THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Bioterrorism and Emerging Infections Education

TULAREMIA

Tularemia, also referred to as Deerfly Fever or Rabbit Fever, is a bacterial zoonosis caused by Francisella tularensis that affects >250 species of wild and domestic mammals, birds, reptiles, fish, and people. Francisella tularensis was originally isolated in 1911 by McCoy and Chapin, from ground squirrels with a plague-like illness in Tulare County, California, during the investigation of a tick-borne plague outbreak in San Francisco. In 1914, Wherry and Lamb established that this plague-like disease, associated with rodents, could infect humans. Dr. Edward Francis defined and characterized the organism, and the disease associated with it. Dr. Francis experienced the disease four times during the course of his laboratory investigations with the organisms.
Tularemia occurs in domestic and wild animals, such as rabbits, prairie dogs and water voles. Humans usually become infected via contact with infected animals or contaminated articles and animal products. Tularemia predominantly presents as a cutaneous infection, but may occur in gastrointestinal and inhalational forms.

History of Development as a Weapon

Francisella tularensis has long been considered a potential biological weapon. In the 1950s and 1960s, the US military developed weapons that would disseminate F. tularensis. From the 1940s to early 1990s, the Soviet Union was also developing tularemia weapons based on strain SCHU SD-40, amongst other strains.

Francisella tularensis is suitable for weaponization because it can be grown relatively easily, is relatively stable in liquid formulation, and is highly stable in a dry formulation. It is also an “appropriate” particle size of bacteria, it has a low LD50 for humans, and is capable of forming secondary foci of infection via infected rodents and can persist in nature for a long period of time.

Three main types of Tularemia biological weapons were developed. These were liquid and dry weapons based on natural unchanged strains, antibiotic resistant tularemia strains, and vaccine subverting tularemia strains. Optimal cultivation conditions were developed in these industrial production programs, including the most suitable temperature, pH value and oxygenation

Traditional production is unlikely for groups that are not highly qualified. However, there are some well-known nutrient media that could be used for small-scale manufacturing. Feasibility of animal techniques should be considered when analyzing the possibility of terrorists producing tularemia

Deployment of a Tularemia Biological Weapon

LD50: 10-50 cells (10-100 according to some other sources)

Q50: about 3-5 kg per km2 (US and USSR data)

Military Deployment: The dry form of the weapon is most likely to be deployed, as it is effective in both deployable forms: line and point sources. Depending on the type of munitions employed, the mortality rate in case of using explosive munitions is estimated to be from 5 to 20%. In military scenarios a tularemia biological weapon most likely would be used as an operational weapon.

Possible Terrorist Application: Not all methods of application may be achievable for terrorist groups, but there are techniques that they could likely use. In terrorist scenarios the probability of use is relatively high if terrorist groups are able to overcome technological hurdles and are capable of deploying the pathogen efficiently.

Possible Scenario for the Deployment of Tularemia: An expert committee from the World Health Organization (WHO) has estimated that an aerosol dispersal of 50 kg of a virulent strain of F. tularensis in a city of 5 million inhabitants would result in approximately 250,000 incapacitated casualties, a number which includes approximately 19,000 deaths. Infected individuals would be ill for several weeks and relapses would be likely in the ensuing weeks or months. The WHO committee assumed that vaccinated individuals would be only partially protected against an aerosol exposure; however, this may not be relevant for US cities, as the rate of vaccination is extremely low in contrast to the former Soviet Union. Based on this study, the Centers for Disease Control and Prevention (CDC) recently estimated the expected economic impact of an F. tularensis aerosol attack to be $5.4 billion for every 100,000 persons exposed. This includes treatment of disease and decontamination of metropolitan area.

Modifications of F. tularensis

Changing the fundamental nature of Francisella tularensis by incorporating plasmids which encode for antibiotic resistance was part of the weaponization work done by both the United States and the Soviet Union. Strains encoding chloramphenicol and tetracycline resistance in F. tularensis were developed, as well as streptomycin-resistant F. tularensis strains. Vaccine subverting preparations were also developed.

Other virulence factors F. tularensis are poorly understood, but it is possible that general virulence could be enhanced through additional laboratory manipulation. For example, the capsule of F. tularensis appears to be involved in its virulence.

