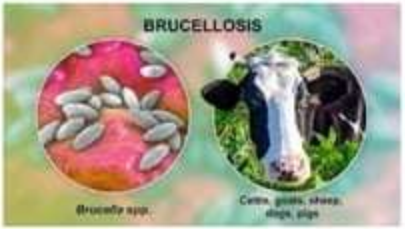
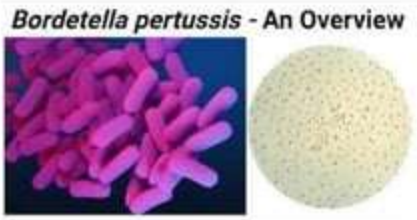


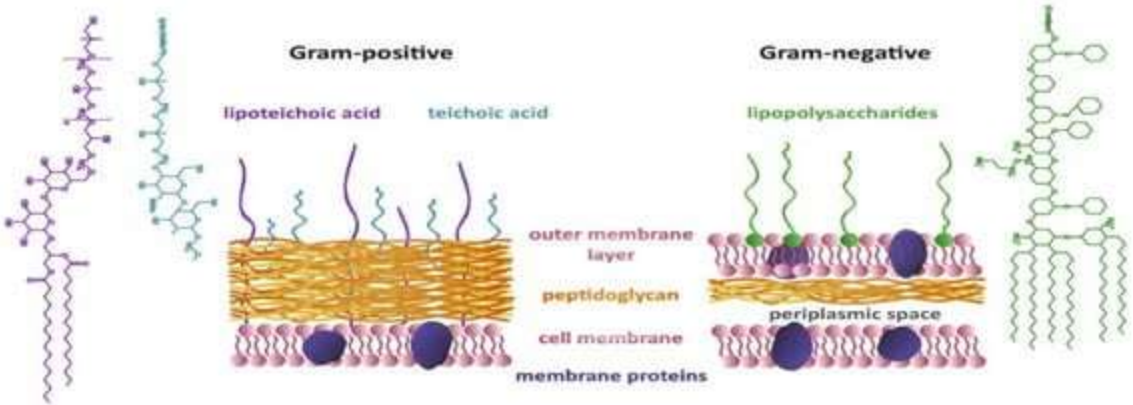
BRUCELLA & BORDETELLA



By

Dr.D.R.Manisha Rani

Bacteria cell walls

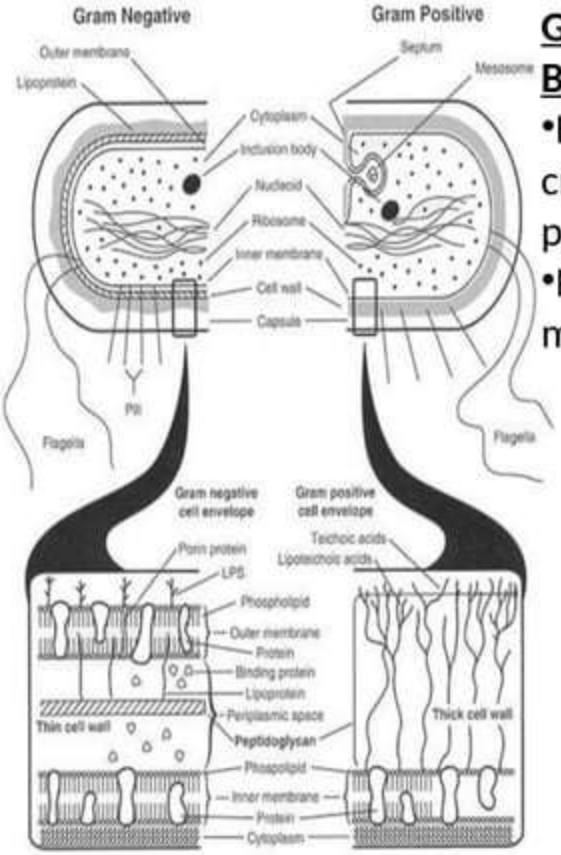


Peptidoglycan: network of sugars cross- linked by short peptides

- Forms the rigid part of the cell wall
- Protects the bacteria against mechanical damage
- Part that picks up the stain in the gram procedure

