



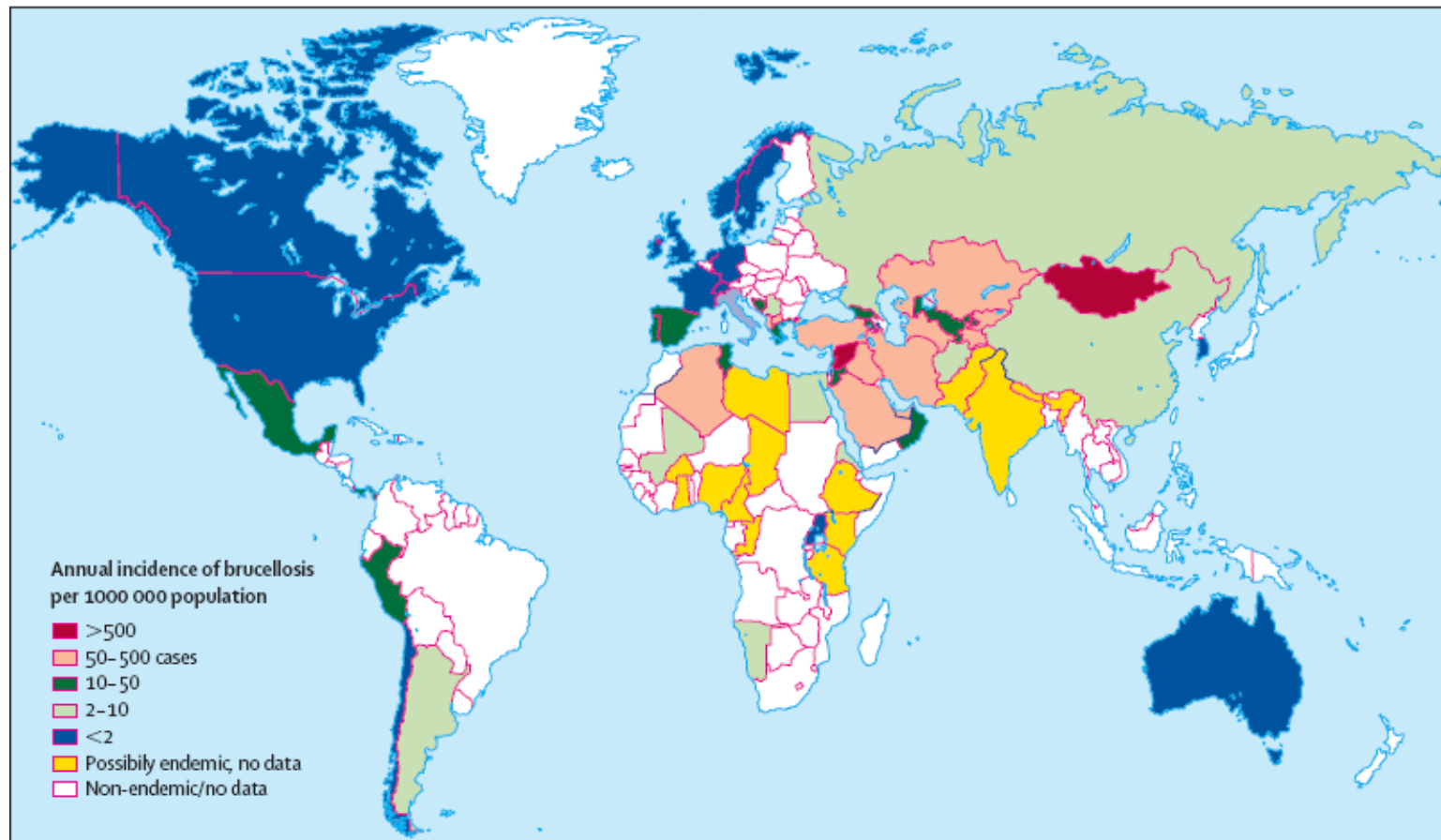
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Brucellosis

Epidemiology of brucellosis

Generality

- is the most common bacterial zoonosis: > 500.000 new cases annually and prevalence rates in some countries exceeding ten cases per 100 000 population (*Pappas G. et al., Lancet ID 2006*)
- remains also underdiagnosed and under-reported (*Godfroid G et al., Vet Res 2005*)
- is an important cause of veterinarian morbidity and mortality, with a potential consequent high economic burden