Epidemiology and Epizootology

Epidemiological Pattern of a Tularemia Weapon

Because the aerosol infectivity will be quite high, and because person to person transmission is not documented for tularemia, the zone of contamination or exposure to an aerosol of tularemia will be roughly equivalent to the zone of infection. With any other mode of deployment other than aerosol (such as food contamination), the zone of infection will be

less than the zone of exposure, as more organisms are required for infection, and not all exposed persons will succumb.

Biovars of Francisella tularensis

Francisella tularensis has been divided into 2 major subspecies (biovars) by virulence testing, biochemical reactions, and epidemiological features. Francisella tularensis biovar tularensis (type A) is highly virulent in humans and animals and is the most common biovar isolated in North America. Under laboratory growth conditions, itproduces acid from glycerol, demonstrates citrulline ureidase activity. Francisella tularensis biovar palaearctica (type B) is relatively avirulent, and is the typical subspecies found in Europe and Asia. In the lab, it does not produce acid from glycerol, and does not demonstrate citrulline ureidase activity.

Natural Reservoirs

Tularemia occurs naturally in rabbits and hares (lagomorphs), as well as many rodents. It has been especially noted to infect microtine rodents such as voles, vole rats, muskrats, and beavers. In addition, a wide variety of other mammals, several species of birds, and even fish have been reported to be infected. F. tularensis can be recovered from contaminated water, soil, and vegetation, which may be associated with the presence of infected amoeba. Epizootics of F. tularensis with extensive die-offs of these animals may be the herald for outbreaks of tularemia in humans, if they are noticed, and naturally occurring human cases can mostly be associated with disease in local animal populations.

Natural Distribution

In 1939, 2,291 cases of tularemia were reported in the U.S. In recent years, up to 200 cases are reported annually in the U.S., occurring mainly in the south-west and on Martha's Vineyard, off the coast of Massachusetts. Tularemia is enzootic in all areas of the continental U.S. F. tularensis has been recovered from over 54 arthropod species, half of which are known to have transmitted the disease to humans. F. tularensis has been isolated from more than 100 types of wildlife and many domestic animals are susceptible to the infection.

Etiology and Pathogenesis

Francisella tularensis is a relatively small (0.2 to 0.7-1 µm), nonmotile, aerobic, gram-negative coccobacillus. Francisella tularensis is Gram-negative in its staining morphology. It is a hardy non–spore-forming organism and can survive for weeks at low temperatures in water, moist soil, hay, straw, and decaying animal carcasses, and even in frozen rabbit meat. It is a facultative intracellular parasite; undergoing phagocytosis but evading intracellular killing.

Francisella tularensis forms a capsule while in the host animal, and under culture conditions. The organism grows best when grown with nutrient media containing blood, as it requires iron and cysteine for optimal growth. According to current knowledge, the capsule is immunogenic but not toxigenic.

The virulence factors of Francisella tularensis are not well defined, nor does it appear to produce any toxins. Recently, pili have been reported on Francisella tularensis, which may contribute to the virulence of the organism. The capsule is considered as a virulence factor

as it protects the bacteria from the bactericidal components of serum and phagocytosis. Three classes of antigens have been found for F. tularensis: a polysaccharide antigen, cell wall and envelope antigens, and a protein antigen that causes a delayed-hypersensitivity reaction with the disease. Note: The LPS of F. tularensis does not exhibit the properties of a classical endotoxin (it fails to induce interleukin-1, etc.)

Cultural characteristics of F. tularensis include aerobic growth, and fastidious growth requirements. It cannot be cultured on ordinary bacterial media but requires the addition of egg yolk, fresh blood or cysteine. It is killed by moist heat at 55°C in 10 minutes, but may remain viable for many years in culture maintained at 10°C, and for many days in moist soil and in water polluted by infected animals.

Pathogenesis

F. tularensis is an intracellular parasite that can survive and multiply within host macrophages as well as in other cells. After cutaneous inoculation, macrophages and circulating mononuclear phagocytes ingest and harbor the organism. F. tularensis multiplies locally, producing a papule that ulcerates after several days. Organisms spread from the local lesion to the regional lymph nodes, where they cause enlarged, tender nodes that may suppurate. Approximately 2 weeks after infection, specific T lymphocytes are activated, and macrophages ingest and kill the organism. From the lymph nodes, the organisms spread via the lymphatic system to various organs and tissues, including lungs, liver, spleen, kidneys, and the CNS. Spreading from the ulcer to the draining nodes may be apparent as erythema. Abscess formation is typical for involved lymph nodes.

