, and causes significant hypersensitivity reactions in up to 10%, including anaphylaxis in 2%. In the event of a known release of a different toxin type, an investigational heptavalent antitoxin versus all known toxin types is available from the Department of Defense. There is less experience with this agent, but it appears to have fewer hypersensitivity reactions [63,64,67].

SLIDE 89: Because of the limited supplies and high risk of hypersensitivity with botulinum antitoxins, routine administration after suspected exposure is currently not recommended in asymptomatic individuals, despite animal data to suggest that this is an effective prophylactic measure. In the event of suspected exposure to aerosolized

botulinum toxin, asymptomatic persons should be under close observation for at least 72 hours after suspected exposure, and antitoxin given immediately upon development of any symptoms [63].

SLIDE 90: Immunization with a pentavalent botulism toxoid versus Types A-E is effective at preventing botulism if given long before exposure, but has no role in post-exposure prophylaxis because it takes months to develop immunity. It is used to immunize laboratory workers and has not been tested versus inhalational exposure[63,64].

SLIDE 91: Because there is no person-to-person transmission of botulism, patients and clinical specimens can be handled following standard precautions.

SLIDE 92: Viral Hemorrhagic Fever (VHF) is a syndrome caused by any of a number of RNA viruses.

SLIDE 93: The Viral Hemorrhagic Fevers, or VHFs, are comprised of a variety of viral illnesses that share a common feature in their ability to produce a febrile hemorrhagic state in infected patients. There has been no known use of these agents as biological weapons but the concern exists that the use of these very pathogenic viruses is feasible, and given the high degree of attention focused on such diseases as Ebola in the media and in movies, there is a great potential for widespread fear.

SLIDE 94: The two families of viruses that are of most concern based on feasibility of production and high mortality are the filoviruses, which include Ebola and Marburg viruses; and the arenaviruses, which include the agents of the South American Hemorrhagic Fevers and Lassa Fever virus. Flaviviruses such as dengue and Yellow Fever viruses and bunyaviruses including Congo-Crimean Hemorrhagic Fever virus are also potential bioweapon agents producing VHF.

SLIDE 95: All of the agents cause sporadic disease or epidemics in areas of endemicity. Routes of transmission are variable, most of the diseases are zoonotic with spread via arthropod bites or contact with infected animals. Person-to-person spread is a major form of transmission for many of the viruses. Filoviruses and Congo-Crimean HF virus are readily transmitted through blood and other body fluids. Nosocomial spread of these diseases to healthcare workers via needle sticks and fluid contact is well-documented and greatly feared in regions with limited healthcare resources. Nosocomial transmission of some arenavirus infections has also been reported. Direct transmission of natural infection between humans via a respiratory route is uncommon for all the VHF's and has been difficult to prove. However, it is likely possible for many, including Ebola HF. All but dengue virus can cause infection as an environmental or laboratory-produced aerosol. Mortality is variable but can be as high as 90% during outbreaks of Ebola.

SLIDE 96: All of the potential agents of VHF are RNA viruses. This is a photo of an electron micrograph of ebola virus.

SLIDE 97: Virus enters the body through mucosal surfaces in contact with infectious fluids, needlesticks or via inhalation. Pathogenesis studies of ebola in animals have shown that a high-grade viremia occurs within 2 days of inoculation and that foci of infection occur in multiple organs, particularly the liver, spleen and lungs. Viral shedding from mucosal surfaces occurs but is preceded by fever and other systemic symptoms. Symptoms appear after a variable incubation period, but it ranges from 2 days to 3 weeks, depending on the disease. Involvement of the vasculature and coagulation system leads to disruptions in fluid and clotting homeostasis that can cause vascular leakage with edema, and significant hemorrhage.

SLIDE 98: Incubation periods are variable and range from 2 days to 3 weeks. Common early symptoms mimic those of other viral diseases such as influenza with fevers, myalgias and malaise. Disease can range from minimally symptomatic to fulminant, and symptomatology varies depending on the specific disease. However, all share the potential for the development of a bleeding diathesis manifested by severe hemorrhage from mucosal surfaces and petechiae. The severity of this hemorrhagic state is variable and may be absent. Bleeding can be further complicated by massive shifts in intravascular volume and edema as a result of disrupted vascular permeability. Shock is the end result in the most severe cases. Thrombocytopenia, leukopenia and hepatitis are common findings in many of the diseases. Diseases most often misdiagnosed as VHF include malaria, typhoid, rickettsial disease, meningococemia, and any cause of disseminated intravascular coagulation (DIC).

SLIDE 99: This photo demonstrates cutaneous ecchymosis and edema in a patient with Congo-Crimean Hemorrhagic Fever.

SLIDE 100: A high index of suspicion is necessary to diagnose a VHF because there are no readily available rapid confirmation tests. An initial diagnosis should be made presumptively on clinical features, especially in an outbreak setting. A confirmed diagnosis can be made retrospectively for most of the VHF agents by serological methods. Virus can be isolated from blood for some of the VHF's, but require a laboratory with capability of highly advanced safety measures (BSL-4). Experimental rapid diagnostic tests such as PCR may be available for some agents at reference laboratories.

SLIDE 101: For all patients with VHF, supportive therapy is the mainstay of management. Intravascular volume support can be complicated by vascular fragility. Rapid fluid shifts into extravascular spaces require aggressive volume resuscitation, however the pulmonary vasculature is also prone to leakage at this time, increasing the risk of ARDS. Routine attention to electrolyte balance, oxygenation and hemodynamic status must be given. Hemorrhage should be addressed with specific therapy as guided by coagulation studies, and by avoidance of invasive procedures if possible. Sedatives and other hepatically cleared drugs should be used judiciously. Experience with antivirals is limited, however ribavirin has been used successfully as specific antiviral therapy for CCHF, Lassa Fever and others. It has no activity versus the filovirus or flavivirus hemorrhagic fevers.

SLIDE 102: Other than oral ribavirin, which has been used investigationaly after high-risk exposure to CCHF and Lassa Fever, there is no specific prophylaxis for asymptomatic persons with suspected exposure to VHF agents. Those potentially exposed should be monitored closely for development of VHF symptoms. Even for the few agents with an available vaccine, immunity takes too long to develop for vaccination to be useful as post-exposure prophylaxis.

SLIDE 103: Yellow Fever is the only VHF with an available licensed vaccine. It has proven to be effective when administered to travelers to endemic areas and serious adverse effects are rare.

SLIDE 104: All patients should be placed in strict respiratory and contact isolation, including the use of face mask and goggles for close contact. Those with the highest potential for spread because of severe cough, hemorrhage, diarrhea, etc. should also be isolated under airborne precautions in a negative pressure room with requirement for use of a HEPA filtered respirator.