From Pappas G. et al.,
Lancet ID 2006;6:91-99



Species	Biotype	Animal Hosts	First described	Human virulence
<i>B. Melitensis</i>	1-3	Goats, sheep, camels	Bruce, 1887	++++
<i>B. Abortus</i>	1-6, 9	Cows, buffalo, yaks, camels	Bang, 1897	++ to +++
<i>B. Suis</i>	1-5	Pigs (1-3), wild hares (2), caribou (4), reindeer (4), wild rodents (5)	Traum, 1914	+
<i>B. Canis</i>	-	Canines	Carmichael and Bruner, 1968	+
<i>B. Ovis</i>	-	Sheep	Van Drimmelen ,1953	-
<i>B. Neotomae</i>	-	Rodents	Stoenner and Lackman, 1957	-
<i>B. Cetaceae</i>	-	Pinnipediae and cetaceae	Ewalt and Ross, 1994	.

The complete sequencing of the *B. melitensis* and *B. suis* genome was achieved in 2002, *B. abortus* in 2001.

Brucella is a cocco-bacillus Gram negative, asporigen, immobile, aerobic, not fermenting, catalase positive, oxidase variable

Pathogenesis

Host immune response

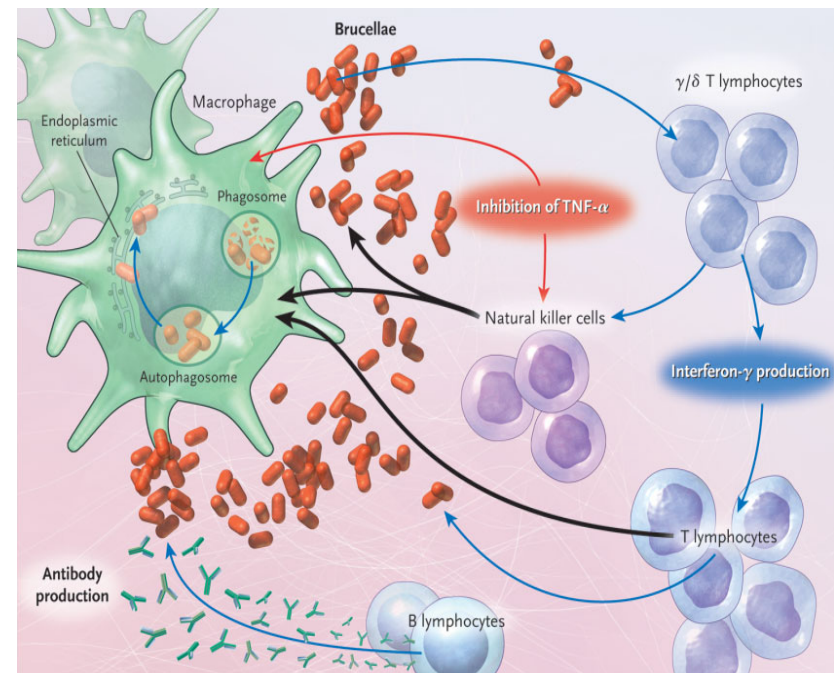
- Antibody response in brucellosis, although extremely useful diagnostically, plays a limited part in the overall host response
- Cellular immunity play a protective role against brucellosis, principally by the IFN- γ actions:
 - by activating macrophages, producing reactive oxygen species and nitrogen intermediates;
 - by inducing apoptosis, enhancing cell differentiation and cytokine production;
 - by converting immunoglobulin G to immunoglobulin G2a;
 - and by increasing the expression of antigen-presenting molecules.
- CD4 lymphocytes play a limited role, principally by facilitating cloning expansion of cytotoxic cells (i.e. CD8)

Escape of host's immune response

After entry, the majority of brucellae are rapidly eliminated by phagolysosome fusion. Of those bacteria, 15-30% survive in autophagosome by impairing generation of reactive oxygen intermediates in macrophages.

The infected cells, through the activity of smooth-LPS, show an altered capacity to present foreign antigens to MHC-II and an inhibition of their apoptosis. This is causing an increased intracellular survive of brucella with a reduced clearance of infected cells. Probably the brucella play also a role of TNF- α down-regulation that is associated with a impaired NK cytotoxic activity

The bacteria replication takes place in the endoplasmic reticulum without affecting host-cell integrity. After replication, brucellae are released with the help of hemolysins and induced cell necrosis.



Route of transmission to human

Brucellosis is an occupational disease in shepherds, abattoir workers, veterinarians, dairy-industry professionals, and personnel in microbiologic laboratories.

Ingestion of infected, unpasteurized animal-milk products, especially raw milk, soft cheese, butter, and ice cream.

