Introduction and etiology of Brucellosis and various approaches to its diagnosis

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Brucellosis

- Chronic infectious disease in cattle
- Causes abortions, birth of dead calves
- Retained placenta
- Infertility
- Reduced milk production
- Infection and swelling of testicles in bulls
- Lameness due to infected bursae

Host Specificities

- ***B. melitensis*** **in cattle & swine**
- ***B. suis*** **in cattle– no abortion.**
- ***B. suis*** **biovar 1 infectious for man**
- ***B. suis*** **biovar 2 non-infectious for man**
- **Role of host factors – tissue tropism erythritol in ruminant placenta**

Impact of Brucellosis

- Economic loss through abortion/infertility
- Economic loss through trade restrictions
- Zoonotic risk to man

Susceptible hosts

- cattle
- buffaloes
- sheep
- goats
- pigs
- dogs
- horses
- Man

Brucella

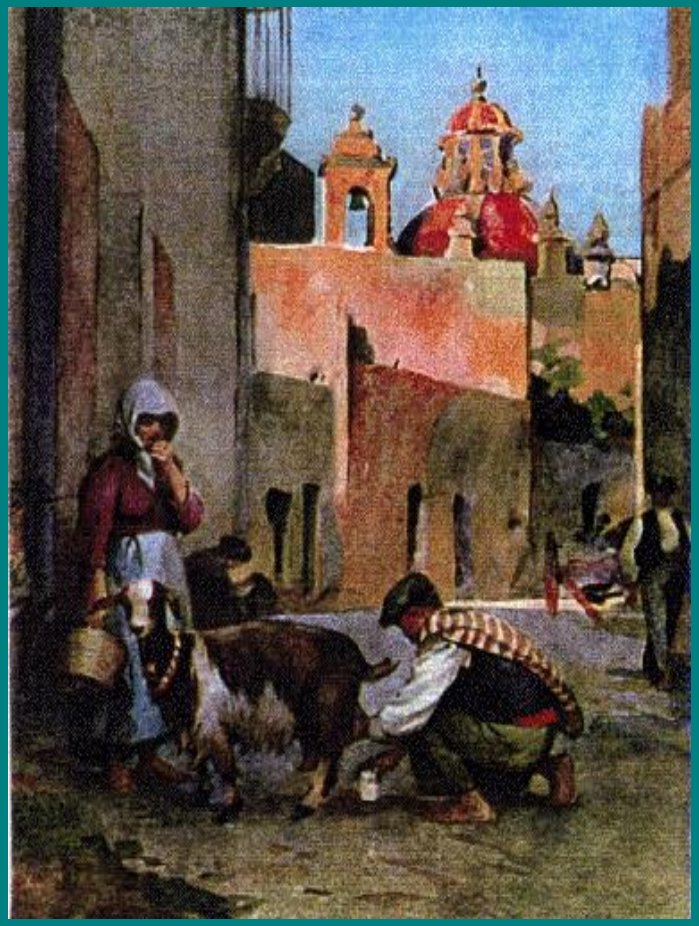
- Gram negative bacteria
- Intracellular parasites
- Short rods, coccobacilli
- Six species
- Nonsporing
- Facultative anaerobe
- Form small colonies on artificial media

Sir David Bruce (1855-1931)

- Army Physician and
- Microbiologist who
- discovered *Micrococcus melitensis* (1887).
- Nomenclature today is credited to Sir David Bruce

Undulant Fever History:

- 1859 – Marston described disease
- 1886 – Bruce discovered causative organism
- 1897 – Hughes wrote classic monograph (844 patients)
- 1897 – Bang causative organism of contagious abortion
- 1918 – Alice Evans noted resemblance between *B. melitensis* & *B. abortus*

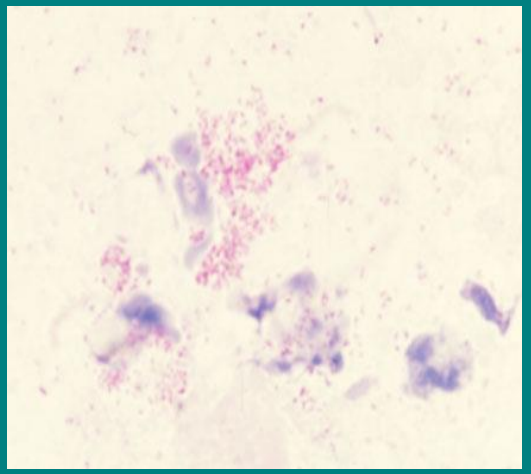


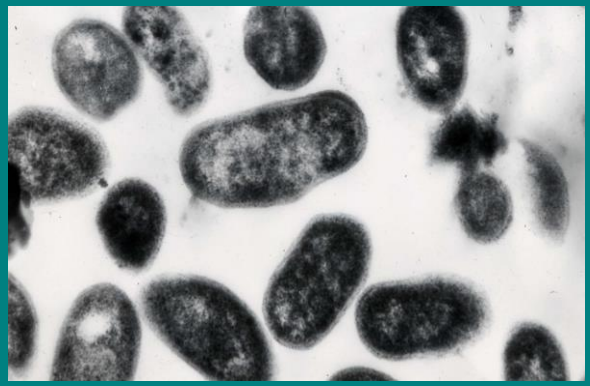
MEDITERRANEAN FEVER COMMISSION, 1904

Standing: Dr. T. Zammit; Capt. Crawford Kennedy, R.A.M.C.; Major J. C. Weir, R.A.M.C.
Seated: Major J. G. McNought, R.A.M.C.; Dr. J. W. H. Eyre; Col. David Bruce, R.A.M.C.;
Major T. McCulloch, R.A.M.C.; Staff Surgeon E. M. A. Clayton, R.N.

Brucellae

- Gram-negative facultative intracellular (extracellular) bacteria.
- Until recently a monospecific genus (>95% genes with 98-100% nucleotide identity)
- Six named species & biovars (differential host specificity & pathogenicity)
- No obvious virulence factors

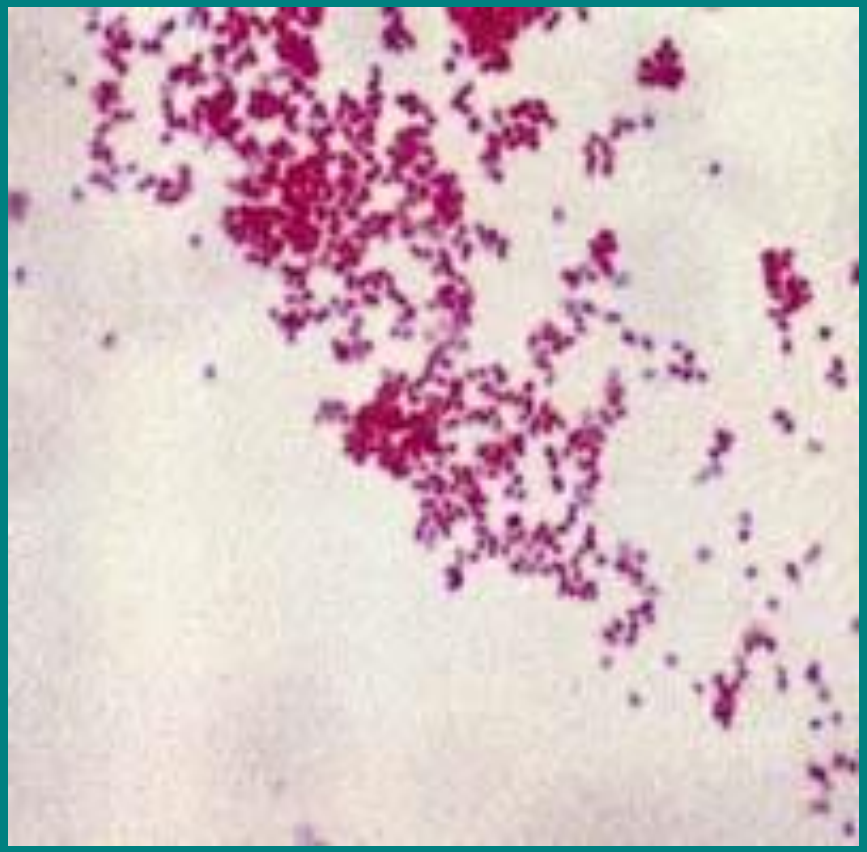




Brucella species	Biovars	Reservoir Host
Brucella melitensis	3	Sheep, goats, cattle
Brucella abortus	7	Cattle
Brucella suis	5	Swine
Brucella canis	1	Dogs
Brucella ovis	1	Sheep
Brucella neotomae	1	Rodents
*Brucella pinnipediae**	Not determined	Otter, seal
*Brucella cetaceae**	Not determined	Dolphin, porpoise