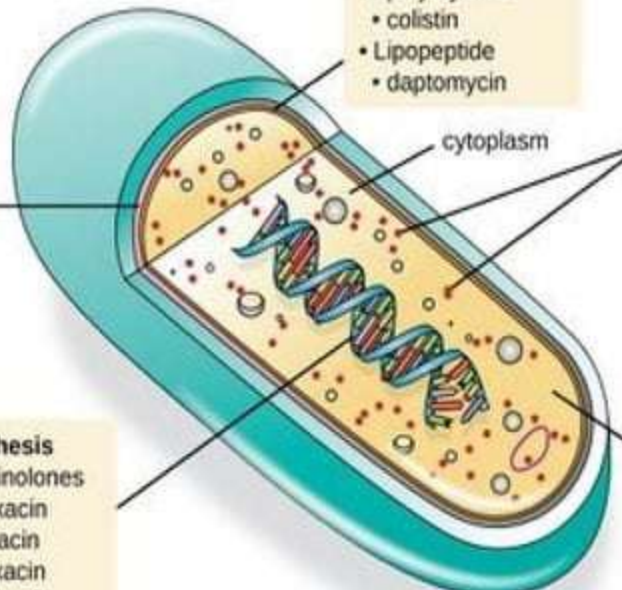
Gram Negative Bacteria

- Small peptidoglycan
- Are stained with crystal violet but decolorized with alcohol after which they pick up the red stain
- LPS on outer membrane toxic for host

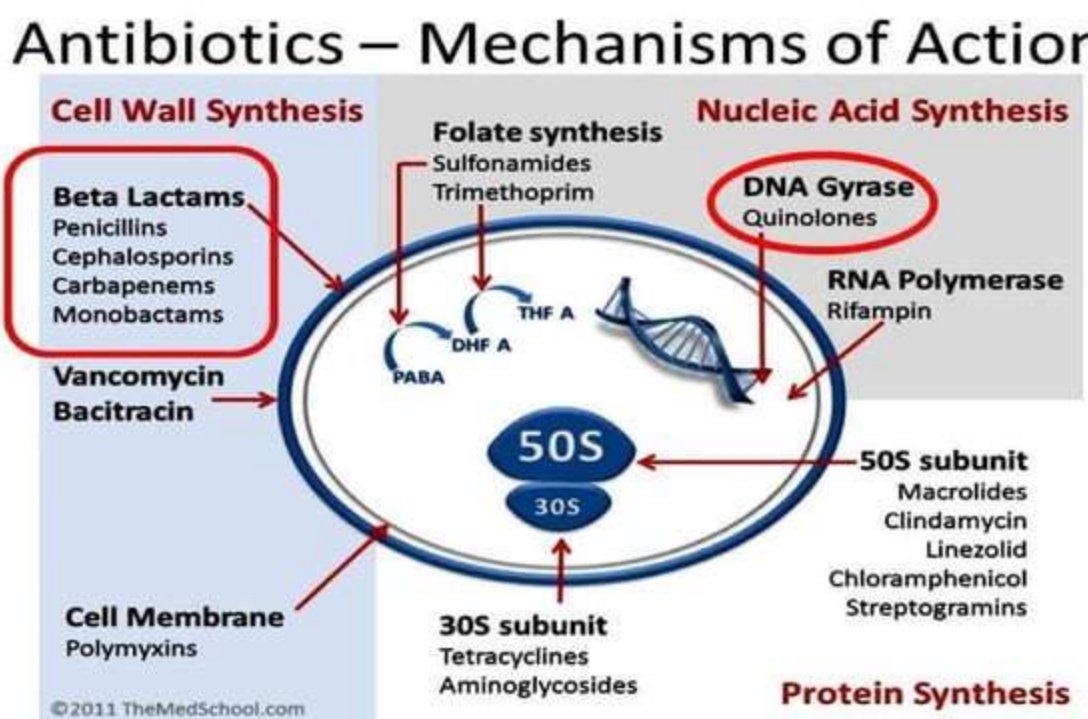


Gram Positive Bacteria

- Large, highly cross- linked petidoglycan
- No outer membrane


Plasma membrane
• Polymyxins
• polymyxin B
• colistin
• Lipopeptide
• daptomycin
Cell wall
• β-lactams
• penicillins
• cephalosporins
• monobactams
• carbapenems
• Glycopeptides
• vancomycin
• Bacitracin
cytoplasm
Ribosomes
• 30S subunit
• aminoglycosides
• tetracyclines
• 50S subunit
• macrolides
• lincosamides
• chloramphenicol
• oxazolidinones
DNA synthesis
• Fluoroquinolones
• ciprofloxacin
• levofloxacin
• moxifloxacin
RNA synthesis
• Rifamycins
• rifampin
Metabolic pathways
• Folic acid synthesis
• sulfonamides
• sulfones
• trimethoprim
• Mycolic acid synthesis
• izoniazid