N.B.: Hard cheese, yogurt, and sour milk are less hazardous, since both propionic and lactic fermentation takes place. Bacterial load in animal muscle tissues is low, but consumption of undercooked traditional delicacies such as liver and spleen has been implicated in human infection

Contact with infected animal parts (such as the placenta by inoculation through ruptures of skin and mucous membranes)

Inhalation of infected aerosolized particles

Limited data support vertical transmission of human brucellosis (*Giannacopoulos I, J Infect 2002; Palanduz A., Int J Inf Dis 2000*) and transmission via breastmilk (*Barroso Espadero D, An Esp Paed 1998; Celebi G., Scand J Inf Dis 2007*)

Reports of transmission of human brucellosis via blood transfusion have also been published (*Al-Kharfy TM, Ann Trop Paediatric 2001; Doganay M, J Hosp Inf 2001*)

After entering the human body and being taken up by local tissue lymphocytes, brucellae are transferred through regional lymph nodes into the circulation and are subsequently seeded throughout the body, with tropism for the reticuloendothelial system. The period of inoculation usually ranges from two to four weeks

Clinical aspects

Detailed patient interviews are crucial for the diagnosis of human brucellosis, especially in urban and non-endemic areas, and in cases of imported brucella, in which travelers acquire the disease abroad and become ill in non-endemic settings.

The clinical features of brucellosis depend on the stage of the disease, and the organs and systems involved. Relapses occur usually in the 10% of the cases, in general in the first year after infection. Clinical differences between species are difficult to determine.

- Fever
- Arthralgia
- Constitutional symptoms (anorexia, malaise, weight loss, asthenia)
- Malodorous and abundant perspiration (almost pathognomonic)

Physical examination is generally nonspecific, though lymphadenopathy, hepatomegaly, or splenomegaly is often present.

Form acute

- Incubation 2-3 week (1 w –several months)
- fever: starting low, become high and undulant

Form chronic

- sometimes chronic from the beginning

Clinical aspects: complications

Practically every organ and system of the human body can be affected in brucellosis (acute or chronic form); the most frequently affected are the following:

Osteoarticular disease is universally the most common (50%) complication of brucellosis

- Peripheral arthritis: is the most common and is non-erosive, since it usually involves the knees, hips, ankles, and wrists in the context of acute infection
- Sacroiliitis: readily diagnosed, also usually in the context of acute brucellosis
- Spondylitis: can be easily diagnosed with plain radiography, in which is present the characteristic Pons sign (a steplike erosion of the anterosuperior vertebral margin - *pseudo-pott*). The lumbar spine is the usual site of involvement.

Reproductive system is the second most common (10%) site of focal brucellosis: in man the most frequent manifestation is an epididymo-orchitis; in pregnancy poses a substantial risk of spontaneous abortion

Hepatitis is common, usually manifesting as mild transaminasemia. Liver abscess and jaundice are rare.

Central Nervous System involvement: present in 5-7% of the cases (Meningitis, encephalitis, meningoenzephalitis, meningovascular disease, brain abscesses, and demyelinating syndromes)

Endocarditis remains the principal cause of mortality in the course of brucellosis. It usually involves the aortic valve and typically requires immediate surgical valve replacement

Laboratory

Blood count:

The blood count is often characterized by mild leukopenia and relative lymphocytosis, along with mild anemia and thrombocytopenia. Pancytopenia in brucellosis is multifactorial and is attributed to hypersplenism and bone marrow involvement

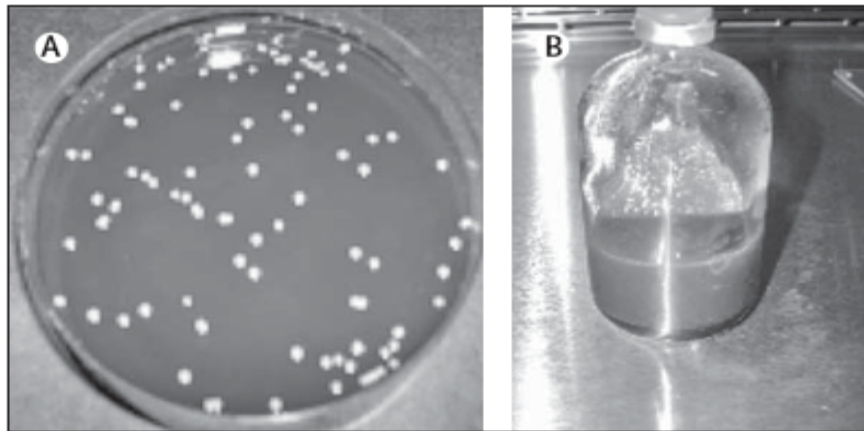
Biochemistry:

The eventual modification of some parameter is function of the clinical complication and the involved organ

Microbiological culture

The absolute diagnosis of brucellosis requires isolation of the bacterium from blood, tissue samples or infected fluids. The sensitivity of blood culture varies, depending on individual laboratory practices and how actively the obtaining of cultures is pursued.

