ASSESSING PATIENTS DURING A MASS CASUALTY

The Need for Clinical Laboratory Services

By Isaac D. Montoya, PhD

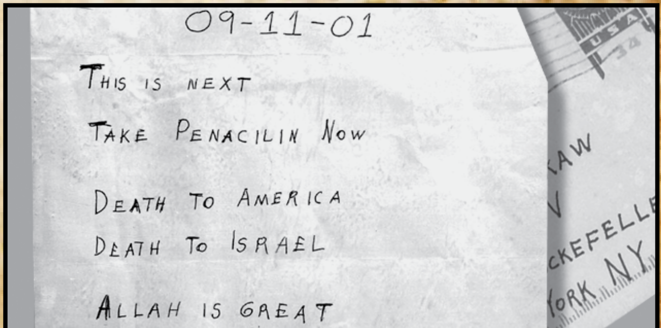
History has recorded many natural epidemics. By World War I, scientists had established germ theory by studying natural epidemics and learned how to use this knowledge for biological sabotage. On September 11, 2001, the United States experienced a terrorist attack that was followed by biological attacks. Since that time the United States has invested heavily in bioterrorism preparedness, however, the role of the clinical laboratory has been taken for granted in this preparation. Studies have demonstrated that without a functioning clinical laboratory, clinicians are relegated to providing first aid. Bioterrorism agents such as anthrax, plague, tularemia, brucellosis, and glanders all require intensive laboratory services for proper diagnosis and treatment. The laboratory's resources are also crucial in preventing the spread of disease.

History

Bioterrorism experts have long warned anyone who would listen that the United States is extremely vulnerable to attack by biological weapons. This warning was fully realized in the fall of 2001, as the World Trade Center and the Pentagon were attacked. Only a week after the terrorist attacks of September 11th, letters containing anthrax spores were mailed to journalist Tom Brokaw at NBC News in New York, the offices of the *New York Post,* and Senator Tom Daschle's office in Washington, D.C. By the end of 2001, anthrax had infected 18 people, five of whom died of the inhaled form of the bacteria, and millions of Americans experienced anxiety as a result of the attacks.

Historians have recorded many natural epidemics that are both frightening and threatening. Epidemics stemming from smallpox and plague are well known and also horrifying. Natural epidemics of such scourges as smallpox and plague are terrifying enough. These naturally occurring events are disastrous, in and of themselves; however, the fact that these diseases are used for warfare is maddening. The use of biological weapons is known to date back centuries, and the recent attacks show us how vulnerable we can be.

A picture of the letter contaminated with anthrax sent to Tom Brokaw's office in 2001.



09-11-01

THIS IS NEXT

TAKE PENACILIN NOW

DEATH TO AMERICA

DEATH TO ISRAEL

ALLAH IS GREAT

Credit: Scott Camazine / Photo Researchers, Inc

During the fourteenth and fifteenth centuries, little was known about what caused disease. According to medieval medical lore, the stench of rotting human bodies transmitted disease. The use of rotting human bodies is one of the earliest known forms of biological warfare. The following well-documented cases illustrate this warfare (Archives):

1340
Attackers hurled dead horses and other animals by catapult at the castle of Thun L'Eveque in Hainault, in what is now northern France. The defenders reported that "the stink and the air were so abominable ... they could not long endure" and negotiated a truce.

1346
As Tartars launched a siege against Caffa, a port on the Crimean peninsula in the Black Sea, they suffered an outbreak of plague. Before abandoning their attack, they sent the infected bodies of their comrades over the walls of the city. Fleeing residents carried the disease to Italy, furthering the second major epidemic of "Black Death" in Europe.

1422
At Karlstein in Bohemia, attacking forces launched the decaying cadavers of men killed in battle over the castle walls. They also stockpiled animal manure in the hope of spreading illness. Yet the defense held fast, and the siege was abandoned after 5 months.

By World War I, scientists understood how microbes such as bacteria and viruses transmit illness, and the germ theory of disease was well established. This knowledge was used by German scientists and military officials in a widespread campaign of biological terrorism. The Germans targeted livestock such as horses, mules, sheep, and cattle that were being shipped from neutral countries to the Allies. Their diseases of choice were glanders and anthrax, both well known to devastate populations of grazing animals resulting in natural epidemics. Simply by infecting a few animals through needle injection and pouring bacteria cultures on animal feed, the Germans hoped to spark devastating epidemics.