Mechanism of Antibiotics



Brucellosis an Important Zoonotic Disease

Brucellosis

Brucellosis,

• Brucellosis, also called **Bang's disease**, **Crimean fever**, **Gibraltar fever**, **Malta fever, Maltese fever**, **Mediterranean fever**, **rock fever**, or **undulant fever**, is a highly contagious zoonosis caused by ingestion of unsterilized milk or meat from infected animals or close contact with their secretions.

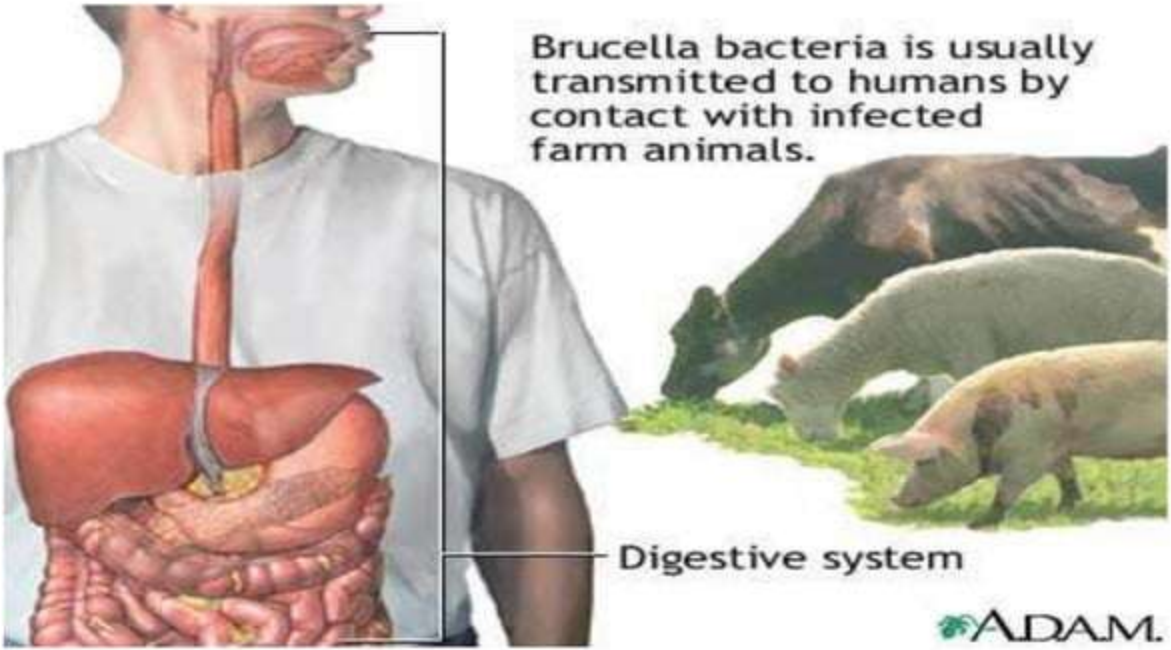
Brucellosis

Brucellosis is a zoonotic infection transmitted to humans contact with fluids from infected animals (sheep, cattle, goats, pigs, or other animals) derived food products such as unpasteurized milk and cheese . The disease is rarely, if ever, transmitted between humans.

Zoonosis

- **Brucellosis**: Disease of domestic and wild animals (zoonosis): Transmittable to humans. It has different non-specific symptoms and signs “
- 1886, **Sir David Bruce isolated Brucella** Melitensis from spleens of malta fever victims.

