Brucella lecture

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http://scholar.cu.edu.eg/?q=hanem/node/add/class
https://cairo.academia.edu/MohamedRefai/Teaching-Documents
Brucellosis has been recognized in the Mediterranean and the Red Sea regions since at least the time of Hippocrates (460-357 BC).
Fresh milk delivery in Malta
History of Brucellosis

▪ Jeffery Allen Marston
  ▪ British Army surgeon
  ▪ Contracted Malta fever
  ▪ Described his own case in great detail

ABOVE: Jeffery Allen Marston (1831-1911) contracted Malta fever and described his own case in great detail. 
Private collection
History of Brucellosis

- In the late 1887,
- Bruce isolated an organism he named *Micrococcus melitensis*
  from individuals with Mediterranean fever.

Sir David Bruce (1855-1931)
British Army physician and microbiologist
History of Brucellosis

- In 1895,
- Bang in Copenhagen isolated what he called Bacillus abortus from cattle that were undergoing spontaneous abortions.

Bernhard Bang (1848-1932)
History of Brucellosis

In 1918, discovered similar morphology and pathology between:

Bang’s *Bacterium abortus*
Bruce’s *Micrococcus melitensis*

Alice Evans (1881-1975)
American bacteriologist
1920 Meyer and Shaw suggested the genus name *Bruceella*

*Pasteurella*

*Salmonella*

*Escherichia*

*Klebsiella*
The Genus Brucella

- Brucella melitensis (Bruce, 1887)
- Brucella abortus (Bang, 1895)
- Brucella suis (Traum, 1914)
- Brucella ovis (Buddle & Boyes 1953)
- Brucella neotomae (Stoenner & Lackman, 1957)
- Brucella canis (Carmichael and Bruner, 1968)
- Brucella marins
- Brucella dolphine
**Brucella melitensis**

- **Distribution**
  - Mediterranean, Middle East, Central Asia, Central America

- **Incidence**
  - Mediterranean, Middle East
    - 78 cases/100,000 people/yr
  - Arabic Peninsula
    - 20% seroprevalence; 2% active cases
  - 100 to 200 U.S. cases annually
  - Unpasteurized cheeses
Brucella abortus

- Distribution
  - Worldwide
  - Eradicated in some countries
- Notifiable disease in many countries
  - World Organization for Animal Health (OIE)
    - Poor surveillance and reporting due to lack of recognition
  - Fever of unknown origin (FUO)
Brucella suis

- Five biovars
  - 1 and 3: Worldwide in swine
  - 1: Cattle in Brazil and Columbia
  - 2: Wild hares, boars in Europe
  - 4: Arctic region (N. America, Russia)
  - 5: Former USSR
- Eradicated from domestic pigs
  - U.S., Canada, much of Europe
- Persistent problem in feral swine
  - U.S., Europe, parts of Australia
Brucella ovis

- Distribution:
- most sheep-raising regions of the world
  - Australia
  - New Zealand
  - North America
  - South America
  - South Africa
  - Many European countries
Brucella canis

- Distribution
  - Probably worldwide
- Prevalence unknown
  - United States: 1 to 19%
  - Mexico: up to 28%
  - Central and South America: 30%
- Human infections
  - Possible but uncommon

Source: Center for Food Security and Public Health, Iowa State University, 2012
Brucella in Marine Mammals

- North Atlantic Ocean
- Mediterranean Sea
- Arctic, including Barents Sea
- Atlantic and Pacific coasts of North America
- Coasts of Peru, Australia, New Zealand, Hawaii, Solomon Islands, Antarctic
Marine Brucella strains isolated from carcasses of seals, dolphins, porpoises and whales

<table>
<thead>
<tr>
<th>B. cetie</th>
<th>Cetaceans</th>
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<tbody>
<tr>
<td>B. pinnipedialis</td>
<td>Pinnipeds</td>
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<tr>
<td>B. microti</td>
<td>Vole (Microtus arvalis)</td>
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<td>B. inopinata</td>
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<tr>
<td>Species</td>
<td>Biovar</td>
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<td><strong>B melitensis</strong></td>
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<td><strong>B neotomae</strong></td>
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<td><strong>B ovis</strong></td>
<td>+</td>
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<tr>
<td><strong>B canis</strong></td>
<td>+</td>
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</tbody>
</table>

*a Dye concentration, 20μg/ml in serum dextrose medium (1:50,000).
*b A, A monospecific antiserum; M, M monospecific antiserum; R, rough Brucella antiserum.
*c Usually positive on primary isolation.
*d Some strains isolated in Canada, Britain, and the United States do not grow on dyes.
*e Some basic fuchsin resistant strains have been isolated in South America and South East Asia.
The following periods of viability have been reported in the available literature.