As Germany engaged biological weapons in World War I, the Japanese used biowarfare on a mass scale prior to and throughout World War II. The Japanese biowarfare was directed against China, in an onslaught that was led by a notorious division of the Imperial Army named Unit 731. This unit occupied Manchuria, beginning in 1936, where Japanese scientists tested scores of human subjects on the lethality of various disease agents including anthrax, cholera, typhoid, and plague. As a result of this testing, as many as 10,000 people died. In October 1940, the Japanese military dropped paper bags filled with plague-infested fleas over the cities of Ningbo and Quzhou in the Zhejiang province. Other attacks by the Japanese involved contaminating water wells and distributing food that had been poisoned.

During the apprehensive era of the Cold War, the Soviet Union and the United States reached new heights with their bioweapons programs. Extensive research efforts took place as both nations explored the use of hundreds of different bacteria, viruses, and biological toxins. And each country experimented with sophisticated ways in which to disperse these agents in fine-mist aerosols, to package them in bombs, and to launch these bombs on missiles.

In the 1990s, the Soviet Union's program began to collapse. As the collapse progressed, salaries declined dramatically, including those of the Soviet Union's scientists. Capitalizing on this opportunity, Iraq recruited bioweapons experts to develop a sophisticated bioweapons program. By the time of the Gulf War cease-fire in 1991, Iraq had weaponized anthrax, botulinum toxin, and aflatoxin, and it had several other lethal agents in various stages of development (Archives). Inspectors from the U.N. Special Commission (UNSCOM) spent exasperating years chasing down evidence of the program, which Iraq repeatedly denied existed. The UNSCOM team found that Iraq's stockpile included Scud missiles loaded to deliver disease. It is known that Iraq unleashed chemical weapons in the 1980s, both during the Iran-Iraq war and against rebellious Kurds in northern Iraq.

In 1984, *Salmonella* was sprinkled on salad bars throughout the county where Indian guru Bagwan Shree Rajneesh was living on a compound in rural Oregon. This supposedly was a trial run for an attack at a later date. The purpose of Rajneesh's scheme was to sicken local citizens and thus prevent them from voting in an upcoming election. Unfortunately, the trial attack resulted in more than 750 cases of food poisoning and 45 hospitalizations. An investigation by the Centers for Disease Control and Prevention concluded that the outbreak was natural. Interestingly enough, it took a year after the investigation, and an independent police investigation, to discover the true source of the poisoning.

The event in Oregon is considered the first bioterrorist act on American soil and almost went unnoticed; yet, a decade later another cult-initiated attack caused a flurry of media coverage and government response. It was 1995 when the apocalyptic religious sect Aum Shinrikyo released sarin gas in a Tokyo subway. This attack killed 12 commuters and injured thousands of others. Between 1993 and 1995, Aum Shinrikyo hired PhD bioweapons experts and tried as many as 10 times to spray *Botulinum* toxin and anthrax in downtown Tokyo. Attacks failed for two reasons: The cult did not sufficiently refine the particle size of its agents, and they were working with an avirulent strain of anthrax.

United States Preparedness

Top U.S. government and public health officials have reported that in spite of an investment of $20 billion in bioterrorism preparedness since 2001, the country is still woefully unprepared to respond to a bioterrorism attack. Critics such as Irwin Redlener from the National Center for Disaster Preparedness at Columbia University contend that bioterrorism preparedness programs are not operated in an effective manner. Bioterrorism preparedness is the primary responsibility of the Department of Health and Human Services

Credit: Arthur Friedlander

Color-enhanced scanning electron micrograph shows splenic tissue from a monkey with inhalational anthrax; featured are rod-shaped bacilli (yellow) and an erythrocyte (red)