Major Transmission of Brucellosis



Other names for Brucellosis

Undulant fever

Malta fever

Gibraltar fever

Mediterranean fever

Characteristics of Bacteria

- Brucella spp are small gram-negative aerobic coccobacilli lacking a capsule, flagella, endospores, or native plasmids.Strict aerobic. Non-motile

- **Oxidase** and **catalase** tests are positive for most members of the genus Brucella.
- Some species require CO2 enrichment for primary isolation in the laboratory.

Morphology

Cococcus bacilli or short rods 0.5-0.7x0.6-1.5 µm.

Non motile, non capsulated and non sporing.

Gram negative and non acid fast.

Bipolar staining is not uncommon.

Culture characteristics

Strict aerobes.B.abortus capnophilic 5-10%co_2

Optimum temp. 37°c (range 20-40°c)

Optimum pH 6.6-7.4.

Colonies small,moist,

- Contd.... Fastedius.Scanty and slow growth on simple media.
- Currently employed media 1 Serum dextrose agar;2 Serum potato infusion agar;3 Trypticase soy agar 4 Tryptose agar.
- Addition of Bacitracin;Polymyxin;and Cycloheximide makes the media selective.
- Liquid Media:Uniform growth.
- Solid Media : small.moist,transluscent,glistening.Mucoid,smooth/rough associated with changes in antigenic structure and virulence.

- **Biochemical reactions**: No carbohydrates are ordinarily fermented,though they possess oxidative capacity. Catalase +ve;Oxidase+ve(except B.neotomae and B.ovis). Urease +ve; Nitrates >Nitrites.IMViC Negative. Resistance :Destroyed by heat at 60 c in 10 minutes. and 1% Phenol in 15 minutes.

  RESISTANCE : Survive in soil for several weeks.Viable for 10 days in refrigerated milk,1 month in icecream,4 months in butter and varying periods cheese depending on pH.may survive many weeks in meat.B.melitensis viable for 6 days in urine,6 weeks in dust,and 10 weeks in water.

- **Antigenic Structure:**

  Two main antigenic determinants. A and M.
- A and M monospecific sera are useful for species identification.
- Antigenic crossreactions with Vibrio cholerae, Esch.coli o 116; o 157; Salmonella serotype group N(O 30 kauffman and White)Ps. Maltophila; Y. enterocolitica;F. tularensis;

Identification of Bacteria

- Other methods for the identification and speciation of Brucella include:

→production of urease and H2S

→sensitivity to dyes, basic fuchsin, thionin, and thionin blue

→use of specific antisera

B. abortus

- **Bacteria is excreted in genital secretions (including semen), milk, colostrum.**
- **Survival time**:

  Cheese at 4°C: 180 days !!!

  Water at 25°C: 50 days

  Meat and salted meat: 65 days

  Manure at 12°C: 250 days !!!!

- **Widespread:** Cattle, Bison, Elk, Deer, Moose, Horse, Sheep, Goat, Swine, Donkey, Dogs, Birds, Hares, Fox, Rats, mice, Camels and Human.

B. abortus

- **Sources of Human Infection:**

  Raw milk and products /Direct contact

- **Portal of entry**: oral mucosa, nasopharynx and conjunctivae, genital then **X** in regional lymph node and spread to RES (nodes of udder, uterus, erythritol…). Placentitis with endometritis. Fetus die with edema /congestion of lung, dissimenated hemorrhages of epicardium and splenic capsule. Bacteria in lung and digestive tract of the fetus.

B. melitensis

- **Goat (1886), Sheep, Cow (1905 in Malta), Swine, Hares, Camels, Buffalo, Impala**.

B. suis

- **Wild pigs, Rats, Swine.**
- **Abortion,metritis, bursitis, spondylitis (Lumbar and sacral), arthritis, orchitis, paralysis.**

Brucella canis

- *Brucella canis* was first described as a cause of abortion in beagles in the USA
- It was subsequently shown to infect dogs in many other countries, irrespective of breed
- An occasional cause of brucellosis in humans

Epidemiology

- Brucellosis occurs worldwide; major endemic areas include countries of the Mediterranean basin, Arabian Gulf, the Indian subcontinent, and parts of Mexico, Central and South America
- Human Infection. melitensis is the species that infects humans most frequently.
- The incubation period ranges from a few days to a fewmonths.
- The disease is manifested as fever accompanied by a widen array of other symptoms

