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UNIVERSITY OF MINNESOTA

College of Veterinary Medicine

VETERINARY CONTINUING EDUCATION

ST. PAUL, MINNESOTA
UNITED STATES OF MINNESOTA

Tuberculosis

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Introduction

Tuberculosis is an ancient, worldwide, chronic bacterial disease of humans and animals that continues to be a major disease problem in developing countries. Annual worldwide tuberculosis-related deaths are estimated at 2 million persons, despite education, vaccination and treatment.

Over 2 billion people, or approximately 1/3 of the current world population, are infected with one of the bacterial species that causes tuberculosis. A vast majority of the human tuberculosis cases are caused by *Mycobacterium tuberculosis* and a small percentage are caused by *Mycobacterium bovis*. There are human endemic cases of tuberculosis in central Africa caused by *Mycobacterium africanum*. Africa, India, Southeast Asia and portions of Central and South America have the highest cases rates.

In the United States, a majority of the tuberculosis infections are in foreign-born persons. According to 2004 WHO data, the case rate for foreign-born persons is 22.8 per 100,000 as compared to 2.6 per 100,000 US-born persons. Mexico, the Philippines, India, China and Viet Nam are the common countries of origin of those infected in the United States. Large cities throughout California, Texas and New York represent 42% of the US cases (1).

Epidemiology

Most tuberculosis is spread from human to human, and in some cases, human to animal by aerosolized respiratory secretions. Although many humans and animals are infected with *Mycobacterium sp.*, transmission from an infected individual to an uninfected individual is dependent on if the infected individual is actively shedding the bacterium in pharyngeal secretions.

Approximately 90% of tuberculosis infected individuals are in good health, immunocompetent and *not* shedding the bacterium in respiratory secretions. Those individuals that are infected and not shedding are classified as latent tuberculosis infections (LTBI). *M. tuberculosis* can infect other vertebrates and has been documented to infect cats, dogs and elephants. Many of the non-human cases of *M. tuberculosis* infection have been traced back to infected, actively shedding humans.

Pathogenesis

The lungs are the target organ of infection in both latent and actively infected individuals. If the host is immunocompetent, the infection will be localized to the lungs and the regional draining lymph nodes of the thoracic cavity (tracheobronchial, mediastinal, and sternal). Infection of the lungs and draining lymph nodes is known as the primary complex and these infections rarely completely resolve. The host's immune response to a tuberculosis infection is initiated by the recruitment of macrophages. As with many higher bacterial infections, the macrophages are unable to clear the bacterial infection and the host responds by recruiting more macrophages. The constant 'tug-of-war' between host and pathogen results in the accumulation of viable and dead (necrotic) macrophages, mineralized debris and ultimately results in the loss of normal tissue architecture resulting in liquefaction and caseation necrosis (2).

In the chronic phases of infection, the immunocompetent host will attempt to 'wall-off' the infection with bands of fibrous connective tissue. A well defined zone of infection encased in a shell

of fibrous connective tissue is known as a tubercle and is a common feature of chronic latent tuberculosis infections. If the bacterium is ingested, Mycobacterium can infect the gastrointestinal tract and the regional draining lymph nodes.

In hosts with a compromised immune system, including the elderly, HIV-infected persons (3), and those with concurrent infections or disease, the potential of a latent tuberculosis infection to manifest into an active infection is increased. An increase in tuberculosis cases in the 1980s and 1990s coincided with the emergence of HIV.

Zoonosis and Bovine Tuberculosis

Mycobacterium bovis, commonly referred to as bovine tuberculosis, is known to infect cattle worldwide. *Mycobacterium bovis* infection in the bovine has a similar pathogenesis to human *Mycobacterium tuberculosis* infections and commonly manifest in pulmonary and occasionally enteric infections. Susceptible cattle are often infected by respiratory secretions of other infected cattle.

Humans are also susceptible to *M. bovis;* however, respiratory exposure from infected cattle is an uncommon route of infection (4). Non-pasteurized infected milk is the most common route of exposure of *M. bovis* infection in humans. Children from countries with endemic bovine TB infections are common victims of bovine tuberculosis (5). Consumption of meat from infected cattle can also result in *M. bovis* infections in humans. Veterinarians, meat packing plant (abattoir) employees and farmers are at an increased risk of *M. bovis* infection. In the United States, routine slaughter exams and the widespread pasteurization of raw milk has all but eliminated the potential for *M. bovis* to infect humans (6).

Additional *M. bovis* animal reservoirs include badgers in Europe, brushtail possums in New Zealand, and most recently, white-tail deer in Michigan. The discovery of *M. bovis* infections in Michigan white-tail deer has challenged the belief that the bacterium can not maintain itself in wild population of ruminants. High deer densities and the practice of feeding and "baiting" deer are thought to maintain the infection in the dense deer populations of eastern Michigan. Cross-contaminated cattle feed is believed to be the initial source of infection (7).

Necropsy exam of cattle

Cattle infected with *Mycobacterium bovis* rarely exhibit respiratory disease and additional clinical signs associated with infection. The 28 beef cows and 2 bulls necropsied at the Minnesota Veterinary Diagnostic Laboratory during the summer and fall of 2005 had adequate body condition and were clinically healthy. A few of the cows had a decreased amount of body condition that coincided with a significant infestation of liver flukes.

Eight of the 30 cattle examined had gross lesions consistent with *Mycobacterium sp.* infection in lymph nodes in the head and thorax. The lymph nodes in the head (mandibular, lateral and medial retropharyngeal, parotid) and thorax (cranial, middle, and caudal mediastinal, right and left tracheobronchial and sternal) were most commonly affected. The lungs and alimentary tracts on all the animals were unremarkable.