* = suggested name

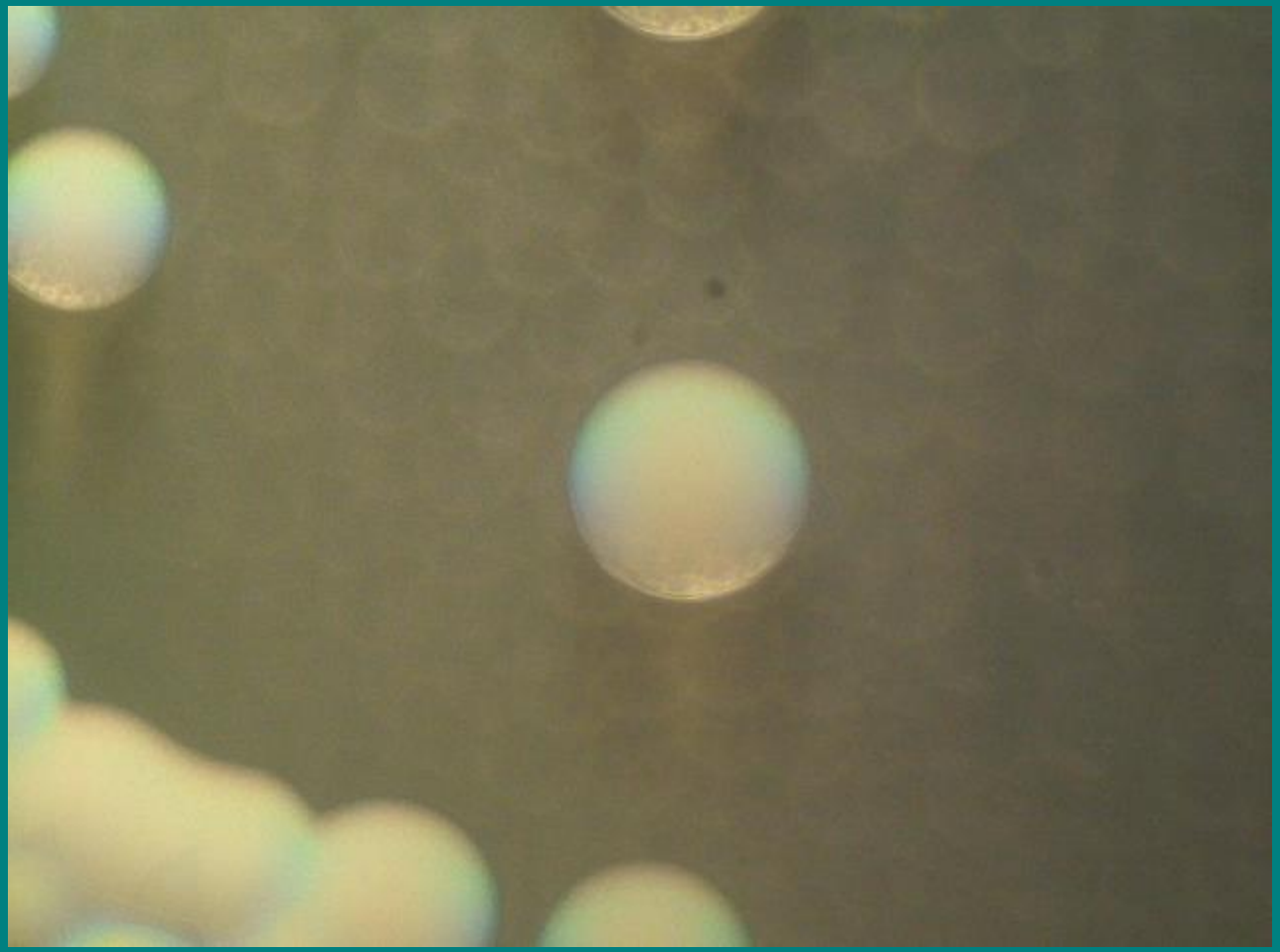
Brucellosis abortus



What is Brucella?

- B.abortus
- B.melitensis
- B.suis
- B.ovis
- B.canis
- B.neotamae
- B.maris

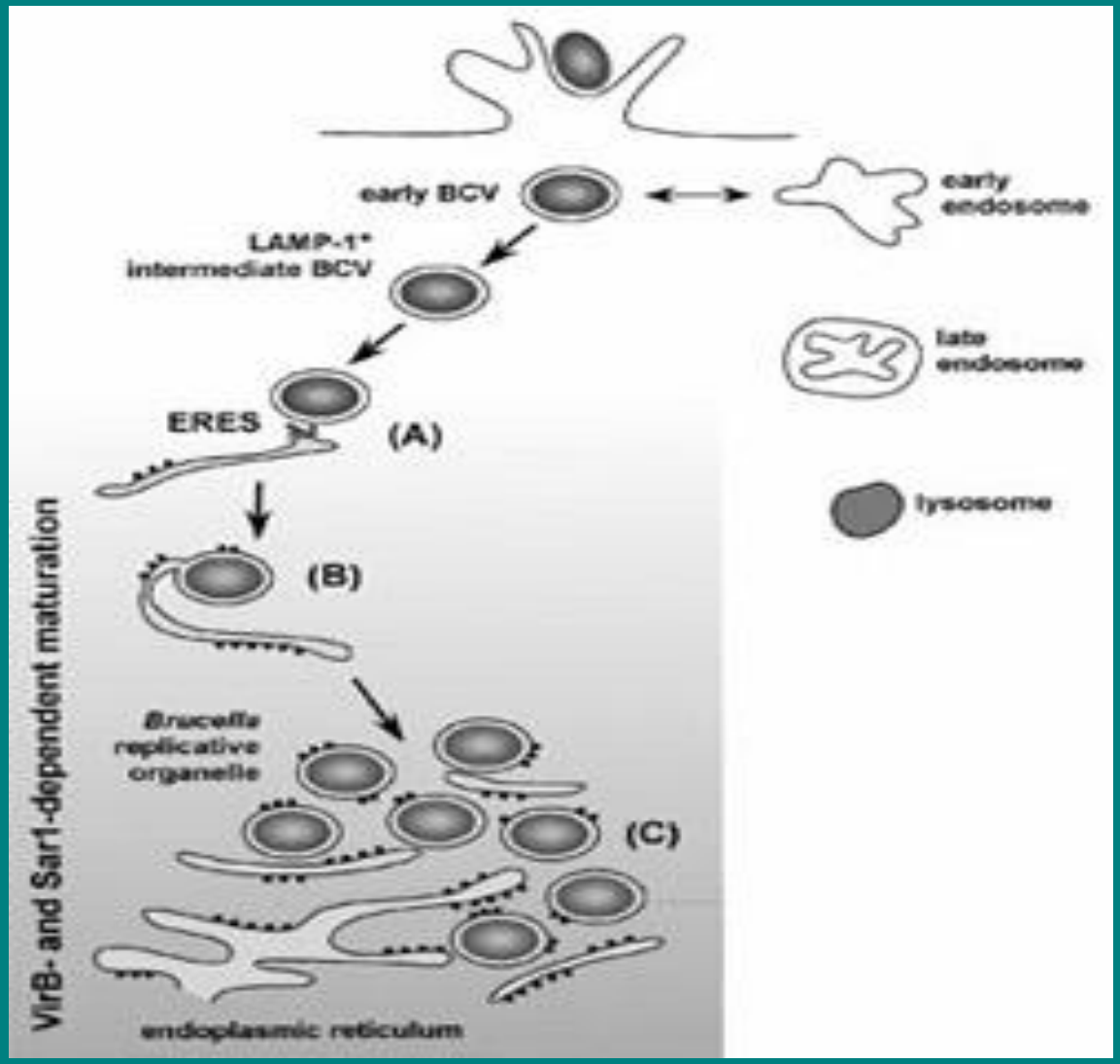
Single colony



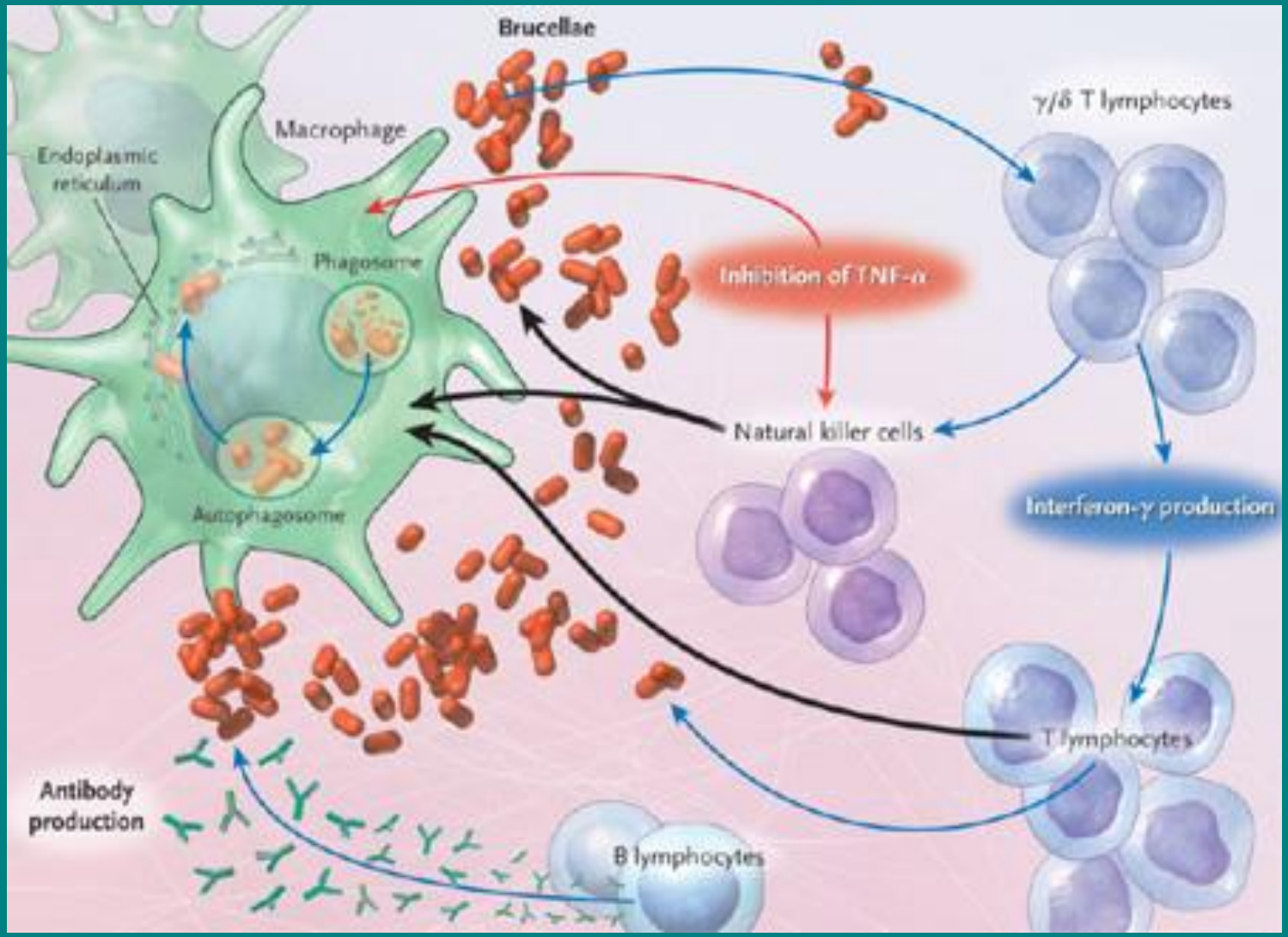
Aetiology

- *Brucella abortus* - Cattle and Buffaloes
- *Brucella melitensis* - Sheep and Goats
- *Brucella suis* - Pigs
- *Brucella canis* - Dogs
- *Brucella ovis* - Ram (Male sheep, but not reported from India)