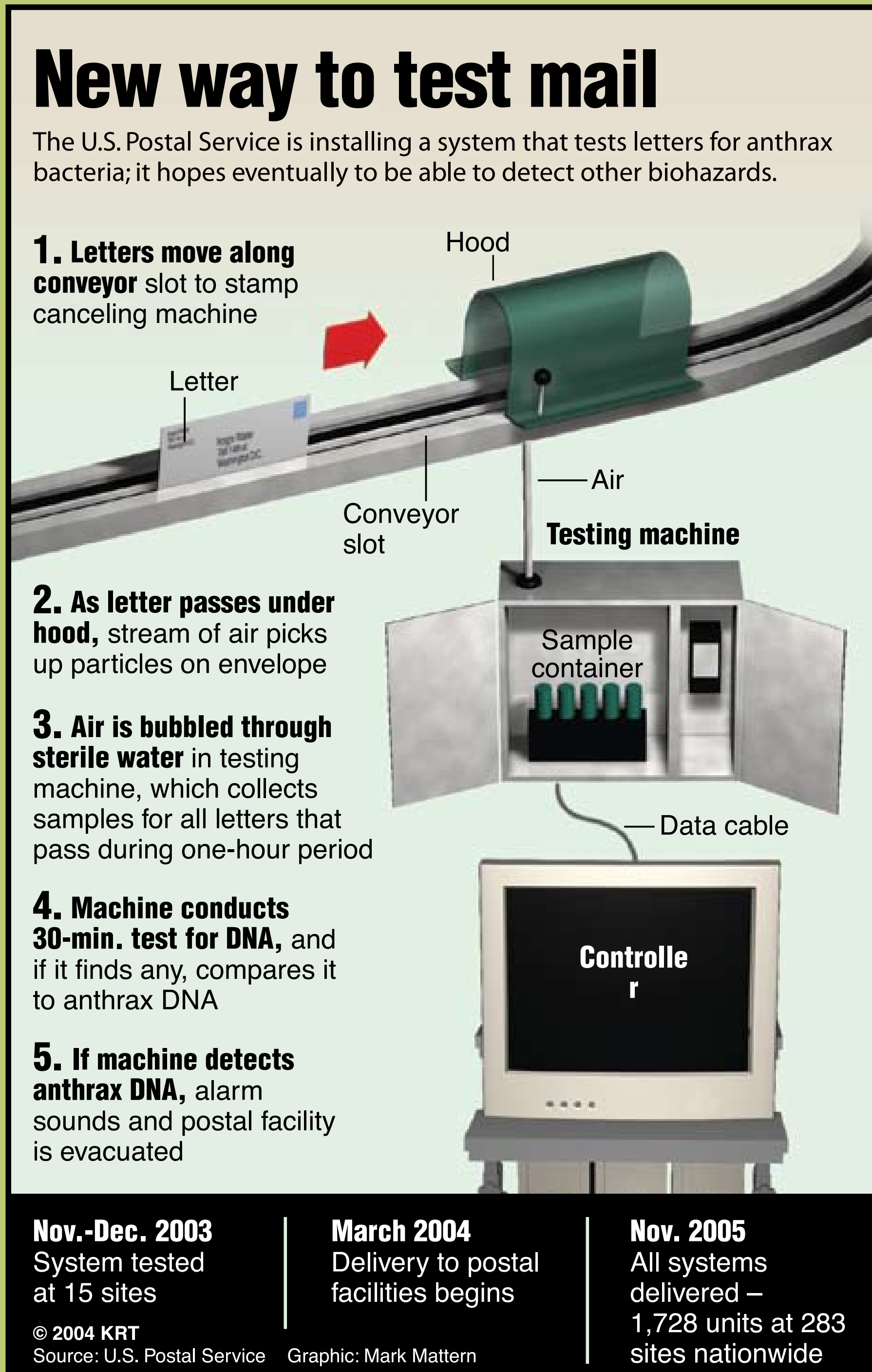
(DHHS). The department's responsibilities include stocking antibiotics, sharing information among laboratories and hospitals, and assisting communities in responding to an emergency.

This is an overwhelming responsibility even for the federal government. Although the government has made advances in addressing this responsibility, problems exist with some of these efforts. For example, a national stockpile of medical equipment and supplies has been amassed and can be delivered to any city within 12 hours of an attack. Unfortunately, once the cities receive the supplies, they have not developed the infrastructure to deliver these supplies to their citizens in a time frame that would save lives. Although the location and exact contents of the national stockpile are secret, DHHS reports that there is enough smallpox vaccine for every U.S. resident and enough antibiotics to treat 60 million people who might be exposed to the most viral form of anthrax.

The Clinical Laboratory

The role of the clinical laboratory in bioterrorism has received no attention, yet the laboratory is a pivotal point in the preparedness strategy. In the event of an attack, and before supplies arrive, physicians and nurses will be faced with massive numbers (hundreds to thousands) of patients presenting with various symptoms that will require supportive therapy until each individual's condition can be assessed for attack impact and for any co-occurring conditions.