Methods of transmission

- Direct inoculation through cuts and skin abrasions from handling animal carcasses, placentas, or contact with animal vaginal secretions
- Direct Conjunctival inoculation
- Inhalation of infectious aerosols
- Ingestion of contaminated food such as raw milk, cheese made from unpasteurized (raw) milk, or raw meat

- Venereal transmission has been suggested, but the data are not conclusive

Incubation period

- Acute or sub acute disease follows an incubation period which can vary from **1 week to 6 or more months**.
- In **most** patients for whom the time of exposure can be identified, the incubation period is between **2 and 6 weeks**
- The length of the incubation period may be influenced by many factors

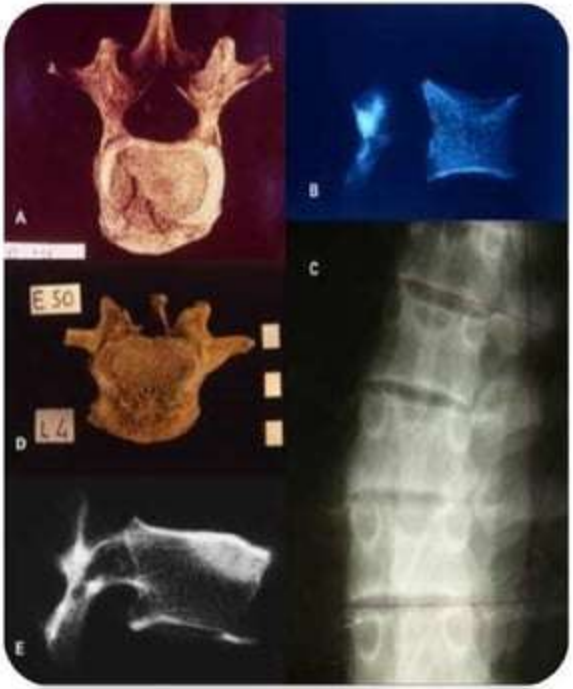
–**virulence** of the infecting strain
–size of the **inoculum**
–**route** of infection
–**resistance** of the host

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

Clinical Manifestation

- Fever
- Night sweats
- Malaise
- Anorexia
- Arthralgia
- Fatigue
- Weight loss
- Depression.



Clinical Manifestations

- The presentation of brucellosis is characteristically **variabl**
- The onset may be **insidious** or **abrupt**
- **Influenza-like** with fever reaching 38 to 40°C

– **Limb and back pains** are unusually severe, night **sweating** and fatigue are marked.

– Anorexia, weakness, severe fatigue and loss of weight, depression

– **Headache**

- The leukocyte count tends to be normal or reduced, with a **relative lymphocytosis**

– Relative leukopenia

- On physical examination, **splenomegaly** may be the only finding.

Clinical features

Often fits one of the three pattern:

febrile illness resembling typhoid, less severe

fever & acute monoarthritis (hip/knee),young child

long lasting fever, hip pain,older man

- Travel to an endemic area
- Occupation

- Consumption of unpasteurized milk

Physical Examination

Physical manifestations may be absent.

- If present,

Focal Features:

Musculoskeletal pain

Osteomyelitis

Septic Arthritis

Minimal lymphadenopathy

Hepatosplenomegaly occasionally.

Systemic Infections with Brucellosis

- Osteoarticular disease, especially sacroileitis — 20 to 30 percent and vertebral spondylitis. Large joints are affected most commonly in children
- Genitourinary disease, especially epididymo-orchitis — 2 to 40 percent of males

- Neurobrucellosis, usually presenting as meningitis — 1 to 2 percent.
- Less common neurologic complications include papilledema, optic neuropathy, radiculopathy, stroke, and intracerebral hemorrhage