pathogenesis



Immune mechanisms



Transmission

- **through mucous membranes of oropharynx, upper respiratory tract, conjunctiva and male or female genital tract.**
- **Through grazing, feed, water etc. contaminated by discharges from infected animals.**
- **Contact with aborted foetus, foetal membranes and infected new born calves.**
- **Intra-herd spread occurs by both vertical and horizontal transmission.**
- **Movement of contaminated tail acts as a common means of spreading the disease.**
- **Milking personnel can act as a means of mechanical transmission.**
- **Spreading of disease from one place to another is through movement of infected animals.**
- **Disease is transmitted through artificial insemination but unlikely through natural services by an infected Bull**

Characteristic symptoms

- Abortion in late gestation (7-9 month in Cattles and Buffaloes, 3-5 months in Sheep and Goats).-abortion storm
- Orchitis.
- Accumulation of fluid in scrotum.
- Retention of placenta.

Gross lesions

- Oedema and thickening of the placenta giving it leathery appearance.
- Necrosis of cotyledons.
- Oedema of foetus, red coloured (sero-sanguinous) fluid in its body cavity.
- Cobbler-stone appearance of foetal lungs due to broncho - pneumonia.
- Enlargement of scrotum due to orchitis in males.
- Induration (firmness) of mammary gland in cows.
- Enlargement of knee joints (hygroma).

Microscopic lesions

- Infiltration of phagocytic cells, epithelioid cells and lymphocytes surrounded by fibrous tissue proliferation.
- Foetal broncho - pneumonia.
- Organism in chorionic epithelial cells.
- In males, proliferation of fibrous tissue which compresses or replaces the epididymis resulting in enlargement of testicles.

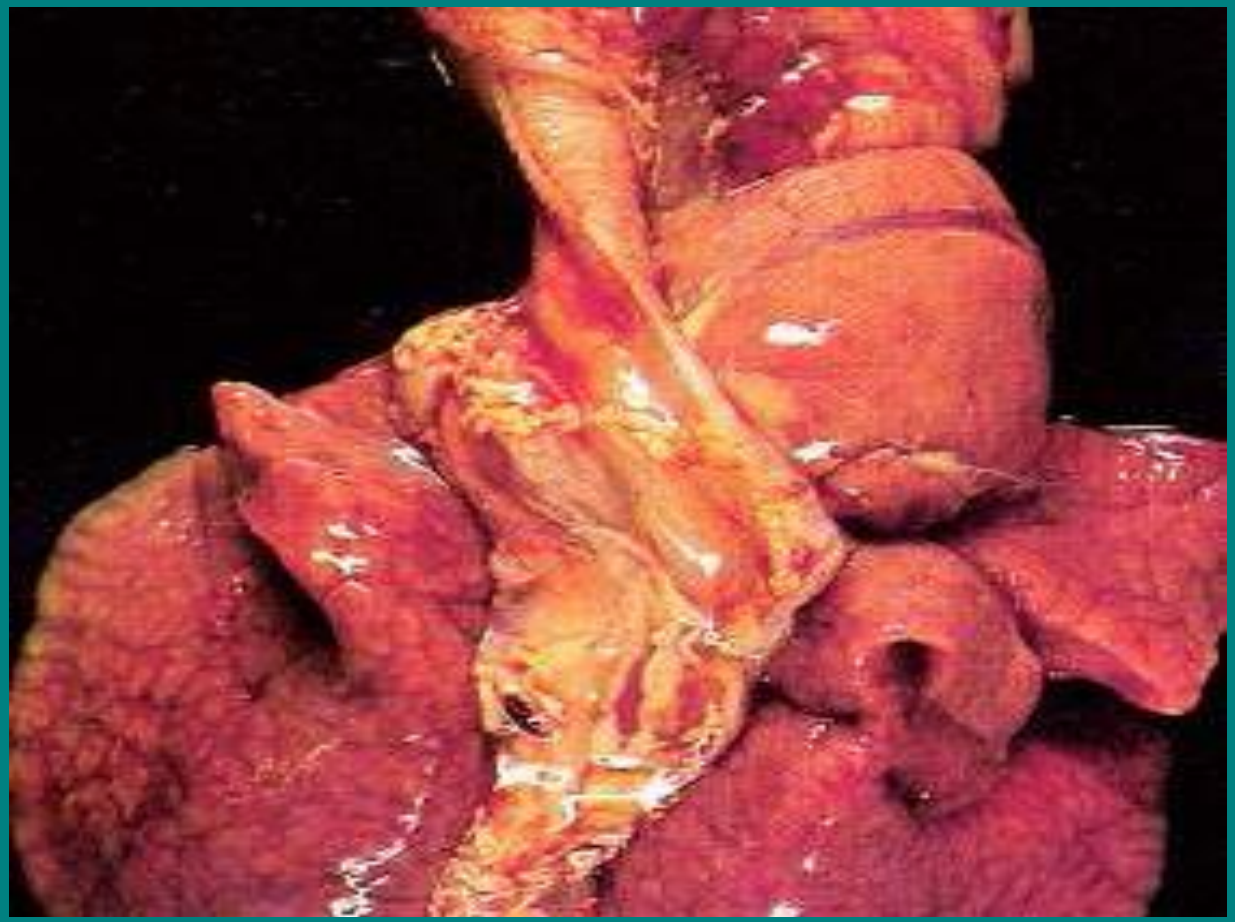
Diagnostic tests currently available

Test Specimenrequired	Test detects	Time taken to obtain result
• Culture and identification of *B. abortus*	Tissue Brucellae	6 days
• Rose Bengal plate test (RBPT)	Serum Antibody	10 min
• Complement fixation test (CFT)	Serum Antibody	4 hrs
• Enzyme-linked immunosorbent assay (ELISA)	Serum Antibody	2 hrs
• Serum agglutination test (SAT)	Serum Antibody	24 hrs
• Bulk milk ring test (BMRT)	Milk Antibody	1 hour
• Individual milk ring test (IMRT)	Milk Antibody	1 hour

Placenta from aborted buffalo due to Brucellosis.

Aborted buffalo foetus after three months of pregnancy

Cobblestone appearance of the foetal lungs



Orchitis in rams

Swelling of testicles

Swelling of joints

Brucellosis in man

- Undulant fever
- Contact with infected animals and discharges
- Consumption of unpasteurized milk
- Inhalation –droplet infection
- Through eyes
- Through cuts and abrasions
- Through live vaccines

Brucellosis in Man

- Primarily presents as “undulant” fever
  - Fever
  - Headaches
  - Weight loss
  - Anorexia
  - Fatigue
  - Depression
- Complications
  - Osteoarthritis
  - Genitourinary
  - Hepatic
  - Pulmonary
  - Psychoneurosis
  - Endocarditis
  - Abortion

- Chronic
- Difficult to treat

Brucellosis – A worldwide zoonosis

- <0.01->200,000 human cases/100,000
- Middle East
- Mediterranean basin
- South-Central America
- South Eastern Europe
- Asia
- Africa
- Caribbean

Clinical diagnosis:

Acute or insidious onset of fever, night sweats, undue fatigue, anorexia, weight loss, headache, and arthralgia

Laboratory criteria for diagnosis

- Isolation of *Brucella* sp. from a clinical specimen, or
- Fourfold or greater rise in *Brucella* agglutination titre between acute- and convalescent-phase serum

Case classification

Probable: a clinically compatible case that is epidemiologically linked to a confirmed case or that has supportive serology

Confirmed: a clinically compatible case that is laboratory confirmed

Human Brucellosis – When to suspect:

- Consumption of unpasteurised dairy produce
- Undercooked meat from infected animals
- Eating aborted foetuses (Ecuador)
- Crushing umbilical cord of newborn lambs/kids between teeth
- Contact - skin lesions; mucous membranes (slaughter house workers; hunters; vets)

Potential Routes of Transmission

- Respiratory
- Animal contact or environmental contamination
- Sexual (among livestock)
- Gastro-intestinal through consumption of animal produce
- Laboratory acquired infection
- Bioterrorism

Brucella as agent for Bioterror

- 100,000 exposed to cloud *B. melitensis*
- 82,500 cases needing extended treatment
- 413 deaths
- Economic impact 477.7 million dollars for every 100,000 exposed!

Symptoms in man

- Acute brucellosis in humans usually begins with intermittent fever, weakness,chills, sweating, headaches, muscle and joint aches and malaise.
- behavioural changes, Characteristically, the fever spikes each day,giving rise to the term ‘undulant fever’
- fever may be chronic and persist for many years

Diagnosis

- Herd/Case History.
- Symptoms and lesions.
- Immunodiagnostic tests
- Isolation of bacteria.
- Demonstration of organisms in aborted materials.
- Detection of bacterial genome by PCR.