It is reasonable to expect exposed patients to experience vomiting, diarrhea, dehydration, and many other symptoms depending on the toxin they have been exposed to. Clinicians will require substantial laboratory work to appropriately sustain patients until it is known what toxic agents patients have been exposed to and until supplies from the federal government arrive. Laboratory tests such as electrolytes, basic chemistries (glucose, renal and liver function tests), complete blood counts, and microbiology cultures are minimal procedures needed to make objective medical decisions.



KRT PHOTOGRAPH BY MIKE STOCKER/ SOUTH FLORIDA SUN-SENTINEL (December 21) MARGATE, FL – Broward County, Florida, technicians Mike Silvestri and Neil Colosi finish checking out a mail box on October 16, 2001, where a postal worker discovered a powdery substance. (FL) NC 2001 (Horiz) (gsb)

Without these tests, clinicians can only provide first aid. After treatment supplies arrive from the federal government, and patients are treated, these same basic tests will be required along with many other tests necessary to assess a patient's complete condition and his or her potential infectivity. In addition, blood products such as plasma or packed cells may be required. Examining the critical elements in the bioterrorism response process, the question begs: Is the laboratory prepared to handle possibly thousands of requests in a 12- to 72-hour period?

For example, will the laboratory have the staff to handle the tremendous volume that accompanies an attack of this magnitude? Will the laboratory have the equipment necessary to process the high volume to testing? Will the laboratory have the reagents and other supplies required for these analyses? What will treatment providers do if laboratory services are available for only a few patients? How will laboratory services be rationed? These are extremely important questions if patient lives are to be saved; yet, little to no attention has been paid to them. It is easy to envision the frustration and chaos that may result in hospitals and clinics if laboratory services are limited.

Clinical Laboratory Dependent Biological Agents

Five of the common bioterror agents are discussed below, as is the critical role of the laboratory in diagnosing and treating these infections.

ANTHRAX

Anthrax is an acute infectious disease caused by the spore-forming bacterium *Bacillus anthracis*. Anthrax most commonly occurs in wild and domestic lower vertebrates (cattle, sheep, goats, camels, antelopes, and other herbivores), but it can also occur in humans when they are exposed to infected animals or tissue from infected animals. Anthrax infection can occur in three forms: cutaneous (skin), inhalation, and gastrointestinal. *B. anthracis* spores can live in the soil for many years, and humans can become infected with anthrax by handling products from infected animals or by inhaling anthrax spores from contaminated animal products. Eating undercooked meat from infected animals can also spread anthrax. Symptoms of the disease vary depending on how the disease was contracted, but symptoms usually occur within 7 days. Symptoms of the three forms include the following:

Cutaneous: Most (about 95%) anthrax infections occur when the bacterium enters a cut or abrasion on the skin, such as when handling contaminated wool, hides, leather, or hair products (especially goat hair) of infected animals. Skin infection begins as a raised itchy bump that resembles an insect bite but within 1 to 2 days develops into a vesicle and then a painless ulcer, usually 1 to 3 cm in diameter, with a characteristic black necrotic (dying) area in the center. Lymph glands in the adjacent area may swell. About 20% of untreated cases of cutaneous anthrax will result in death. Deaths are rare with appropriate antimicrobial therapy.

Inhalation: Initial symptoms may resemble a common cold. After several days, the symptoms may progress to severe breathing problems and shock. Inhalation anthrax is usually fatal.

Intestinal: The intestinal disease form of anthrax may follow the consumption of contaminated meat and is characterized by an acute inflammation of the intestinal tract. Initial signs include nausea, loss of appetite, vomiting, and fever, followed by abdominal pain, vomiting of blood, and severe diarrhea. Intestinal anthrax results in death in 25% to 60% of cases.

Diagnosis—Anthrax is diagnosed by isolating *B. anthracis* from the blood, skin lesions, or respiratory secretions or by measuring specific antibodies in the blood of persons with suspected cases. The isolation of the bacteria is done in the microbiology department of a clinical laboratory while the measurement of antibodies is done in the immunology department of a laboratory.

Treatment—Clinicians will prescribe antibiotics that have been shown to be effective against *B. anthracis* by the clinical laboratory. To be effective, treatment should be initiated early. If left untreated, the disease can be fatal (Prevention, 2006).

