

Brucella and Bordetella

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Bordetella

Bordetella

- **Gram-negative coccobacilli**
- **Strict aerobes**
- **Catalase, oxidase positive**
- **Do not ferment carbohydrates**
- **H₂S, indole, citrate and VP negative**
- **Parasite in respiratory tract of human beings, animals or birds.**

Disease

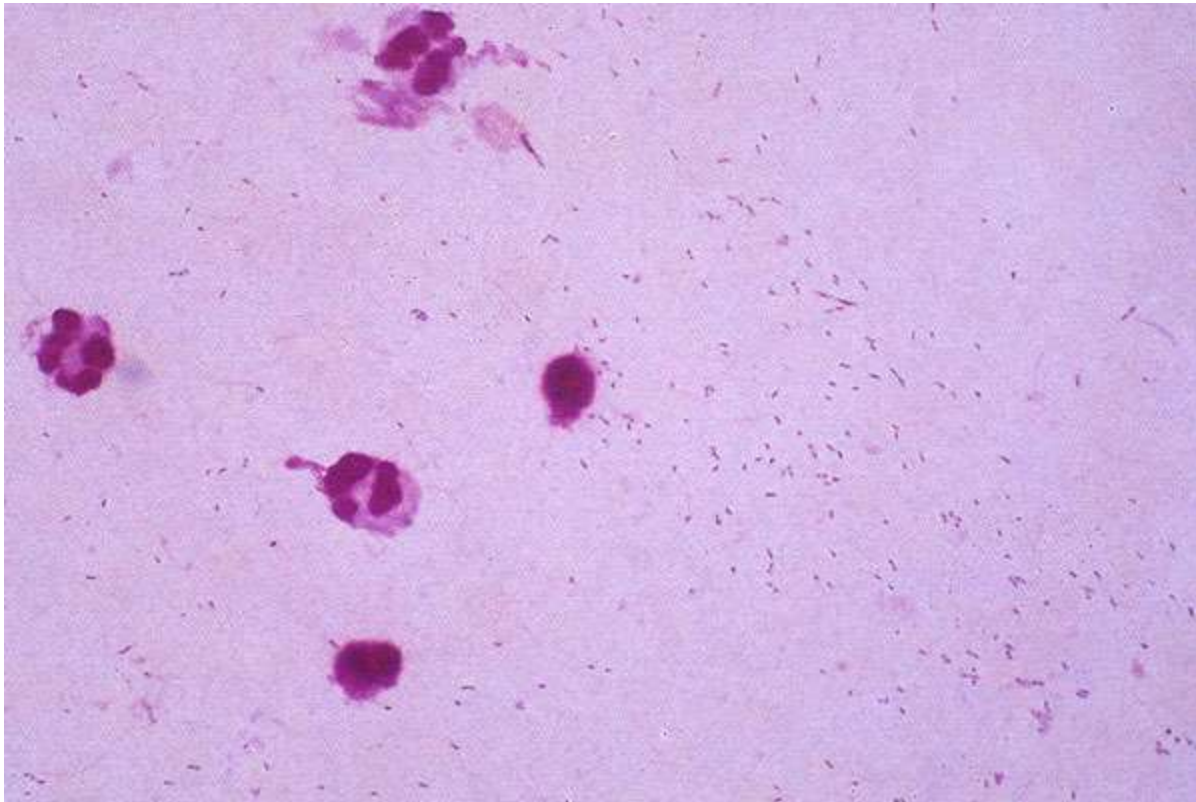
- Causative agent of
pertussis/
whooping cough/
100days fever

Bordetella pertusis

Morphology

- Gram-negative
- **Metachromatic granules on toluidine blue stain**
- small, ovoid, coccobacilli
- Non motile
- Non sporing
- Capsulated
- Arranged in clumps
- **Thumb print appearance**

Gram stain Bordetella



Cultural characteristics

- Strict aerobes
- Grows on complex media
- Growth best 35 -36⁰ C
- **Bordet Gengou glycerin potato blood agar/ Regan lowe media**
- Charcoal blood agar
- Colonies on Bordet gengou glycerin potato blood agar – small , doom shaped, smooth, viscid, glistening resembling **bisected pearls or mercury drops**
- **Confluent growth- aluminium paint appearance**

Biochemical Reactions

Biochemically inactive

- Sugars not fermented
- Indole and VP negative
- Catalase positive usually
- Oxidase positive
- Nitrates not reduced to nitrites

Pathogenesis

- Infection initiated by attachment to ciliated epithelium of NP (med by surface adhesins)
- Local cellular invasion with intra-cellular persistence
- **No systemic dissemination**
- Systemic manifestations due to toxin

Pathogenesis

- Incubation period 1 to 2 weeks
- **Three stages** : catarrhal, paroxysmal, convalescent
- **Catarrhal** : fever, cough, sneezing
(2 wks) maximum infective stage
- **Paroxysmal** : characteristic inspiratory gasp, paroxysm of cough, posttussive vomiting
- Most complications
(whooping cough 2-4 weeks)
- **Convalescent** : decreased severity and frequency of coughing(2-4 wks)

- **Complications :**
- **Pressure symptoms:** subconjunctival hemorrhage, subcutaneous emphysema
- **Respiratory:** broncho- pneumonia, lung collapse
- **Neurological:** convulsions, coma

Lab diagnosis

- **Direct demonstration of bacilli in respiratory secretions by fluorescent antibody techniques.**
- **Culture :** different methods (dacron/Ca-alginate swab)
- **Nasopharyngeal aspirate- Best specimen**
- **The prenasal swab**, swabs to be plated without delay
- The cough plate method
- The post nasal (per oral) swab

