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BIOLOGICAL AGENTS
Brucellosis[1]

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AETIOLOGY

BRU CELLOSIS IS CAUSED BY organisms of the genus Brucella; particularly B. melitensis, B. suis and B. abortus. These organisms are small Gram-negative, aerobic, coccobacilli and are facultative intracellular parasites which grow within monocytes and macrophages[1].

EPIDEMIOLOGY

Brucellosis is primarily a disease of domestic animals, but humans may be infected through ingestion of animal products (fresh milk and milk products), direct contact with infected animals, or inhalation of aerosols of the microorganism'.

Although the human disease is now uncommon in most developed countries, it remains hyperendemic in the Middle East (Iran, Iraq, Saudi Arabia, Kuwait), in Northern Africa (Algeria, Tunisia, Malta, Egypt, Morocco), some European countries (France, Spain, Greece), South America (Mexico, Peru), and Asia (China, parts of the former USSR)'.

Brucellae are killed by pasteurisation and simple disinfectants. The organisms are stable at low temperatures and may remain viable for weeks in infected tissue, water, unpasteurised dairy products, and soil[2,3].

Man-to-man transmission has not been documented[3].

PATHOLOGY

Organisms which have entered the body are engulfed by phagocytes, proliferate within them, and are carried to the regional lymph nodes. Infected lymphocytes may die, releasing bacteria and stimulating the activation of local mononuclear cells. If this immune response is insufficient to control the infection, infected cells may disseminate via the bloodstream. They then localise in reticuloendothelial tissue (especially the spleen, liver, bone marrow and lymph nodes) and also in the kidney. The infection induces a granulomatous reaction; caseation and necrosis occasionally result.

Bacteria may remain dormant in tissue and bone marrow, which makes relapse possible, and also reduces the efficiency of antibiotic therapy.

ANTIGENICITY

No exotoxins or antiphagocytic antigens have been detected. Two major surface antigens, A and M occur in differing amounts in different species. An outer membrane protein, the L antigen, has also been detected.

A 'smooth' (S) form of Brucella appears to be associated with virulence and is more resistant to phagocytic killing than 'rough' strains. B. abortus also demonstrates inhibition of neutrophil

degranulation which consequently suppresses the anti-bacterial activity of polymorphonuclear leukocytes. The factors which cause this inhibition are of low molecular weight (less than 1000 Da).

A possible virulence factor which is only produced in vivo and enhances intracellular survival has been detected but not characterised[2].

CLINICAL MANIFESTATIONS

The incubation period in humans is one to six weeks, with an average of two weeks3 1 The disease may last for several days to months or even years[5]. Mortality is low (usually less than 2 per cent in untreated cases, although this figure is higher for B. melitensis infections[5].

Onset may be insidious or abrupt[5]. Common features include loss of appetite and weight loss, back-ache and headache, malaise, weakness, irregular fever (especially with B. melitensis infections [2], profuse sweating, arthralgia, chills, indistinct gastrointestinal and nervous symptoms, and depression[2.1.5]. Enlarged lymph nodes, spleen and liver, and localised spondylitis may also be present[2]. Cough occurs in about one-quarter of patients, but chest x-rays are usually normal.

Complications are not uncommon and may include vertebral osteomyelitis, osteoarticular involvement, meningoencephalitis, epi-didymo orchitis, endocarditis, interstitial nephritis, and (rarely) prepatellar bursitis [2.5]. Genitourinary problems occur in about 2-20 per cent of cases.

Relapses are common, especially upon re-exposure to the pathogen.

A BW attack would probably be by aerosol and would produce primarily pulmonary symptoms. This form of the disease seldom occurs naturally.

Pulmonary Brucellosis

Symptoms associated with pulmonary brucellosis are non-specific. Most cases of pulmonary brucellosis involve fever, cough, mucopurulent sputum, and abnormal signs in the chest[6]. Chest X-rays may reveal pneumonic patches or consolidation, pleural effusion, granuloma of the lung and interstitial pneumonitis. Physical examination may reveal rats, wheezing, and diminished entry of air into the lungs.

DIAGNOSIS

Laboratory Diagnosis

Isolation of the pathogen from blood, bone marrow, or other tissue provides positive identification. However, Brucella is relatively slow-growing and fastidious in the laboratory' and this form of identification may be slow. Serology is more useful and maybe done using ELISA on IgG or IgM. Fluorescent antibody analysis is useful for genus identification.

Agglutination tests are frequently performed on sera: titres of above 1:80 are usually indicative of infection (antibodies to F tularensis, Yersinia enterocolitica, and Vibrio cholera may cross-react with the agglutinating antigen and give a false-positive reaction).

More rapid diagnostic systems are being developed.

Differential diagnosis

Brucellosis could be mistaken for tuberculosis, typhoid fever, visceral leishmaniasis, malaria, EBV or cytomegalovirus mononucleosis, infective endocarditis or Q fever[6].