	Incubation time	Requires blind sub-cultures	Sensitivity (disease stage)
Ruiz-Castañeda	7-21 days	Yes	70-80% (acute); <50% (chronic)
Lysis centrifugation	2-4 days	No	>90% (acute); 70% (chronic)
Bone marrow culture	4-7 days	Depends on method and media used	97% (acute); 90% (subacute); 50% (chronic)



For blood culture, the brucellae are cultured in standard biphasic (solid and liquid) mode or with the Castaneda bottle (which incorporates both solid and liquid mediums in the same container). Blood-culture sensitivity may be improved by a lysis-centrifugation technique .

Bone marrow cultures are considered the gold standard for the diagnosis of brucellosis, since the relatively high concentration of brucella in the reticuloendothelial system makes it easier to detect the organism. Bone marrow cultures may provide a higher sensitivity, yield faster culture times, and may be superior to blood cultures when evaluating patients with previous antibiotic use

Since brucellosis constitutes one of the most common laboratory-acquired infections, special care should be taken when using the lysis centrifugation method to avoid infection from contaminated aerosols.

Agglutination tests

Rose Bengal test

is used as a screening test, and positive results are confirmed by the SAT. The sensitivity of this test is very high and the specificity is fairly high

Is based on the reactivity of antibodies against smooth-LPS. These antibodies tend to persist in patients long after recovery; therefore, in endemic areas, high background values could occur that may affect the diagnostic value of the test.

it is possible cross-reaction with other Gram-negative bacteria such as *Yersinia enterocolitica* O:9, *Vibrio cholerae*, *Escherichia coli* O:157, and *Francisella tularensis*.

SAT (serum agglutination test)

Titers above 1:160 are considered diagnostic in conjunction with a compatible clinical presentation. However, in areas of endemic disease, using a titer of 1:320 as diagnostic may be more specific.

A major drawback is that is not suitable for patient follow-up, since titers can remain high for a prolonged period

It is not able to diagnose *B. canis* infections and it is possible a cross-reactions of class M immunoglobulins with *Francisella tularensis*, *Escherichia coli* (O116, O157), *Salmonella urbana*, *Yersinia enterocolitica* O:9, *Vibrio cholerae*, *Xanthomonas maltophilia*, and *Afipia clevelandensis*

Coombs' test may be more suitable for confirmation of brucellosis in relapsing patients or patients with persisting disease,

ELISA

The sensitivity may be high, especially when the detection of specific IgM antibodies is complemented with the detection of specific IgG antibodies. The specificity, however, seems to be less than that of the agglutination tests.

Since ELISA for brucella is based on the detection of antibodies against smooth-LPS, the cut-off value may need adjustment to optimise specificity when used in endemic areas, and this may influence sensitivity

PCR

Adequate comparisons of the different PCR systems and primers are still lacking, and results may presumably depend on the nature of the clinical specimen, the sample preparation procedure, and the duration and stage of illness.

could be particularly useful in patients with specific complications such as neurobrucellosis, or other localised infections, since serological testing often fails in such patients

may be used as an alternative to culture in the confirmation of brucellosis, to monitor treatment efficacy, and to diagnose relapsing patients.

appears to be useful in species differentiation and biotyping of isolates

Treatment

Treatment of human brucellosis should involve antibiotics that can penetrate macrophages and can act in the acidic intracellular environment.

There is a general need for combined treatment, since all monotherapies are characterized by unacceptably high relapse rates.

Since brucella does not contain any plasmids and human beings are end hosts, these factors may contribute to the absence of any pronounced degree of antibiotic resistance.

There is no evidence to suggest that drug resistance has an important part in treatment failure and relapse

Antibiotic Treatment	1° choice	alternative	2° choice
	Doxycyclin ± Rifampicin 6 weeks + gentamicin for 2 weeks	Doxycyclin for 6 weeks + gentamicin for 2 weeks	Doxy ciclin + Rifampicin for 6 weeks or Tetracilin for 6 weeks + gentamicin for 2 weeks

In case of children less than 8 year-old or pregnant women, also if data available are limited, the therapeutic approach more usefull seems to be TMP-SMX for 6 months

A human vaccine has not been developed for brucellosis