Complications and Brucella

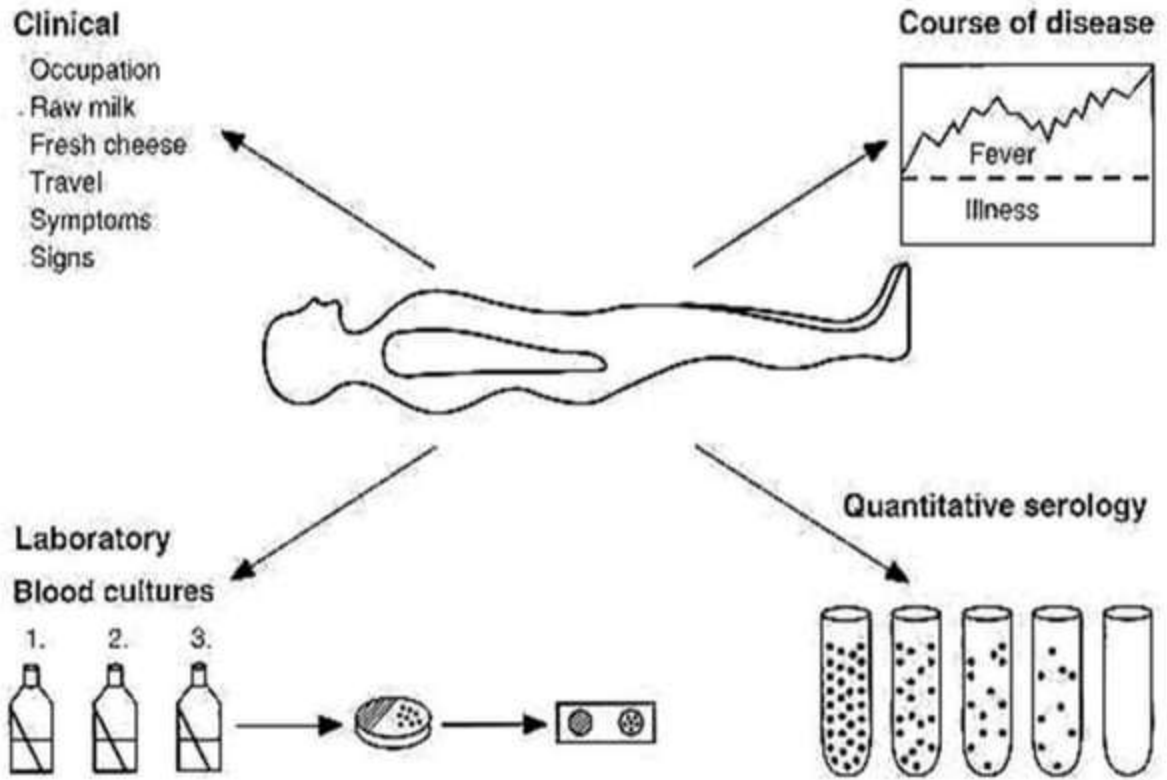
- **Endocarditis** — 1 percent.Most cases of endocarditis are left-sided, and about two-thirds occur on previously damaged valves.
- **Hepatic abscess** — 1 percent
- Other less common complications include pneumonitis, pleural effusion, empyema,, or abscess involving the spleen, thyroid, or epidural space, uveitis.
- A few cases of Brucella infection involving prosthetic devices such as pacemaker wires and prosthetic joints have been reported

Differential Diagnosis

- Tuberculosis
- Toxoplasmosis
- CMV
- HIV infection

Laboratory Diagnosis

History and Physical Exam



Investigations

- Total counts-Normal/reduced
- Thrombocytopenia
- ESR/CRP-Normal/Increased
- CSF/Body fluid analysis-Lymphocytosis, low glucose levels, elevated ADA
- Biopsied samples of lymph node, liver-non caveating granuloma without acid fast bacilli.

Serological Tests

- Most serological studies for diagnosis of Brucellosis are based on antibody detection

These include:

- Serum agglutination (standard tube agglutination)
- ELISA **Rose Bengal agglutination**
- Complement fixation
- Indirect Coombs
- Immunecapture-agglutination (Brucellacapt

PCR

- Polymerase chain reaction (PCR) shows promise for rapid diagnosis of Brucella spp in human blood specimens
- Positive PCR at the completion of treatment is not predictive of subsequent relapse
- PCR testing for fluid and tissue samples other than blood has also been described

Management

- The World Health Organization recommends the following for adults and children older than 8 years:
  - Doxycycline 100 mg PO bid and rifampin 600-900 mg/d PO: Both drugs are to be given for 6 weeks (more convenient but probably increases the risk of relapse).
  - Doxycycline 100 mg PO bid for 6 weeks and streptomycin 1 g/d IM daily for 2-3 weeks: This regimen is believed to be more effective, mainly in preventing relapse.