Since most infections are acquired by direct inoculation or from the bite of a contaminated arthropod, the main form of the disease is the ulceroglandular form.

Transmission

Humans and other mammals become infected with F. tularensis by a variety of modes. Most commonly, bites by infective arthropods such as ticks; handling infectious animal tissues or fluids; direct contact with or ingestion of contaminated water, food, or soil; and inhalation of infective aerosols are the causes of infection. Note: person-to-person transmission has not been documented

Transmission by Vectors

F. tularensis has been recovered from over 54 arthropod species, half of which are known to have transmitted the disease to humans. Ticks are an efficient reservoir as well as a vector for tularemia. In the United States, Dermacentor andersoni (the wood tick), Amblyomma americanum (the lone-star tick) and Dermacentor variabilis (the dog tick) as well as biting flies (e.g., deer flies, Chrysops discalis) and, less commonly, mosquitoes are all known vectors for F. tularensis. The house fly may also play a factor in disease transmission via mechanical transmission during which the flies transmit contaminated material on their legs (tarsal pads).

Clinical Manifestations

Commonly, liver, spleen and lymph nodes are enlarged. Miliary lesions are frequently observed in the liver and occasionally on spleen and lymph nodes. Francisella organisms can

usually be recovered from necropsy specimens following standard laboratory protocols. Laboratory personnel and persons performing necropsy should be very cautious when performing procedures which may generate aerosols, as aerosols of Francisella are highly infectious.

Specific recommendations for laboratory diagnostic procedures are available on the CDC website at: http://www.bt.cdc.gov/agent/tularemia/index.asp.
Clinical Manifestations of Tularemia infection from a biological attack

The most common forms of tularemia resulting from a biological weapon attack will be typhoidal and pneumonic. Tularemic meningitis, gastrointestinal disease, and bacterial endocarditis may result from infection. However, these same complications could result secondarily from typhoidal or pneumonic forms as well. The incubation period is 2-5 days. Other forms of tularemia as described below may also occur following an aerosol attack, and should not be dismissed as natural until further surveillance is performed.

Symptoms of the pneumonic form will include fever, headache, muscle pain, breathing difficulty, cough, and pleural pains. Symptoms of the typhoidal form will include fever without visible foci on skin or even without lymphadenopathy.

Clinical Manifestations of Natural Infections

Clinical manifestations of natural infections may include a pneumonic form, a typhoidal form, an ulceroglandular form, a glandular form, an oculoglandular form and a gastrointestinal form.

Pneumonic tularemia: Pneumonic tularemia follows deposition of bacteria-bearing particles into the alveolar spaces by an aerosol infection. Macrophages ingest the bacteria, which reside within the phago-lysome and replicate. Eventually macrophages will lyse. Intracellular bacteria are transported by macrophages to mediastinal lymph nodes. Once multiplication has begun, disease follows rapidly. Patients present with fever, headache, muscle pain, shortness of breath, cough, and pleural pains. Chest X-ray may reveal spotted infiltrates in lungs, lobular pneumonia, and pleural exudation.

Typhoidal tularemia: Typhoidal tularemia is tularemia without an obvious site of inoculation. It is also referred to as systemic or septicemic tularemia. Symptoms of typhoidal tularemia include fever without visible foci on skin or without lymphadenopathy. This form of disease could be very difficult to diagnose without collecting a thorough medical history.

Ulceroglandular tularemia: Ulceroglandular tularemia occurs following the deposition of Francisella bacteria into the skin through cuts or abrasions. After the bacteria innoculate skin tissues, infection results in local ulcer and associated lymph node enlargement. This is the most common form of tularemia.

Glandular tularemia: Glandular tularemia typically presents as lymphadenopathy in the absence of an ulcer, usually as a result of contact with contaminated animal tissue or insect bite.

Oculoglandular tularemia: Oculoglandular tularemia is direct contamination of the eye via contaminated hands or possibly by aerosol exposure. Conjunctivitis and swelling of associated lymph nodes may occur.

Gastrointestinal (GI) tularemia: Gastrointestinal tularemia occurs as a result of ingestion of Francisella bacteria into the upper or lower gastrointestinal tract. Depending on the focus of infection, this can result in either the oro-pharyngeal (upper GI tract) or ileocecal form (lower GI tract).