Serological tests

- SAT-serum agglutination test
- RBPT-Rose Bengal Plate agglutination test
- MRT-Milk ring test
- BPAT-Brucella Plate agglutination Test
- CFT-Complement Fixation Test
- AGPT-Agar gel precipitation test
- ELISA-Enzyme linked immunosorbant assay

Diagram showing agglutination process

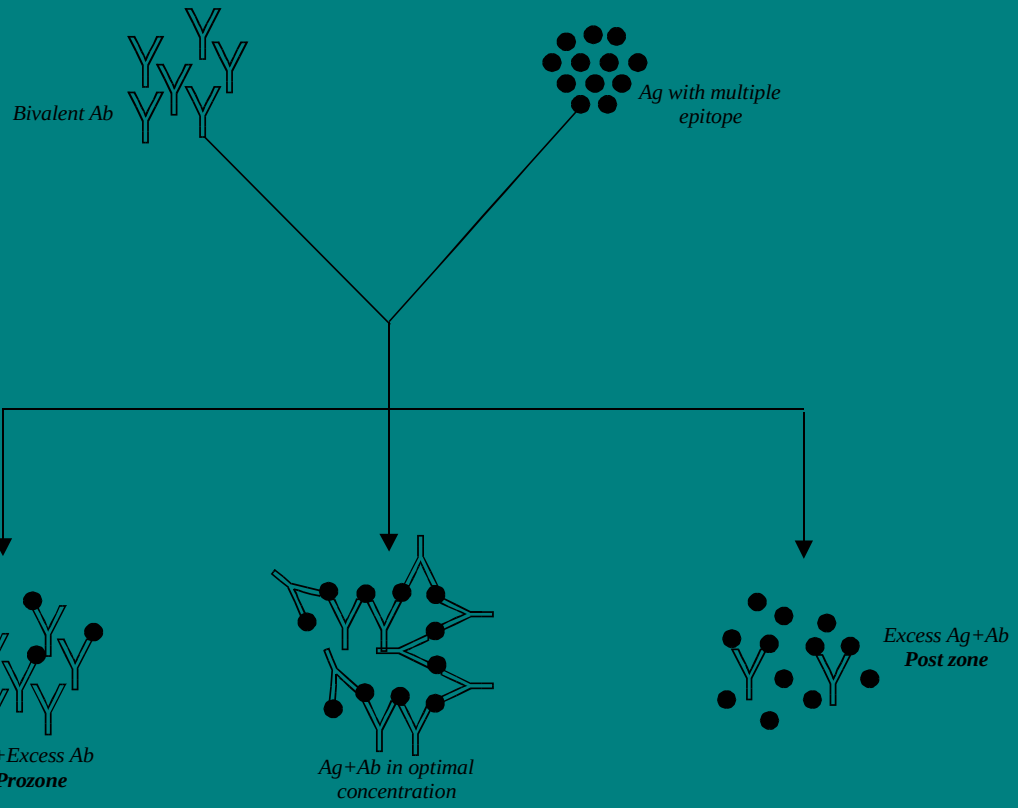
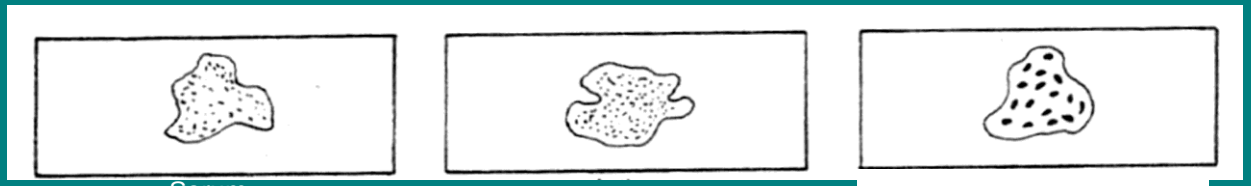
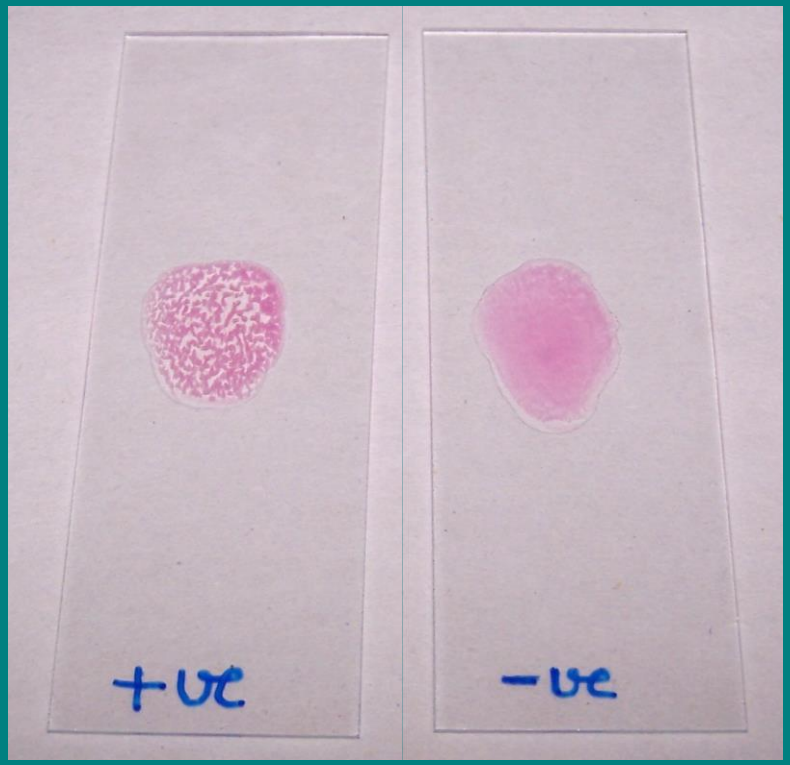