PLAGUE

Fleas that become infected with the bacteria *Yersinia pestis* typically transmit plague. Fleas transmit the plague bacteria to humans and other mammals during the feeding process. Fleas may become infected by feeding on rodents, such as chipmunks, prairie dogs, ground squirrels, mice, and other mammals that are infected with the bacteria *Yersinia pestis*. The bacteria *Yersinia pestis* are maintained in the blood systems of rodents. Infected persons transmit *Yersinia pestis* by coughing plague-containing droplets into the air, which is then breathed by non-infected persons.

The typical sign of the most common form of human plague are swollen and very tender lymph glands, accompanied by pain. The swollen gland is called a "bubo" (hence the term "bubonic plague"). Bubonic plague should be suspected when a person develops a swollen gland, fever, chills, headache, extreme exhaustion, and has a history of possible exposure to infected rodents, rabbits, or fleas. A person usually becomes ill with bubonic plague 2 to 6 days after being infected. When bubonic plague is left untreated, plague bacteria invade the bloodstream. When plague bacteria multiply in the bloodstream, they spread rapidly throughout the body and cause a severe and often fatal condition. Infection of the lungs with the plague bacterium causes the pneumonic form of plague, a severe respiratory illness. The infected person may experience high fever, chills, cough, breathing difficulty, and expel bloody sputum. If plague patients are not given specific antibiotic therapy, the disease can progress rapidly to death. About 14% (1 in 7) of all plague cases in the United States are fatal.

Diagnosis—*Yersinia pestis* must be isolated in the microbiology section of the clinical laboratory. Laboratory tests that should be done include blood cultures for plague bacteria and microscopic examination of the lymph gland, blood, and sputum samples.

Treatment—A patient diagnosed with suspected plague should be hospitalized and medically isolated. Antibiotic treatment should begin as soon as possible after laboratory specimens are taken. Streptomycin is the antibiotic of choice. Gentamicin is used when streptomycin is not available. Tetracyclines and chloramphenicol are also effective. Persons who have been in close contact with a plague patient, particularly a patient with plague pneumonia, should be identified and evaluated. The U.S. Public Health Service requires that all cases of suspected plague be reported immediately to local and state health departments, and that CDC confirm the diagnosis. As required by the International Health Regulations, the CDC reports all U.S. plague cases to the World Health Organization (Prevention, 2006).

TULAREMIA

Tularemia is a potentially serious illness that occurs naturally in the United States. It is caused by the bacterium *Francisella tularensis* and is found in animals (especially rodents, rabbits, and hares). People can contract tularemia in many different ways. The most common include:

- Being bitten by an infected tick, deerfly, or other insect.
- Handling infected animal carcasses.
- Eating or drinking contaminated food or water.
- Breathing in the bacteria, *F. tularensis.*

Symptoms of tularemia usually appear 3 to 5 days after exposure to the bacteria, but can take as long as 14 days. Tularemia may include these symptoms:

- Sudden fever
- Chill
- Headaches
- Diarrhea
- Muscle aches
- Joint pain
- Dry cough
- Progressive weakness

Other symptoms of tularemia depend on how a person was exposed to the tularemia bacteria. These symptoms can include ulcers on the skin or mouth, swollen and painful lymph glands, swollen and painful eyes, and a sore throat. People can also catch pneumonia and develop chest pain, bloody sputum, trouble breathing, or even cessation of breathing. Tularemia is not known to be spread from person to person. People who have tularemia do not need to be isolated. People who have been exposed to the tularemia bacteria should be treated as soon as possible. The disease can be fatal if it is not treated with the right antibiotics.

Diagnosis—Rapid diagnostic testing for tularemia is not widely available. Physicians who suspect inhalational tularemia in patients presenting with atypical pneumonia, pleuritis, and hilar lymphadenopathy should promptly collect specimens of respiratory secretions and blood and alert the laboratory to the need for special diagnostic and safety procedures.

F. tularensis may be identified through direct examination of secretions, exudates, or biopsy specimens using Gram stains, direct fluorescent antibodies, or immunohistochemical stains. Microscopic demonstration of *F. tularensis* using fluorescent-labeled antibodies is a rapid diagnostic procedure performed in designated reference laboratories in the National Public Health Laboratory Network; test results can be available within several hours of receiving the specimens, if the laboratory is alerted and prepared.

Growth of *F. tularensis* in culture is the definitive means of confirming the diagnosis of tularemia. It can be grown from pharyngeal washings, sputum specimens, and even fasting gastric aspirates in a high proportion of patients with inhalational tularemia. It is only occasionally isolated from blood.