Treatment
Several different antibiotic regimens have been used to successfully treat human brucellosis. Combinations of antibiotics appear to reduce the possibility of relapse more than treatment with one antibiotic alone[1].

The current WHO-recommended therapy is a combination of rifampicin and doxycycline[13], although they also recommend treatment with streptomycin and tetracycline. However, both of these regimens are associated with relatively high rates of relapse [11].

RECOMMENDED THERAPY
100 mg of oral doxycycline every 12 hours for 6 weeks, plus 1g of streptomycin IMI for first 2 weeks of treatment [11].

- Relapse occurred in only 4 per cent of cases
- 96 per cent of patients became afebrile and asymptomatic within the first week of treatment
- Adverse side-effects were minimal
  Or
- 100 mg of doxycycline bd plus 300 mg of rifampicin bd for 42 days.
- May have a higher relapse rate than the above regimen [11]

Other combinations of drugs may also prove to be effective.

Several antibiotics have been shown to be associated with unacceptably high rates of relapse when used alone, despite good in vitro results. These include ceftriaxone, ciprofloxacin, rifampicin, erythromycin, chloramphenicol, and ampicillin [10.15].

SUSCEPTIBILITY OF POPULATION
The susceptibility of the general population is high, although most individuals have some degree of resistance or acquired partial immunity[3] Susceptibility to B. melitensis and B. suis appears to be 50-80 per cent' - Traditionally, more males than females have been infected, although this is most likely due to occupational factors. Secondary spread is not considered significant.

PREVENTION
Although vaccination of livestock is possible, no effective vaccine is available for human use (some individuals in China and the former USSR have been vaccinated with animal vaccines).

Both live attenuated and non-living vaccines have been investigated.

Live vaccines
The most promising live vaccines are those derived from B. abortus, because of its lower pathogenicity in humans and cross-reactivity with other Brucella species[17].

Trials in China have been performed using strain 104M (isolated in the former USSR - is claimed to be antigenically and immunogenically stable). The vaccine contains 7-10 x 109 cells and is

administered by scarification. Although side effects are minor (headache, weakness, erythema at site of inoculation), severe effects occur if the vaccine is delivered subcutaneously [17,18].

Another B. abortus strain, 19BA, has been used in the former USSR. Approximately 2-3 x 106 cells are injected subcutaneously or via scarification and are reported to provide protection for about one year [17,18]. However, severe reactions often occur, particularly after revaccination [19].

A vaccine-derived from B. melitensis has been tested on humans, but the margin between an innocuous dose and one producing a febrile illness was considered too small for safe use [17 20].

Non-living vaccines

An acetic acid-hydrolysed cell wall preparation of S-type Brucella consisting of a protein-polysaccharide derivative has been used in the former USSR [17,10]. The immunogenicity of this vaccine appears to be enhanced if the patient has previously been exposed to live vaccine[11]. A recommended dose of 1 mg is recommended, with boosters every 11-12 months[22]. Although the vaccine is claimed to be safe, there appears to be no literature concerning Western trials.

A French vaccine, consisting of phenol extraction of delipidated B. melitensis and B. abortus, has also been tested. This vaccine is non-toxic, and two does of 1 mg should be given 15 days apart. However, the efficacy and duration of immunity still needs to be established[1]. Limited trials suggest that a good immunity is acquired, and that a booster should be given every year.

Passive immune approaches do not appear to be successful in humans, although experiments using animal models are currently being performed.

POTENTIAL AS BIOLOGICAL WARFARE AGENT

Advantages as a Biological Warfare Agent

A BW attack would most likely be in aerosol form which would result in pulmonary brucellosis. This form of the disease is very rare in nature and may be more severe than the usual forms of brucellosis. The symptoms are non-specific, which would make diagnosis solely by clinical observation difficult.

Although the mortality is generally low, an attack with Brucella may result in a high degree of morbidity, and a severe drain on manpower and medical resources, particularly if relapses and complications occur. The long (and variable) incubation period may also lead to a drawn-out appearance of the disease.

Antibiotic therapy should be aggressive and prompt, and the right drugs used to minimise the chances of relapse.

Vaccines are currently not available for human use (although they are probably being used in the former Soviet Union).

The organism is relatively stable in the environment and may persist for some time.

Disadvantages as a BW

Although stable in the environment, the pathogen can be killed by simple sterilisation. Man-to-man transmission is not likely.

FUTURE DIRECTIONS

More work should be done on human vaccines, and the French vaccine should undergo further testing. Other killed vaccines should also be tested (it would appear that live vaccines produce too many severe side effects to be considered suitable for human use).

Because cell-mediated immunity is the most important in brucellosis (the pathogen is intracellular, so humoral immunity only has a limited effect), better adjuvants need to be developed to enhance the effect of killed vaccines.

Antibiotic therapy should be continued to be studied, as well as better means of delivery. Liposome-encapsulated antibiotics may show some promise.

Passive immune therapies, using monoclonal antibodies are also undergoing testing.

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