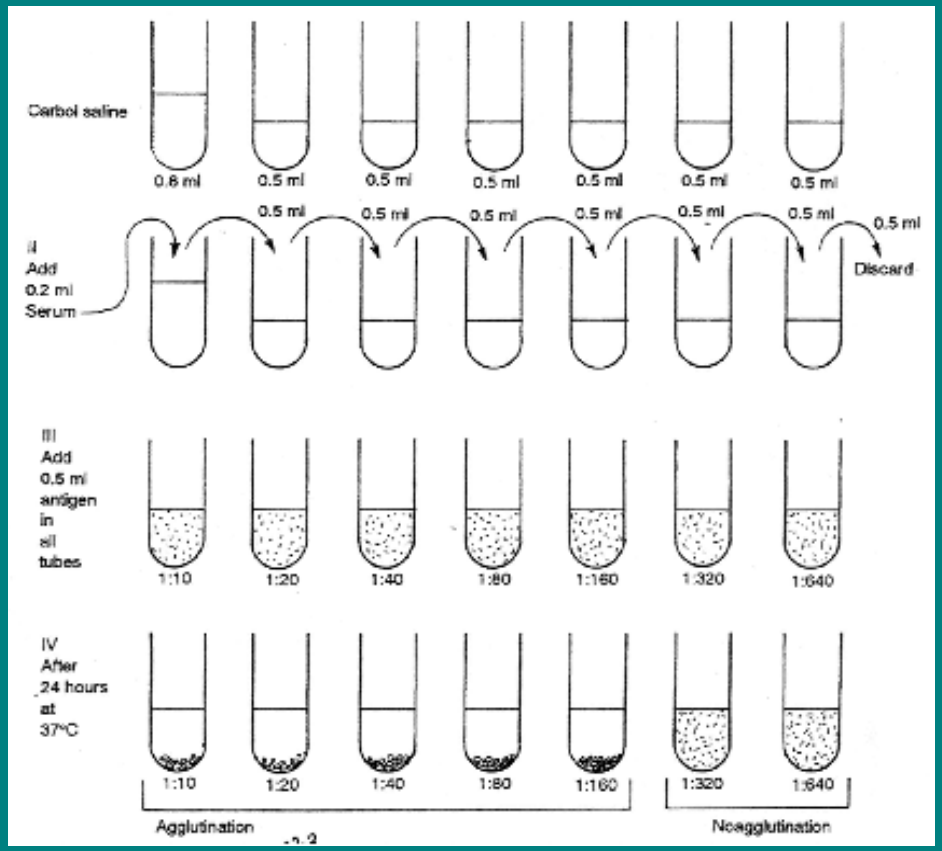
Plate agglutination test

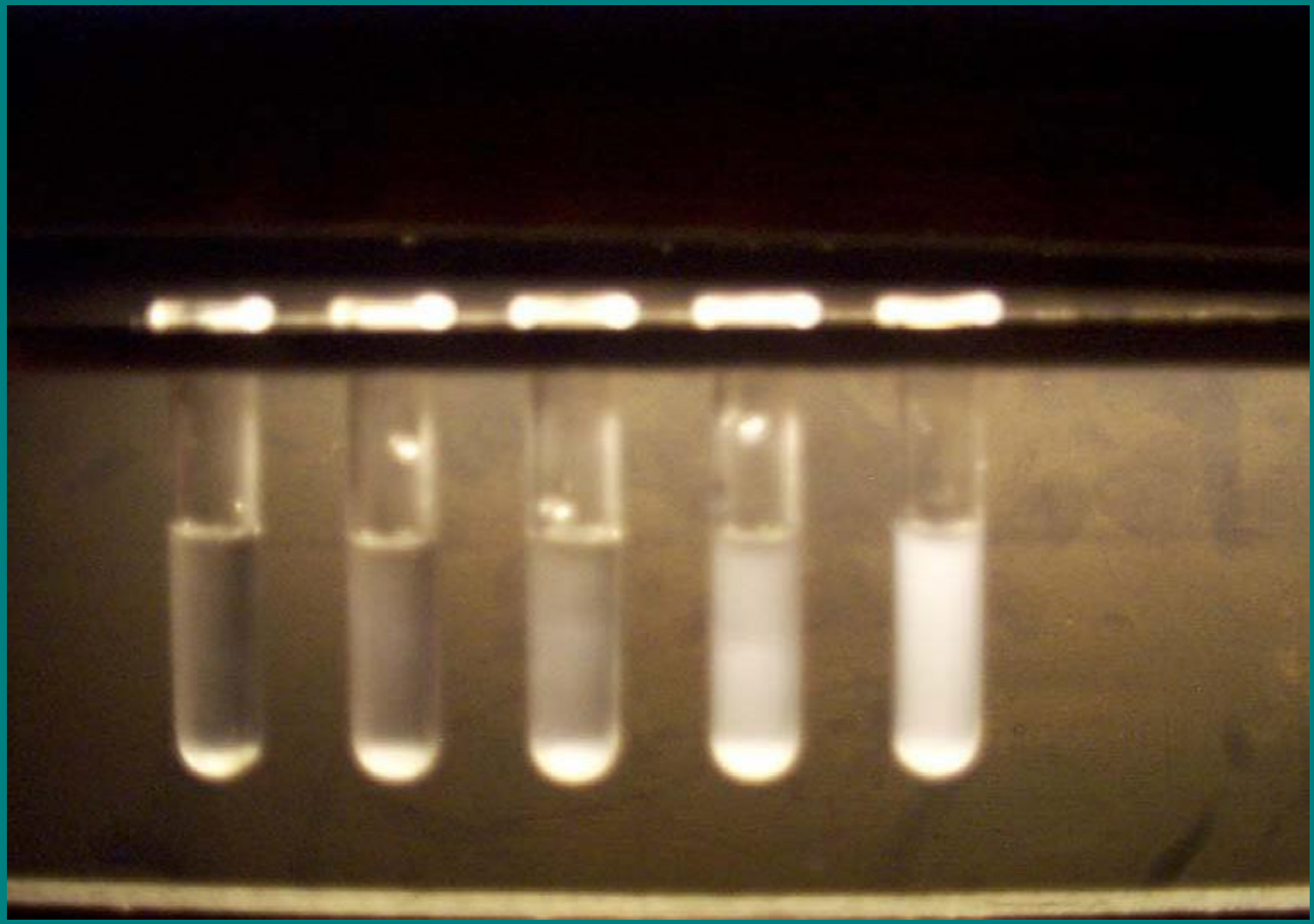


RBPT

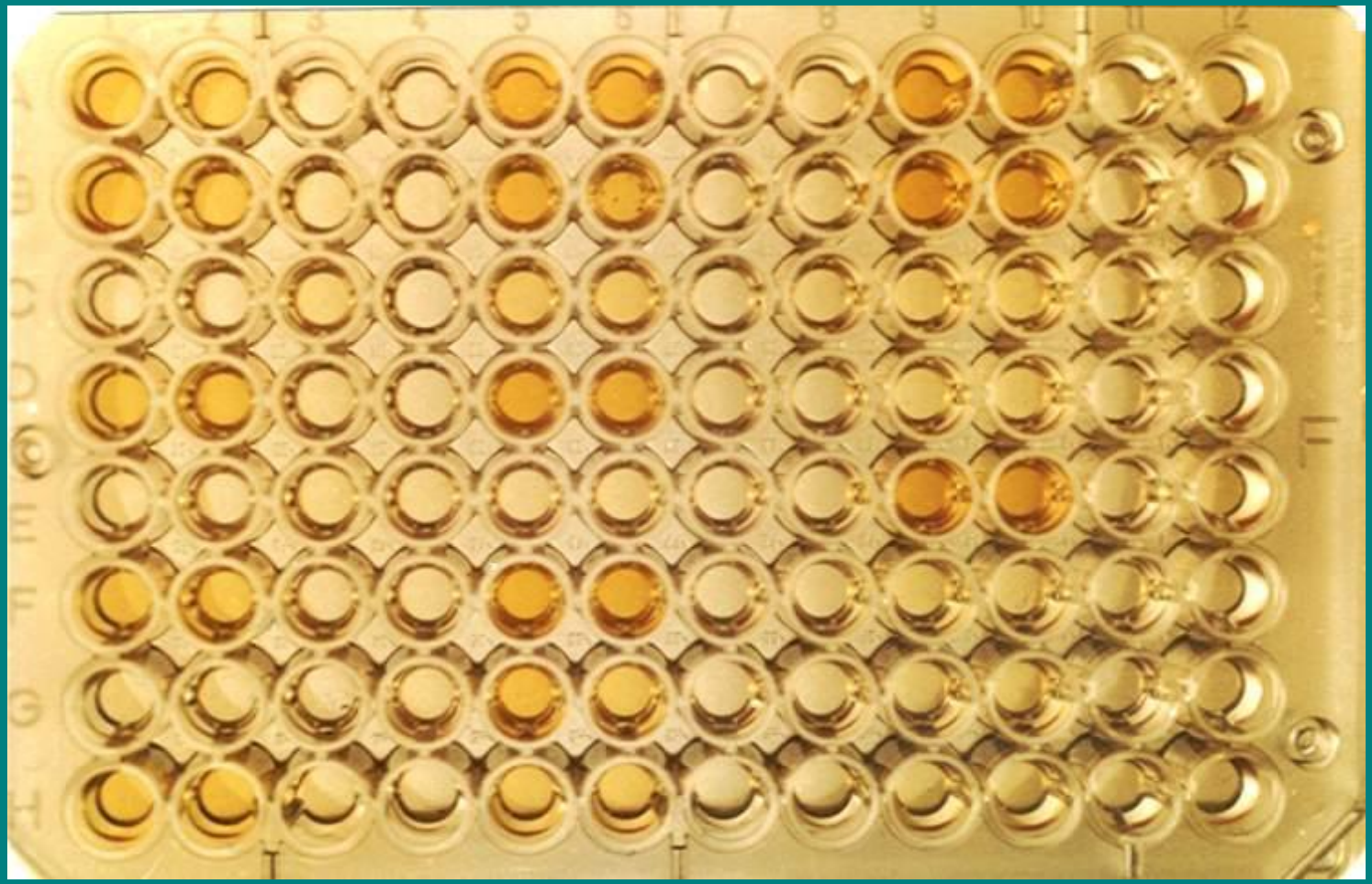


Diagrammatic illustration of tube agglutination test

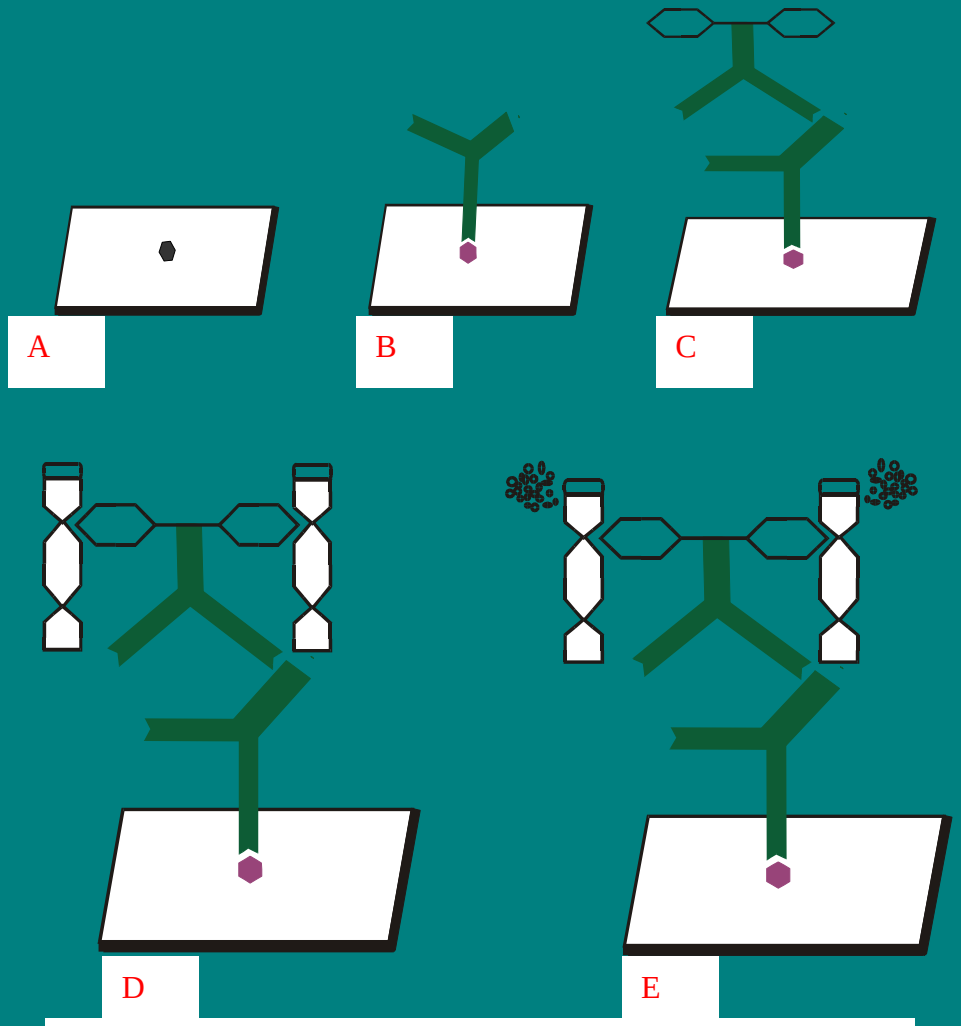




ELISA



enzyme labeled avidin-biotin techniques-



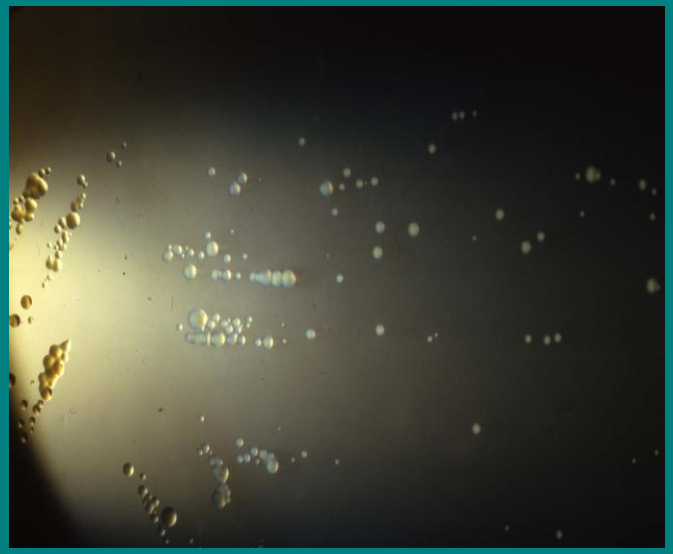
A. Antigen coated in well, B. Primary antibody binds with antigen,
C. Secondary antibody labeled with biotin binds with B,
D. Avidin conjugated with Peroxidase binds with C, and
E. Peroxidase reaction with substrate.

Typical diagnostic samples

- Blood cultures:
  - 15-70% positive
- Bone marrow
- Others
  - Tissues
  - Fluids – CSF; synovial; pleural effusion; abscess drains

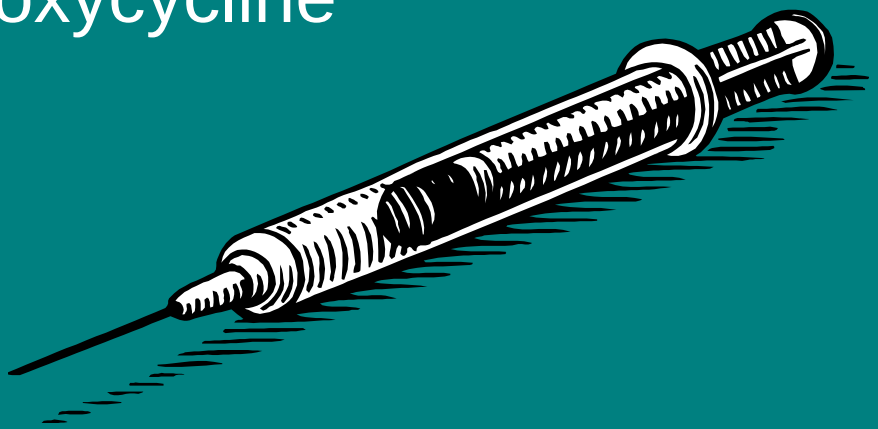
Identification

- Small Gram negative coccobacilli (poorly staining)
- Oxidase positive
- Urease positive
- Non-motile
- No growth on MacConkey
- No requirement for X & V factors
- Take culture to containment level 3!!!



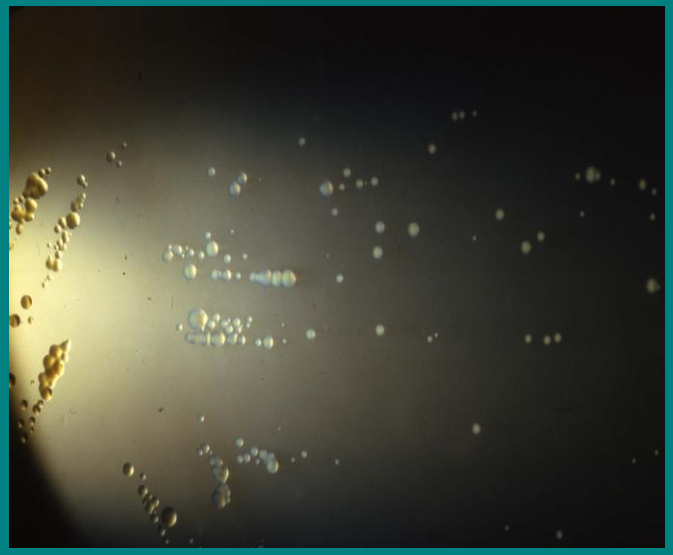
Treatment

- Prolonged course
- Rifampicin + Doxycycline
- Streptomycin + Doxycycline
- Gentamicin + Doxycycline

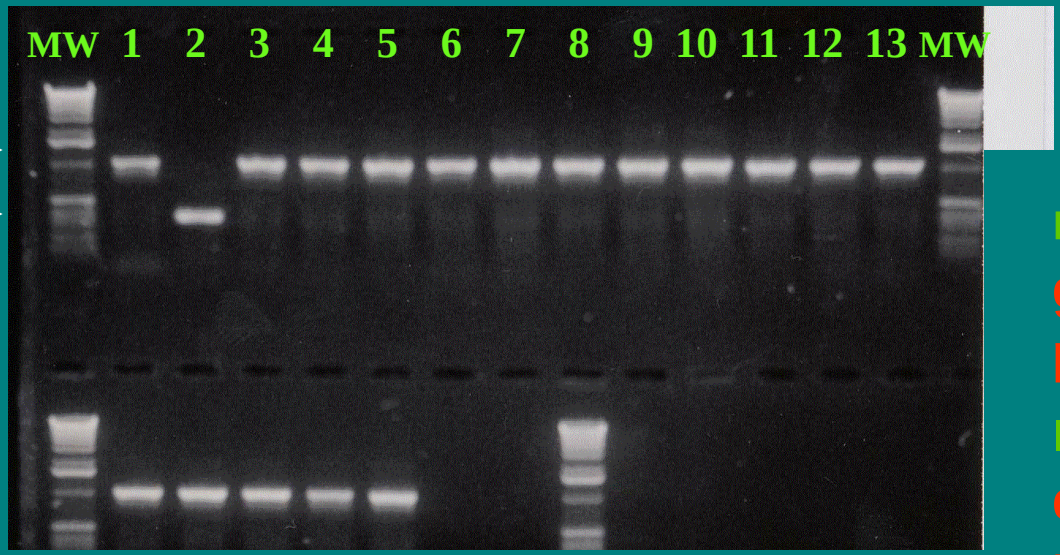


Confirmation:

- Classical Biotyping (CO_2; H_2S; Urea; Dye & phage susceptibilities; Agglutination monospecific antisera (A+M)