Treatment—The clinical laboratory will determine which antibiotics are best suited for the treatment of tularemia. A vaccine for tularemia is under review by the Food and Drug Administration, but it is not currently available in the United States.

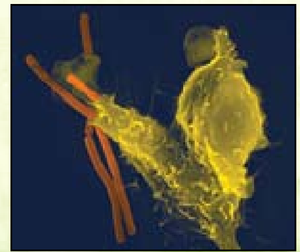
Tularemia as a Weapon—*Francisella tularensis* is very infectious. A small number (10–50 or so organisms) can cause disease. If *F. tularensis* were used as a weapon, the bacteria would likely be made airborne for exposure by inhalation. People who inhale an infectious aerosol would generally experience severe respiratory illness, including life-threatening pneumonia and systemic infection, if they are not treated. The bacteria that cause tularemia occur widely in nature and could be isolated and grown in quantity in a laboratory, although manufacturing an effective aerosol weapon would require considerable sophistication (Prevention, 2006).

BRUCELLOSIS

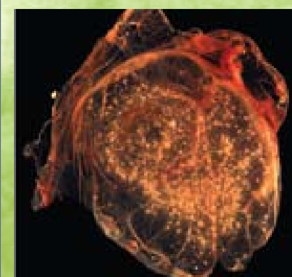
Brucellosis is an infectious disease caused by the bacteria of the genus *Brucella*. These bacteria are primarily passed among animals, and they cause disease in many different vertebrates. Various *Brucella* species affect sheep, goats, cattle, deer, elk, pigs, dogs, and several other animals. Humans become infected by coming in contact with animals or animal products that are contaminated with these bacteria. In humans, brucellosis can cause a range of symptoms that are similar to the flu and may include fever, sweats, headaches, back pains, and physical weakness. Severe infections of the central nervous system or lining of the heart may occur. Brucellosis can also cause long-lasting or chronic symptoms that include recurrent fevers, joint pain, and fatigue.

Humans are generally infected in one of three ways: eating or drinking something that is contaminated with *Brucella*, breathing in the organism (inhalation), or having the bacteria enter the body through skin wounds. The most common way to be infected is by eating or drinking contaminated milk products. When sheep, goats, cows, or camels are infected, their milk is contaminated with the bacteria. If the milk is not pasteurized, these bacteria can be transmitted to persons who drink the milk or eat cheeses made from it. Inhalation of *Brucella* organisms is not a common route of infection, but it can be a significant hazard for people in certain occupations, such as those working in laboratories where the organism is cultured. Inhalation is often responsible for a significant percentage of cases in abattoir (slaughterhouse) employees.

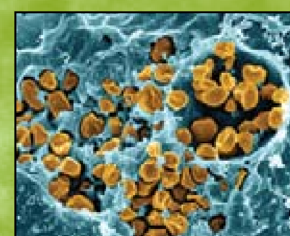
Direct person-to-person spread of brucellosis is extremely rare. Mothers who are breast-feeding may transmit the infection to their infants. Sexual transmission has also been reported. For both sexual and breast-feeding transmission, if the infant or person at risk is treated for brucellosis, their risk of becoming infected will probably be eliminated within 3 days. Although uncommon, transmission may also occur via contaminated tissue transplantation.



▲ Neutrophil with Anthrax



▲ Smallpox



▲ Brucellosis

▲ Tularemia

▲ Brucella

▲ Scanning electron micrograph depicting a mass of Yersinia pestis bacteria (the cause of bubonic plague) in the foregut of the flea vector

Diagnosis—Brucellosis is diagnosed in a microbiology section of a clinical laboratory by finding *Brucella* organisms in samples of blood or bone marrow. Also, blood tests in the immunology section of the clinical laboratory can be done to detect antibodies against the bacteria. If this method is used, two blood samples should be collected 2 weeks apart.

Treatment—Treatment can be difficult. Doctors can prescribe effective antibiotics as identified by the clinical laboratory. Usually, doxycycline and rifampin are used in combination for 6 weeks to prevent reoccurring infections. Depending on the timing of treatment and severity of illness, recovery may take a few weeks to several months. Mortality is low (less than 2%) and is usually associated with endocarditis (Prevention, 2006).

GLANDERS

Glanders is an infectious disease that is caused by the bacterium *Burkholderia mallei.* Glanders is primarily a disease affecting horses, but it also affects donkeys and mules and can be naturally contracted by goats, dogs, and cats. Human infection, although not seen in the United States since 1945, has occurred rarely and sporadically among laboratory workers as well as those in direct and prolonged contact with infected domestic animals. However, it is still commonly seen among domestic animals in Africa, Asia, the Middle East, and Central and South America.