<table>
<thead>
<tr>
<th>Item</th>
<th>Milk under refrigeration</th>
<th>Butter</th>
<th>Refrigerated cream</th>
<th>Meat at 0°C to -20°C</th>
<th>Faeces on walls</th>
<th>On grass</th>
<th>Manure pit</th>
<th>Pond water</th>
<th>Slurry (temperature about 12°C)</th>
<th>Liquid manure in summer</th>
<th>Liquid manure in winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38 days</td>
<td>38-142 days</td>
<td>8-10 days</td>
<td>at least 65 days</td>
<td>1 day</td>
<td>53 days</td>
<td>1-5 days</td>
<td>less than 4 hours</td>
<td>3 months</td>
<td>8 months</td>
<td>108 days</td>
</tr>
</tbody>
</table>
Portals of entry

• **Oral entry** - most common route
  – Ingestion of contaminated animal products (often raw milk or its derivatives)
  – Contact with contaminated fingers

• **Aerosols**
  – Inhalation of bacteria
  – Contamination of the conjunctivae

• **Percutaneous** infection through skin abrasions or by accidental inoculation
Human Brucellosis

* Intermittant typhoid
* Typho-malarial fever
* Remittent fever
* Mediterranean fever
* Rock of Gibraltar fever
* Neopolitan fever
* Undulant fever
* Cyprus fever
* Malta fever
<table>
<thead>
<tr>
<th>Species</th>
<th>Biovar/Serovar</th>
<th>Natural Host</th>
<th>Human Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. abortus</em></td>
<td>1-6, 9</td>
<td>cattle</td>
<td>yes</td>
</tr>
<tr>
<td><em>B. melitensis</em></td>
<td>1-3</td>
<td>goats, sheep</td>
<td>yes</td>
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<tr>
<td><em>B. suis</em></td>
<td>1, 3</td>
<td>swine</td>
<td>yes</td>
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<td></td>
<td>2</td>
<td>hares</td>
<td>yes</td>
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<td>4</td>
<td>reindeer, caribou</td>
<td>yes</td>
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<td>5</td>
<td>rodents</td>
<td>yes</td>
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<td><em>B. canis</em></td>
<td>none</td>
<td>dogs, other canids</td>
<td>yes</td>
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<tr>
<td><em>B. ovis</em></td>
<td>none</td>
<td>sheep</td>
<td>no</td>
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<tr>
<td><em>B. neotomae</em></td>
<td>none</td>
<td>Desert wood rat</td>
<td>no</td>
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<tr>
<td><em>B. maris (?)</em></td>
<td>none</td>
<td>marine mammals</td>
<td>?</td>
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</tbody>
</table>
Reservoirs of infection
Occupations at high risk

1. Farmers
2. Veterinarians
3. Artificial inseminators
4. Meat inspectors
5. Abattoir workers
6. Laboratory workers
Brucellosis in Animals

- 1. abortion or weak neonates,
- 2. reduced milk yield,
- 3. decreased weaning weight,
- 4. extended birthing interval
- 5. lower fertility
Clinical Signs: Cattle & Bison

- Third trimester abortions with *B. abortus*
- Retained placenta
  - Once expelled will have a leathery appearance
- Endometritis
- Birth of dead or weak calves
  - Respiratory distress and lung infections
- Low milk yield
Clinical Signs: Sheep & Goats

- *B. melitensis* causes late term abortions
  - Retained placenta
  - Birth of dead or weak lambs/kids
- Goats - articular and periarticular hygroma localizations
- *B. ovis* causes abortions, fertility problems
  - Orchitis, epididymitis
  - Abnormal breeding soundness exam
  - Organisms present in semen
Clinical Signs: Swine

- *B. suis*
- Prolonged bacteremia
- Abortion, early or late gestation
- Fertility problems
  - Sows temporary
  - Boars, unilateral or bilateral orchitis
- Lameness, posterior paralysis, spondylitis, metritis, abscesses
Clinical Signs: Horses

- *B. abortus* most common
  - Susceptible to *B. suis*
- Fistulous Withers or Poll Evil
  - Inflammation of the supraspinous bursa
  - Exudative process
    - Fills with clear viscous liquid
    - Can eventually rupture
Clinical Signs: Dogs

- Susceptible to
  - *B. melitensis*, *B. abortus*, and *B. suis*
- *B. canis* causes abortions
  - Last trimester of pregnancy
  - Prolonged vaginal discharge
  - Bacteremia
  - Failure to conceive, stillbirths, prostatitis, epididymitis
Clinical Signs: Wildlife

- **Elk**
  - Abortions, no retained placenta
- **Moose**
  - Debilitated, death
- **Predators not clinical, but are vectors**
  - Coyotes, crows, vultures, bears
    - Aid in disease spread by carrying infected tissues away from abortion site

Center for Food Security and Public Health, Iowa State University, 2008
Transmission in Humans

- Ingestion
  - Raw milk, unpasteurized dairy products
  - Rarely through undercooked meat
- Mucous membrane or abraded skin contact with infected tissues
  - Animal abortion products
  - Vaginal discharge, aborted fetuses, placentas
Control of brucellosis in animals

1. Prevention of exposure of animals to infection
2. Rapid recognition of infected animals
3. Measures to be taken in infected herds
4. Vaccination
2. Rapid recognition of infected animals

* Abortion, if it occurs

* Presence of the organism in the body by bacteriological examination of each abortion or premature birth

* Presence of antibodies in diagnostic titres by regular serological testing of animals
Diagnosis of brucellosis

*Accordingly, diagnosis depends on:

1. **Isolation of Brucella** which is conclusive if +, but not when -

2. **Detection of antibodies** which is conclusive if -, but it is not 100% conclusive when +(false +, false -)
Isolation of brucellae

- Isolation is the most definitive diagnosis when it is positive.