- Genus-specific PCR
- Multiplex AMOS PCR - Abortus, Melitensis, Ovis & Suis.
- PCR over deletions to identify vaccine strains



S19 *ery* gene PCR



- Wild type ery gene gives a PCR product of 1063 bp
- S19 has a 702 bp deletion which results in a 361 bp PCR product

1 - *B. abortus* biovar 1 wild type control

2 - S19 control

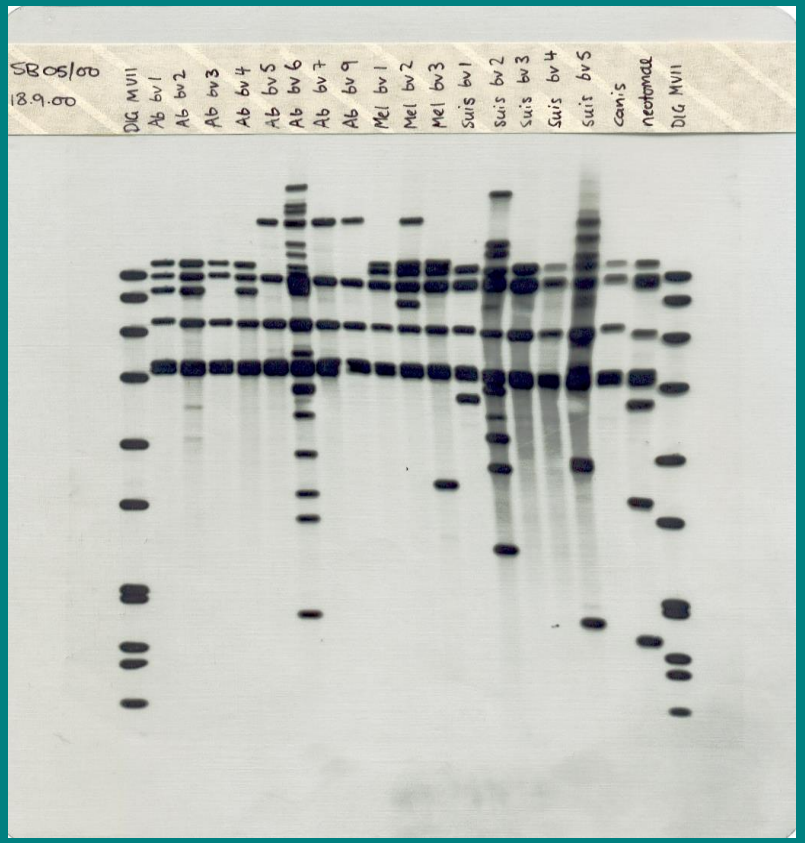
3 to 13 - *B. abortus* Field isolates from NI

Approaches:

- Methods that index chromosomal DNA polymorphisms – RFLP
  - Digestion & hybridisation with probe (*IS711*)
    - *Eco*R1 digest used to characterise marine mammal isolates
  - Infrequent cutter + PFGE
    - *Xba*1, *Pac*1 or *Spe*1 species differentiation (not biovars)
  - Ribotyping – poor discrimination, operons <0.1% chromosome
    - Used to show marine isolates belong to Brucellae and that they represented new subgroup (*Hin*dIII rDNA profiles)

Discrimination reliant on multiple copies at diverse locations

IS711 Fingerprinting



- Fails to produce the same level of characterisation as the phenotypic approach. BUT are more:
  - Rapid
  - Safe
  - Objective
- Identification of 3 groups within marine mammal isolates.
- Techniques now in use in reference centre work
- Continue validation with further isolates and genetically related bacteria.