Treatment

Drugs against Brucella

- Tetracycline's
- Aminoglycosides
  - *Streptomycin since 1947*
  - *Gentamicin*
  - *Netilmicin*
- **Rifampicin**
- **Quinolones - ciprofloxacin**
- **?3rd generation cephalosporins**

Pregnancy and Brucellosis

- Premature labor and fetal wastage
- Rifampin — 900 mg once daily for six weeks
  - Rifampin — 900 mg once daily plus trimethoprim-Sulphmethoxazole(TMP-SMX; 5 mg /kg of the trimethoprim component twice daily) for four weeks

Prevention

- Control of disease in domestic animals
  - immunization using B. abortus strain 19 and B. melitensis strain Rev 1
  - Routine pasteurization of milk
- In labs strict biosafety precautions

BORDETELLA

- Introduction
- The genus Bordetella is named after Jules Bordet, who along with Octave Gengou,identified the small ovoid bacillus in the sputum of children suffering from whooping cough.
- The bacillus is now known as Bordetella pertussis.

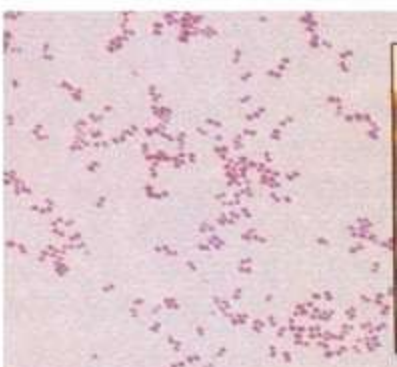
- This child has pertussis. It is difficult for him to stop coughing and to get air. Coughing spasms with a "**whooping**" sound that follows the cough are typical. The sound means child is trying to catch his breath before the next round of coughing

Bordetella

- Genus Bordetella includes gram negative coccobacilli formerly included in the genus Hemophilus.
- Three important spp
- B.pertussis - causes whooping cough
- B.parapertussis – causes milder form of whooping cough
- B.bronchiseptica – causes diseases in animals

Bordetella pertussis

- Morphology & Identification:
- Gram negative cocco bacilli, capsulated, non motile.
- With Toluidine blue stain bipolar metachromatic granules seen
- *Thumbprint appearance*



Bordetella pertussis (Gram sta

Bordetella pertussis

- Culture:
- Requires enriched media like *Bordet-Gengou(glycerol –potato-blood agar)* with PenicillinG 0.5µg/ml used
- Incubated in aerobic moist environment at 35-37^{0}C
- Small opaque greyish colonies resembling *bisected pearls* or *mercury drops*
- Hemolysis present
- Identified by Immunofluorescence

Bordetella pertussis

- Biochemical Reactions:
- Biochemically inactive
- Oxidase and catalase positive
- Forms acid but no gas from glucose and lactose

Bordetella pertussis

- Antigenic Structure
- 1.Agglutinogens – capsular heat labile antigens. Help organisms in attachment to respiratory cells. Used for serotyping
- 1-14 factors present
- 1-6 only in B.pertussis
- Factor 7 found in all three species.
- Factor 12 for B.bronchiseptica and factor 14 for B.parapertussis

Bordetella pertussis

- 2.Pertussis Toxin:
- Heat labile exotoxin.
- Major virulence factor. Responsible for many signs and symptoms of pertussis
- Also known as lymphocytosis producing factor, histamine sensitising factor and islet activating protein
- Has two units A and B.A unit active made up of S1and B unit consists of five polypeptide chains
- Can be toxioded
- Regulated by bvg (bordetella virulence gene)

Bordetella pertussis

- 3.Tracheal Cytotoxin:

  Derived from the peptidoglycan of cell wall. It causes damage to respiratory epithelial cells

- 4.Lipopolysaccharide:

  Endotoxin of the cell wall ,heat stable lipopolysaccharide.

- 5.Adenylate Cyclase:

  This enzyme when taken up by phagocytic cells inhibits their bactericidal activity.