Clinical Manifestations in Domesticated Animals

Tularemia has been reported in sheep, dogs, cats, pigs, and horses. It is reported that cattle appear to be resistant. The incubation period is 1-10 days. In most domestic animals, as in humans, the disease is characterized by sudden onset of high fever, lethargy, anorexia, stiffness, reduced mobility, or other signs associated with septicemic disease. If left untreated, prostration and death may occur in a few hours or days. During periods of heavy tick infestation, either tularemia or tick paralysis should be suspected. Mortality may be up to 15% in outbreaks in untreated lambs and subclinical cases may be common.

Clinical Manifestation in Pets

Pets are most likely to acquire tularemia from eating infected carcasses and also from tick or deer fly bites. Cats with tularemia may have symptoms including high fever, a lack of appetite and being sluggish. Dogs with the disease may have a high fever, nasal and eye discharge and skin sores at the site of the insect bite.

Diagnosis and Treatment

In humans, differential diagnosis needs to be made to exclude influenza, Anthrax, Plague, and Q fever, as well as from acute pneumonia.

Diagnosis of acute tularemia infection should be confirmed by culture and identification of the bacterium, and may also involve direct or indirect fluorescent antibody test, or a positive antibody titer test. Direct examination of clinical samples using fluorescent labeled antibodies and growth of F. tularensis in culture are definitive confirmation of diagnosis. Standard protocols for laboratory based tests can be found on the CDC website, Rapid diagnostic tests are not widely available in clinical laboratories, although with recent preparedness activities, they are available in state and regional labs, and clinical laboratory staff have increased awareness of tularemia. Advanced techniques such as PCR, ELISA and other antigen detection assays are not commonly available in clinical labs, but are highly used in research and reference labs.

Specific recommendations for laboratory diagnostic procedures are available on the CDC website at: http://www.bt.cdc.gov/agent/tularemia/index.asp.

Tularemia Vaccine

A live vaccine strain was based on the avirulent strain 15 of F. tularensis. Interestingly, strain 15 was obtained by the United States in an exchange with the Soviet Union for Strain Schu SD4. The US used the Soviet strain to develop a vaccine, while the former Soviet Union used the American strain to develop weapons. This vaccine is not completely effective

in preventing disease but can lessen the severity of the disease, and was administered to large numbers of people in the former Soviet Union in the 1930s and 1940s. Vaccination is recommended for people at significantly increased risk of exposure to the organism; however, this vaccine is not currently available. The FDA has rescinded the IND status of this vaccine, and is reviewing its safety. Other vaccine candidates are in development. Inactivated or component vaccines do not elicit protective cellular immunity to date.

Treatment and Control

Recommended antibiotics for single cases include treptomycin, gentamicin, chloramphenicol, and tetracycline.. However, it should be noted that tetracycline and chloramphenicol have been associated with relapses. In mass casualty settings, ciprofloxacin or doxycycline are recommended, although they may be less efffective. Longer treatment regimens may be necessary since the organism is intracellular. Recovery from the disease confers long-lasting immunity and is generally protective against future infection.

Specific recommendations for adult and pediatric therapy for both contained and mass casualty settings are available on the CDC website at:
http://www.bt.cdc.gov/agent/tularemia/index.asp

Isolation/Decon Precautions: In the hospital, standard precautions are recommended, as person-to-person transmission has not been documented.

Risk of infection during necropsy or to laboratory personnel, especially if there is a possibility of aerosolizing the bacteria, is significant and special procedures and facilities are necessary. Further information regarding these special precautions is available on the CDC website at http://www.bt.cdc.gov/agent/tularemia/index.asp

Environmental control is difficult and is limited to reducing tick infestation and to rapid diagnosis and treatment of human cases.

Sources of further information

Useful Websites:

CDC Emergency Preparedness & Response: Tularemia
http://www.bt.cdc.gov/agent/tularemia/

Consensus Statement: Tularemia as a Biological weapon - Medical and Public Health Management http://jama.ama-assn.org/cgi/content/full/285/21/2763?#SEC6

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15. University of Pittsburg Medical Center, Center for Biosecurity. Tularemia Fact Sheet.
http://www.upmc-biosecurity.org/pages/agents/tularemia.html