PCR-Gene RFLP Typing:

- Polymorphic alleles digested with RE's
  - OMP typing:
    - *Omp*2 locus (*omp2a* and *omp2b* 36 kDa porin proteins)
      - Used to classify marine isolates
      - Dolphins/Porpoises have 2 *omp2b* genes
      - Otter/Seal one *omp2a* & one *omp2b*
      - Terrestrial mammals one of each or two *omp2a*
    - *Omp*25
      - Very homogeneous
    - *Omp*31
      - Moderate differentiation
      - Absent from all *B. abortus* biovars

Fails to differentiate all nomenspecies/biovars

AFLP

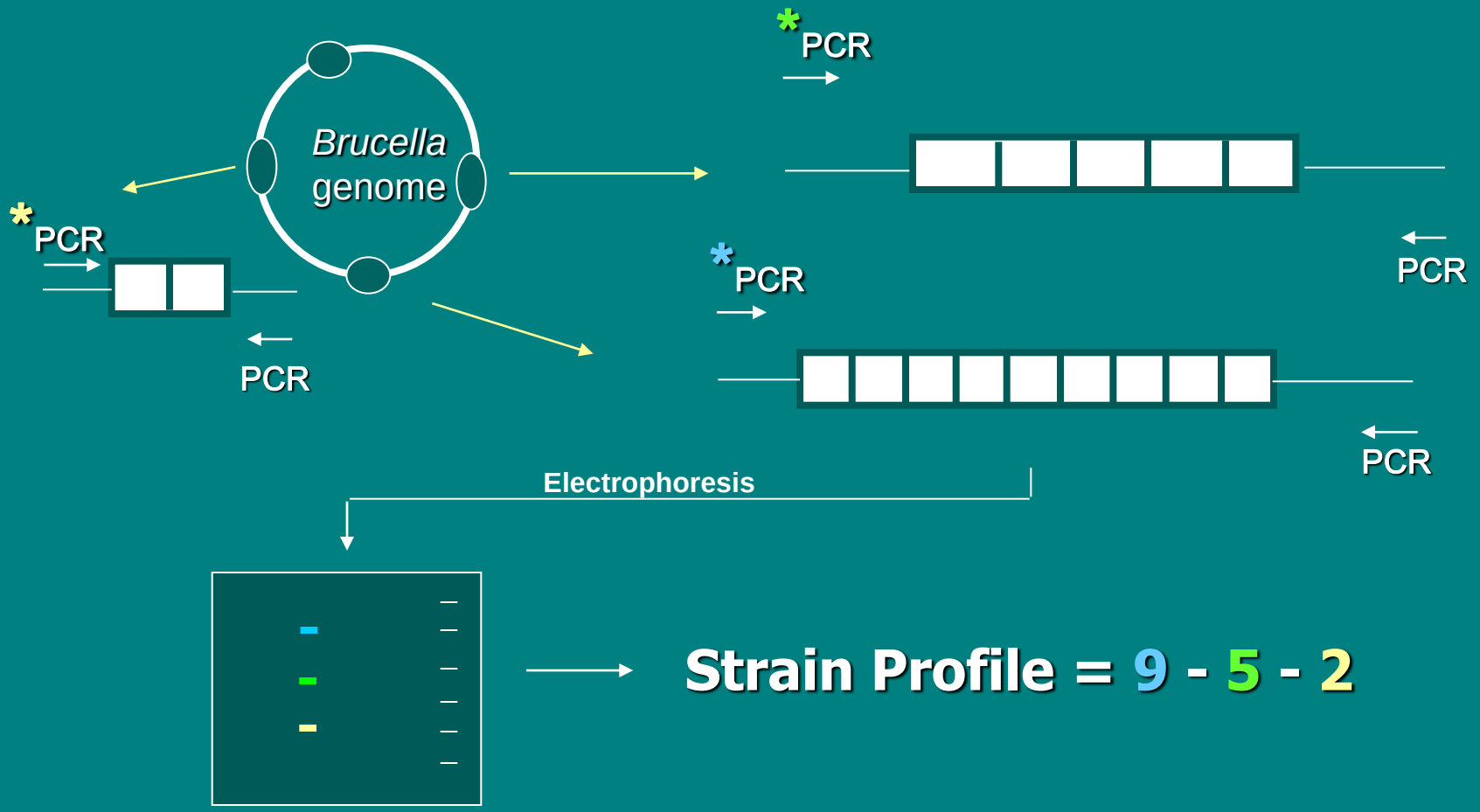
- Restriction-ligation – DNA fragments tagged with specific adapters
  - Primers complementary to adapters + DNA used to amplify subset of tagged restricted fragments

Unable to differentiate all nomenspecies/biovars

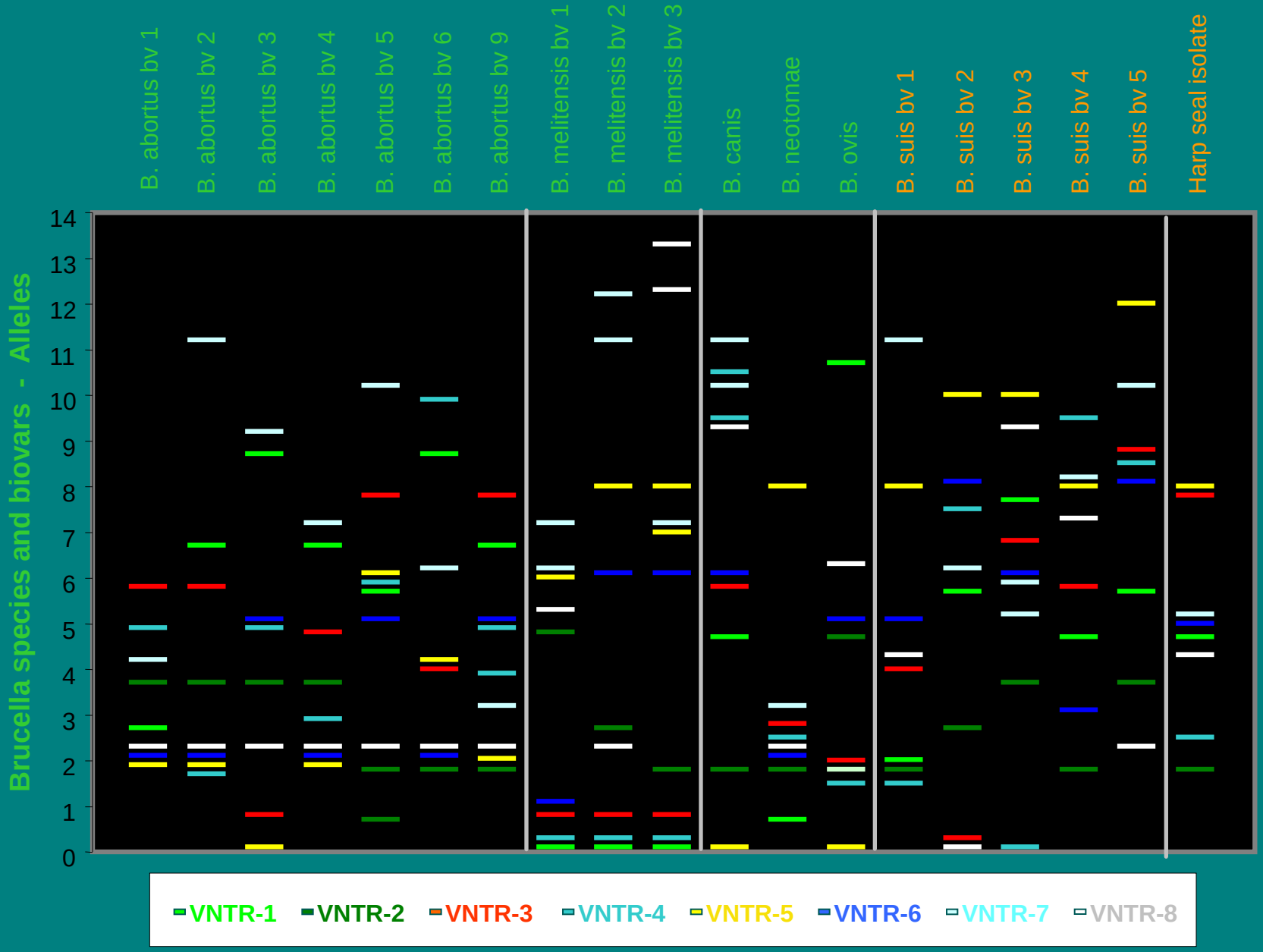
Multi-locus microsatellite fingerprinting