- Failure to isolate the organism does not mean negative result.
Isolation is important because it helps in completing the identification by:

- biotyping,
- serotyping,
- phage typing,
- nuclear sequencing,
- restriction endonuclease fragmenting
- hybridisation.
<table>
<thead>
<tr>
<th>Species</th>
<th>Biovar</th>
<th>CO₂ Requirement</th>
<th>H₂S Production</th>
<th>Growth on Dyes</th>
<th>Agglutination in Serum</th>
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<td>Thionine</td>
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<td>B canis</td>
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* Dye concentration, 20μg/ml in serum dextrose medium (1:50,000).
* A, A monospecific antiserum; M, M monospecific antiserum; R, rough Brucella antiserum.
* Usually positive on primary isolation.
* Some strains isolated in Canada, Britain, and the United States do not grow on dyes.
* Some basic fuchsine resistant strains have been isolated in South America and South East Asia.
Serological Diagnosis of brucellosis

- Although the serological diagnosis is not 100% reliable when positive
- It is the main tool for the rapid recognition of infected herd and individual animals
- the serological diagnosis is 100% reliable when negative
<table>
<thead>
<tr>
<th>Serological tests</th>
<th>Immunoglobulins</th>
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<tbody>
<tr>
<td></td>
<td>IgM</td>
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<tr>
<td>Standard Aggl. Test</td>
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<tr>
<td>Rose Bengal Plate Test</td>
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<tr>
<td>Buffered acidified Test</td>
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<tr>
<td>Mercaptoethanol Test</td>
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<td>Rivanol Test</td>
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<td>Complement fixation</td>
<td>-</td>
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<tr>
<td>Milk Ring Test</td>
<td>+</td>
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</tbody>
</table>
Rose Bengal plate test

MRT
Milk ring test
Tube Agglutination Test
A positive serology means:

- field strain infection
- vaccination infection
- residual vaccination titre
- cross-reactivity with other organisms, like Yersinia, Salmonella, Pasteurella etc
- human errors.
Polymerase chain reaction (PCR)

- PCR is particularly useful in case of tissues and fluids contaminated with non-viable or low numbers of Brucella organisms in diagnosis,
- It can detect Brucella DNA.
- A good sensitivity of PCR was reported by Fekete et al. (1990a and 1990b), Baily et al. (1992) and Da Costa et al. (1996).
Vaccination
Natural infection gives life-long immunity

This means the best immunity is achieved by using live vaccines
ATTENUATED BRUCELLA VACCINES

- *Brucella abortus* Strain 19
  Spontaneous loss of virulence
- *Brucella suis* 2
  by in-vitro transfer
- *Brucella melitensis* Rev 1
  Selective mutagenesis
- *Brucella abortus* RB51
  through antibiotics
Brucella abortus Strain 19 (S19) vaccine

1. It is Brucella abortus biovar 1
2. Can be smooth or rough
3. Does not revert to virulence
4. Rarely persists in the body for long
5. Is not excreted
Brucella melitensis Rev 1 vaccine (Elberg, 1955)

- Streptomycin independent variant of streptomycin-dependent mutant of B. melitensis biovar 1
- Genetically stable
- Low virulence, good immunogenicity
- Effective protection in small ruminants
Vaccines through genetic engineering

- aim: to produce alternative vaccines that are:
  * safe and
  * do not induce antibodies, which interfere with the serodiagnosis of field infection.
Brucella cell components acting as antigens

- 1. Purified extracts
- 2. Cell wall fractions
- 3. Lipopolysaccharide (LPS)
- 4. O-polysaccharides (OPS)
- 5. Outer membrane proteins (OMPs)
  - * conserved in all Brucella species
- 6. Ribosomal fractions
- 7. DNA
Conclusion about Vaccines through genetic engineering

- Subunit vaccines proved to be not effective in protecting animals from subsequent infection
  (Confer et al., 1987 and Winter et al., 1988).
The failure to obtain an effective subunit or recombinant monovalent Brucella vaccine

- This problem is in great extent related to the antigen processing and presentation events which are rather complex (Schurig, 1994).
A good, strong and long-lasting immunity against Brucella requires that:

1. the vaccinal strain persists a time longe enough in lymphoid organs to produce the desired immunity

2. the vaccinal strain has a low but real residual virulence linked to ability to multiply and resist
* It is a laboratory-derived rough mutant of the virulent strain 2308 of Brucella abortus
* Rifampin and penicillin resistant
* It contains the same OMP as S19 and S2308
Thank you

Refai