Bordetella pertussis

- 6.Filamentous Haemagglutinin:
- Present on the surface of the bacilli.
- Mediates attachment to the epithelial cells
- Antibodies directed to it are protective
- FHA is used in acellular pertussis vaccine
- 7.Haemolysin
- 8.Heat Labile Toxin

Bordetella pertussis

- Variation :
- Reversible phenotypic modulation occurs when grown at 28^{0}C versus 38^{0}C in the presence of $MgSO_4$ so that hemolytic,toxin producing virulent bacteria is converted into nonhemolytic ,nontoxin producing avirulent bacteria

Bordetella pertussis

- Pathogenesis
- Predominantly a childhood disease- whooping cough
- Source of infection is patient in the early stage of disease,mode of infection is droplets
- Incubation period 1-2 weeks
- Has three stages- catarrhal , paroxysmal and convalscent stage each lasts two weeks

Clinical presentation of Bordetella pertussis

with antibiotics to inhibit growth of normal flora. More rapid diagno-

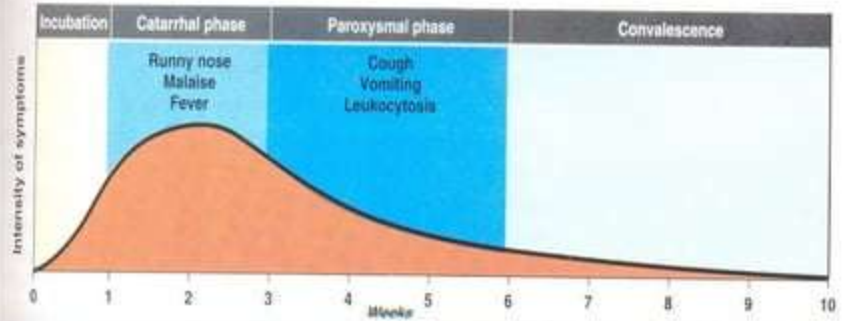


Figure 13.7
Clinical presentation of *Bordetella pertussis* disease.

Bordetella pertussis

- Complications:
- Pressure effects due to coughing like subconjuctival hemorrhage, lung collapse
- Broncho pneumonia ,convulsions and collapse

Bordetella pertussis

- Laboratory diagnosis:
- Microscopy demonstration of bacilli in respiratory secretions in nasal swabs by fluorescent antibody technique
- Culture of per nasal swab , post nasal swab or cough plate sample on Bordet – Gangue medium incubated at 37^0C at high humidity.
- Typical colonies, pearl like appear after 48-72hrs confirmed by microscopy and Immunofluorescence or by slide agglutination with specific antisera

Bordetella pertussis

- PCR
- Most sensitive method to diagnose pertussis.
- Serology
- Rise in titer may be demonstrated in paired sera samples

Bordetella species

Gram (–) rods

Bordetella pertussis (Gram stain)

Bordetella pertussis on Regan-Lowe medium

- **Gram-negative**
- Small coccobacilli that grow singly or in pairs
- Encapsulated
- Aerobic
- Culture on Regan-Lowe agar

Bordetella pertussis

- Pertussis (whooping cough)

PENICILLINS
CEPHALOSPORINS
TETRACYCLINES
AMINOGLYCOSIDES

Macrolides	1	Erythromycin

FLUOROQUINOLONES

Other	2	Trimethoprim/ sulfamethoxazole

Ciliated cells of the respiratory system infected with *Bordetella pertussis* (colorized scanning electron micrograph)

Bordetella pertussis

- Treatment : **Erythromycin** is the drug of choice
- Prevention:
- A killed whole bacterial vaccine is normally administered as DPT combination. Three I.M injections 4-6weeks apart starting at 6weeks and a booster dose at the end of first year.
- B.pertussis acts as an adjuvant for toxoids
- An acellular vaccine consisting of filamentous hemagglutinins is also available and is recommended for booster shots.

Bordetella parapertussis

- Responsible for 5% of whooping cough cases and produce mild disease
- *B.parapertussis* grows on common laboratory media and can be distinguished from *B. pertussis* in that *B. pertussis* is oxidase positive but urease negative, while *B. parapertussis* is oxidase negative and urease positive.

Bordetella bronchiseptica

- causes diseases in animals and cause 0.1% cases of whooping cough in humans.
- Motile with peritrichate flagella
- *B. bronchoseptica* is positive for both urease and oxidase


Thank you