Growth on charcoal blood agar



Treatment

- **Macrolide**- Erythromycin, Azt, Clarithromycin is drug of choice
- Chloromphenicol, Cotrimoxazole also useful
- Non immunized contacts – Erythromycin prophylaxis

Prophylaxis- active immunization

- **Killed vaccine**
- Administered with tetanus and diphtheria toxoid (triple vaccine)
- Three injections at 6, 10, 14 weeks of life
- Booster dose at 18 mths
- More adverse effects in age > 7years(CI)

- **Acellular vaccine** – Less reactogenic (mainly contains- PT, pertectin, FHA)

- **90% protection rate**

Chemoprophylaxis

- For household contacts- Erythromycin (DOC)

Pseudo-whooping cough

- By other respiratory pathogens_ Adenovirus, Mycoplasma pneumoniae

Brucella

Brucellosis

- Zoonotic disease of economic importance
- primarily affecting goats, sheep, cattle, buffaloes, pigs
- Transmitted to humans by contact with infected animals or through their products
- In humans it causes Malta fever/Undulant fever
Mediterranean fever

- Pathogenic types in humans - *B. melitensis, abortus, canis and suis*
- *B. abortus* -- CO₂ supplementation (5-10%) ; capnophilic
- Growth on simple media, growth slow and scanty
- lipopolysaccharide of cell wall: major antigen; contains the A and M antigens
 - Useful for species identification by the agglutination test
- Antigenic cross reaction
 - *V. cholerae*, *E. coli* O: 116, O:157, *Salmonella* serotypes, *Yersinia enterocolitica*, *Francisella tularensis*

Transmission

- Infection can be transmitted to humans through –
 1. **Contact**– brucellae in vaginal discharges, placenta, urine, manure, carcasses enter through skin, mucosa, conjunctiva; especially important as occupational hazard
 2. **Ingestion** – Most common method of transmission , unpasteurized milk or dairy products, rarely contaminated vegetables or water
 3. **Inhalation** – of dried material of animal origin such as dust from wool

- *Brucella melitensis* – predominantly in goats and sheep
- *Brucella abortus* – cattle
- *Brucella suis* – swine

Clinical features

- Latent infection – only serological but no clinical evidence
- Acute/subacute brucellosis
- Chronic brucellosis

Clinical Features

- Acute or insidious onset, continued, intermittent or irregular fever of variable duration, profuse sweating, particularly at night, fatigue, anorexia, weight loss, headache, arthralgia, and generalized aching
- Local infection of organs may occur.
- Possible complications: arthritis, uveitis, sacroiliitis, spondylitis (10% of cases), meningitis (5%), and epididymoorchitis
- Cardiovascular
Endocarditis resulting in death
- Most recover entirely within 3 to 12 months

Diagnosis

- History of animal contact is pivotal
 - Occupation
 - Raw milk
 - Fresh cheese
 - Travel
- In endemic area, it should be in the DDx of any nonspecific febrile illness

Lab diagnosis

- Direct demonstration
- Culture
- Serology
- Hypersensitivity tests
- Animal inoculation
- PCR
- Typing

Castaneda method

- **Both liquid and solid media are placed within the same container**
- No need of subculture on solid media
- To see the growth on solid phase, liquid is allowed to spread over the solid slant at a regular interval (every 48 h)
- Advantages :
 - reduce manipulations & materials,
 - chances of contamination is less
 - Prevent laboratory acquired infections

Bone marrow culture:

- **gold standard**
- High concentration of Brucella in RES
- Disadvantages:
 - Invasive, Painful
 - Results not universally reproducible

Culture :

lymph node aspirate, CSF, urine, sputum, breast milk, abscesses, vaginal discharge

Serological test

- 1. Standard agglutination test**
- 2. ELISA**
- 3. CFT**

Standard agglutination test

- **Tube agglutination test**
 - Equal vol of serial dilutions of pt's serum & standardised ag (killed suspension of standard strain of Brucella abortus) are mixed
 - Incubated at 37C for 24 hrs or 50C for 18 hrs
 - Titre of 160 or more – significant
 - Blocking abs or nonagglutinating abs – removed by heating serum at 55C for 30 min or using 4% saline as diluent for the test

- SAT identifies mainly the IgM antibody; IgG and IgA may act as blocking abs
- CFT is more useful in chronic cases as it detects the IgG ab also
- ELISA – sensitive, specific ; can detect IgM & IgG separately

- **Brucellin skin test** – delayed hypersensitivity type skin test with brucella ag
- **Not useful in diagnosing acute brucellosis**
- Only indicates prior sensitisation with the ag
- Brucellin testing may lead to rise in titre of antibodies

- Demonstration of brucellae by microscopy in clinical specimens – **immunofluorescence**
- **Rapid methods for detection of brucellosis in herds of cattle**
 - Rapid plate agglutination test
 - Rose bengal card test- detects antibodies

Milk ring test

- Sample of whole milk is mixed well with a drop of stained brucella ag (conc. suspension of killed Br abortus stained with haematoxylin)
- Incubated in water bath at 70C for 40-50 min
- **If Abs +, bacilli are agglutinated and rise with cream to form blue ring at the top, leaving milk unstained**
- **Whey agglutination test** – another method for detecting abs in milk

Treatment

- **Doxycycline for 45 days with streptomycin IM** daily for first 2 weeks in adults
- In children – **cotrimoxazole**
- **Doxy** along with rifampicin for prophylaxis
- Prevention – pasteurisation of milk
- Detection of infected animals, elimination by slaughter