Burkholderia mallei is an organism that is associated with infections in laboratory workers, because so very few organisms are required to cause disease. The organism has been considered as a potential agent for both biological warfare and biological terrorism. Glanders is transmitted to humans by direct contact with infected animals. The bacteria enter the body through the skin and through mucosal surfaces of the eyes and nose. The sporadic cases that have been documented are in veterinarians, horse caretakers, and laboratorians.

Along with animal exposure, cases of human-to-human transmission have been reported. These cases included two suggested cases of sexual transmission and several cases in family members who cared for the patients. There is no vaccine available for glanders. In countries where glanders is endemic in animals, prevention of the disease in humans involves identification and elimination of the infection in the animal population.

The symptoms of glanders depend upon the route of infection with the organism. The types of infection include localized, pus-forming cutaneous infections, pulmonary infections, bloodstream infections, and chronic suppurative infections of the skin. Generalized symptoms of glanders include fever, muscle aches, chest pain, muscle tightness, and headache. Additional symptoms have included excessive tearing of the eyes, light sensitivity, and diarrhea.

Localized infections: If there is a cut or scratch in the skin, a localized infection with ulceration will develop within 1 to 5 days at the site where the bacteria entered the body. Swollen lymph nodes may also be apparent. Infections involving the mucous membranes in the eyes, nose, and respiratory tract will cause increased mucus production from the affected sites.

Pulmonary infections: In pulmonary infections, pneumonia, pulmonary abscesses, and pleural effusion can occur. Chest X-rays will show localized infection in the lobes of the lungs.

Bloodstream infections: Glanders bloodstream infections are usually fatal within 7 to 10 days.

Chronic infections: The chronic form of glanders involves multiple abscesses within the muscles of the arms and legs or in the spleen or liver.

Diagnosis—The disease is diagnosed in the laboratory by isolating *Burkholderia mallei* from blood, sputum, urine, or skin lesions. Serologic assays are not available.

Treatment—Because human cases of glanders are rare, there is limited information about antibiotic treatment of the organism in humans. Sulfadiazine has been found to be effective in experimental animals and in humans. *Burkholderia mallei* is usually sensitive to tetracyclines, ciprofloxacin, streptomycin, novobiocin, gentamicin, imipenem, ceftrazidime, and the sulfonamides. Resistance to chloramphenicol has been reported (Prevention, 2006).

Conclusion

A report by the Lewin Group found that laboratory results influence 60% to 70% of objective medical decisions (Group, 2005). This is because the clinical laboratory serves as a bridge between the basic sciences and clinical care via the patient's clinician. The selection, generation, and translation of biological activity into a meaningful form of basic information for diagnosis, prognosis, and management of patient care is critical. Emphasis must be placed on accuracy, precision, and specificity of laboratory results so that clinicians can assess and treat patients appropriately.

In the event of a biological attack, multiple body systems are affected. For example, the circulatory system may experience abnormal bleeding while the respiratory system experiences stress. Very quickly these could lead to kidney and liver involvement resulting in a patient who is gravely ill and requires significant resources that are already in short supply. If the attack involves radiation, the issues remain the same. Multiple bodily systems are involved, and without laboratory data to provide accurate evaluations of system functioning (or damage), the clinician can do no more than provide first aid.

A successful response to a bioterrorist attack hinges on sufficient clinical laboratory services being available and medications to treat the sustained injuries in an effort to treat and prevent the spread of infections.

References

Archives, T.N. (2006). America's historical documents, U.S. Government.

Group, T.L. (2005). The value of diagnostics: Innovation, adoption and diffusion into health care. Washington, D.C.: 209–212.

Prevention, C.f.D.C.a. (2006). Division of Bacterial and Mycotic Diseases. ■

Earn CE Credit
To earn CE credit, complete the exam for this article on page 59 or complete the exam online at www.acfei.com (select "Online CE").

About the Author

Isaac D. Montoya, PhD, CHS, CLS, CMC, is a clinical professor in the College of Pharmacy at the University of Houston. He is also a senior research scientist at Affiliated Systems, a health-care "think tank" located in Houston. Before his current position, Dr. Montoya served as Director of Clinical Laboratories at several major teaching hospitals.