Normal lymph nodes are round to oval with a grey cortex supported by variable amounts of adipose and medullary connective tissue. Most of the affected lymph nodes were not significantly enlarged, ranging in size from 2 to 5 cm. *Mycobacterium sp.* infected lymph nodes had variable-sized yellow caseonecrotic granulomas scattered throughout the lymph node cortex and many were surrounded by a partially organized fibrous capsule. A few of the affected nodes contained well-organized granulomas consisting of a mineralized core surrounded by firm, yellow caseous material, necrotic debris and variable amounts of hemorrhage encased in a fibrous shell. Mineralization is a significant feature of chronic tuberculosis granulomas. Any lung, liver and lymph nodes that contain mineralized foci should be submitted for culture and histopathological exam.

As described previously, granulomas have unique features and are relatively specific for tuberculosis infection; however, similar lesions of the head lymph nodes can be seen with *Actinobacilus lignieresii* (wooden tongue) and Actinomyces bovis (lumpy jaw) infections. Foreign bodies (wire, grass awns) of the jaw and upper airway can result in chronic abscesses that have similar features of a granuloma. Pulmonary abscesses associated with *Mycoplasma sp.* infections can have similar appearances to tuberculosis granulomas; however, *Mycoplasma sp.* abscesses rarely organize or mineralize and they are not commonly observed in draining lymph nodes of the thorax or the pleura. Generalized lymphadenopathy secondary to lymphsarcoma will produce striking lesions. Lymphosarcoma affected lymphnodes have a homogeneous texture and color (white to yellow) with soft, necrotic cores in the large, more severely affected lymph nodes. Lymphosarcoma can have similar appearances to tubercles and suspect lesions should be submitted for histopathological exam and culture.

Overall, necropsy exam of the cattle from positive cattle herds was underwhelming. *Mycobacterium bovis* was isolate from a few small lymph nodes of the head and chest and mineralized foci appeared to correlate well with positive culture results.

Diagnostics

The Mantoux skin test is the staple of tuberculosis diagnosis in humans (8). This test detects prior exposure to *Mycobacterium sp.* by injecting purified protein extract in the dermis and examining the wheal 48-72 hours later for swelling indicative of a cell mediated (memory) immune response. Although the Mantoux test has been widely used, there are a few limitations. Many individuals throughout the world have been vaccinated against tuberculosis in an attempt to control the spread in endemic countries. The name of the vaccine is bacilli Calmette-Guerin (BCG) and it is an avirulent strain of *Mycobacterium bovis.* The vaccine contains living BCG organisms; however, it does not completely prevent infections and, more importantly, it interferes with diagnostic skin (Mantoux) test. The caudal tail-fold and comparative cervical test in cattle is analogous to the Mantoux test.

Gamma interferon tests are the newest tests to detect both active and latent tuberculosis infections in human and animals (9). This test requires a blood sample from which lymphocytes are isolated. If the circulating lymphocytes have been previously exposed to *M. tuberculosis,* they will release gamma interferon when stimulated with *M. tuberculosis* purified protein derivative in vitro. The gamma interferon released by the sensitized lymphocytes can be easily detected by a bioassay. The gamma interferon test has several advantages:1) it can be used in BCG vaccinated persons, 2) it requires only one visit to the testing station, and 3) it is more rapid than the Mantoux test.

Radiographs of the chest are commonly performed on persons who test positive with the Mantoux test. Radio-dense circular areas near the hilus and mainstem bronchi are lesions consistent with tuberculosis. If tuberculosis is suspected, sputum cultures are initiated. Sputum cultures and acid fast staining of sputum smears have been used for decades in the United States, and although the culture techniques have been improved, there are many limitations. Microscopic exams and acid fast stains are rapid but they lack sensitivity and are only useful as a presumptive test. Bacterial cultures are labor-intensive and slow but continue to be the gold standard test due to its high degrees of sensitivity and specificity.

Many molecular diagnostic (PCR) tests have been developed to expedite tuberculosis diagnosis in sputum and tissue samples. Because many laboratories have developed their own primers, probes and PCR techniques, there are wide variations in assay performance. Nevertheless, PCR techniques will continue to improve and assist in rapid tuberculosis diagnosis (10).

RFLP genotyping of the *Mycobacterium bovis* isolates from the infected Minnesota herds were similar to strains isolated in Mexico and Texas and *not* similar to strains isolated in deer or cattle in Michigan and Manitoba.

Treatment

Treatment for tuberculosis has been relatively successful when available. Two drugs have dominated treatment: 1) Isonicotinic acid hydrazide (isoniazid) and 2) rifampin. Effective treatment in both humans and animals requires extended treatment over many months. In those individual with latent tuberculosis infection, 90% of treated individuals never develop active infection. Treatment is not recommended for individuals with active tuberculosis infections as drug resistance is thought to occur. Animals, including elephants (11), have been successfully treated; however, animal treatment in the United States has been banned.

Summary

Tuberculosis will continue to be one of the top 10 infectious diseases in the world over the next 20 years. Human infection is dominated by exposure of naïve person to actively-infected individuals. Other vertebrates can be infected with the human tuberculosis and potentially spread the bacterium but this has been demonstrated to be an uncommon route of infection. Bovine tuberculosis will continue to infect cattle worldwide and consumption of non-pasteurized milk is the greatest risk of exposure and infection to humans.

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