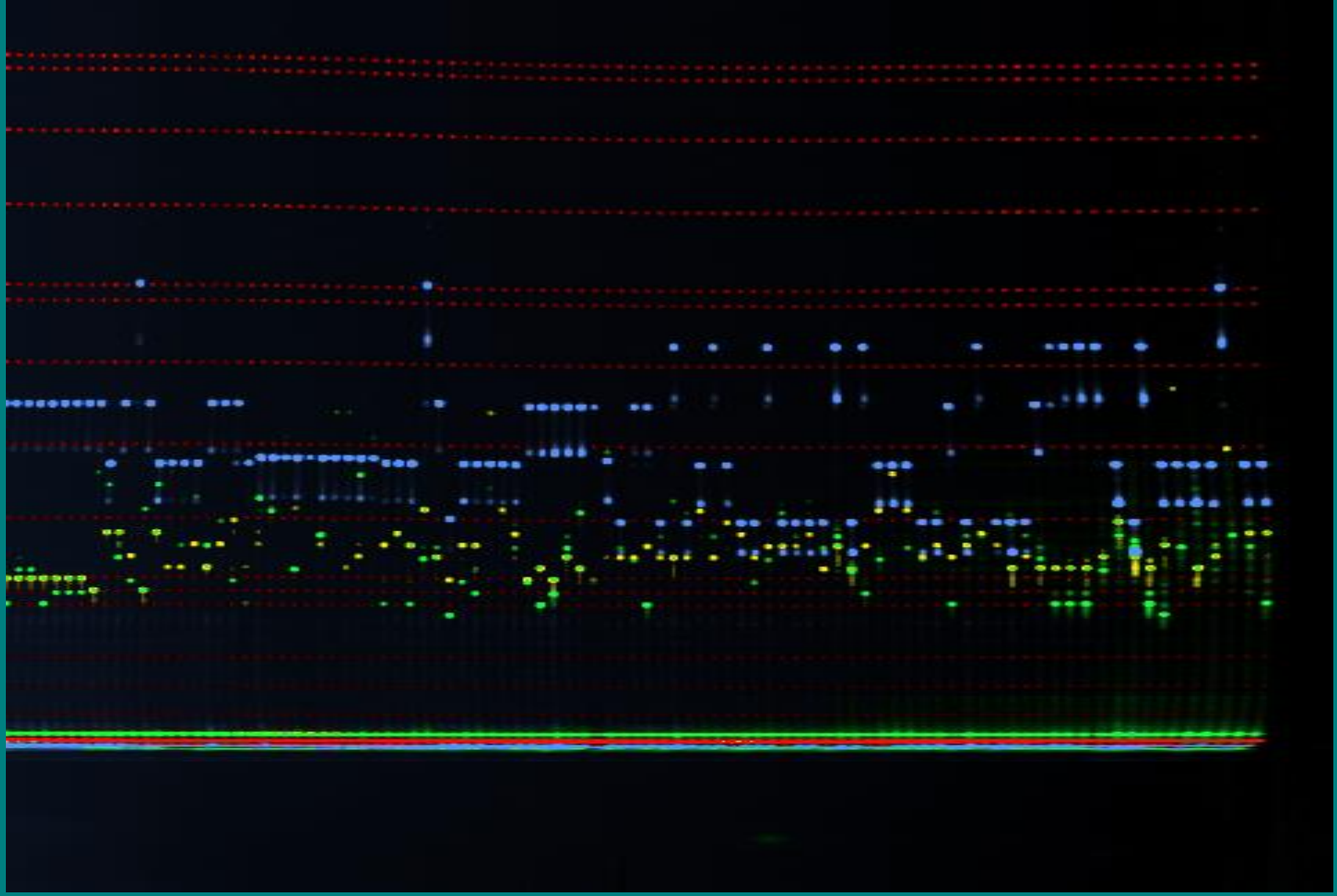
- PCR based using sequence tandemly repeated variable numbers in different strains
- Different loci with different mutation rates
- Permits multiple levels of discrimination
- Discrimination of species, biovars and among unrelated isolates within biovars
- Multiple isolates give constant patterns

Variable Number of Tandem Repeat (VNTR) Typing



VNTR profiling for brucellosis:

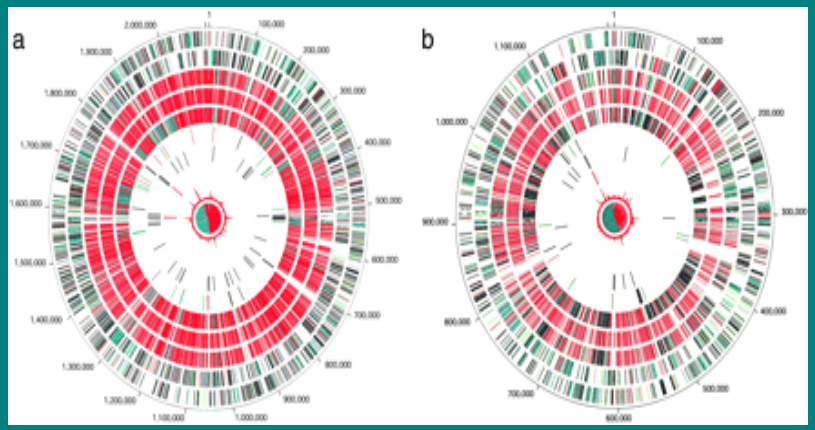




Genomic organisation:

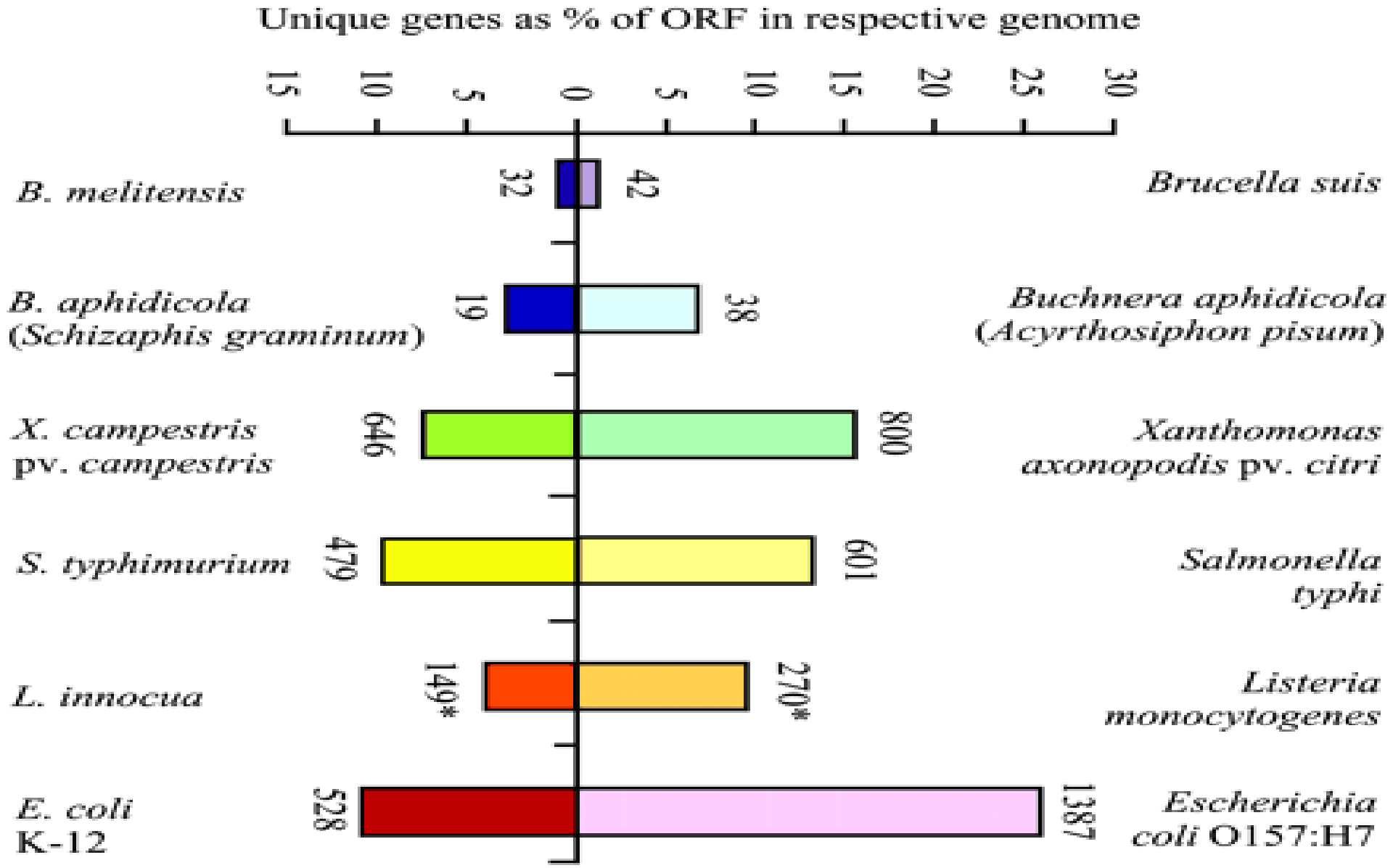
- Brucellae usually have two chromosomes, chromosome I 2.1 Mb and chromosome II 1.2 Mb
- Chr I classic bacterial circular chromosome, genes for transcription, translation & protein synthesis
- Chr II has cluster plasmid-like replication genes (cf plasmids in *Agrobacterium & Rhizobium spp*) membrane transport & energy metabolism genes
- Distinct chromosomal origins?
- Chr II has features of megaplasmid, could it have been captured from an ancestral *Brucella?*
- *B. suis* biovar 3 has single 3.3 Mb chromosome

Whole Genome Comparisons



- *B. melitensis* (DelVecchio *et al.* Proc Nat Acad Sci 2002 99; 443-8).
- *B. suis* (Paulsen *et al.* PNAS 2002 99; 131; 48-53).
- *B. abortus* (Halling *et al.* 2005 J. Bacteriol. 187; 2715-26).
- *B. ovis* (currently underway, TIGR)

Comparative Genomic Analysis of closely related bacteria with different host specificity / pathogenicity



Tsolis 2002 PNAS 99 12503-5

Brucella spp. persist in different cell types:

- Infect oropharynx to genital tract
- Cellular tropisms
  - Non professional placental trophoblasts
  - Professional phagocytes
  - Foetal lung tissue
  - Reticuloendothelial system